Environmental Assessment

1. **Date:** June 7, 1984

2. **Applicant:** The Upjohn Company

3. **Address:** Kalamazoo, Michigan 49001

4. **Description of the proposed action:**

Lincomycin will be added to the diets of growing-finishing swine at a level of 20 grams per ton for increased rate of weight gain. This action will increase the efficiency of pork production. Lincomycin is manufactured at two locations; Portage, Michigan in an industrial complex of The Upjohn Company located in a semi-rural area and in an industrial complex located in a semi-rural area near Arecibo, Puerto Rico.

Growing-finishing swine fed lincomycin are located primarily in the ten mid-west corn-belt states. The vast majority of these swine are raised in rural areas under conditions of confinement rather than on pastures or open fields. Floors of confinement facilties are generally paved concrete or slatted. Resultant wastes may be handled as liquids or solids and are eventually distributed on crop land for the fertilizer value.

5. **Introduction of substances into the environment:**

Manufacture

Information relative to the manufacture of lincomycin by formulation and manufacture of the premixes are covered in three attachments regarding these activities as follows:

Attachment -

A. Lincomycin hydrochloride Ag Grade
Environmental Impact Analysis Report
(for Kalamazoo, MI - Feb. 15, 1984)

B. Lincomycin Hydrochloride Ag Grade
Environmental Impact Analysis Report
(for Barceloneta, PR - Feb. 15, 1984)

C. Lincomix® 20 Premix
and
Lincomix® 50 Premix
Environmental Impact Analysis Report
(for Kalamazoo, MI - Jan. 1, 1984)

Animal Use

Lincomycin will enter the environment at the use site via hog wastes. The number of swine marketed in the United States each year is about 80 million. The estimated number of hogs that will receive lincomycin for growth promotion is approximately 3.25% or 2.60 million. For growth promotion hogs will be fed lincomycin at a level of 20 grams per ton of feed from weaning to market weight, for a total maximum gain of about 200 pounds per hog. Two hundred pounds of gain will require about 600 pounds of feed containing a total of six grams of lincomycin (600 pounds of feed x 10 mg lincomycin per pound). Assuming no inactivation of lincomycin in the intestinal tract of the hog, 2.6 million hogs could excrete 15,600 kg of lincomycin in their wastes. The major site area where this lincomycin will enter the environment is the ten corn-belt states where the vast majority of hogs in the U.S. are raised. The majority of commercially raised hogs are fed in confinement facilities (as opposed to pasture or open fields). In confinement facilities floors are generally paved concrete or slatted and resultant wastes are collected and distributed on crop land for the fertilizer value.

6. Fate of emitted substances in the environment:

Updated information regarding this subject for both manufacture of lincomycin by fermentation and manufacture of the Lincomix premixes is covered in the three submitted reports indicated under item 5 above. Based on information previously submitted under NADA 97-505 by The Upjohn Company (EIAR - for swine dysentery February 5, 1975, EIAR - for mycoplasmal pneumonia April 14, 1980, EIAR amendment - for chicken feeds April 28, 1978) it was stated in the FONSI report of October, 1980 under Manufacture of Lincomycin that "the manufacture of the lincomycin premixes do not have a significant impact on the quality of the environment when produced according to the procedures described in the application".

Regarding the use of lincomycin in swine, the above cited FONSI report of October, 1980 sets forth a "worst case analysis" which involves lincomycin concentrations in soil and water from paved feeder-pig lots that do not utilize run-off collection or treatment of wastes. Based on these same assumptions, parameters, and calculations the concentrations of lincomycin in the run-off and in agricultural soils would be 1.2 ppm and 0.16 ppm, respectively from swine fed the 20 gram level of lincomycin for growth promotion. These amounts are only half of what was projected for swine fed the 40 gram level of lincomycin (Case #1 - least severe) and as such should be of no concern from an environmental standpoint. This conclusion is reinforced based on the FONSI report, involving higher feeding levels of lincomycin, and which was stated in the conclusions that "Simple dilution and inactivation of the antimicrobial properties of the drug residues in the field and in receiving waters appear to preclude long-term irreversible environmental effects".

The FONSI report of October, 1980 did request additional information on lincomycin and studies were conducted by The Upjohn Company to satisfy this request. Following is a listing of the Technical Reports of the studies with a condensation of the results and conclusions:

I. Physical and Chemical Properties of Lincomycin Hydrochloride (U-10,149A) Technical Report No. 524-9760-83-006. K.T. Koshy. May 16, 1983. (Appendix Tab A).

1. Description

1.1 Name: Lincomycin hydrochloride, U.S.P.

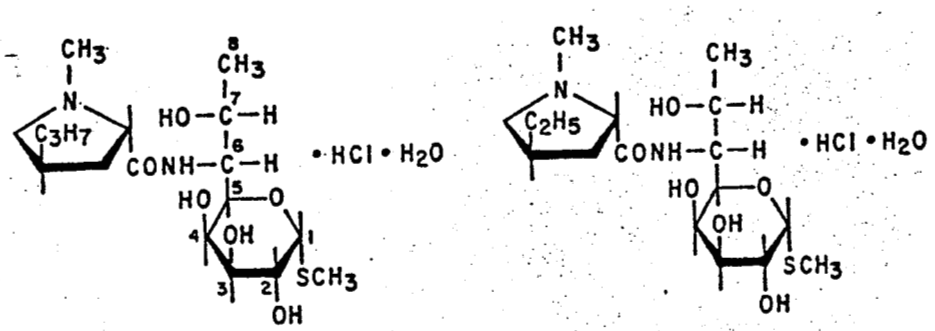
Chemical Name: D-erythro-a-D-galcato-Octopyranoside, methyl 6,8-dideoxy-6[[(1-methyl-4-propyl-2-pyrrolidinyl) carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2s-trans)-.

Methyl 6,8-dideoxy-6-(1-methyl-trans-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-erythro-a-D-galacto-octopyranoside monohydrochloride monohydrate.

1.2 Formula and Molecular Weight

Crystalline lincomycin is obtained as the hydrochloride monohydrate by the addition of acetone to an aqueous-hydrochloric acid solution of lincomycin. The USP XX specifies that it has a potency equivalent to not less than 790 µg of lincomycin base ($C_{18}H_{34}N_2O_6S$) per mg of the hydrochloride monohydrate.

SCHEME I



LINCOMYCIN A LINCOMYCIN B

Lincomycin hydrochloride USP may contain the 4-ethyl analog on the pyrrolidine ring as an impurity which is designated as lincomycin B. The USP XX specifies that it contains not more than 5% of lincomycin B.

$C_{18}H_{34}N_2O_6S.HCl.H_2O$ F.W. = 461.01

1.3 Appearance, Color and Odor

Lincomycin hydrochloride is a white or practically white, crystalline powder. It has a characteristic pungent odor.

2. Physical Properties

2.1 Spectra

2.11 Ultraviolet Spectra.
The ultraviolet spectrum of a 0.0217 M solution of lincomycin hydrochloride monohydrate in water displayed a very high end absorption from 260 NM down to 233 NM with no characteristic peaks or valleys.

2.12 Infrared Spectra.
A mineral oil mull of lincomycin hydrochloride monohydrate showed significant infrared absorption bands characteristic for alcohols, secondary amide and oyclic ethers. Table I shows assignments for the significant infrared absorption bands:

Table I. Infrared Band Assignments for Lincomycin Hydrochloride, Monohydrate

Wave numbers (cm^{-1})	Structural Feature	Assignment
3529, 3489, 3453, 3380, 3339, 3290, 3228, 3199, 3076 3046, 3023	Alcohols and secondary amide	O-H stretch and N-H stretch
2751 broad	Amine sale	N-H stretch
1658	Secondary amide	C=O stretch
1567	Secondary amide	Amide II
1107, 1092, 1077 1042	Alcohols and cyclic ether	C-O stretch

2.13 NMR Spectra and 2.14 Mass Spectra.
Those two spectra further confirm the chemical structure and configuration of lincomycin hydrochloride monohydrate shown above (see 1. Description).

2.2 Crystal Properties

2.21 Melting Range
Lincomycin hydrochloride melts with decomposition at about 148°C.

2.22 Polymorphs
Lincomycin hydrochloride exists in two polymorphic forms(1). As prepared commercially, the monohydrate is the predominant species and is designated as Form II: Form I contains varying amounts of water. Both can be rendered anhydrous by drying. The two forms retain their particular infrared characteristics in the anhydrous state. Form II is thermodynamically more stable than Form I. It also has greater bulk density. (See section 2.24 for X-ray diffraction patterns of the two forms.)

2.23 Thermal Analysis

Differential Scanning Calorimetric (DSC) and thermgravimetric analysis (TGA) curves for lincomycin hydrochloride are shown in Figures 7 and 8 (Appendix A) respectively[2]. The curves were generated from a DuPont thermal analyzer (Model No. 1090, DuPont De Nemours and Co., Wilmington, Delaware). The sample was contained in aluminum pans and the analysis was conducted under an atmosphere of nitrogen. The heating rate for the DSC and TGA curves were 2° and 5°C/min respectively. The long shallow endotherm in the DSC curve from about 136-145°C is probably associated with release of water. The melting endotherm in the DSC peaks at 152.2°C. The TGA curve indicates gradual loss of water of crystallization and also a crystalline transition stage. The compound appears to lose all water before the beginning of the melting endotherm after which it undergoes decomposition.

2.24 X-Ray Diffraction

Figures 9 and 10 (Appendix A) are X-ray diffraction patterns[3] of crystalline lincomycin hydrochloride forms I and II respectively. With the aid of the X-ray diffraction patterns of the two forms containing varying amounts of water and their infrared spectra, the authors were able to establish conditions under which the transition from one form to the other takes place. Transitions in the X-ray diffraction pattern of Form I appeared at about the 4% water level, showing a definite shift which could be attributed to larger interplanar spacings as the water level is increased. Between 0.67% and 3.34% water the patterns were identical and were characterized by major peaks at 5.55° and 11.20° 2θ, corresponding to d = 15.9Å and 7.89Å. At 6.66% water the X-ray diffraction pattern was different, showing a shift of these peaks to 5.05° and 10.30° 2θ corresponding to d= 17.48Å and 8.58Å. At 3.83% and 5.35% water combinations of the two patterns were found. The infrared spectrum of Form I also changed with water content. These changes were not as readily discernable as those observed by X-ray, and indicated hydrogen bonding to have resulted in band broadening at higher water contents.

Table IV shows the X-ray diffraction patterns of the two forms of lincomycin hydrochloride.

Table IV. Powder X-Ray Diffraction Data of Lincomycin Hydrochloride Polymorphs.

Form I			Form II		
2θ	d-spacing(A)	Intensity*	2θ	d-spacing(A)	Intensity*
5.55	15.92	1	6.30	14.03	2
6.40	13.81		8.50	10.40	
6.90	12.81	4	9.45	9.36	5
7.45	11.87		10.35	8.55	
11.15	7.94	2	12.75	6.94	4
13.00	6.81		14.15	6.26	1
13.95	6.35	5	15.00	5.91	
14.40	6.15		15.70(w)	5.64	
17.00	5.22	3	15.95(w)	5.56	

Table IV. Powder X-Ray Diffraction Data of Lincomycin Hydrochloride Polymorphs. (continued)

Form I			Form II		
2θ	d-spacing(A)	Intensity*	2θ	d-spacing(A)	Intensity*
17.85	4.97		16.80	5.28	
18.40(b)	4.82		17.25	5.14	
19.40	4.58		17.85	4.97	
20.15	4.41		18.25(w)	4.86	
21.05	4.22		19.15	4.63	
21.95(sh)	4.05		19.80	4.48	
22.35	3.98		21.00	4.23	
23.15	3.84		21.55	4.12	
23.95	3.72		22.00	4.04	
25.80	3.45		22.85	3.89	
27.10	3.29		24.35	3.65	
			25.40	3.51	
			25.80	3.45	
			26.35	3.38	
			27.75	3.21	
			29.20	3.06	
			29.60	3.02	
			30.80	2.90	

Note: b = broad
w = weak
sh = shoulder

*Five strongest peaks (1 = the most intense peak)

d-spacing Å = $\left(\frac{n\lambda}{2 \sin \theta}\right)$

(1) Struck, W. A. Internal communication, The Upjohn Company, Kalamazoo, Michigan 49001.

(2) Bergren, M. S. Personal communication, The Upjohn Company, Kalamazoo, Michigan 49001.

(3) Knuth, M. D. and Zipplem, K. Internal communication, The Upjohn Company, Kalamazoo, Michigan 49001.

2.3 Solubility

Lincomycin hydrochloride is extremely water soluble. It forms a syrup with water and the solubility is estimated to be between 500-1000 mg/ml[1]. The solubilities of lincomycin hydrochloride and several other antibiotics were determined by Marsh and Weiss[2] in a number of solvents. Their data are shown in Table V.

Table V. Solubilities of Lincomycin Hydrochloride in Common Organic Solvents (From Ref. 2)

Solvent	Solubility (mg/ml)*
Methanol	>20
Ethanol	>20
Isopropanol	4.83
Isoamyl alcohol	1.06
Cyclohexane	0.02
Benzene	0.08
Petroleum ether	0.01
Isooctane	0.02
Carbon tetrachloride	0.02
Ethyl acetate	0.03
Isoamyl acetate	0.05
Acetone	0.07
Methyl ethyl ketone	0.03
Diethyl ether	0.01
Ethylene chloride	0.01
1,4-Dioxane	1.37
Chloroform	0.06
Carbon disulfide	0.03
Pyridine	>20
Formamide	>20
Ethylene glycol	>20
Propylene glycol	>20
Dimethyl sulfoxide	>20

206

*The experimental design was such that if all the material appeared to be in solution, the solubility was considered to be greater than 20 mg/ml.

(1) Forist, A. A. Internal communications, The Upjohn Company, Kalamazoo, Michigan.

(2) Marsh, J. R., and Weiss, P. J., Assoc. Office of Anal. Chem., 50, 457 (1967).

