ENVIRONMENTAL ASSESSMENT OF ALBENDAZOLE
A BROAD-SPECTRUM ANTHELMINTIC

1. DATE: March 7, 1989

2. APPLICANT: SmithKline Animal Health Products

3. ADDRESS: Applebrook Research Center
1600 Paoli Pike
West Chester, PA 19380

4. DESCRIPTION OF THE PROPOSED ACTION:

A. Description of the Requested Approval

SmithKline Animal Health Products is requesting approval for use of albendazole as a broad spectrum anthelmintic. Albendazole will be administered as a single dose treatment for helminth infections of all classes of cattle, including dairy cows, replacement heifers, and beef cattle. It will also be used as an anthelmintic for sheep and ultimately for goats.

B. Need for the Proposed Action

Albendazole is an anthelmintic substance which is effective against all classes of helminths which commonly infect domestic animals, viz. gastrointestinal roundworms, lungworms, tapeworms, as well as liver flukes. The mode of action of albendazole has not been confirmed, however, it is presumed to be similar to other 2-amino substituted benzimidazole anthelmintics which eliminate helminths via interfering with the polymerization of microtubulin.[1]

The increased spectrum of activity of albendazole over the other 2-amino substituted benzimidazole anthelmintics is probably due to the fact that albendazole has greatly enhanced absorption and distribution. No other single chemical entity is known which possesses such a wide spectrum of activity, thereby negating a need for combination therapy and offering significant therapeutic benefits to the livestock owner. Further, such a drug, which is both safe and efficacious, is urgently needed to combat the deer liver fluke Fascioloides magna which yearly infects, debilitates and kills sheep in ever widening areas of the country[2].

C. Location where Product will be Produced

Albendazole, the active ingredient in the product which is the subject of the proposed action, is manufactured in Cuernavaca, Mexico, by Laboratorios Julian de Mexico, S. A., a wholly-owned subsidiary of SmithKline Beckman Corporation.

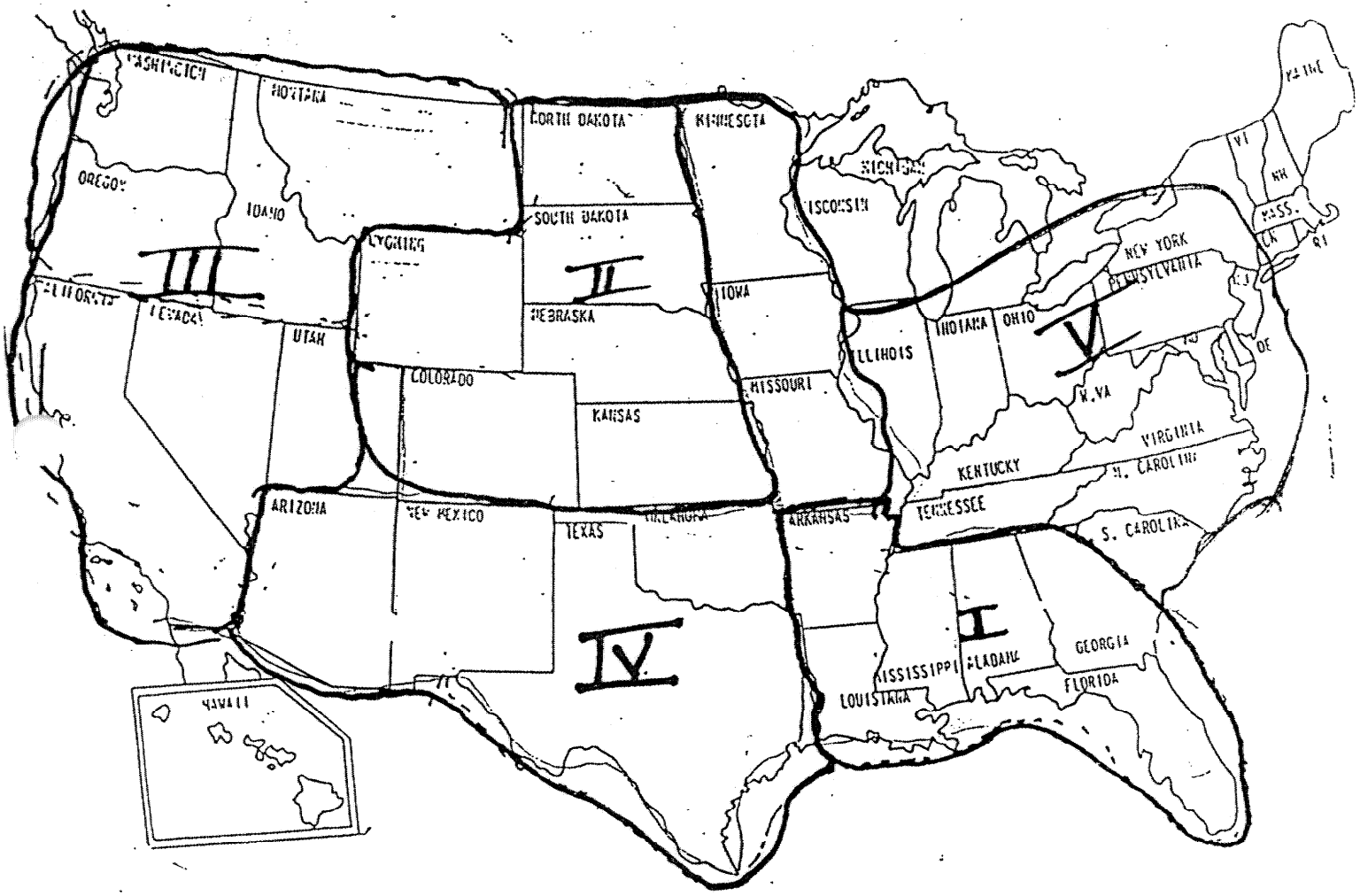
Formulations of albendazole for various modes of administration will be prepared at Norden Laboratories, Lincoln, Nebraska; and at SmithKline Animal Health Products, Omaha, Nebraska.

D. Environments which will be Affected

The geographic areas of predominant use of albendazole will coincide with the areas of greatest domestic ruminant population and severity of parasite infection. The heaviest use of albendazole (See Figure 1) will be in the Southeastern states (I) due to the numbers of cattle and the prevalence of internal parasites including heavy infections of flukes in low-lying Gulf coast areas of those states. Next in order of use will be the Midwest (II) with its high concentration of cattle, followed by the far West and the Pacific Northwest, especially in fluke endemic areas (III) and the arid Southwest with its abundant feedlot and range populations (IV). Finally, the Northeast (V), with its low numbers of cattle, sheep and goats probably represents the area of smallest utilization.

Figure 1

Geographic Areas of Predominant Use of Albendazole



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

A. Description

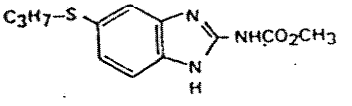
Albendazole is a member of a well known and widely used chemical class of compounds, the benzimidazoles, and is related in chemical structure and pharmacologic properties to other drugs commercially available in the United States, namely thiabendazole, fenbendazole, oxibendazole and mebendazole. Other related chemicals on international markets include the veterinary anthelmintics, parbendazole and oxfendazole. Both thiabendazole and mebendazole are currently approved for use in man in the U.S.

Substance: Albendazole

CAS Nomenclature and Number: Methyl [5-(propylthio)-1H-benzimidazol-2-yl]carbamate, 54965-21-8

Chemical Formula: $C_{12}H_{15}N_3O_2S$

Structural Formula:



Molecular Weight: 265.342

Appearance: White to buff solid

Melting Point: 208-210°C

Stability:

Prolonged heating of albendazole in aqueous acids or bases leads to hydrolysis of the carbamate group producing 2-amino-5-propylthio-1H-benzimidazole.

6. INTRODUCTION OF SUBSTANCES INTO THE ENVIRONMENT

Introduction of albendazole into the environment can occur from three sources: (A) the albendazole chemical production facility, (B) and (C) the albendazole finished dosage form production facilities, and (D) the sites of intended use in cattle, sheep and goats.

A. Description of Albendazole Chemical Production Facility and Calculation of Environmental Exposure

Albendazole, the active ingredient in the product which is the subject of the proposed action, is manufactured by Laboratorios Julian de Mexico S.A., a wholly-owned subsidiary of SmithKline Beckman Corporation. It is produced by chemical synthesis in which wastes are minimized as much as possible. Solvents are better than 90 percent recovered and recycled.

Laboratorios Julian de Mexico operates under regulations issued by the Secretaria de Desarrollo Urbano y Ecologia (SEDUE) (Secretariat of Urban and Ecological Development), and the Secretaria de Trabajo Provision Social (Secretariat of Work and Social Security). These regulations cover personal safety, risk analysis, handling of air emissions and the characteristics of the waste streams to be discharged. The Secretariat makes an annual inspection of the plant for compliance at the work site.

In addition to the above agencies, additional environmental agencies and regulations that control the plant operation are as follows:

- Reglamento para la Prevencion y Control de las Aguas (Regulation for the Prevention and Control of Waters)
- Secretaria de Salud (Department of Health)
- Secretaria de Salubridad y Asistencia (Secretariat of Health and Welfare).
- Reglamento para la Prevencion y Control de la Contaminacion Atmosferica Originada por la Emision de Humos y Polvos (Regulation for the Prevention and Control of the Atmospheric Contamination Originated by the Emission of Vapors and Dusts.

Specific environmental control practices utilized at the plant are as follows:

i. Solvent Liquid Streams

Spent toluene, propanol and acetone streams generated in the process are recovered and recycled for process reuse. Residuals remaining after solvent recovery and solidified carbonized residues from the distillation process are sent to an Industrial Cemetery in San Luis Potosi, Mexico. The Secretaria de Salubridad y Asistencia (Secretariat of Health and Welfare) authorized Ing. Salvador Aldrett to construct and operate the site on September 22, 1982. A copy of the authorization is found in Appendix I. As a result of approval of albendazole for cattle, sheep and goats in the United States, approximately 4,520 additional kilograms of residual solids will be disposed of in compliance with the above regulations.

ii. Process Washes

Process liquors and washes containing organics and inorganics from the reactions are pretreated with hydrogen peroxide to reduce the chemical oxygen demand of the stream prior to discharge. Process liquors containing sodium sulfite are electrolytically oxidized to reduce chemical oxygen demand. Alkaline and acid waste streams are collected in their respective receptacles and then used to neutralize each other. The resulting sediment is combined with the treated process liquors and treated washes, then the general plant waste streams are discharged to Empresa para el Control de la Contaminacion del Aqua en la Zona de Civac (Enterprise for the Control of the Contamination of Water in the Area of Civac) (ECCACIV), a State-run biological treatment plant serving the industrial city CIVAC. Wastes to ECCACIV are controlled and must be in compliance with the Reglamento para la Prevencion y Control de las Aguas (Regulation for the Prevention and Control of Waters) Chapter 2, Articles 6, 7 and 13; and the Registro de las Descargas (Discharge Registry) authorized by the Secretaria de Recursos Hidraulicos (SARH) (Hydraulic Resources Department) Chapter 2, Articles 8 and 13. Approximately 200,000 additional liters of mother liquors and 600,000 additional liters of aqueous waste streams will be discharged to ECCACIV in compliance with the above regulations.

iii. Air Emissions

Air emissions generated during the production process, consisting of organics such as toluene, methanol, propanol, etc., will be controlled by condensers. In addition, there is an odex system connected to each reactor and centrifuge to further treat condenser and reactor emissions, with a caustic scrubbing solution, prior to discharge to the atmosphere. Air emissions are in compliance with the Reglamento para la Prevencion y Control de la Contaminacion Atmosferica Originada por la Emision de Humos y Polvos (Regulation for the Prevention and Control of the Atmospheric Contamination Originated by the Emission of Vapors and Dusts) Chapter 2, Articles 20 and 21.

Dust emissions are controlled by a dust extraction system in the powder handling areas to comply with the above mentioned dust regulations.

iv. Calculation of Environmental Exposure

Approximately 200,000 additional liters of aqueous mother liquors, 600,000 additional liters of aqueous waste streams, and 4,520 additional kilograms of residual solids will be processed in compliance with the above mentioned regulations. All waste treatment systems minimize environmental exposure.

v. Employee Protection

Personnel working in the plant are provided with safety helmets, safety glasses/goggles, uniforms, safety shoes and gloves as their normal protective equipment. If conditions warrant, the operators have at their disposal Scott Air Pak® air breathing systems, air suits, aprons and boots. The working directions are written advising the operators what safety equipment must be used to handle each operation. In addition, Material Safety Data Sheets are available on site for all chemicals handled in the plant, Appendix II.

By signing this environmental assessment, the SmithKline Beckman Animal Health Products representative certifies compliance with all emissions requirements in Mexico.

B. Description of Albendazole Finished Pharmaceutical Formulations Production Facility

All pharmaceutical formulations of albendazole will be manufactured at Norden Laboratories, 602 West Cornhusker Highway, Lincoln, Nebraska 68521. Environmental control practices followed at this facility are as follows:

i. Liquid Waste Stream

Waste liquids generated from the production of albendazole dosage forms come from the washing of empty equipment after use. Very small percentages of all product raw materials are included in this waste. This waste is regulated by the City of Lincoln Waste water Ordinance, Sections 17.58.050 and 17.58.065. This waste is treated by the City of Lincoln Waste water System, NPPES Permit No. NE0111112 and regulated by the Nebraska Department of Environmental Control under Title 128, Chapter 2, Titles 118, 119, and 127, as subject to regulations under Section 307b of the Clean Water Act, 40 CFR, Part 439. Norden's disposal of waste water is in compliance with the above referenced laws and regulations.

ii. Solid Waste

Solid waste is disposed of at the City of Lincoln municipal landfill. The municipal landfill is regulated by the Nebraska Department of Environmental Control, Title 128, Rules and Regulations Governing Hazardous Waste in Nebraska, Code Section 4, 10, 12, and 14 per CFR 40, Part 261 and Title 132, Rules and Regulations Pertaining to Solid Waste Management. Disposal of solid waste by Norden is conducted in accordance with the above referenced laws and regulations. This waste includes small quantities of waste and containers from the manufacturing process and outdated and returned finished goods.

iii. Air Emissions

Air emissions that are generated from the production of albendazole dosage forms are controlled by the Nebraska Department of Environmental Control Title 129 Rules and Regulations governing air pollution in Nebraska. OSHA 29 CFR 1910 Subpart Z, Section 1910.000 Air Emissions, City of Lincoln Air Pollution Control Ordinance Chapter 8.64 and the air pollution control regulations and standards for the City of Lincoln, Nebraska are regulated by the Lancaster County Health Department.

Dust collectors are used to control the release of dust into the atmosphere in the production area. The dust collectors exhaust are HEPA filtered and returned to the building air supply for recirculation, all in compliance with the above laws and regulations. The powder waste from the dust collection system is disposed of with the solid wastes as described above. Norden Laboratories meets all the local, state and federal regulations per Section 112 of the Clean Air Act.

iv. Employee Protection

Material Safety Data Sheets (see Appendix II) are available for employees who work in the production areas. Employee protective clothing and other precautions will be implemented during the manufacturing operations as appropriate, to assure compliance with OSHA standards, CFR 29, Part 1900 to 1910 and OSHA's Hazard Communication, CFR 29, Part 1910.

v. Calculation of Environmental Exposure

The amount of albendazole contained in the liquid waste is negligible. Approximately 100 kilograms of dust per year containing approximately 10 kg of albendazole and small amounts of returned goods will be disposed of at the City of Lincoln municipal landfill.

By signing this environmental assessment, the SmithKline Beckman Animal Health Products representative certifies that Norden Laboratories complies with the cited emissions requirements.

C. Description of Albendazole Medicated Article (Premix) Formulations Production Facility

All Type A Medicated Articles (medicated premix) formulations of albendazole will be manufactured at our facility located at 4444 South 76th Street, Omaha, Nebraska 68127. Environmental control practices followed at this plant are as follows:

i. Liquid Waste Stream

Waste liquids are generated from the manufacturing of albendazole Type A Medicated Articles as a result of washing the empty production equipment after use and small quantities from the analytical laboratory. This liquid waste water contains very small amounts of albendazole and inert carrier. This waste is regulated by the City of Omaha Municipal Code Chapter 31. Treatment of this waste by the City of Omaha Waste Water System, NPDES Permit No. NE0036358, is regulated by the Nebraska Department of Environmental Control under Title 128, Chapter 2, Titles 118, 119 and 127 as subject to regulations under Section 307b of the Clean Air Act, 40 CFR Part 439. This liquid waste requires no pretreatment and is in compliance with the above referenced laws and regulations.

ii. Air Emissions

Air emissions from the production of albendazole Type A Medicated Articles that escape our production system consist of dust which contains albendazole and the inert carrier. Only negligible amounts of this dust escape outside the plant. The dust is contained inside the plant by keeping the manufacturing system closed as much as possible and using the central dust collector (MAC Model 72AV25, bag filter, 25,000 CFM) to extract dust that escapes the system. The dust collected by the system is deposited in a central container and is disposed of at the City of Omaha County landfill by the Tecrep Company. This system meets the requirements of the Nebraska Department of Environmental Control, Title 129, Rules and Regulations Governing Air Pollution in Nebraska and OSHA Safety and Health Standards (29 CFR 1910) Subpart 2, Section 1910.000 (Air Emissions). Air emissions associated with the production of albendazole Type A Medicated Articles contain no hazardous materials regulated by the State of Nebraska; therefore, the state does not require a permit.

iii. Dry Solid Waste

Dry solid waste is disposed of at the City of Omaha County landfill by Browning-Ferris Industries Waste Systems. This municipal landfill is regulated by the Nebraska Department of Environmental Control, Title 128, Rules and Regulations Governing Hazardous Waste in Nebraska, Code Section 4, 10, 12 and 14 per CFR 40, Part 261 and Title 132, Rules and Regulations pertaining to Solid Waste Management. The dry solid waste consists of flush material used to clean equipment, floor sweepings, dust from the dust collector (approximately 100 lbs/year) which contains approximately 22 pounds of albendazole and outdated and returned goods. This waste is disposed of in accordance with the above referenced laws and regulations.

iv. Employee Protection

Material Safety Data Sheets (Appendix II) are available for employees who work in the production area. In addition, employees in the production and packaging areas wear protective clothing and dust respirators as needed, to assure compliance with OSHA standards, CFR 29, Part 1900 to 1910 and OSHA's Hazard Communication, CFR 29, Part 1910. Employee training and industrial hygiene programs are routine plant operations.

v. Calculation of Environmental Exposure

The amount of albendazole contained in liquid waste is negligible. Approximately 100 lbs/year, of dust containing approximately 22 lbs of albendazole and small quantities of returned goods, will be disposed of at the City of Omaha landfill on a yearly basis in accordance with the referenced laws and regulations.

By signing this environmental assessment, the SmithKline Beckman Animal Health Products representative certifies that SmithKline Animal Health Products complies with the cited emissions requirements.

D. Introduction through the Target Animals

Decisions regarding the environmental safety of albendazole 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 excreted residues in feedlot and agricultural soils.

i. Theoretical Amounts of Albendazole and Metabolites Eliminated by Target Animals

It is estimated that approximately 4 to 5 million doses of albendazole (approximately 20 tons) per year will be administered to cattle, sheep and goats.

Although the major use of albendazole will be in pastured and feedlot cattle, the "worst case" environmental concentrations and exposures to albendazole metabolites are expected to occur when the drug is used in feedlot cattle. As a consequence, residues of albendazole metabolites will be introduced into agricultural soils, since feedlot manure is introduced into this environment as a fertilizer. In order to determine the expected concentrations of albendazole 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[3]	136 days
Average starting weight for feedlot steers and heifers[3]	678 lb or 308 kg
Average spreading rate of manure onto agricultural soil[4]	20 tons/acre
Manure excreted per 1000 lb live weight for finishing cattle[4]	8.5 tons/year
Manure excreted per 1000 lb live weight adjusted for 60% loss of moisture[4] due to exposure to weather in feedlot	3.4 tons/year

Albendazole will be administered at the dose level of 10 mg/kg b.w. The only expected area of concentrated use for the product would be in feedlots. Calculations performed to estimate the expected residues of albendazole and metabolites as runoff from the feedlot and in agricultural soils follow.

(1) 308 kg (Average weight of animal) X 10 mg/kg b.w. (drug dosage) = 3,080 mg ABZ equivalents/animal

Since, on the average, manure is not removed from the feedlot more than once per feedout period, the expected concentration of albendazole and metabolite residues can be calculated in feedlot manure. Albendazole and metabolite residue concentrations are based on a complete elimination of the albendazole dose and uniform mixing of the dose into the total amount of manure eliminated during the feedout period.

