ENVIRONMENTAL ASSESSMENT

NADA 140-854

OXFENDAZOLE, 9.06% ORAL SUSPENSION
OXFENDAZOLE, 22.5% ORAL AND INTRARUMINAL SUSPENSION

1. **DATE**

April 1990

2. **NAME OF APPLICANT/PETITIONER**

Syntex Animal Health, Inc.

3. **ADDRESS**

3401 Hillview Avenue
Palo Alto, CA 94304

4. **DESCRIPTION OF PROPOSED ACTION**

Syntex Animal Health, Inc. is requesting approval for the use of oxfendazole as a broad-spectrum anthelmintic. Oxfendazole will be administered as a single-dose treatment for helminth infections of all classes of cattle, including non-lactating dairy cows, replacement heifers and beef cattle. Retreatment may be employed (at four to six weeks following initial treatment) in those situations, such as pastures, where reinfection with helminths is a common occurrence.

Oxfendazole is an anthelmintic substance which is effective against all classes of helminths which commonly infect domestic animals (i.e., gastrointestinal roundworms, lungworms, and tapeworms). The mode of action of oxfendazole is similar to that of other 2-amino substituted benzimidazoles in that it eliminates helminths by interfering with the polymerization of microtubulin [1]. The broad spectrum of activity of oxfendazole is related to its level of absorption and distribution. Oxfendazole possesses a wide spectrum of activity, thereby negating the need for combination therapy and offering significant therapeutic benefit to the livestock owner.

Oxfendazole, the active ingredient in the products which are the subject of the proposed action, is manufactured at the facility of Syntex Pharmaceuticals International Limited, Bahamas Chemical Division, Grand Bahama Island; and at the facility of Syntex Chemicals, Inc. (a subsidiary of Syntex USA), Boulder, Colorado, USA. The drug substance will be shipped by Syntex to Diamond Scientific Co., Des Moines, Iowa, USA and/or Coopers Animal Health, Inc., Kansas City, Kansas, USA for manufacturing and packaging of the final dosage forms. The products will be distributed throughout the United States for use in cattle.

The following environments may be affected by the proposed action:

- The environments adjacent to the facilities located in the Bahamas; Boulder, Colorado; Des Moines, Iowa; and Kansas City, Kansas.

- Feedlots and other cattle environments receiving residues of oxfendazole contained in animal wastes.

- Agricultural lands receiving animal wastes containing oxfendazole residues.

- The heaviest use of oxfendazole will be in the larger feedlots located throughout the midwestern, southwestern, and western plains states. Areas of the next greatest use will be the Mississippi, Missouri, and Ohio River valleys where smaller feedlots are more common and animals may be raised on pasture.

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

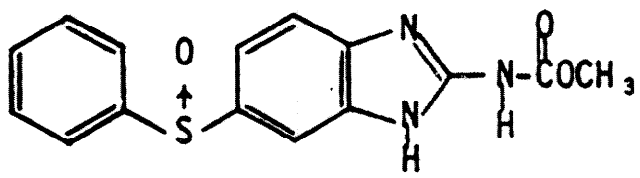
Oxfendazole is a member of a well-known and widely used chemical class of compounds, the benzimidazoles, and is related in chemical stucture and pharmacological properties to other drugs commercially available in the United States, such as thiabendazole, fenbendazole, oxibendazole, mebendazole and albendazole. Other related compounds available on the international market include febantel and triclabendazole. Both thiabendazole and mebendazole are currently approved for use in humans in the United States.

SUBSTANCE: Oxfendazole

CAS REGISTRY NUMBER: 53716-50-0

CAS NOMENCLATURE: Methyl (5-phenylsulfinyl)-2-benzimidazole carbamate

STRUCTURAL FORMULA:



MOLECULAR FORMULA: $C_{15}H_{13}O_3N_3S$

MOLECULAR WEIGHT: 315.34

MELTING POINT: 245-265 ° C (with decomposition)

SOLUBILITY: Solubility in water is 3.11 - 4.63 mg/L

OCTANOL/WATER PARTITION COEFFICIENT: Log K_{ow} 1.953 +/- 0.162

UV SPECTRUM: A typical UV spectrum tracing for the oxfendazole reference standard (lot B-6-JK-001) is presented in Appendix 1. At 295 nm, E% = 549.5. At 226 nm, E% = 1,447.5.

IR SPECTRUM: A typical IR spectrum tracing for the oxfendazole reference standard (lot B-6-JK-001) is presented in Appendix 2.

IMPURITIES: Oxfendazole active ingredient contains not less than 97.0% of $C_{15}H_{13}O_3N_3S$, calculated on a dried basis, not more than 2.0% of methyl(5-phenylsulfonyl)-2-benzimidazole carbamate, and not more than 1.0% of any other individual foreign related substance.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

Introduction of oxfendazole into the environment can occur from three sources: 1) the oxfendazole drug substance manufacturing facilities, 2) the oxfendazole formulated drug product manufacturing facilities, and 3) the sites of intended use of the formulated drug products in cattle and adjacent agricultural lands.

INTRODUCTION OF SUBSTANCES THROUGH THE MANUFACTURING PROCESS

For those facilities at which oxfendazole drug substance will be manufactured by Syntex (Syntex Pharmaceuticals International Limited and Syntex Chemicals, Inc.), the introduction of substances into the environment will be negligible because manufacturing will be carried out in modern facilities, well equipped for waste water disposal and air pollution control, which comply with all applicable environmental laws and regulations.

At Syntex Pharmaceuticals International Limited, Bahamas Chemical Division, Grand Bahama Island, oxfendazole is manufactured in compliance with those regulations promulgated under the Environmental Health Services Act, 1985. A local environmental assessment for the facility at Syntex Pharmaceuticals International Limited, Bahamas Chemical Division, Grand Bahama Island, is presented in Appendix 3.

At Syntex Chemicals, Inc., Boulder, Colorado, oxfendazole is manufactured in compliance with the applicable environmental laws and regulations listed below:

Federal Laws

- Clean Air Act, as amended
- Resources Conservation and Recovery Act, as amended
- Water Pollution Control Act, as amended
- Emergency Planning and Community Right-To-Know Act of 1986
- Occupational Safety and Health Act of 1970, as amended

State Laws and Regulations, as amended

- Colorado Water Quality Act
- Colorado Water Quality Regulations
- Colorado Water Quality Standards
- Colorado Air Quality Act
- Colorado Air Pollution Control Regulations
- Colorado Discharge Permit System Regulations
- Colorado Disposal Sites and Facilities Law
- Colorado Hazardous Waste Act
- Colorado Hazardous Waste Management Regulations
- Colorado Standards for Owners and Operators of Hazardous Waste Treatment, Storage and Disposal Facilities

A local environmental assessment for the facility at Syntex Chemicals, Inc., Boulder, Colorado, USA is presented as Appendix 4.

For those facilities at which oxfendazole formulated drug product will be manufactured and packaged for Syntex Animal Health, Inc. (Diamond Scientific Co. and Coopers Animal Health, Inc.), the introduction of substances into the environment will be negligible because manufacturing and packaging will be carried out in modern facilities, well equipped for waste water disposal and air pollution control, which comply with all local, state and federal environmental laws and regulations.

Diamond Scientific Co., Des Moines, IA, and Coopers Animal Health, Inc., Kansas City, KS have indicated their compliance with all applicable environmental laws and regulations in local environmental assessments prepared by each individual contract manufacturer as presented in Appendix 5 (Diamond Scientific Co.) and Appendix 6 (Coopers Animal Health, Inc.).

At all manufacturing locations, Material Safety Data Sheets (MSDS) are available for all employees involved in the production of the drug substance and the formulated products.

INTRODUCTION OF SUBSTANCES THROUGH TREATED ANIMALS

Theoretical Amounts of Oxfendazole and Metabolites Eliminated by Target Animals

Decisions regarding the environmental safety of oxfendazole and its metabolites are based on the relationship between the residue concentration expected in the environment and residue levels expected to have no adverse effect on aquatic and terrestrial resources based on appropriate environmental-effects screening tests. Environmental residues are estimated from drug use and properties governing the behavior of the expected residues in feedlot and agricultural soils.

For practical purposes, the product will be introduced into the environment only when it is excreted by treated animals. Handling, distribution and storage of the finished product will not result in environmental exposure because the product is marketed in closed plastic containers.

Although the major use of oxfendazole will be in pastured and feedlot cattle, the "worst case" environmental concentrations and exposures to oxfendazole and its metabolites are expected to occur when the drug is used in feedlot cattle. As a consequence, residues of oxfendazole and its metabolites will be introduced into agricultural soils when feedlot manure is introduced into this environment as a fertilizer. In order to determine the expected concentrations of oxfendazole and its metabolites in the feedlot and agricultural soils, and how they relate to the environmental studies, the following information was considered.

Average days to finish for feedlot steers and heifers [2]	136 days
Average starting weight for feedlot steers and heifers [2]	678 lb /308 kg
Average spreading rate of manure onto agricultural soil [3]	20 tons/acre
Manure excreted per 1,000 lb. live weight for finishing cattle [3]	8.5 tons/year
Manure excreted per 1,000 lb. live weight adjusted for 60% loss of moisture due to exposure to weather in feedlot [3]	3.4 tons/year

Oxfendazole will be administered at a dose level of 4.5 mg/kg of body weight. The only expected area of concentrated use for the formulated drug product will be the feedlot. Although retreatment at four to six weeks following initial treatment, if reinfection is suspected, is provided for on the product label, treatment is not likely to be repeated in the feedlot due to the fact that feedlot conditions are not conducive to reinfection. If a second dose of oxfendazole were to be administered to cattle in the feedlot, the ultimate effect would be a doubling of the estimated concentrations presented below. Calculations performed to estimate the expected residues of oxfendazole and its metabolites as runoff from the feedlot and in agricultural soils follow.

(1) 308 kg (average weight of animal) X 4.5 mg/kg (drug dosage) = 1,386 mg OFZ equivalents/treated animal

Since, on the average, manure is not removed from the feedlot more than once per feedlot period, the expected concentration of oxfendazole and metabolite residues can be calculated in feedlot manure. Oxfendazole and metabolite residue concentrations are based on a complete elimination of the oxfendazole dose and

uniform mixing of the dose into the total amount of manure eliminated during the feedlot period.

In addition, the total amount of oxfendazole equivalents eliminated in the average feedlot per animal per feedlot period is calculated as follows:

(2) 136 days X (3.4 tons / 365 days) =

1.2668 tons of manure/animal/feedlot period*

(* this figure is based on a 1,000 lb animal for a 136 day feedlot period)

The maximum concentration of oxfendazole equivalents in the manure would be:

(3) $\frac{\text{1,386 mg OFZ equivalents/animal}}{\text{1.2668 tons of manure/animal}}$ =

1,094 mg OFZ equivalents / ton of manure

or

0.547 mg OFZ equivalents / lb of manure

or

1.2 mg OFZ equivalents / kg of manure

Theoretical Amounts of Oxfendazole and Metabolites in Feedlot Runoff and in Agricultural Soil Under Two Extreme Conditions

FEEDLOT RUNOFF

Under "worst case" conditions, assuming all of the applied oxfendazole and metabolite residues are contained in a two inch rainfall in the feedlot where each animal occupies 200 square feet of space [4], the concentration of these residues in the runoff (without taking water/soil equilibration into account) can be estimated as follows:

(4) $\frac{\text{200 sq ft}}{\text{animal}}$ X 2 inch rainfall X $\frac{\text{1 ft}}{\text{12 inches}}$ X 28.317 L / cu ft =

943.9 L water

(5) $\frac{\text{1,386 mg OZF equivalents / animal}}{\text{943.9 L water}}$ = 1.468 mg OFZ equivalents / L

or

1.468 ppm OFZ in feedlot runoff

AGRICULTURAL SOIL

The average spreading rate of manure per acre of agricultural soil was estimated to be 20 tons per acre. Since 20 tons of manure equals 40,000 lb, and the concentration of oxfendazole equivalents per pound of manure is 0.547 mg per pound, the following relationship is established.

(6) $\frac{40{,}000 \text{ lb manure}}{\text{acre}} \times \frac{0.547 \text{ mg OFZ equivalents}}{\text{lb}} =$

$$\frac{21{,}880 \text{ mg OFZ equivalents}}{\text{acre}}$$

It is assumed that manure will be mixed into the top six inches of soil, equivalent to a soil weight of 9.09×10^5 kg/acre (1.47 gm/cc) [5]. Therefore,

(7) $\frac{21{,}880 \text{ mg OFZ equivalents/acre}}{9.09 \times 10^5 \text{ kg soil/acre}} \times \frac{1{,}000 \text{ ug}}{\text{mg}} =$

$$\frac{24.07 \text{ ug OFZ equivalents}}{\text{kg of soil}}$$

or

24 ppb OFZ equivalents in soil

7. FATE OF SUBSTANCES EMITTED INTO THE ENVIRONMENT

Inasmuch as the primary manner of environmental exposure will be through excretion of oxfendazole by treated animals into the environment, Syntex has undertaken or commissioned a number of studies to determine the fate of oxfendazole in the environment.

Water Solubility Of Oxfendazole And Oxfendazole Amine

The water solubility of oxfendazole was studied at Syntex Research by a number of methods. When the data from this study were considered in conjunction with reports of the dissociation constants of oxfendazole, the solubility of oxfendazole was found to be as follows:

pH	Solubility (ppm)
5.22	2.12
5.68	2.47
6.28	2.33
6.96	2.35
7.58	2.29
8.13	2.34

It is clear from these data that oxfendazole is relatively water-insoluble.

A more detailed summary of this study is presented in Appendix 7.

The major hydrolytic degradation product of oxfendazole is 2-amino-5(6)-phenylsulfinyl benzimidazole (oxfendazole amine). Its solubility was studied by Method 3.01, IV B, Undersaturation/Oversaturation, as described in the FDA Environmental Assessment Technical Handbook. Triplicate suspensions of the compound were prepared in aqueous buffer mixtures at pH 5.0, pH 6.9, and pH 8.9.

Equilibration solubility was reached at pH 5.0 within seven days, allowing an estimate of the solubility of 46 ppm. At pH 6.9 and pH 8.9, true equilibration was not achieved even at 28 days. As a result, the solubility of oxfendazole amine at these pH values can be estimated only to be greater than 50 ppm.

A more detailed summary of this study is presented in Appendix 8.

Determination of Apparent pK1a and pK2a Values from Solubility Variation with pH Studies

The solubility and pKa values of oxfendazole were determined by the general solubility method. The pK1a of the conjugate acid of oxfendazole was calculated to be 3.54 ± 0.28 and the pK2a was calculated to be 9.81 ± 0.31. Solubility over the pH range of environmental interest is listed under Water Solubility, above.

A more detailed summary of this study is presented in Appendix 9.

Octanol/Water Partition Coefficients

A study was conducted to determine the tendency of oxfendazole to distribute between n-octanol and water. The purpose of this study was to evaluate the potential for oxfendazole to accumulate in lipoid tissue and to sorb onto soil particles.

The n-octanol/water partition coefficient (K_{OW}) observed in this study was about 90, or $\log K_{OW} = 1.95$. This value is far below those values ($\log K_{OW} \geq 4$) considered likely to result in bioconcentration or to sorb significantly (FDA Environmental Assessment Technical Assistance Document 3.02, March 1987).

A more detailed summary of this study is presented in Appendix 10.

Vapor Pressure

The vapor pressure of oxfendazole was measured by a method which utilizes electron impact mass spectrometry. From the results of this study, it can be stated that the ambient vapor pressure of oxfendazole is between 10^{-6} and 10^{-11} Torr. Thus, the vapor pressure of oxfendazole is below a level of environmental concern.