2.4 Partition Coefficient

The octanol/water partition coefficient at pH 2, 7 and 9 and between water and a few other solvents are shown in Table VI.

Table VI. Partition Coefficient of Lincomycin Hydrochloride Between Water and a Few Organic Solvents.

Solvent Pair	P.C. $C_{organic}/C_{water}$
Butanol:water (pH 10)[1]	2.5
CH_2Cl_2:water (pH 9.9)[1]	0.38
Butyl acetate:water (pH 9.6)[1]	0.19
Methyl ethyl Ketone:water (pH 9.6)[1]	0.77
n-octanol:water (pH 2)[2]	0.0031
n-octanol:water (pH7)[2]	2.55
n-octanol:water (pH 9, Borate)[2]	0.20
n-octanol:water (pH 9, THAM (tris))[2]	2.98

(1) Forist, A. A. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

(2) Koshy, K. T. and Knuth, D. W. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

2.5 Ionization Constant, pK

The pH of a 1% solution of production lots of lincomycin hydrochloride in water is in the range 4.7-4.9. It has a pKa of 7.6.

2.6 Optical Rotation

The USP XX specifies that lincomycin hydrochloride has a specific rotation between +135° and +150° in an aqueous solution containing 20 mg per ml, calculated on the anhydrous basis.

3. Chemical Stability

3.1 Modes of Degradation

Vigorous acid hydrolysis of lincomycin was performed by Herr and Slomp[1]. Two products were isolated; methyl mercaptan, isolated and identified as its 2,4-dinitrophenyl thioether, and an amino acid identified as n-propylhygric acid. Milder hydrolysis using hydrazine hydrate under reflux conditions efficiently cleaved the amide bond[2] without destroying the stereochemistry of the sugar moiety. The resulting compounds were identified as L-trans-4-n-propylhygric acid and methyl 6-amino-6,8-dideoxy-1-thio-D-erythro-α-D-galacto-octopyranoside (Structure V, Scheme II)[3,4] on page 10 of this report.

3.2 Stability in Aqueous Solution

Forist and Royer[5] have reported the stability of lincomycin hydrochloride at 70° in 0.1 N HCl and in 0.1 N NaOH. The degradation in both instances followed pseudo-first-order kinetics and the calculated half-lives were 40 and 25 hours in the acid and base respectively. Forist et al.[6] also studied the stability of lincomycin hydrochloride in 0.1 N HCl at 70° and at 37°. There was no degradation at 37° for at least 48 hours. The half-life at 70° was 39 hours. The principal degradation products were methyl mercaptan and 1-dethiomethyl-1-hydroxylincomycin.

Clindamycin hydrochloride is a synthetic analog of lincomycin hydrochloride in which the 7-position hydroxyl group is replaced by a chlorine atom. Oesterling[7] has reported a detailed study of the aqueous stability of clindamycin hydrochloride in the pH range 0.44-11.66. From the results of this study and the ones reported earlier (1-6), the following conclusions depicted in Scheme II (page 10) may be postulated regarding the stability and mode of degradation in aqueous solutions:

1) Lincomycin hydrochloride solutions adjusted to pH 1-6 are stable at room temperature.

2) The major degradation in buffers pH 0.4-4 at elevated temperatures is via the thioglycoside hydrolysis to form 1-dethiomethyl-1-hydroxylincomycin (Structure IV) and methyl mercaptan (Structure III).

3) The degradation is minimal in the pH range 3-6.

4) Above pH 9, the degradation is predominantly via the amide linkage producing (Structure II) and (Structure V).

(1)Herr, H. R., and Slomp, G., J. Amer. Chem. Soc., 89, 2444 (1967).

(2)Schroeder, W., Bannister, B., and Hoeskma, H., Ibid, 89, 2448 (1967).

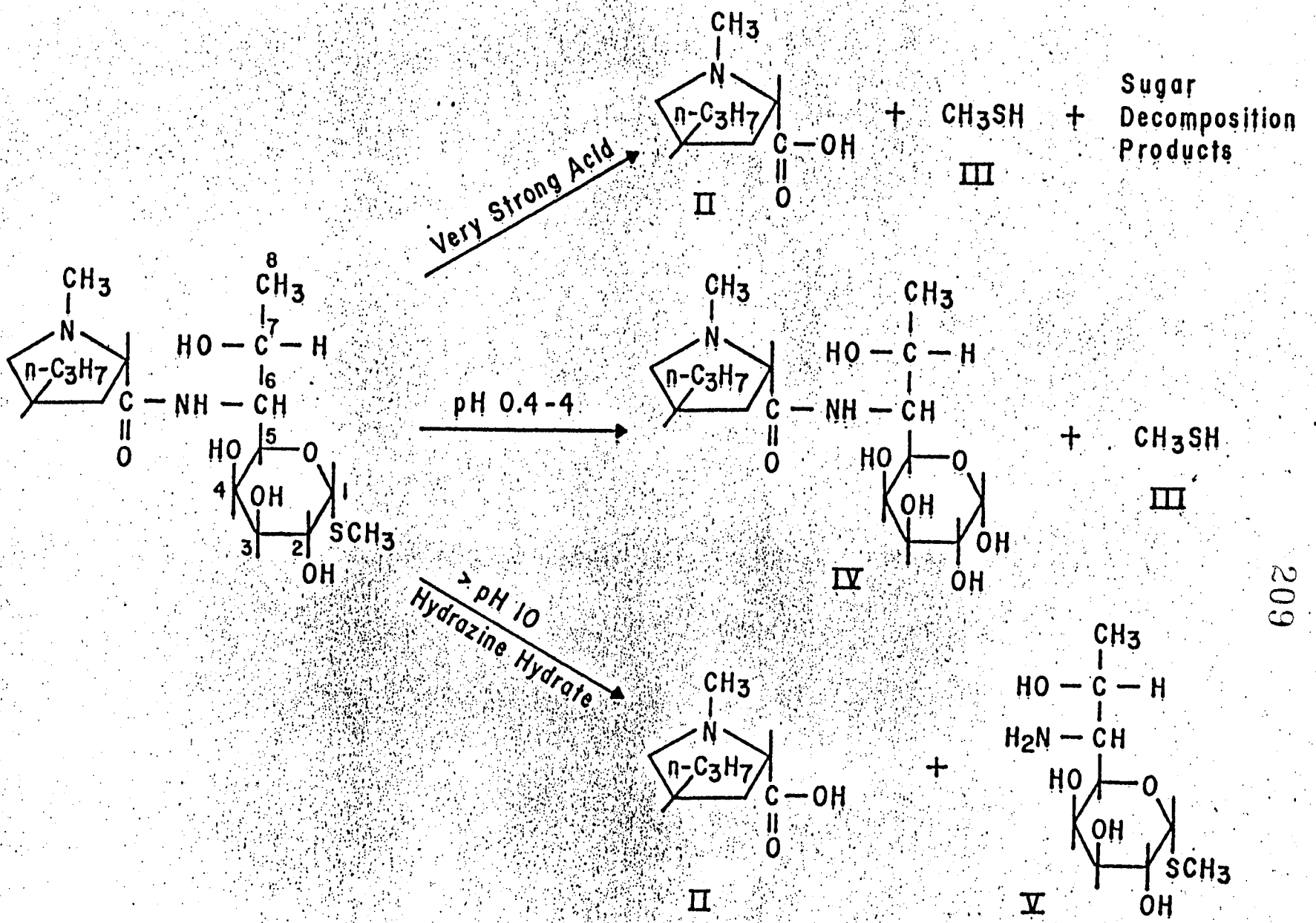
(3)Slomp, G., and MacKeller, F. A., Ibid, 89, 2454 (1967).

(4)Magerlein, B. J., Birkenmeyer, R. D., Herr, H. R. and Kagan, F., Ibid, 89, 2459 (1967).

(5)Forist, A. A., Royer, M. E. Internal communication, The Upjohn Company, Kalamazoo, Michigan 49001.

(6)Forist, A. A., Brown, L. W., and Royer, M. E., J. Pharm. Sci. 54, 476 (1965).

(7)Oestering, T. O., Ibid, 59, 63 (1970).



Scheme II. Chemical Degradation Pathways of Lincomycin

4. Methods of Analysis

Information under this title can be found in the Appendix Tab A (Technical Report No. 524-9760-83-006 pages 17-25).

5. Metabolism and Pharmacokinetics

In preliminary reports on the absorption and excretion of lincomycin HCl in man and rats, Lewis and Meyer[1] reported the following observations: 1) Lincomycin is solely absorbed from the small intestine, 2) about 35-40% of the administered oral dose is excreted in the feces after 12 hours, 3) the antibiotic is not degraded by stomach acidity, gastric enzymes or by bacterial action in the caecum or large intestine.

Vavra et al.[2] have reported on the absorption and excretion of lincomycin HCl in normal adult human volunteers after oral, intramuscular and intravenous routes of administration. Lincomycin given orally as a single 500 mg dose to 50 normal adults produced an average serum concentration that peaked at 4 hours at 3.4 ± 0.4 µg/ml and remained at or about 1.1 ± 0.1 µg/ml for at least 12 hours. In oral, multiple-dose studies (500 mg every 6 hours), lincomycin serum levels did not appear to build up with time. High serum levels of 5.7 ± 1.2 µg/ml were obtained within 4 hours after the first dose with subsequent nadir values ranging between 2.4 and 3.6 µg/ml for 174 hours, the entire duration of the study.

With single intramuscular doses of 100, 200 and 600 mg, the following respective peak levels were obtained within the first hour after dosing: 2.7, 3.8 and 11.6 µg/ml. In the case of the 600 mg dose, detectable amounts of lincomycin were present in sera from 18 of the 20 subjects as late as 24 hours after dosing. When 600 mg was administered every 8 hours, high concentrations of the antibiotic were present in the serum for 97 hours, the duration of the study.

The authors also reported serum levels after single and multiple 300 and 600 mg dose intravenous administration. With the 300 mg dose infused every 12 hours for 74 hours, the high level was 9.5 µg/ml and the low level 1.6 µg/ml with essentially no accumulation of the antibiotic in the serum. However, at 600 mg every 6 hours, the average high level was 17.5 µg/ml and the low level 8.2 µg/ml during a 74-hour period. The urinary excretion from the single and multiple oral dose serum level studies decribed above was 3 to 5% of the dose after 24 hours. Higher urine recoveries were seen after parenteral administration of the antibiotic.

Eberts et al.[3] studied the fate of tritium-labeled lincomycin in man and has postulated a kinetic model for its metabolism and excretion. ^{3}H-lincomycin HCl was administered to two panels of five subjects each. The oral dose was a single 500 mg capsule including 250 µCi of ^{3}H-lincomycin HCl. The I.M. dose was 2 ml of a 300 mg/ml solution containing 50 µCi of ^{3}H-lincomycin HCl. Their conclusions were:

1) The mean peak-plasma level of 1.7 µg/ml (0.64-4.10) in the subjects was achieved within 2-4 hours. In the I.M. group the mean level was 10.5 µg/ml (8.2-12.9) achieved within 0.5-1 hour.

2) The mean recovery of the radioactive dose in the P.O. study was: urine, 8.6% (4.9-19.9); feces, 50.3% (13.6-78.9); total, 59.0% (18.5-84.4). In the I.M. study recovery was: urine, 55.3% (48.1-62.6); feces, 38.1% (36.6-40.3); total, 93.4% (86.3-99.5).

3) When lincomycin was administered either P.O. or I.M., it was excreted via urine and feces as unchanged lincomycin plus an inactive metabolite(s). However, since the plasma disappearance rate exceeded the combined urinary and fecal excretion rates, an additional lincomycin compartment was suggested.

   From these observations the kinetic model shown in Scheme III (on page 13 of this report) was developed utilizing the analog computer data simulator. It is proposed as the simplest model consistent with the experimental data.

4) This complicated transport mechanism permitted calculation of only a minimal plasma half-life of 6.67 ± 1.77 hr. The primary volume of distribution, instantaneously equilibrated with plasma, was estimated to be 42.4 ± 6.2 L. The volume of the secondary compartment could not be estimated.

5. The bulk of the I.M. dose was equilibrated instantaneously throughout the primary volume of distribution; however, a variable amount showed delayed absorption with an estimated maximal absorption half-time of 1.20 hr. Absorption of the P.O. dose appeared to be of an exponential-growth type and could not be described by simple first-order models. Paucity of data in this phase and the limited capacity of the analog computer precluded estimation of absorption half-time of the oral dose.