In addition, the total amount of albendazole (ABZ) equivalents eliminated in the average feedlot per animal per feedout period is calculated as follows:

(2) $136 \text{ days} \times \frac{3.4 \text{ tons}}{365 \text{ days}} = 1.2668 \text{ tons}^{*}$ manure/animal/feedout period

The maximum concentration of ABZ equivalents in the manure would then be:

(3) $\frac{3080 \text{ mg ABZ equivalents/animal}}{1.2668 \text{ tons manure/animal}} =$ 2431 mg ABZ equivalents/ton manure or approx. 1.2 mg ABZ equivalents/lb manure or approx. 2.7 mg ABZ equivalents/Kg (ppm) manure

ii. Theoretical Amounts of Albendazole and Metabolites in Feedlot Runoff and in Agricultural Soil Under Two Extreme Conditions

a. Feedlot Runoff

Under "worst case" conditions assuming all of the applied albendazole and metabolite residues are contained in a two inch rainfall in the feedlot where each animal occupies 200 square feet of space[5], the concentration of these residues in the runoff (without taking water/soil equilibration into account) can be estimated as follows:

(4)

$$\frac{200 \text{ Sq. Ft.}}{\text{animal}} \times 2 \text{ inch rainfall} \times \frac{1 \text{ Ft.}}{12 \text{ in}} \times \frac{28.317 \text{ Liter}}{\text{Cu. Ft.}} = 943.9 \text{ Liters Water}$$

(5)

$$\frac{3080 \text{ mg ABZ equivalents/animal}}{943.9 \text{ Liters Water}} = 3.263 \text{ mg ABZ equivalents/Liter}$$

or

3.263 ppm ABZ equivalents

b. Agricultural Soil

The average spreading rate of manure per acre of agriculture soil was estimated to be 20 tons per acre. Since 20 tons of manure equals 40,000 lb, and the concentration of ABZ equivalents per pound of manure is 1.2 mg, the following relationship is established.

(6)

$$\frac{40{,}000 \text{ lb of manure}}{\text{acre}} \times \frac{1.2 \text{ mg ABZ equivalents}}{\text{lb manure}} = \frac{48{,}000 \text{ mg ABZ equivalents}}{\text{acre}}$$

If it is assumed that manure will be mixed into the top six inches of soil, this is equivalent to a soil weight of 9.09×10^5 kg/acre (1.47 g/cc)[11]. Therefore:

*This figure is based on a 1,000 lb. animal for a 136 day period.

(7)

$$\frac{48{,}000 \text{ mg ABZ equivalents/acre}}{9.09 \times 10^5 \text{ kg soil/acre}} \times \frac{1000 \text{ µg}}{\text{mg}} = 52.8 \text{ µg ABZ equivalents/kg soil or approximateiy 53 ppb ABZ equivalents in soil}$$

Properties governing the chemical fate and degradative pathways for excreted albendazole and metabolites are presented in Section 7, Fate of Emitted Substances. Considerations of toxicity to aquatic and terrestrial organisms are summarized in Section 8, Environmental Effects of Released Substances.

7. FATE OF EMITTED SUBSTANCES IN THE ENVIRONMENT

A. Metabolism in Target Animals

Albendazole when administered to cattle and sheep is rapidly metabolized via sulfur oxidation followed by carbamate hydrolysis. Cattle metabolism studies following oral administration indicate that the majority of the albendazole dose excreted has been identified as three metabolites; sulfoxide (SO), sulfone (SO_2), and 2-aminosulfone ($2NH_2SO_2$). Metabolism in sheep has been shown to follow a similar pattern[6].

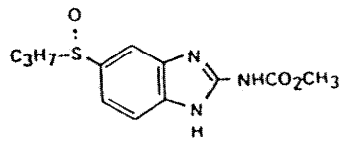
The chemical names and structures of these major metabolites are described as follows:

- Substance: Sulfoxide (Metabolite C)

  Methyl[5-(propylsulfinyl)-1H-benzimidazol-2-yl] carbamate

  Chemical Formula: $C_{12}H_{15}N_3O_3S$

  Structural Formula:



Molecular Weight: 281.342

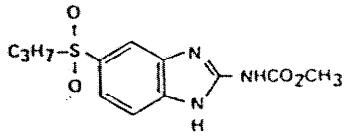
Melting Point: 186.5 (decomposition)

- Substance: Sulfone (Metabolite A)

Methyl[5-(propylsulfonyl)-1H-benzimidazol-2-yl] carbamate

Chemical Formula: $C_{12}H_{15}N_3O_4S$

Structural Formula:



Molecular Weight: 297.342

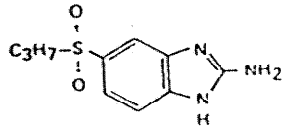
Melting Point: > 290°C

- Substance: 2-Aminosulfone (Metabolite I)

5-(propylsulfonyl)-1H-benzimidazol-2-amine

Chemical Formula: $C_{10}H_{13}N_3O_2S$

Structural Formula:



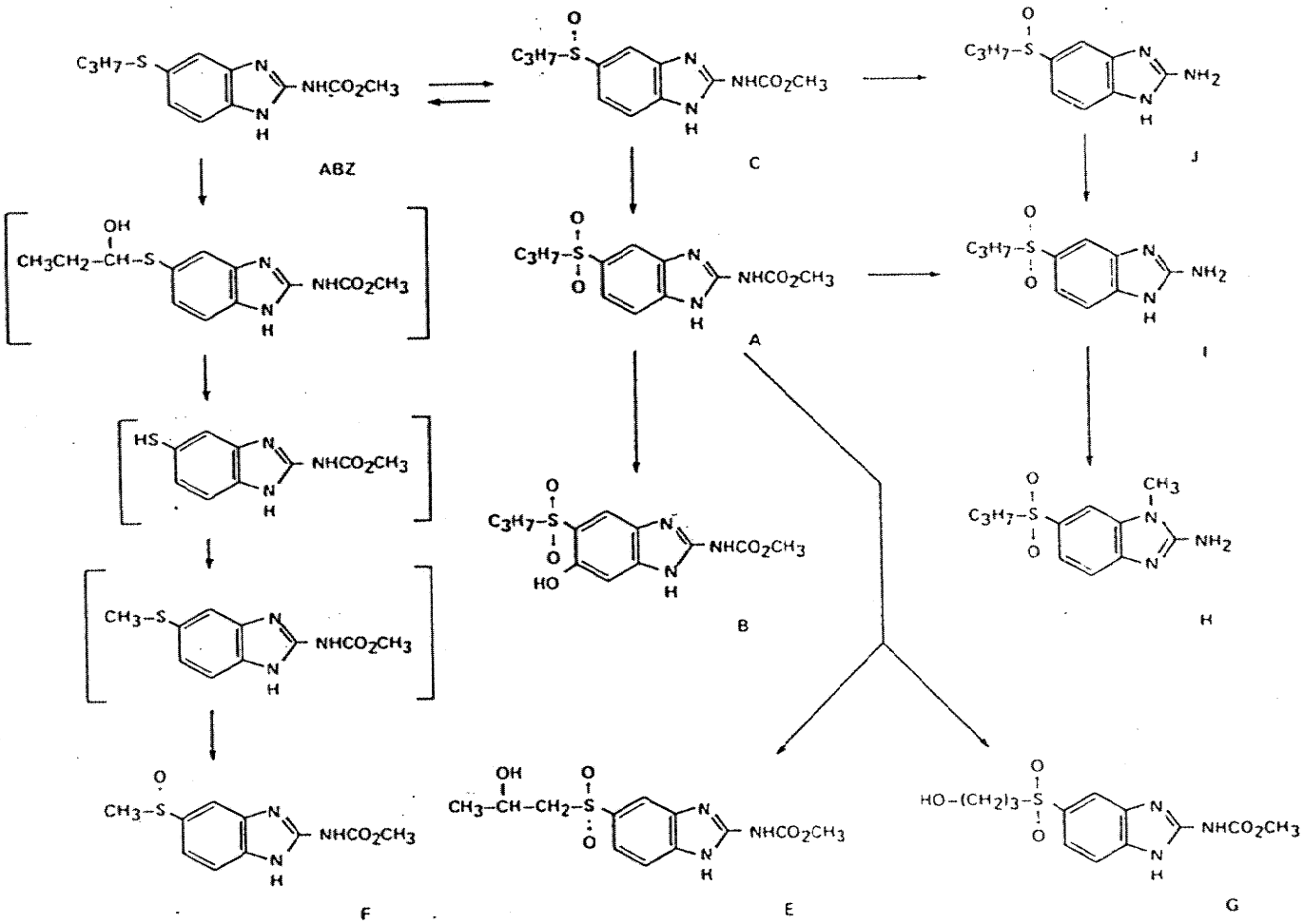
Molecular Weight: 239.304

Melting Point: 222-223.5°C

A proposed metabolic pathway is presented in Figure 2 which includes major and minor metabolites as well as postulated intermediates.

Figure 2

Proposed Metabolic Pathway of Albendazole[7]



Balance-excretion studies have shown that the majority of albendazole residues entering the environment come from urinary and fecal waste from albendazole treated animals[6,10]. Excretion profiles for albendazole treated cattle and sheep are presented in Table 1.

Table 1

Balance-Excretion Profiles for Albendazole Treated Cattle and Sheep

	% Urinary Excretion	% Fecal Excretion	Total %
Cattle (0-120 hrs)	54	17	71
Sheep (0-216 hrs)	51	*	-

* Not determined

Excreta can be further characterized as to the percentages of parent albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites with respect to the dose administered. A summary of urine and fecal metabolic data (adjusted to 100% excretion) is presented in Table 2.

Table 2

Comparison of Major Urinary and Fecal Metabolites for Cattle and Sheep

Percentage of ABZ and Its Major Metabolites

		ABZ	SO_2	SO	$2NH_2SO_2$	Total	% Other++
Cattle							
	Urine	0.4	5.2	20.1	19.9	45.6	30.4
	Feces	0.4	3.4	4.5	3.1	11.4	12.6
	Total	0.8	8.6	24.6	23.0	57.0	43.0
Sheep							
	Urine+	< 0.7	1.7	20	13	35.4	-

+ Calculation not based on total excretion due to absence of fecal data.

++Minor metabolites and endogenous radioactivity.

Approximately 60% of albendazole residues in excreta are accounted for as albendazole and the three major metabolites, sulfoxide, sulfone and 2-aminosulfone. The remainder contains numerous minor metabolites such as metabolites E, F and G in Figure 2.

It is seen here that the parent albendazole comprises less than 0.8% of the excreted dose in the environment. These data confirm that the metabolites are qualitatively similar in cattle and sheep.

Based on the calculated concentration of albendazole and its metabolite residues in feedlot runoff (3.263 ppm) and in amended agricultural soil (53 ppb), and the percentage of these residues in excreta (Table 2), the maximum possible concentrations of albendazole and its metabolite residues in feedlot runoff and in agricultural soil can be estimated as follows (Table 3):

Table 3

Estimated Concentration (ppb) of Albendazole and Its Metabolites from Cattle Excreta

	ABZ	SO_2	SO	$2NH_2SO_2$
Agricultural Soil[a]	0.4	4.6	13.0	12.2
Feedlot Runoff[b]	26.1	280.6	802.7	750.5

[a] = µg/kg soil [b] = µg/L water

B. Fate Studies

The fate of albendazole and its metabolites in the environment is markedly influenced by properties affecting chemical behavior. Earlier studies, conducted prior to the publication of the FDA Environmental Assessment Technical Assistance Handbook[8], established that albendazole binds to soil, but provided little indication of a pathway for degradation. Recent studies performed according to FDA protocols verify the results from earlier studies and provide evidence for a pathway of degradation.

i. Water Solubilities

Water solubility studies for albendazole and its major metabolites were conducted according to FDA Technical Assistance Document 3.01. Results are summarized in Table 4.

Table 4

Water Solubility (in Parts per Million)

Chemical	pH = 5.0	pH = 7.0	pH = 9.0
Albendazole (ABZ)	0.579	0.530	0.564
ABZ Sulfone	8.05	6.82	7.51
ABZ Sulfoxide	71.7	67.5	72.1
ABZ 2-aminosulfone	1343	511	488

It is clearly demonstrated that the albendazole 2-aminosulfone is the most soluble major metabolite in water as compared to albendazole which is relatively water insoluble, Appendix III.

ii. Dissociation Constants

Dissociation constants were determined for the albendazole major metabolites according to FDA Technical Assistance Document 3.04. Results are summarized in Table 5.

Table 5

CHEMICAL	DISSOCIATION CONSTANT (pk)
Albendazole (ABZ)	Not Determined
ABZ Sulfone	6.78
ABZ Sulfoxide	7.87
ABZ 2-Aminosulfone	9.35

A study summary is located in Appendix IV.

iii. Octanol/Water Partition Coefficients

An octanol/water partitioning study was performed to evaluate the potential for the absorption of albendazole and its metabolites by animals and plants. Results of this study are summarized in Table 6.

Table 6

Chemical	Octanol/Water Partition Coefficient [a]*	% **
Albendazole (ABZ)	501	0.2
ABZ Sulfone	26	3.6
ABZ Sulfoxide	14	6.5
ABZ 2-Aminosulfone	0.62	60.8

*[a] = concentration of solute in octanol / concentration of solute in water

**% = percent solute in water phase

These results suggest that albendazole is more lipid soluble than its metabolites, which agrees with the water solubility data presented in Table 4.

A study summary is located in Appendix V.

iv. UV-Visible Absorption Spectra

A UV-visible absorption study was conducted for albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites at pH 5, 7, and 9, in conjunction with aquatic photodegradation studies. The extinction coefficients obtained for these chemicals are summarized in Table 7:

Table 7

Summary of UV-Visible Absorbance Spectra for Albendazole And its Major Metabolites

	pH	Extinction Coefficient	Lambda max. (nm)
Albendazole	5	1.24×10^4	301.6
	7	1.11×10^4	295.0
	9	7.59×10^3	294.0
Sulfoxide	5	2.16×10^4	291.5
	7	2.36×10^4	291.5
	9	1.72×10^4	292.4
Sulfone	5	1.5×10^4	290.8
	7	1.10×10^4	291.0
	9	1.53×10^4	292.0
2-Aminosulfone	5	9.84×10^3	288.5
	7	1.22×10^4	290.4
	9	1.13×10^4	290.6

Albendazole and its major metabolites adsorb in the UV region in which photodegradation characteristically occurs, Appendix VI.

v. Soil Adsorption/Desorption

Soil adsorption/desorption studies (Appendix VII) were conducted at Springborn Life Sciences, Inc. according to FDA Technical Assistance Document 3.08 to determine the adsorption and desorption coefficients of albendazole and its sulfoxide metabolite. Testing of the sulfone and 2-aminosulfone metabolites was not pursued. Testing was performed using three soils varying in pH, organic matter and cation exchange capacity, to determine the adsorption coefficients (K_d) and adsorption coefficients based upon the organic carbon content of the soil (K_{OC}). Additional testing was conducted to define the equilibrium period.

A summary of the results is presented in Table 8:

Table 8

Results of Adsorption/Desorption Studies

Albendazole

	Adsorption 0.01M $CaCl_2$			Desorption 0.01M $CaCl_2$			
Soil	K_d	K_{oc}	1/n	K_d	K_{oc}	1/n	pH
TXSTLM[a]	109.6	13400	1.21	275423	33588200	2.86	8.0
ILSTLM[b]	501.2	27500	0.770	1047	57500	0.915	5.5
NYLM[c]	141.3	7800	0.936	912	50100	1.33	6.5

Sulfoxide

	Adsorption 0.01M $CaCl_2$			Desorption 0.01M $CaCl_2$			
Soil	K_d	K_{oc}	1/n	K_d	K_{oc}	1/n	pH
ILSTM[b]	52.6	2900	0.611	38.1	2100	0.576	5.5
NYLM[c]	6.3	350	0.771	6.5	360	0.657	6.5
TXSTLM[a]	1.2*	150*	*	*	*	*	8.0

*Screening tests demonstrated that < 25% adsorbed, therefore, advanced tests were not required.

[a] Texas Silty Loam (16% Sand, 65% Silt, 19% Clay and Organic Matter 1.4%)

[b] Illinois Silty Loam (20% Sand, 59% Silt, 21% Clay and Organic Matter 3.1%)

[c] New York Loam (48% Sand, 30% Silt, 22% Clay and Organic Matter 3.1%)

These results demonstrate that albendazole strongly binds to all soils with extremely minimal desorption observed. Albendazole sulfoxide is more mobile than albendazole, with a tendency to remain in solution.

Several previous studies conducted by SmithKline Beckman Animal Health Products to determine the potential for runoff of albendazole and urinary metabolites as a mixture all concluded that albendazole and urinary metabolites bind significantly to a variety of soil types under static and dynamic testing conditions. These studies are discussed in parts vi, vii and viii which immediately follow.

vi. Biodegradation in Soil

Excreta from cattle dosed with ^{14}C-albendazole were mixed into soil at a level of 1 ppm urinary and 1 ppm fecal radioactivity and incubated under aerobic and anaerobic conditions. The distribution of radioactivity demonstrated that 69.2% of the ^{14}C-radiolabel was extensively bound to soil components upon initial contact and the degree of binding increased over time. Upon measurement of $^{14}CO_2$, little degradation (< 2%) was observed over 120 days. Therefore, according to this study, most of the albendazole metabolite residues coming in contact with feedlot soil through cattle excretion are not readily available for runoff into streams, Appendix VIII.

vii. Runoff

^{14}C-Albendazole and urine from a calf treated with ^{14}C-albendazole were mixed with a variety of soil types and the distribution of residues under dynamic and static conditions were determined. Dynamic interaction simulates fast moving water conditions from soil to streams and static interaction simulates the equilibration of standing water in contact with soil.

Under dynamic conditions, albendazole quickly binds to soil after 72 hrs (76.4 to 98.57%). Under static conditions, binding time is increased; however, by 4 weeks 68.72 to 85.46% of the ^{14}C-albendazole is bound to the soil.

The urinary metabolites exhibited a similar binding pattern under dynamic and static conditions. Under dynamic conditions 65.6 to 78.67% of the radioactivity was bound to the soil after 72 hrs. Under static conditions, 53.91 to 65.70% of the radioactivity was bound to soil after 4 weeks.

These data demonstrated that the extent of binding for albendazole was somewhat greater than that for the urinary metabolites, but the binding characteristics (patterns) for both were similar, Appendix IX.

viii. Mobility

Excreta from cattle dosed with ^{14}C-albendazole were mixed with several soil types and subjected to simulated percolation studies with the leachate being monitored for radioactive residues. Between 82 and 99% of the applied radioactivity was retained on the columns regardless of soil types which indicates the relative immobility of the residues, Appendix X.

ix. Photodegradation

Aqueous photolysis studies (Appendix XI) were conducted at SmithKline Beckman Animal Health Products according to FDA Technical Assistance Document 3.10 to determine the potential for aqueous photodegradation and hydrolysis, and half-lives of albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites at pH 5, 7 and 9. A summary of the study results is presented in Table 9.

Table 9

Photolytic Half-Lives (Days) of Albendazole and its Major Metabolites

	Measured[a]				Calculated[b]		
Albendazole							
pH	5	7	9		5	7	9
Day[c]	0.537	0.937	0.729	Summer	0.106	0.192	0.052
Day & night[d]	0.525	0.516	0.704	Winter	0.382	0.989	0.155
Albendazole Sulfoxide							
Day[c]	0.573	0.852	0.197	Summer	0.143	0.150	0.076
Day & night[d]	0.525	0.902	0.096	Winter	0.832	0.438	0.450
Albendazole Sulfone							
Day[c]	1.52	0.608	0.103	Summer	0.444	0.190	0.048
Day & night[d]	1.31	0.516	0.050	Winter	1.30	0.553	0.191
Albendazole 2-Aminosulfone							
Day[c]	4.54	1.55	1.29	Summer	0.547	0.190	0.268
Day & night[d]	4.12	1.60	1.44	Winter	1.62	0.554	1.65

a The half-life calculated after correcting the measured rate (k_p) for the rate at flat water body (k_{pE}) in the environment by using the equation $k_{pE} = k_p/2.2$.

b Half-life calculated based on the quantum efficiency of light received during the experiment

c Based on an average day length of 11.74 hrs. (between sunrise to sunset) during the time of experiment for albendazole and its metabolites (September 26 and October 8).

d Calculated using k_p values from the Statistics Reports of Studies A-3032-88, A-3033-88, A-3034-88 and A-3035-88. The half-life values are based on a day length of 24 hrs.

Based on the half-lives calculated using quantum efficiencies of light, it was clearly demonstrated that albendazole and its three major metabolites undergo rapid degradation to 50% of their starting concentrations within a day in mid-summer and less than two days in mid-winter. The experimental (measured) half-lives are slightly longer which reflects upon the season (beginning of fall) and also on poor weather conditions (especially albendazole and the 2-aminosulfone metabolite) under which the studies were conducted. An environmental hazard assessment is conservatively estimated using the measured rate of degradation of the test chemicals based on total hours (after correcting for flat water body).