A more detailed summary of this study is presented in Appendix 11.

UV-Visible Absorption Spectrum

A UV-visible absorption spectrum analysis was conducted to determine the ability of oxfendazole to absorb radiation in the UV-visible spectrum. Absorbance maxima were observed at 228 nm and 295 nm, with band widths of 31.8 and 19.3, respectively.

A more detailed summary of this study is presented in Appendix 12.

Soil Sorption and Desorption

Soil sorption/desorption studies were conducted by Biospherics Incorporated (Maryland) according to CVM/FDA Environmental Assessment Technical Handbook Document 3.08. A screening test demonstrated that oxfendazole readily sorbed to and did not readily desorb from three representative soil samples. Data from this screening study, in which oxfendazole was partitioned between 100 mL of 0.01 M calcium chloride solution containing 20 gm of soil, are summarized below:

Adsorption:

Soil Type	% Adsorbed	Kd	Koc
Sandy Loam	53±1	6.02 ± 0.14	512± 12
Silt Loam	43 ± 2	3.87 ± 0.29	470 ± 35
Clay Loam	76 ± 1	16.73 ± 1.3	1673 ± 130

Desorption

Soil Type	% Desorbed*	Kd	Koc
Sandy Loam	33	22.8 - 25.5	1938 - 2168
Silt Loam	45	16.7 - 13.4	2028 - 1627
Clay Loam	19	43.7 - 57.3	4370 - 5730

* The sum of two rinses.

Based on these results, an advanced test was conducted. The kinetic portion of this study indicated that equilibrium was achieved within 22 hours. The material balance portion of this study indicated that from 106% to 111% oxfendazole could be recovered after equilibration. The isotherm portion of this study indicated that adsorption on all three soils followed the Freundlich isotherm. Distribution coefficients from Freundlich plots are summarized below:

Soil Type	K	1/n	Koc	% Organic Matter
Sandy Loam	6.4	0.90	544	2.0
Silt Loam	4.5	0.87	546	1.4
Clay Loam	15.7	0.93	1570	1.7

These data demonstrate that oxfendazole would have low mobility in soil, being most readily adsorbed by the clay loam, less readily by the sandy loam, and least readily by the silt loam soil.

A more detailed summary of this study is presented in Appendix 13.

Biodegradation in Soil

In a study conducted by Biospherics Incorporated, the biodegradation of radiolabeled oxfendazole was determined in three different types of soil. Glucose was used as a reference known to biodegrade in order to validate the viability of the soil micro-organisms. The soils selected were appropriate since they had pH values within the range that oxfendazole does not change form.

After 68 days on test, at least 60% of the ^{14}C-glucose was metabolized. However, oxfendazole degraded only minimally to carbon dioxide in the three types of soil. The sulfone analogue of oxfendazole was the major degradation product found on recovery of oxfendazole initially added to the soil.

A more detailed sumary of this study is presented in Appendix 14

Hydrolysis of Oxfendazole

Two studies were carried out in which the hydrolysis of oxfendazole was studied.

In the first study, conducted by Biospherics Incorporated, the hydrolysis of ^{14}C-labelled oxfendazole was determined in buffer solutions at pH 5, 7, and 9 over a 28-day period. This study found that little, if any, degradation of oxfendazole took place at either pH 5 or 7. Therefore, neither hydrolysis rate constants or half-lives could be estimated for these pH values. However, at pH 9, the hydrolytic rate constant was determined to be 3.94×10^{-2} day^{-1} and the half-life was determined to be 17.6 days.

A more detailed summary of this study is presented in Appendix 15.

In the second study, conducted by Syntex Research, the hydrolysis of oxfendazole in aqueous buffer solution was studied at 80°C as a function of pH. When the data from this study are combined with those from the Biospherics study, the variation of hydrolytic half-life of oxfendazole with pH is as follows:

pH	T 1/2 (days)
5.17	2,983
6.58	946
7.10	383
7.82	157
8.32	51.9
8.56	33.8
9.16	17.6

A more detailed summary of this study is presented in Appendix 16.

Impact of Hydrolysis on the Concentrations of Oxfendazole in Feedlot Manure

The effect of hydrolysis on the concentrations of oxfendazole in feedlot manure over a period of 136 days is presented in the following table:

pH of Manure	T 1/2 (days)	C (initial)/C	C (ppb)
5.17	2983	1.032	1163
6.58	946	1.105	1086
7.19	383	1.279	938
8.56	33.8	16.26	73.8

T 1/2 = half-life
C (initial) = initial concentration of oxfendazole in manure (1,200 ppb)
C = final concentration of oxfendazole in manure after 136 days

A more detailed summary of these calculations is presented in Appendix 17.

Impact of Hydrolysis on the Concentrations of Oxfendazole in Agricultural Soils

The effect of hydrolysis on the concentrations of oxfendazole in agricultural soils of various pH levels is presented below:

pH	Maximum Soil Concentration (ppb) *
5.17	297
6.58	103
7.19	49.8
8.56	24.1

*Based on annual application of manure containing 1200 ppb of oxfendazole equivalents to achieve an initial concentration of 24.07 ppb of oxfendazole equivalents when the manure is mixed into the top six inches of agricultural soil.

A more detailed summary of these calculations is presented in Appendix 18.

Photolytic Decomposition of Oxfendazole in Aqueous Solution

In a study designed to conform to Method 3.10 in the FDA Environmental Assessment Technical Assistance Document, the photodegradation of oxfendazole in aqueous solution was characterized. This study was carried out at Syntex Research in Palo Alto, California, using solutions of oxfendazole at nominal concentrations of 1.5 ppm (approximately 1/2 maximum solubility). Solutions of oxfendazole and a reference actinometer (p-nitroacetophenone/pyridine) were exposed to natural sunlight.

After compensation for changing solar radiation by comparison with the photodegradation of the reference compound, the environmental summer season half-lives of oxfendazole (T 1/2) were calculated and were as follows:

pH	T 1/2 (days)
5.0	0.100
6.9	0.121
8.9	0.073

A more detailed summary of this study is presented in Appendix 19.

Photolytic Decomposition of Oxfendazole Amine in Aqueous Solution

The major product from hydrolysis of oxfendazole is the amine, 2-amino-5(6)-phenylsulfinyl benzimidazole. This study was conducted at Syntex Research to characterize the photodegradation of this amine. The study was designed to conform to Method 3.10 in the FDA Environmental Assessment Technical Assistance Document.

Solutions of oxfendazole amine at nominal concentrations of 11 ppm were exposed to natuaral sunlight at pH 5.11, pH 6.97, and pH 9.05. The reference chemical was p-nitroacetophenone/pyridine. Test solutions of oxfendazole and the referenece actinometer were exposed for four-hour periods, beginning about one hour before solar noon.

After compensation for changing solar radiation by comparison with the photodegradation of the reference compound, the photodegradation summer half-lives of oxfendazole amine are as follows:

pH	T 1/2 (days)
5.11	0.28
6.97	0.095
9.05	0.085

A more detailed summary of this study is presented in Appendix 20.

Summary of the Environmental Fate Studies Applied to the Degradation of Oxfendazole in The Environment

- **Aquatic Environment**

Feedlot runoff concentrations of oxfendazole have been calculated without providing for soil adsorption or desorption (soil adsorption/desorption data presented above). It has been demonstrated that oxfendazole readily adsorbs to and does not readily desorb from common agricultural soils. Extensive adsorption combined with limited desorption would result in less than the total administered dose of oxfendazole in feedlot runoff. Such reduction in the concentration of oxfendazole in feedlot runoff is not addressed in the evaluation of the effect of excreted oxfendazole on the aquatic environment. The total administered dose of oxfendazole has been used to estimate the "worst case" concentration.

The estimated "worst case" concentration of oxfendazole in feedlot runoff is 1.468 ppm. This "worst case" estimate is the highest concentration of oxfendazole in the aquatic environment, as feedlot runoff will be significantly diluted as it enters bodies of water such as streams, rivers, ponds and lakes.

Upon entry into the aquatic environment, oxfendazole and its major degradation product (oxfendazole amine) rapidly decompose through the process of photodegradation. Photolytic half-lives of oxfendazole and oxfendazole amine have been measured in hours (Appendices 19 and 20)). These results demonstrate that any oxfendazole, or its major degradation product, entering the aquatic environment from feedlot runoff will be significantly diluted and then rapidly degraded.

Terrestrial Environment

Initially manure is slightly acidic (approximately pH 6.0). As manure accumulates in the feedlot, acid forming bacteria cause the material to become more acidic , thus lowering the pH to approximately pH 5.5. However, microorganisms in the manure begin to metabolize inorganic nitrogen to ammonium nitrogen, causing the pH to rise rapidly to a level of approximately pH 8.5. As decomposition continues, ammonia is released to the atmosphere or converted to nitrates. As the nitrates are lost through leaching or through the action of denitrifying bacteria, the feedlot material approaches neutrality or remains slightly alkaline (pH 7.1 - 7.2).[6] Oxfendazole begins to hydrolyze in manure as the pH of the manure increases and then stabilizes at approximately pH 7.1.

Oxfendazole in feedlot manure, at pH 7.1, will begin to degrade to oxfendazole amine as manure accumulates in the feedlot over the feeding period. It is estimated that as much as 20% of the oxfendazole in feedlot manure may hydrolyze to oxfendazole amine before the manure is removed from the feedlot and applied to agricultural soil (Appendix 17).

When twenty tons of feedlot manure, containing 1,200 ppb of oxfendazole equivalents, is mixed with the top six inches of one acre of agricultural soil, the initial soil concentration of oxfendazole is 24.07 ppb. Oxfendazole in agricultural soil will hydrolysis to oxfendazole amine at a rate dependent upon the pH of the soil. The pH of agricultural soils ranges from pH 5.5 to pH 8.4. [7] The hydrolysis of oxfendazole in agricultural soil at various pH levels ranging from pH 5.17 to pH 8.56 following an unlimited number of annual applications of manure has been calculated (Appendix 18). It is apparent from these calculations that oxfendazole concentrations in agricultural soils, initially well below observed environmental effect levels (see Section 8, below), will degrade with time (more slowly at lower pH levels) and are not expected to exceed a maximum concentration of 297 ppb at pH 5.17 or, more realistically, 49.8 ppb at pH 7.19, following an unlimited number of annual applications of manure containing 1,200 ppb of oxfendazole.

Although the oxfendazole product label indicates that cattle may be treated a second time, at four to six weeks after the initial dose, if reinfection is suspected, retreatment is not expected to occur in the feedlot ("worst case" situation) due to the fact that feedlot conditions are not conducive to reinfection. However, if a second

dose of oxfendazole were to be administered to a group of cattle in a feedlot, the ultimate effect of this action on the concentrations of oxfendazole in feedlot runoff and agricultural soils would be a doubling of the estimated concentrations presented above. The increased concentrations due to retreatment would still result in oxfendazole concentrations well below observed terrestrial effect levels.

8. EFFECTS OF SUBSTANCES EMITTED INTO THE ENVIRONMENT

OCCUPATIONAL SAFETY

Oxfendazole is a 2-substituted benzimidazole with broad anthelmintic activity against a variety of gastrointestinal and lung nematodes and tapeworms in cattle and other animals. Oxfendazole has been evaluated in numerous mammalian species for ocular and dermal irritation, oral toxicity (acute, subchronic, chronic and reproductive) carcinogenicity, and safety in domestic animals. Oxfendazole is not considered to be an ocular or dermal irritant. Additionally, oxfendazole has met all of the human food safety and target animal safety criteria for anthelmintics administered orally and intraruminally.

Employees involved in the manufacturing of oxfendazole active ingredient and formulated drug product are informed with regard to the nature of the oxfendazole compound and are provided with standard protective clothing (safety helmets, safety glasses, goggles or face shields, uniforms, safety shoes and gloves), as appropriate. If conditions warrant, the operators have at their disposal supplied breathing air hoods or supplied breathing air suits, aprons, and boots. Each employee involved in the production of oxfendazole drug substance or formulated drug product has at his/her disposal an oxfendazole Material Safety Data Sheet (MSDS) which provides information with regard to the drug substance, safety precautions to be employed, and the established Permissible Exposure Limit (PML) . A copy of the oxfendazole MSDS is presented in Appendix 21.

Based on the information presented above and further summarized below, oxfendazole formulated drug products, when used in accordance with established procedures and good veterinary practices, do not constitute a risk or hazard to human health.

OCULAR AND DERMAL IRRITATION STUDIES

- Ocular Irritation Study

Six male rabbits (New Zealand Albino) were used to evaluate the ocular irritancy of oxfendazole. For each animal, 0.1 gm of dry oxfendazole was placed into the conjunctival sac of the left eye. The right eye was untreated and served as a control. The eyes were scored for signs of irritation at 1, 2, 3, and 7 days after dosing.

There were no signs of irritation in either eye of any of the animals at any of the observation points. Therefore, oxfendazole was not considered to be an eye irritant when tested in the rabbit eye.

• Dermal Irritation Study

Three male and three female rabbits (New Zealand Albino) were used to evaluate the skin irritancy of oxfendazole. The oxfendazole formulation was applied to intact and abraded skin (four application sites per rabbit, two intact and two abraded) of each rabbit. The test sites were occluded for four hours, following which the dressings were removed and the test sites scored for erythema and edema. The test sites were then washed and scoring was repeated at 24 and 48 hours after dosing.

No evidence of erythema or edema was seen on the treated skin of any of the rabbits at any of the observation points. The oxfendazole formulation did not cause primary skin irritation. Therefore, oxfendazole was not considered to be a skin irritant when tested in rabbits and would not be expected to cause primary skin irritation in humans in case of dermal exposure.

HUMAN FOOD SAFETY STUDIES

The following studies were performed in order to determine the safety of oxfendazole residues in food:

In mice and rats, single oral doses of 6,400 mg/kg were well tolerated, while in dogs a single oral dose of 1,600 mg/kg produced only emesis and signs of slight gastrointestinal irritation.

In subchronic studies in rats, dietary concentrations of oxfendazole up to 100 ppm were well tolerated over a 90-day feeding period. No gross or microscopic changes related to treatment could be found. In a similar 90-day study in dogs, daily doses of up to 6 mg/kg produced no meaningful changes.

In teratology studies, oxfendazole was found to be without effect at 10 mg/kg per day in rats, 108 mg/kg per day in mice, and greater than or equal to 0.625 mg/kg per day (the highest dose tested) in rabbits. In reproduction studies in rats, a dietary concentration of 10 ppm of oxfendazole was found to be the no-observed-effect level (NOEL).

When oxfendazole was administered for one year to dogs and rats in chronic toxicity evaluations, the no-observed-effect level (NOEL) was 13.5 mg/kg per day for dogs and 0.7 mg/kg per day for rats.

Carcinogenicity studies in mice and rats have been conducted. In mice, no evidence of carcinogenic effect was observed at any of the treatment levels and the highest level (1,000 ppm or 150 mg/kg per day) was considered to be the no-observed-effect level (NOEL). In rats, no evidence of carcinogenic effect was observed at any of the treatment levels (highest level was 100 ppm). The no-observed-effect level (NOEL) was 10 ppm, or 0.7 mg/kg per day.

ENVIRONMENTAL EFFECT STUDIES

Microbial Growth Inhibition

Oxfendazole was tested by Syntex Research for activity against a range of bacteria, fungi, mycoplasma, and several soil micro-organisms. Oxfendazole was not active against the soil micro-organisms at concentrations greater than or equal to the maximim solubility of the compound (9 ppm). Discs containing 100 mcg of oxfendazole produced no zones of inhibition when placed on nutrient agar plates inoculated with representative bacteria, fungi, or mycoplasma.

