ENVIRONMENTAL ASSESSMENT

NADA 141-036

Pirlimycin Hydrochloride for the Intramammary Treatment of Clinical Mastitis in Lactating Dairy Cattle.

THE UPJOHN COMPANY

June 1993

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Pirlimycin Hydrochloride Aqueous Gel
Environmental Assessment Report

1. Date

16 March 1993

2. Name of applicant/petitioner

The Upjohn Company

3. Address

The mailing address of The Upjohn Company's headquarters is as follows:

7000 Portage Road
Kalamazoo, MI 49001
Telephone Number: (616) 323-4000

4. Description of the proposed action

4.1 Request approval - need for the action

This environmental assessment is provided to obtain approval of the New Animal Drug (NADA 141036) pirlimycin hydrochloride. Pirlimycin hydrochloride in an aqueous gel will provide the dairy industry an efficacious antibiotic product for intramammary infusion in lactating dairy cattle for the treatment of clinical and subclinical mastitis. An estimated population of one million cows is anticipated to benefit from this product the first year of introduction.

4.2 Location where the product will be produced

The drug substance will be produced, and the formulation and packaging of the drug product will be done at The Upjohn Company's main pharmaceutical manufacturing complex in Kalamazoo (Portage), Michigan.

4.3 Location where the product will be used

The ultimate use of the finished product will be on dairy farms. Finished products will be stored in distribution centers throughout the United States prior to transportation for sale at veterinary clinics and animal health care outlets.

4.4 Locations where product will be disposed

Disposal of drug product may result during manufacturing activities, from the discarding of returned goods or from end-user disposal of individual units of empty or partly empty finished product containers. The present infrastructure at the proposed manufacturing site provides for the following recovery and/or ultimate disposal mechanisms:

- Unused quantities of material for disposal at the manufacturing site will be crushed and shredded and placed in an approved sanitary landfill or incinerated in an on-site approved incinerator. This incinerator is being operated as a Resource Conservation and Recovery Act (RCRA) interim status treatment storage and disposal facility under #MID000820381 in compliance with 40 CFR 264, Subpart 0 requirements. Additionally, 40 CFR 265.1(b) and Section 3005(e) of RCRA provide for the continued operation of an existing facility that meets certain conditions, until final administrative disposition of the owner's and operator's permit application is made.

  A hazardous waste RCRA Part B/Act 64 permit application has been submitted to the Waste Management Division of the Michigan Department of Natural Resources (MDNR) in Lansing, Michigan. The Upjohn facility is grandfathered by MDNR until action is taken on the permit application. MDNR action on the Act 64 permit is expected in 1993. The State air permit issued on 15 July 1980 (#242-80) was revised to incorporate the Act 64 requirements and was approved on 26 May 1993.

  All necessary permits are in place for the manufacture of this product to begin, as an existing interim status facility in accordance with Section 3005(e) of RCRA and Michigan Act 64 licensing requirements.

  This incinerator is a two-stage system: the primary chamber rotary kiln operates at a minimum of 700°F; the secondary chamber, where final destruction of the product and of the off-gasses occurs, operates at 1,800°F. The incinerator is equipped with a pollution control equipment train designed to remove gaseous and particulate pollutants. The pollution control equipment consists of a quench section, an acid-gas pre-scrubber, a Venturi scrubber, an entrainment separator, an induced draft fan, and an exhaust stack.

- Individual empty flexitubes or partly empty end products disposed by consumers will be handled along with commercial or agricultural garbage by the community's solid waste management system and disposed of in an approved sanitary landfill. Only minute traces of the active ingredient, pirlimycin hydrochloride, would be expected to remain with empty product containers.

4.5 Type of environment present at and adjacent to manufacturing locations

The proposed facility for the synthesis of the drug substance and the pirlimycin hydrochloride aqueous gel formulation will be located within the confines of Upjohn's existing facilities. The Portage site complex consists of approximately 80 buildings including chemical/ pharmaceutical manufacturing operations, offices, laboratories, utility operations, and various support buildings (see Section 15.1 in the Appendix). The plant site occupies a portion of approximately 810 hectares lying south of Bishop Road, east of Portage Road, north of Center Street, and west of Sprinkle Road in Portage, Michigan. The area is relatively flat and rural with the nearest school located approximately three kilometers to the southwest. The area is largely characterized by agriculture, forest land, undeveloped open spaces and suburban development in a temperate climate. The plant is located in terms of the Universal Transverse Mercator Coordinate System (UTM), in Zone 16 at 619.1 Km east north which corresponds to latitude 42°12'42" north and longitude 85°33'25" west.

5. Identification of chemical substances that are the subject of the proposed action

5.1 Chemical process

The materials listed below are used in the chemical process for the synthesis of pirlimycin hydrochloride, U-57930E, the final active drug:

acetone
ammonium persulfate
BOC-4-ethylpipecolic acid (an intermediate)
i-butyl chloroformate
7-Cl-MTL (an intermediate)
di-t-butoxy carbonyl anhydride
ethyl acetate
4-ethylpicolinic acid (an intermediate)
4-ethylpipecolic acid (an intermediate)
4-ethylpyridine (purchased raw material)
4-ethylpyridine-2-carboxamide (an intermediate)
formamide
heptane
gaseous hydrogen chloride
hydrogen
isopropyl alcohol
methanol
methylene chloride
N-methylmorpholine

phosphoric acid
platinum on carbon (catalyst)
sodium bicarbonate
sodium bisulfite
sodium carbonate
sodium hydroxide
Solka flock (filter aid)
sulfuric acid
tetrahydrofuran
toluene
water

5.2 Pharmaceutical formulation

Copies of Material Safety Data Sheets for the ingredients used in the formulation of the drug product have been included. (See Section 15.2 of the Appendix.) The following describes the main properties of the components used in pirlimycin hydrochloride aqueous gel formulation:

5.2.1 Pirlimycin Hydrochloride

1. Identity of substance

1.1 International non-proprietary name

Pirlimycin Hydrochloride

1.2 IUPAC name

Methyl(2S-Cis)-7-chloro-6,7,8-trideoxy-6-[[(4-ethyl-2-piperidinyl)carbonyl]amino]-1-thio-L-threo-α-D-*galacto*-octopyranoside hydrochloride hydrate

1.3 CAS name

L-threo-.alpha.-D-galacto-Octopyranoside, methyl 7-chloro-6,7,8-trideoxy-6-[[(4-ethyl-2-piperidinyl)carbonyl]amino]-1-thio-, monohydrochloride, (2S-cis)

1.4 Classification

Therapeutic:

Synthetic Antibiotic

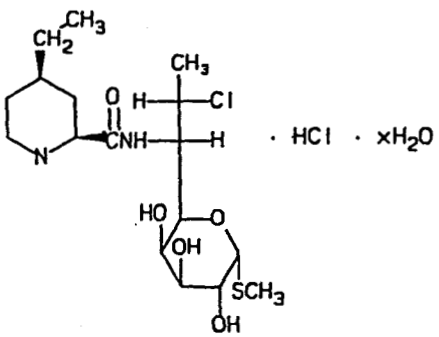
Pharmacological:

Intramammary infusion in lactating cattle for control of bovine mastitis

1.5 Synonyms and abbreviations

Pirlimycin Hydrochloride
Pirlimycin HCl
Upjohn Laboratory Code: U-57930E
PIRSUE™ Aqueous Gel
Chemical Abstracts Registry Number 78822-40-9

1.6 Structural formula



Pirlimycin Hydrochloride
(U-57930E)

1.7 Molecular formula

$C_{17}H_{31}O_5N_2ClS \cdot HCl \cdot XH_2O$

1.8 Molecular weight

447.42 (anhydrous)

1.9 Degree of impurity

Proposed Bulk Drug Specifications:

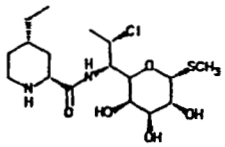
Not less than 879 µg pirlimycin/mg pirlimycin hydrochloride, excluding the solvent on an anhydrous basis.