There was some loss of test chemical in dark controls at some sampling periods, however, the loss did not seem to be time-dependent and therefore, hydrolysis was not considered to be a significant variable.

x. Summary of Fate Studies Applied to the Degradation of Albendazole and its Metabolites

Soil Adsorption/Desorption: Using the minimal soil binding and maximum desorption data for albendazole (Table 8), the data from Table 3 can be adjusted to reflect the maximum "worst case" estimated runoff concentrations of albendazole that could be available after equilibration with feedlot and agricultural soil as presented in Table 10.

Feedlot runoff concentrations are calculated using the lowest K_d measured for adsorption (109.6 for ABZ) and assume that 2 inches of rainfall are in equilibrium with the top 6 inches of soil. Amended agricultural soil concentrations are calculated using the lowest K_d measured for desorption (K_d = 912 for ABZ) and also assume 2 inches of rainfall are in equilibrium with 6 inches of soil. This will result in the "worst case" estimated concentration of albendazole available for runoff. Detailed calculations for these data in Table 10 are shown in Appendix XII.

The estimated "worst case" concentrations for the major metabolites in feedlot runoff and agricultural soil are presented in Table 10 (previously presented in Table 3) based on limited absorption to soils.

Table 10

"Worst Case" Concentrations (ppb) of Albendazole and Its Metabolites Available for Runoff after Equilibration with Soil

	ABZ	SO	SO_2	$2NH_2SO_2$
Feedlot Runoff (Adsorption)				
Water Phase	.054 µg/L	802.7 µg/L	280.6 µg/L	750.5 µg/L
Soil Phase	5.92 µg/Kg			
Agricultural Soil (Desorption)				
Water Phase	.0004 µg/L			
Soil Phase	0.3648 µg/Kg	13.0 µg/Kg	4.6 µg/Kg	12.2 µg/Kg

Under this assumption, feedlot runoff will result in the highest estimated concentrations (worst case) of albendazole and its major metabolites in an aquatic system since further dilution would occur upon entry into lakes, streams, ponds, etc. Rainfall runoff from amended agricultural soil containing albendazole and its major metabolites would be insignificant.

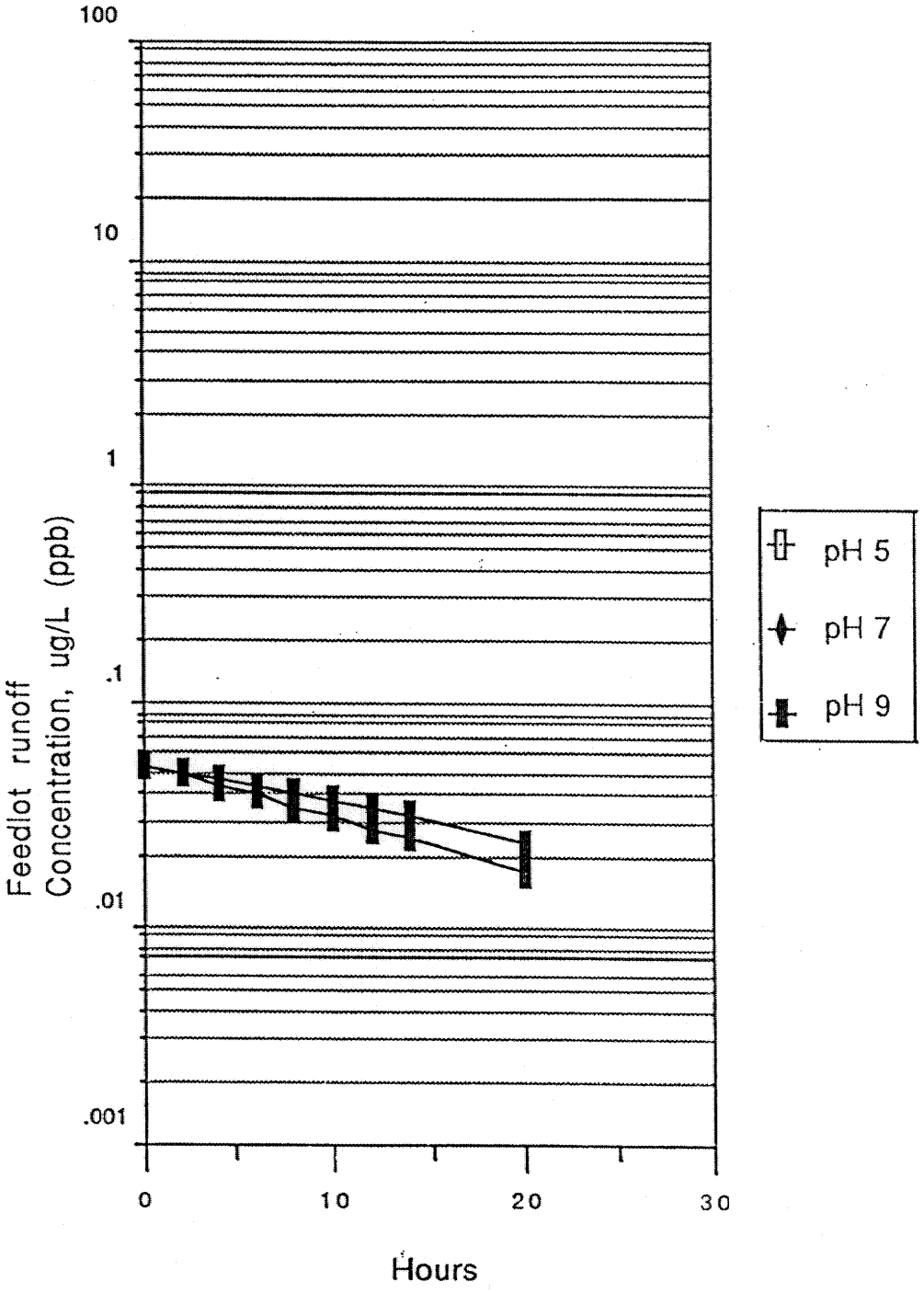
Results from the biodegradation in soil (Appendix VIII), runoff (Appendix IX) and mobility (Appendix X) studies correlate well with the soil adsorption/desorption studies (Table 8), and indicate that the residue levels in Table 10 would be a "worst case" scenario.

Photodegradation: As a worst case assumption, the photodegradation of albendazole and its major metabolites when applied to the feedlot runoff concentrations (after soil adsorption/desorption for albendazole in the feedlot, Table 10) result in the estimated degradation presented in Figures 3 through 6 based on degradation rates from actual measured data.

These figures demonstrate that any albendazole and major metabolites entering an aquatic environment from feedlot runoff rapidly degrade to insignificant concentrations in the aquatic environment.

For ABZ-sulfoxide, ABZ-sulfone and ABZ 2-aminosulfone, photolytic breakdown is expected to accelerate the disappearance of feedlot residues and prevent accumulation. These metabolites are not expected to tightly bind to soil or persist in the environment. For the less soluble parent drug, binding to soil is expected but photolysis will accelerate its disappearance. Based upon solubility in water, properties of adsorption/desorption, photolytic breakdown and available pathways for dissipation, the concentration of albendazole and its metabolites reaching aquatic and terrestrial environments is not expected to be significant.

Figure 3. AQUATIC PHOTODEGRADATION OF ALBENDAZOLE



Note: The plots for pH 5 and 7 appear to be superimposed

Figure 4. AQUATIC PHOTODEGRADATION OF ALBENDAZOLE SULFOXIDE

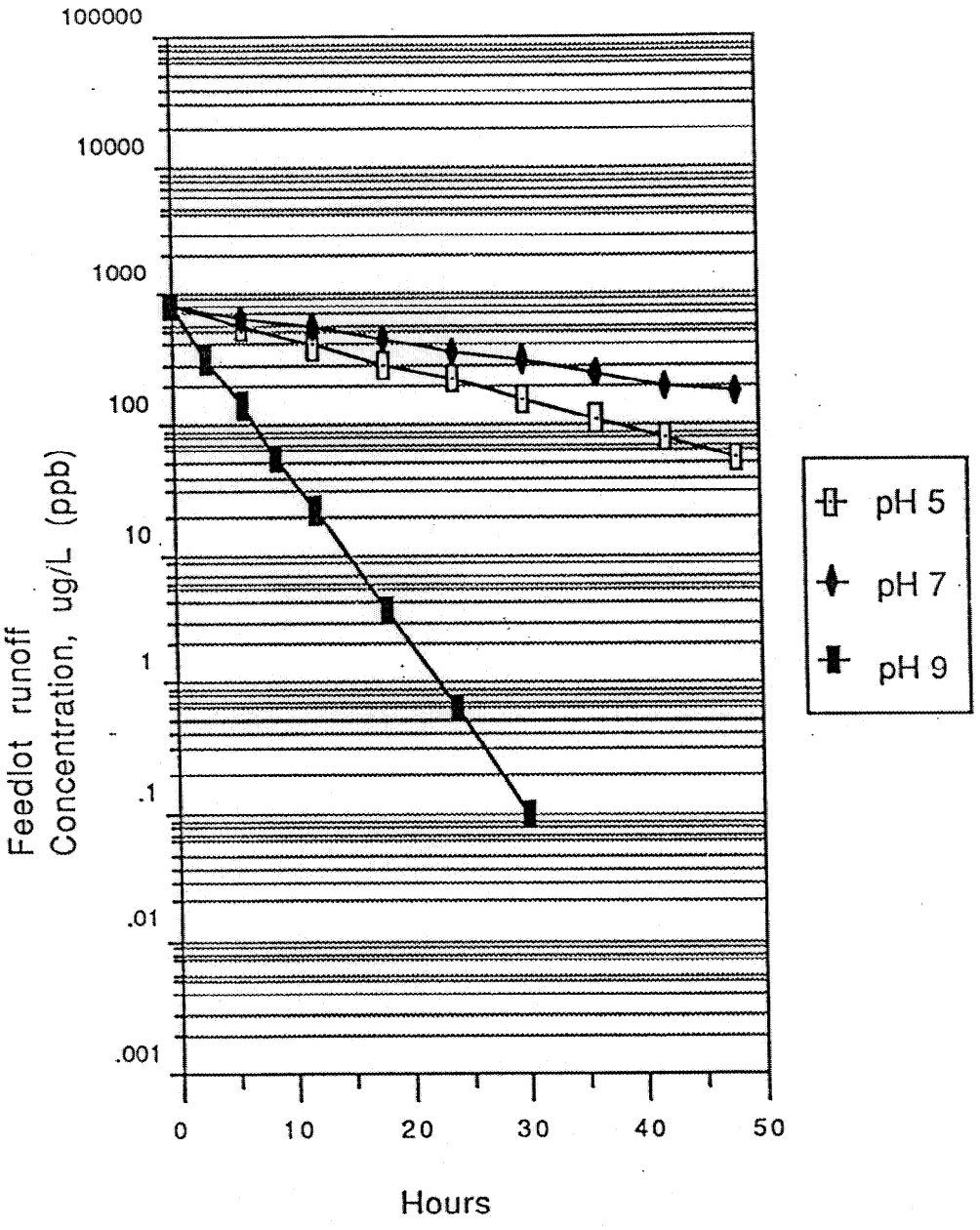


Figure 5. AQUATIC PHOTODEGRADATION OF ALBENDAZOLE SULFONE

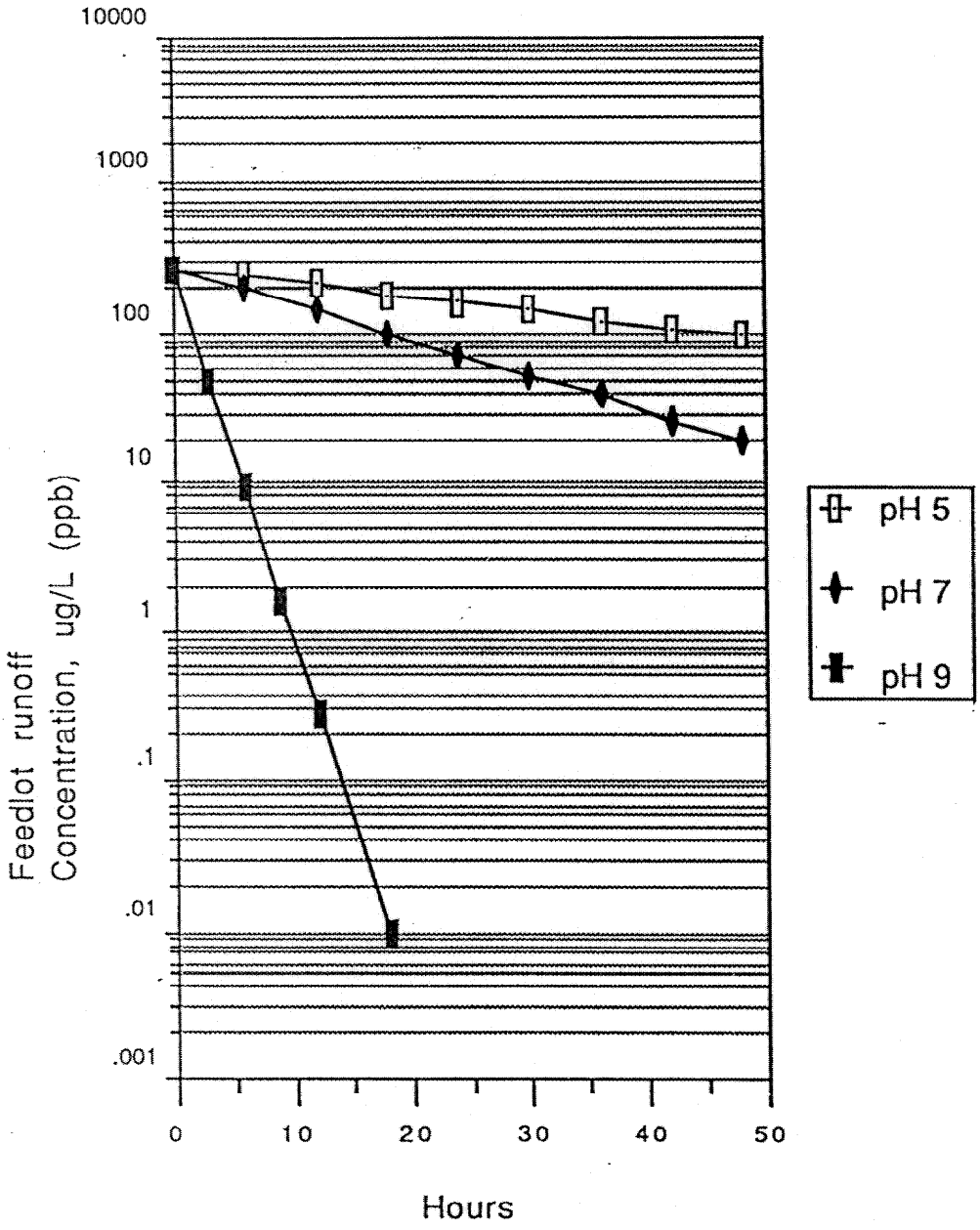
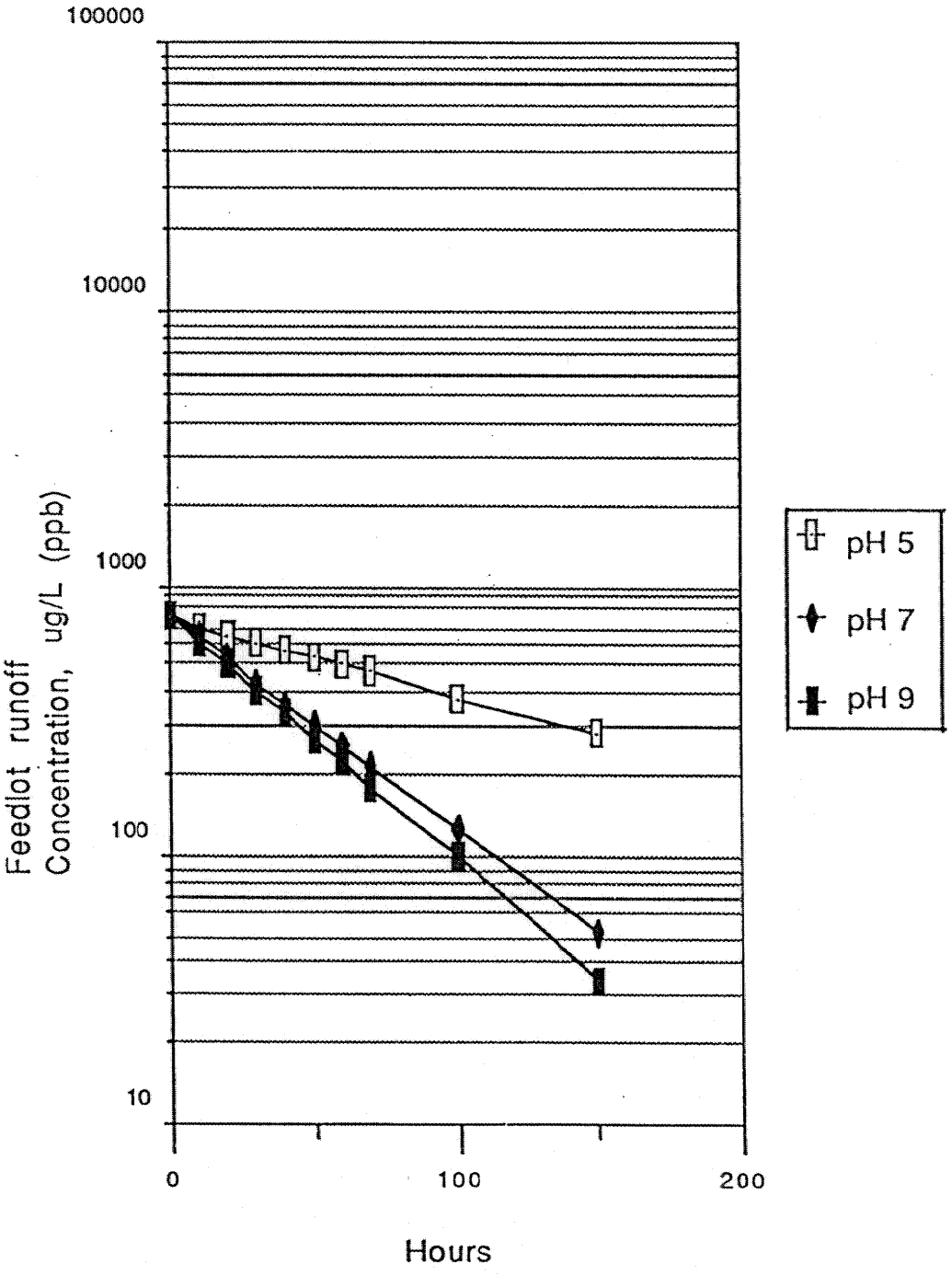


Figure 6. AQUATIC PHOTODEGRADATION OF ALBENDAZOLE 2-AMINOSULFONE



Fate studies demonstrated the rapid degradation of albendazole and its major metabolites when released into the environment.

8. ENVIRONMENTAL EFFECTS OF RELEASED SUBSTANCES:

A. Occupational Safety

Albendazole is a 2-aminosubstituted benzimidazole with broad anthelmintic activity against a variety of gastrointestinal and lung nematodes, tapeworms and flukes in cattle and sheep and helminths in man. Albendazole has met the human food safety and animal safety criteria for anthelmintics administered orally, and its use does not constitute a risk or hazard to human health.

i. Dermal and Eye Irritation Studies

a. Eye Sensitivity Study

Six male Pel Freez Rabbits, weighing 2.5 to 3 Kg, were divided into two groups of three rabbits each. Albendazole (100 mg) as a powder, was placed in the conjunctival sac of the left eye of each of the three rabbits in group I. The right eye left untreated and served as a control. The animals were observed throughout the first day and again at 24, 48 and 72 hours for evidence of irritation.

In group II, three rabbits received 100 mg of the powdered compound in the conjunctival sac of the left eye. The powder was allowed to remain in contact with the eye for 4 minutes, then it was washed and the rabbits were observed in the same manner as in group I.

Results: There were no signs of irritation in the eyes of treated rabbits.

b. Skin Sensitivity Study

Male Pel Freez Rabbits, weighing 2.5 - 3 kg were used. The hair of each rabbit's back was clipped. Nair was then applied to completely depilate the area 24 hours prior to the test. The right side of each of three rabbits was abraded using a sterile 23 gauge needle just prior to application of the compound. The left side was not abraded. Albendazole (500 mg) was applied to 12-ply folded gauze pads. One pad was placed on the abraded site and one pad was applied to the non-abraded site of each rabbit. The pads were covered, then Saran Wrap™ was wrapped around the rabbits trunk. Readings were taken at 24, 48 and 72 hours.