A more detailed sumary of this study is presented in Appendix 22.

Earthworm Toxicity

The subacute toxicity of oxfendazole for earthworms (Lumbricus terrestris) was evaluated in a study conducted by Analytical Bio-Chemistry Laboratories, Inc., in accordance with FDA Environmental Assessment Technical Assistance Document 4.12. Mortality and signs of sublethal toxicity, at soil concentrations up to 971 ppm, were observed and recorded over a 28-day period. The results indicate that oxfendazole is not expected to have an effect on earthworms at concentrations as high as 971 ppm in soil.

A more detailed summary of this study is presented in Appendix 23.

Daphnia Acute Toxicity

The acute toxicity of oxfendazole for Daphnia magna was evaluated in a well-controlled study conducted by Analytical Bio-Chemistry Laboratories, Inc. Quadruplicate groups of five first-instar larvae of Daphnia magna, less than 24 hours of age, were exposed to concentrations of zero to 1.0 mg oxfendazole per liter of water (nominal) in 200 ml static systems for a duration of two days.

Actual measured concentrations ranged from less than 0.1 mg/L to 0.86 mg/L, The 48-hour EC_{50} was calculated to be 0.52 mg/L, with a 95% confidence interval of 0.4 to 0.76 ppm. The slope of the dose-response was determined to be 2.7. The no-observed-effect-concentration (NOEC) was 0.12 ppm.

A more detailed summary of this study is presented in Appendix 24.

A second static bioassay was conducted by Analytical Bio-Chemistry Laboratories, Inc., to determine the effect of 2-amino-5(6)-phenylsulfinyl-benzimidazole (oxfendazole amine), the hydrolytic degradation product of oxfendazole, on neonate Daphnia magna. No mortality or abnormal effects were observed at concentrations of zero, 0.01, 0.1, 1.0, or 10.0 mg/L of the test substance.

A more detailed summary of this study is presented in Appendix 25.

Freshwater Fish Acute Toxicity (Bluegill Sunfish)

Bluegill sunfish (Lepomis macrochirus) were selected as a representative species of warm-freshwater fish to determine the acute toxicity of oxfendazole to freshwater fish. Groups of ten sunfish, averaging 0.54 gm in weight and 28 mm in length, were exposed to concentrations of oxfendazole (97% purity) ranging from less than 0.2 ppm to 2.7 ppm. Fish were observed for mortality and signs of sublethal effects daily for the test period of four days.

No mortality or sublethal effects were observed during the four-day observation period. Therefore, the 96-hour LC_{50} for oxfendazole was greater than 2.7 ppm, the highest concentration tested.

A more detailed summary of this study is presented in Appendix 26.

Freshwater Fish Acute Toxicity (Rainbow Trout)

Rainbow trout (Salmo gairdneri) were selected as a representative species of cold-freshwater fish to determine the acute toxicity of oxfendazole to freshwater fish. Groups of ten trout were exposed to concentrations of oxfendazole (97% purity) ranging from less than 0.2 ppm to 2.5 ppm. Fish were observed for mortality and signs of sublethal effects daily for the test period of four days.

No mortality or sublethal effects were observed during the four-day observation period. Therefore, the 96-hour LC_{50} for oxfendazole was greater than 2.5 ppm, the highest concentration tested.

A more detailed summary of this study is presented in Appendix 27.

Seed Germination and Root Elongation (Preliminary Study)

A preliminary study was conducted by Analytical Bio-Chemistry Laboratories, Inc. to determine the effects of oxfendazole on seed germination and root elongation of six angiosperms of agronomic importance:

- Wheat (Triticum aestivum)
- Perennial Ryegrass (Lolium perenne)
- Cucumber (Cucumis sativus)
- Soybean (Glycine max)
- Lettuce (Lactuca sativa)
- Tomato (Lycopersicon esculentum)

Six replicates, each of 50 seeds, of each of the above species were exposed either to deionized water (control) or to oxfendazole suspended in deionized water at a concentration of 1,000 ppm during germination. Germination, defined as radicle length of greater than 3 mm, and radicle growth were determined at intervals during the test. Cucumber, soybean, lettuce, and tomato showed no statistically significant effects from exposure to oxfendazole at 1,000 ppm. Wheat showed a difference in radicle growth and ryegrass showed a statistically significant difference in seed germination at the 1,000 ppm concentration.

Due to the results obtained with the wheat and perennial ryegrass, a definitive study was undertaken and the results of that study are reported separately.

A more detailed summary of this preliminary study is presented in Appendix 28.

Seed Germination and Root Elongation (Definitive Study)

This definitive study was undertaken to further define the effect of oxfendazole on wheat and ryegrass germination. This study was carried out by Analytical Bio-Chemistry Laboratories, Inc. in accordance with FDA Environmental Assessment Technical Assistance Document 4.06.

Seeds of perennial ryegrass were exposed to oxfendazole suspensions of 1,000, 91.0, 8.3, 0.75, and 0.07 ppm while wheat seeds were exposed to 1,000, 100, 10.0, 1.0, and 0.1 ppm. Each treatment group consisted of six replicates of 50 seeds each.

Analysis of variance (ANOVA) for percent seed germination showed no differences among treatments for either wheat ($p < 0.05$) or perennial ryegrass ($p < 0.05$). ANOVA of the wheat radicle length data detected no significant differences among treatments ($p < 0.05$). ANOVA of the perennial ryegrass mean radicle length indicated a statistically significant difference did exist among treatments at $p < 0.05$. Application of Tuckey's HSD critical range indicated a significant difference between the 0.75 ppm oxfendazole concentration and the 8.3 ppm group. However, these results do not correlate with the preliminary study (at 1,000 ppm) and are not dose-related. No significant adverse effects on seed germination and root elongation will occur in the proposed agricultural setting.

A more detailed summary of this study is presented in Appendix 29.

Seedling Growth

The effect of oxfendazole on seedling growth was determined in a study carried out by Springborn Life Sciences. Six species of angiosperms were selected for study, three monocots and three dicots. Representative monocots included wheat (Triticum aestivum), corn (Zea mays), and perennial ryegrass (Lolium perenne). Representative dicots included cucumber (Cucumis sativus), pinto bean (Phaseolus vulgaris), and soybean (Glycine max).

After germination, seedlings were transplanted to plastic pots in groups of five, with up to eight replicates per treatment. Support media, consisting of washed silica sand, were treated with either control or graded concentrations of 99.3% pure oxfendazole. Pots were exposed to a 16-hour light/8-hour dark cycle and were fed by subirrigation for the 21-day test period. Shoot length was measured periodically and shoot weight and root weight (oven-dry) were measured at termination.

Results were as follows:

Species	NOEC (mg/kg)	LOEC (mg/kg)
Cucumber	7.56	10.0
Pinto Bean	0.912	1. 82
Ryegrass	7.56	10.0
Wheat	102.0	N.D.
Corn	102.0	N.D.
Soybean	10.0*	48.5**

N.D. - Not determined due to conflicting results from preliminary and definitive studies.

* Determined in the preliminary study.
** Determined in the definitive study.

A more detailed summary of this study is reported in Appendix 30.

Since a No-Observed-Effect-Concentration was not identified for soybean in the first definitive study carried out by Springborn, a second definitive study was carried out on this species. The second study was conducted in a manner similar to that of the first definitive study. In the second study, concentrations ranged from 3.6 mg oxfendazole per kg of support medium to 110 mg/kg. Statistical analysis of the data generated established that none of the concentrations tested affected plant survival, shoot length, or shoot and root weights measured at 21 days after transplantation.

A more detailed summary of this study is presented in Appendix 31.

Environmental Hazard Assessment

AQUATIC ENVIRONMENT

Under "worst case" conditions (assuming that all oxfendazole administered to feedlot cattle is excreted into feedlot manure and extracted from the manure, by a two inch rainfall, into feedlot runoff), the estimated feedlot runoff concentration of oxfendazole is 1.468 ppm. This is the highest concentration of oxfendazole in any aquatic environment as feedlot runoff will be significantly diluted as it enters bodies of water such as streams, rivers, ponds and lakes (secondary aquatic environments). Upon entry into these secondary aquatic environments, oxfendazole and its major degradation product (oxfendazole amine) rapidly decompose through the process of photodegradation. Dilution and photochemical decomposition in secondary aquatic environments reduces the environmental concentrations of oxfendazole and it major metabolite such that effects from oxfendazole or vertebrate or invertebrate populations are expected to be transient and would not be considered to be significant.

AQUATIC EFFECT LEVELS

- Daphnia Toxicity >> NOEC (48 hr.) = 120 ppb
- Trout Toxicity >> LC50 (96 hr.) > 2,500 ppb
- Bluegill Toxicity >> LC50 (96 hr.) > 2,700 ppb

TERRESTRIAL ENVIRONMENT

Under "worst case" conditions (assuming that all oxfendazole administered to feedlot cattle is excreted in feedlot manure and that the manure which accumulates over a feeding period is mixed into the top six inches of soil at the rate of 20 tons of manure per ace of land) the total initial concentration of oxfendazole and its major degradation product (oxfendazole amine) in agricultural soil is calculated to be 24.07 ppb. Following unlimited annual applications of manure, the build-up of oxfendazole is not expected to exceed 297 ppb in soil at pH 5.17, 103 ppb in soil at pH 6.58, and, most realistically, 49.8 ppb in soil at pH 7.19. In the event that a second dose of oxfendazole is administered to cattle in the feedlot, maximum agricultural soil concentrations would increase by a factor of two (i.e. 594 ppb in soil at pH 5.17, 206 ppb in soil at pH 6.58, and, most realistically, 99.8 ppb in soil at pH 7.19)

TERRESTRIAL EFFECT LEVELS

- Microorganisms >>

  NOEC ≥ 9,000 ppb (maximum solubility.)

- Seedling Growth (pinto bean most sensitive) >>

  NOEC = 912 ppb

- Seed Germination/Root Elongation >>

  NOEC > 1,000,000 ppb

- Earthworm Toxicity >>

  NOEC (28 days) > 971,000 ppb

The comparison of calculated environmental concentrations of oxfendazole in the aquatic and terrestrial environments in conjunction with an analysis of the fate of such residues indicates that the use of oxfendazole products in feedlot cattle (the "worst case" situation) is not expected to have a significant impact on the environment.

9. USE OF RESOURCES AND ENERGY

There is expected to be no significant depletion of natural resources associated with the approval of this New Animal Drug Application. There is expected to be no effect on the depletion of natural resources due to the manufacture of the drug substance or the final dosage forms. There will be negligible demands for the use of energy or petrochemicals.

The indirect effect of approval of this New Animal Drug Application will be a saving of natural resources and energy due to the beneficial effect of the use of these products on the health and performance of animals treated with oxfendazole resulting in the more efficient use of feed resources and the reduced need for additional veterinary care.

10. MITIGATION MEASURES

In light of the data presented above, there is no need to take measures to avoid or mitigate potential environmental effects of the use of oxfendazole in cattle. No significant adverse environmental effects following the use of oxfendazole in cattle have been identified.

Material Safety Data Sheets (MSDS's) are available for employees who work in raw material and formulated product manufacturing areas. Additionally, employees in production and packaging areas wear protective clothing and dust respirators, as appropriate, to ensure compliance with all applicable standards.

No other mitigating measures are necessary since oxfendazole does not pose a known threat to the environment.

11. ALTERNATIVES TO THE PROPOSED ACTION

None. No potential significant adverse environmental impacts have been identified.

12. LIST OF PREPARERS

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Springborn Life Sciences, Inc.
(Springborn Laboratories, Inc.)
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Wareham, Massachusetts 02571

13. CERTIFICATION

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

Paul F. Kopeck 4/30/90

Signature of Official Date

Paul F. Kopeck
Director
Regulatory Affairs and Corporate Compliance
Syntex Animal Health

14. REFERENCES

[1] Lacey, E., Brady, R.L., Prichard, R.K. and Watson, T.R., Veterinary Parasitology 23: 105-119, 1987.

[2] Feedstuffs, October 31, 1988, p. C-1.

[3] Ensminger, M.E. and Olentine, Jr., C.G., Feeds & Nutrition, The Ensminger Publishing Company, 1978, p. 544 ff.

[4] Dyer, I.A. and O'Mary, C.C., The Feedlot, Lea & Febiger, 1972, p. 154.

[5] Goring and Hamaker, Organic Chemicals in the Soil Environment, Volume 1, 1972.

[6] Merkel, J.A., Managing Livestock Waste, Ari Publishing Company, 1981, p. 314.

[7] Sheets, T.J., Crafts, A.S. and Drever, H.R., "Soil Effects on Herbicides - Influence of Soil Properties on the Phytotoxicities of the s-Triazine Herbicides", Agricultural and Food Chemistry, 10:6, 1962, p. 459.

15. APPENDICES - Attached

APPENDIX 1

1.0
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0.6
0.5
0.4
0.3
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0.1
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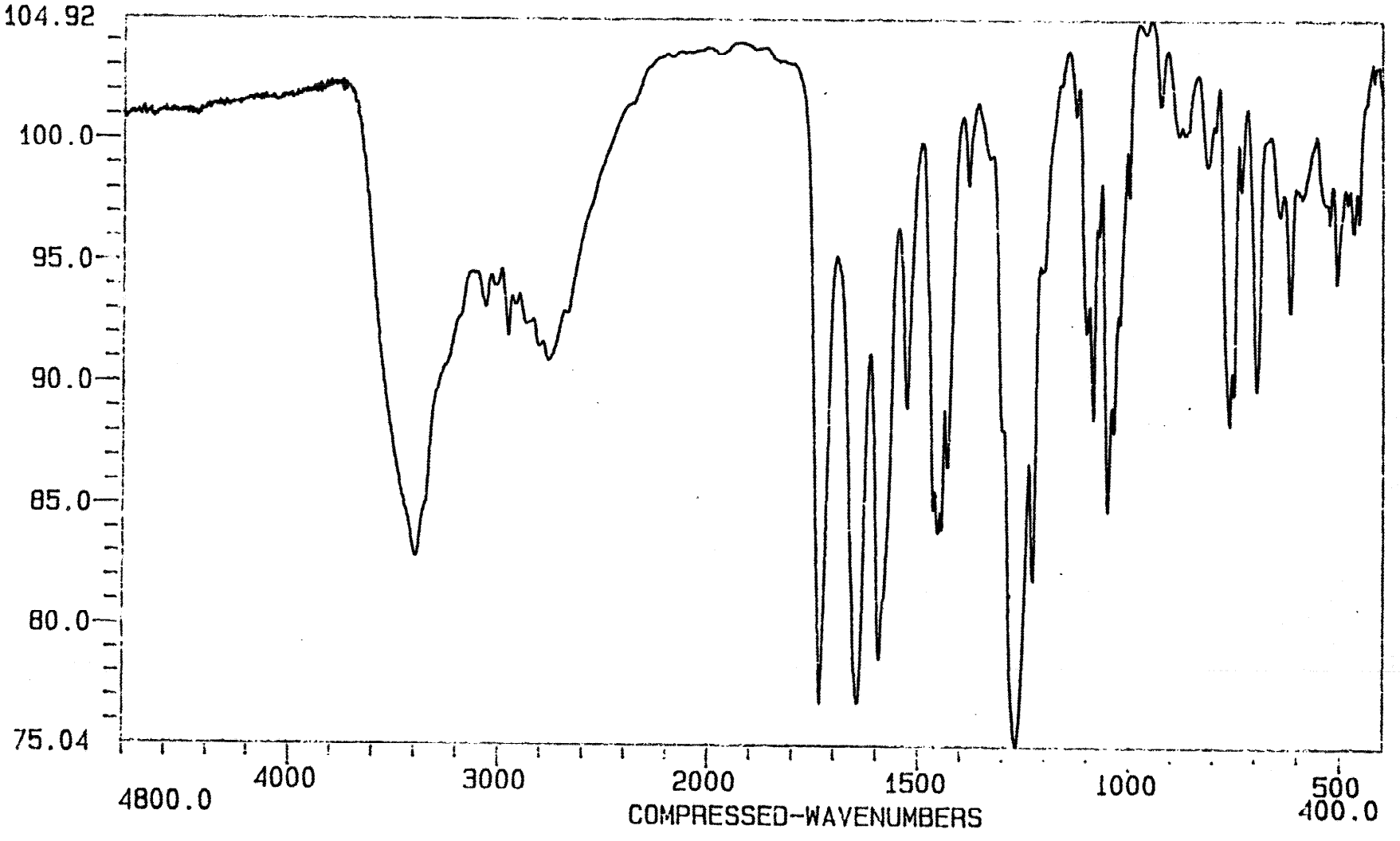
APPENDIX 2