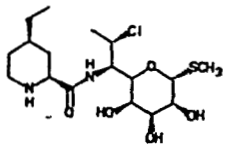
1.10 Qualitative and quantitative composition of impurities

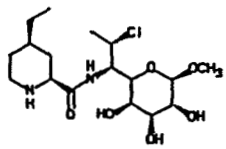
Proposed Bulk Drug Impurities Specifications:

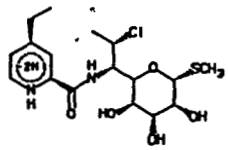
Total Impurities: Not More Than 2.0%
Single Impurity: Not More Than 1.0%

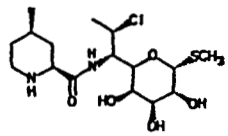
Potential Process Impurities and Degradation Products include:

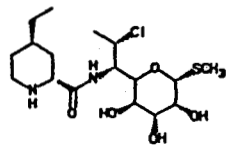
Iso-Pirlimycin 

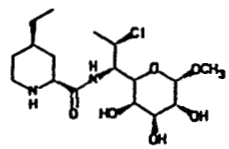
6S-Pirlimycin 

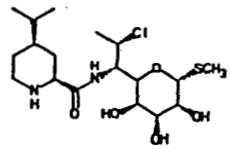
1S-MeO-Pirlimycin 

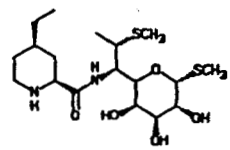
Δ-pip-Pirlimycin 

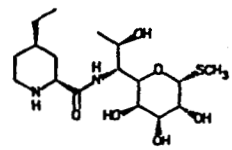
Me-Pirlimycin 

Trans-Pirlimycin 

1R-MeO-Pirlimycin 

IPr-Pirlimycin 

7-MeS-Pirlimycin 

7R-OH-Pirlimycin 

1.11 Description of physical properties

Melting point:

The melting range of pirlimycin hydrochloride is 210.5° - 212.5°C with subsequent decomposition.

Solubility:

Solubility of pirlimycin hydrochloride in aqueous solutions is pH dependent. The maximum solubility occurs at pH 4.5 and is 70 g/liter. The solubility of pirlimycin is lowest at pH 13, around 3 g/liter. Organic solvents such as dimethyl

formamide, methanol, and propylene glycol all have pirlimycin hydrochloride solubilities in excess of 100 g/liter. Solubility is less than 10 gram/liter for other organic solvents, such as methylene chloride and ethylacetate.

Optical rotation:

The specific optical rotation is controlled between +170° and +190°.

5.2.2 Benzyl Alcohol

CAS Reg. No.:	100-51-6
Molecular Formula:	C_7H_8O
Molecular Weight:	108.14
Description:	Colorless liquid

5.2.3 Citric Acid Anhydrous Powder

CAS Reg. No.	77-92-9
Molecular Formula:	$C_6H_8O_7$
Molecular Weight:	210.14
Description:	Colorless, translucent powder

5.2.4 Carboxymethylcellulose (CMC) Sodium

CAS Reg. No.	9004-32-4
Molecular Formula:	Not available
Molecular Weight:	Not available
Description:	Solid polymer

5.2.5 Sodium Hydroxide Solution

CAS Reg. No.	1310-73-2
Molecular Formula:	NaOH
Molecular Weight:	40.0
Description:	Clear liquid

5.2.6 Purified Water

CAS Reg. No.	7732-18-5
Molecular Formula:	H_2O
Molecular Weight:	18.0
Description:	Clear liquid

5.2.7 Sodium Citrate USP

CAS Reg. No.	6132-04-3
Molecular Formula:	$C(OH)(CO_2Na)(CH_2CO_2Na)_2 \cdot 2H_2O$
Molecular Weight:	294.10
Description:	Odorless, crystalline material

5.2.8 10% Hydrochloric Acid

CAS Reg. No.	7647-01-0
Molecular Formula:	HCl
Molecular Weight:	36.47
Description:	Corrosive, toxic, colorless liquid

6. Introduction of substances into the environment - control systems

Portions of the materials listed in Section 5 will be released to the environment as a result of the proposed action. These will be generated from the manufacturing site in the form of air emissions, liquid waste streams and solid wastes.

6.1 Chemical processing

Although at this time the processes and control devices in Buildings 173 and 225 for manufacture of pirlimycin HCl are not currently permitted, The Upjohn Company is under a consent decree with the Michigan Department of Natural Resources (MDNR) dated 15 March 1991, which has required that an inventory be taken of all equipment with the potential to emit an air contaminant and control equipment by 1 July 1991 and that completed permits be in place for this equipment in accordance with the schedule set forth in the consent decree. That inventory was submitted to MDNR on 1 July 1991. Where applicable, air pollution control equipment for all particulate sources and LAER (Lowest Achievable Emission Rate) controls must be installed on the VOC (Volatile Organic Compound) portion of the process by 1 April 1995. A state-of-the-art VOC regional control system, a cryogenic condenser system with a 95% control efficiency, will be installed and operated upon approval of the air permit application. The pirlimycin HCl production facility is part of Region III in the Fine Chemical Division. The permit application for all subject equipment in this Region was submitted to the MDNR on 21 April 1992 in compliance with the 1 May 1992 submittal date within the consent decree.

As in the pharmaceutical formulation manufacturing area, adequate protection will be provided to employees by preventing unnecessary exposure to resulting emissions. All solvent tanks and reactors are equipped with approved safety vent systems. Material Safety Data Sheets (MSDS) will be available on-site. Employees associated with the manufacture of pirlimycin will have appropriate MSDSs available for their review. Employee protective clothing (e.g., gloves, uniforms and safety shoes) and protective equipment (e.g., safety glasses, safety hats and approved respirators) will be used during manufacture to assure compliance with applicable occupational safety requirements.

Aqueous waste streams containing traces of soluble solvents, inorganic and organic salts and other dissolved impurities from the process are sent to a pre-treatment clarifying system where aqueous wastewater is neutralized prior to discharge into an approved chemical process water management (CPWM) system. Our CPWM operations are conducted in accordance with this facility's Underground Injection Control permits granted pursuant to the Safe Drinking Water Act.

Used solvent mixtures are directed to the Solvent Recovery and Distribution (SRD) unit at the Portage site where approximately 60% of the acetone, 40% of ethyl acetone, 60% of methylene chloride, 73% of the tetrahydrofuran, and 40% of toluene used in the chemical process are returned to SRD for recycling, either to be reprocessed for use within the Portage site facility or sent to an off-site approved facility for ultimate disposal. The waste solvent storage areas have received interim authorization from EPA as a hazardous waste facility. The discarded heavy metal catalyst will be isolated and returned to an outside reclaimer for regeneration and reprocessing. No significant increase of solid waste generation resulting from the proposed project is envisioned.

6.2 Pharmaceutical formulation

The pharmaceutical formulation of the proposed product will consist of mixing the bulk pirlimycin with other formulation components to form a consistent aqueous gel.

- Air: Air emissions from the formulation process for PIRSUE Aqueous Gel are permitted with the Michigan Department of Natural Resources under permit #1232-91. Particulate emissions are controlled by a wet dynamic scrubber (Type W Roto-Clone) with an efficiency of 92.2%. There are no Volatile Organic Compound emissions from the process.

- Liquid: Liquid waste streams resulting from the pharmaceutical formulation and packaging facility will consist of residue wastewaters from sanitary use and washing operations which will be discarded to the municipal sewer system for tertiary treatment at the City of Kalamazoo Water Reclamation Plant.

  In response to federal and state requirements governing the City of Kalamazoo's Industrial Pre-treatment Program (IPP), The Upjohn Company has been issued an Administrative Order as a Significant Industrial User of the City of Kalamazoo Water Reclamation Plant. These discharge limitations were in effect as of 31 January 1989, with final compliance with the limitations achieved by 31 December 1989. All discharges from the production of pirlimycin hydrochloride are permitted under these categorical pre-treatment standards agreed upon between The Upjohn Company and the City of Kalamazoo Water Reclamation Plant.

