FIRST GUARD™
(Colistimethate Sodium)
Sterile Powder

1. **Date:** June 1996

2. **Name of Applicant:** ALPHARMA INC.

3. **Address:** One Executive Drive
Fort Lee, NJ 07024

4. **Description of Proposed Action**

4.1 Request Approval

This environmental assessment is submitted to the new animal drug application (NADA) for the sterile powder First Guard™ (Colistimethate Sodium) for the treatment of colibacillosis in day-old chicks to prevent mortality in young chickens infected with *Escherichia coli* (early chick mortality).

The ultimate purpose is to increase the availability of poultry for human consumption. Economic benefits are to reduce the cost of poultry production, thereby reducing the cost of poultry products to the consumer.

4.2 Location and Environment Adjacent to Manufacturing Location

The sterile bulk drug product is produced at ALPHARMA INC.'s subsidiary, DUMEX-ALPHARMA A/S in Copenhagen, Denmark. This plant follows all applicable Danish environmental requirements (Appendix I).

Sterile bulk drug product is shipped tc the Cherry Hill, New Jersey plant of Marsam Pharmaceuticals, Inc., where it is aseptically filled into vials, packaged and labeled. The facility meets all applicable local and state environmental requirements (Appendix II).

4.3 Location Where Product will be Used

The finished, packaged product will be warehoused prior to transportation to the hatchery as the end user. At the hatchery, the drug will be constituted and injected subcutaneously into each chick. The broiler-intensive areas where this product will be used primarily are Delaware, Maryland, North Carolina, South Carolina, Alabama, Mississippi, Georgia, Louisiana, Arkansas, and California. Chicks are removed from the hatchery and taken to facilities where they are grown-out (see Section 7 for excreta handling).

4.4 Locations Where Product will be Disposed

Disposal of product may result during manufacturing activities in the form of discarded off specification lots, from the discarding of returned goods, or from end user disposal of individual units of empty or partly empty finished product vials. Bulk quantities of material for disposal will be generated only at the manufacturing site and will be handled with other compatible waste materials resulting from current operations. The present infrastructure provides for a recovery and/or ultimate disposal mechanism. Returned goods are received at the facility, where they are destroyed.

Individual empty or partly empty end products disposed by producers will be handled along with garbage by the community's solid waste management system. Only minute traces of product would be expected to remain with empty product containers.

5. Identification of Chemical Substances that are the Subject of the Proposed Action

The following summary describes the main properties of the drug:

Colistimethate Sodium (Sterile)

- CAS No. 30 387-39-4
- Empirical formula: $C_{58}H_{105}N_{16}Na_5O_{28}S_5$
- M.W. 1670
- White Fine Powder

Adjusted for potency via sterile, dry-mixing dilution with a sterile sugar.

The bulk drug product is imported as a sterile bulk drug product ready for packaging from DUMEX-ALPHARMA A/S, Copenhagen, Denmark. The MSDS is in Appendix III.

[N.B. Colistimethate sodium has been available as a human medicine product (USP 23, p. 422, 1995) for over 20 years.]

6. Introduction of Substances into the Environment

Colistimethate sodium could be released to the environment in the packaging process for colistimethate sodium at the Marsam facility in Cherry Hill, New Jersey. A statement from Marsam, included as Appendix II, confirms compliance with all local, county, state and federal environmental rules and regulations. All individuals who handle the product will have access to the MSDS (Appendix III).

The yearly estimated market volume is 27,900 vials of First Guard. Each vial will contain 10 grams of active ingredient, which is to be constituted according to label directions. The use of this volume of product will result in minute traces of drug in the empty containers.

Most commercial hatcheries utilize the following procedures: The product is reconstituted and injected into the chick using an automatic injection unit. This unit is cleaned and

reused. The syringes and empty vials are placed in the regular trash generated at the hatchery and are transported to a Class II landfill for disposal. The sharps are placed in a "sharps box", which is sent to an approved incinerator for destruction.

Introduction via the chicken litter is discussed in the next section.

7. Fate of Emitted Substances in the Environment

First Guard (colistimethate sodium) Sterile Powder is intended for use in one day old chicks to control colibacillosis due to *Escherichia coli* suspectable to colistin.

Colibacillosis, an infectious disease, occurs in all types and age groups of poultry where it is responsible for major economic losses to the poultry industry. Egg transmission of pathogenic *E. coli* is common and can result in high mortality of chicks unless effective treatment is given early.

First Guard (colistimethate sodium) Sterile Powder will be given in the hatchery as a subcutaneous injection. Chicks will then immediately be shipped to broiler houses, where they will be kept for approximately 6 weeks (a "grow-out") prior to slaughter. A typical broiler house will have approximately 25,000 birds per grow-out. With a dose of 0.2 mg per bird, the broiler houses would be exposed to 5 g of colistin per grow-out, or approximately 35 g/year (7 grow-outs). On a total U.S. basis, where approximately 6.2 billion broilers are raised annually, total First Guard use is calculated to be 279 kg colistin activity. This assumes 90% of broilers will be dosed with an antibiotic and the projected market share for First Guard is 25%. Thus, less than 300 kg of colistin activity will be used in the U.S. annually.