SYNTEX ANALYTICAL
RESEARCH FT-IR

PRESEAUX, NOTEBK#12213-24
REF#018352
KBR-RF

SYNTEX ANALYTICAL RESEARCH FT-IR
PC/IR PEAK TABLE for IR018352
Printed on: Mon Oct 31 14:43:24 1988

Peak #	Peak Location (cm-1)	Start Frequency (cm-1)	End Frequency (cm-1)	Peak Value(%T)
1	3393.22	3416.37	3373.93	82.854
2	2955.32	2966.90	2943.75	91.952
3	2762.42	2818.36	2721.91	90.926
4	1732.30	1767.02	1697.57	76.774
5	1643.56	1651.28	1614.62	76.803
6	1593.40	1612.70	1583.76	78.628
7	1525.89	1539.39	1502.74	89.048
8	1464.16	1487.31	1460.30	84.880
9	1454.51	1460.30	1448.73	83.962
10	1444.87	1448.73	1437.15	84.054
11	1431.36	1437.15	1398.57	86.635
12	1267.39	1296.33	1236.53	75.036
13	1226.88	1236.53	1209.52	81.971
14	1099.56	1116.93	1091.85	92.125
15	1084.13	1091.85	1074.49	88.574
16	1049.41	1062.91	1039.76	84.860
17	1035.91	1039.76	1024.33	88.045
18	760.05	787.06	754.26	88.377
19	750.40	754.26	738.83	89.592
20	694.46	715.68	677.10	89.767

END OF PEAK TABLE

APPENDIX 3

ENVIRONMENTAL ASSESSMENT

OXFENDAZOLE

1. DATE: August 1989

2. NAME OF APPLICANT: Syntex Pharmaceutical International Limited (Bahamas Chemical Division)

3. ADDRESS: Freeport, Grand Bahama Island, BAHAMAS

4. DESCRIPTION OF PROPOSED ACTION:

The Environmental Assessment Report is pursuant to Title 21 CRF 25.31, covering the manufacturing of the bulk drug substance, oxfendazole, which will be manufactured by Bahamas Chemical Division, Freeport, Grand Bahama Island, Bahamas. Oxfendazole will be used in the manufacture of an oral medication for the treatment of internal parasites in cattle and sheep. The use, consumption, and disposal of the drug substance are addressed in the environmental assessment for the drug product.

The location, where the present action and activities consequent to it are likely to occur, is the bulk drug substance manufacturing facility at Freeport, Grand Bahama Island, Bahamas.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES:

The identity of oxfendazole, the active ingredient in the drug product as well as the manufacture and control procedures are described elsewhere in this Drug Master File.

5a. CHEMICAL NAMES

(1) Oxfendazole (USAN)

(2) 2-Methoxycarbonylamino-5-phenylsulfinylbenzimidazole

(3) Methyl-5-(phenylsulfinyl)-2-benzimidazole carbamate

(4) Methyl(5-phenylsulfinyl)-1H-benzimidazole carbamate

(5) Carbamic acid,[5-(phenylsulfinyl)-1H-benzimidazol-2-yl]-,methyl ester

5b. SYNTEX CODE NUMBER

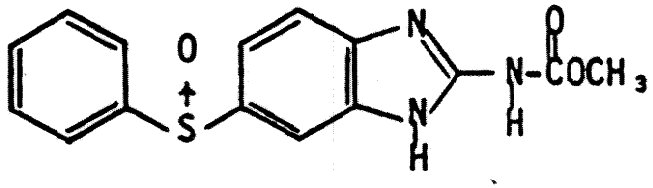
RS-8858

5c. MOLECULAR WEIGHT

315.34

5d. STRUCTURAL FORMULA

$C_{15}H_{13}O_3N_3S$



5e. PHYSICAL DESCRIPTION

Oxfendazole is a white, grey or tan powder.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

a. The identities of components, reagents, and solvents used in the manufacture of oxfendazole drug substance are described elsewhere in this Drug Master File.

b. Procedures for handling components derived from manufacture.

The components used and/or produced in the manufacture of oxfendazole drug substance are either recycled back into the process, or disposed of in accordance with the laws of the site of manufacture.

c. The disposal techniques and controls exercised over the materials are as follows:

1) Bulk Solvents

Spent methanol streams (about 250,000 kg/year) generated in the process are recovered and analyzed to determine compliance with appropriate specifications. If they comply, the solvents are reused in the process. Unusable solvents may either be burned in an on site rotary kiln incinerator or biotreated in an activated sludge biotreatment basin.

2) Solid Waste

Filter cloths (about 100 kg/year) used in a filtration step in the manufacturing of oxfendazole are incinerated in the onsite rotating kiln incinerator.

3) Wastewater

Process aqueous wastes containing both organics and inorganics from the reactions are neutralized, and biotreated in an activated sludge biotreatment basin. Both the primary and secondary sludge from the biobasins are incinerated in the onsite rotary kiln incinerator. The treated water is discharged into Hawksbill Creek.

4) Airborne Emissions

Air emissions generated during the production process, consisting of organics such as methyl alcohol will be controlled by both the reactor condenser and a plant ventilation system brine cooled condenser followed by a wet scrubber.

Dust emissions are controlled by a dust extraction system in the powder handling areas. The extraction system contains a bag house to capture the dust.

Consequently, the treatment of emissions from these facilities reduces or eliminates the discharge of emissions into the environment.

5) Employee Protection

Personnel working in the plant are provided with safety helmets, safety glasses, goggles or face shields, uniforms, safety shoes and gloves. If conditions warrant, the operators have at their disposal supplied breathing air hoods, aprons, and boots. Directions are written at each appropriate step of the operating procedure advising the operators what safety equipment must be used during that step of the operation. Each operating procedure includes a Safety and Health Section advising the operator of potential hazards of all chemicals used in that operation. In addition, Material Safety Data Sheets are available for all the chemicals handled on the plant.

6) Summary

The pollution control devices in use and the waste disposal methods used by the facility serve to minimize release of environmental emissions resultant from the production of oxfendazole. Emissions from this facility are in compliance with the laws and regulations listed below.

7) Laws and Regulations

Environmental Health Act of 1987

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

The facility located in Freeport, Grand Bahama Island, Bahamas, is a fully equipped chemical manufacturing facility which minimizes the release of emissions into the environment by standard good manufacturing practices. The use of these practices and the treatment/disposal techniques in use result in minimal emissions to the environment. Because minimal emission of substances into the environment is expected to occur, the concentration of substances in the environment is expected to be negligible.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

Based on the information given in items 6 and 7 above, the quantities of substances released into the environment, as a consequence of the manufacture of oxfendazole, will be negligible. No effects on the environment from either short-term or long-term production are expected.

9. USE OF RESOURCES AND ENERGY:

The raw materials used in the manufacture of oxfendazole are readily available. The production of the drug substance and the energy use involved therein do not cause a depletion of any natural resources that are in critically short supply.

10. MITIGATION MEASURES:

Bahamas Chemical Division takes all necessary measures to achieve compliance with the regulations of applicable government authorities. In fact, the facility has taken action which exceeds the basic environmental requirements they must meet. In light of the information presented, no such considerations are necessary.

11. ALTERNATIVE TO THE PROPOSED ACTION:

No potential adverse environmental effects have been identified as a result of the proposed action. Therefore, alternatives have not been considered.

12. LIST OF PREPARERS:

Mr. Bob DeGreve
Environmental, Health and Safety Manager
Bahamas Chemical Division
Freeport, Grand Bahama Island
BAHAMAS

13. CERTIFICATION:

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of Bahamas Chemical Division.

Alan Wallace
Director, Engineering and Maintenance

Date: 15 August 1989

14. REFERENCES:

Not applicable.

15. APPENDICES:

Not applicable.

APPENDIX 4

ENVIRONMENTAL ASSESSMENT

OXFENDAZOLE

1. DATE: August 1989

2. NAME OF APPLICANT: Syntex Chemicals, Inc.

3. ADDRESS: Boulder, Colorado, USA

4. DESCRIPTION OF PROPOSED ACTION:

The Environmental Assessment Report is pursuant to Title 21 CRF 25.31, covering the manufacturing of the bulk drug substance, oxfendazole, which will be manufactured by Syntex Chemicals, Inc., Boulder, Colorado, USA. Oxfendazole will be used in the manufacture of an oral medication for the treatment of internal parasites in cattle and sheep. The use, consumption, and disposal of the drug substance are addressed in the environmental assessment for the drug product.

The location, where the present action and activities consequent to it are likely to occur, is the bulk drug substance manufacturing facility at Boulder, Colorado, USA.

5. IDENTIFICATION OF CHEMICAL SUBSTANCES:

The identity of oxfendazole, the active ingredient in the drug product as well as the manufacture and control procedures are described elsewhere in this Drug Master File.

5a. CHEMICAL NAMES

(1) Oxfendazole (USAN)
(2) 2-Methoxycarbonylamino-5-phenylsulfinylbenzimidazole
(3) Methyl-5-(phenylsulfinyl)-2-benzimidazole carbamate
(4) Methyl(5-phenylsulfinyl)-1H-benzimidazole carbamate
(5) Carbamic acid,[5-(phenylsulfinyl)-1H-benzimidazol-2-yl]-,methyl ester

5b. SYNTEX CODE NUMBER

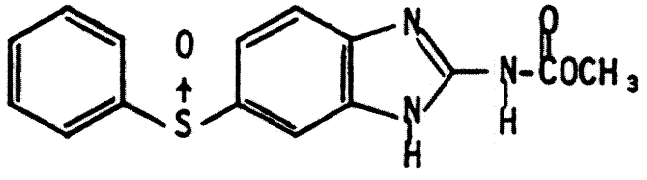
RS-8858

5c. MOLECULAR WEIGHT

315.34

5d. STRUCTURAL FORMULA

$C_{15}H_{13}O_3N_3S$



5e. PHYSICAL DESCRIPTION

Oxfendazole is a white, grey or tan powder.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT:

a. The identities of components, reagents, and solvents used in the manufacture of oxfendazole drug substance are described elsewhere in this Drug Master File.

b. Procedures for handling components derived from manufacture.

The components used and/or produced in the manufacture of oxfendazole drug substance are either used in subsequent steps, recycled back into the process, or disposed of in accordance with the waste disposal laws of the sites of manufacture.

c. The disposal techniques and controls exercised over the materials are as follows:

1) Bulk Solvents

Spent methanol, streams generated in the process are recovered and analyzed to determine compliance with appropriate specifications. If they comply, the solvents are reused in the process. Unusable solvents may either be burned in an on site industrial boiler operated in accordance with 40 C.F.R. 266, Subpart D, or sent off site to a permitted hazardous waste treatment facility. Spent methylene chloride is also analyzed for compliance with specifications and reused in the process if applicable. Spent methylene chloride that is off specification is sent off site to a permitted hazardous waste treatment facility to be recycled.

2) Solid Waste

Approximately 66,000 kilograms of inorganic salts will be produced as a by-product from the manufacture of oxfendazole and will be sold to a recycler to be reintroduced into the marketplace. Approximately 24,000 kilograms of diatomaceous earth filter cake will be generated. This waste is sent to a permitted, hazardous waste landfill.

3) Wastewater

Aqueous discharges from processing operations are pumped to an on site aerobic biological wastewater treatment facility to reduce the chemical oxygen demand. Routine monitoring of incoming waste streams consists of the measurement of pH, flammability, and toxic organics. Treated wastewater is discharged to the City of Boulder's publicly owned treatment works (POTW). The discharge of treated wastewaters into the City of Boulder's system is governed by City of Boulder Ordinance No. 4667. Approximately 1,800,000 liters of treated wastewater will be discharged annually to the Boulder POTW from the manufacture of oxfendazole. Sludge from the primary clarifier is sent to a permitted hazardous waste landfill.

4) Airborne Emissions

All emissions of volatile organic compounds generated during the production process are controlled by condensors. In addition, process equipment vents are manifolded and ducted through wet scrubbers employing caustic scrubbing solutions, and finally through activated carbon adsorption units before discharge to the atmosphere. Consequently, the treatment of emissions from these facilities reduces or eliminates the discharge of these gasses into the environment.

5) Employee Protection

Personnel working in the plant are provided with safety helmets, safety glasses, goggles or face shields, uniforms, safety shoes and gloves. If conditions warrant, the operators have at their disposal supplied breathing air hoods, supplied breathing air suits, aprons, and boots. Directions are written at each appropriate step of the operating procedure advising the operators what safety equipment must be used during that step of the operation. Each operating procedure includes a Safety and Health section advising the operator of potential hazards of all chemicals used in that operation. In addition, Material Safety Data Sheets are available for all the chemicals handled on the plant.

6) Summary

The pollution control devices in use and the waste disposal methods used by the facility serve to minimize release of environmental emissions resultant from the production of oxfendazole. Emissions from this facility are in compliance with laws and regulations listed below.

7) Laws and Regulations

Federal Laws

- Clean Air Act, as amended
- Resource Conservation and Recovery Act, as amended
- Water Pollution Control Act, as amended
- Emergency Planning and Community Right-to-Know Act of 1986
- Occupational Safety and Health Act of 1970, as amended

State Laws and Regulations, as amended

- Colorado Water Quality Act
- Colorado Water Quality Regulations
- Colorado Water Quality Standards
- Colorado Air Quality Act
- Colorado Air Pollution Control Regulations
- Colorado Discharge Permit System Regulations
- Colorado Disposal Sites and Facilities Law

- Colorado Hazardous Waste Act

- Colorado Hazardous Waste Management Regulations

- Colorado Standards for Owners and Operators of Hazardous Waste Treatment, Storage, and Disposal Facilities

- Colorado Hazardous Waste Notification and Permit Rules

Local Rules

- City of Boulder Ordinance No. 4667 for Industrial Wastewater Dischargers

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT:

The facility located in Boulder, Colorado, USA, is a fully equipped chemical manufacturing facility which minimizes the release of emissions into the environment by standard good manufacturing practices. The use of these practices, the laws and regulations in effect, and the treatment/disposal techniques in use result in minimal emissions to the environment. Because minimal emission of substances into the environment is expected to occur, the concentration of substances in the environment is expected to be negligible.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

Based on the information given in items 6 and 7 above, the quantities of substances released into the environment, as a consequence of the manufacture of oxfendazole, will be negligible. No effects on the environment from either short-term or long-term production are expected.