  Projecting to the fifth year of production, the pharmaceutical formulation and packaging of pirlimycin hydrochloride aqueous gel will not impact these standards. Since the production of this product as a percentage of total production at the manufacturing site represents 0.06% in 1993, is projected to rise to 0.1% in 1995, and to remain flat after 1995, it will not cause us to be in violation of the

existing parameters set forth in our Administrative Order with the City of Kalamazoo Water Reclamation Plant.

- Solid: Solid wastes will consist mainly of cardboard, paper and plastics which will be temporarily stored in containers presently located at the proposed facilities. Recycling of appropriate waste materials will be done where possible. Ultimate disposal of non-recycled solid wastes will be into a sanitary landfill.

6.3 Effect of the approval of the proposed action - statement of compliance

Approval of the proposed action will initially result in the modification of existing facilities and the installation of expanded utilities. In turn, air emissions will be exhausted to the atmosphere, liquid wastewater streams will be discharged and solid wastes will be generated. As a long term effect, the approval action will result in the use of resources confined to raw materials and utilities in the manufacturing area. These will be done in compliance with applicable requirements enforced at local and federal levels as appropriate. The following regulations or standards are cited as applicable to the proposed action:

1. Federal Food, Drug and Cosmetic Act, PL 75-717, as amended.
2. Clean Air Act PL 91-604, as amended.
3. Clean Water Act PL 95-217, as amended.
4. Safe Drinking Water Act PL 93-523.
5. Resources Conservation and Recovery Act of 1976 PL 94-580, as amended.
6. Occupational Safety and Health Act of 1970, as amended.
7. Standards from the American National Standards Institute.
8. National Fire Protection Agency Standards.
   a. National Electrical Code Standards
   b. Life Safety Requirements
9. Act #348 of 1965, Michigan Air Pollution Act, as amended.
10. Act #245 of 1929, Michigan Water Resource Commission Act, as amended.
11. Act #399 of 1976, Michigan Safe Drinking Water Act, as amended.
12. Act #136 of 1969, Michigan Liquid Industrial Waste Disposal Act, as amended.
13. Act #315 of 1969, Michigan Mineral Well Act, as amended.
14. Act #641 of 1978, Michigan Solid Waste Management Act.
15. Act #64 of 1979, Michigan Hazardous Waste Management Act, as amended.
16. Act #368 of 1978, Public Health Code.
17. Chapter 28 of the Kalamazoo City Code (Services and Waste Water) as amended by ordinance No. 1190.
18. Michigan Occupational Safety and Health Act of 1970, as amended. (Local regulation applicable to the State of Michigan.)

6.4 Use and disposal of products

The intended use of this product is the treatment of mastitis in lactating dairy cattle. The indication for use will read as follows: "Pirlimycin is indicated for the treatment of clinical and subclinical mastitis in lactating dairy cattle. Pirlimycin has been proven effective only against Staphylococcus species such as *Staphylococcus aureus* and Streptococcus species such as *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, and *Streptococcus uberis*. Cows with systemic clinical signs caused by mastitis should receive other appropriate therapy under the direction of a licensed veterinarian."

The label dose is 50 mg of pirlimycin administered in each affected quarter twice at a 24-hour interval.

Estimates of the number of finished units (FLEXITUBES) and the total amount of pirlimycin hydrochloride used each year over the next five years are summarized as follows:

Year	No. Flexitubes	Total Pirlimycin HCl
1st	0.95 M	47.5 kg
2nd	1.4 M	70.0 kg
3rd	1.75 M	87.5 kg
4th	2.1 M	105.0 kg
5th	2.25 M	112.5 kg

The dairy industry currently treats less than two quarters per cow (average 1.18 quarters), and in a two-year study, the incidence of clinical mastitis was consistently less than 10 cases per 100 cows (Appendix 15.4, 1).

It is anticipated that the unused or discarded products from veterinary clinics, animal health care outlets and ultimate users will become a small increment of a consumer's current refuse or will be returned to The Upjohn Company for disposal in accordance with established procedures. On the dairy farms, used/empty plastets will be handled along with commercial agricultural garbage.

Residues of pirlimycin in milk, urine and feces of the treated dairy cow are the major sources of pirlimycin entering the environment. The anticipated environmental effects from the release of this product through milk discard and animal waste is addressed in Section 7 below.

7. Fate of emitted substances in the environment

7.1 Target animal disposition and metabolism

Pirlimycin hydrochloride will be provided as an aqueous gel and will be administered by intramammary infusion to dairy cattle identified as suffering from clinical mastitis. Dairy herds range in size from small (50 or less) to very large (6,000), with an average of 100 cows. On the average for most herds, only 10% of the herd will be treated for mastitis per month, and usually only one quarter per cow will be treated (Appendix 15.4,1). In either the 100 or 6,000 cow herd, assume that all mastitis cases are treated with pirlimycin hydrochloride aqueous gel and that all four quarters are treated. The total amount of drug involved would be 50 mg/quarter/cow/treatment x four quarters x two treatments x 600 cows/month or 240,000 mg/month in the 6,000 cow herd or 50 mg/quarter x four quarters x two treatments x 10 cows/month or 4,000 mg/month in a 100 cow herd. Thus, approximately 4 g of drug per month could enter the environment from a 100 cow dairy farm or 240 g of drug per month from a 6,000 cow dairy farm through milk discard and animal waste.

The disposition and metabolism of pirlimycin hydrochloride aqueous gel has been studied in the dairy cow at a treatment rate of 200 mg/quarter for two treatments in all four quarters (Appendices 15.4, 2 and 15.4, 3). About 50% of the administered dose was absorbed into the animal across the udder membrane/blood barrier and excreted in urine (10%) and feces (24%) during the six day post-treatment period. The excretion half-lives in urine and feces were 45 hours and 51 hours, respectively. The excreted products (expressed as a percent of the dose) were pirlimycin itself (19%), pirlimycin sulfoxide (1%), and two biologically inactive metabolites (13%).

The other 50% of the administered dose was eliminated in the milk, 99% of which appeared in the first four milkings following the second treatment. The milk elimination half-life over this period was calculated to be seven hours. The residue in milk was found to be almost exclusively (>95%) pirlimycin itself. Thus, the release of pirlimycin into the environment will come mostly from milk discard and from animal excreta.

7.2 Stability of pirlimycin in manure and soil

Since pirlimycin and its metabolites will be excreted in urine and feces, the stability of their biological activity in these media was measured (Appendix 15.4, 4). The disappearance of biological activity in unshaken incubation systems appeared to involve the conversion of pirlimycin and the sulfoxide to the polar metabolites observed in the feces, which were devoid of antimicrobiol activity and identified to be nucleotide adducts likely produced by microflora. However, this conversion appeared to be partially reversible. It was concluded that neither the chemical activities nor the microorganism metabolizing activities alone in dairy cow urine and feces were capable of degrading pirlimycin through destruction of the pirlimycin molecule.

The manure from the treated animals will be spread on soil for fertilizer in most cases. The discarded milk may be either dumped onto soil, poured down the drain, or fed to other animals. Ultimately, the residues may be exposed to the action of soil microorganisms. Thus, a study of the ability of these organisms to biodegrade pirlimycin to CO_2 was conducted (Appendix 15.4, 5). Three types of soil were used: clay from California, sandy clay loam from New Jersey, and silty clay loam from Wisconsin. Each soil was fortified with pirlimycin at 500 µg/g and the production of CO_2 above background followed for 42 days. Only New Jersey soil gave any indication of biodegradation of pirlimycin to CO_2, and then only to the extent of 5%. California and Wisconsin soils gave no indication of significant biodegradation as measured by the increased production of CO_2 above background (untreated controls). The results suggest that pirlimycin is marginally biodegraded to CO_2 in soil. Importantly, however, pirlimycin showed no tendency to inhibit the ability of microorganisms in the soils tested to degrade glucose to CO_2 (Appendix 15.4, 5).