First Guard will be dosed in one day old chicks at a concentration of 0.2 mg potency/bird. The radiolabeled drug total residue study, performed for the human food safety evaluation, shows that 32.6% of the 0.2 mg First Guard dose has been excreted after 28 days. A total of 36.8% excreted is calculated for a 42 day grow-out [32.6 + (14 days x 0.3% per day) = 36.8%], which is equivalent to 0.073 mg per bird.

The fresh feces was evaluated as part of the total residue study to determine the nature of the ^{14}C-CMS equivalent material excreted. The feces was solubilized in $CHCl_3$:MeOH, or 0.5 M Sulfuric acid, and the fractions tested for microbiological activity. A microbiologically active fraction equivalent to 0.074% of the total dose was isolated in the 0 to 12 hour fecal collection only. No later collections showed any microbiological activity. The 0.074% of the total 2 mg dose is equivalent to 0.148 μg per bird.

The concentration of colistin in dried manure and manure-treated soils was calculated using the following formulas:

Amount of Dried Manure in 42-day Grow-Out:

130 g/day x 42 days = 5.46 kg fresh (75% H_20)

5.46 kg + 1.8 kg litter (1/3 Total) - 4.10 kg H_20 = 3.16 kg Dried Manure

Total Concentration in Dried Manure:

$$\frac{\text{Colistin}}{3.16 \text{ kg DM}} = \text{ppb in Manure}$$

(This is a maximum value; there has been no adjustment for dilution with droppings from untreated birds.)

Total Concentration in Soil: (based on dry manure values)

$$\frac{\text{(Manure Conc.) x kg/Acre}}{\text{Soil/Wt/Acre}}$$

$$\frac{\text{(Colistin) } (4.6 \times 10^3)}{910{,}500} = \text{ppb in Soil}$$

The formulas were used to calculate the colistin concentrations in dry manure and soil for the three cases: total colistin dosed, total colistin excreted, and total microbiologically active colistin, as shown in the table below.

	Colistin Conc. in Dry Manure and Soils		
Environmental Sample	Total Dosed	Total Excreted	Microbiologically Active
Dry Manure	63 ppb	23 ppb	0.047 ppb
Soil	0.4 ppb	0.2 ppb	0.00028 ppb

The total amounts of colistin introduced into the environment annually, based on a projected usage of 300 kg per year and the above data, is 222 g of active colistin per year out of 109 kg total colistin-related material excreted.

The environmental studies, listed in Appendices IV to IX, were all performed with colistin base because colistimethate sodium is completely metabolized to colistin prior to excretion from the chicken. Neat colistin is water soluble (Appendix IV) with a basic pKa of 11.5 (Appendix V). It has a low octanol/water partition coefficient of 10 (Appendix VI), indicating that it is water soluble with very little potential for bioaccummulation. Colistin has no natural light absorption, thereby ruling out any photo degradation (Appendix X).

The major route of introduction of colistin to the environment is via the fecal matter/manure. The adsorption-desorption of colistin base in three soils plus chicken manure (Appendix VII) shows that colistin base has a low mobility from chicken manure. The material that is solubilized from the chicken manure will be washed onto the soil, where it will be immobilized. Colistin base adsorbed onto the soil will not be leached off under any environmental circumstances.

Soil biodegradation studies (Appendix VIII) were done with three soil types, plus chicken manure. The test concentrations used were 10 ppm (10 μg colistin base/g soil), which are 8×10^4 times higher than the calculated 0.12 ppb expected in bird manure treated soil. The mineralization of colistin to CO_2 in the three soils was low at 6.86%, 2.37% and 3.90%. The percentage mineralization in chicken manure was higher at 18.7%. The absolute amount mineralized at 21, 42 and 90 days is listed in the table below.

Colistin Mineralization to CO_2 — 10 ppm Start Concentration

Test Day	21 day		42 day		90 day	
Sample	%	Absolute ppb	%	Absolute ppb	%	Absolute ppb
Soil A	2.55	255	3.71	371	6.86	686
Soil B	0.52	52	1.61	161	2.37	237
Soil C	2.12	212	2.18	218	3.90	390
Chicken Manure	5.83	583	8.57	857	18.7	1870

The actual concentrations in manure and soil are 23 ppb and 0.12 ppb, respectively.

The absolute values in the table above show that the concentrations of total colistin in the manure or manure-treated soils would be mineralized within 21 days.

The binding of colistin to soils and its mineralization in manure or soils clearly shows that all of the colistin will be inactivated within the bird raising site.

8. Environmental Effects of Released Substances

The mammalian toxicity of CMS USP is very low. In the dog, treated orally for 13 weeks, the no-observed-effect level is 1.6% in the diet, or 457 mg/kg body weight/day. The rat has a NOEL of 0.5% CMS USP in the diet, or 387 mg/kg body weight/day, for both the 13-week toxicity study and the 2 generation reproduction/teratology study in rats.

The NOEL of 0.5% CMS USP (387 mg/kg/day) obtained in the 13-week oral toxicity study in the rat, corresponds to 297 mg/kg/day of colistimethate sodium. Using the 297 mg/kg/day value as the NOEL, along with a safety factor of 1000, the safe concentration is:

$$SC = \frac{297 \times 60}{1000 \times 50} = 35.64 \text{ mg/kg/day} = 36 \text{ ppm}$$

The safe concentration in muscle is administratively limited to 5.0 ppm of colistimethate sodium (as the molecular entity with a molecular weight of 1620) because no chronic toxicity testing has been performed with the product. The safe concentrations of total colistin for human consumption is 15 ppm for liver and 30 ppm for skin/fat.