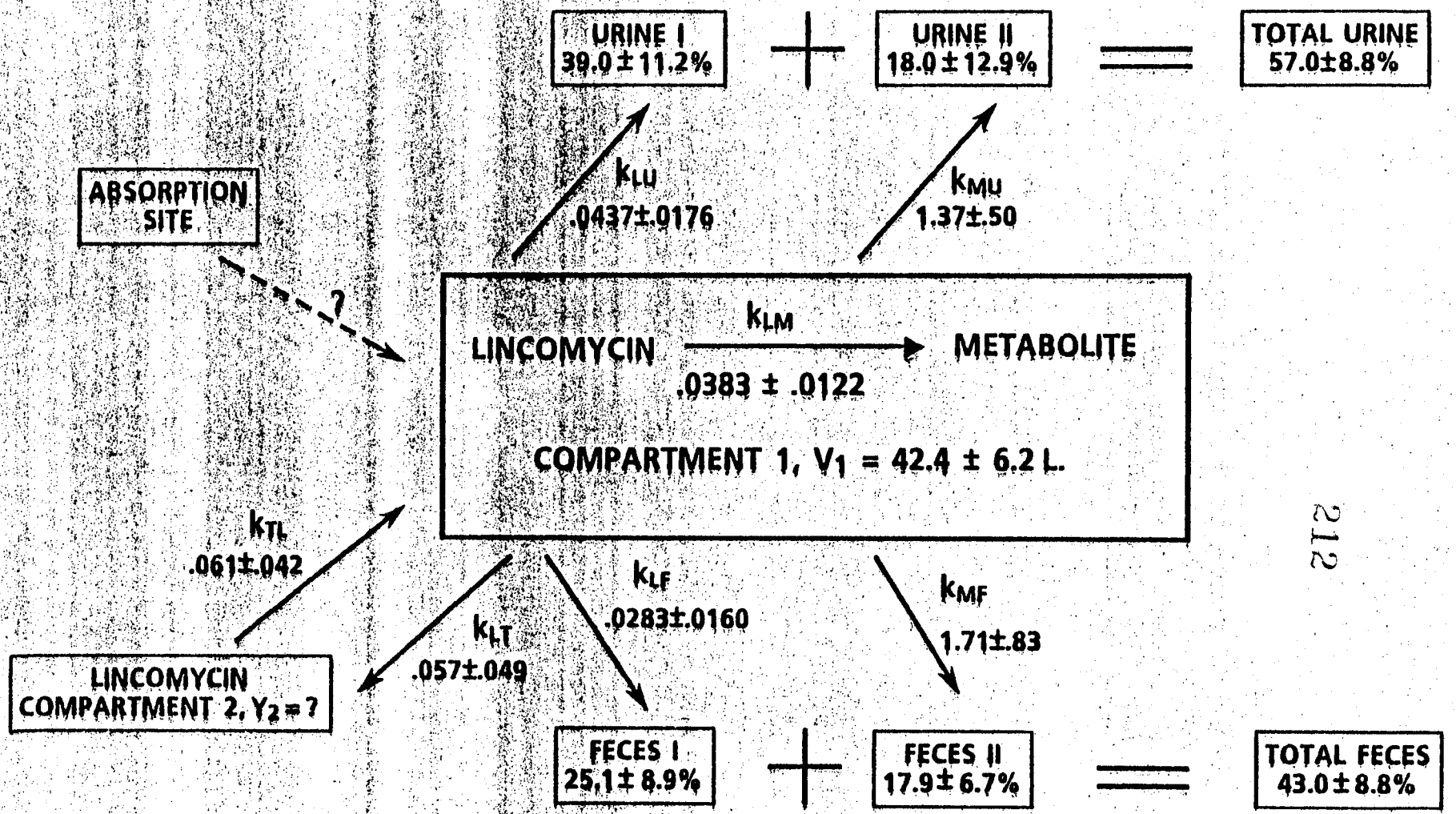
6. It was calculated that from 7-32% of the oral dose was absorbed. The absorption-efficiency distribution was variable but appeared to center around 7% and averaged about 10% with tailing to higher values. Thus, the results of this study were comparable to the results of earlier clinical studies.

7. Although the percent of the absorbed dose excreted in urine (51-66%) vs. feces (34-49%) was relatively constant, the amount of lincomycin vs. metabolite in either urine or feces was highly variable from subject to subject.

In a subsequent report on the characterization of the urinary excretion products in dog and man, Eberts and Meeks[4] have made the following conclusions; the primary urinary excretory product of lincomycin administered orally or intramuscularly to dog or man is unmetabolized drug. In the dog, this amounted to 74% (P.O.) and 80-85% (I.M.) of the faction of the dose found in urine, and 11% (P.O.) and 33-45% (I.M.) of the administered dose. Comparable figures for man were 56% (P.O.) and 83% (I.M.) based on urinary excretion, and 8% (P.O.) and 49% (I.M.) based on the administered dose. Although none of the metabolites were fully characterized, they possessed little or no bioactivity.

The above findings were confirmed by Daniels and Van Eyk[5] in a dog metabolism study using ^{14}C-lincomycin HCl. However, they had evidence to suspect that lincomycin sulfoxide and N-demethyl lincomycin to be minor metabolites (<3% of the dose).

THE RA : AND EXTENT OF TRANSPORT OF LINCOMYCIN



Scheme III.

(1)Lewis, C., and Meyer, C. E. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

(2)Vavra, J. J., Sokolski, W. T. and Lawson, J. R. Antimicrobial Agents Chemotherapy, 176 (1963).

(3)Eberts, F. S., Jr., Baker, R. H., Jr., Meeks, R. C., and Vliek, R. W. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

(4)Eberts, F. S., Jr., and Meeks, R. C. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

(5)Daniels, E. G., and Van Eyk, R. L. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

II. **Determination of the Octanol-Water Partition Coefficient of Lincomycin HCl at pH 2, 7 and 9.**
Technical Report No. 524-9760-83-001. D. W. Knuth and K. T. Koshy. April 6, 1983. (Appendix Tab B).

The K_{OW} value of lincomycin-HCl monohydrate is pH dependent. The mean K_{OW} values obtained at the pH values tested are:

pH2 = 0.003
pH7 = 2.55
pH9 = 0.201 in borate buffer
pH9 = 2.98 in Tris-buffer

These values indicate that lincomycin-HCl preferentially transfers to the aqueous phase. The K_{OW} values can be used to project the potential extent of accumulation of lincomycin-HCl in a tissue or an organism by calculating the bioconcentration factor (BCF) according to the following equations:

(1) For flowing water ecosystems (Voerman, 1969):
$\log BCF = 0.124 + 0.542 \log K_{OW}$.
(2) For static water ecosystem (Fujita et al. 1954):
$\log BCF = 0.7235 + 0.635 \log K_{WO}$.

Using the above two equations, lincomycin-HCl potential bioaccumulation in tissues of aquatic animals can be calculated to be:

			Bioaccumulation Factor in Ecosystems	
pH	K_{OW}	$\log K_{OW}$	flowing	Static
2.0	0.003	-2.52	0.057	0.134
7.0	2.550	0.406	2.208	9.690
9.0 (borate)	0.201	-0.696	0.558	1.934
9.0 (Tris)	2.980	0.474	2.404	10.703

It has been considered that if the bioconcentration factor is 1000 or higher, then the bioaccumulation of the chemical in the environment is a matter of concern. A factor value of 100 to 1000 suggests that the bioaccumulation of the chemical or drug may be important when considered along with environmental persistence, mobility, and toxicity of the chemical. A value of less than 100, indicates that the significant bioaccumulation of the chemical in the environment is unlikely to occur.

From the calculated bioaccumulation factors, lincomycin is not expected to bioaccumulate to any significant amounts in the tissues of environmentally exposed organisms. Although other properties of lincomycin-HCl such as metal chelation, deposition in bones...etc. might seriously affect the projected bioaccumulation potential of lincomycin-HCl in tissues, available evidence does not support this possibility.

References

Voeman, S. (1969). In: Bulletin of Environmental Contamination and Toxicology. Vol. 4, pp. 64-67.

Fujita, T., Iwasha, J. and Bansch, C (1964). J. Amer. Chem. Soc. 86, 5175-5180.

III. Vapor Pressure of Lincomycin Hydrochloride.
K. Thomas Koshy. October, 1982 (Appendix Tab C).

While not determined experimentally, supportive evidence suggests that lincomycin hydrochloride has a vapor pressure value of less than $5X10^{-4}$ torr at ambient temperature (21-22°C) and pressure. Based on the phsyical/chemical properties of the antibiotic, it is adequate to expect that lincomycin hydrochloride will have no significant vapor pressure at ambient temperatures.

IV. Sorption/Desorption of U-10,149A (Lincomycin) in Three Soil Types at 0.2, 1.0, 5.0 and 25 mg/liter.
Technical Report No. 524-9760-83-002. D. B. Johnson and B. L. Cox. March 21, 1983. (Appendix Tab D).

(1) Approximately 30-50% of lincomycin-HCl was sorbed by the three tested soils. The soils tested were: sandy clay loam, clay, and clay loam. The soils were spiked with lincomycin-HCl at 25 ppm.

(2) About 6 hours were required for the antibiotic to reach soil/water equilibrium in all three soils.

(3) Approximately 40-60% of the sorbed lincomycin HCl could be desorbed from the tested soils.

(4) the K_{OW} coefficients ranged from 0.12 to 1.59 for the three soils.

(5) The very low K_{OW} values coupled with the high water solubility of lincomycin-HCl indicate that the antibiotic would not be appreciably sorbed to soil. Therefore, lincomycin-HCl would be expected to leach from the tested soils.

(6) During the course of the sorption/desorption study, preliminary evidence indicates that about 10% of the sorbed lincomycin-HCl might be metabolized, in soil, to a more polar compound. This polar compound is not readily leached from the tested soils and does not possess antimicrobial activity.

V. **Proposed Degradation of Lincomycin in Soil.**
R. E. Hornish. March 23, 1983. (Appendix Tab E).

In a study designed to demonstrate the eco-fate of lincomycin under normal use conditions, manure (feces + urine) from a pig fed a diet which contained 100 g of lincomycin per 907 kg was added to a Michigan clay loam soil at a concentration equal to normal manure application rates. After mixing in the soil, and assay of the soil for lincomycin, within one day no lincomycin could be detected. In addition, manure from a pig fed a diet which contained no lincomycin was added to this same soil type at the same application rate, but spiked with lincomycin at a concentration of 10 ppm, which is five times the expected application rate. In this latter study, only 20% remained after seven weeks, and all lincomycin was undetectable after 11 weeks.(1)

Based on data from this soil inactivation study it was calculated and reported in the FONSI report of October, 1980 that the half-life biological activity for lincomycin is equal to about 25.5 days.

(1)Lincomycin Degradaton in the Ecosystem: Research Report #524-9660-012, G. L. Stahl and M. J. DeGeeter, October 11, 19874. Submitted to NADA 97-505 Feburary 5, 1975.

VI. **Minimum Inhibitory Concentration (MIC) In Vitro for Lincomycin (U-10, 149A) Against Organisms Commonly Found in the Environment.**
Technical Report No. 524-9760-83-004. A. R. Barbiers. April 26, 1983. (Appendix Tab F).

The minimum inhibitory concentration (MIC) for lincomycin was determined *in vitro* against pure cultures of beneficial bacteria, fungi, and blue-green algae normally found in the environment. The MIC's were determined by the use of the agar plate dilution technique commonly used to test the susceptibility of pathogenic organisms to antimicrobial agents. The MIC's for each organism are listed below.

Table I. Minimum Inhibitory Concentration In Vitro for Lincomycin Against Tested Organisms

Test Organism	MIC mcg/ml
Aspergillus carbonarius, UC-1511	>1000.0
Chatomium cochliodes, UC-7217	>1000.0
Fusarium roseum, UC-7170	>1000.0
Penicillium notatum, UC-1296	>1000.0
Trichoderma viride, UC-4021	>1000.0
Streptomyces albus, UC-2043	>1000.0

Table I (continued)

Test Organism	MIC mcg/ml
Psuedomonas fluorescens, UC-3049	>1000.0
Clostridium butyricum, UC-9385	1.56
Clostridium perfringens, UC-247	0.78
Clostridium perfringens, UC-6509	0.78
Cellulomonas sp., UC-6274	16.0
Arthrobacter globiformis, UC-3604	16.0
Flavobacterium heparinum, UC-6284	80.0
Cytophaga johnsonae, UC-9386	40.0
Bacillus subtillis (Difco)	12.0
Bacillus cereus (Difco)	12.0
Azobacter vinelandii, UC-3144	500.0
Nostoc sp., ATCC 27895	>1000.0

As discussed previously under Item 6 (Fate of emitted substances in the environment) a "worst case analysis" for lincomycin introduced into the environment through the proposed action, involves concentrations in soil and water from paved feeder-pig lots that do not utilize run-off collection or treatment of wastes. Under these conditions the concentrations of lincomycin in the run-off and in agricultural soils is projected to be 1.2 ppm and 0.16 ppm respectively. Comparing those values with the 18 MIC's in Table I (above), for various micro-organisms normally found in the environment, only two, (Clostridium perfringens - UC-247 and UC-6509), show MIC's for lincomycin below the 1.2 ppm level projected for the run-off. All 18 MIC's exceeded the 0.16 ppm of lincomycin that are projected to be contained in agricultural soils. From this it would appear that the antimicrobial effects of lincomycin introduced into the environment as a result of the proposed action would at worst be minimal or non-existent.

VII. Effect of Lincomycin (U-10,149A) on the Sulfur Transformation Test. Technical Report No. 524-9760-83-008. A. R. Barbiers and M. A. Clasby. August 12, 1983. (Appendix Tab G).

The effect of lincomycin on sulfur transformation was determined by a modified time-contact evaluation of various concentrations of lincomycin in contact with an anerobic sulfate-reducing bacteria, Desulfavibrio vulgaris subsp. vulgaris. Lincomycin-HCl tested at a concentration of 10 g/ml or less had no inhibitory effect on the activity and growth of the test organism. However, lincomycin-HCl concentrations of 50 g/mg and above were inhibitory. While no definite MIC value for lincomycin-HCl against the organism was established the data indicate that the MIC value falls between 10 and 50 g lincomycin per ml of culture. As discussed in Section VI above the projected "worst case" concentrations of lincomycin introduced into the environment for the proposed action would be 1.2 ppm and 0.16 ppm for run-off an agricultural soils, respectively. The results of this study indicates that the environmental levels of lincomycin are well below the most sensitive MIC value for this organism and as such should pose no environmental threat.

VIII. Effect of Lincomycin (U-10, 149A) on the Nitrogen Transformation Test. Technical Report. No. 524-9760-83-007. A. R. Barbiers and M. A. Clasby. July 12, 1983. (Appendix Tab G).

The results of this study indicates that lincomycin causes between 55 and 70% inhibition of the nitrogen transformation process based on total means at Day 22. Values obtained from lincomycin where levels ranged from 10-1000 ppm were similar, indicating there was no dose response relationship due to lincomycin concentration. On Day 8, the control differed significantly from all levels of lincomycin for both the total and cumulative ammonia production. On Day 17, the control was significantly different from all levels for the cumulative ammonia values.

From this study, there is an inconsistent, inhibitory effect (maximum of 70%) of lincomycin on the ammonification process. Most significant was the complete absence of a dose response correlation, as there were no statistically significant differences in the range of lincomycin concentrations from 10 ppm to 1000 ppm.

IX. Effect of Lincomycin (U-10,149A) on the Cellulose Decomposition Test. Technical Report No. 524-9760-83-005. A. R. Barbiers and M. A. Clasby. May 4, 1983. (Appendix Tab G).