Pirlimycin is a member of the lincomycin family of antibiotics. Lincomycin has been shown to have a low affinity for binding to soils, with K_{oc} coefficients ranging from 0.12 to 1.59 for three types of soil (Appendix 15.4, 6). Similar soil sorption/desorption studies have been conducted for pirlimycin in three soil types (Appendix 15.4, 7). The binding of pirlimycin to soil ranged from slightly bound in silt loam (K_{oc}=2.78 x 10^2 - 8.36 x 10^2) to tightly bound in silty clay loam (K_{oc} = 5.66 x 10^3 - 1.12 x 10^6). Pirlimycin binding to clay loam (K_{oc} = 3.55 x 10^2 - 3.46 x 10^3) was intermediate between these two extremes. Therefore, the mobility of pirlimycin in soil is variable, given that it appears to depend on soil type.

7.3 Physical/chemical properties

A. **Water Solubility.** The aqueous solubility was determined at various pH values and reached a maximum in the range of pH 4 to pH 5 (Appendix 15.4, 8) as the following summary table shows:

Aqueous Medium	Solubility		
	Temp °C	pH*	mg/mL
0.1 N HCl	25	0.9	48.0
water for injection	25	4.6	64.9
0.2 M acetate buffer	37	4.0	69.1
0.2 M acetate buffer	37	5.0	69.7
pH 7 USP buffer	37	6.7	39.7
pH 8 phosphate buffer	37	7.2	37.4
0.1 N NaOH	25	12.5	3.0
* pH of saturated solution			

B. **Dissociation Constant.** The pKa of pirlimycin was determined to be 8.38 at zero ionic strength and 8.77 at an ionic strength of 0.1 (Appendix 15.4, 8). Thus, pirlimycin will exist primarily as a protonated salt at environmentally relevant pH values.

C. **Octanol/Water Partition Coefficient (K_{ow}).** In water, pirlimycin hydrochloride produces a moderately acidic aqueous solution in which essentially all of the compound exists in the ionized form. The K_{ow} was measured in pH 7 phosphate buffer at 37°C (pirlimycin HCl produced a shift in the pH to 6.81) and was calculated to be 0.60. The partition coefficient, which reflects partitioning of the neutral species (pirlimycin free base), was calculated to be 60 (Appendix 15.4, 8). A second measurement of the K_{ow} was carried out at ambient temperature in various buffers at pH 5.4, 7.0, and 8.9. The mean K_{ow} values obtained, which reflect the partitioning of total pirlimycin, ranged from 0.024 to 7.58 over the range of pH values used. The corrected partition coefficients for the neutral species only ranged from 11.7 to 29.1 (Appendix 15.4, 9).

D. **UV-VIS Absorption Spectrum.** No absorption maxima were observed above 240 nm for either aqueous or ethanol solutions of pirlimycin. An absorption increase was observed on scanning

down from 240 nm to 200 nm. However, the maxima occurs at a wavelength considered to be end absorption (Appendix 15.4, 10). This is consistent with the structure of pirlimycin which contains essentially no UV absorbing chromophore other than the carbonyl portion of the amide linkage.

E. **Vapor Pressure.** The vapor pressure was estimated to be less than 1×10^{-2} Torr (Appendix 15.4, 11). Moreover, pirlimycin hydrochloride has a melting point range of 210.5°C to 212.5°C with decomposition. Thus, pirlimycin is not likely to enter the atmosphere.

7.4 Hydrolytic stability

Aqueous stability was examined in two ways (Appendix 15.4, 8). First, the stability of pirlimycin was examined at various pH values and temperatures. Second, it was examined following exposure to UV light at room temperature. At room temperature, in solutions ranging from 0.1 N HCl to pH 9 buffer, pirlimycin showed less than 5% degradation over six weeks. A half-life of about five days was estimated for its stability in 0.1 N NaOH solution. At a temperature of 70°C, all solutions of pirlimycin were unstable, with greater than 40% degradation occurring after three weeks. When exposed to UV light at room temperature, the half-lives of pirlimycin in pure water, 0.1 N HCl and 0.1 N NaOH were 6.7, 3.5 and 0.5 days, respectively.

7.5 Expected Emitted Concentration (EEC)

The route of entry into the environment for pirlimycin hydrochloride will be through the disposition of manure (urine and feces) and milk disposal. As discussed in section 7.1, treatment of 10% of a 6,000 cow herd monthly would use 240 g of pirlimycin which could enter the environment.

Soil

In a typical month, a lactating cow will produce approximately 960 kg of waste material (142 lbs dry weight) including manure, urine and discarded milk (30 kg feces and urine/day x 30 days) + (20 kg milk/day x 30 days x 10% (mastitis incidence rate)). The 6,000 cow dairy will produce 5,760,000 kg of waste material monthly on a wet weight basis (850,000 kg dry weight). If 240 g of pirlimycin were present in the total waste of the entire 6,000 cow herd (5.76×10^6 kg), this results in a pirlimycin concentration in the total waste of 0.28 mg/kg on a dry weight basis or 4.1×10^{-2} mg/kg on a wet weight basis.

Dairy manure management practices range from daily spreading operations to storage systems that typically spread during the spring

and fall of the year. Dairy farm operations range in size from 110 to 400 acres (Reference 14.1). If the average dairy farm is assumed to be approximately 200 acres and 1/3 of that land is fertilized by the manure mixture, then approximately 66 acres/application could be affected.

The recommended application rates for bedded systems is 10 tons/acre (10,000 kg/acre), with typical application rates being between 15 and 20 tons/acre (Reference 14.2) Incorporation rates vary from 2-4 inches (chisel), 4-6 inches (disc), and 6-8 inches (plow) (Reference 14.2). At a manure application rate of 15,000 kg/acre in 2-4 inches of soil a concentration of 7.7×10^{-4} mg/kg could occur in the soil (4.1×10^{-2} mg pirlimycin/kg manure x 15,000 kg manure = 615 mg pirlimycin incorporated into 798,000 kg soil/acre).

This concentration is well below the NOEC for the earthworm ($\geq$ 1,000 mg/kg), the most sensitive plant species - cucumber (NOEC 19 mg/kg) and the most sensitive soil microorganism tested *Clostridium perfringens,* which had an MIC of 0.13 µg/mL and would be considered a worst case (see Section 8).

Water

In a 2-inch rainfall (equals 0.166 cu ft water/sq ft land), approximately 1.3×10^7 liters of rain would fall on the 66 acres (43,560 sq ft/acre x 66 acres x 0.166 cu ft rain/sq ft x 28.3 liters/cu ft water = 1.3×10^7 liters). If all of the pirlimycin from a single application (615 mg/acres x 66 acres = 40,509 mg pirlimycin = 40 g) was contained in the runoff (1.3×10^7 L), the concentration would be 3.0×10^{-3} mg/L. But on average, only 52% (see Section 7.6) of the available pirlimycin in the soil would be expected to be lost to runoff due to the sorption/desorption characteristics of this compound. Taking this into account, the concentration of pirlimycin in the runoff water would be 1.56×10^{-3} mg/L. This level is below the minimum inhibitory concentration (MIC) of the most sensitive species tested, a freshwater green alga (MIC = 2.9×10^{-2} mg/L).

7.6 Long-term environmental accumulation

Since pirlimycin may have a potential to persist in the environment, the expected concentrations in soil and water after 10 years of product use was assessed.

Potential for accumulation in soils

If a twice yearly application rate were considered, a concentration of 1.5×10^{-3} mg pirlimycin/kg soil could exist if no runoff were to occur and with incorporation rates of 2-4 inches (soil concentration after one application = 7.7×10^{-4} mg/kg x 2 = 1.5×10^{-3} mg/kg). After 10 years, if no degradation and no loss to runoff were to occur, a concentration of 1.5

x 10^{-2} mg/kg (10 x 1.5 x 10^{-3}) could exist in the soil. This level is below the MIC of the most sensitive soil species tested (*Clostridium perfringens* MIC = 0.13 mg/L).