Results: No irritation was observed on either the abraded or non-abraded side in any of the rabbits at 24, 48 and 72 hours after topical application of 1000 mg of the compound (500 mg at each side).

ii. Human Safety Studies

The following studies were performed in order to determine the safety of albendazole residues in food with the resulting no-observed-effect levels (NOEL). Details of each study may be found in the Freedom of Information Summary for Use of Albendazole in Cattle[9]. The results of these studies can also be used as guidance in determining safe levels of occupational exposure.

Study	NOEL
Teratogenicity Study in Mice	30 mg/kg/day
Teratogenicity Study in Rats	5 mg/kg/day
Teratogenicity Study in Rabbits	5 mg/kg/day
Three Generation Reproduction Study in Rats	150 ppm
Chronic/Carcinogenicity Study in Rats	7.0 mg/kg/day
Chronic/Carcinogenicity Study in Mice	25 mg/kg/day
Six Month Dog Study	5 mg/kg/day
Peri/Postnatal Reproduction Study in Rats	20 mg/kg/day

The mutagenicity of albendazole was studied in the Salmonella (Ames) Test, and in the Chinese Hamster Ovary Cells Tests - with and without exogenous metabolic activation. In these tests no evidence of mutagenicity was observed.

B. Environmental Effects Studies

Effects of exposure to albendazole and its metabolites have been examined using representative aquatic and terrestrial organisms. Earlier studies were performed using agricultural soils treated at levels equivalent to those estimated to occur following incorporation from feedlot wastes and mixtures of parent drug and metabolites. Mixtures were prepared in proportion to the expected metabolic profile following excretion from treated animals. More recent studies of toxicity to aquatic organisms have been performed using the pure parent drug or metabolites.

i. Earthworm Toxicity

Manure from cattle administered four 15 mg/kg doses of albendazole over a three week period was mixed into soil at the rate of 15 and 30 tons per acre and the effect of the soil residues on earthworms was determined. After 60 days, the numbers of adult worms, eggs and young worms were not different between manure/soil mixtures and soil mixed with manure from untreated cattle (control). It was therefore concluded that albendazole residues had no effect on the condition or reproduction of earthworms at the concentrations tested, Appendix XIII.

ii. Housefly Toxicity

Manure from cattle administered four 15 mg/kg doses of albendazole over a three week period was mixed into soil at the rate of 15 and 30 tons per acre and the effect of these soil residues on the housefly was determined. In both the F_0 and F_1 generations, there were no consistent treatment-related differences in the average number of pupae or adults between the control and the medicated treatments at either application rate, Appendix XIV.

iii. Microbial Growth Inhibition

The following studies were designed to assess the possible impact of albendazole and metabolite residues on microbial growth.

a. Antibacterial and Antifungal Activity:

The major albendazole metabolites excreted into the environment exhibit very weak activity against algae and a moderate to weak activity against fungi. Albendazole exhibited greater antifungal activity, but is of no environmental consequence since only trace quantities are eliminated into the environment by treated animals. No activity was exhibited against any of the bacterial species tested, Appendix XV. Albendazole and metabolite concentrations tested were far in excess of the maximum level of residues anticipated in soil. Table 11 summarizes the MIC values for albendazole against various organisms.

Table 11

Minimum Inhibitory Concentration for Albendazole and Its Bovine Urinary Metabolites to Soil Microflora

	Albendazole (µg/disc)	Sulfoxide Metabolite (µg/disc)	2-Aminosulfone Metabolite (µg/disc)
Bacterial Species			
Aerobacter levanicum	NI	NI	NI
Arthrobacter globiformis	NI	NI	NI
Bacillus subtilis	NI	NI	NI
Pseudomonas fluorescens	NI	NI	NI
Streptomyces albus	NI	NI	NI
	(µg/disc)	(µg/disc)	(µg/disc)
Fungal Species			
Aspergillis niger	0.1	10	10
Chaetomium globosum	1	10	NI
Penicillium chrysogenum	0.1	3	3
Trichoderma viridi	NI	NI	NI
	(ppm)	(ppm)	(ppm)
Algal Species			
Microcystis aeruginosa	NI	50	50
Selenastrum capricornutum	NI	NI	NI

NI - No inhibition at any dose tested. Bacterial and fungal species were tested at 30, 10, 3, 1, 0.3 or 0.1 µg/disc; algal species were grown in liquid culture containing 50, 10 or 1 ppm of the test compounds.

b. Soil Enzymatic Function:

The soil functions studied included effects on degradation of cellulose, protein and starch in soils and on nitrification and aerobic nitrogen fixation. Albendazole and its metabolites were incorporated into three types of soil in a proportion that simulated that found in excreta from cattle treated with the product. The two levels tested, 10 and 50 ppm, which are both exaggerated levels (200 to 1000 times), were necessary in order to obtain any measurable responses.

- Degradation of Starch and Cellulose:

Results from studying starch, cellulose and protein degradation as well as nitrogen fixation and nitrification were variable. No clear patterns emerged different from those expected from the minimum inhibitory concentration data above, Appendix XV.

iv. Plant Bioaccumulation and Toxicity

a. Rotational Crop Effect

The objective of this study was to assess the secondary bioaccumulation of albendazole metabolites in three representative food crops; spinach, beets and wheat. Urine and feces from a steer dosed with ^{14}C-albendazole at 15 mg/kg b.w. (1.5 times the recommended dose) was mixed into soil at a level of 2.62 ppm total residues expressed as albendazole equivalents, which is 50 times the estimated worst case concentration in soil from treated animals. The results of the uptake of radioactivity are summarized in Table 12.

Table 12

Mature Plants	Average ppm
Spinach	0.130
Beet foliage	0.103
Beet root	0.013
Wheat straw	0.751
Wheat seed	0.019

Immature Plant	Average ppm
Spinach	0.113
Beet	0.085
Wheat	0.151

These results confirm that under exaggerated conditions, there is a minor uptake of radioactivity by plants grown in soil fertilized with excreta from treated animals, Appendix XVI.

b. Growth Yield Effect

Studies were performed to determine the effect of exaggerated levels of albendazole metabolite residues in soil on crop growth (height and weight). Manure from cattle administered four 15 mg/kg doses of albendazole over a three week period was mixed with soil at the rates of 15 and 30 tons/acre. Corn, cucumber, green bean, tomato, wheat, barley and fescue were grown in treated and control manure/soil mixtures.

For all crops no significant differences were observed between the control and treated crops, Appendix XVII. Therefore, it is concluded that albendazole and its metabolite residues have no phytotoxic effect on these crops.

v. Daphnia Acute Toxicity

Effects of albendazole and its metabolites on the fresh water crustacean, Daphnia magna, have recently been established according to FDA Technical Assistance Document 4.08, Appendix XVIII. In 48 hour static acute exposures, the following EC_{50} and no-observed-effect concentrations or NOEC's were derived. Results were reported based on mean measured concentrations of each test article over a 48 hour period and are presented in Table 13.

Table 13

	EC_{50}	NOEC*
ABZ	24 µg/L	17 µg/L
Sulfoxide	30 mg/L	11 mg/L
Sulfone	> 14 mg/L**	6.1 mg/L
2-Aminosulfone	110 mg/L	53 mg/L

* NOEC is equivalent to zero immobilization relative to controls.

** Estimated, above water solubility of 6.82 to 8.05 ppm

vi. Fresh Water Fish Acute Toxicity

The effects of a mixture of the three urinary metabolites of albendazole on fresh water fish have also been investigated. Static, acute exposures were performed with the cold water rainbow trout, Salmo gairdneri, and warm water bluegill sunfish, Lepomis macrochirus. From 96 hour exposures, the LC_{50} of the mixture to bluegill sunfish was estimated to be 222 ppm, and to rainbow trout 86 ppm. The metabolite mixture (SO_2, SO, $2NH_2SO_2$) was prepared to simulate the proportion of sulfoxide, sulfone and 2-aminosulfone expected to be excreted from cattle, Appendix XIX.

vii. Bioaccumulation Study in Bluegill Sunfish

Radiolabelled metabolites, prepared from urine from a steer dosed with ^{14}C-albendazole and ^{14}C-albendazole itself were mixed in a 95:5 ratio respectively, and added to water at a concentration of 100 ppb (based on albendazole equivalents). Bluegill sunfish, introduced to this environment, were sampled at various time periods for radioactive residues in edible and non-edible tissues.

The results, expressed as albendazole equivalents are:

After one week, residue levels were 90 ppb in edible and 1179 ppb in non-edible tissues.

At 22 days, levels were 22 ppb in edible and 174 ppb in non-edible tissues.

After 44 days levels were 30 ppb in edible and 116 ppb in non-edible tissues.

After 44 days, since no plateau value was obtained, the fish were transferred to a tank free of radiolabelled urine and levels in both edible and non-edible portions declined to 13 and 29 ppb, respectively.

After 22 days, the bioaccumulation ratios were 1.86 in non-edible tissues and 0.23 in edible tissues.

After transfer to an environment free of the test substances, carbon-14 levels were rapidly reduced to < 20 ppb for edible tissues and < 40 ppb for non-edible tissues.

From these results, it is concluded that albendazole metabolites do not bioaccumulate at levels higher than those present in the environment, and deplete rapidly when fish no longer are exposed. Soil studies have shown that binding of these residues to the soil would result in only miniscule amounts available in runoff into streams, Appendix XX.

C. Environmental Hazard Assessment

i. Hazard Assessment in Terrestrial Ecosystem

Based upon the concentration of albendazole and its major metabolites in amended agricultural soil from Table 3 the following can be summarized for terrestrial exposure.

Earthworm and Housefly Toxicity - Levels tested showing no apparent effect were 4 to 9 fold higher than the maximum estimated total albendazole and metabolite concentration in amended agricultural soil of 53 ppb and substantially higher than the concentrations for individual compounds in Table 3.

Antibacterial and Antifungal Activity - The concentration of albendazole needed to produce inhibition was significantly higher than the amount estimated to be present in soil. The major metabolites required levels significantly higher than those estimated to be present in soil to produce any inhibitory effect.

Soil Enzymatic Function - Results from studying starch, cellulose and protein degradation as well as nitrogen fixation and nitrification were variable. No clear patterns emerged different from those expected from the minimum inhibitory concentration data above.

Rotational Crop Effect - Bioaccumulation of radioactivity in three representative food crops, spinach, beets and wheat, from levels in amended agricultural soil, approximately 50 times the estimated exposure concentration, was significantly less than the concentration in the soil.

Growth Yield Effect - At concentrations approximately 50 times the estimated exposure in amended agricultural soil, no significant differences were observed between control and treated crops.

ii. Hazard Assessment in Aquatic Ecosystems

Based on the estimated concentration of albendazole and its metabolites in feedlot runoff (worst case exposure) and the photodegradation results, aquatic exposure can be estimated.

Daphnia Acute and Fresh Water Fish Acute Toxicity - without considering the rapid photodegradation of albendazole and its metabolites in water the following comparisons can be made with the NOEC or LC_{50} data and the estimated exposure from Table 10 converted from µg/L to mg/L.

	Fish LC_{50} (mg/L)	Daphnid NOEC (mg/L)	Estimated Runoff (mg/L)
Albendazole		0.017	0.000054
Sulfoxide	86-222	11	0.8027
Sulfone	(As mixture)	6.1	0.2806
2-Aminosulfone		53	0.7505

These data demonstrate the margin of safety to daphnids and fish in comparison to the highest levels of albendazole and its metabolites estimated to occur in feedlot runoff.

Obviously when the rapid photodegradation is considered as in Figures 7-10, any possible effect on daphnids or fresh water fish would be transient.

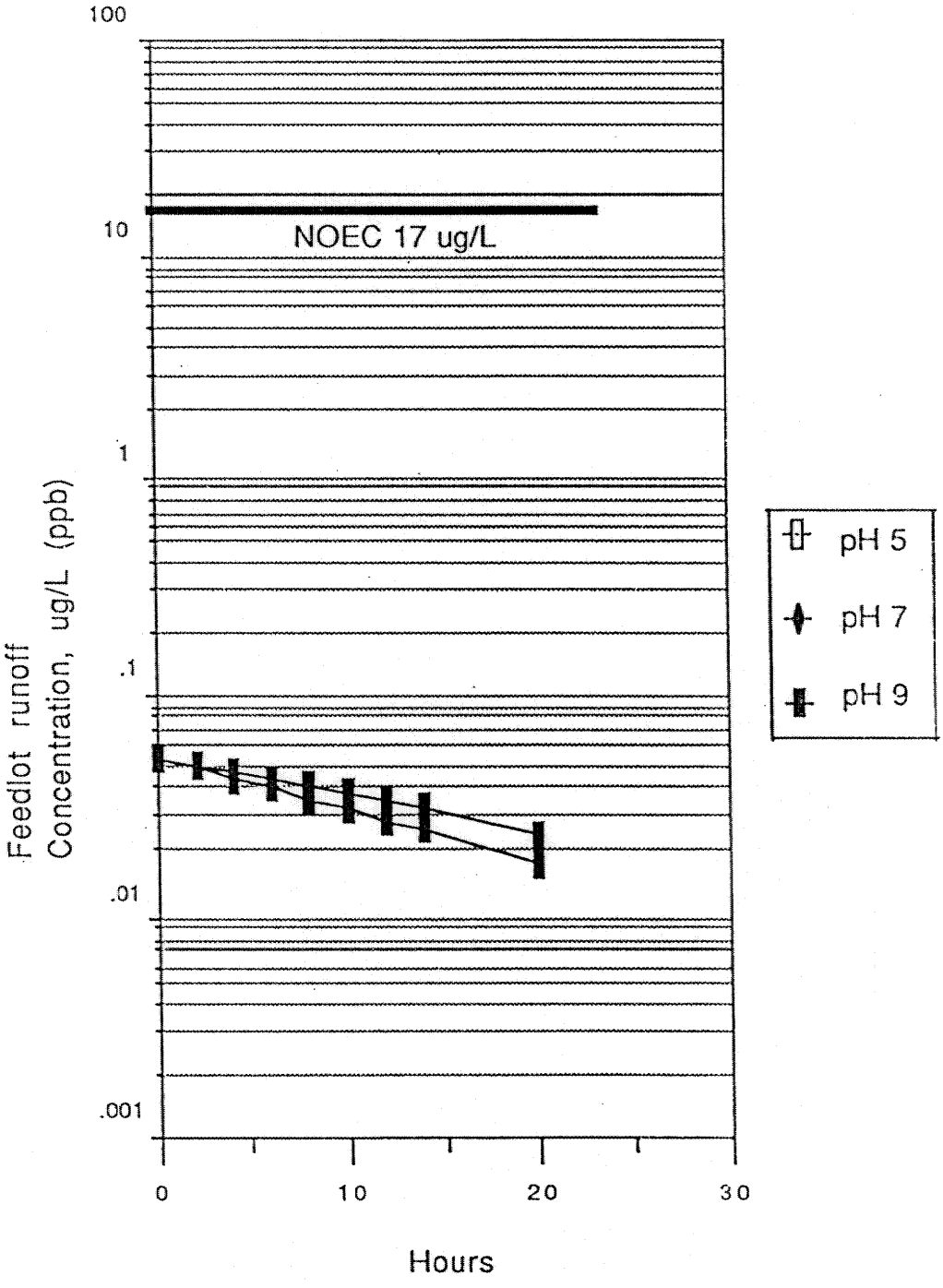
9. USE OF RESOURCES AND ENERGY CONSUMPTION:

As previously stated (in Section 6), a very small proportion of the raw materials utilized in the manufacturing process of albendazole are not recoverable. The recoverable solvents are recycled and re-utilized in the manufacturing process. Energy sources (gas, oil and electricity, etc.) are nonrecoverable resources and are considered normal requirements of manufacturing/production.

10. MITIGATION MEASURES:

Material Safety Data Sheets are available for employees who work in the production area. In addition, employees in the production and packaging areas wear protective clothing and dust respirators as needed, to assure compliance with OSHA standards as discussed in Section 6. No other mitigation measures are necessary since albendazole does not pose any known harm to the environment.

Figure 7. HAZARD ASSESSMENT IN AQUATIC ECO-SYSTEM
Comparison of Aquatic Photodegradation of
Albendazole with NOEC for Daphnia Magna



Note: The plots for pH 5 and 7 appear to be superimposed

Figure 8. HAZARD ASSESSMENT IN AQUATIC ECO-SYSTEM Comparison of Aquatic Photodegradation of Albendazole Sulfoxide with NOEC for Daphnia Magna.

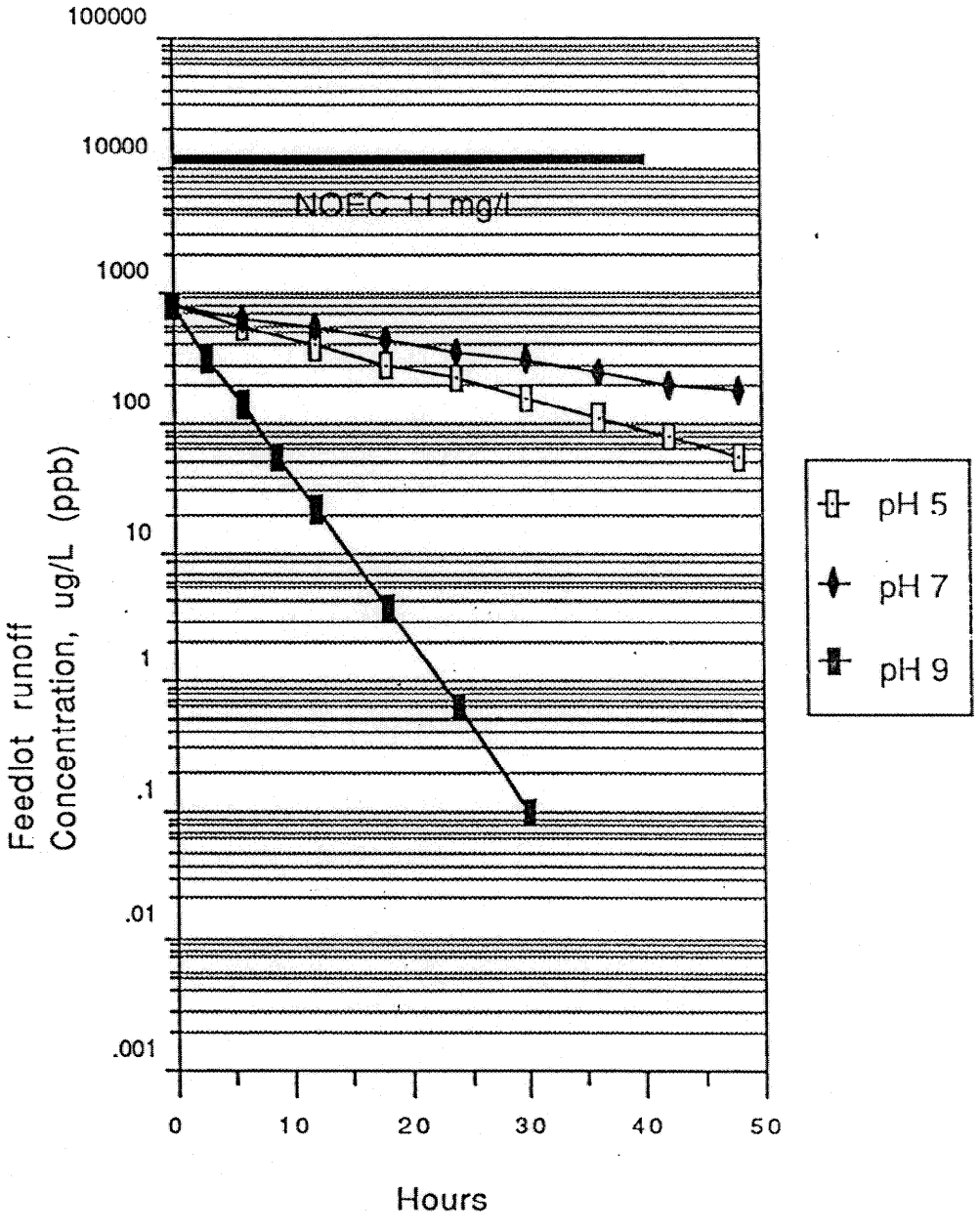


Figure 9. HAZARD ASSESSMENT IN THE AQUATIC ECO-SYSTEM.
Comparison of Aquatic Photodegradation of Albendazole Sulfone with NOEC for Daphnia Magna.