The effect of lincomycin on the microbial decomposition of cellulose was determined by using a cellulase-producing organism (Trichoderma reesei, ATCC 26921) and measuring the evolved carbon dioxide.

Results from this study indicate that lincomycin at 500 ppm and 1000 ppm had no effect on the microbial decomposition of cellulose.

X. The Effect of Lincomycin in Soil on the Earthworm (Lumbricus terrestris). Technical Report No. 524-9760-83-003. T. S. Arnold, March 23, 1983. (Appendix Tab H).

The effect of lincomycin on the health state and survival rate of earthworms (Lumbricus terrestris) was determined by exposing earthworms to 1000 ppm lincomycin in a soil media for 28 days.

The results of this study indicated that lincomycin at 1000 ppm in a soil media had no deteramental effects on the survival or health state of earthworms.

7. The effects on the environment of released substances as a result of the proposed action:

The addition of 20 grams of lincomycin to each ton of complete feed to increase the rate of weight gain of swine, does not pose any harmful conditions to humans or other organisms within the ecosystem. At the sites of production of lincomycin hydrochloride and the ultimate use-level premixes, all environmental regulations, Federal, State and local, are adhered to in the manufacture and handling of the product and all

generated wastes. The production of lincomycin hydrochloride for swine usage, and especially for the proposed action, has little effect on the environment since this use represents only a small fraction of the total fermation production. A "worst case analysis" as set forth in item VI above, which considers the concentration of lincomycin in soil and water-run-off from paved swine feed lots which do not utilize a collection system, indicates that levels of .16 ppm and 1.2 ppm lincomycin respectively can be expected. Studies have been conducted to determine the chemical and physcial properties of lincomycin whereby these properties pertain to the fate of lincomycin in the environment following the proposed action. Conclusions from these studies:

a. The relatively low vapor pressure of lincomycin at ambient temperatures poses no significant harmful effect to air quality.

b. Lincomycin is highly soluble in water. However, the potential for bioaccumulation in the tissue of aquatic animals has been calculated over a water pH range of from 2.0 to 9.0 and the bioaccumulation factor has been established at approximately 10 or less for both flowing and static waters. A bioaccumulation factor of less than 100 indicates that the chemical is not likely to accumulate to any significant level in the environment.

c. The absorption/desorption potential of lincomycin in soils was found to reach a maximum of 50% sorption when the soils were spiked with 25 ppm lincomycin. About 6 hours was required for equilibrium to be reached between soil and water. As much as 60% of the sorbed lincomycin was desorbed in this test system. The n-octanol/water partition coefficient (K_{OW}) of lincomycin hydrochloride was found to be very low in this study (range 0.12 to 1.59). The high water solubility along with the very low K_{OW} of lincomycin indicates that the antibiotic would not be appreciably sorbed to soil and would be expected to be leached from soils very easily. This study provided some indication that about 10% of the sorbed lincomycin might be metabolized, in soil, to a more polar compound which is not readily leached from the tested soils, however, it possess no antimicrobial activity. Therefore, the low bioaccumulation factor and very low K_{OW} of lincomycin provides a high margin of safety to the terrestrial ecosystem as a result of the proposed action.

8. Utilization of natural resources and energy:

Pork production will be more efficient as a result of the proposed action by increasing the rate at which pigs gain weight. Therefore, there will be no increased demand on natural resources such as land, energy or water. There is not expected to be increased demands on natural resources as a result of the production of lincomycin hydrochloride (refer to manufacturing information previously described).

9. Disruptions of the physical environment:

There are no disruptions of the physical environment identified with the proposed action.

10. Mitigation measures:

Mitigating measures as a result of the proposed action does not apply.

11. Alternatives to proposed action:

No alternatives to the proposed action have been identified.

12. List of preparers:

R. E. Bloss, Ph.D., Animal Nutritionist
R. A. Evans, B.S., Animal Scientist
A. B. Spradling, Ph.D., Organic and Fermentation Chemist
J. C. Prue, B.S., Pharmacist
R. A. Amador, M.S., Mechnical Engineer License #5387
A. W. Neff, Ph.D., Analytical and Residue Chemist
C. J. Farho, D.V.M., Regulatory Affairs

13. Certification:

The undersigned official certifies that the information presented is true, accurate, and complete to the best of the knowledge of The Upjohn Company.

Charles J. Farho

Charles J. Farho, D.V.M., Manager,
Product Support and FDA Liaison

LINCOMYCIN HYDROCHLORIDE AG GRADE

Environmental Impact Analysis Report

A. Date: February 15, 1984 220

B. Name of Applicant: The Upjohn Company

C. Address: Portage Road, Kalamazoo, Michigan 49001

D. Environmental Information:

1. Describe the proposed action:

Continued production of lincomycin hydrochloride for the Lincomix products. Lincomycin hydrochloride is an antibiotic produced by fermentation in the multiple-product fermentation plant of The Upjohn Company at Kalamazoo, Michigan. It has been produced at this location for many years.

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

a. Production of lincomycin hydrochloride for swine usage has little effect on the environment since this use represents only a small fraction of the total fermentation production.

b. All non-contact cooling water is discharged to a ground water recharge pond, and all other wastewaters are sent to the Kalamazoo municipal sewage systems. Used solvents are recovered by distillation, and small quantities may be sold or burned if recovery by distillation is not feasible. Solid waste is sent to a suitable landfill operation. There are no effects on public health, endangered species, historical places, or other human values. There is no possibility of food contamination.

c. The following regulations are cited as being applicable to the proposed action:

1. Resource Conservation and Recovery Act of 1976 - Public Law 94-580.

2. US EPA Effluent Guideline for the Pharmaceutical Industry.

3. Michigan Solid Waste Management Act 641.

4. Michigan Hazardous Waste Management Act 64.

5. City of Kalamazoo Plumbing and Sewer Code.

6. Michigan Air Pollution Act 348.

d. All manufacturing and waste disposal operations meet local, state, and federal requirements.

3. Describe the probable adverse environmental effects that cannot be avoided.

   None.

4. Evaluate alternatives to the proposed action.

   There are no feasible alternatives to the proposed action.

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

   The fermentation plant uses relatively large quantities of water, most of which is used for cooling purposes and is returned to the ground unpolluted. Industrial wastewaters are discharged to the Kalamazoo waste treatment plant.

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

   Irreversible commitment of resources is limited to the raw materials and utilities used in manufacturing.

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

   None.

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

   No requirement.

9. Risk - benefit analysis.

   Benefit to the public is in the form of a useful drug. This benefit should far outweigh the small adverse effects produced.

E. The proposed action has been reviewed and approved by the Environmental Engineering Unit of The Upjohn Company.

F. Certification:

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

A. B. Spradling
Fine Chemical Division
The Upjohn Company
Kalamazoo, MI 49001

LINCOMYCIN HYDROCHLORIDE AG GRADE
Environmental Impact Analysis Report

A. DATE: February 15, 1984

B: NAME OF APPLICANT: THE UPJOHN MANUFACTURING COMPANY

C. ADDRESS: Km 60.0, Barceloneta, Puerto Rico 00617

D. ENVIRONMENTAL INFORMATION:

1. Describe the Proposed Action:

Continued production of Lincomycin Hydrochloride for Lincomix products. Lincomycin Hydrochloride is an antibiotic produced by fermentation in the multiple-product Fermentation Plant of The Upjohn Manufacturing Company at Barceloneta, Puerto Rico. It has been produced at this location for many years.

2. Discuss the probable impact of the proposed action on the environment, including primary and secondary consequences.

a) Production of Lincomycin Hydrochloride for swine usage will have minimal impact on the environment since this represents only a small fraction of the total current fermentation production. The liquid waste consists mainly of residual wastewater from fermentation and residual solvent from extraction and chemical processes. The spent beer from the fermentation operation is discharged into the Barceloneta regional wastewater treatment system. Residual solvents are reused in the processes and/or reprocessed at an off site facility. Solid wastes are sent to the Barceloneta sanitary landfill or to the Toa Baja sanitary landfill. Fermentation off gases do not represent any harm to the environment.

There are no significant adverse effects on public health, endangered species, historical places, or other human values. There is no possibility of food contamination. Use of natural resources and energy for this product is a small increment of present total usage.

b) The following regulations are cited as being applicable to the proposed action:

(1) The Federal Clean Air Act, PL95-95, as amended.
(2) The Federal Clean Water Act, 92-500, as amended.
(3) The Federal Resource Conservation and Recovery Act of 1976 - Public Law 94-580, as amended.
(4) Puerto Rico Public Law 9, the Environmental Public Policy Act of 1970.
(5) Puerto Rico Public Law 163, of May 3, 1949 as amended, the Puerto Rico Aqueduct and Sewer Authority.

c) All manufacturing and waste disposal operations meet local and Federal emission requirements.

3. Describe the probable adverse environmental effects that cannot be avoided.

None

4. Evaluate alternatives to the proposed action.

Resources and facilities are being used efficiently to produce a quality product with minimal environmental impact. No other alternatives are contemplated.

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

The proposed operation will have no additional materially adverse effect on the environment. The fermentation plant uses relatively large quantities of water, most of which is used for non-contact cooling purposes and is returned unpolluted to the ground. The spent beer from fermentation is discharged into the sanitary sewer systems which is permitted by local and Federal authorities. Residual solvent is sent to an authorized facility for recovery and/or disposal as permitted by local and Federal authorities. The solid wastes are handled jointly with wastes from current operations and disposed as per instructions from local authorities and Federal requirements. The use of waters, discharge of waste waters and disposal of solid waste should not have a significant environmental effect either in the short-term or the long-term basis.

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

Irreversible commitment of resources is limited to the raw materials and utilities used in manufacturing. These are only small increments of current use.

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

None

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

   None

9. Risk - Benefit Analysis:

   Benefit to the public will be the availability of an antibiotic which is expected to fill agricultural needs. This benefit should far out-weigh any small potential risks to the environment.

E. The proposed action has been reviewed and approved by the Environmental Engineers of The Upjohn Manufacturing Company.

F. CERTIFICATION

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

Ramon A. Amador - Group Manager E&M
Chemical Manufacturing Division
The Upjohn Manufacturing Company
Barceloneta, Puerto Rico 00617

/lb
RAA-EnvImpAna

LINCOMIX® 20 Premix
and
LINCOMIX® 50 Premix

ENVIRONMENTAL IMPACT ANALYSIS REPORT

A. Date: 01/24/84

B. Name of Applicant: The Upjohn Company

C. Address: Portage Road, Kalamazoo, Michigan 49001

D. Environmental Information:

1. Describe the proposed action:

Manufacture of the dry powder mixture, LINCOMIX® 20 Premix and LINCOMIX® 50 Premix, in the agricultural premix facilities of The Upjohn Company in Kalamazoo, Michigan.

2. Discuss the probable impact of the proposed action on the environment, including primary and secondary consequences.

a. There are no by-products formed in the manufacturing process. This is a non-continuous batch process scheduled on an intermittent basis throughout the year.

Dust generated in the manufacturing process is exhausted through an inertial wet collector. Equipment is cleaned with a vacuum cleaner and washed down with water. Waste water from clean up and dust collector is discharged into the Sanitary sewer system of the City of Kalamazoo. Solid waste, consisting primarily of defective packaging material, is incinerated or sent to a State-approved sanitary landfill. There are no significant adverse effects on public health, endangered species, historical places, or other human values. There is no possibility of food contamination. Use of natural resources and energy for this product is a very small increment of present total usage.

b. The following regulations are cited as being applicable to the proposed action:

1. Resource Conservation and Recovery Act of 1976 - Public Law 94-580.

2. Clean Water Act, of 1977 as amended.

3. Michigan Solid Waste Management Act 641.

Environmental Impact Analysis Report (Continued)

4. Michigan Hazardous Waste Management Act 64.

5. City of Kalamazoo Plumbing and Sewer Code.

6. Michigan Air Pollution Act 348.

c. All manufacturing and waste disposal operations will, when applicable permits are granted by the State, meet local, State and Federal emission requirments.

3. Describe the probable adverse environmental effects that cannot be avoided.

None.

4. Evaluate alternatives to the proposed action.

Resources and facilities are being used efficiently to produce a quality product with minimal environmental impact. No other alternatives are contemplated.

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

The proposed operation will have no additional adverse effect on the environment. The liquid wastes are discharged into the sanitary sewer systems which is permitted by local and federal authorities. The use of waters, discharge of waste waters and air emmisions and disposal of solid waste should not have a significant environmental effect either in the short-term or on a long-term basis.

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

Irreversible commitment of resources is limited to the raw materials and utilities used in manufacturing. These are small increments of current use.

LINCOMIX® 20 Premix
and
LINCOMIX® 50 Premix

Environmental Impact Analysis Report (Continued)

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

   None.

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

   Manufacture of the product for marketing under approved indications is currently in progress and will continue. Other than this situation there is no time requirement.

9. Risk - benefit analysis.

   Benefit to the public will be the availability of an antibiotic, lincomycin, which is beneficial to animal health. Indirectly this will benefit the public through lower food cost and increased food supplies. This benefit far out-weighs any small potential risks to the environment.

E. The proposed action has been reviewed and approved by the Environmental Affairs Unit of The Upjohn Company.

F. Certification:

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

John C Prue

John C. Prue
Pharmaceutical Manufacturing
The Upjohn Company
Kalamazoo, Michigan 49001

Date 1/24/84

INDEX

Appendix Tab

230

Appendix
Tab

b. Effects on waste stabilization processes (e.g. in pit storage, lagoon, runoff retention basins, etc.).

G. Effect of Lincomycin (U-10,149A) on the Sulfur Transformation Test. Technical Report No. 524-9760-83-008. August 12, 1983.

Effect of Lincomycin (U-10,149A) on the Nitrogen Transformation Test. Technical Report No. 524-9760-83-007. July 12, 1983.

Effect of Lincomycin (U-10,149A) on the Cellulose Decomposition Test. Technical Report No. 524-9760-83-005. May 4, 1983.

c. Effects on representtive invertebrate populations present in waste and/or feedlot runoff.