There are at least three mechanisms which will lower this potential concentration: runoff due to rainfall, degradation by soil microorganisms, and degradation by chemical action.

Rainfall runoff would remove an estimated 52% of the total initial concentration applied to the soil (see discussion below on amount of compound lost to runoff). When this 52% loss is taken into account, the end of the year concentrations would be approximately 5.8 x 10^{-4} mg/kg (0.52 x 1.5 x 10^{-3} = 7.8 x 10^{-4} lost to runoff, 7.2 x 10^{-4} remaining). This level is approximately 0.4% of the MIC for the most sensitive species tested (*Clostridium perfringens* MIC = 0.13 mg/L).

In the New Jersey soil, a degradation of 5% after 42 days was observed, indicating a possible long term degradation rate not observed in the other two soil types. This would approximate a 42% degradation rate over one year. At this rate, with no other degradation occurring and no loss to runoff, after 10 years a concentration of 2.5 x 10^{-3} mg/kg might be observed (beginning concentration of 1.5 x 10^{-3}, 42% lost each year, and an additional 1.5 x 10^{-3} added yearly). If losses due to runoff were included, a concentration of 1.5 x 10^{-3} mg/kg would result. This is approximately 1% of the MIC for the most sensitive species tested (*Clostridium perfringens* MIC = 0.13 mg/L).

Potential for accumulation in water

Sorption/desorption studies indicate that pirlimycin binding to soil is variable (Section 7.2). Percent sorbed values ranged from 47-89% in $CaCl_2$ to 80.8-98.8% in DDI water. The percent desorbed from the amount sorbed to the soil ranged from 22.4-67.5% in $CaCl_2$ and from 6.97 - 35.4% in DDI water. Thus, on the average, a sorption rate of 75% and a desorption rate of 36% might be expected. This would result in approximately 52% of the yearly concentration of pirlimycin applied to the 66 acres being lost to runoff (25% not sorbed plus 36% of the 75% of the sorbed (27%) = 52%).

In a given year, 4.0 x 10^{4} mg of pirlimycin could be applied to the 66 acres (0.041 mg pirlimycin/kg waste x 15,000 kg waste/acre x 66 acres) presented in the earlier scenario (Section 7.5). If 52% of the amount

applied to the soil (2.08×10^4 mg) is contained in the runoff and the average rainfall is 42 inches (2.83×10^8 L) then a concentration of 7.3×10^{-5} mg/L could occur in the receiving waters in the first year.

After 10 years with no further degradation occurring, a concentration of 7.3×10^{-4} mg/L could be expected. This is approximately 2% of the MIC of the most sensitive species tested (freshwater green alga MIC = 2.9×10^{-2} mg/L).

Pirlimycin in aqueous solutions has been shown to be unstable when exposed to UV light. This is a potential pathway for the degradation of pirlimycin. However, the importance of this pathway in natural waters is unknown.

7.7 Summary

Pirlimycin would likely enter the environment in small amounts either intact or as the less biologically active sulfoxide metabolite through such mechanisms as milk discard and elimination of animal wastes. It does not appear to be degraded biologically or chemically in the manure in which it is excreted. It does not appear to be biodegraded to CO_2 by soil microorganisms, but neither does it inhibit their ability to convert glucose to CO_2. It is unlikely to be volatilized into the atmosphere and would appear to be somewhat retained in soil, although it could eventually leach into the aquatic environment. Although pirlimycin is rapidly hydrolyzed above pH 9, it is slowly degraded at neutral or acidic pH. Under normal environmental conditions, pirlimycin appears to be potentially persistent and variably mobile in soil and therefore terrestrial and aquatic toxicological effects were evaluated (Section 8).

8. Environmental effects of released substances

8.1 Mammalian effects

The mammalian toxicity of pirlimycin is low. In the beagle dog treated orally for 90 days with pirlimycin, the No-Observed-Effect-Level (NOEL) was 16 mg/kg/day (Appendix 15.4, 12). In the rat treated orally for 90 days, the NOEL was 10 mg/kg/day (Appendix 15.4, 13). Summaries of the 90-day rat and dog studies are found in Section 8.4.

Assume for the moment that 10% of the average dairy herd of 100 cows would be treated every month with pirlimycin at a rate of 50 mg/quarter/cow/treatment x four quarters x two treatments x 10 cows. This results in a total drug use of 4,000 mg of pirlimycin. Absorption, Distribution, Metabolism, and Excretion (ADME) studies have shown that 50% of the dose was eliminated in the milk and that the other 50% was absorbed and eventually excreted in urine and feces (Appendix 15.4, 3). The milk residues were essentially all unmetabolized pirlimycin, but only half of the manure residues were pirlimycin (Appendix 15.4, 3). Next assume 85% of all infused pirlimycin is excreted by six days post last treatment (Appendix 15.4,3). In feces and urine (manure), 19.5% of the total administered dose is parent pirlimycin (Appendix 15.4,3).

Two scenarios apply here for the fate of these residues in regards to the toxicity of pirlimycin to mammals. First, a cow excretes approximately 30 kg of manure daily and usually 10% of a given herd is treated for mastitis at any one time. For the 100 cow herd, the 10 treated cows would excrete 1,800 kg of manure over six days and maximum treatment would use 4,000 mg pirlimycin (Section 7.1) The concentration of pirlimycin in the manure of these 10 treated cows is 0.43 mg/kg on the average (19.5% x 4,000 mg pirlimycin/1,800 kg manure). The same concentration would be calculated following treatment of 10% of a 6,000 cow herd (19.5% x 240,000 mg/108,000 kg manure). This level is 4 % of the lowest NOEL (10 mg/kg in the rat). In actual practice, the manure from treated cows would be mixed with manure from non-treated cows to dilute the average concentration by an order of magnitude or more. Therefore, manure from treated cows should not be harmful to domestic mammals.

Second, if discarded milk is fed directly to other animals, residue data indicate that the maximum concentration of pirlimycin in the first milking after treatment in all four quarters is in the range 5.50 to 10.0 mg/L (Appendix 15.4, 14). If this were by remote chance the sole source of food, and the daily intake is 0.05 L/kg, an exposure rate could reach 0.05 L/kg/day x 10 mg/L = 0.5 mg/kg/day. However, the actual exposure would be considerably lessened as subsequent milkings containing diminishing concentrations of residue are fed. The maximum exposure rate is at least one to two orders of magnitude lower than the NOEL (10 mg/kg/day) for rats and dogs exposed chronically. Further, the concentration would be greatly diluted as more milk (which would have lower levels or pirlimycin residue) is discarded. Therefore, milk from treated cows should not be harmful to domestic mammals.

8.2 Terrestrial effects

The soil sorption/desorption characteristics of pirlimycin suggest that since pirlimycin may be somewhat retained in soil, its terrestrial effects needed evaluation. These effects were addressed in terms of its toxicity towards microorganisms and macroorganisms.

A - Microorganisms. The biological activity of pirlimycin is traditionally measured against *M. luteus* with a minimum inhibitory concentration of 0.02 µg/mL. The metabolites of pirlimycin have very little activity against *M. luteus*; the MIC for pirlimycin sulfoxide is 2.84 µg/mL (or 1/142 the activity of pirlimycin) and >17 µg/mL for the polar metabolites excreted in cow urine and feces (Appendix 15.4, 15). The Limit Of Detection (LOD) of the various substances against *M. luteus* are summarized as follows:

Compound	LOD µg/mL
Pirlimycin	0.02
Pirlimycin Sulfoxide	2.84
Metabolite 3-4*	> 42
Metabolite 4-4*	> 45
Metabolite 6-6*	> 17
* Identified as nucleotide adducts of pirlimycin and pirlimycin sulfoxide.	