9. USE OF RESOURCES AND ENERGY:

The raw materials used in the manufacture of oxfendazole are readily available. The production of the drug substance and the energy use involved therein do not cause a depletion of any natural resources that are in critically short supply.

10. MITIGATION MEASURES:

Syntex Chemicals, Inc. takes all necessary measures to achieve compliance with the regulations of applicable government authorities and cited in the Code of Federal Regulations Title 21, part 211. In fact, the facility has taken action which exceeds the basic environmental requirements they must meet. In light of the information presented, no such considerations are necessary.

11. ALTERNATIVE TO THE PROPOSED ACTION:

No potential adverse environmental effects have been identified as a result of the proposed action. Therefore, alternatives have not been considered.

12. LIST OF PREPARERS:

Mr. John Sipkowski
Environmental Specialist
Syntex Chemicals, Inc.
Boulder, Colorado

13. CERTIFICATION:

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of Syntex Chemicals, Inc.

Ellen B. Arnold, Ph.D.
Director, Environmental Affairs

Date: 8/3/89

14. REFERENCES:

Not applicable.

15. APPENDICES:

Not applicable.

0411V

APPENDIX 5

Oxfendazole 9.06% Suspension and Oxfendazole 22.5% Suspension

Environmental Assessment

Introduction

The following is an abbreviated Environmental Assessment, pursuant to 21 CFR 25.31a, covering the manufacture of Oxfendazole 9.06% Suspension and Oxfendazole 22.5% Suspension.

1. Date: March 17, 1987

2. Name of Applicant: Diamond Scientific Co.

3. Address: 2538 S.E. 43rd Street
   Des Moines, Iowa 50304

4. Description of Proposed Action: The proposed action is the manufacture, processing, packaging, labeling and control of the following products: oxfendazole 9.06% suspension and oxfendazole 22.5% suspension.

   a. Requested approval - Need for the action

      This environmental assessment is necessary for the approval of the New Animal Drug Application for oxfendazole 9.06% suspension and oxfendazole 22.5% suspension to be marketed by Syntex Animal Health, Inc., a subsidiary of Syntex Agribusiness, Inc.

   b. Production location

      The bulk active chemical will be supplied by Syntex to Diamond Scientific Co. 2538 S.E. 43rd Street, Des Moines, Iowa, where the final product will be formulated and packaged.

   c. Location where product will be used

      Finished product will be returned to Syntex Animal Health, Inc., Des Moines, Iowa, for sale and distribution. Location of ultimate use will be specified by Syntex.

   d. Location where product will be disposed

      Negligible quantities of product generated during equipment clean-up or production waste are disposed through municipal waste water system or solid waste landfill facilities. In the event a production batch is determined to be outside of acceptance specifications and disposal is required, arrangements will be made with the solid waste landfill servicing Diamond to dispose of the failed batch in compliance with local waste disposal regulations or incinerate the product using an on-site incinerator.

e. Environments present at and adjacent to the manufacturing facility

Diamond Scientific Co. is located in a one level brick, tile and concrete structure at 2538 S.E. 43rd Street, Des Moines, Polk County, Iowa 50317. The main building at this site houses the administration offices, manufacturing facilities, warehouse, engineering and maintenance facilities. This structure is located on approximately 34 acres of land and contains about 142,000 square feet. A separate building immediately adjacent to the south of the main building is used for biological quality control and related activities and contains approximately 12,100 square feet of floor area; 4,100 square feet of this building is used for animal testing. Additional smaller buildings are located on the site and are used for animal housing. Also, a separate 3,696 square foot modular office building is located north of the main plant. The main buildings and the additions are equipped with sprinkler systems.

5. Identification of Chemical Substances

a. Nomenclature

Oxfendazole is also known as methyle 5-(phenylsulfinyl) - 2-benzimidazole carbamate.

b. CAS registry number

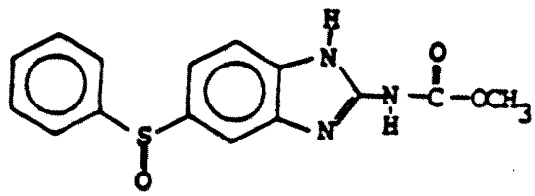
No. 53716-50-0

c. Molecular weight

315.34

d. Molecular Formula and Structural Formula

$C_{15}H_{13}O_3N_3S$



e. Physical Description

Oxfendazole is a white-gray powder which may have a slight color.

f. Impurities

Not applicable - Syntex to supply for product.

6. Introduction of Substances into the Environment

a. Substances that could enter the environment

The following is a list of the substances used in these two formulations: oxfendazole; polyoxyl 40 stearate; polyethylene glycol 8000; citric acid, monohydrate; sodium citrate, dihydrate; xanthan gum; methylcellulose; colloidal silicon dioxide; sorbic acid; simethicone; methylparaben and purified water.

b. Controls exercised

Insignificant amounts of the listed substances will enter the environment at the production site. Because of the high cost of pharmaceutical materials, as well as GMP requirements for strict accounting for their use, the manufacturing process is designed and expected to result in minimal residues, less than 1.0% of the materials used in manufacturing.

c. Application and compliance with emission requirements (Federal
d. State, Local and Occupational)

Diamond Scientific Co. will comply with all applicable federal, state, and local regulations as follows:

Air effluent: No vapors or fumes are generated by the manufacturing process. An Air Pollution Control Permit has been issued to Diamond Scientific Co. by the City of Des Moines. References: Chapter 5, Polk County Air Pollution and Control Division.

Water effluent: No effluent is generated in the manufacturing process with the exception of negligible amounts resulting from equipment clean up. All pertinent approvals for Diamond Scientific have been received from the Des Moines Municipal Sewage Treatment Facility. Reference: Chapter 16, Municipal Code of Des Moines.

Solids effluent: Due to the careful controls employed in the manufacture of the finished products, an insignificant amount of material from the process can be expected to be released into the environment. The only other solid waste would be generated from defective packaging material which would be disposed of through incineration in an on-site incinerator or normal solid waste collection procedures. The disposal of solids complies with all local waste disposal regulations. Reference: Chapter 100-108, Department of Water, Air, and Waste Management, State of Iowa.

Containers and distribution: No release of the product or its ingredients into the environment should occur because of faulty containers or improper distribution as both are controlled under CGMPs.

e. Effect of approval of Proposed Action

From discussions above, no deleterious effects are anticipated due to the approval of the proposed action.

7. Fate of emitted substances in the Environment

Not applicable - Syntex to supply for product.

8. Environmental effects of released substances

Not applicable - Syntex to supply for product.

9. Use of resources and energy

The raw materials used in the formulation of the final dosage form are readily available. The production of the oxfendazole suspension products and the energy use involved therein do not cause depletion of any natural resources which are in critically short supply, although the materials are irretrievable once used.

No disruption of the physical environment is anticipated other than the usual effects of operating the pharmaceutical and chemical manufacturing facilities which are already in place.

10. Mitigation measures

Diamond Scientific Co. will take all the necessary measures to remain in compliance with the statutes noted in this report.

11. Alternatives to the proposed action

Not applicable - Syntex to supply for product.

12. List of preparers

Thomas Froke
Regulatory Affairs Coordinator

Louis Van Daele
Director of Pharmaceutical Operations

13. Certification

The undersigned offical certifies that the information presented is true, accurate and complete to the best of the knowledge of Diamond Scientific Co.

3/17/87
Date

Thomas Froke
Diamond Scientific Co. Offical

APPENDIX 6

Environmental Assessment

1. Date: March 11, 1987

2. Name of Applicant: Coopers Animal Health Inc. (Coopers)

3. Address: 2000 South 11th Street
Kansas City, KS 66103-1438

4. Description of Proposed Action: The proposed action is the manufacture, processing, packaging, labeling and control of the following products: oxfendazole 9.06 percent suspension and oxfendazole 22.5 percent suspension.

4a. Requested Approval - Need for the Action

This environmental assessment is necessary for the approval of the New Animal Drug Application for oxfendazole 9.06 percent suspension and oxfendazole 22.5 percent suspension to be marketed by Syntex Animal Health, Inc., a subsidiary of Syntex Agribusiness, Inc.

4b. Production Location

The bulk active chemical will be supplied by Syntex to Coopers Animal Health Inc. where the final product will be formulated and packaged. These activities will occur at the Coopers facility located at 2000 South 11th Street, Kansas City, Kansas 66103-1438 (FDA Drug Establishment Registration Number 191748).

4c. Location Where Product will be Used

Finished product will be returned to Syntex Animal Health, Inc., Des Moines, Iowa, for sale and distribution. Location of ultimate use will be specified by Syntex.

4d. Location Where Product will be Disposed Of

Product not meeting specifications, dust and refuse from formulating and packaging activities, will be removed by an approved solid waste handling organization and deposited in an approved waste location.

5. Identification of Chemical Substances

5a. Nomenclature

Oxfendazole is also known as methyl 5-(phenylsulfinyl)-2-benzimidazole carbamate.

5b. CAS Registry Number

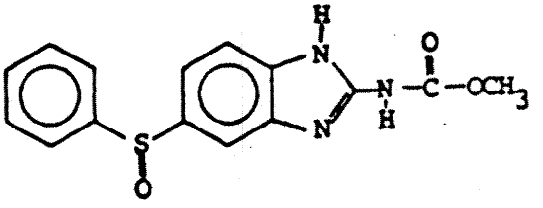
No. 53716-50-0

5c. Molecular Weight

315.34

5d. Molecular Formula and Structural Formula

$C_{15}H_{13}O_2N_3S$



5e. Physical Description

Oxfendazole is a white-gray powder which may have a slight color.

6. Introduction of Substances into the Environment

6a. Substances that Could Enter the Environment

The following is a list of the substances used in these two formulations: oxfendazole; polyoxyl 40 stearate; polyethylene glycol 8000; citric acid, monohydrate; sodium citrate, dihydrate; xanthan gum; methylcellulose; colloidal silicon dioxide; sorbic acid; simethicone; methylparaben and purified water.

6b. Controls Exercised and Applicable Emission Requirements

There are no significant emissions into the environment anticipated from the manufacturing, labeling and packaging of oxfendazole 9.06 percent and 22.5 percent suspension. As far as a citation of applicable emissions requirements, we cite the Code of Ordinances, City of Kansas City, Kansas, Amended Chapter 2A, which provides the following limits of emission of particulate matter into the environment: 0.550 lbs/hr. for a 100 lb solids process weight.

6c. Coopers Animal Health Inc. is currently and will continue to be in compliance with all applicable emissions requirements (including occupational) at the federal, state and local level. FDA approval of Coopers as a manufacturing site for Syntex will not have an effect on compliance with current emission requirements at the production site (i.e., Coopers Animal Health Inc., 2000 South 11th Street, Kansas City, KS 66103).

9. Use of Resources and Energy

Since Coopers will not manufacture the bulk drug but only formulate and package the drug products, the use of resources and energy is finite, but insignificant and not accurately quantifiable.

10. Mitigation Measures

Since the effect on the environment of this proposed action is insignificant, Coopers is not aware of any feasible mitigation measures.

12. List of Preparers:

Donald A. Buss
Director, Regulatory Affairs
Coopers Animal Health Inc.
(Qualifications include technical education and 15 years' experience in regulatory compliance and related matters)

13. Certification

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of the individuals responsible for preparation and certification of this Environmental Assessment.

(Date) MARCH 11, 1987

(Signature of Responsible Official) Donald A. Buss

(Title of Responsible Official) Director, Regulatory Affairs

APPENDIX 7

Water Solubility of Oxfendazole

The aqueous solubility of oxfendazole was determined utilizing three methods. The first method was the undersaturation/ oversaturation method utilizing 33 mg of 98.95% pure oxfendazole and 2 liters of deionized water. The experiment was not replicated. The second method was the time-dependent equilibrium method where oxfendazole was dissolved in methanol and in acetone and deposited on the side of a two-liter flask. Water was added and the amount dissolved was measured over one month. The experiment was not replicated. In the third method, the solubility of oxfendazole was determined in a pH 6.25 buffer system. The sample was equilibrated for 5 and 24 hours. Samples were taken in triplicate and analyzed by HPLC.

The mean solubility established in the over/under saturation method is 3.87 mg/L ± 0.46 with a range of 3.11-4.63 mg/L at 90% C.I. The solubility resulting from the time-dependent method ranged from 5.33 mg/L (filtered) to 9.05 mg/L (unfiltered) at 4 weeks for the methanol solution and from 9.70 mg/L at 1 week to 6.78 mg/L at 4 weeks for the acetone method. The solubility measured from the pH 6.25 buffer ranged from 4.56-5.11.

Data were obtained from an additional study by the under-saturation/oversaturation method conducted at an ionic strength of $\mu = 0.45$. The experiment was replicated three times. Solubility ranged from 1.76 mg/L to 2.02 mg/L.

APPENDIX 8

Water Solubility of Oxfendazole Amine

The major hydrolytic degradation product of oxfendazole is 2-amino-5(6)-phenylsulfinyl benzimidazole; its solubility was studied by Method 3.01, IV B, Undersaturation/Oversaturation, as described in the FDA Environmental Assessment Technical Handbook. The study was carried out at Syntex Research.

Triplicate suspensions of the test compound were prepared in aqueous buffer mixtures at pH 5.0, pH 6.9, and pH 8.9. To achieve an initial condition of oversaturation, a set of samples is heated to 50°C for one hour while undersaturated solutions are maintained at 24°C. When the difference in concentration between the oversaturation and undersaturation solutions is less than 5%, true equilibration solubility has been reached.

Solution samples were filtered and analyzed by HPLC.

Equilibration solubility was reached at Day 7 for the samples at pH 5.0; solubility was estimated to be 46 ppm. However, at pH 6.9 and 8.9, the difference between under- and over-saturation exceeded 5% up to Day 28 when the experiment was terminated. The solubility of oxfendazole amine at pH 6.9 and pH 8.9 exceeds 50 ppm but cannot be estimated with greater precision since equilibration solubility was not achieved.