H. The effects of Lincomycin in Soil on the Earthworm (Lumbricus terrestris). Technical Report No. 524-9760-83-003. March 23, 1983.

AGRICULTURAL RESEARCH AND
DEVELOPMENT LABORATORIES,
THE UPJOHN COMPANY

TECHNICAL REPORT NO. 524-9760-83-006

PATHOLOGY/TOXICOLOGY NO. _____

TRIAL OR STUDY NO. _____

DATE: May 16, 1983

TECHNICAL REPORT

TITLE: Physical and Chemical Properties of Lincomycin Hydrochloride (U-10,149A)

K. T. K.

AUTHOR: K. T. Koshy

ABSTRACT: This report is a compilation of the physical and chemical properties of lincomycin hydrochloride (U-10,149A). The data for this document was gathered from published and unpublished reports from within the company. The authors of these reports are acknowledged for their contributions. This report includes the following information on lincomycin:

1. Structural description
2. Ultraviolet, IR, NMR and mass spectra
3. Crystal properties
4. Solubility, partition coefficient, pKa and optical rotation
5. Chemical stability
6. Qualitative and quantitative methods of analysis
7. Metabolism and pharmacokinetics.

en

Table of Contents

234

1. Description

1.1 Name: Lincomycin hydrochloride, U.S.P.

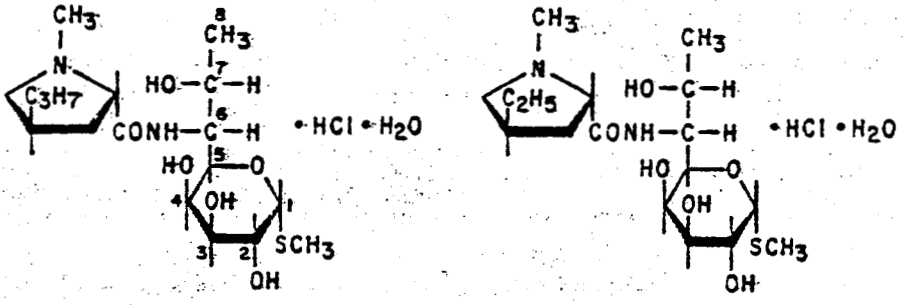
Chemical Name: D-*erythro*-α-D-*galacto*-Octopyranoside, methyl 6,8-dideoxy-6-[[(1-methyl-4-propyl-2-pyrrolidinyl) carbonyl]amino]-1-thio-, monohydrochloride, monohydrate, (2*S*-*trans*)-.

Methyl 6,8-dideoxy-6-(1-methyl-*trans*-4-propyl-L-2-pyrrolidinecarboxamido)-1-thio-D-*erythro*-α-D-*galacto*-octopyranoside monohydrochloride monohydrate

1.2 Formula and Molecular Weight

Crystalline lincomycin is obtained as the hydrochloride monohydrate by the addition of acetone to an aqueous-hydrochloric acid solution of lincomycin. The USP XX specifies that it has a potency equivalent to not less than 790 µg of lincomycin base ($C_{18}H_{34}N_2O_6S$) per mg of the hydrochloride monohydrate.

SCHEME I



LINCOMYCIN A

LINCOMYCIN B

Lincomycin hydrochloride USP may contain the 4-ethyl analog on the pyrrolidine ring as an impurity which is designated as lincomycin B. The USP XX specifies that it contains not more than 5% of lincomycin B.

$C_{18}H_{34}N_2O_6S \cdot HCl \cdot H_2O$ F.W. = 461.01

1.3 Appearance, Color and Odor

Lincomycin hydrochloride is a white or practically white, crystalline powder. It has a characteristic pungent odor.

235

2. Physical Properties

2.1 Spectra

2.11 Ultraviolet Spectra

Figure 1 is the ultraviolet spectrum of a 0.1% (0.0217 M) solution of lincomycin hydrochloride monohydrate in water recorded on a Cary Model 15 recording spectrophotometer. It has very high end absorption from 260 nm on down with no characteristic peaks or valleys. The UV absorption is sufficient below 254 nm to enable the use of commonly available detectors for the high performance liquid chromatographic analysis of lincomycin hydrochloride (see Section 4.33).

2.12 Infrared Spectra

The infrared spectrum of a mineral oil mull of lincomycin hydrochloride is shown in Figure 2 (2). Table I shows assignments for the significant infrared absorption bands (2).

2.13 NMR Spectra

Proton NMR spectra of lincomycin and some related compounds were analyzed by Slomp and MacKeller (3). Carbon-13 NMR spectral analysis and spin-lattice relaxation times of lincomycin and related compounds were analyzed by Mizsak et al. (4). Figures 3 and 4 are the proton and C-13 NMR spectra of lincomycin hydrochloride respectively (5). Tables II and III are the corresponding chemical shifts in the proton and C-13 spectra (5).

2.14 Mass Spectra

The spectrum of lincomycin hydrochloride obtained by direct probe mass spectrometry is shown in Figure 5 (6). It is a simple spectra showing a weak ion at 406 representing M^+ less HCl and the water of crystallization. The most likely structures of the other fragment ions are indicated on the spectrum. The predominant fragment ion m/z 126 is characteristic of lincomycin and all lincomycin A related compounds.

Lincomycin can be easily derivatized to the tetra trimethylsilyl ether (see Section 4.32) and the tetra acetate and subjected to GLC/mass spectrometry. A spectrum of the trimethylsilyl derivative is shown in Figure 6 (7). The spectrum shows a weak molecular ion at m/z 694 and few fragment ions, the most predominant of which is at m/z 126.

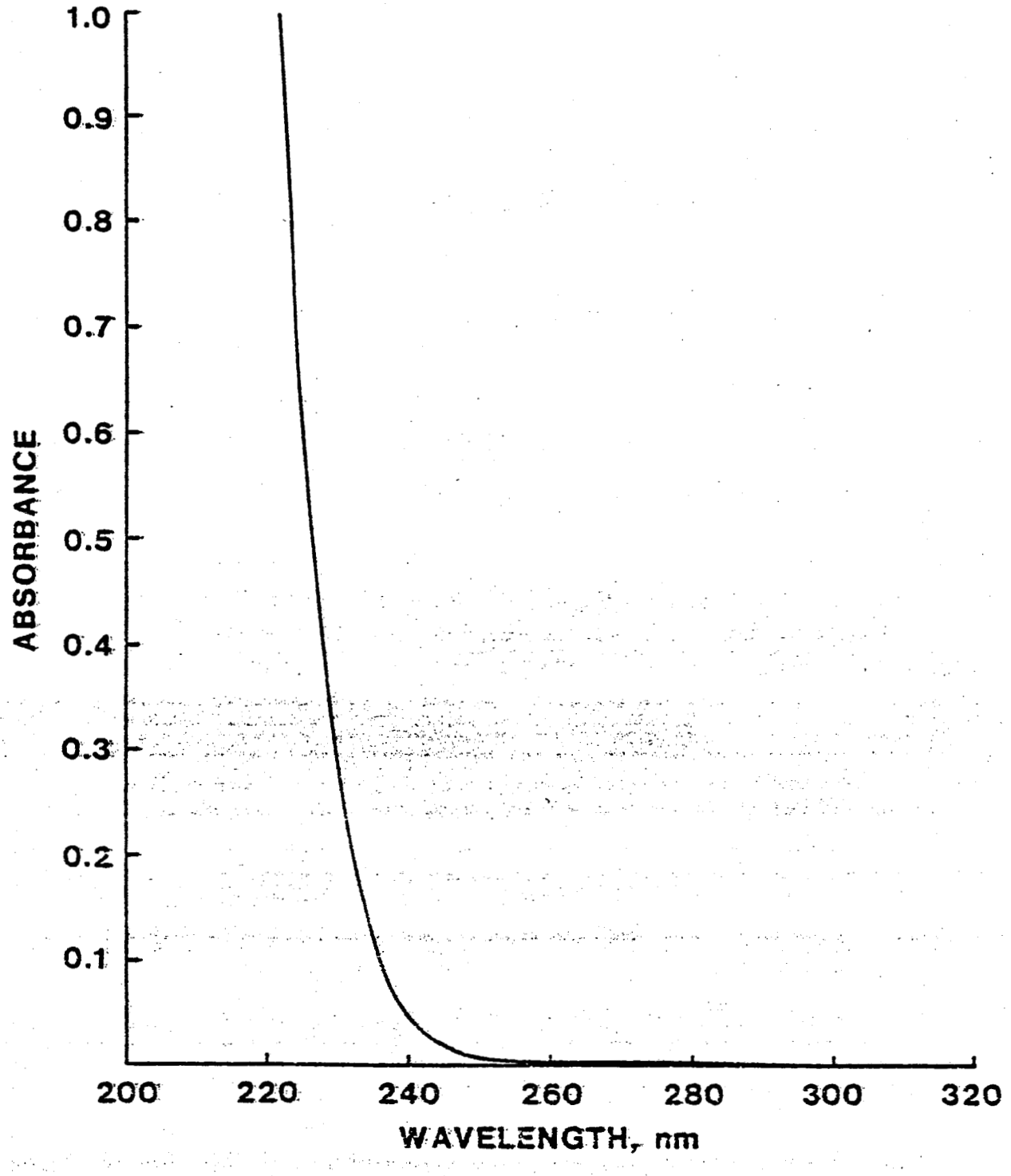


Figure 1. The ultraviolet spectrum of lincomycin hydrochloride in water (0.0217 M), 1 cm cell