The concentration of total colistin-related entities excreted after 42 days is 23 ppb in the dried manure. This value is 2.6×10^6 times lower than the NOEL in the rat, and establishes the safety margin to mammals eating the dried manure.

The subcutaneous toxicity of First Guard (colistimethate sodium) Sterile Powder to day-old chicks has been determined using a 10 times dose, followed by a 7 day observation period. No deleterious effects were seen after 7 days.

The MIC values for the organisms used in the Microbial Growth Inhibition test (Appendix IX) are listed in the table below:

TABLE I

List of Test Organisms Used for Microbial Growth Inhibition Test of Colistin Base

Genus & Species[a]	Representative Type	ATCC Number	MIC mg/Liter
Pseudomonas fluorescens	Bacterium	12842	61.52
Bacillus megaterium	Bacterium	6459	6.15
Azotobacter chroococcum	Nitrogen-Fixing Bacterium	4412	4.28
Anabaena flos-aquae	Nitrogen Fixing Blue-Green Alga	22664	>61.52
Aspergillus clavatus	Fungus	9192	>61.52
Penicillium canescens	Fungus	10419	>61.52
Chaetomium globosum	Fungus	44699	>61.52

[a] Obtained from ATCC (American Type Culture Collection, Rockville, MD)

The most sensitive bacterium MIC is 4.28 mg/liter (4 ppm), and it is 200 times higher than the 23 ppb concentration of total colistin-related entities in the dried manure.

The amount of microbiologically active colistin has been determined as a maximum of 0.047 ppb in the dried manure and 0.28 ppt in the soil after a manure treatment. All of the colistin concentration values in dried manure or manure-treated soil are a minimum of 200 times lower than any effect level in any of the tested species.

9. Use of Resources and Energy

Natural Resources

No change in the use and accessibility of natural resources will result from the proposed action.

Energy

There is no direct impact on the energy supply or utilization that stems from the use of the product.

Other

There are no expected effects upon endangered or threatened species, nor upon properties listed in or eligible for listing in the National Register of Historic Places.

10. Mitigation Measures

Material Safety Data Sheets (Appendix III) provide information on potential hazards, personal protective equipment, safe handling practices and emergency procedures.

The only potential adverse consequence would result from contact with the skin or mucous membranes. In order to avoid these adverse effects, the labels bear the warning:

> **WARNING: Not for human use. Keep out of reach of children. Avoid contact with skin. Direct contact with skin or mucous membranes may cause irritation.**

11. Alternatives to the Proposed Action

No alternatives to the proposed action have been identified.

12. List of Preparers

Paul F. Duquette	Director of Product Development M.S. Animal Science 23 years experience in animal science research
Sondra C. Flick	Director of Regulatory Affairs B.S. Animal Science, M.B.A. 27 years experience in veterinary product development and animal drug regulatory affairs
Alex MacDonald	Pharma Science Inc. Ph.D. Chemistry 29 years experience in veterinary product development

13. Certification

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

Larry A. Muir

Larry A. Muir Ph.D.
Vice President, Science & Technology

July 3, 1996
Date

Environmental Assessment
Appendices

I. DUMEX Statement of Compliance

II. Marsam Statement of Compliance

III. Material Safety Data Sheet (MSDS)

IV. Water Solubility

V. Dissociation Constant

VI. Octanol-Water Partition Coefficient

VII. Adsorption-Desorption

VIII. Biodegradation in Soils

IX. Microbial Growth

X. UV-Vis Spectra (Huntingdon)

The documents (IV through X in the public file) will be limited to the abstract. The whole report will be held confidential.

ABBREVIATED ENVIRONMENTAL ASSESSMENT FORMAT
(Alternate Manufacturing Site for Animal Drugs)

1. Date: **11 October 1993.**

2. Name of applicant or petitioner: **A/S DUMEX (Dumex Ltd).**

3. Address: **Prags Boulevard 37, DK-2300 Copenhagen S, Denmark.**

4. Description of the proposed action:
Manufacture of a sterile bulk product (Colistimethate sodium USP standardised with mannitol, AADA 61-316) for A.L. Laboratories, Inc.

5. Identification of the chemical substances that are the subject of the proposed action:
Colistimethate sodium USP, sterile bulk.

6. Introduction of substances into the environment for the site(s) of production:

   a. List the substances expected to be emitted;

   b. Describe the control used to limit or eliminate emissions;

   c. Cite the applicable Federal, state and local emission regulations and laws (including occupational) for the manufacturing site.

   d. Provide a statement certifying compliance with the cited regulations;

   e. Discuss the effects the approval will have upon compliance with current emission requirements and provide an estimate of the maximum yearly market volume of the drug product.

Alternative to a-e for manufacturing that occurs in a foreign country:
Provide a letter (from the appropriate foreign government office responsible for administering environmental laws and regulations) certifying that the plant is in compliance with the applicable environmental and occupational requirements of the foreign country.

7-11. Documentation for items 7-11 of the EA format in 21 CFR 25.31a, concerning the fate, effects, resources and energy use, mitigation and alternatives, need not be provided.

12. List of preparers:
Dorthe Jensen, M.Sc. (Pharmacy), R.Ph., Manager, Regulatory Compliance.
Alex Juel Nielsen, M.Sc. (Chemical Engineer), Vice President, Production and Engineering.