The biological activity of pirlimycin against various nonpathogenic organisms has been determined. The MIC of pirlimycin against the various fungi listed below was found to be > 1000 µg/mL, the highest concentration tested (Appendix 15.4, 16):

Absidia sp. F83
Alternaria sp. F85
Aspergillus niger F92
Cladosporium sp. F86
Fusarium roseum UC 7170
Monascus ruber F88
Sordaria sp. F90
Zygorhincus sp. F91
Acremonium sp. F84
Asper. carbonarius UC 1511
Chaetomium cochliodes UC 7217
Chrysosporium parvum F87
Penicillium notatum UC 1296
Trichoderma virde UC 4021
Scopulariopsis brumpti F89

The activity of pirlimycin against various bacteria is summarized in the following table (Appendix 15.4, 16):

Bacteria	MIC (µg/mL)
Arthrobacter globiformis UC 3604	1.0
Azotobacter vinelandii UC 3144	1024
Bacillus cereus ATCC 6633	1.0
Bacillus subtilus ATCC 11778	0.25
Cellulomonas sp. UC 6274	0.5
Clostridium butyricum UC 9385	0.5
Clostridium perfringens UC 247	0.13
Clostridium perfringens UC 6509	0.13
Cytophaga johnsonae UC 9386	0.5
Flavobacterium heparinium UC 6284	0.5
Pseudomonas fluorescens UC 3049	>1024

Two of the Gram-negative bacterial species, *Azotobacter vinelandii* and *Pseudomonas fluorescens*, were not inhibited except at extremely high concentrations of pirlimycin. However, the Gram-negative *Flavobacterium* and *Cytophaga sp.* were inhibited at concentrations of 0.5 µg/mL. The Gram-positive bacteria, as expected, were inhibited at various concentrations below 1 µg/mL. The most sensitive organism tested was *Clostridium perfringens* which had an MIC of 0.13 µg/mL.

In addition, the biological activity of the two principal metabolites, pirlimycin sulfoxide and pirlimycin adenylate, were tested against some of these organisms (Appendix 15.4, 17). The MICs were as follows:

Fungi	MIC, µg/mL		
	Adenylate	Sulfoxide	Pirlimycin
Aspergillus carbonarius UC 1511	>1000	>1000	>1000
Chaetomium cochliodes UC 7217	>1000	>1000	>1000
Fusarium roseum UC 7170	>1000	>1000	>1000
Penicillium notatum UC 1296	>1000	>1000	>1000
Trichoderma virde UC 4021	>1000	>1000	>1000

Bacteria	MIC, µg/mL		
	Adenylate	Sulfoxide	Pirlimycin
Streptomyces albus UC 2043	>1000	>1000	>100
Arthrobacter globiformis UC 3604	128	64	1
Azotobacter vinelandii UC 3144	512	>1024	4
Bacillus cereus ATCC 6633	128	256	1
Bacillus subtilus ATCC 11778	32	32	0.25
Cellulomonas sp. UC 6274	256	>1024	4
Cytophaga johnsonae UC 9386	128	512	1
Flavobacterium heparinium UC 6284	16	32	0.3
Pseudomonas fluorescens UC 3049	>1024	>1024	>1024

Assume that the manure from treated and untreated cows would be mixed and spread over the land as fertilizer. As noted in section 7.5, a maximum potential concentration of pirlimycin in total waste material is 0.041 mg/kg on a wet weight basis. Even if this manure were diluted no further and used directly, the concentration of pirlimycin is well below the MIC for even the most sensitive organism listed above. Furthermore, dilutions will occur as a result of soil mixing and rainfall as was discussed in section 7.5 above, to concentrations on the order of 7.7×10^{-4} mg/kg. This low concentration provides a margin of safety >100 for pirlimycin to the most sensitive bacterium tested, *Clostridium perfringens*.

In addition, pirlimycin does not inhibit the metabolic activity of the soil microorganisms which normally metabolize glucose to CO_2, although pirlimycin itself is only slowly degraded to CO_2 (Appendix 15.2, 6). Therefore, pirlimycin appears to have no adverse effect on environmentally important bacteria and fungi.

B - Macroorganisms. The toxicological effect of pirlimycin was tested against earthworms, *Lumbricus terrestris*, in a 28-day subacute test at concentrations up to 1,000 mg/kg in soil (Appendix 15.4, 18). The No-Observed-Effect-Concentration (NOEC) for earthworm survival was ≥1,000 mg/kg and because no mortality was observed at this exposure rate, the 28-day LC_{50} was estimated to be >1,000 mg/kg. The NOEC for earthworm weight loss was estimated to be between 100 and 1,000 mg/kg in the 28-day subacute test, although this was not a specific objective of the earthworm toxicity study. Therefore, pirlimycin will have no adverse effect on macroorganisms such as earthworms.

C - Plant Toxicology. The effects of pirlimycin on plants of economic and ecological importance was addressed in terms of seed germination and root elongation (Appendix 15.4, 19) and seedling growth and survival (Appendix 15.4, 20). The results of these studies are tabulated as follows:

Species	Seed Germination[1]		Root Elongation[1]		Seedling Growth[4]		Classification
	NOEC[2]	LOEC[3]	NOEC[2]	LOEC[3]	NOEC[2]	LOEC[3]	
Corn	550	940	230	550	6.9	13	insensitive
Cucumber	19	>19	0.4	0.83	0.42	0.85	sensitive
Ryegrass	130	>130	61	130	0.73	1.7	weakly sensitive
Soybean	420	840	81	110	1.7	3.1	weakly sensitive
Tomato	130	>130	22	61	0.73	1.7	weakly sensitive
Wheat	550	940	550	940	13	25	insensitive

[1] Seeds exposed to aqueous solutions of pirlimycin contained in a Petri dish. The evaluation was based on the percent of seeds germinated (with a root length > 3 mm) and the root length after four to six days exposure compared to controls at a level of significance $p > 0.05$.
[2] No-Observable-Effect-Concentration, expressed in mg/L.
[3] Lowest Observable Effect Concentration, expressed in mg/L.
[4] Seedling plants exposed to an aqueous solution of pirlimycin by sub-irrigation. The evaluation was based on the shoot length and the dry weights of the shoots and roots measured against controls over a 21-day exposure.

The most sensitive species tested was the cucumber, which gave an NOEC of 0.4 mg/L for both root elongation and seedling growth. Assume that the manure from the treated cows would be used to fertilize the soil growing cucumbers, the expected maximum concentration of pirlimycin in the soil/manure mixture was estimated (Section 7.5) to be 7.7×10^{-4} mg/kg. This concentration is <500 times that of the NOEC, providing a large safety margin for pirlimycin's toxic effect on this plant species.

D - Summary. The results of these terrestrial toxicology studies clearly show that pirlimycin will present no adverse effects on the terrestrial environment at the concentrations which might be encountered under the most extreme conditions around the site of manufacturing nor around the sites of end product use.

8.3 Aquatic effects

The water/octanol partition coefficient gives an estimate of a compound's ability to remain associated with the aqueous environment and from this a biological concentration factor (BCF), using K_{ow}, may be calculated using the following formula (Appendix 15.4, 21):

$$\log_{10}BCF = 0.76 \log_{10} K_{ow} - 0.23$$

The calculated BCFs are 0.40 and 13.2 for the hydrochloride and free base pirlimycin, respectively. Since these BCFs are less than 100, pirlimycin

should remain in the aqueous environment and no bioaccumulation should occur (Appendix 15.4, 21). Since the pirlimycin metabolites are even more polar, and more water soluble than pirlimycin, these arguments should apply to the metabolites as well.