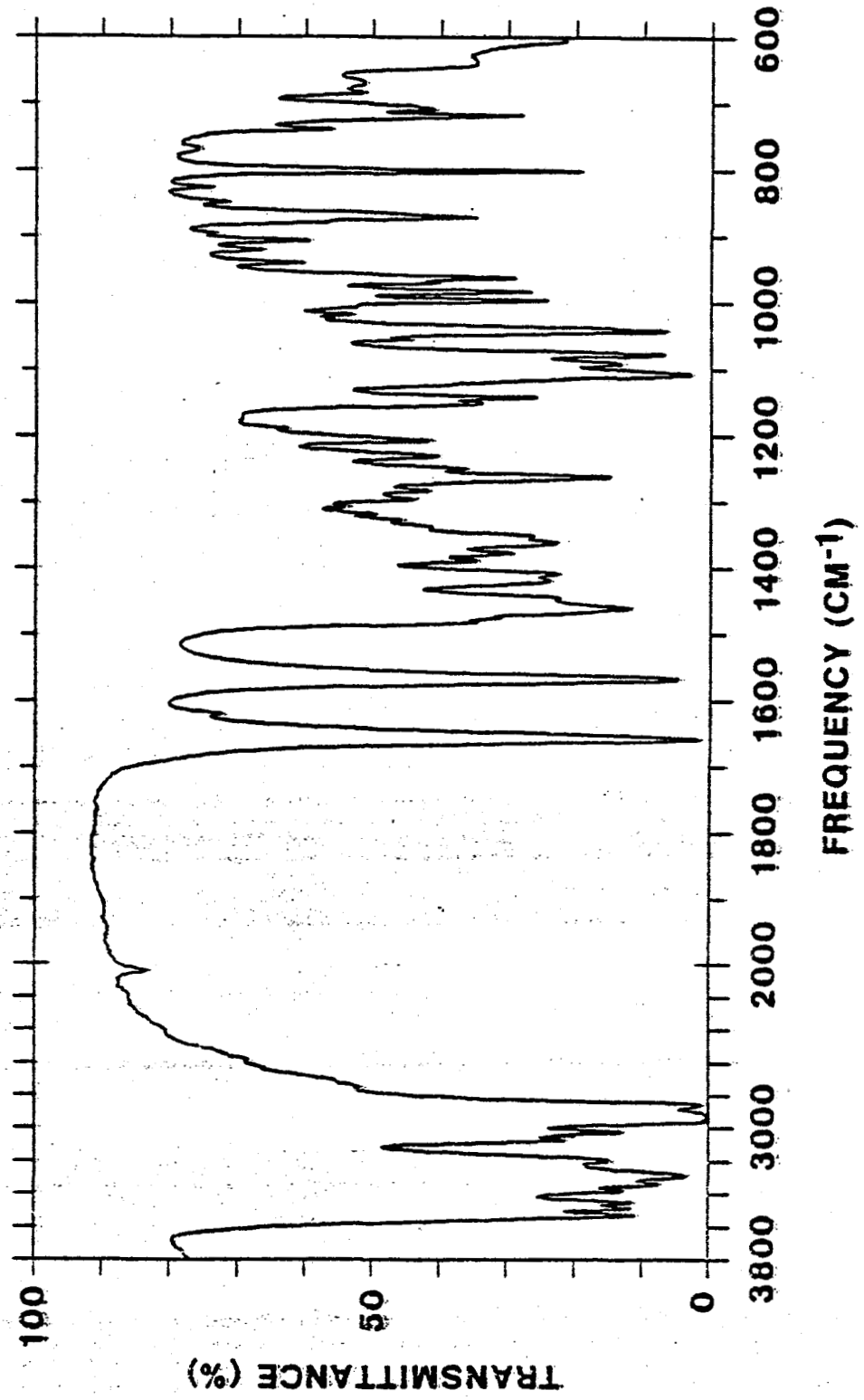


Figure 2. Infrared spectrum of a mineral oil mull of lincomycin hydrochloride

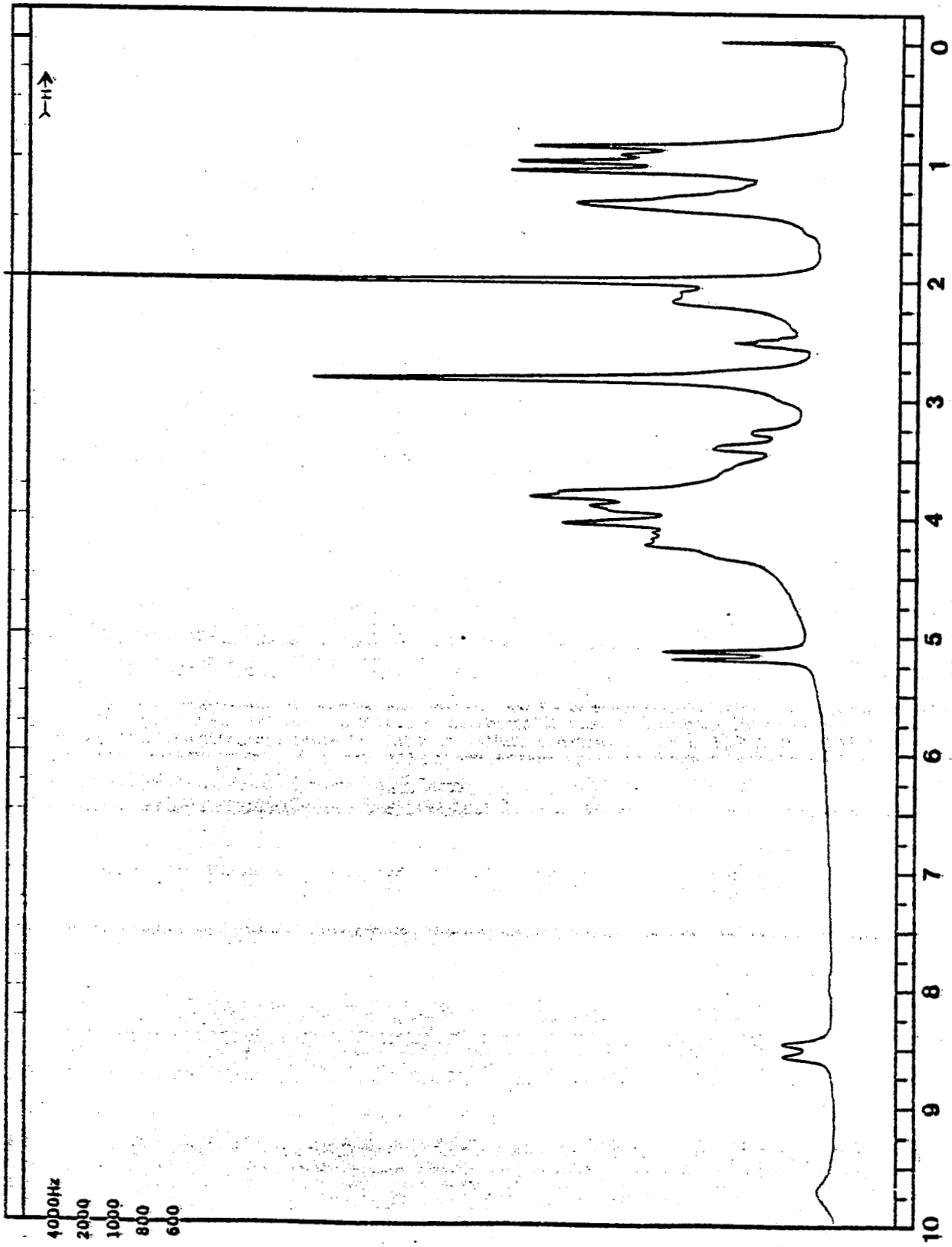


Figure 3. 80 MHz proton NMR spectrum of lincomycin hydrochloride in dimethyl sulfoxide

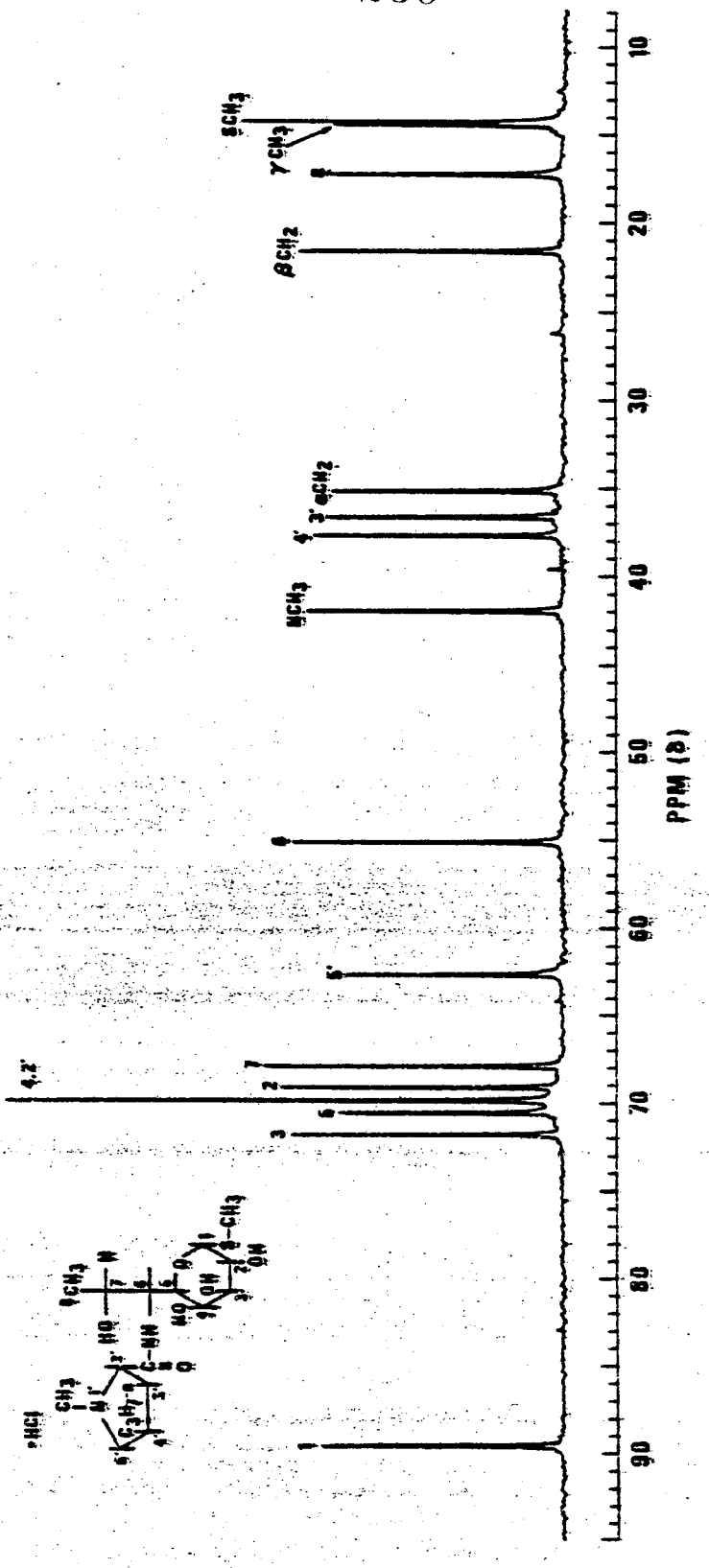


Figure 4. Carbon-13 NMR spectrum of lincomycin hydrochloride

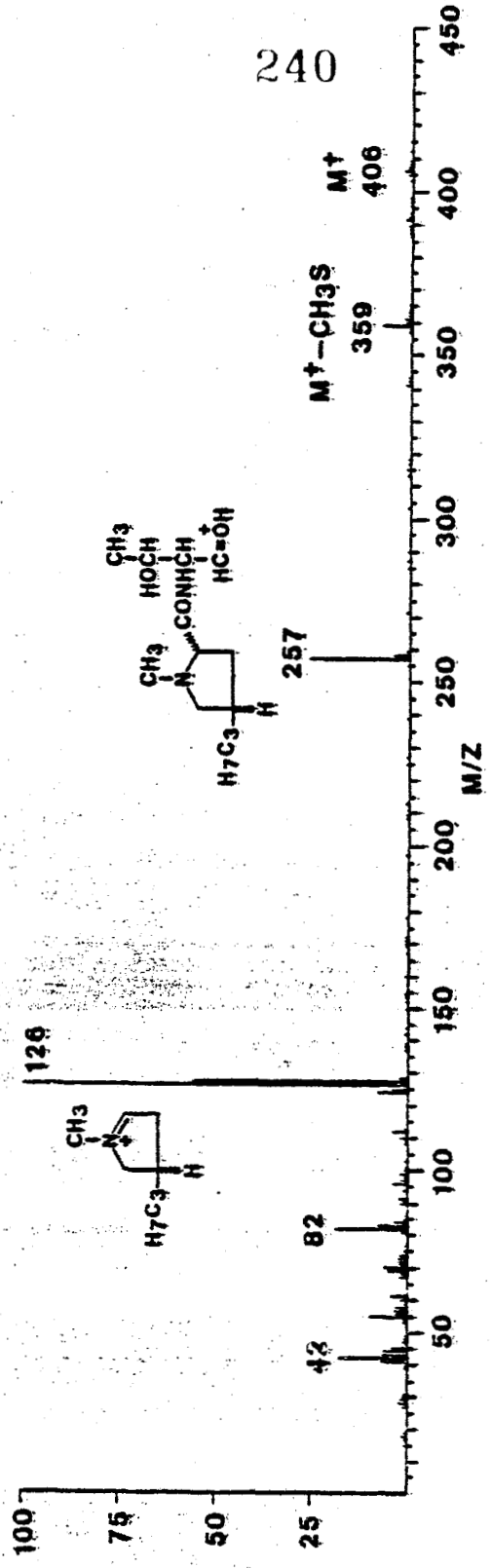


Figure 5. Mass spectrum of lincomycin hydrochloride (direct probe)

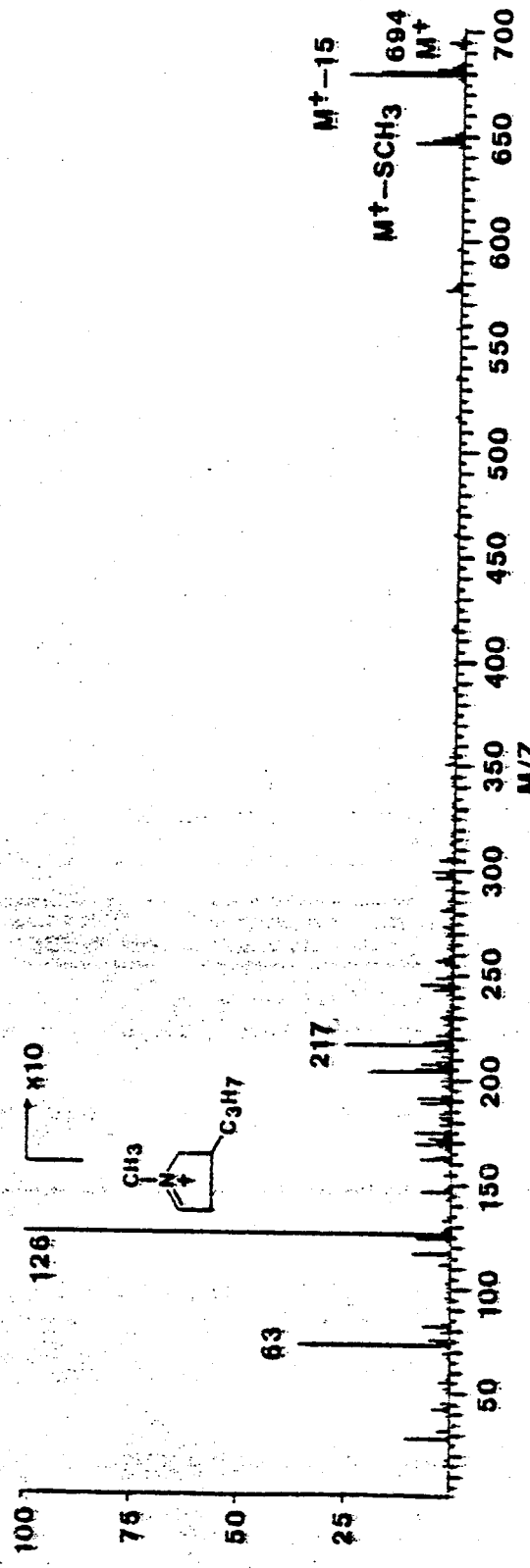


Figure 6. Mass spectrum of tetra trimethylsilyl lincomycin

2.2 Crystal Properties

2.21 Melting Range

Lincomycin hydrochloride melts with decomposition at about 148°.

2.22 Polymorphs

Lincomycin hydrochloride exists in two polymorphic forms (8). As prepared commercially, the monohydrate is the predominant species and is designated as Form II: Form I contains varying amounts of water. Both can be rendered anhydrous by drying. The two forms retain their particular infrared characteristics in the anhydrous state. Form II is thermodynamically more stable than Form I. It also has greater bulk density. (See section 2.24 for X-ray diffraction patterns of the two forms.)

2.23 Thermal Analysis

Differential Scanning Calorimetric (DSC) and thermogravimetric analysis (TGA) curves for lincomycin hydrochloride are shown in Figures 7 and 8 respectively (9). The curves were generated from a DuPont thermal analyzer (Model No. 1090, DuPont De Nemours and Co., Wilmington, Delaware). The sample was contained in aluminum pans and the analysis was conducted under an atmosphere of nitrogen. The heating rate for the DSC and TGA curves were 2° and 5°C/min respectively. The long shallow endotherm in the DSC curve from about 136-145°C is probably associated with release of water. The melting endotherm in the DSC peaks at 152.2°C. The TGA curve indicates gradual loss of water of crystallization and also a crystalline transition stage. The compound appears to lose all water before the beginning of the melting endotherm after which it undergoes decomposition.