13. Certification: Include a statement signed by the responsible official of the applicant's firm certifying that the information presented is true, accurate, and complete to the best knowledge of the firm.

(Date): **11 October 1993**

(Signature of responsible official): **Alex Juel Nielsen** Alex J. Niels

(Title of responsible official): **Vice President, Production and Engineering**

14. References:

Miljøkontrollen
Københavns kommune

Certified to be an Exact Copy 000026
By Dorthe Jensen Date 9 November 93

A/S Dumex
Dalslandsgade 11
2300 København S

Date: 30 SEP. 1993

Our ref.:
930877-4
S 881029
CSØ/ven

Statement concerning the relations between

A/S Dumex and existing environmental legislation.

Referring to your letter of September 30th 1993 The Agency of Environmental Protection, Copenhagen, hereby confirm that A/S Dumex, Dalslandsgade 11, DK-2300 København S, operates in accordance with given environmental permissions and existing environmental laws.

Yours sincerely

The Agency of Environmental Protection
City of Copenhagen

Jørgen Høg
Head of office

Carsten Sølyst
Case officer

Miljøkontrollen
Flæsketorvet 68
1711 København V.

Telefon 33 66 58 00
Telefax 31 31 66 21
Giro nr. 3 96 82 00

Ekspeditionstid:
Mandag til torsdag kl. 8.15 - 15.45
Fredag kl. 8.15 - 15.15

By Dorthe Jensen Date 9 November 1993

000027

Arbejdstilsynet
Kreds
København og Frederiksberg
Svanevej 12
2400 København NV

Telefon 31 81 55 55
Telefax 35 82 35 15

Dumex
Dalslandsgade 11
2300 København S
Att.: Alex J. Nielsen

Dato 5. oktober 1993 | Deres ref. | Deres jour.nr.
Vores ref CH/LS | Vores jour.nr. 101-35220/004 99-01/93

Concerning occupational environment co-operation with medical factory Dumex

As local labour inspection attempt to confirm our longterm and positiv co-operation with fine results about occupational environment in all sections.

Christopher Harajchi
Labour Inspector
(chem.ing.)

MARSAM
PHARMACEUTICALS INC.

PRODUCT: FIRST GUARD™ (colistimethate sodium) Sterile Powder

SITE OF PRODUCTION: Marsam Pharmaceuticals Inc.
Building 31, Olney Avenue
Cherry Hill, New Jersey 08034

SUBSTANCES EXPECTED TO BE EMITTED: Colistimethate Sodium
Mannitol

Marsam Pharmaceuticals Inc. receives bulk Sterile FIRST GUARD and subdivides it into sterile containers which are stoppered in the filling area. The total emission resulting from this process is not significant and is roughly comparable to that from the production of the FDA approved human drug product, Coly-Mycin M Parenteral (Parke-Davis).

Based on the most recent industrial Pretreatment Inspection Report, issued by the Camden County Municipal Utilities Authority, dated October 4, 1990, Marsam Pharmaceuticals Inc. is in compliance with all applicable environmental rules and regulations.

The approval of the proposed action will have no adverse effect on compliance with current emission requirements at this production site.

Marsam Pharmaceuticals Inc. is in compliance with all applicable federal, state and local rules and regulations regarding the environment.

Marsam Pharmaceuticals Inc. has available to its employees Material Safety Data Sheets (MSDS) on the raw materials and finished products, and trains all employees in the location and understanding of the MSDS's.

MAXIMUM YEARLY MARKET VOLUME: 27,900 vials

Davis R. Reese
Executive Director, Scientific and
Regulatory Affairs

61096at

Building 31, Olney Ave. P.O. Box 1022 Cherry Hill, New Jersey 08034, (609) 424-5600
Telex: 5106012909 Marsam Pharma UQ
Facsimile: 609-751-8784

AL-452
Common Name

MATERIAL SAFETY DATA SHEET
A. L. LABORATORIES, INC.

Address:
400 State Street
Chicago Heights, IL 60411

Emergency and Information
Telephone Calls:
(708) 758-0111

A. Hirsch
Responsible Party

1-29-92
date prepared

SECTION 1 - IDENTITY

COMMON NAME	AL-452
SYNONYMS	n/a
CAS NUMBER	8068-28-8; 1264-72-8; 1066-17-7
RTECS NUMBER	GH1650000; TR1500000; GH1575000
CHEMICAL FAMILY	Polymyxin
THERAPEUTIC CATEGORY	Antibacterial
FORMULA	$C_{58}H_{105}N_{16}NA_5O_{28}S_5$ (A) $C_{58}H_{103}N_{16}NA_5O_{28}S_5$ (B)

SECTION 2 - HAZARDOUS INGREDIENTS

	NAME	PERCENT	THRESHOLD LIMIT VALUE (UNITS)
PRINCIPAL HAZARDOUS COMPONENT(S)/[Chemical & Common Name(s)]	AL-452	33-45% Active	Not Established

SECTION 3 - PHYSICAL AND CHEMICAL CHARACTERISTICS
(Fire & Explosion Data)