However, because pirlimycin is not tightly bound to all soil types, the potential exists for pirlimycin to reach the aquatic environment from discarded unused product or as leachates from animal waste/soil mixtures. Aquatic toxicology was therefore evaluated in a battery of tests against freshwater fish (Appendices 15.4, 22 and 15.4, 23), daphnia (Appendix 15.4, 24), and an algal species (Appendix 15.4, 25). The results are summarized as follows:

Species	NOEC[1]	LC_{50}[2]	EC_{50}[3]	MIC[4]
Rainbow Trout	190	>970		
Bluegill Sunfish	380	>990		
Daphnia Magna	100-130		190	
Freshwater Green Alga	0.014			>0.029

[1] No-Observable-Effect-Concentration, in mg/L
[2] Lethal Concentration at 50% mortality in 96 hours, in mg/L
[3] Effective Concentration causing 50% immobilization in 48 hours, in mg/L
[4] Minimum Inhibitory Concentration for a minimum significant difference of 15% compared to untreated controls, in mg/L

Although it is difficult to assess potential concentrations of pirlimycin in the aquifer that might be under dairy farms (the most likely site for environmental exposure), it should be readily apparent that pirlimycin will not present an adverse effect on freshwater fish and daphnia. The only potential effect might be against the alga tested, which showed a MIC >0.029 mg/L, or 29 mg/m^3. If all of the prescribed doses of 4,000 mg for the treatment of 10 cows (50 mg/quarter/day x four quarters x two days) were directly dispersed into a closed aquifer, the volume needed to achieve this concentration would be 138 m^3. This would equate to a backyard swimming pool with dimensions of 6 m x 12 m with an average depth < 2 m. This volume is very small compared to the total volume of an aquifer, therefore, pirlimycin should present no potential threat to the aqueous environment.

The estimated concentration of pirlimycin in run-off water, Section 7.5, was 0.003 mg/L (worst case). The MIC for the most sensitive aquatic species/organism tested, a freshwater green alga, was 0.029, or approximately 10 times the expected environmental concentration. This estimate again illustrates a substantial safety margin for the toxicity of pirlimycin to aquatic species.

8.4 Summary of effects

Mammalian	NOEL[1] (mg/kg/day)	EEC[2] (mg/kg soil)	Safety Factor[3]
Dog	16	7.5×10^{-4}	2×10^{4}
Rat	10	7.5×10^{-4}	1.3×10^{4}
Terrestrial	**MIC (µg/mL)**	**EEC[2] (mg/kg soil)**	**Safety Factor[3]**
Bacteria			
Arthrobacter globiformis UC 3604	1.0	7.5×10^{-4}	1.3×10^{3}
Azotobacter vinelandii UC 3144	1024	7.5×10^{-4}	1.4×10^{6}
Bacillus cereus ATCC 6633	1.0	7.5×10^{-4}	1.3×10^{3}
Bacillus subtilus ATCC 11778	0.25	7.5×10^{-4}	3.3×10^{2}
Cellulomonas sp. UC 6274	0.5	7.5×10^{-4}	6.7×10^{2}
Clostridium perfringens UC 9385	0.5	7.5×10^{-4}	6.7×10^{2}
Clostridium perfringens UC 6509	0.13	7.5×10^{-4}	1.7×10^{2}
Cytophaga johnsonae UC 9386	0.5	7.5×10^{-4}	6.7×10^{2}
Flavobacterium heparinium UC 6284	0.5	7.5×10^{-4}	6.7×10^{2}
Pseudomonas fluorescens UC 3049	>1024	7.5×10^{-4}	1.4×10^{6}
Terrestrial	**NOEC (mg/kg)**	**EEC[2] (mg/kg soil)**	**Safety Factor[3]**
Lumbricus terrestris	>1000	7.5×10^{-4}	1.3×10^{6}
Plants	**LOEC[5] (mg/L)**	**EEC[2] (mg/kg soil)**	**Safety Factor[3]**
Corn	13	7.5×10^{-4}	1.7×10^{4}
Cucumber	0.85	7.5×10^{-4}	1.1×10^{3}
Ryegrass	1.7	7.5×10^{-4}	2.3×10^{3}
Soybean	3.1	7.5×10^{-4}	4.1×10^{3}
Tomato	1.7	7.5×10^{-4}	2.3×10^{3}
Wheat	25	7.5×10^{-4}	3.3×10^{4}
Aquatic	**NOEC (mg/L)**	**EEC[6] (mg/L water)**	**Safety Factor[3]**
Rainbow trout	190	2.9×10^{-4}	6.6×10^{5}
Bluegill sunfish	380	2.9×10^{-4}	1.3×10^{6}
Daphnia magna	100-130	2.9×10^{-4}	3.4×10^{5}
Freshwater green alga	0.014	2.9×10^{-4}	5×10

[1] No-Observed-Effect-Level (mg/kg/day)
[2] Expected Emitted Concentration (Soil) (mg/kg)
[3] Safety Factor = NOEL, MIC, NOEC or LOEC/EEC
[4] No-Observed-Effect-Concentration (mg/kg)
[5] Lowest Observed Effect Concentration (mg/L)
[6] Expected Emitted Concentration (Water)

From the safety factors present in the above table, the low toxicity of pirlimycin provides a very high safety margin for terrestrial and aquatic organisms.

Thus, while pirlimycin may be slowly degraded and exhibit variable mobility in soil, the pattern of use for this product suggests that the levels to which it might accumulate will not be deleterious to the most sensitive soil and aquatic organisms tested. Therefore, The Upjohn Company suggests that its use should not pose an environmental threat.

9. Use of resources and energy

Projecting to the fifth year of production, the use of natural resources and energy for the formulation and packaging of this product will be 0.0006% of present total plant usage in 1993, up to 0.001% in 1995 and is forecast to remain flat after 1995. This usage will be handled by the existing infrastructure. The resources committed will be the materials listed in Section 5, the utilities used in manufacturing and minor miscellaneous support materials.

Under the authority of the National Historic Preservation Act of 1966, as amended, The Upjohn Company has received an opinion letter from the State Historic Preservation Officer that, since this activity does not involve the alteration, demolition or construction of building or any earth-disturbing projects, historic property determination is not required (see Appendix 15.3). No effects are anticipated for endangered or threatened species.

10. Mitigation measures

Adherence to all applicable state and federal regulations as outlined in Section 6.3 above shall be followed to avoid potential adverse impact associated with the proposed action.

Measures taken to avoid potential adverse environmental impacts associated with the proposed action include:

10.1 use of condenser and air filtration systems to prevent emission levels from exceeding limits established by federal and local regulations;

10.2 injection of aqueous waste into an approved chemical process water management system;

10.3 recovery and reuse or use of spent solvents for manufacturing or fuel, respectively;

10.4 recycling and/or disposal of solid wastes in a sanitary landfill.

Reference is made to item 6 above for additional information.

11. Alternatives to the proposed action

Resources and facilities are being used effectively to produce a quality product with minimal environmental impact. The alternative of no action, resulting in deprivation to the dairy industry of a beneficial therapy, is not contemplated.

12. List of preparers

Enclosed is a list of those persons, and corresponding qualifications, that participated in the preparation of this assessment. No government agency was consulted for this specific evaluation other than for routine implementation of ongoing environmental programs conducted at existing facilities.

José A. Alvarez	Environmental Engineer MCE - June, 1981 BSCE - June, 1974 Registered Professional Engineer - License # 7221 P.R. 15 years experience as Environmental Engineer
Mark W. Gauthier	PIM Specialist B.S. Biology - February 1981 Professional Experience: 6 years
Rex E. Hornish	Senior Research Scientist III Ph.D. - Organic Chemistry 1970 Professional Experience: 19 years
Jeffrey S. Mehring	Corporate Toxicologist Ph.D. - Agriculture Professional Experience: 22 years
Richard F. Gendernalik	Health and Safety Regulatory Affairs M.S., Aquatic and Terrestrial Ecology Professional experience: 13 years
Clifford E. Sacks	Associate Director Fine Chemical Process Research & Development Ph.D. - Chemistry 1979 Professional Experience: 12 years
Susan I. Shedore	Environmental Technician A.A. - December, 1990 Corporate Experience: 21 years

Joseph A. Robinson	Acting Associate Director Biostatistics and Environmental Research Ph.D. - Microbiology and Public Health - 1982 Professional Experience: 8 years
John W. Hallberg	Clinical Research Scientist II, DVM, PhD - 1982, 1984 Worldwide Animal Health Clinical Research and Product Development Professional Experience: 10 years

13. Certification

The undersigned officials certify that the information presented is true, accurate, and complete to the best of their knowledge.