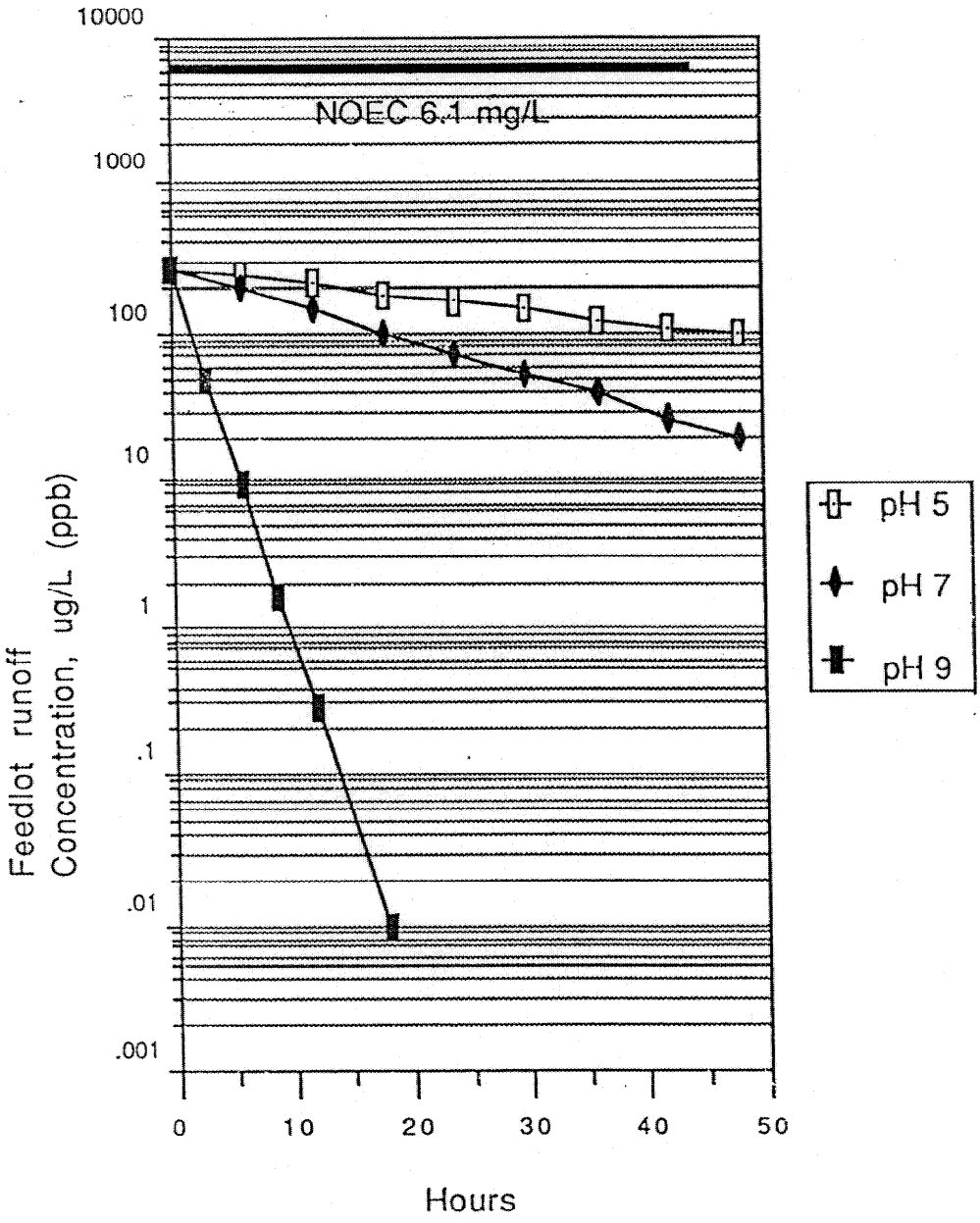
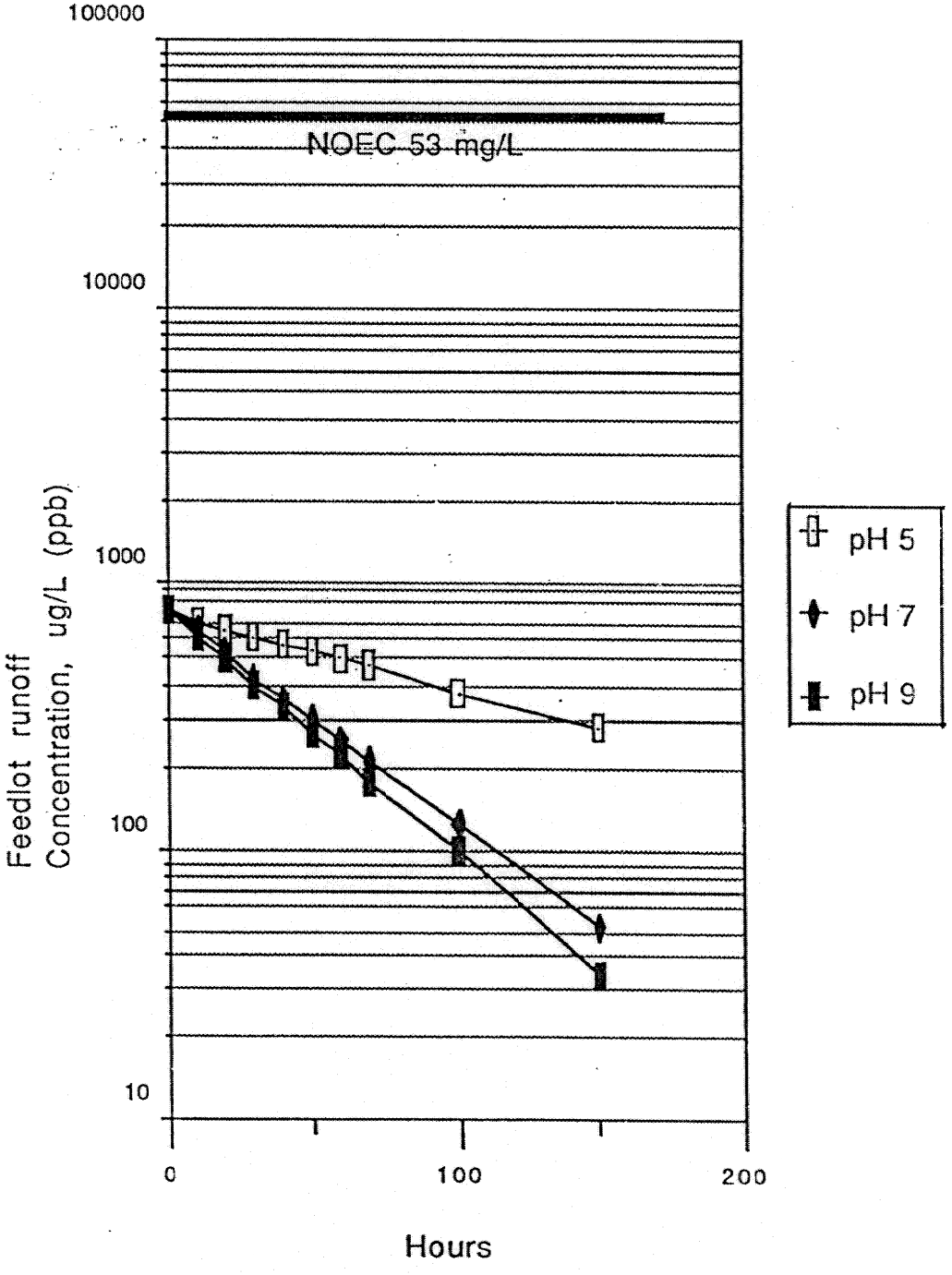


Figure 10. HAZARD ASSESSMENT IN THE AQUATIC ECO-SYSTEM. Comparison of Aquatic Photodegradation of Albendazole 2-Aminosulfone with NOEC for Daphnia Magna.



11. ALTERNATIVES TO THE PROPOSED ACTION:

There are no known adverse environmental effects and no alternatives to the proposed action are needed. Treatment of waste resulting from the manufacturing process is in compliance with federal, state and local regulations.

Albendazole offers to livestock producers a method to combat liver fluke and at the same time rid their animals of tapeworms and roundworms without the need for administration of additional therapeutic agents, thus enhancing the health of these animals.

There is no currently approved anthelmintic agent for use against the deer liver fluke (Fascioloides magna) which also infects domestic ruminants and that meets the requirements of the Food, Drug and Cosmetic Act. No other single anthelmintic has activity against such a wide variety of helminths at such low doses. This negates the need for use of combination drugs or simultaneous dosing with different pharmaceutical preparations in those areas where flukes and other helminths are endemic.

12. LIST OF PREPARERS

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Six months in present position at SKBAHP
Ten years as a manager in Drug Metabolism & Bioanalytical Services
Extensive experience with laboratory methodology and management

Constance Gombatz
Senior Biochemist
BS in Biology/Chemistry
Immaculata College
4 years in present position at SKBAHP
14 years in Drug Metabolism and Bioanalytical Services
Extensive experience in laboratory methodology, instrumentation and technical document preparations.

Robert Foster
President, Springborn Life Sciences, Inc.
He holds an M.S. degree in aquatic biology and a B.S. in biology, both from Eastern Michigan University. With 15 years of industrial and contract laboratory experience, Mr. Foster specializes in ascertaining the environmental safety of the manufacture, use, and disposal of chemical products. He also functions as technical regulatory intermediary to product or process development deliberations with manufacturers and state/federal regulatory agencies responsible for evaluating the hazard to aquatic organisms of commercial development and use. He is author of numerous publications in environmental toxicology, is a charter member of the Society of Environmental Toxicology and Chemistry (SETAC).

Theodore I. Wishousky, Ph.D.
Manager, Regulatory Liaison and Product Registration
Doctorate in Analytical Chemistry, Villanova University
Three years in Regulatory Affairs at SKBAHP
Five years as an investigator in Drug Metabolism and Bioanalytical Services, SKBAHP
Nine years as a scientist in human drug metabolism
Extensive experience with laboratory methodology and instrumentation.

Preparer of Human Food Safety in Section 8.
V. J. Theodorides, D.V.M., Ph.D.
Director, Human Safety Evaluation, SKBAHP, 8/88 - Present
Director, Human Safety Evaluation, SKBAHP
and Antiparasitic Research, 9/84 - 8/88
Manager of Parasitology & Toxicology, SKBAHP, 9/82 - 9/84
Manager of Chemotherapy, SKBAHP, 7/70 - 9/82
Assoc. Director of Chemotherapy, SKBAHP, 8/68 - 7/70
Group Leader, SKF, 7/67 - 8/68
Senior Microbiologist, SKF, 7/65 - 7/67
Visiting Professor - U. of Penn, 1967 - Present
Senior Parasitologist, AHP Pfizer, Terre Haute, 6/63 - 6/65
Lecturer, Boston Univ., Boston, 9/59 - 5/63
Assistant Professor, Univ. Thessaloniki, Greece, 1/56-7/57

Preparers of Manufacturing and Production Information in Section 6:
R. Richard Unangst
Director Pharmaceutical Technical Services and Quality Assurance
B. S. in Pharmacy, Philadelphia College of Pharmacy and Science
Registered Pharmacist in Pennsylvania
Six years experience as Manager of Pharmaceutical Technical Services and Quality Assurance for SKBAHP.
Ten years experience as Manager of Pharmaceutical Development for SKAHP.
Six years experience as Pharmaceutical Chemist for SK&F Labs.

Gaetano J. Celenza
Manager Environmental Engineering for SmithKline Chemicals
B. S. in Chemical Engineering, Drexel University
Registered Professional Engineers in Pennsylvania and New York
Certified diplomat of the American Academy of Environmental Engineers
Twenty years of Environmental Engineering experience in the consulting and engineering industry

13. CERTIFICATION

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

C. John DiCuollo

C. John DiCuollo, Ph.D. *
Group Director, Worldwide
Regulatory Affairs
and Product Development
SmithKline Beckman Animal
Health Products

3/10/89
Date

* Responsible for occupational health and environmental affairs, assuring that SmithKline Beckman Animal Health Products facilities Worldwide (SmithKline Animal Health Products, Norden Laboratories and support divisions of SmithKline Beckman Corporation) comply with local, state, federal and corporate standards as they apply to environmental affairs, hazardous waste disposal and worker safety.

14. **REFERENCES:**

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APPENDIX I

Authorization to Construct and Operate an Industrial Cemetery

18/18/88 11:42 T 91 73 162529 1065 TELEX MEX 67

SECRETARIA
DE
SALUBRIDAD Y ASISTENCIA

DEPENDENCIA: SUBSECRETARIA DE MEJORAMIENTO DEL AMBIENTE.
DIRECCION GENERAL DE SANEA-
DIRECCION: MIENTO DEL SUELO Y
MESA: PROGRAMAS ESPECIALES
NUMERO DEL OFICIO: 406
EXPEDIENTE: 4664

ASUNTO: Se otorga autorización para la construcción y operación de un Cementerio Industrial.

"AÑO DEL GRAL. VICENTE GUERRERO"

México, D. F., 22 SET. 1982

C. ING. SALVADOR ALDRETT LEON
Mier y Terán Nº 385
San Luis Potosí, S.L.P.
Código Postal 78000

En relación a sus escritos S/N de fechas 10 de Marzo, 30 de Junio y 6 de Septiembre del presente año, en donde solicita y entrega requerimientos para llevar a cabo la construcción y operación de un Cementerio Industrial en el Municipio de San Luis Potosí, S.L.P., me permito informar a Usted, que después de haber analizado el proyecto, no tenemos inconveniente en autorizarlo.

Por las características especiales de los desechos a disponer, deberá informar a esta Dirección General a mi cargo, los inicios de la construcción y operación del Cementerio indicado. Así como la forma que garantice el transporte de los materiales del sitio de su generación al de disposición final.

A T E N T A M E N T E .
SUFRAGIO EFECTIVO. NO REELECCION
EL DIRECTOR GENERAL

DR. MANUEL SIRVENT RAMOS

C.c.p.- C. Ing. César Maciel Azcárate. Director de Ingeniería Sanitaria y Saneamiento del Suelo. Pte.
C.c.p. C. Ing. Fidel Cortés Caballar. Encargado de la Subdirección de Saneamiento del Suelo. Pte.

MSR/ OMA/ FCC/ rmrg

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SmithKline Beckman Animal Health Products

A SMITHKLINE BECKMAN COMPANY

APPROVAL: 01/13/89
SKB00100

MATERIAL SAFETY DATA SHEET PAGE: 1

ALBENDAZOLE
AHP00005 02/22/89

SMITHKLINE BECKMAN ANIMAL HEALTH PRODUCTS
1600 PAOLI PIKE
WEST CHESTER, PA 19380
PHONE:(215) 251-7424 OR (215) 251-7414

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1. SUBSTANCE IDENTIFICATION

SUBSTANCE:
ALBENDAZOLE (CAS NUMBER 54965-21-8)
TRADE NAMES/SYNONYMS:
(5-(PROPYLTHIO)-1H-BENZIMIDAZOL-2-YL-)CARBAMIC ACID METHYL ESTER, VALBAZEN, ZENTEL, ANALGON, ALBAZENE, MONIL, VALBOVINO, ALBENDAZOLUM, ABZ, SK&F NO. 62979
CHEMICAL FAMILY:
BENZIMIDAZOLE CARBAMATE ANTHELMINTIC
MOLECULAR FORMULA:
C12H5N3O2S
MOLECULAR WEIGHT:
265.342
SUMMARY OF HAZARDS:
PHYSICAL HAZARDS:
DUST MAY BE EXPLOSIVE.
HEALTH HAZARDS:
BIRTH DEFECTS RESULTED IN LABORATORY ANIMALS, PREGNANT FEMALES SHOULD AVOID CONTACT.
MAY CAUSE ALLERGIC REACTIONS, AVOID SKIN CONTACT.

---

2. COMPONENTS AND CONTAMINANTS

COMPONENT:
ALBENDAZOLE (100%)
OTHER CONTAMINANTS:
NOT APPLICABLE
EXPOSURE LIMITS:
OSHA PERMISSIBLE EXPOSURE LIMIT:
NONE ESTABLISHED
ACGIH THRESHOLD LIMIT VALUE:
NONE ESTABLISHED
SMITHKLINE BECKMAN PERMISSIBLE IN-HOUSE EXPOSURE LIMIT:
0.3 MG/CUBIC METER

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3. PHYSICAL DATA

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ALBENDAZOLE
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DESCRIPTION:
ODORLESS, OFF-WHITE MICRONIZED POWDER.
ODOR THRESHOLD:
NOT APPLICABLE
MELTING POINT:
208-210 DEGREES F
SOLUBILITY (SOLVENT - SOLUBILITY):
WATER - INSOLUBLE AT PH 5, 7 AND 9 (LESS THAN 1 MG/L)
DIMETHYL SULFOXIDE - SOLUBLE
STRONG ACIDS AND BASES - SOLUBLE
BOILING POINT:
NOT APPLICABLE
SPECIFIC GRAVITY:
NOT APPLICABLE
VAPOR PRESSURE:
NOT APPLICABLE
VAPOR DENSITY:
NOT APPLICABLE
PERCENT VOLATILES:
NOT APPLICABLE
EVAPORATION RATE:
NOT APPLICABLE

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4. FIRE AND EXPLOSION DATA

FIRE AND EXPLOSION HAZARDS:
USE EXTREME CAUTION, DUST MAY FORM EXPLOSIVE MIXTURES WHEN MIXED IN AIR. KEEP DUST GENERATION TO A MINIMUM AND AVOID SPARKS.
EXTINGUISHING MEDIA:
USE WATER, CARBON DIOXIDE, FOAM OR DRY POWDER SUITABLE FOR SURROUNDING FIRE.
SPECIAL FIREFIGHTING PROCEDURES:
FIRES OF THIS MATERIAL CAN BE EXPECTED TO EMIT HIGHLY TOXIC FUMES. SELF-CONTAINED BREATHING APPARATUS IS RECOMMENDED FOR FIREFIGHTERS.
FLASH POINT:
NOT DETERMINED
LOWER EXPLOSION LIMIT:
NOT DETERMINED
UPPER EXPLOSION LIMIT:
NOT DETERMINED
AUTOIGNITION TEMPERATURE:
NOT DETERMINED

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MATERIAL SAFETY DATA SHEET **PAGE: 3**

ALBENDAZOLE
AHP00005 **02/22/89**

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5. TRANSPORTATION

DEPARTMENT OF TRANSPORTATION HAZARD CLASSIFICATION:
NOT ESTABLISHED
DEPARTMENT OF TRANSPORTATION LABELING REQUIREMENTS:
NOT ESTABLISHED

---

6. TOXICITY

LETHALITY:
THIS MATERIAL PRODUCES MODERATE ORAL LETHALITY FOLLOWING A SINGLE TREATMENT IN LABORATORY ANIMALS. LD50 VALUES ARE:
MOUSE - GREATER THAN 3000 MG/KG;
RAT - BETWEEN 1320 AND 2400 MG/KG;
HAMSTER - GREATER THAN 10000 MG/KG;
RABBIT - GREATER THAN 500 MG/KG
LETHALITY IN RATS WAS DELAYED APPROXIMATELY 1 WEEK AFTER TREATMENT AND SYMPTOMS OF POISONING INCLUDED REDUCED BODY WEIGHTS AND FOOD CONSUMPTION, BOTH SIGNS OF GENERAL TOXICITY.
MUTAGENICITY:
THIS MATERIAL WAS NOT MUTAGENIC IN TWO LABORATORY TESTS (AMES TEST AND CELL-MEDIATED CHINESE HAMSTER OVARY TEST).
CARCINOGENICITY:
THIS MATERIAL IS NOT LISTED AS A CARCINOGEN BY IARC, NTP OR OSHA. LIFETIME STUDIES WITH MICE AND RATS DEMONSTRATED NO EVIDENCE OF CARCINOGENICITY.
REPRODUCTIVE EFFECTS:
TERATOGENIC EFFECTS (BIRTH DEFECTS) OR TOXICITY TO DEVELOPING OFFSPRING OCCURRED IN STUDIES WITH RATS, RABBITS, SHEEP AND SWINE. THESE EFFECTS USUALLY OCCURRED AT DOSE LEVELS THAT ALSO PRODUCED MATERNAL TOXICITY. RAT REPRODUCTIVE TOXICITY STUDIES SHOWED ONLY MINIMAL EFFECTS ON MALE OR FEMALE REPRODUCTION.
OTHER EFFECTS:
NO EVIDENCE FOR SKIN OR EYE IRRITATION IN RABBITS.
NUMEROUS SUBCHRONIC AND CHRONIC TOXICITY STUDIES HAVE BEEN CONDUCTED WITH THIS MATERIAL. AT HIGH DOSE LEVELS, OFTEN PRODUCING LETHALITY, ADVERSE EFFECTS WERE NOTED THAT MAY REPRESENT TARGET ORGAN TOXICITY:
IN THE TESTES OF RATS AND MICE;
IN BLOOD OR BLOOD FORMING ORGANS OF RATS, MICE AND DOGS;
IN LIVER OF RATS AND MICE.

---

7. HEALTH HAZARDS AND FIRST AID

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MATERIAL SAFETY DATA SHEET **PAGE: 4**

ALBENDAZOLE
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PRIMARY ROUTES OF EXPOSURE:
AVOID BREATHING DUST, SKIN CONTACT. PREGNANT FEMALES SHOULD AVOID ALL CONTACT.