2.24 X-Ray Diffraction

Figures 9 and 10 are X-ray diffraction patterns (10) of crystalline lincomycin hydrochloride forms I and II respectively. With the aid of the X-ray diffraction patterns of the two forms containing varying amounts of water and their infrared spectra, the authors were able to establish conditions under which the transition from one form to the other takes place. Transitions in the X-ray diffraction pattern of Form I appeared at about the 4% water level, showing a definite shift which could be attributed to larger interplanar spacings as the water level is increased. Between 0.67% and 3.34% water the patterns were identical and were characterized by major peaks at 5.55° and 11.20° 2θ, corresponding to d = 15.91Å and 7.89Å. At 6.66% water the X-ray diffraction pattern was different, showing a shift of these peaks to 5.05° and 10.30° 2θ corresponding to d = 17.48Å and 8.58Å. At 3.83% and 5.35%

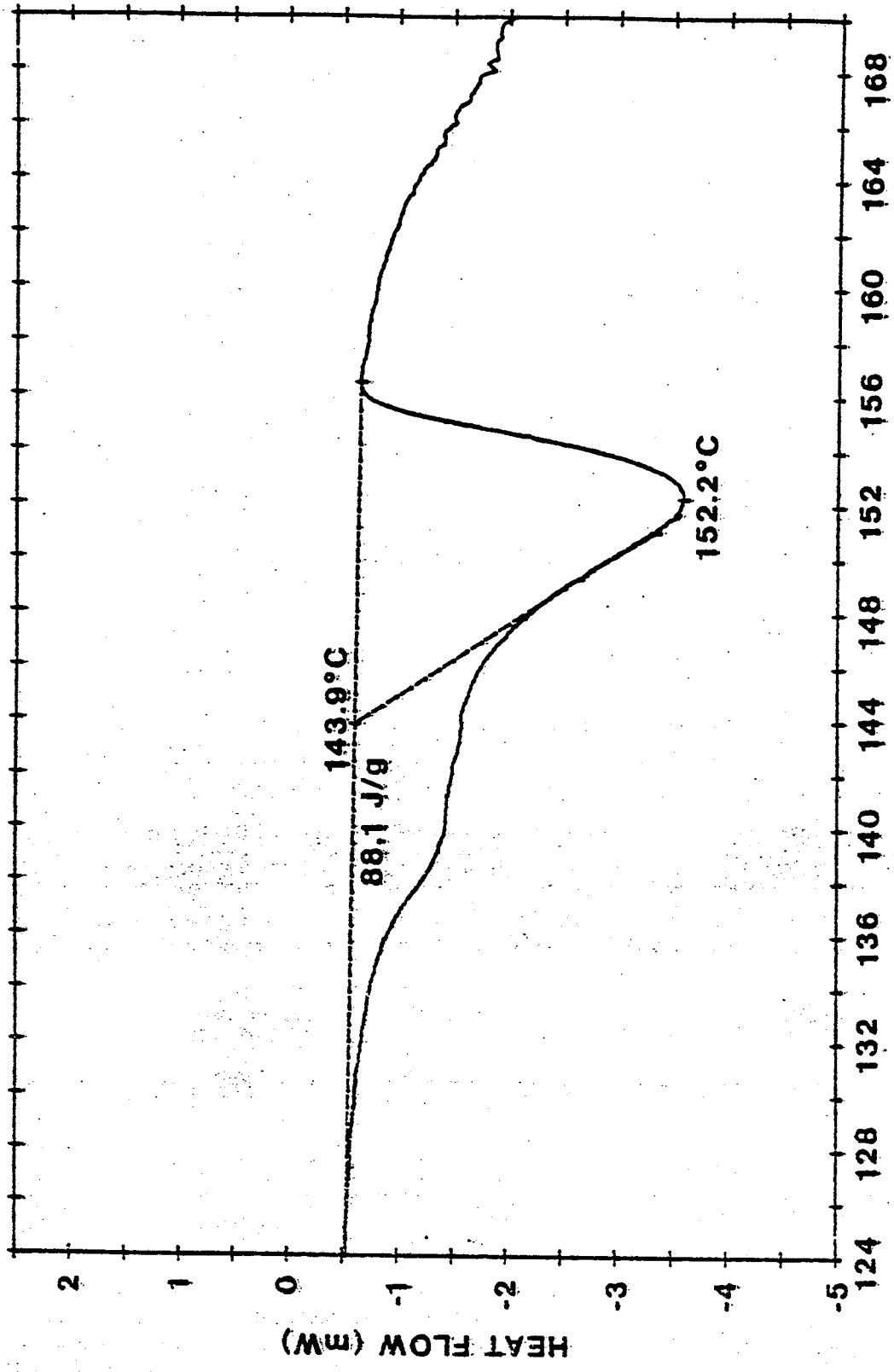


Figure 7. Differential Scanning Calorimetric curve for lincomycin hydrochloride

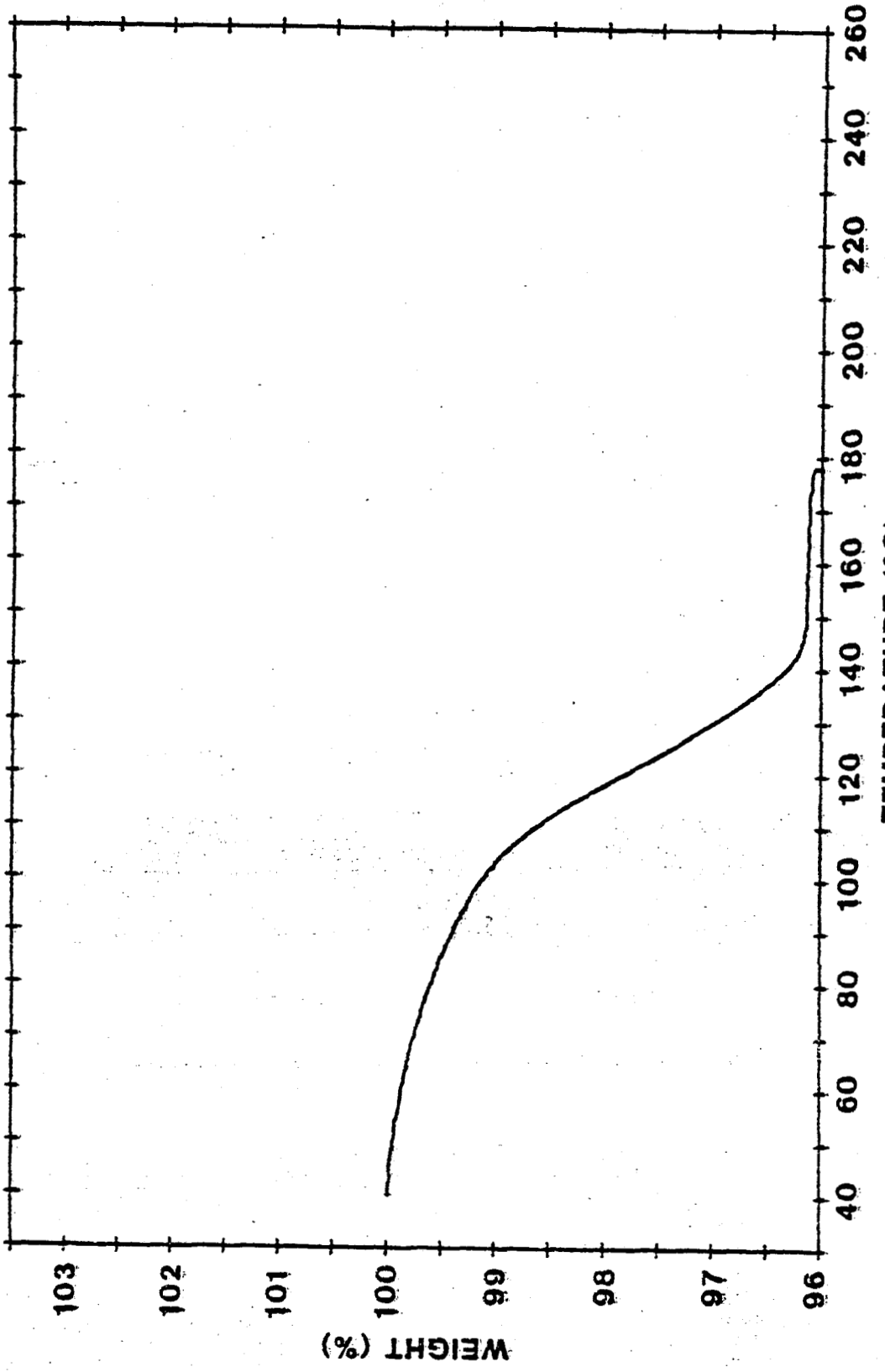


Figure 8. Thermogravimetric analysis curve for lincomycin hydrochloride

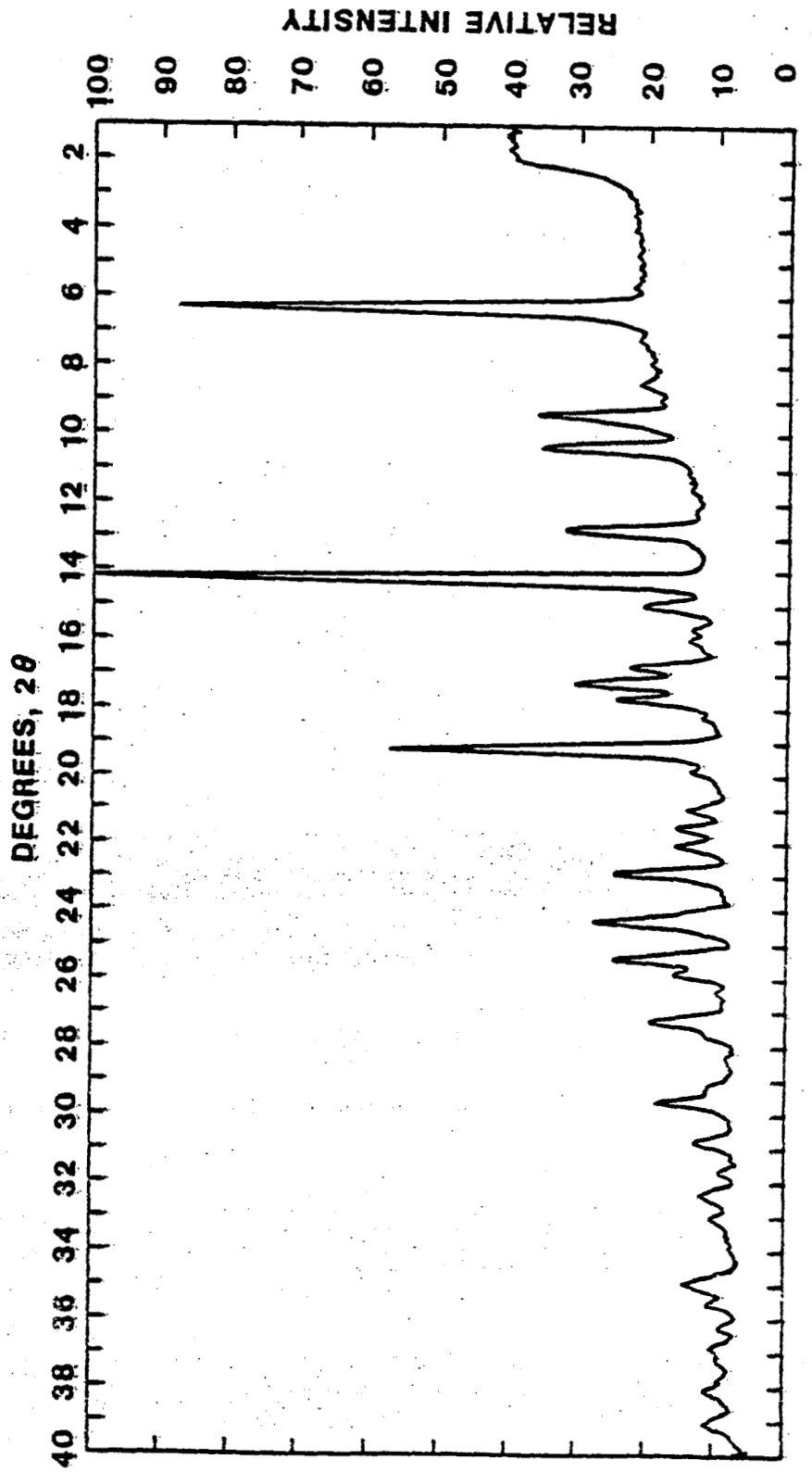


Figure 9. X-ray powder diffraction pattern of lincomycin hydrochloride, Form II

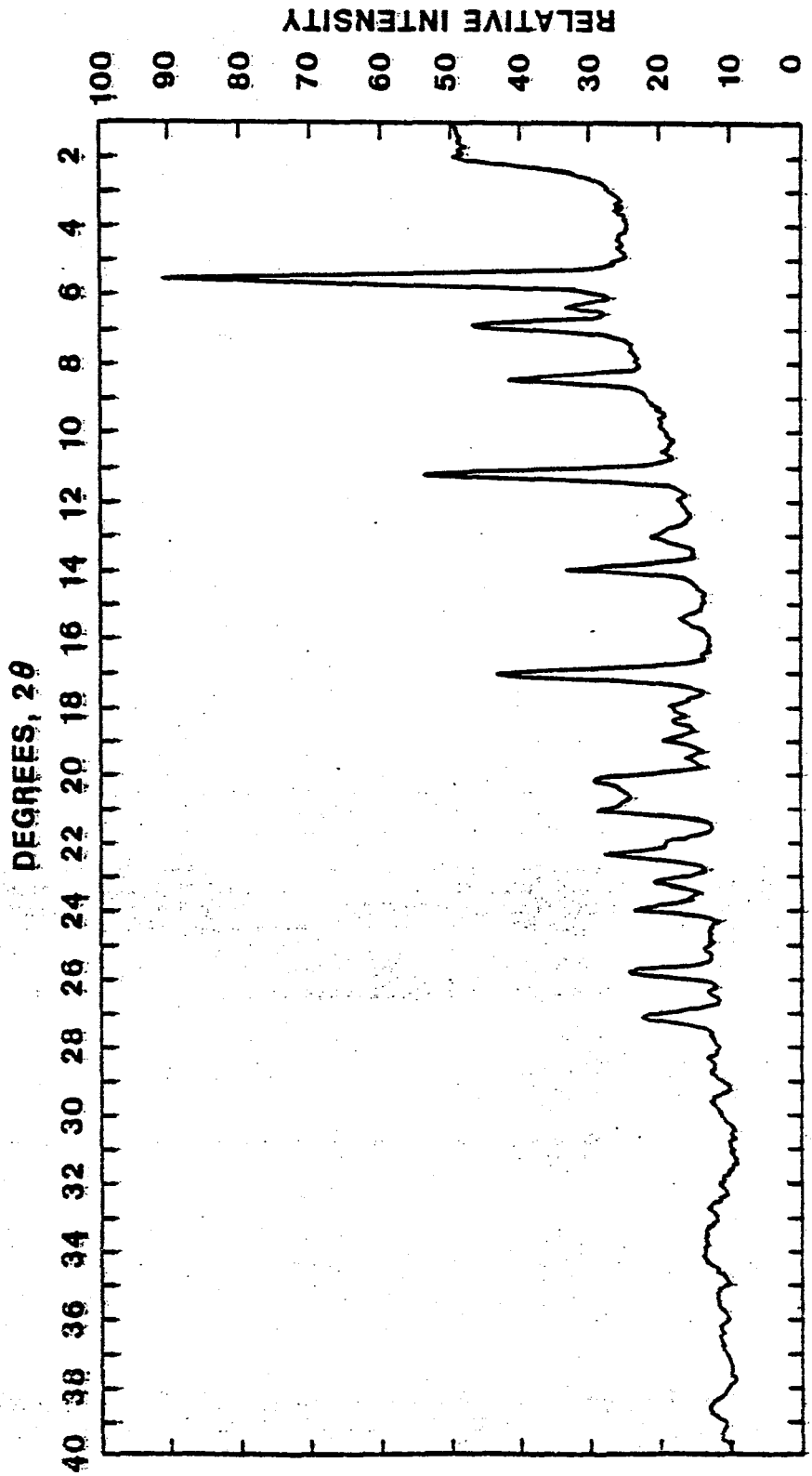


Figure 10. X-ray powder diffraction pattern of lincomycin hydrochloride, Form I

water combinations of the two patterns were found. The infrared spectrum of Form I also changed with water content. These changes were not as readily discernable as those observed by X-ray, and indicated hydrogen bonding to have resulted in band broadening at higher water contents.