R. S. Senger — March 30 1993 (Date)
Corporate Environmental Manager
(Telephone: 616/323-5341)

J. S. Mehring — 30 MAR 93 (Date)
Health and Safety Regulatory Affairs Manager
(Telephone: 616/329-5535)

14. References

14.1 The Penn State Dairy Reference Manual, 2nd Ed. Cooperative Extension Service, 213 Borland Laboratory, Penn State University, University Park, PA 16802, 1980

14.2 Dr. Stu Melvin, Agricultural Engineer, Iowa State University, telephone conversation, May 1992.

15. Appendices

15.1 Map of Upjohn's Portage Site Complex

15.2 Material Safety Data Sheets

As previously mentioned, enclosed are copies of Material Safety Data Sheets for the main raw materials used in the formulation of pirlimycin hydrochloride aqueous gel.

15.3 9-3-91 Letter from Michigan Deptartment of State

15.4 Technical Report Summaries

1. J.M.Booth, G.J.Rowlands, "Monitoring the Incidence of Clinical Mastitis," International Symposium on Bovine Mastitis, National Mastitis Council and American Association of Bovine Practitioners, Indianapolis, IN, September 13-16, 1990.

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3. R.E.Hornish, J.M.Nappier, F.S.Yein, M.J.Zaya, M.H.Yurkanin, "Absorption, Distribution, Metabolism, and Excretion of ^{14}C-Pirlimycin Hydrochloride (U-57930E) in the Lactating Dairy Cow. Part II. Metabolite Profiles," Upjohn Technical Report 782-9760-88-002, 6 January 1989.

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COMMON NAME---------- PIRLIMYCIN
UPJOHN ID NUMBER---------- P-000000-77-4

MSDS RECIPIENT---------- AG. R&D - AN.HLTH.CLIN.DEV.
UNIT 9690-190-00

MANUFACTURER---------- THE UPJOHN COMPANY
7171 PORTAGE RD
KALAMAZOO, MI 49001

EMERGENCY TELEPHONE------- 616-323-7555 (8:00 A.M. - 4:30 P.M.)
616-323-6722 (24 HOURS)

SECTION 1 - MATERIAL IDENTIFICATION

COMMON NAME---------- PIRLIMYCIN
UPJOHN ID NUMBER---------- P-000000-77-4

SYNONYMS---------- EDP NUMBER - 268132
PIRLIMYCIN HYDROCHLORIDE
U-57,930E
VOLCANOMYCIN HCL HYDRATE

MOLECULAR FORMULA--------- C17-H32-CL-N2-O6-P5
CHEMICAL FAMILY---------- ANTIBIOTIC

SECTION 2 - PHYSICAL DATA

APPEARANCE---------- WHITE AMORPHOUS POWDER
MELTING RANGE---------- 210.5 - 212.5 C WITH DECOMPOSITION
ODOR---------- ODORLESS
SOLUBILITY IN WATER------- WATER SOLUBLE, SOLUBLE IN DIMETHYL FORMAMIDE, METHANOL

SECTION 3 - FIRE AND EXPLOSION DATA

FLASH POINT---------- NOT ESTABLISHED
METHOD---------- NOT ESTABLISHED
FLAMMABLE LIMITS
LEL---------- NOT ESTABLISHED
UEL---------- NOT ESTABLISHED

SECTION 4 - REACTIVITY

STABILITY---------- STABLE.
HAZARDOUS DECOMPOSITION PRODUCTS-- NONE.
HAZARDOUS POLYMERIZATION-- WILL NOT OCCUR.

SECTION 5 - HEALTH HAZARD

PERMISSIBLE EXPOSURE LIMIT-NOT ESTABLISHED (29 CFR 1910).
THRESHOLD LIMIT VALUE----- NOT ESTABLISHED (ACGIH, 1984-85).
UPJOHN EXPOSURE GUIDELINE- NOT ESTABLISHED (1985).
EFFECTS OF OVEREXPOSURE--- NOT ESTABLISHED; MAY CAUSE SKIN AND EYE IRRITATION OR ALLERGIC SKIN REACTION.
TOXICITY---------- ORAL LD50 (RAT): 2524 MG/KG.
IM (RABBIT): SEVERE IRRITATION.
OCULAR (RABBIT): SEVERELY IRRITATING.
DERMAL (RABBIT): MODERATELY IRRITATING.
AMES TEST: NOT MUTAGENIC.
V79 ASSAY: NOT MUTAGENIC.
MICRONUCLEUS TEST: NOT CLASTOGENIC OR MUTAGENIC.
MEDICAL CONDITIONS
AGGRAVATED BY EXPOSURE---- HYPERSENSITIVITY TO PIRLIMYCIN.

COMMON NAME---------- PIRLIMYCIN
UPJOHN ID NUMBER---------- P-000000-77-4

SECTION 6 - FIRST AID

EMERGENCY AND FIRST AID PROCEDURES
INHALATION---------- REMOVE TO FRESH AIR.
EYES---------- FLUSH WITH WATER FOR 15 MINUTES.
SKIN---------- WASH WITH SOAP AND WATER.

SECTION 7 - SPILL, LEAK AND DISPOSAL PROCEDURES

STEPS TO BE TAKEN IN CASE MATERIAL IS RELEASED OR SPILLED-- VACUUM OR SWEEP UP SPILLED MATERIAL AND PLACE IN CONTAINER.
WASTE DISPOSAL METHOD----- INCINERATE OR SANITARY LANDFILL. DISPOSE OF IN ACCORDANCE WITH FEDERAL, STATE AND LOCAL REGULATIONS.

SECTION 8 - SPECIAL HANDLING

RESPIRATORY PROTECTION---- APPROVED RESPIRATOR.
VENTILATION---------- LOCAL EXHAUST: RECOMMENDED.
PROTECTIVE GLOVES---------- RUBBER.
EYE PROTECTION---------- SAFETY GLASSES.
OTHER PROTECTIVE EQUIPMENT-- COVERINGS FOR OTHER EXPOSED AREAS OF SKIN.

SECTION 9 - SPECIAL PRECAUTIONS

PRECAUTIONS TO BE TAKEN IN HANDLING AND STORING-- AVOID CONTACT WITH EYES, SKIN AND CLOTHING. WASH THOROUGHLY AFTER HANDLING.

SECTION 10 - CHEMICAL NAME

(2S-CIS)-7.CHLORO-6,7,8-TRIDEOXY-6-{{4-ETHYL-2-PIPERIDINYL)CARBONYL)AMINO}-1-THIO-L-THREO-ALPHA-D-GALACTO-OCTOPYRANOSIDE HYDROCHLORIDE HYDRATE

SECTION 11 - PRODUCT INGREDIENTS

PIRLIMYCIN HYDROCHLORIDE-- 5 MG/ML.
BENZYL ALCOHOL---------- 94.5 MG/10 ML.
CITRIC ACID POWDER-------- 105 MG/10 ML.
CARBOXYMETHYLCELLULOSE---- 200 MG/ML.
SODIUM HYDROXIDE-WATER SOLUTION-- Q. S.

SECTION 12 - MSDS PREPARATION INFORMATION

REVISED BY---------- RONALD J. TRZOS
ENVIRONMENTAL SERVICES
MARCH 9, 1987

SECTION 13 - UPJOHN DISCLAIMER

THE ABOVE INFORMATION IS BELIEVED TO BE CORRECT BUT SHOULD ONLY BE USED AS A GUIDE. UPJOHN DISCLAIMS ANY EXPRESS OR IMPLIED WARRANTY AS TO THE ACCURACY OF THE ABOVE INFORMATION AND SHALL NOT BE HELD LIABLE FOR ANY DIRECT, INCIDENTAL OR CONSEQUENTIAL DAMAGES RESULTING FROM RELIANCE ON THE ABOVE INFORMATION.