APPENDIX 9

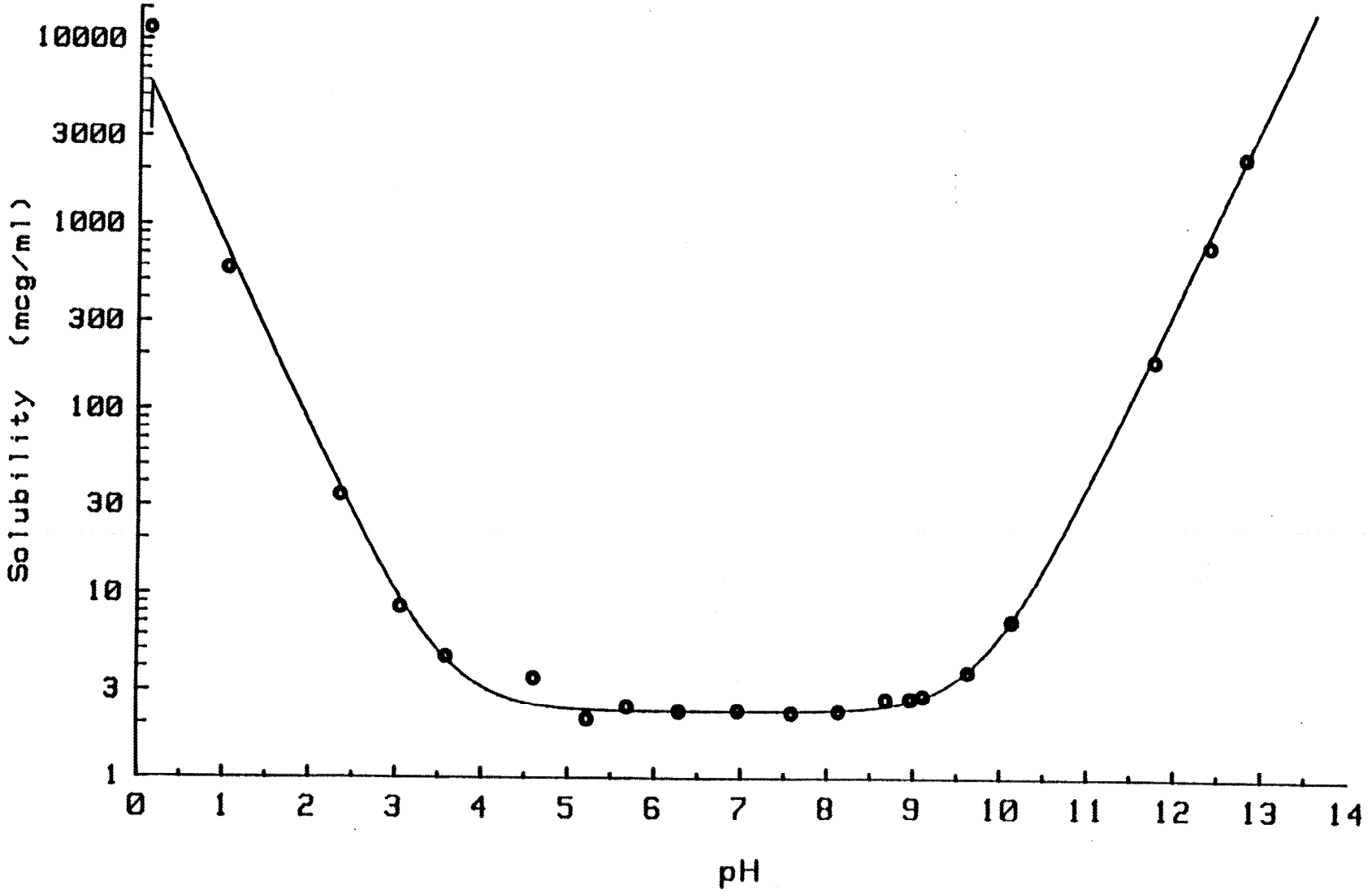
Determination of Apparent pK1a and pK2a Values of Oxfendazole from Solubility Variation with pH Studies

The solubility and pKa values of oxfendazole were determined by the general solubility method (Reference 1). Solubility determinations were made in hydrochloric acid (pH $\leq$ 3), potassium hydroxide (pH $\geq$ 11), and in buffers (pH 3-11). The ionic strength of the buffers was 1.0. Oxfendazole was suspended in 1-2 ml of solution and equilibrated for two days at constant temperature (25 ± 0.5°C). Based on the measured solubility below pH5 and above pH8, respectively, the pK1a was calculated to be 3.54 ± 0.28 and the pK2a was calculated to be 9.81 ± 0.31.

The solubility profile of oxfendazole over a range of pH values is displayed in Figure 1.

1. A. Albert and E. P. Serjeant, "Ionization Constants of Acids and Bases", Chapman and Hall, London, 1971.

pH Versus Solubility Profile
Oxfendazole Ionic strength 1.0M 25C



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APPENDIX 10

Octanol/Water Partition Coefficients

A study was designed to determine the tendency of oxfendazole to distribute between n-octanol and water. Test solutions of ^{14}C-oxfendazole (purity NLT 96.7%) were prepared at concentrations of 0.5 and 5 µg/ml (1.6×10^{-6}M and 1.6×10^{-5}M) in 99.7% pure n-octanol. Duplicate 20 ml samples of these test solutions were then mixed with respective duplicate samples of boiled distilled-deionized water. The resulting mixtures were shaken for 20 hours at 25°C. After centrifugation and separation of the two phases, duplicate samples of each solvent were measured for radioactivity by liquid scintillation counting.

The results were as follows:

Table (X)

Oxfendazole Concentration	Rep	DPM/ml n-octanol	DPM/ml Water	DPM/ml Total	% Recovery	Partition Coefficient (log Kow)
0.5 µg/ml	1	114,300	1,264	115,564	99.66	1.956
	2	114,728	1.256	115,984	100.02	1,961
	Mean	114,514	1,260	115,774	99.84	1.959
5.0 µg/ml	1	1,133,382	12,756	1,146,138	98.96	1.949
	2	1,140,018	12,932	1,152,950	99.55	1.945
	Mean	1,136,700	12,844	1,149,544	99.26	1.947

Even though oxfendazole has been shown to be amphoteric, it is stable in form over the pH range of interest, 5 to 9. Therefore, the log Kow of approximately 1.95 is representative of the partition coefficient of oxfendazole under conditions to which it would be exposed in the environment.

APPENDIX 11

Vapor Pressure

The first attempt to measure the vapor pressure of oxfendazole was made by measuring the amount of ^{14}C-oxfendazole in small, equilibrated volumes of nitrogen. This work indicated that the vapor pressure of oxfendazole is approximately 2.2×10^{-6} Torr. A second study, by an independent method, was undertaken to attempt to validate the first study.

The second study to determine the vapor pressure of oxfendazole was conducted with a method developed by Syntex which utilizes electron impact mass spectrometry instrumentation. Five micrograms of solid oxfendazole were placed in the mass spectrometer chamber under a pressure of 2×10^{-6} Torr. Measurements of total ion current and intensity were obtained as the temperature was stepped by increments of 50°C to 300°C. From the data collected, the vapor pressure at ambient temperature (21.85°C or 295°K) was estimated by calculation to be 12×10^{-11} to 2×10^{-11} Torr. This is far below that which is measurable by the gas saturation method, which estimated the vapor pressure of oxfendazole at 2.2×10^{-6} Torr at 25°C. In view of the lack of validation, it is prudent to conclude only that the vapor pressure of oxfendazole is between 10^{-6} and 10^{-11} Torr and thus below the level of environmental concern.

APPENDIX 12

Ultraviolet-Visible Absorption Spectrum of Oxfendazole in Water

A study was conducted to determine the ability of oxfendazole to absorb radiation in the UV-visible spectrum. Oxfendazole, of 97% purity, was prepared in a methanol stock solution that was serially diluted with distilled-deionized water to solutions of 10^{-4}M and 10^{-5}M concentrations. Samples of each concentration were tested, in triplicate, for peak absorbance in a scanning UV-visible spectrophotometer.

Absorbance peaks were found at 228 nm and 295 nm.

Absorbance Peak (nm)	Band-Width (nm)	Molar Extinction Coefficient (X 10^4)
228	31.8 ± 0.4	3.6225 ± 0.1394
295	19.3 ± 0.9	1.6512 ± 0.0772

APPENDIX 13

Soil Adsorption and Desorption

A study was conducted to determine the ability of oxfendazole to sorb and desorb from three different types of soil. Oxfendazole (either 99% pure drug ^{14}C-labelled in the imidazole ring or mixtures of the ^{14}C-labelled drug + various amounts of 99.8% pure drug that was non-labelled) was diluted with 0.01 M $CaCl_2$ to make up test solutions. One-hundred ml of these test solutions were then added to triplicate 20 gram samples of each soil type. The nominal test solution concentrations were: 1) 2.85 ppm for the screening test, and 2) 0.04, 0.2, 1.0, and 5.0 ppm for the advanced test. The actual oxfendazole concentrations were measured via liquid scintillation counting and the average concentrations in the screening and advanced test solutions were found to be: 1) 2.79 ppm, and 2) 0.06, 0.22, 1.02, and 5.02 ppm, respectively. Appropriate blanks for the test solutions and the soils were used as controls for this study.

The three soils tested were a sandy loam and a silt loam from California and a clay loam from Mississippi. These three soils had the following characteristics: sandy loam = 55% sand, 29% silt, 16% clay, 2.0% organic matter, pH 5.4, C.E.C. of 15 meq/100 g; silt loam = 43% sand, 52% silt, 5% clay, 1.4% organic matter, pH 7.0, C.E.C. of 18 meq/100 g; clay loam = 23% sand, 47% silt, 30% clay, 1.7% organic matter, pH 7.4 and C.E.C. of 19 meq/100 g.

The screening test was run for 16 hours and demonstrated that oxfendazole was readily sorbed to and not readily desorbed from these three soils. The average % adsorption, Kd (distribution adsorption coefficient), and Koc (Kd adjusted for % organic matter) for the above soils were: 53%, 6.0, and 512 for the sandy loam; 43%, 3.9, and 470 for the silt loam, and 76%, 16.7 and 1,673 for the clay loam. Subsequent testing for the loss of oxfendazole from these soils upon two cycles of desorption with untreated 0.01 M $CaCl_2$ solutions resulted in total oxfendazole losses from soils of 33%, 45%, and 19%, respectively. Based upon these results, an advanced test was undertaken.

The results of the advanced test demonstrated that the equilibrium between oxfendazole partitioning into water and soil was reached in these three soil types in about 22 hours. At 22 hrs, the sandy loam adsorbed from 52-65% of the various oxfendazole doses, while the silt loam and clay loam, respectively, adsorbed from 44-58% and 76-81% of the oxfendazole present. As would be expected, there was a distinct tendency for the lower oxfendazole doses to be more completely sorbed by the uniform level of sorbant (20 g soil). The K's and Koc's calculated from the isotherm portion of the advanced test data were reported to

be 6.4 and 544 for the sandy loam, 4.5 and 546 for the silt loam, and 15.7 and 1,570 for the clay loam. These data demonstrate that oxfendazole would be predicted to generally have a low mobility in soil, with this drug most readily adsorbed by the clay loam, less so by the sandy loam, and least by the silt loam soil.

The material balance portion of the advanced test demonstrated that, by the end of the test (111 hrs), the mean ^{14}C recoveries for these three soils and their respective aqueous phases ranged from 106-111%. Therefore, degradation of oxfendazole to volatile products did not seem to occur.

APPENDIX 14

Biodegradation in Soil

The degradation of oxfendazole was studied at Biospherics Incorporated in three different types of soil. The characteristics of the three soil types are listed below:

		%					
Soil Type	Origin	Sand	Silt	Clay	Organic Matter	pH	C.E.C. meq/100g
Sandy Loam	GA	77	11	12	2.0	6.3	10.4
Silt Loam	AK	15	72	13	1.2	6.4	11.0
Silty Clay Loam	AK	18	49	33	2.2	7.4	19.0

Since the pK1a of oxfendazole has been determined to be 3.54 ± 0.28 and the pK2a to be 9.81 ± 0.31, oxfendazole would not undergo a change in form in the pH range of the soils tested; thus, the soils selected for study were appropriate test soils.

Triplicate samples of each soil were prepared and dosed as follows:

1. ^{14}C-oxfendazole (99.8% pure) : 10 ppm
2. 10 ppm ^{14}C-oxfendazole + 500 ppm glucose
3. 0.7 ppm ^{14}C-oxfendazole + 500 ppm glucose
4. 500 ppm glucose
5. Nontreated controls

Samples were placed in flasks which were connected to inlet and outlet trapping systems. Radioactivity produced from the sample was measured by liquid scientillation counting of the traps.

At the end of the 68-day test period, a mass-balance approach was taken with the oxfendazole-treated soils to attempt to account for all of the radioactivity. About 85% of the radioactivity was accounted for by this technique.

At least 60% of the ^{14}C-glucose in the positive control flasks had been metabolized to gaseous products; therefore, the three soils contained healthy microbial populations. During the same 68-day test period, less than 0.5% of the ^{14}C-oxfendazole was found to be converted to ^{14}C-Co_2 or other volatile products in any of the three soil treatments.

Total recovery of radioactivity from oxfendazole-treated soils averaged about 95%. Depending on soil type, 40% to 65% of this radioactivity was nonextractable from the soil by use of methanol and acetone. TLC of the extracted radioactivity showed that 75% to 80% of the extractable residues were parent (oxfendazole) and 8% to 15% consisted of the sulfone derivative.

It may be concluded from this report that oxfendazole was degraded only minimally in the types of soil tested in this study and that the major degradation product of oxfendazole in soil is the -sulfone analog.

APPENDIX 15

Hydrolysis of Carbon-14 Labelled Oxfendazole (Benzimidazole Carbamate)

In a study conducted by Biospherics Incorporated, the hydrolysis of ^{14}C-labelled oxfendazole was determined in buffer solutions at pH 5, 7, and 9 over a 28-day period. Each of the respective pH solutions, maintained in triplicate, contained 2.64 mg ^{14}C-oxfendazole/L buffer and was kept at 25 ± 1°C. Samples were taken for determination of oxfendazole radiocarbon levels at from 1- to 3-day intervals over the 28-day test period. Additional samples were taken periodically to determine the types and proportions of degradation products present in each of the respective ph solutions. These determinations were carried out by means of TLC separation and autoradiographic comparison with Rf values of cold standards of specific possible degradation products.

Total radiocarbon recovery rates ranged from 95-98% at the beginning of the test and were still 96-97% of initial levels at 28 days, indicating that no radioactivity was lost through volatilization during the study.

Little, if any, degradation of oxfendazole was observed at either pH 5 or pH 7; therefore, neither hydrolysis rate constants nor half-lives could be estimated at these pH values. However, at pH 9, oxfendazole degraded rather rapidly, with only 30.2% of the total radioactivity remaining as oxfendazole at day 28. At pH 9, the rate constant was determined to be 3.94×10^{-2} day^{-1} and the half-life was calculated to be 17.6 days.

As determined by TLC analysis of the degradation products found at pH 9 at day 28, about two-thirds of the radioactivity remained at the origin (polar components which represent the hydroxyphenylsulfinyl and the 2-amino products). Less than 2.5% of the radioactivity chromatographed as other, less polar oxfendazole analogues, such as the sulfone and the -thio analogue (fenbendazole).

APPENDIX 16

Chemical Reactivity of Oxfendazole in Solution

In this study conducted by Syntex Research, the hydrolysis of oxfendazole was determined in aqueous buffer solution at 80°C as a function of pH. Results indicate that the degradation of oxfendazole follows simple first-order kinetics over more than four half-lives. At all pH values, the only degradation product observed was 2-amino-5 phenylsulfinyl-benzimidazole. The observed rate constant was independent of pH below pH 2.5, decreased from above pH 2.5 to pH 4, became independent of pH again between pH 4 and pH 6, and then showed a linear increase with increasing pH to give a slope close to unity. In the pH range of 6 to 12.5, oxfendazole shows a linear dependence on hydroxide ion activity, with an inflection at approximately pH 10 where the dissociation of oxfendazole affects the rate of hydrolysis.

Using the data from this trial and the data from the Biospherics Incorporated trial (see Appendix (x)-7a, above), the hydrolytic half-lives of oxfendazole, as they vary with pH, are as follows:

pH	Half Life (Days)
5.17	2983
6.58	946
7.19	383
7.82	157
8.32	51.9
8.56	33.8
9.16	17.6

APPENDIX 17

IMPACT OF HYDROLYSIS ON THE CONCENTRATIONS OF OXFENDAZOLE IN FEEDLOT MANURE

The effect of hydrolysis on the concentration of oxfendazole in feedlot waste will vary greatly with pH. One may estimate the amount converted at various assumed pH values from the rate data reported in Document # AER 10,008, *Environmental Half-life of Oxfendazole in Cattle Manure and Agricultural Soils*. The concentration at the end of 136 days, the assumed manure residence time, is given in the Table below.