SKIN CONTACT:

EFFECTS OF EXPOSURE:
THIS MATERIAL WAS NON-IRRITATING IN RABBITS BUT DERMATITIS RESULTING FROM MECHANICAL IRRITATION HAS BEEN REPORTED IN HUMANS. IN ADDITION, RARE INSTANCES OF IDIOSYNCRATIC HYPERSENSITIVITY, A TYPE OF ALLERGIC REACTION, HAVE BEEN NOTED IN HUMANS FOLLOWING SKIN CONTACT.

FIRST-AID:
REMOVE CONTAMINATED CLOTHING AND WASH WITH SOAP AND WATER. IF SIGNS OF IRRITATION SUCH AS REDNESS OR SWELLING DEVELOP, SEE A PHYSICIAN.

EYE CONTACT:

EFFECTS OF EXPOSURE:
THIS MATERIAL WAS NON-IRRITATING IN RABBITS AND EYE IRRITATION IS NOT EXPECTED IN HUMANS.

FIRST-AID:
FLUSH EYES WITH A LARGE AMOUNT OF WATER. IF SIGNS OF IRRITATION SUCH AS REDNESS OR SWELLING DEVELOP, SEE A PHYSICIAN.

INHALATION:

EFFECTS OF EXPOSURE:
THE EFFECTS OF BREATHING DUST HAVE NOT BEEN DETERMINED.

FIRST-AID:
MOVE EXPOSED SUBJECT TO FRESH AIR. CLEAR NOSE BY BLOWING. SEE A PHYSICIAN IF SUBJECT EXPERIENCES DIFFICULTY BREATHING. IF BREATHING HAS STOPPED, PERFORM ARTIFICIAL RESPIRATION AND SEEK IMMEDIATE MEDICAL ASSISTANCE.

INGESTION:

EFFECTS OF EXPOSURE:
THIS MATERIAL IS NOT EXPECTED TO PRODUCE SIGNIFICANT TOXICITY FOLLOWING INGESTION IN HUMANS SINCE IT HAS BEEN USED THERAPEUTICALLY AT DAILY DOSES UP TO 400 MG IN ADULTS.

FIRST-AID:
IN THE EVENT OF OVEREXPOSURE TO THIS MATERIAL BY INGESTION, INDUCE VOMITTING BUT ONLY IF THE SUBJECT IS FULLY CONSCIOUS.

CONDITIONS AGGRAVATED BY EXPOSURE:
NONE KNOWN

---

8. REACTIVITY

CONDITIONS TO AVOID:
AVOID HIGH TEMPERATURES.

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ALBENDAZOLE
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INCOMPATABILITY:
NONE KNOWN.
STABILITY:
STABLE AT ROOM TEMPERATURE FOR UP TO 3 YEARS.
HAZARDOUS POLYMERIZATION:
NONE KNOWN.
HAZARDOUS DECOMPOSITION PRODUCTS:
OXIDES OF SULFUR.

9. STORAGE AND DISPOSAL

STORAGE:
STORE IN A COOL, DRY PLACE. DO NOT STORE ABOVE 40-45 DEGREES C FOR LONGER THAN 6 MONTHS.
DISPOSAL:
OBSERVE ALL FEDERAL, STATE AND LOCAL REGULATIONS WHEN DISPOSING OF THIS MATERIAL.

10. SPILLS AND LEAKS

OOP OR SHOVEL SPILLED MATERIAL INTO A DRUM OR APPROPRIATELY SIZED CONTAINER R DISPOSAL. KEEP DUST GENERATION TO A MINIMUM. FOR LIQUIDS CONTAINING THIS MATERIAL, USE ABSORBANT MATERIAL AND PLACE IN A SEALED CONTAINER FOR DISPOSAL.

11. PROTECTIVE EQUIPMENT

LABORATORY:
RESPIRATORS:
A DUST MASK SHOULD BE USED WHEN HANDLING SMALL QUANTITIES OF THIS MATERIAL.
GLOVES:
WEAR IMPERVIOUS GLOVES WHEN HANDLING THIS MATERIAL.
EYE PROTECTION:
WEAR SAFETY GLASSES WITH SIDESHIELDS WHEN HANDLING THIS MATERIAL.

HYGIENE PRACTICES:
WASH HANDS AND ARMS THOROUGHLY AFTER HANDLING THIS MATERIAL. CLEAN UP SPILLED MATERIAL IMMEDIATELY.
VENTILATION:
USE A FUME HOOD WHEN WORKING WITH DUST.
OTHER PROTECTIVE EQUIPMENT:
WEAR LAB COAT WITH LONG SLEEVES.
AREAS WHERE LARGE QUANTITIES ARE USED (PRODUCTION OR FORMULATION):

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AHP00005 **02/22/89**

RESPIRATORS:
WEAR A CARTRIDGE RESPIRATOR WITH A HIGH EFFICIENCY PARTICULATE FILTER, OR IF CONDITIONS WARRANT, USE A POSITIVE PRESSURE SUPPLIED AIR RESPIRATOR. DUST LEVELS SHOULD BE KEPT BELOW 0.3 MG/CUBIC METER.

GLOVES:
WEAR IMPERVIOUS GLOVES WHEN HANDLING THIS MATERIAL.

EYE PROTECTION:
WEAR SAFETY GLASSES WITH SIDESHIELDS, GOGGLES OR A FULLFACE RESPIRATOR.

HYGIENE PRACTICES:
SHOWER AT THE END OF EACH SHIFT WHEN HANDLING THIS MATERIAL IN BULK QUANTITIES. WEAR PROTECTIVE CLOTHING THAT IS LAUNDERED OR DISCARDED AFTER EACH USE. CLEAN UP SPILLS IMMEDIATELY.

VENTILATION:
USE WITH ADEQUATE MECHANICAL VENTILATION. USE LOCAL EXHAUST IN AREAS WHERE DUST IS GENERATED.

OTHER PROTECTIVE EQUIPMENT:
WEAR CLOTHING WITH LONG SLEEVES TO AVOID SKIN CONTACT.

12. LABEL INFORMATION

ALBENDOZOLE
CAUTION !
DUST MAY BE EXPLOSIVE - KEEP DUST TO A MINIMUM, AVOID SPARKS
POSSIBLE BIRTH DEFECTS - PREGNANT FEMALES SHOULD AVOID INGESTION,
(BASED ON ANIMAL DATA) BREATHING DUST, SKIN CONTACT, EYE CONTACT
MAY CAUSE ALLERGIC REACTION - AVOID BREATHING DUST, SKIN CONTACT

CREATION DATE: 01/13/89 REVISION DATE: 02/09/89

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APPENDIX III

Water Solubility Determinations of Albendazole And Its Sulfoxide, Sulfone and 2-Aminosulfone Metabolites

INTRODUCTION

The objective of these studies (conducted by Hazleton Laboratories America, Inc. for SmithKline Beckman Animal Health Products) was to determine the solubility of albendazole and its major metabolites, sulfoxide, sulfone and 2-aminosulfone, in pH 5, 7 and 9 aqueous buffers.

METHODS

Albendazole: The water solubility of the test material was determined at 25°C by the column elution method as described in the Environmental Technical Assistance Document No. 3.01 and OECD Guidelines for Testing Chemicals, Section 1, No. 105. Into each of three round bottom flasks was weighed 3.0 g of 250 µm glass beads. Approximately 0.15 g of the test material, which was dissolved in chloroform, was added to each of the round bottom flasks. The solvent was completely evaporated in a rotary evaporator and the dry carrier material was poured into a microcolumn that contained a pH 5, 7 or 9 buffer. The three systems were allowed to equilibrate for at least 2 hours. The temperature of the microcolumns was maintained at 25°C by use of a thermostat-controlled circulating pump that circulated water through a jacket surrounding each microcolumn. The flow through each column was started and the flow rate was adjusted to approximately 25 mL/hour. Samples from each column were withdrawn until equilibration was established (approximately 24 hours), as defined by five successive samples whose concentrations did not differ by more than ± 30% in a random fashion. These samples were separated from each other by time intervals corresponding to the passage of at least 10 bed volumes of eluant through the column.

Sulfoxide: The water solubility of the test material was determined at 25°C by the flask method as described in the documents referenced above. Approximately 0.02 g of the test material was added to triplicate test systems (nine tubes for each shaking time of 24, 48 and 72 hours, each containing 10 mL of pH 5, 7 or 9 buffer). The tubes were capped and agitated in a 25°C water/shaker bath. At 24 and subsequently, 48 and 72 hours, nine tubes were removed and re-equilibrated for an additional 24 hours in a 25°C water bath. After re-equilibration, the tubes were centrifuged for approximately 30 minutes at 10,000 rpm and the concentration of the test material in the clear aqueous phase was determined by HPLC.

Sulfone: The water solubility of the test material was determined at 25°C by the column elution method as described in the documents referenced above. Into each of three round bottom flasks was weighed 0.75 g of silica gel. Approximately 0.04 g of test material that was dissolved in a methanol/chloroform solution was added to each of the round bottom flasks. The solvent was completely evaporated in a rotary evaporator and the dry carrier material was poured into a microcolumn that contained a pH 5, 7 or 9 buffer. The three systems were allowed to equilibrate overnight. The temperature of the microcolumns was maintained at 25°C using a thermostat-controlled circulating pump that circulated water through a jacket surrounding each microcolumn. The flow through each column was started and the flow rate was adjusted to approximately 25 mL/hour. Samples from each column were withdrawn until equilibration was established (approximately 24 hours), defined by five successive samples whose concentrations did not differ from each other by more than ± 30% in a random fashion. These samples were separated from each other by time intervals corresponding to the passage of at least 10 bed volumes of the eluant through the column.

2-Aminosulfone: The water solubility of the test material was determined by the flask method as described in the documents referenced above. Approximately 0.1 g of the test material was added to triplicate test systems (nine tubes for each shaking time of 24, 48 and 72 hours, each containing 10 mL of pH 5, 7 or 9 buffer). The tubes were capped and agitated in a 25°C water/shaker bath. At 24 and subsequently, 48 and 72 hours, nine tubes were removed and re-equilibrated for an additional 24 hours in a 25°C water bath. After re-equilibration, the tubes were centrifuged for 30 minutes at 10,000 rpm and the concentration of the test material in the clear aqueous phase was determined.

RESULTS

Average water solubilities determined for albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites are summarized below:

Water Solubility in ppm (mg/L)

Chemical	pH5	pH7	pH9
Albendazole	0.579	0.530	0.564
Sulfoxide	71.7	67.5	72.1
Sulfone	8.05	6.82	7.51
2-Aminosulfone	1343	511	488

CONCLUSIONS

The 2-aminosulfone metabolite is the most water soluble metabolite, as compared to albendazole which is relatively water insoluble.

APPENDIX IV

Determination of Dissociation Constants for the Sulfoxide, Sulfone and 2-Aminosulfone Metabolites of Albendazole

INTRODUCTION

The objective of this study (conducted by Hazleton Laboratories America, Inc.) was to determine the dissociation constants (K) of the major metabolites of albendazole: sulfoxide, sulfone and 2-aminosulfone. The dissociation constant for albendazole was not determined since the solubility of this test material was < 5 ppm.

METHODS

The method used for dissociation constant determinations was described in:

- Environmental Science and Technology 3, II, pp.1, 186-1, 188 (1969)
- Organization for Economic Cooperation and Development Guideline for Testing of Chemicals, Section 1, No. 112
- Environmental Assessment Technical Assistance Document 3.04

Solutions of the test materials, sulfoxide, sulfone and 2-aminosulfone metabolites of albendazole were titrated and the pH of the solutions and milliliters of NaOH added were recorded after each addition of NaOH. A plot of pH/mL versus milliliters of NaOH and a plot of ΔpH/ΔmL versus milliliters of NaOH were generated. The pK values were calculated for 10 points near the equivalence point. Titration was carried out past the equivalence point. The equivalence point was defined by the section of the titration curve in which a small addition of NaOH resulted in a large change in pH. The test materials were titrated in triplicate. The pK and K values were determined as follows:

$$pK = pH - \log \frac{[B^-]}{[HB]}$$

$$K = \text{antilog } (-pK)$$

Where: pH = pH of test solution at any point
[B-] = Concentration of ion
[HB] = Concentration of starting material

RESULTS

Results of these determinations are summarized below:

Chemical	pK	K	Standard Deviation
Sulfoxide	7.87	1.34×10^{-8}	1.16×10^{-9}
Sulfone	6.78	1.67×10^{-7}	9.61×10^{-9}
2-Aminosulfone	9.35	4.46×10^{-10}	3.30×10^{-11}

APPENDIX V

Octanol/Water Partitioning Study: Albendazole and Its Major Metabolites

INTRODUCTION

The purpose of these studies performed at SmithKline Beckman Animal Health Products was to determine the lipid/water partitioning coefficients for albendazole and its major metabolites, sulfoxide, sulfone and 2-aminosulfone.

METHODS

^{14}C-Labelled albendazole, sulfoxide, sulfone and 2-aminosulfone were dissolved in water equilibrated n-octanol and the concentrations confirmed utilizing liquid scintillation counting techniques. Equal volumes of n-octanol equilibrated water were added to each albendazole and metabolite solution and the replicate samples were agitated on a horizontal shaker at room temperature ($23^{o}C$). After 72 hours, the phases were separated by centrifugation and both layers assayed for radioactivity.

RESULTS

The average partition coefficients and percent solutes for albendazole, sulfoxide, sulfone and 2-aminosulfone are:

Chemical	[a]	%
Albendazole	501	0.2
Sulfone	26	3.6
Sulfoxide	14	6.5
2-Aminosulfone	0.62	60.8

where: $[a] = \frac{\text{conc. of solute in octanol}}{\text{conc. of solute in water}}$;

% = percent solute in water phase

CONCLUSION

Albendazole has the lowest water solubility and highest lipid affinity while the 2-aminosulfone was the most water soluble.

APPENDIX VI

UV-Visible Absorbance Spectra for Albendazole and its Major Metabolites

INTRODUCTION

The objective of this study (conducted by SmithKline Beckman Animal Health Products) was to determine the ultraviolet-visible absorption spectra for albendazole and its major metabolites, sulfoxide, sulfone and 2-aminosulfone.

METHODS

The UV/VIS spectra of the test materials were determined by the aqueous system method as described in the Environmental Assessment Technical Assistance Document No. 3.05. The ultraviolet-visible absorption spectra (190 - 900 nm) were determined in conjunction with the photodegradation studies summarized in Appendix XI at pH 5, 7 and 9 by placing aliquots of the initial buffered test solutions in quartz sample cells. The spectra were obtained with a Perkin Elmer 'Lambda-Array' 3840 Spectrophotometer, interfaced to a PE 7300 PC and a PE PR200 printer.

RESULTS

The average values obtained for albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites are summarized below:

Chemical	pH	Extinction Coefficient[a]	Lambda max. (nm)
Albendazole	5	1.24×10^4	301.6
	7	1.11×10^4	295.0
	9	7.59×10^3	294.0
Sulfoxide	5	2.16×10^4	291.5
	7	2.36×10^4	291.5
	9	1.72×10^4	292.4
Sulfone	5	1.5×10^4	290.8
	7	1.10×10^4	291.0
	9	1.53×10^4	292.0
2-Aminosulfone	5	9.84×10^3	288.5
	7	1.22×10^4	290.4
	9	1.13×10^4	290.6

[a]Average of 3 replicate determinations.

APPENDIX VII

Determinations of Adsorption and Desorption Coefficients for Albendazole and its Sulfoxide Metabolite

INTRODUCTION

The objective of this study (conducted at Springborn Life Sciences, Inc.) was to determine the partitioning of albendazole and its sulfoxide metabolite between sorbed and solution phases. Adsorption and desorption coefficients were determined under varying soil conditions and employed to derive isotherms utilizing the Freundlich equation.

METHODS

The procedures followed for determination of the sorption and desorption coefficients for albendazole and its sulfoxide metabolite are detailed in the Environmental Assessment Technical Assistance Document No. 3.08.

^{14}C-Albendazole sulfoxide: The equilibrium phase test was performed at solution: soil ratios of 20 to 1 for Illinois silt loam soil (ILSTLM) and 5 to 1 for New York loam soil (NYLM) for equilibrium periods of 2, 4, 16, 24 and 48 hours. A 25 mg/L solution was prepared by addition of ^{14}C-albendazole sulfoxide and analytical grade albendazole sulfoxide to 0.01 M $CaCl_2$. Three tubes were prepared at the appropriate solution: soil ratio for each soil type to serve as control blanks and triplicate samples were prepared for each equilibrium duration containing 40 mL of a 25 mg/L albendazole sulfoxide solution to serve as soil-less controls. All tubes were shaken for the time periods (above) at 130 rpm and aqueous samples removed for radioassay after centrifugation. The adsorption phase test was performed at solution: soil ratios of 20 to 1 for ILSTLM and at 5 to 1 for the NYLM soil for an equilibrium period of 16 hours. ^{14}C-Albendazole sulfoxide and analytical grade albendazole sulfoxide solutions were prepared in 0.01 M $CaCl_2$ at 25, 10, 5 and 2.5 mg/L. Triplicate 2 g soil samples of ILSTLM and triplicate 8 g samples of NYLM were placed in tubes and 40 mL of albendazole sulfoxide concentration added to individual tubes. Six soil-less controls for each test concentration and three blank controls for each soil type were prepared.

The desorption phase test was performed by adding a volume of 0.01M $CaCl_2$ (equal to the volume removed from the adsorption phase) to the tubes containing soil (except for the soil-less controls). ^{14}C-Albendazole sulfoxide retained in the soil phase was allowed to desorb from the soil for the same equilibration period. After shaking for the required time interval, the aqueous phase was sampled and assayed as above. A second desorption phase was performed with fresh 0.01 M $CaCl_2$, as described above and the soil was then assayed by combustion.

^{14}C-Albendazole:

The adsorption and desorption of ^{14}C-albendazole using three soil types were tested in equilibrium studies. The screening test was performed with two aqueous phases using a ^{14}C-albendazole nominal concentration of 2.0 mg/L of 0.01M $CaCl_2$ or deionized (DI) water, and a solution: soil ratio of 5:1 to determine the necessity of the advanced test. The equilibrium phase of the advanced test was performed using a solution:soil ratio of 100:1 for Texas Silt Loam (TXSTLM), ILSTLM and NYLM at a nominal concentration of 0.20 mg/L. The time required to reach equilibrium and an approximation of the adsorption coefficient was determined. A definitive test was performed using four aqueous ^{14}C-albendazole nominal concentrations (0.25, 0.13, 0.06, and 0.03 mg/L) at a solution:soil ratio of 100:1 as in the equilibrium phase and for the established equilibration time (24 hrs.) to further define the adsorption and desorption coefficients. All data generated in the definitive test was evaluated using the Freundlich equation and adsorption and desorption isotherms were plotted.

Results:
Albendazole

Adsorption 0.01M $CaCl_2$				Desorption 0.01M $CaCl_2$			
Soil	K_d	K_{oc}	1/n	K_d	K_{oc}	1/n	pH
TXSTLM	109.6	13400	1.21	275423	33588200	2.86	8.0
ILSTLM	501.2	27500	0.770	1047	57500	0.915	5.5
NYLM	141.3	7800	0.936	912	50100	1.33	6.5

Sulfoxide

Adsorption 0.01M $CaCl_2$				Desorption 0.01M $CaCl_2$			
Soil	K_d	K_{oc}	1/n	K_d	K_{oc}	1/n	pH
ILSTLM	52.6	2900	0.611	38.1	2100	0.576	5.5
NYLM	6.3	350	0.771	6.5	360	0.657	6.5
TXSTLM	1.2*	150*	*	*	*	*	8.0

*Screening tests demonstrated that < 25% adsorbed, therefore, advanced tests were not required.

K_d = Concentration insoil (µg/g)/Concentration in water (µg/mL).

$K_{oc} = K_d \times 100 / \%$ Organic carbon
Where the organic carbon content was calculated by dividing the % organic matter by 1.7.

Conclusions

Greater than 51% of the initial 14C-albendazole sulfoxide added adsorbed onto ILSTLM and greater than 43% adsorbed onto NYLM. Approximately 55% and 56% of the adsorbed 14C-albendazole sulfoxide subsequently desorbed from ILSTLM and NYLM soils, respectively.

Greater than 36% of the initial 14C-albendazole added adsorbed onto TXSTLM, greater than 92% adsorbed onto ILSTLM and greater than 59% adsorbed onto NYLM.

APPENDIX VIII

Biodegradation of Albendazole Urinary and Fecal Metabolites in Feedlot Soil

INTRODUCTION

Fortification studies were conducted (at Bio/dynamics, Inc.) to determine the stability and binding of albendazole and its metabolites when incorporated into a feedlot-type soil matrix.