BOILING POINT	n/a
SPECIFIC GRAVITY (H_2O = 1)	n/a
VAPOR PRESSURE (mm Hg)	n/a
PERCENT VOLATILE BY VOLUME (%)	n/a
VAPOR DENSITY (AIR = 1)	n/a
EVAPORATION RATE	n/a
SOLUBILITY IN WATER	Freely soluble
REACTIVITY IN WATER	n/a
APPEARANCE AND ODOR	A white to cream-colored fine powder; odorless
FLASH POINT	n/a
FLAMMABLE LIMITS IN AIR % BY VOLUME	LOWER n/a UPPER n/a
EXTINGUISHER MEDIA	Water spray, dry chemical, carbon dioxide or foam as appropriate for surrounding fire and materials.
AUTO-IGNITION TEMPERATURE	n/a
SPECIAL FIRE FIGHTING PROCEDURES	As with all fires, evacuate personnel to safe area. Firefighters should use self-contained breathing equipment and protective clothing.

n/a = not applicable

UNUSUAL FIRE AND EXPLOSION HAZARDS

This material is assumed to be combustible. As with all dry powders it is advisable to ground mechanical equipment in contact with dry material to dissipate the potential buildup of static electricity. When heated to decomposition material emits toxic fumes. Emits toxic fumes under fire conditions.

SECTION 4 - PHYSICAL HAZARDS

STABILITY

( ) Unstable (X) Stable

CONDITIONS TO AVOID

Material is stable from a safety point of view.

INCOMPATIBILITY (MATERIALS TO AVOID)

Carbenicillin sodium, cephaloridine, cephalothin sodium, cephazolin sodium, and erythromycin lactobionate.

HAZARDOUS DECOMPOSITION PRODUCTS

When heated to decomposition material emits toxic fumes of NO_x, SO_x, and Na_2O. Emits toxic fumes under fire conditions.

HAZARDOUS POLYMERIZATION

( ) May Occur (X) Will Not Occur

SECTION 5 - HEALTH HAZARDS

THRESHOLD LIMIT VALUE

None established

SIGNS AND SYMPTOMS OF OVEREXPOSURE

[AL-452 CAS RN: 8068-28-8
LDLO: 90 mg/Kg/18H-I intramuscular-child;
TDLo: 56700 µ/Kg/9D-I intramuscular-human;
LD_{50}: 5450 mg/Kg oral-rat;
LD_{50}: 86 mg/Kg intraperitoneal-rat;
LD_{50}: 87 mg/Kg subcutaneous-rat;
LD_{50}: 44 mg/Kg intramuscular-rat;
LD_{50}: 126 mg/Kg intraperitoneal-mouse;
LD_{50}: 138 mg/Kg subcutaneous-mouse;
LD_{50}: 222 mg/Kg intravenous-mouse;
LD_{50}: 748 mg/Kg intramuscular-mouse;
LD_{50}: 465 mg/Kg intramuscular-rabbit;
LD_{50}: 444 mg/Kg intramuscular-guinea pig;
Mutation Data [RTECS]]
[CAS RN: 1066-17-7
LD_{50}: 236 mg/Kg intraperitoneal-mouse;
LD_{50}: 115 mg/Kg subcutaneous-mouse;
LD_{50}: 8800 µ/Kg intravenous-mouse;
Mutation Data [RTECS]]
[CAS RN: 1264-72-8
LD_{50}: 72200 µ/Kg subcutaneous-rat;
LD_{50}: 793 mg/Kg oral-mouse;
LD_{50}: 21800 µ/Kg intraperitoneal-mouse;
LD_{50}: 53500 µ/Kg subcutaneous-mouse;
LD_{50}: 5978 µ/Kg intravenous-mouse]

n/a = not applicable

AL-452
Common Name

The usual adult dose is 2-4 mg/Kg daily in 4 divided doses (usually intramuscular or intravenous). Possible allergic reaction to dust if inhaled, ingested or in contact with skin. Adverse effects include flushing, dizziness, or drowsiness.

ACUTE: Eye, skin and/or respiratory tract irritation
CHRONIC: Possible hypersensitization

PRECAUTIONS TO CONSIDER: Persons developing hypersensitivity (anaphylactic) reactions must receive immediate medical attention. Material may be irritating to mucous membranes and respiratory tract. As a general rule, when handling USP Reference Standards avoid all contact and inhalation of dust, fumes, mists, and/or vapors associated with the material. Keep container tightly closed and use with adequate ventilation; wash thoroughly after handling. Individuals working with chemicals should consider all chemicals to be potentially hazardous even if their individual hazards may be uncharacterized or unknown. Patients intolerant to one polymyxin may be intolerant to AL-452.

MEDICAL CONDITIONS AGGRAVATED BY EXPOSURE: Hypersensitivity to material

CHEMICAL LISTED AS CARCINOGEN OR POTENTIAL CARCINOGEN:

NATIONAL TOXICOLOGY PROGRAM	( ) Yes	(X) No
I.A.R.C. Monographs	( ) Yes	(X) No
OSHA	( ) Yes	(X) No
OTHER	n/a	

ACGIH TLV: n/a

OTHER EXPOSURE LIMIT(S) USED: n/a

OSHA PERMISSIBLE EXPOSURE LIMIT: Not established

OTHER EXPOSURE LIMIT USED: Not established

EMERGENCY AND FIRST AID PROCEDURES: Remove from exposure. Remove contaminated clothing. Persons developing serious hypersensitivity reactions must receive immediate medical attention. Upon eye or skin contact, flush affected area with copious quantities of water. Obtain medical attention. If not breathing give artificial respiration. If breathing is difficult give oxygen.