CONCENTRATION OF OXFENDAZOLE IN FEEDLOT MANURE AT THE END OF 136 DAYS

pH OF MANURE	$t_{½}$ (days)	k (per day)[1]	C_o/C[2]	C (ppb)[3]
5.17	2983	0.0002323	1.032	1163
6.58	946	0.0007326	1.105	1086
7.19	383	0.001809	1.279	938
8.56	33.8	0.02050	16.26	73. 8

$t_{½}$ = half-life
k = degradation constant
C_o = initial concentration
C = final concentration

NOTES:

[1] From rate equation: $C = C_o \times \exp(-kt)$

therefore $\ln C = \ln C_o - kt$ and $k = \ln (C_o / C) / t$

At $t_{½}$, C is one-half C_o therefore $C_o / C = 2$ and $\ln (C_o / C) = \ln 2$

$\ln 2 = 0.693$

$k = 0.693 / t_{½}$: $t_{½}$ is taken from column two

[2] From the equation above: $k = \ln (C_o / C) / t$

therefore $\ln (C_o / C) = kt$ and $C_o / C = e^{kt}$

with k taken from column three and t = 136 days

[3] When C_o = 1200 ppm: $C = 1200 / (C_o / C)$

(C_o / C) is taken from column four

The initial concentration of oxfendazole in the manure is based on a one time dose of 4.5 mg/kg, an animal weight of 308 kg, a residence time of 136 days, and a dry basis manure excretion rate of 3.4 tons/1000# animal-year.

The pH of feedlot manure has been measured for a Colorado feedlot by Hansen, et al (1). The measurements were made over a one year period, and represented 6 different types of rations. The mean value and standard deviation derived from 139 samples collected from three locations within the feedlot were 7.09 and 0.07, respectively.

REFERENCES:

1. Hansen, R.W., Harper, J.M., Stone, M.L., Ward, G.M. and Kidd, R.A. *Manure Harvesting Practices: Effects on Waste Characteristics and Runoff*, Report # EPA-600/2-76-292

APPENDIX 18

BUILDUP OF OXFENDAZOLE IN AGRICULTURAL SOILS WITH VARYING pH VALUES UPON REPEATED APPLICATION OF MANURE FROM TREATED CATTLE

The buildup of oxfendazole in agricultural soils from yearly application of manure containing oxfendazole, taking into account the loss of oxfendazole due to ongoing hydrolysis, can by calculated as follows:

Using the values below the concentration of oxfendazole in agricultural soils after the addition of manure is calculated to be 24.07 ppb. A detailed explanation of this calculation is found in the text.

Oxfendazole administered -	4.5	mg/kg body weight
Feedlot period -	136	days
Concentration in manure -	1.2	mg/kg manure
Amount of manure used -	20	tons/acre
Weight of top 6" of topsoil -	9.09×10^5	kg/acre

After one year the concentration in soil with a pH of 5.17 providing for hydrolysis could be calculated as follows:

$C_{n=1} = C_o \times e^{-kt}$

$C_o = 24.07$ ppb (the initial concentration of oxfendazole in soil)

$k = 0.0002323$ (from calculation of K in Doc. # AER 10546: *Impact of Hydrolysis on the Concentration of Oxfendazole in Feedlot manure)*

$t = 365$ days

$C_{n=1} = 24.07 \times 0.919 = 22.12$ ppb

The following year the concentration would decrease:

$C_{n=2} = C_{n=1} \times 0.919 = C_o \times (0.919)^2$

Each subsequent year the concentration would be:

$C_{n=x} = C_o \times (0.919)^x$

After n years the accumulated oxfendazole concentration in the soil, accounting for the loss through hydrolysis would be:

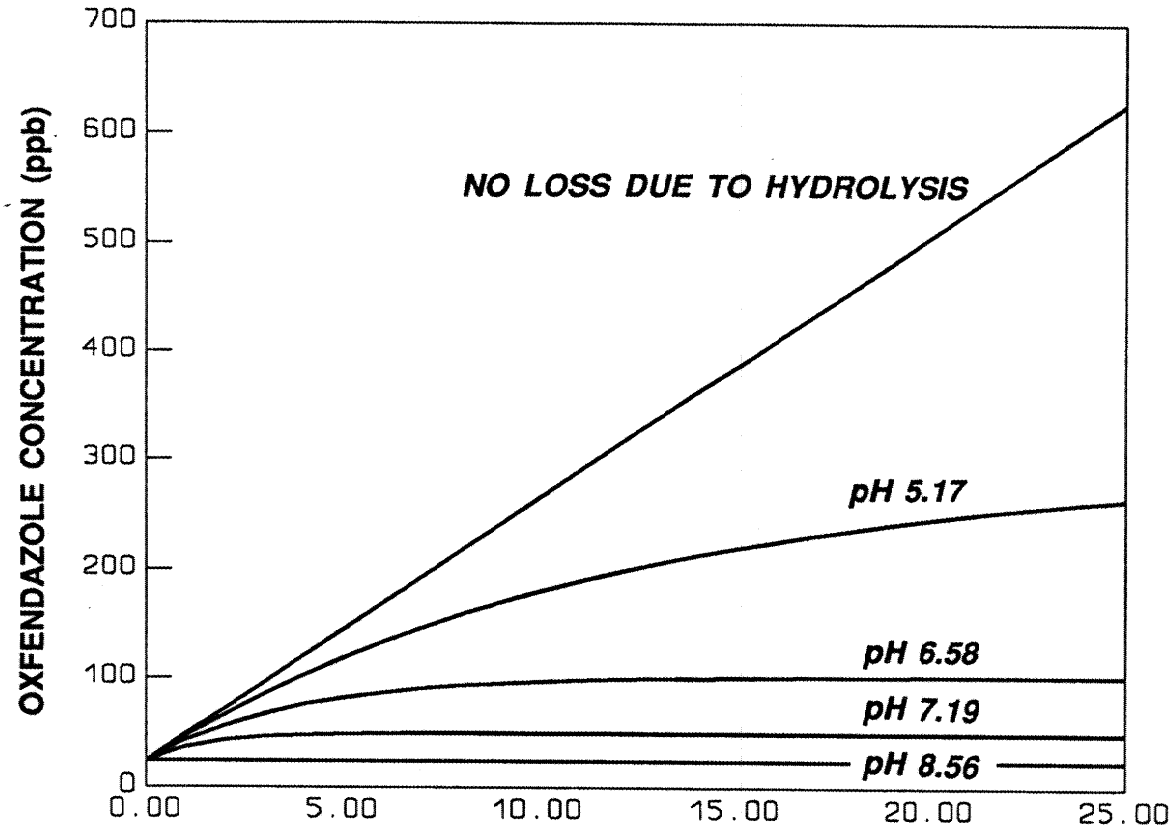
$$C_n = C_o + C_o \times 0.919 + C_o \times (0.919)^2 + \ldots C_o \times (0.919)^{n-1}$$

$$= C_o \times (1 + 0.919 + (0.919)^2 + \ldots \times (0.919)^{n-1})$$

The cumulative effect of the application of manure over a period of 25 years for soils of varying pH levels is given in Figure 1. The level of oxfendazole that would result in the absence of hydrolytic degradation is given for comparison.

Figure 1

The Effect of Hydrolysis on Oxfendazole Concentration in Agricultural Soils by pH



The maximum concentration with this loading can be determined by the limit:

$$\lim C_n = C_o (a / 1 - r)$$

Therefore the maximum concentration of oxfendazole in an agricultural soil with a pH of 5.17 would be:

$$C_n = 24.07 (1 / 1 - 0.919) = 297 \text{ ppb}$$

By utilizing the same procedure the maximum concentrations at other pH values can be determined. Values calculated for soils at several pH levels are given below:

pH	max. concentration (ppb)
5.17	297
6.58	103
7.19	49.8
8.56	24.1

APPENDIX 19

Photolytic Decomposition of Oxfendazole in Aqueous Solution

The photodegradation of oxfendazole was characterized by exposure to sunlight of aqueous solutions at pH 5, pH 7, and pH 9, in conformance with Technical Assistance Document 3.10. Solutions of oxfendazole at nominal concentrations of 1.5 ppm (1/2 maximum) were exposed to sunlight for three hours, starting one hour before solar noon. In each case, the extent of photodegradation exceeded three half-lives since the concentration of oxfendazole was reduced, after exposure, to less than 20% of the starting concentration.

The reference chemical used in this study was p-nitroacetophenone in an aqueous 0.2 M pyridine solution containing 1% acetonitrile. Actinometers made from this material were exposed to sun at exactly the same intervals as the oxfendazole solutions.

After determination of the half-lives of oxfendazole, compensation for changing solar radiation was made by comparing the degradation rates of oxfendazole to those of the actinometer. In all cases, oxfendazole degraded more rapidly than the actinometer and the environmental summer season photodegradation rates for oxfendazole were calculated to be as follows:

pH	t 1/2
5.0	0.100 days
6.9	0.121 days
8.9	0.073 days

APPENDIX 20

Photolytic Decomposition of Oxfendazole Amine in Aqueous Solution

The major endproduct of hydrolytic degradation of oxfendazole is the amine, 2-amino-5(6)phenylsulfinylbenzimidazole. A study was carried out by Syntex Research at Palo Alto, CA, to characterize the rate of photodegradation of oxfendazole amine in solution. Oxfendazole amine was studied at a nominal concentration of 11 ppm, less than one-half its measured solubility, at pH 5.11, pH 6.97, and pH 9.05. The study was carried out to conform with Method 3.10 in the FDA Environmental Assessment Technical Assistance Document.

The reference compound used in this study was p-nitroacetophenone in an aqueous 0.2 M pyridine solution containing 1% acetonitrite. Actinometers containing this material were exposed to sun at exactly the same intervals as oxfendazole amine solutions; e.g., for a four-hour period beginning one hour before solar noon. Since degradation at pH 5.11 was slower, exposure of the solutions at this pH were exposed on a second day to achieve two half-lives.

In all cases, the oxfendazole amine degraded faster than did the actinometer. Calculated half-lives for summer season, clear-sky conditions at 40 degrees North latitude are as follows:

pH	t 1/2
5.11	0.28 days
6.97	0.095 days
9.05	0.085 days

APPENDIX 21

Material Safety Data Sheet

SYNTEX

Date: 30 May 1981 Revised: 13 May 1987

SUPPLIER OF DATA: Syntex (U.S.A.) Inc.
3401 Hillview Avenue
Palo Alto, CA 94304

In emergency, call: Environmental Health & Safety, (415) 855-5050

WARNING STATEMENT

Warning: Chronic overexposure may cause reproductive system and blood disorders. Avoid ingestion, inhalation, skin contact, and eye contact. Material intended for veterinary manufacturing use only.

MATERIAL IDENTIFICATION

COMMON NAME: Oxfendazole

CHEMICAL NAME: methyl (5-phenylsulfinyl)-2-benzimidazole carbamate

FORMULA: $C_{15}H_{13}N_3O_3S$

SYNONYMS: CAS-53716-50-0, RS-8858

Disclaimer of Warranty on Back Page

HEALTH HAZARD

EYE

Based on animal studies, oxfendazole is not expected to cause eye irritation.

SKIN

Based on animal studies, oxfendazole is not expected to cause skin irritation or to produce skin sensitization.

SYSTEMIC

Test animals tolerated well a single oral dose of oxfendazole of 6 g/kg body weight.

Based on animal studies, overexposure to oxfendazole dust may lead to blood abnormalities. These are manifested by decreases in the numbers of red and/or white blood cells or shifts in the ratios of the various types of white blood cells.

Chronic overexposure to oxfendazole may produce liver damage. This can be detected by an elevation in the levels of certain liver enzymes in the blood.

In animal studies, very high doses of oxfendazole produced teratogenicity and embryotoxicity. However, at doses of 10 mg/kg or less, oxfendazole was not embryotoxic or teratogenic in rats, mice, sheep or cattle. Studies carried out with large numbers of rams indicate that oxfendazole did not functionally impair reproduction or cause any testicular changes.

Oxfendazole is not carcinogenic in rodents.

PERMISSIBLE EXPOSURE LIMIT

0.35 milligram/m^3 (Syntex internal PEL, 8 h time-weighted average)

FIRST AID

EYE CONTACT

Flush eyes thoroughly with water for at least 15 minutes.

SKIN CONTACT

Wash contacted area thoroughly with copious amounts of soap and water.

INHALATION

Remove from source of exposure to fresh air. Contact supervisor and physician.

INGESTION

Give moderate amounts of water. Contact supervisor and physician.

wp/5049r-69

SPECIAL PROTECTION

EYE PROTECTION

Wear safety glasses with side shields, chemical splash goggles or full face shield. The choice of eye protection should be appropriate for the job activity being conducted.

RESPIRATORY PROTECTION

If airborne exposure levels of oxfendazole are above the PEL, respiratory protection should be used. The following represent the minimum recommended respiratory protection: for levels up to 3.5 mg/m^3, a properly fitted NIOSH/MSHA-approved half-mask air-purifying respirator with dust/mist filters; for levels up to 17.5 mg/m^3, a full facepiece air-purifying respirator with dust/mist filters; for exposures up to 350 mg/m^3, "hood type" powered-air purifying respirators, and for exposures above 350 mg/m^3, atmosphere-supplying respirators.

SKIN PROTECTION

Hands, wrists, arms and other potentially exposed skin surfaces should be completely protected. Skin protection may include gloves impervious to any solvents being used, lab coat, coveralls, Tyvek or other impervious disposable clothing (e.g., coveralls and sleeve covers), and boot covers. The choice of skin protection should be appropriate for the job activity.

VENTILATION

Dust control ventilation (e.g. local exhaust) should be used where workplace levels cannot be maintained below the PEL by improved handling techniques or by isolating the dust emission source.

OTHER

Employees working with oxfendazole should wash hands and face before all breaks. Showers are recommended at the completion of the work shift for workers in operations with direct exposure to bulk oxfendazole. Work clothing, including work shoes, should not be taken home. All protective equipment should be cleaned thoroughly.

FIRE PROTECTION

FLASH POINT
Not applicable

MINIMUM EXPLOSIVE CONCENTRATION
Classes as a strong-to-severe dust explosion hazard. As a finely divided material suspended in air in heavy concentration, explosion may occur if subjected to a source of ignition.

MINIMUM IGNITION ENERGY
Unknown

EXTINGUISHING MEDIA
Water, multipurpose dry chemical or halon fire extinguisher.

SPECIAL FIRE FIGHTING PROCEDURES
Wear full protective clothing and NIOSH/MSHA approved positive pressure self-contained breathing apparatus. Thoroughly wash all equipment after use.

REACTIVITY

STABILITY
Stable.

INCOMPATIBILITY
Strong oxidizing agents (e.g. peroxides, permanganates, nitric acid, etc.) may produce violent reaction.

HAZARDOUS DECOMPOSITION PRODUCTS
Combustion may produce carbon dioxide and carbon monoxide and oxides of nitrogen and sulfur.

HAZARDOUS POLYMERIZATION
Will Not Occur

ENVIRONMENTAL PROTECTION

PRECAUTIONS IF MATERIAL IS RELEASED OR SPILLED

First cordon off area. Do not attempt to sweep up powdered materials, use either wet cleanup methods with an appropriate solvent such as slightly acidic or basic aqueous solutions or industrial vacuum cleaners equipped with high efficiency particulate filters. Use appropriate personal protective equipment.

WASTE DISPOSAL METHODS

All waste residuals should be contained and stored separately from other facility discharges. Dispose of any residual wastes according to prescribed federal, state, and local guidelines; e.g. approved chemical wastes incineration, secured chemical landfill, or approved aqueous discharge of rinsewaters resulting from spill cleanups to municipal or on-site wastewater treatment systems.