METHODS

Urine and feces from calves administered ^{14}C-albendazole at a dosage level of 15 mg/kg body weight were incorporated into feedlot soil at a rate of 1 ppm of urinary radioactivity and 1 ppm fecal radioactivity and incubated in a sealed glass chamber. After 28 days, half of the chambers were converted to anaerobic systems and half remained as aerobic systems. At intervals of 0, 7, 14, 28, 60, 90 and 120 days, the air above the soil was drawn through gas wash bottles containing Aquasol 2® and Oxifluor CO_2® to trap organic volatile components and $^{14}CO_2$ respectively. Aliquots of soil at various intervals were extracted with ethyl acetate and water buffered at pH 5.

RESULTS

The amount of radioactivity present as organic volatiles amounted to < 0.1% of the initial radioactivity present in the soil. The amount of radioactivity present as $^{14}CO_2$ in the chamber air ranged from 0.51 to 1.63% in the aerobic systems and 0.28 to 0.65% in the anaerobic systems. The amount of radioactivity still present in the soil at day 120 ranged from 80 - 98% of the initial radioactivity in the aerobic systems and 108 - 131% in the anaerobic systems.

The average distribution of radioactivity in the soil extracts are summarized below:

Interval (Days)		Organic Soluble	Water Soluble	Bound	Total Recovery
0		15.0	15.8	69.2	100.0
7		12.4	11.6	83.8	107.8
14		11.4	11.3	80.6	103.3
21		11.2	11.8	80.8	103.8
28		9.0	10.7	76.6	96.3
60	Aerobic	12.4	11.1	74.7	98.2
	Anaerobic	10.1	8.8	72.9	91.8
90	Aerobic	7.2	8.7	88.4	104.3
	Anaerobic	11.2	6.1	67.5	84.8
120	Aerobic	8.1	8.2	88.5	104.8
	Anaerobic	11.5	5.0	79.5	96.0

CONCLUSIONS

The albendazole residues present in cattle excreta are largely bound to soil components upon initial contact, with the degree of binding increasing over time.

APPENDIX IX

SIMULATED RUNOFF EXPERIMENTS OF ^{14}C-ALBENDAZOLE AND ITS METABOLITES IN CALF URINE - COMPARISON OF SOIL TYPES

INTRODUCTION

The purpose of this study (conducted at Bio/dynamics, Inc.) was to determine and compare the runoff characteristics of ^{14}C-albendazole and urinary metabolites of ^{14}C-albendazole in both dynamic and static water/soil interactions using three soil types with varying organic and clay content.

METHODS AND MATERIALS

Soils:

Silt loam, silty clay loam and a high organic matter silt loam soil, used in these experiments were analyzed by United States Testing Company, Inc., Memphis, Tennessee and the soil characterized as follows:

Source:	West Chester, PA	E. Millstone, NJ	Clarksdale Miss.
Type:	Silt Loam (High Organic)	Silt Loam	Silty Clay Loam
Organic Matter %:	6.5	1.8	2.5
Sand Content %:	41.2	40.0	9.8
Silt Content %:	49.2	51.2	52.2
Clay Content %:	9.6	8.8	38.0
Water Reten. @1/3 Bar,%:	32.1	28.4	39.5
pH	7.8	6.4	6.3
Cation Exchange Capacity meq/100g:	17.1	12.25	33.3

TEST MATERIALS

- ^{14}C-Albendazole (Specific Activity = 2077 dpm/µg) at a concentration of 1 mg/mL in acidic methanol.
- Urine (0-48 hour) from a calf treated with ^{14}C-albendazole.

^{14}C - Albendazole - Dynamic Interaction:

All three soil types were fortified with ^{14}C-albendazole at 20 ppm, homogenized, placed in glass vessels with 2400 mL water and shaken on an equipoise shaker for 24 hours. Aliquots of water were removed, the suspended soil particles allowed to settle, and the water analyzed for radioactivity. Vessels were returned for two additional 24 hour intervals to ascertain that equilibrium was established in the system. The soil was then separated from the water and analyzed for radioactivity.

^{14}C - Albendazole - Static Interaction:

All three soil types were placed in jars with 2400 mL of distilled water, fortified with ^{14}C-albendazole at 2.0 ppm, and stirred for 10 minutes, 2 times per week, at approximately 45 rpm to simulate mild movements of water under pond conditions. At intervals of 0, 1, 2, 3 and 4 weeks post-treatments, aliquots of the aqueous were removed, centrifuged to remove suspended soil and the aqueous assayed for radioactivity. After four weeks, soil aliquots were assayed for radioactivity.

^{14}C-Albendazole Urinary Residues - Dynamic Interaction:

All three soil types were fortified with ^{14}C-albendazole urinary residues at 20 ppm, homogenized with 2400 mL water, shaken on an equipoise shaker for 24 hours and assayed as previously described for dynamic interaction testing.

^{14}C-Albendazole Urinary Residues - Static Interaction:

All three soil types were placed in jars with 2400 mL of distilled water, fortified with ^{14}C-albendazole urinary residues at 2.0 ppm, stirred for 10 minutes, two times per week at ~ 45 rpm and assayed as previously described for static interaction testing.

RESULTS:

The results of dynamic and static soil/water interaction with ^{14}C-albendazole and urinary residues of ^{14}C-albendazole are summarized as follows:

Time Post-Treatment	% of Radioactivity					
	Loam		Silt Loam		Silty Clay Loam	
	Aqueous	Soil	Aqueous	Soil	Aqueous	Soil
SUMMARY OF RADIOACTIVITY AFTER DYNAMIC WATER-SOIL INTERACTION WITH ^{14}C-ALBENDAZOLE						
24 hr	15.86	-	19.87	-	5.40	-
48 hr	18.71	-	19.54	-	7.73	-
72 hr	19.66	85.6	20.10	76.40	5.80	98.57
SUMMARY OF RADIOACTIVITY AFTER DYNAMIC WATER-SOIL INTERACTION WITH THE CALF URINARY METABOLITES OF ^{14}C-ALBENDAZOLE						
24 hr	31.91	-	53.40	-	33.82	-
48 hr	23.62	-	44.70	-	30.95	-
72 hr	25.67	65.6	36.44	66.20	19.94	78.67
SUMMARY OF RADIOACTIVITY AFTER STATIC WATER-SOIL INTERACTION WITH ^{14}C-ALBENDAZOLE						
Initial	82.48	-	54.26	-	14.43	-
1 week	49.08	-	39.59	-	13.55	-
2 weeks	33.70	-	31.91	-	18.44	-
3 weeks	25.46	-	23.70	-	15.39	-
4 weeks	20.27	68.72	16.76	82.28	10.93	85.46
SUMMARY OF RADIOACTIVITY AFTER STATIC WATER-SOIL INTERACTION WITH CALF URINARY METABOLITES OF ^{14}C-ALBENDAZOLE						
Initial	91.19	-	85.50	-	81.50	-
1 week	66.78	-	67.04	-	61.25	-
2 weeks	48.04	-	54.39	-	32.84	-
3 weeks	43.31	-	43.21	-	31.23	-
4 weeks	37.95	53.91	34.34	65.70	34.54	55.98

CONCLUSIONS

All three soil types exhibited significant binding with the ^{14}C-albendazole and its urinary metabolites. The higher clay content silty clay loam soil, however, exhibited a higher degree of binding of both the ^{14}C-albendazole and the ^{14}C-labeled urinary metabolites.

APPENDIX X

Soil Mobility of Albendazole and Its Metabolites from Cattle Excreta

INTRODUCTION

The objective of this study (conducted by Raltech Scientific Services) was to determine whether exaggerated levels of albendazole and its metabolites could migrate in agricultural soils as a result of rainfall.

METHODS

An albendazole metabolite mixture was obtained from a steer orally dosed with ^{14}C-albendazole at 15 mg/kg. Urine and feces collected from this animal were mixed together as a homogenate and applied to 5 g of the soils to be tested. After drying, the soil/metabolite mixture was assayed for radioactivity and placed onto previously prepared columns containing soil. Four different soil types were studied, spanning a range of textural properties, organic matter content and pH. Prior to preparing the columns, the soils were air-dried and sieved through a 20 mesh screen to promote a relatively uniform aggregate size. They were then sieved to 30 mesh prior to packing the soil columns. As controls, 2,4-D was employed as a control for substances which easily migrate through soil and DDT as a control to monitor substances which are retained by soil. Two aliquots (125 mL each) of distilled water were allowed to percolate through each of the columns (2 cm head) and each leachate aliquot was collected separately. The leachate volumes were determined and the radioactivity present measured by counting 1 mL aliquots. After percolation, the soil columns were cut into 2.54 cm segments and the soils air-dried. The dried segments were weighed, pulverized, mixed and assayed for radioactivity by combustion by scintillation counting. Since column lengths were 30 cm, approximately 12 column segments were available for assay for each soil type.

RESULTS

The distribution of albendazole and albendazole metabolites was similar for all soil types. Between 82% and 99% of the applied radioactivity was retained in the soil columns while 1% to 18% of the radioactivity appeared in the leachates. The bulk of the albendazole derived radioactivity appeared in the first four column segments, with successive segments containing decreasing amounts. The reference compounds, 2,4-D and DDT were found primarily in the leachate and the first column segment, respectively.

CONCLUSIONS

The pattern observed for albendazole derived radioactivity indicated that most of the material was relatively immobile on each of the soil types.

APPENDIX XI

Photolytic Degradation of Albendazole and its Major Metabolites, Sulfoxide, Sulfone and 2-Aminosulfone

INTRODUCTION

The objectives of this study (conducted at SmithKline Beckman Animal Health Products) were to determine the susceptibility of albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites to aquatic photodegradation and to calculate their aquatic photodegradation rates and half-lives.

METHODS

The susceptibility of the test materials to aquatic photodegradation was determined using the method described in the Environmental Assessment Technical Assistance Document No. 3.10.

Meteorological Measurements: Daily temperature and relative humidity measurements were recorded along with sunrise/sunset times and sky conditions throughout the study.

Actinometers: Aqueous solutions containing Para-Nitroacetophenone (PNAP, 2×10^{-5} M) and 11×10^{-2} M or 9.7×10^{-2}M pyridine were prepared in sterile water.

The actinometer solutions were exposed to natural sunlight along with test solutions to provide a marker of sunlight intensity.

Preparation of test solutions:

^{14}C-Albendazole and its major metabolites, ^{14}C-sulfoxide, ^{14}C-sulfone and ^{14}C-2-aminosulfone were prepared (triplicate) in pH 5, 7 and 9 buffers and aerated with bacteria-free air until saturation. A second set of samples, prepared identically but covered with aluminum foil, served as controls.

Sample treatment:

The test solutions were exposed to sunlight along with the actinometers and sampled after various exposure times. These samples were analyzed by HPLC in order to determine the concentration of the test compound remaining after each specific length of exposure.

RESULTS

Based on the results, photolysis rates and half-lives of albendazole and its three major metabolites were calculated.

Photodegradation Rate and Half-Life

The photolysis rate measured in a tube (11x100mm) will be faster than the rate at which a test chemical will photolyze at the flat water body surface in the environment. The internal reflections of the incident sunlight in a test tube increase the path length of light. To convert the photolysis rate measured in a tube (k_p) to a rate at the surface of a flat water body (k_{pE}) the following equation is used:

$$k_{pE} = k_p/2.2$$

k_{pE}, which represents a first-order photolysis rate constant for a water body in sunlight is deduced by the equation:

$$k_{pE} = \phi_E \Sigma \varepsilon\lambda L\lambda$$

Where ϕ_E is the reaction quantum yield which represents the fraction of photons absorbed that actually affects photodegradation of test chemical; $\varepsilon\lambda$ is the molar absorptivity; $L\lambda$ is the solar irradiance in water and the summation is taken over the range λ = 290 to 800 nm. $L\lambda$ is the solar irradiance at shallow depths for a water body under clear sky conditions and is a function of latitude and season of the year.

The reaction quantum yield for the test chemical, ϕ^c_E is given by:

$$\phi^c_E = \frac{k^c_p \; \Sigma\varepsilon^a\lambda L\lambda}{k^a_p \; \Sigma\varepsilon c\lambda L\lambda} \quad (\phi^a_E)$$

Where k^c_p/k^a_p is the ratio of measured photolysis rate constants for test chemical (c) and p-nitroacetophenone-pyridine (PNAP-PYR) actinometer (a); $\Sigma\varepsilon^c\lambda L\lambda$ is the light absorbed by the test chemical; and $\Sigma\varepsilon^a\lambda L\lambda$ is the light absorbed by the actinometer; and ϕ^a_E is the reaction quantum yield for the actinometer.

The ratio of rate constants k^c_p/k^a_p can be determined by measuring the concentration of the test chemical and actinometer as a function of time (t) in sunlight, where:

$$\ln (C_o/C_t)^c = (k^c_p/k^a_p) \quad \ln (C_o/C_t)^a,$$

assuming that the loss of chemical is only due to photolysis.

The term $\Sigma\varepsilon^c\lambda L\lambda$ for PNAP-PYR actinometer has been tabulated as a function of latitude and season of the year in Table 2 of EPA (1985)[12]. The term $\Sigma\varepsilon^c\lambda L\lambda$ for the test chemical can be calculated from the experimentally measured molar absorptivities (between 297.5-800 nm) and $L\lambda$ values listed in Table 5 EPA (1985)[12].

ϕa_E for PNAP-PYR actinometer can be determined using the equation:

$$\phi a_E = [PYR],$$

where [PYR] is the molar concentration of pyridine used during the experiment. The reaction quantum yield can be used to calculate maximum photolysis rates and minimum half-lives for winter and summer months.

Maximum Environmental Photolysis Rates and Minimum Half-Lives

The maximum photolysis rate constants and minimum half-lives at the flat water body surface under clear sky conditions in summer and winter months can be calculated using the equations:

$$k_{pE} = \phi^{c}_{E} \cdot \Sigma \varepsilon^{c}\lambda L\lambda \quad \text{and}$$

$$t_{1/2} = 0.693/k_{pE}$$

In order to calculate $\varepsilon^{c}\lambda L\lambda$ for summer and winter, $L\lambda$ values can be obtained from Table 5 EPA (1985)[12] and $\varepsilon^{c}\lambda$ values are the same that were used to calcuate ϕ^{c}_{E}.

Photolytic Half-Lives (Days) of Albendazole and its Major Metabolites

	Measured[a]				Calculated[b]		
Albendazole							
pH	5	7	9		5	7	9
Day[c]	0.537	0.937	0.729	Summer	0.106	0.192	0.052
Day & night[d]	0.525	0.516	0.704	Winter	0.382	0.989	0.155
Albendazole Sulfoxide							
Day[c]	0.573	0.852	0.197	Summer	0.143	0.150	0.076
Day & night[d]	0.525	0.902	0.096	Winter	0.832	0.438	0.450
Albendazole Sulfone							
Day[c]	1.52	0.608	0.103	Summer	0.444	0.190	0.048
Day & night[d]	1.31	0.516	0.050	Winter	1.30	0.553	0.191
Albendazole 2-Aminosulfone							
Day[c]	4.54	1.55	1.29	Summer	0.547	0.190	0.268
Day & night[d]	4.12	1.60	1.44	Winter	1.62	0.554	1.65

a The half-life calculated after correcting the measured rate (k_p) for the rate at flat water body (k_{pE}) in the environment by using the equation $k_{pE} = k_p/2.2$.

b Half-life calculated based on the quantum efficiency of light received during the experiment

c Based on an average day length of 11.74 hrs. (between sunrise to sunset) during the time of experiment for albendazole and its metabolites (September 26 and October 8).

d Calculated using k_p values from the statistics report of studies A-3032-88, A-3033-88, A-3034-88 and A-3035-88. The half-life values are based on a day length of 24 hours.

Conclusions

Based on the half-lives calculated using quantum efficiencies of light, it was clearly demonstrated that albendazole and its three major metabolites undergo rapid degradation to 50% of their starting concentrations within a day in mid-summer and less than two days in mid-winter. The experimental (measured) half-lives are slightly longer which reflects upon the season (beginning of fall) and also on poor weather conditions (especially albendazole and 2-aminosulfone) under which the studies were conducted. An environmental hazard assessment is conservatively estimated using the measured rate of degradation of the test chemicals based on total hours (after correcting for flat water body).

There was some loss of test chemical in dark controls at some sampling periods, however, the loss did not seem to be time-dependent and therefore, hydrolysis was not considered to be a significant variable.

APPENDIX XII

Calculation of the Estimated Concentrations of Albendazole and Its Metabolites Adjusting for Soil Adsorption/Desorption

Adsorption:

Using the total cattle excretion data from Table 2 of the environmental assessment for albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites, runoff calculations are adjusted to a total mg basis as follows:

3080 mg Abz equivalents/animal X $\frac{0.8}{100}$ Abz (% in cattle excreta) = 24.64 mg

3080 mg Abz equivalents/animal X $\frac{8.6}{100}$ Abz-SO_2 (% in cattle excreta) = 264.88 mg

3080 mg Abz equivalents/animal X $\frac{24.6}{100}$ Abz-SO (% in cattle excreta = 757.68 mg

3080 mg Abz equivalents/animal X $\frac{23}{100}$ Abz-$2NH_2SO_2$ (% in cattle excreta) = 708.40 mg

Desorption:

Using estimated concentrations from Table 3 of the environmental assessment for albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites, the total mg expected in agricultural soil are calculated as follows:

0.4 ppb Abz x 4162.6 Kg soil/1000 µg/mg = 1.665 mg Abz
13 ppb Abz-SO x 4162.6 Kg soil/1000 µg/mg = 54.11 mg Abz-SO
4.6 ppb Abz-SO_2 x 4162.6 Kg soil/1000 µg/mg = 19.15 mg Abz-SO_2
12.2 ppb Abz-$2NH_2SO_2$ x 4162.6 Kg soil/1000 µg/mg = 50.78 mg Abz-$2NH_2SO_2$

Parameters:

Runoff:
200 sq ft per animal — 308 Kg animal
10 mg/Kg dose per animal — 3080 mg dosed per animal
2 inches of rainfall on 200 ft^2

$$\frac{200\ ft^2}{animal} \times 2\ inch\ rainfall \times \frac{1\ ft}{12\ in} \times \frac{28.317\ L}{ft^3} = 943.9\ Liters\ Water$$

Agricultural Soil:
Top 6 inches of soil available
200 sq ft x 0.5 ft = 100 ft^3
100 ft^3 x 28.317 L/ft^3 = 2831.7 Liters
approximately a mean surface density of soil as 1.47 g/cc[11]
2831.7 Liters soil x 1.47 Kg/Liter soil = 4162.60 Kg soil

Calculations:

The following calculations estimate the maximum runoff (adsorption) and agricultural soil (desorption) concentrations for albendazole. All K_d values are from Appendix VII and text page 24.

For Runoff

X = concentration of test substance in water or soil.

Albendazole: Total Abz available is 24.64 mg
4162.6 Kg soil x 109.6 X = mass of Abz in soil
943.9 L water x 1 X = mass of Abz in water
(4162.6 kg x 109.6 X) + (943.9 L x 1 X) = 457164.86 X

457164.86 X = 24.64 mg (total Abz available)
X = 0.000054 mg/L = 0.054 µg/L
concentration of Abz in water = 0.054 µg/L
and 0.054 µg/L x 109.6 = concentration of Abz in soil
= 5.92 µg/Kg

For Agricultural Soil

X = concentration of test substance in water or soil.

Albendazole: Total Abz available is 1.665 mg
4162.6 Kg soil x 912 X = mass of Abz in soil
943.9 L water x 1 X = mass of Abz in water
(4162.6 Kg x 912 X) + (943.9 L x 1 X) = 3797235.1 X

3797235.1 X = 1.665 mg (total Abz available)
X = 0.0000004 mg/L = 0.0004 µg/L
concentration of Abz in water = 0.0004 µg/L
and 0.0004 µg/L x 912 = concentration of Abz in soil
= 0.3648 µg/Kg

APPENDIX XIII

Evaluation of the Potential Effect of Albendazole Residues Contained in Cattle Manure to Earthworms

INTRODUCTION

The purpose of this project (conducted by Raltech Scientific Services, Inc.) was to determine whether manure from cattle dosed with albendazole four times at 15 mg/Kg effected on the general condition of earthworms and their reproductive activity.

METHODS AND MATERIALS

CATTLE MANURE

Manure obtained from heifers treated with albendazole four times at dose levels of 15 mg/Kg and collected within a three week period was mixed into soil at application rates of 15 and 30 tons per acre.

Soil Source and Analysis:

Soil for the project was obtained from lot 84, Fahey Heights subdivision, County MN, south of Oregon, Wisconsin. A representative sample of the soil was sent to the State Soil Lab for analysis and type determination. The soil was a sandy loam (55% sand, 35% silt and 10% clay) with a pH of 6.4.