1. INHALATION: May cause irritation of respiratory tract. Avoid inhalation. Remove to fresh air.
2. EYES: May cause irritation. Flush with copious quantities of water.
3. SKIN: May cause irritation. Flush with copious quantities of water.
4. INGESTION: May cause irritation. Flush with copious quantities of water.

n/a = not applicable

SECTION 6 - SPECIAL PROTECTION INFORMATION

RESPIRATORY PROTECTION (SPECIFY TYPE)	NIOSH approved respirator
VENTILATION	Adequate
LOCAL EXHAUST	Recommended
MECHANICAL (GENERAL)	Recommended
OTHER	n/a
PROTECTIVE GLOVES	Rubber
EYE PROTECTION	Safety goggles
OTHER PROTECTIVE CLOTHING OR EQUIPMENT	Appropriate laboratory apparel, protect exposed skin

SECTION 7 - SPECIAL PRECAUTIONS AND SPILL/LEAK PROCEDURES

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORAGE	Store in tight container as defined in the United States Pharmacopeia. This material should be handled and stored per label and other instructions to ensure product integrity.
OTHER PRECAUTIONS	Avoid contact with eyes, skin or clothing. Avoid breathing dust or mist. Use with adequate dust control. Wash thoroughly after handling. Wear fresh clothing daily. Wash contaminated clothing before reuse. Do not permit eating, drinking or smoking near material.
STEPS TO BE TAKEN IN CASE MATERIAL IS SPILLED OR RELEASED	Wear approved respirator and chemically compatible gloves. Vacuum or sweep up spillage. Avoid dust. Place spillage in appropriate container for waste disposal. Wash contaminated clothing before reuse. Ventilate area and wash spill site.
WASTE DISPOSAL METHODS	Dispose of waste in accordance with all applicable Federal, State and local laws.

NOTICE: Use is intended by persons having technical skill and at their own discretion and risk. The information has been developed by A.L. staff from sources considered reliable but has not been independently verified by A.L. Therefore, A.L. cannot guarantee the accuracy of the information in these sources nor should the statements contained herein be considered an official expression. NO REPRESENTATION OR WARRANTY, EXPRESS OR IMPLIED, INCLUDING THE WARRANTIES OF MERCHANTABILITY AND FITNESS FOR A PARTICULAR PURPOSE is made with respect to the information contained herein.

n/a = not applicable

Appendix IV

Determination of Water Solubility of ^{14}C-Colistin Base
(Shake Flask Method)

Data Guideline

United States Food and Drug Administration (US FDA)
FDA Technical Assistance Document 3.01
Water Solubility

Summary

Colistin is a strong water-soluble base. Solubility values were obtained with pH 9 buffer and reagent water, as shown in the table below. The strong water-soluble base character of colistin precludes any solubility determination in pH 5 and pH 7 buffers.

Matrix	**Solubility ± Standard Deviation (mg/mL)**
pH 9	2.55 ± 0.13
Reagent Water	43.8 ± 3.6

Appendix V

Dissociation Constant of Colistin Base in Water

Data Guideline

United States Food and Drug Administration (US FDA)
FDA Technical Assistance Document 3.04
Determination of Dissociation Constant

Summary

The dissociation constant of colistin base was determined spectrophotometrically at 206 nm over the pH range of 8.64 to 12.87. The dissociation constant (pK_a) value for colistin base was determined to be 11.53.

Appendix VI

Determination of Octanol/Water Partition Coefficient of ^{14}C-Colistin Base
(Shake Flask Method)

Data Guideline

United States Food and Drug Administration (US FDA)
FDA Technical Assistance Document 3.02
n-Octanol/Water Partition Coefficient

Summary

The test substance, ^{14}C-Colistin Base, was found to have an octanol/water partition coefficient of less than 10 in the pH 5, 7, and 9 buffers. Based on the data obtained from this study, the test chemical, ^{14}C-Colistin Base, can be considered highly mobile in water.

Appendix VII

Adsorption-Desorption of Colistin Base

Data Guideline

United States Food and Drug Administration (US FDA)
FDA Technical Assistance Document 3.08
Sorption/Desorption

Summary

The adsorption-desorption of the test chemical, ^{14}C-Colistin Base, was examined in chicken manure and three soil types: sandy loam (Mutchlers, soil A), silt loam (East of Quanset, soil B), and silt loam (Lower Level, soil C). The characterization of each soil and chicken manure is listed below:

	Soil A	Soil C	Soil D	Chicken Manure
% Organic	1.4	2.8	2.3	60.7
% Sand	67.3	45.3	23.3	—
% Silt	17.2	33.2	55.2	—
% Clay	15.5	21.5	21.5	—
Texture	Sandy Loam	Loam	Silt Loam	—
Cation Exchange Capacity (meq/100 g)	14.0	20.1	18.5	19.3
pH	5.2	5.7	6.6	7.3
Bulk Density (g/cm^3)	1.37	1.36	1.18	0.34

The adsorption-desorption of colistin base with each soil and chicken manure is listed below:

Sample	Mean ± SD	Mean ± SD	Mean ± SD	K_d		K_{oc}	
	Percent Adsorbed (A)	Percent Desorbed (D)	Percent Not Desorbed (R)	ADS	DES	ADS	DES
Soil A	96.0 ± 1.5	4.8 ± 2.5	95.2 ± 2.5	4452	5067	546909	622561
Soil C	97.1 ± 1.3	3.6 ± 2.1	96.4 ± 2.1	8883	8126	545647	499153
Soil D	96.9 ± 2.0	3.4 ± 2.1	96.6 ± 2.1	11583	11341	866183	848145
Chicken Manure	82.5 ± 3.6	14.1 ± 2.8	85.9 ± 2.8	519	600	1472	1699

The mean ^{14}C-mass balance for the test system was 97.9 ± 7.8%, indicating that there was no significant loss of ^{14}C-activity during the conduct of the isotherm determination test. HPLC analysis of the soil and manure extracts demonstrated that the test chemical was stable under the test conditions.