PHYSICAL PROPERTIES

BOILING POINT:	Not applicable
MELTING POINT:	245-265 degrees C with decomposition
MOLECULAR WEIGHT:	315.34
SOLUBILITY:	Very soluble at low and high pH.
VAPOR PRESSURE:	Nil
SPECIFIC GRAVITY:	Information not available.
PERCENT VOLATILE:	Nil
VAPOR DENSITY:	Information not available.
EVAPORATION:	Nil
APPEARANCE, COLOR, ODOR:	White-grey powder. Slight characteristic odor.

wp/5049r-72

LABELING

Bulk containers of oxfendazole or oxfendazole-containing formulations should have affixed the following warning label (in addition to the product identity label):

Warning: Chronic overexposure may cause reproductive system and blood disorders. Avoid ingestion, inhalation, skin contact, and eye contact. Material intended for veterinary manufacturing use only. Read and understand the Material Safety Data Sheet for additional information before working with this product.

* *

The above information is based on data available to use and is believed to be correct. However, NO WARRANTY is expressed or to be implied regarding the accuracy of this information, the results to be obtained from the use thereof, or the hazards connected with the use of material. Since the information contained herein may be applied under conditions beyond our control and with which we may be unfamiliar, we do not assume any responsibility for the results of its use. This information is furnished upon the condition that the persons receiving it shall make their own determinations of the effects, properties and protections which pertain to their particular conditions.

APPENDIX 22

Microbial Growth Inhibition

In a test conducted by Syntex Research, the activity of oxfendazole against several genera of bacteria was tested using a serial-dilution technique. Organisms tested included:

Staphylococcus aureus	ATCC 6538P
Staphylococcus aureus	ATCC 14154
Streptococcus pyogenes	ATCC 8668
Klebsiella pneumoniae	ATCC 10031-2
Proteus vulgaris	ATCC 9484
Escherichia coli	ATCC 25922-1
Pseudomonas aeruginosa	ATCC 10145

Even at concentrations exceeding the solubility of oxfendazole, the compound did not affect the replication of these bacteria.

In addition, 100 mcg quantities of oxfendazole were applied to 13 mm microbial discs, which were then placed on plates of solid media inoculated with the following organisms:

Staphylococcus aureus	ATCC 6538P
Streptococcus pyogenes	ATCC 8668
Escherichia coli	"Smith"
Pseudomonas aeruginosa	ATCC No. 10145
Candida albicans	Clinical isolate
Trichophyton mentagrophytes	Tm 78
Mycoplasma spp.	Ms 21

No zones of inhibition were observed on any of the culture plates, following incubation.

A second study was conducted at Syntex Research to determine the effect of oxfendazole against representative soil organisms. Oxfendazole was incorporated into nutrient agar, prior to pouring, to yield final drug concentrations of 30, 10, 3, 1, and 0 µg/ml (ppm). After drying, plates were inoculated with organisms according to Table 1. In addition to the organism, incubation conditions and media are also listed in Table 1.

No inhibition of growth was observed. Therefore, one can conclude that oxfendazole has no effect on the soil or environmental organisms described even at concentrations equal to its maximum solubility ($\geq$9 ppm).

Table 1

Organism	ATCC Number	Incubation Temperature	Length of Incubation (Days)	Growth Medium
Nostoc species	27895	25°C[1]	13	Medium BG-11[2]
Arthrobacter terregens	13345	25°C	3	Nutrient Agar
Azotobacter chroococcum	9043	25°C	3	Nutrient Agar
Cytophaga johnsonae	29589	25°C	2	Nutrient Agar
Pseudomonas facilus	17695	25°C	2	Nutrient Agar
Streptomyces galilaeus	14969	25°C	2	Nutrient Agar
Bacillus mycoides	6462	30°C	1	Nutrient Agar
Cellulomonas flavigena	484	30°C	1	Nutrient Agar
Flavobacterium species	29790	30°C	1	Nutrient Agar
Clostridium absonum	27555	37°C[3]	1	Reinforced Clostridial Agar
Aspergillus bicolor	36104	30°C	7	Modified Emmons Agar[4]
Chaetomium piluliferoides	34564	30°C	2	PDA[5]
Fusarium chlamydosporum	15615	30°C	2	PDA
Penicillium inflatum	48995	30°C	3	PDA
Trichoderma harzianum	48134	30°C	2	PDA

[1] Grown Under Continuous Fluorescent Light

[2] Medium BG-11 for Blue-Green Algae (ATCC medium 616)

[3] Anaerobic Incubation

[4] Modification of Sabouraud's Dextrose Agar (1% Dextrose)

[5] Potato Dextrose Agar

APPENDIX 23

Subacute Toxicity of Oxfendazole to Earthworms

In this study, conducted in compliance with Protocol 4.12 of the FDA Environmental Assessment Technical Assistance Handbook, oxfendazole was evaluated for subacute toxicity to earthworms, Lumbricus terrestris. Earthworms were randomly assigned to replicated control and treatment groups (mean measured concentrations of 0, 80, 146, 288, 544, and 971 mg oxfendazole/kg of soil) and observed for 28 days. Mortality and signs of sublethal toxicity (lethargy, burrowing activity, and visible lesions) were recorded on days 7, 14, 21, and 28; weights were measured on days 0 and 28. Soil conditions were measured on days 0, 7, 14, 21, and 28. Concentrations of oxfendazole in soil were determined on days 0, 14, and 28. Soil moisture levels were approximately 30% during the test and soil pH was consistent throughout the study period, ranging from 6.1 to 6.9. Two of 40 worms died in the control group and in the 544 ppm group (5% mortality); 2.5% mortality was observed in the high-dose group (971 ppm). One individual in each of the control, 80, 288, and 544 ppm groups exhibited a lesion. Earthworm burrowing time and incidence of abnormalities were not affected by oxfendazole treatment. All but one replicate of earthworms (rep. D at 544 ppm) showed positive weight gains; ANOVA indicated no significant difference among the groups with respect to weight gain over the 28-day period.

The results of the study indicate that oxfendazole is not expected to have an effect on earthworms at concentrations up to 971 mg oxfendazole per kg soil (971 ppm).

APPENDIX 24

Acute Toxicity of Oxfendazole to Daphnia magna

ABC Labs conducted a well-controlled study in which quadruplicate groups of five first-instar larvae of Daphnia magna (<24 hr old) were exposed to varying concentrations of oxfendazole of 97% purity. Actual concentrations, measured by HPLC, averaged <0.1, 0.06, 0.12, 0.23, 046, and 0.86 mg oxfendazole/L in samples taken on day 0 and day 2.

Lethal and sublethal effects were monitored each day of the 2-day test, while temperature, dissolved oxygen concentrations, and pH of representative test solutions were monitored periodically and did not appear to vary significantly.

The 48-hour EC_{50} for oxfendazole was calculated to be 0.52 ppm, with a 95% confidence interval of 0.4 to 0.76 ppm. The slope of the dose-response line was determined to be 2.7.

APPENDIX 25

Acute Toxicity of 2-amino-5(6)-phenylsulfinyl-benzimidazole for Neonate *Daphnia magna*

ABC Labs conducted a 48-hour static bioassay to determine the effect of this hydrolytic product of oxfendazole on neonate *Daphnia magna*. Daphnids were exposed to concentrations of 0, 0.01, 0.1, 1.0, and 10.0 mg of 2-amino-5(6)-phenylsulfinyl-benzimidazole per liter (ppm). They were observed at 24 and 48 hours for mortality or abnormal effects. None were observed; therefore, at concentrations $\leq$10.0 ppm, this hydrolytic degradation product of oxfendazole is not toxic to neonate daphnids.

APPENDIX 26

Fresh-Water Fish Acute Toxicity (Bluegill Sunfish)

Bluegill sunfish (Lepomis macrochirus) were selected as a species representative of warm-freshwater fish to determine the acute toxicity of oxfendazole for freshwater fish. The study was conducted by ABC Labs in Missouri. Bluegill sunfish, averaging 0.54 g live-weight and 28 mm in length, were exposed to varying concentrations of oxfendazole, of 97% purity. Groups of ten fish were exposed to concentrations ranging from <0.2 to 2.7 ppm. Observations for mortality and signs of sublethal effects -- such as loss of equilibrium, quiescence, surfacing, discoloration, and remaining on the bottom of the test vessels -- were made daily during the test period of four days. Water temperature, dissolved oxygen concentrations and pH of representative test solutions were monitored periodically and did not vary significantly. No mortality or sublethal abnormal effects were observed at any time or at any concentration tested.

The 96-hour LC_{50} for oxfendazole for bluegill sunfish was >2.7 mg/L (ppm), the highest concentration tested.

APPENDIX 27

Freshwater Acute Toxicity (Rainbow Trout)

Rainbow trout (Salmo gairdneri) were selected as a representative species of cold-freshwater fish in the determination of the acute toxicity of oxfendazole to freshwater fish. ABC Labs exposed groups of ten trout, averaging 0.4 g and 31 mm, to concentrations of oxfendazole, 97% pure, varying from <0.2 to 2.5 mg/L (ppm). Observations were made daily over the four-day test period for mortality and for signs of sublethal abnormalities: loss of equilibrium, quiescence, surfacing, dark discoloration, and remaining on bottom of the test vessel. Water temperature, dissolved oxygen concentration, and pH of representative solutions were monitored periodically, and did not vary significantly among samples.

No mortality or abnormalities were noted at any of the test concentrations = <0.2, 0.57, 0.95, 1.7, 2.1, and 2.5 mg/L (ppm). Therefore, the 96 hour LC_{50} for oxfendazole is >2.5 ppm, the highest concentration tested.

APPENDIX 28

Phase I for Determining the Toxicity of Oxfendazole on Seed Germination and Root Elongation of Wheat (Triticum aestivum), Perennial Ryegrass (Lolium perenne), Cucumber (Cucumis sativus), Soybean (Glycine max), Lettuce (Lactuca sativa)

A preliminary study was conducted by ABC Labs to determine the toxicity of oxfendazole for six angiosperms of agronomic importance, listed above in the title. Six replicates, each of 50 seeds, of each of the test species were exposed either to deionized water (control) or to oxfendazole suspended in deionized water at a concentration of 1000 ppm during germination. Germination, defined as radicle length of >3 mm, and radicle elongation were determined at intervals. Percent germination and radicle growth data were analyzed and group means were compared using a paired T-test at 0.05% alpha level. Statistically significant differences occurred between the treatment and controls for germination of perennial ryegrass and for radicle length of wheat.

Because of these findings, a definitive study was conducted using wheat and perennial ryegrass. The results are reported in another appendix.

APPENDIX 29

Bioassay for Determining the Toxicity of Oxfendazole on the Seed Germination and Root Elongation of Wheat (Triticum aestivum)and Perennial Ryegrass (Lolium perenne)

This study was conducted by ABC Labs to evaluate the potential toxicity of oxfendazole at a series of concentrations up to 1000 ppm on the germination rate and radicle growth of wheat and perennial ryegrass. In a preliminary test with six different angiosperms of agronomic importance, wheat had demonstrated a difference in radicle length and the data indicated a decrease in seed germination for ryegrass. Therefore, this definitive study was carried out to better define these effects.

The design of the study was in agreement with TAD 4.06 in FDA's Environmental Assessment Technical Handbook. Ryegrass seeds were exposed to oxfendazole of 98.7% purity in suspensions of 1000, 91.0, 8.3, 0.75 and 0.07 ppm; wheat seeds were exposed to 1000, 100, 10.0, 1.0, and 0.1 ppm. Each treatment and control group consisted of six replicates of 50 seeds each. Following pre-treatment preparation consisting of rinsing, chlorine soaking, further rinsing, and then soaking in deionized water, seeds were placed in germination dishes on two layers of filter paper to which the appropriate test suspension (25 ml) had been added. Dishes were then placed in a dark incubator in a randomized complete block design.

Data collected included percent germination and radicle length in addition to recording any visible abnormalities such as discoloration, lesions, or fungal growth. Germination was defined as emergence of a radicle at least 3 mm in length from the seed coat. When average radicle length in the control group was greater than 20 mm, testing was concluded and photographs were taken of each test dish.

Analysis of variance (ANOVA) for percent germination of wheat and for wheat radicle length showed no significant difference among treatments ($p < 0.05$). ANOVA for ryegrass germination data also found no statistically significant differences among treatments ($p < 0.05$). ANOVA of the data for ryegrass radicle lengths indicated that a significant difference existed among treatments. Applying Tuckey's HSD critical range to the treatment means showed a significant difference between treatment at 0.75 ppm (greater growth) and at 8.3 ppm (lesser growth). These results are not consistent with the results of the preliminary study and are not dose-related. Some visible abnormalities were observed in both wheat and ryegrass, but incidence was low and did not appear to be treatment-related.

Based on the results of the preliminary and definitive studies, one would not expect significant adverse effects on seed germination or root elongation in the proposed agricultural setting.

APPENDIX 30

Seedling Growth Studies

The potential effects of oxfendazole on seedling growth was determined in a study conducted by Springborn Life Sciences. A total of six species of angiosperms was selected for study, including three monocot species and three dicot species. The three monocots were wheat (Triticum aestivum), corn (Zea mays) and ryegrass (Lolium perenne), and the three dicots were cucumber (Cucumis sativus), pinto bean (Phaseolus vulgaris), and soybean (Glycine max).

Seeds not previously treated with fungicides or insecticides were selected and germinated in moistened paper towels in the dark. The towels were kept moist during the germination period.

After germination, the seedlings were transplanted into 5-inch diameter polypropylene pots, five seedlings per pot or replicate. Three replicates per treatment (15 plants) were used in a preliminary study while five replicates (25 plants) were used in the definitive study.

Washed silica sand, 20-40 mesh, was used as the support medium; oxfendazole was added in varying concentrations to the support medium as a solution in formic acid diluted in methanol.

Pots were placed in environmental chambers, using a 16-hour light:8-hour dark cycle. A nutrient solution was provided by subirrigation on alternate days throughout the 21-day test. Seedling shoot length was measured periodically until termination. At 21 days, roots and shoots of each plant were separated and oven-dried prior to weighing.

Results obtained during the preliminary phase were used to select an appropriate range of treatment concentrations for the definitive test.

No-observed-effect concentrations (N.O.E.C.) and lowest-observed-effect concentrations (L.O.E.C.)were calculated and can be summarized as follows:

Species	NOEC (mg/kg)	LOEC (mg/kg)
Cucumber	7.56	10.0
Pinto Bean	0.912	1.82
Ryegrass	7.56	10.0
Wheat	102	N.D.
Corn	102	N.D.
Soy Bean	10*	48.5**

N.D. - Not determined due to conflicting results from preliminary and definitive tests.

* Determined in the preliminary study.
** Determined from results in the definitive study.

APPENDIX 31

Soybean Seedling Growth Study

Since a No-Observed-Effect-Concentration was not established in the definitive study described previously (Appendix y-8), a repeat study was conducted on soybeans only. Materials and methods were essentially identical to those utilized in the six-species definitive seedling-growth study. In the second test, only soybeans were evaluated and six target concentrations were tested: 4.0, 8.0, 16.0, 32.0, 64.0, and 128.0 mg oxfendazole/kg support medium.

Data on seedling survival were analyzed by Fisher's Exact Test while growth parameters were analyzed by Dunnett's one-sided test. Data from the test groups were compared to those from the solvent-control group and no effects were observed at any of the concentrations tested on any of the measurements ($p < 0.05$). Actual concentrations tested, determined by analysis, were 3.6, 6.6, 7.6, 26, 40, and 110 mg oxfendazole per kg of support medium.

Thus, the N.O.E.C. of oxfendazole on soybean seedling growth was determined to be equal to or greater than 110 mg/kg.