The manure for each treatment was mixed in a V-shell blendor for 5 minutes, then manually mixed and divided equally into five replicate glass jars. The experimental design summarizing the treatments, each consisting of five replicates, was as follows:

- Medicated manure applied at 15 tons per acre
- Control manure applied at 15 tons per acre
- Medicated manure applied at 30 tons per acre
- Control manure applied at 30 tons per acre
- Soil plus CSMA media
- Soil only

One hundred milliliters of tap water was added to each jar. Fifty redworms were added to each jar and the soil was covered with a damp cheese cloth.

MAINTENANCE

The test containers were held at 67°F. Moisture levels were maintained by keeping the surface and cheese cloth damp as required. After 14 days, the containers were emptied and the number of worms was recorded. After returning the worms to their respective containers, 5 g of CSMA fly larval medium was added to the surface of each jar as a source of food. At weekly intervals, thereafter, 5 g of CSMA medium and 20 mL water were added to each jar. After 60 days, the experiment was terminated. Jars were emptied and the number of adults, eggs and young worms were recorded.

RESULTS

There were no significant differences in the number of adult worms, the average number of eggs or young worms between the control and medicated manure treatments at 15 or 30 tons per acre.

CONCLUSIONS

There were no treatment-related effects between medicated and control treatment groups which could be attributable to manure from albendazole treated cattle. Consequently, albendazole and its metabolites have been shown to be free of any environmental effects on adult earthworms, eggs and young worms at the concentrations tested.

APPENDIX XIV

Evaluation of the Potential Adverse Effect of Albendazole Residues Contained in Cattle Manure to Housefly Eggs and Larvae

INTRODUCTION

The purpose of this project (conducted by Raltech Scientific Services, Inc.) was to determine whether manure from cattle drenched with albendazole four times at 15 mg/Kg had any effect on the development of housefly eggs and larvae.

METHODS AND MATERIALS

CATTLE MANURE

Manure obtained from heifers treated with albendazole four times at dose levels of 15 mg/Kg was collected within a three week period and incorporated into soil at application rates of 15 and 30 tons per acre.

LARVAL MEDIA

All media were prepared the day prior to housefly egg collections.

CSMA

This is a standard media used as a reference comparison. A 2500 gram quantity of CSMA Standard Fly Larval Medium was incorporated into manure and then mixed into a suspension of 80 mL of nondiastatic dimalt and 45 g of active dry yeast in 8 liters of deionized water. The medium was mixed thoroughly and equal quantities were transferred to five glass battery jars (15-1/2 cm diameter by 21 cm depth) per treatment and covered with cloth. The experimental design for the study, employing five replicates per treatment, is summarized as follows:

- medicated manure applied at 15 tons per acre
- control manure applied at 15 tons per acre
- medicated manure applied at 30 tons per acre
- control manure applied at 30 tons per acre
- CSMA media with no manure

EGGS (F_o GENERATION)

The morning following media preparation, eggs were collected from the food dishes of mature F58W strain houseflies from an in-house breeding colony. Two hundred viable eggs were counted onto lined filter paper for each replicate. The larval media prepared the previous day was thoroughly mixed and the eggs were washed with water into a 1-cm wide by 2.54-cm deep trench in the center of the media. The eggs were then covered with the media and jar openings were covered with cloths.

PUPAE AND ADULTS

Since mature larvae migrate to the surface to pupate, a two inch layer of vermiculite was placed in each jar of medium three days after seeding the eggs to facilitate the removal of the pupae. After six days the mixture of vermiculite and pupae was screened to recover the pupae. The remaining media was also examined for pupae and the total number of pupae for each replicate were counted, recorded and placed in a screened cage, 24 X 24 cm, fitted with a sleeve opening. The adult emergence was also counted and recorded for the five replicates of each treatment.

F_1 GENERATION

The adult houseflies from each of the five replicates per treatment were combined in order to obtain enough eggs for replicate media jars for the F_1 generation. The flies were placed in 30 X 30 cm screened cages, each fitted with a sleeve opening, and provided with a diet of non-fat liquid milk. After mating had occurred, the F_1 eggs were collected as described for the F_0 generation and seeded into standard CSMA media containing no manure. Five replicate media jars, each containing 200 eggs were prepared for each of the treatments from the F_0 generation. The same procedures were used to obtain F_1 pupae and adults as described for the F_0 generation.

RESULTS

Manure from heifers drenched with albendazole at 15 mg/kg of body weight four times within a 3-week period and applied to media at a rate of 15 tons/acre produced fewer F_0 housefly pupae and adults than manure applied at the same rate from untreated heifers. However, in the F_1 generation of houseflies, there was no difference in the number of pupae produced between treated and untreated manure media; further, there was a greater number of F_1 adults produced from the generation cultured in the treated manure than from the untreated manure. These results do not show a consistent treatment-related effect from the F_0 to the F_1 generation.

Treated manure applied at 30 tons/acre as compared to untreated manure applied at the same level did not affect the numbers of F_0 housefly pupae and adults, or the numbers of F_1 pupae and adults.

CONCLUSION

There were no effects on the F_0 and F_1 generations which could be attributed to manure from albendazole treated cattle. Consequently, albendazole and its metabolites have been shown to be free of any environmental effects on housefly eggs and larvae at the concentrations tested.

APPENDIX XV

Determination of the Effects of Albendazole and its Major Metabolites on Soil Microflora and Enzymatic Functions

INTRODUCTION

Studies were performed to assess the possible impact of albendazole and metabolite residues on microbial growth and enzymatic functions.

METHODS

Antibacterial and Antifungal Activity:
The effect of albendazole and its sulfoxide and 2-aminosulfone metabolites on the growth of bacterial fungal species was determined by the inhibition zone method. Test platings were prepared by spreading a small amount of inoculum from an actively growing culture over the surface of the medium. Filter paper discs containing 30, 10, 3, 1, 0.3 or 0.1 µg of the test compounds were placed on the agar surface along with control discs. Algal species were grown in liquid culture containing 50, 10, 1 or 0 ppm of the test compounds. Growth inhibition in algal species was determined statistically (Dunnett's procedure) by comparing the cell populations of the test groups to those in the control group.

Soil Enzymatic Function:

The effect of albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites on the degradation of cellulose, protein and starch in soils, upon nitrification and upon aerobic nitrogen fixation by Azotobacter vinlandii were evaluated. Degradation was followed by measuring $^{14}CO_2$-evolved from ^{14}C-substrates; nitrification was determined by measurement of nitrate formed from the ammonium ion; nitrogen fixation was evaluated by acetylene reduction to ethylene. Degradation and nitrification were followed in soils both immediately after incorporation of the test mixture at 10 and 50 ppm and after aerobic aging of such soils for 30 days.

RESULTS

Antibacterial and Antifungal Activity:
The minimum inhibitory concentrations for albendazole, sulfoxide and 2-aminosulfone to soil microflora are summarized as follows:

	Albendazole (µg/disc)	Sulfoxide Metabolite (µg/disc)	2-Aminosulfone Metabolite (µg/disc)
Bacterial Species			
Aerobacter levanicum	NI	NI	NI
Arthrobacter globiformis	NI	NI	NI
Bacillus subtilis	NI	NI	NI
Pseudomonas fluorescens	NI	NI	NI
Streptomyces albus	NI	NI	NI
	(µg/disc)	(mg/disc)	(µg/disc)
Fungal Species			
Aspergillis niger	0.1	10	10
Chaetomium globosum	1	10	NI
Penicillium chrysogenum	0.1	3	3
Trichoderma viridi	NI	NI	NI
Algal Species			
Microcystis aeruginosa	NI	50	50
Selenastrum capricornutum	NI	NI	NI

NI - No inhibition at any dose tested as listed under methods.

Soil Enzymatic Function:

Results from studying starch, cellulose and protein degradation as well as nitrogen fixation and nitrification were variable. No clear patterns emerged different from those expected from the minimum inhibitory concentration data above.

APPENDIX XVI

Evaluation of the Effect of Albendazole Soil Residues On Rotational (Secondary) Crop Uptake

INTRODUCTION

The objective of this study was to assess the secondary bioaccumulation of ^{14}C-albendazole equivalents in three representative food crops; spinach, beets and wheat.

METHODS AND MATERIALS

Crops were grown in flats containing soil fertilized at a rate of 24 tons per acre with urine and feces from a steer dosed with ^{14}C-albendazole at 15 mg/Kg (equivalent to 2.62 ppm albendazole equivalents).

Each of the three crops (nine flats per crop) were grown separately. Three flats were harvested as immature plants and five were harvested as mature plants. Two control flats containing soil with untreated manure were seeded separately with each crop.

Single 5/8" diameter soil cores were removed monthly from each treated flat to monitor the radioactivity level. (Initial measurements involved removal of 5 soil cores from various locations in each treated flat).

Harvest:

Wheat - Immature plants were cut ~ 1 inch above the soil, 21 days after planting. Mature plants were cut ~ 4 inches above the soil, 114 days after planting.

Spinach - Immature spinach (41 days after planting) was cut leaving ~ 1 to 2 inches of stem with the leaf. Mature plants were cut 64 days after planting, leaving ~ 1 to 2 inches of stem with the leaves.

Beets - Immature leaves were removed 48 days after planting leaving 1 to 2 inches of stem on the leaf. Mature plants were harvested 79 days after planting. The leaves plus stems were cut 1/2 to 1 inch above the soil and removed before the beet root was dug out.

RESULTS

Mature crop results are summarized below:

Mature plant	Albendazole Equivalents Mean ppm	Std. Deviation	Mean 95% Confidence Int.
Spinach	0.130	0.0122	0.121 - 0.139
Beet foliage	0.103	0.0230	0.087 - 0.119
Beet root	0.013	0.0018	0.012 - 0.014
Wheat straw	0.751	0.1142	0.669 - 0.833
Wheat seed	0.019	0.0039	0.016 - 0.022

Calculation of albendazole equivalents normalized to a dry weight basis assuming 20% and 90% water in straw and beans, respectively, results in concentrations of the same magnitude for each: Spinach = 1.3 ppm; Beet greens = 1.0 ppm; Wheat straw = 0.9 ppm

Immature crop results are summarized as follows:

Immature Plant	Albendazole Equivalents Mean (ppm)	Std. Deviation
Spinach	0.113	0.0044
Beet	0.085	0.0050
Wheat	0.151	0.0148

The yield (biomass) from plants grown in soil containing ^{14}C-albendazole and its ^{14}C-labeled bovine excretion products are summarized as follows:

	Wheat (weight per flat-g)	Beet[1] Ave. weight per plant-g)	Spinach[1] Ave. weight per plant-g)
Control	194	53.3	26.5
	171	31.1	23.6
Average	182	42.2	25.0
Treatment	223	68.2	19.9
	216	63.3	17.5
	248	41.6	18.2
	209	76.6	22.2
	242	74.1	27.3
Average	228	64.8	21.0

[1]Yields were expressed on a weight per plant basis because the number of plants per flat varied.

CONCLUSIONS

There was a slight uptake of ^{14}-carbon from albendazole and its metabolite degradation products. The degree of uptake in all plants was considered minor. No significant differences in biomass were noted between control and treated plants.

APPENDIX XVII

GREENHOUSE PHYTOTOXICITY EVALUATION OF ALBENDAZOLE RESIDUES IN CATTLE MANURE ON SEVEN CROPS

INTRODUCTION

The objective of this project (conducted by Raltech Scientific Services, Inc.) was to determine if manure from cattle treated four times with albendazole at 15 mg/kg and applied to soil at 15 and 30 tons per acre had a phytotoxic effect on the growth of seven selected crops.

METHODS AND MATERIALS

Soil Source and Analysis:

Soil for the project was obtained from lot 84, Fahey Heights subdivision, County MN, south of Oregon, Wisconsin. A representative sample of the soil was sent to the State Soil Lab for analysis and type determination. The soil was a sandy loam (55% sand, 35% silt and 10% clay) with a pH of 6.4.

Treatment and Sample Collection:

Treatment of animals and sample collection were conducted by SmithKline Beckman Animal Health Products personnel. Manure from two heifers drenched four times with albendazole at 15 mg/kg over a three week period was collected and assayed for moisture content.

Soil Preparation and Experimental Design:

The sandy loam soil was passed through a mechanical shredder and put into 2-1/4 square foot flats in a greenhouse. The urine and feces mixtures were spread over the soil in the flats at rates which corresponded to 15 and 30 tons per acre. For each crop, five replicate sets of flats were planted, each set containing five flats:

- medicated manure applied at 15 tons per acre
- control manure applied at 15 tons per acre
- medicated manure applied at 30 tons per acre
- control manure applied at 30 tons per acre
- soil with no manure

Positioning of the flats in the greenhouse varied to minimize light intensity or temperature effects. All flats were watered weekly.

RESULTS

Wheat - No differences were found in plant heights 25 days after planting between treated or control flats at either 15 or 30 tons per acre.

After 40 days, the cut plant weights were greater in the treated flats at both application rates than their respective control flats.

Barley - No differences were found in plant heights 25 days after planting, between treated or control flats at either 15 or 30 tons per acre.

After 40 days, the cut plant weights were greater in the treated flats at both application rates than their respective control flats.

Corn - No differences were found in plant heights 30 days after planting, between treated or control flats at either 15 or 30 tons per acre.

After 48 days, the cut plant weights were not different at either application rate than their respective control flats.

Beans - No differences were found in plant heights 30 days after planting, between treated or control flats at either 15 or 30 tons per acre.

After 46 days, the cut plant weights were not different at either application rate than their respective control flats.

Tomatoes - No differences were found in plant heights 40 days after planting between treated or control flats at 15 tons per acre or between treated or control flats at 30 tons per acre. The higher rate of manure application, 30 tons per acre, produced taller plants than the lower rate of application in both treated and control flats.

Cucumbers - No differences were found in plant heights 48 days after planting, between treated or control flats at either 15 or 30 tons per acre.

The cut plant weights were not different at either application rate than their respective control flats.

Fescue - At the first cutting of the fescue (50 days after planting), average plant weights between treated or control flats were similar at both application rates. The second cutting, one week later, showed no significant differences in plant weights among any of the treatments.

In all cases, the average height and/or weight of plants in untreated flats (no manure) was, as expected, much lower than any of the treated groups.

CONCLUSIONS

Exaggerated levels of albendazole metabolite residues in soil produced no statistically significant differences (Duncan's multiple range test) in crop growth (height or weight) between controls and treated plants at both application levels. Therefore, it is concluded that albendazole and its metabolite residues are not expected to have any phytotoxic effect on these crops.

APPENDIX XVIII

Static Acute Toxicity of Albendazole and Its Major Metabolites to Daphnids

INTRODUCTION

The objective of this study (conducted at Springborn Life Sciences) was to estimate the acute toxicity (EC_{50}) of albendazole and its major sulfoxide, sulfone and 2-aminosulfone metabolites, to daphnids (Daphnia magna) under static conditions.

METHODS AND MATERIALS

The toxicity test was conducted using the methods described in the Environmental Assessment Technical Assistance Document No. 4.08.

Stock solutions of albendazole and its sulfoxide, sulfone and 2-aminosulfone metabolites were prepared at several concentrations and twenty daphnids were impartially distributed to each concentration (five daphnids per replicate) within thirty minutes after the test solutions had been prepared. Daphnids were not fed during the exposure.

The number of immobilized daphnids in each replicate test vessel was recorded at 24 and 48 hours of exposure. Biological observations, physical characteristics, temperature, pH, and dissolved oxygen concentrations were measured and recorded at 0, 24 and 48 hours.

The biological-response to concentration data (immobilization) derived from the toxicity tests were used to estimate 24 and 48 hour median effect concentrations (EC_{50}) and 95% confidence intervals. The EC_{50} is defined as the concentration of the test material in dilution water which caused immobilization of 50% of the test organism population at the stated time interval. EC_{50} values were empirically estimated as being greater than the highest concentration tested when no test concentrations caused 50% or more immobilization.

The no-observed-effect concentration (NOEC), defined as the highest concentration tested at and below which there were no toxicant-related immobilization or physical and behavioral abnormalities, with respect to the control organisms, during the 48 hour exposure period was also determined.

RESULTS

The results of these tests are summarized below:

Chemical	EC_{50}	NOEC
Albendazole	24 µg/L	17 µg/L
Sulfoxide	30 mg/L	11 mg/L
Sulfone	>14 mg/L*	6.1 mg/L
2-Aminosulfone	110 mg/L	53 mg/L

* Estimated, above water solubility of 6.82 to 8.05 ppm.

CONCLUSIONS

Relatively high levels of all metabolites were required for Daphnia toxicity. ABZ sulfone was the least toxic metabolite to Daphnia while Albendazole showed the highest level of toxicity.

APPENDIX XIX

Rainbow Trout and Bluegill Sunfish Toxicity Study with the Major Metabolites of Albendazole

INTRODUCTION

The purpose of this study (conducted at Raltech Scientific Services) was to determine the toxicity of the major sulfoxide, sulfone and 2-aminosulfone metabolites of albendazole, in Rainbow trout and Bluegill sunfish.

METHODS

Static, acute exposures were performed with the cold water Rainbow trout, Salmo gairdneri and warm water Bluegill sunfish, Lepomis macrochirus.

A mixture of sulfoxide, sulfone and 2-aminosulfone (the three major bovine urinary metabolites of albendazole) were combined in a 45:45:10 ratio to simulate the approximate proportions of each found in the urine. Ten Bluegill sunfish were exposed to the metabolites at concentrations of 0, 115, 135, 158, 186, 219, and 257 and to dilution water alone (control). Ten Rainbow trout were exposed to the metabolites at concentrations of 0, 30, 50, 80, 100, 125, 150, 180, 210, 250 and 300 ppm and to control dilution water. The number of test organisms affected or killed was recorded daily and at more frequent intervals during the initial phase of the test.

RESULTS

The preliminary test with Bluegill sunfish indicated that the LC_{50} was less than 300 ppm. All ten fish exposed to this level of mixed metabolites died within 96 hours and the first fish was dead at the 64 hour observation. Fish exposed to concentrations between 219 to 257 ppm exhibited abnormal/erratic behavior after 45 hours. The LC_{50} value for Bluegill sunfish as calculated from the mortality data obtained was 222 ppm with lower and upper 95% confidence limits of 201 and 250 ppm. The LC_{50} value for Rainbow trout was calculated by probit analysis to be 86 ppm with 95% lower and upper confidence limits of 53 and 110 ppm.

CONCLUSIONS

Relatively high levels of all metabolites were required to produce toxicity.

APPENDIX XX

Bioaccumulation Study of Albendazole and its Major Urinary Metabolites in Bluegill Sunfish

INTRODUCTION

The purpose of this study (conducted at Raltech Scientific Services, Inc.) was to assess the extent of bioaccumulation of albendazole and its major urinary metabolites in bluegill sunfish.

METHODS

A mixture of radiolabelled metabolites was prepared from bovine urine following ^{14}C-albendazole administration. This mixture and ^{14}C-albendazole were incorporated into 2000 L of water in a 95:5 ratio such that the resulting nominal concentration of albendazole and its metabolites, expressed as albendazole equivalents, was 100 ppb. Bluegill fish (150) were added and maintained for 44 days at 21°C. Both temperature and carbon-14 content of the water were monitored frequently during the experiment to ensure that the radiochemical activity remained at > 80% of the initial value and that temperature variations > 1°C did not occur. Additional water was added as required to compensate for evaporation. Fish (3 to 4) were removed twice weekly and dissected into edible and non-edible portions. These portions were each weighed and homogenized in 2.0 mL of water and the carbon-14 content was determined by combustion and scintillation counting.

RESULTS

The carbon-14 concentration reached a maximum (for a single fish) within the first week of incubation (90 ppb edible; 1179 ppb non-edible) and then declined markedly until at 22 days the levels reached mean values of 22 ppb for edible tissues and 174 ppb for non-edible tissues. After 44 days, the edible portions had mean levels of 30 ppb and non-edible levels of 116 ppb. At this time it became apparent that no plateau value would be obtained and the fish were transferred to a second tank free of radiolabelled test substance. Levels in both edible and non-edible portions declined to 13 and 29 ppb, respectively. The test was terminated after 76 days.

CONCLUSIONS

The transient nature of the accumulated substances was demonstrated by the decline of carbon-14 levels in tissues following initial accumulation. After 22 days the bioaccumulation ratios were 1.86 for non-edible portions and 0.23 for edible portions. After transfer to an environment free of the test substances, carbon-14 levels were rapidly reduced to < 20 ppb for edible tissue and < 40 ppb for non-edible portions. These results indicate that accumulation of albendazole and its urinary metabolites in bluegill sunfish is a short-lived effect. Due to the binding properties of albendazole and it metabolites to soil components, very little of these residues are expected to run off into streams. The trace amounts which might leach into streams would not bioaccumulate in fish. Therefore, there would be no hazard to humans and other animals which may use fish as a food source.