The mobility of a chemical through soil can be directly related to its adsorption properties. K_{oc} values can be used to rank and compare chemicals with respect to their leaching potential. K_{oc} values greater than 5000 denote immobility of a chemical in soil; K_{oc} values between 2000 and 5000 denote slight mobility of a chemical in soil; K_{oc} values between 500 and 2000 denote low mobility; K_{oc} values between 150 and 500 denote medium mobility; K_{oc} values of 50 to 150 denote high mobility; and K_{oc} values less than 50 denote very high mobility of a chemical through soil. The adsorption K_{oc} value of 1472 for colistin base in chicken manure denotes a low mobility in this matrix, and indicates its potential movement and subsequent bioavailability in manure. Since the K_{oc} values for colistin base were > 5000 for all soil types, it can be considered immobile in soil.

Appendix VIII

Aerobic Biodegradation of ^{14}C-Colistin Base in Soils and Chicken Manure

Data Guideline

United States Food and Drug Administration (US FDA)
FDA Technical Assistance Document 3.12
Aerobic Biodegradation in Soil

Summary

The aerobic soil biodegradation of the test chemical, ^{14}C-Colistin Base, was examined in chicken manure and three soil types: sandy loam (Mutchlers, soil A), silt loam (East of Quanset, soil B), and silt loam (Lower Level, soil C). A reference chemical, ^{14}C-D-glucose, was tested concurrently with these soils and manure to monitor the viability of the microbial population. For each of these matrices, three replicates contained the test chemical, ^{14}C-Colistin Base, three replicates contained the reference chemical, ^{14}C-D-glucose, and three replicates served as blank controls and contained no chemical. Analysis of extractable and nonextractable ^{14}C-residues at 0, 21, 42, and 90 days was conducted to evaluate the decline of the parent test chemical, as well as the formulation of the decline of degradation products. During the 90-day incubation, all systems were continuously aerated. The flasks were weighed periodically (days 0, 21, 42, 64, and 85) and the moisture content was adjusted to approximately 70% of field capacity, when necessary. The percent biodegradability was calculated as a function of the $^{14}CO_2$ production in the test systems as compared to the amount of ^{14}C-activity applied.

After the 90-day aerobic incubation, a mean of 53.1, 56.9, 45.0 and 58.3% of the applied reference compound (^{14}C-D-glucose) was biodegraded to $^{14}CO_2$ in soils A, B, C and chicken manure, respectively, which verified the microbial inoculum in these three soil types was viable and active.

For the test chemical (^{14}C-Colistin Base), a mean of 6.86, 2.37, 3.90, and 18.7% of applied ^{14}C-activity was mineralized to $^{14}CO_2$ after the same incubation period in soils A, B, C and chicken manure, respectively. This indicated that the test chemical was slowly mineralized in all matrices tested. The ^{14}C-organic volatiles were also trapped and quantitated during the study. In all cases, the production of ^{14}C-organic volatiles was negligible (0 - 0.064% of applied ^{14}C-activity).

At days 0, 21, 42, and 90, the test soils containing the test chemical were extracted, and the soil extracts were analyzed by HPLC. The post-extracted soils were combusted to determine the nonextractable ^{14}C-residues. In general, the extractable ^{14}C-residues decreased with time while the nonextractable ^{14}C-residues increased.

Comparison of the HPLC profiles of the test chemical in the dosing solution with the acid-hydrolyzed soil extracts indicated that the ^{14}C-activity in the soil extracts could be attributed to the parent test chemical and that there did not appear to be any significant degradation of the test chemical into new degradate components.

The overall ^{14}C-mass balance is taken as the summation of total $^{14}CO_2$, total volatile ^{14}C-activity, total extractable ^{14}C-activity, and total nonextractable ^{14}C-activity. The mean ^{14}C-mass balance for test chemical was 110.0, 101.7, 105.6, and 108.9% for soils A, B, C and chicken manure, respectively.

In conclusions, the test chemical ^{14}C-Colistin Base was slowly mineralized in three soils and one manure. No evidence of major biotransformation products was seen in any of the matrices tested. The mineralization appeared to result from the biodegradation of the colistin.

Appendix IX

Microbial Growth Inhibition with Colistin Base

Data Guideline

United States Food and Drug Administration (US FDA)
FDA Technical Assistance Document 4.02
Microbial Growth Inhibition

Summary

Colistin base was incorporated into an agar medium in a series of varying concentrations. Triplicate series of the test plates were inoculated with pure cultures of microorganisms. After an appropriate incubation period, the presence or absence of microbial growth on the agar surface was noted. Absence of visual growth was used as an indication of possible inhibitory effects, and for each organism a minimum inhibitory concentration (MIC) value was attempted. The MIC is defined as the lowest concentration of the test chemical that completely inhibits the growth of the test microorganism.

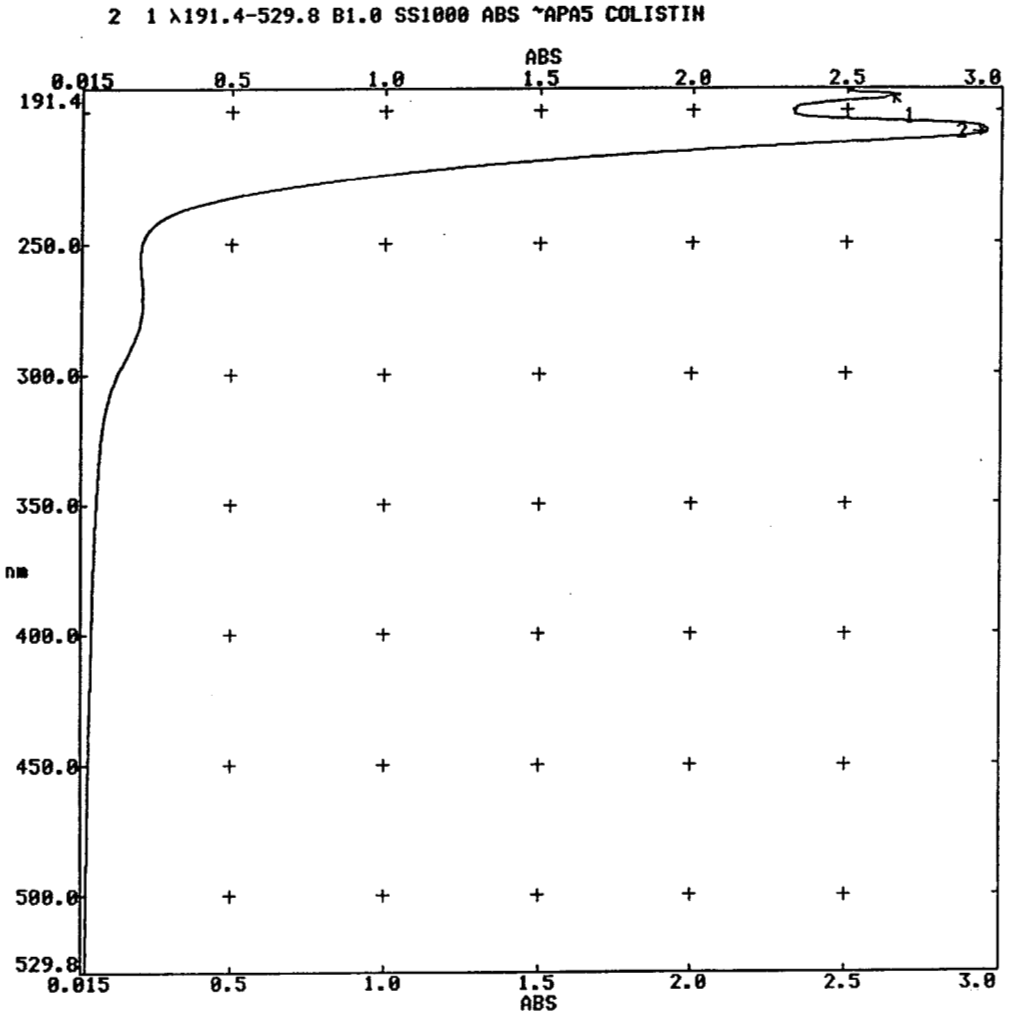
Concentrations of 61.52, 6.15, 0.61, 0.06, and 0 mg/L colistin base in agar media were prepared and the growth of microorganisms on the appropriate media tested. No inhibition was observed for *Anabaena flos-aquae*, *Aspergillus clavatus*, *Penicillium canescens* and *Chaetomium globosum* at any concentration of colistin base tested up to and including 61.52 mg/L. Hence, no MIC was determined for these organisms.

However, *Pseudomonas fluorescens* was inhibited at 61.52 mg/L, while *Bacillus megaterium* and *Azotobacter chroococcum* were inhibited at 6.15 mg/L. Additional tests were performed at 40.58, 30.43, 20.29, 10.14, and 0 mg/L for *Pseudomonas fluorescens* and at 4.28, 3.21, 2.14, 1.07, and 0 mg/L for *Bacillus megaterium* and *Azotobacter chroococcum*. The MIC values were 40.58 mg/L, 6.15 mg/L, and 4.28 mg/L, respectively, for *Pseudomonas*, *Bacillus*, and *Azotobacter*. The concentrations of the stock solutions and dilutions spiked with radiolabeled test chemical were confirmed by LSC analysis.

Appendix X

UV-VIS Spectra of Colistin
Data Guideline

A Philips Scientific PV8700UV/VIS scanning spectrophotometer was used to scan a 0.5 mg/ml solution of colistin in HPLC-grade water over the spectra range of 191.4 to 529.8 μm. Absorbance maxima were found at 207.9 and 193.7 nm, as shown on the Spectra, below.



SAMPLE Colistin 0.5mg/ml REFERENCE water

CELL PATH 1cm OPERATOR Christine

19.4.96

2 1 λ191.4-529.8 B1.0 SS1000 ABS ^APA5 COLISTIN

	1	2	3	4	5	6	7	8
λ	193.7	207.9						
ABS	2.642	2.955						