Table IV shows the X-ray diffraction patterns of the two forms of lincomycin hydrochloride.

2.3 Solubility

Lincomycin hydrochloride is extremely water soluble. It forms a syrup with water and the solubility is estimated to be between 500-1000 mg/ml (11). The solubilities of lincomycin hydrochloride and several other antibiotics were determined by Marsh and Weiss (12) in a number of solvents. Their data is shown in Table V.

2.4 Partition Coefficient

The octanol/water partition coefficient at pH 2, 7 and 9 (13) and between water and a few other solvents (11) are shown in Table VI.

2.5 Ionization Constant, pK

The pH of a 1% solution of production lots of lincomycin hydrochloride in water is in the range 4.7-4.9. It has a pKa of 7.6.

2.6 Optical Rotation

The USP XX specifies that lincomycin hydrochloride has a specific rotation between +135° and +150° in an aqueous solution containing 20 mg. per ml, calculated on the anhydrous basis.

3. Chemical Stability

3.1 Modes of Degradation

Vigorous acid hydrolysis of lincomycin was performed by Herr and Slomp (14). Two products were isolated; methyl mercaptan, isolated and identified as its 2,4-dinitrophenyl thioether, and an amino acid identified as n-propylhygric acid. Milder hydrolysis using hydrazine hydrate under reflux conditions efficiently cleaved the amide bond (15) without destroying the stereochemistry of the sugar moiety. The resulting compounds were identified as L-trans-4-n-propylhygric acid and methyl 6-amino-6,8-dideoxy-1-thio-D-erythro-α-D-galacto-octopyranoside (V, Scheme II) (16, 17).

3.2 Stability in Aqueous Solution

Forist and Royer (18) have reported the stability of lincomycin hydrochloride at 70° in 0.1 N HCl and in 0.1 N NaOH. The degradation in both instances followed pseudo-first-order kinetics and the calculated half-lives were 40 and 25 hours in the acid and base respectively. Forist et al. (19) also studied the stability of lincomycin hydrochloride in 0.1 N HCl at 70° and at 37°. There was no degradation at 37° for at least 48 hours. The half-life at 70° was 39 hours. The principal degradation products were methyl mercaptan and 1-dethiomethyl-1-hydroxylincomycin.

Clindamycin hydrochloride is a synthetic analog of lincomycin hydrochloride in which the 7-position hydroxyl group is replaced by a chlorine atom. Oesterling (20) has reported a detailed study of the aqueous stability of clindamycin hydrochloride in the pH range 0.44-11.66. From the results of this study and the ones reported earlier (14-19), the following conclusions depicted in Scheme II may be postulated regarding the stability and mode of degradation in aqueous solutions.

1) Lincomycin hydrochloride solutions adjusted to pH 1-6 are stable at room temperature.

2) The major degradation in buffers pH 0.4-4 at elevated temperatures is via the thioglycoside hydrolysis to form 1-dethiomethyl-1-hydroxylincomycin (IV) and methyl mercaptan (III).

3) The degradation is minimal in the pH range 3-6.

4) Above pH 9, the degradation is predominantly via the amide linkage producing (II) and (V).

4. Methods of Analysis

4.1 Identification Tests

Lincomycin hydrochloride is identified by comparison of the infrared spectrum and by gas liquid chromatographic retention times of the sample with that of an authentic standard.

4.2 Qualitative Methods

4.21 Paper Chromatography

Paper chromatographic systems that are reported (1) for the identification of lincomycin are shown in Table VII. Descending chromatography on Whatman No. 1 filter paper was used. Approximately 20 µg of lincomycin were spotted on the paper and developed without equilibration in the solvent vapors. The antibiotic was located on the developed strips by bioautography on trays of agar seeded with Sarcina lutea.

4.22 Thin Layer Chromatography

Thin layer chromatographic data of lincomycin base on commercial silica gel GF254, 250 µ plates in common organic solvents is shown in Table VIII. The detection of lincomycin was by exposure of the plates to iodine vapor followed by spraying with soluble starch.

4.3 Quantitative Methods

4.31 Colorimetric Method

An automated colorimetric method for lincomycin was reported by Prescott (21). The method involves the acid hydrolysis of

Scheme II. Chemical Degradation Pathways of Lincomycin

lincomycin to liberate methane thiol which is distilled and reacted with a 0.01% aqueous solution of 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB) at pH 8. Full color development is almost instantaneous at room temperature and is measured with a spectrophotometer. The method was developed for automated analysis of fermentation beers and for production samples. Most of the ingredients of the fermentation media do not interfere except for blackstrap molasses. For this reason the standard curve for the assay is generated by the hydrolysis of lincomycin HCl dissolved in spent fermentation beer. The method is not specific as all lincomycin analogs and other compounds containing a hydrolyzable thiol group give full molecular response in the assay.

4.32 Gas Chromatographic Method

Principle of Assay Method: A gas liquid chromatographic (GLC) method (22) is used for the assay of lincomycin hydrochloride monohydrate in bulk materials and pharmaceutical preparations. The GLC method is official in the USP XX. The lincomycin hydrochloride monohydrate is converted to the free base and extracted into a chloroform/imidazole solution. The lincomycin is silylated and subjected to GLC using n-dotricontane ($n\text{-}C_{32}$) as the internal standard. Using the appropriate instrument conditions below, the elution of the lincomycin derivative relative to the internal standard is 0.7.

Instrument and Column Conditions: Instrument and column may vary providing equivalent chromatography and results are produced.

Instrument:	Hewlett-Packard 402 or equivalent
Column:	Glass, 3 mm x 122 cm
Column packing:	3% OV-17 on 100-120 mesh Gas Chrom Q.
Detector:	Flame ionization, 270°C
Integrator:	Hewlett-Packard 3390A or equivalent
Column temperature:	255°C
Helium:	Approximately 60 ml per min
Sample volume:	0.5 to 1.5 mcl

Internal Standard Solution: Prepare a chloroform solution containing approximately 8.5 mg of dotricontane ($n\text{-}C_{32}$) per ml.

Chloroform/Imidazole Solution: Prepare a chloroform solution containing 20 mg of imidazole per ml of solution.

Reference Standard Preparation: Accurately weigh about 110 mg of lincomycin hydrochloride monohydrate reference standard and transfer to a suitable flask. Pipette 10.0 ml of internal standard solution into the flask, add 90 ml of chloroform/imidazole solution and shake vigorously until solution is complete. The flask can be immersed in an ultrasonic bath to hasten dissolution of the reference material.

Sample Preparation: Accurately weigh about 110 mg of the lincomycin hydrochloride sample and treat it the same way as the reference standard. The formulated products are handled according to the nature of the individual product. In general, the final sample preparation will contain approximately 1 mg of lincomycin and 0.85 mg of the C_{32} hydrocarbon per ml of chloroform.

Derivatization: Transfer approximately 4 ml aliquots of the sample preparation and the reference standard preparation into separate 15 ml centrifuge tubes. To each tube add 1 ml of N,O-bistrimethylsilylacetamide (BSA) containing 1 percent trimethylchlorosilane (TMCS) and swirl gently to mix. Position glass stoppers loosely in the tubes and place in a heating block or bath at 65°C for 30 min. Mix the contents of the tubes and chromatograph aliquots of the sample and reference standard preparations. Figure 11 shows a typical chromatogram showing the base-line separation of lincomycin A and B.

Calculations: The concentration of lincomycin base in the sample is calculated on the "as is" basis using the following equation:

mcg/mg: (Bulk material)

$$\frac{R(Sa)}{R(Std)} \times \frac{Wt(Std)}{Wt(Sa)} \times \frac{F2}{F3} \times 1000 \times C$$

where,

$R(Sa) = \frac{\text{Area of the lincomycin sample peak}}{\text{Area of the internal standard peak}}$

$R(Std) = \frac{\text{Area of the lincomycin standard peak}}{\text{Area of the internal standard peak}}$

Wt(Std) = Weight of lincomycin hydrochloride monohydrate reference standard in mg

Wt(Sa) = Weight of sample (in mg)

C = Assigned potency of lincomycin hydrochloride reference standard (in mg)

F2 = ml of internal standard solution added to the sample preparation

252

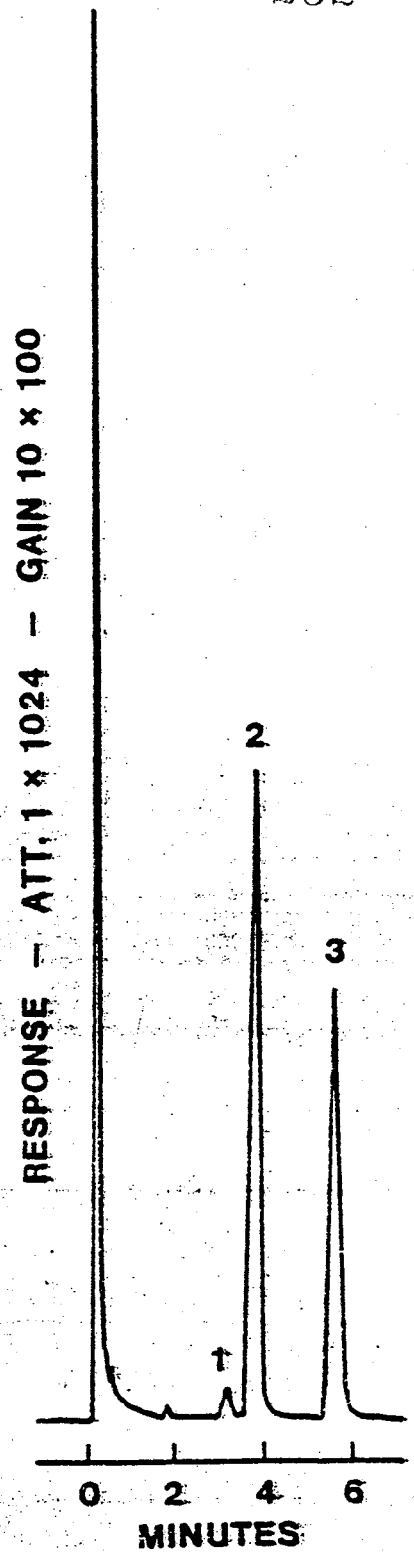


Figure 11. Gas-liquid chromatogram of tetra trimethylsilyl lincomycin.
Key: 1, lincomycin; 2, lincomycin A;
and 3, internal standard.

F3 = ml of internal standard solution added to the reference standard preparation

The concentration of lincomycin B is calculated using the formula

$$\% \text{ Lincomycin B} = \frac{B}{B+A} \times 100$$

where,

B = Area of the lincomycin B derivative
A = Area of the lincomycin A derivative

4.33 High Performance Liquid Chromatographic Method (HPLC)

The GLC procedure described earlier is used for the routine analysis of bulk lincomycin hydrochloride and all formulated products. The HPLC procedure (23) described below was developed for the rapid analysis of lincomycin in the fermentation beer during its production. It is readily adaptable for the analysis of the bulk drug and formulated products. The procedure utilizes a C_{18}-bonded microparticulate silica column, pH 8.2 phosphate buffer/acetonitrile as the mobile phase and a variable wavelength detector operated at 200 nm. Figure 12 shows typical chromatograms of a standard mixture of lincomycin A and B and that of a beer sample. The precision of the assay is ±2% relative standard deviation at the 0.06 mg/ml level. The detection limit can vary from 0.008 mg/ml to 0.02 mg/ml depending on the stability of the detector. Since the procedure involves injection of the fermentation beer, the frit in front of the guard column is replaced each morning. The guard column also needs cleaning or repacking every two or three days.

Chromatographic Conditions

Pump:	Any good quality pump capable of operation at 2500 psi
Detector:	A variable wavelength detector capable of smooth operation at 200 nm at 0.05 AUFS
Recorder or Integrator:	Any suitable strip chart recorder or a Hewlett Packard[1] computing integrator or equivalent (Model 3390A)
Injector:	Loop or other type of injector capable of reproducibly injecting 10 µl
Column:	Whatman[2] ODS-3, 4.6 mm x 25 cm or equivalent with a suitable guard column

1. Hewlett-Packard Inc., Palo Alto, California
2. Whatman Inc. Clifton, New Jersey

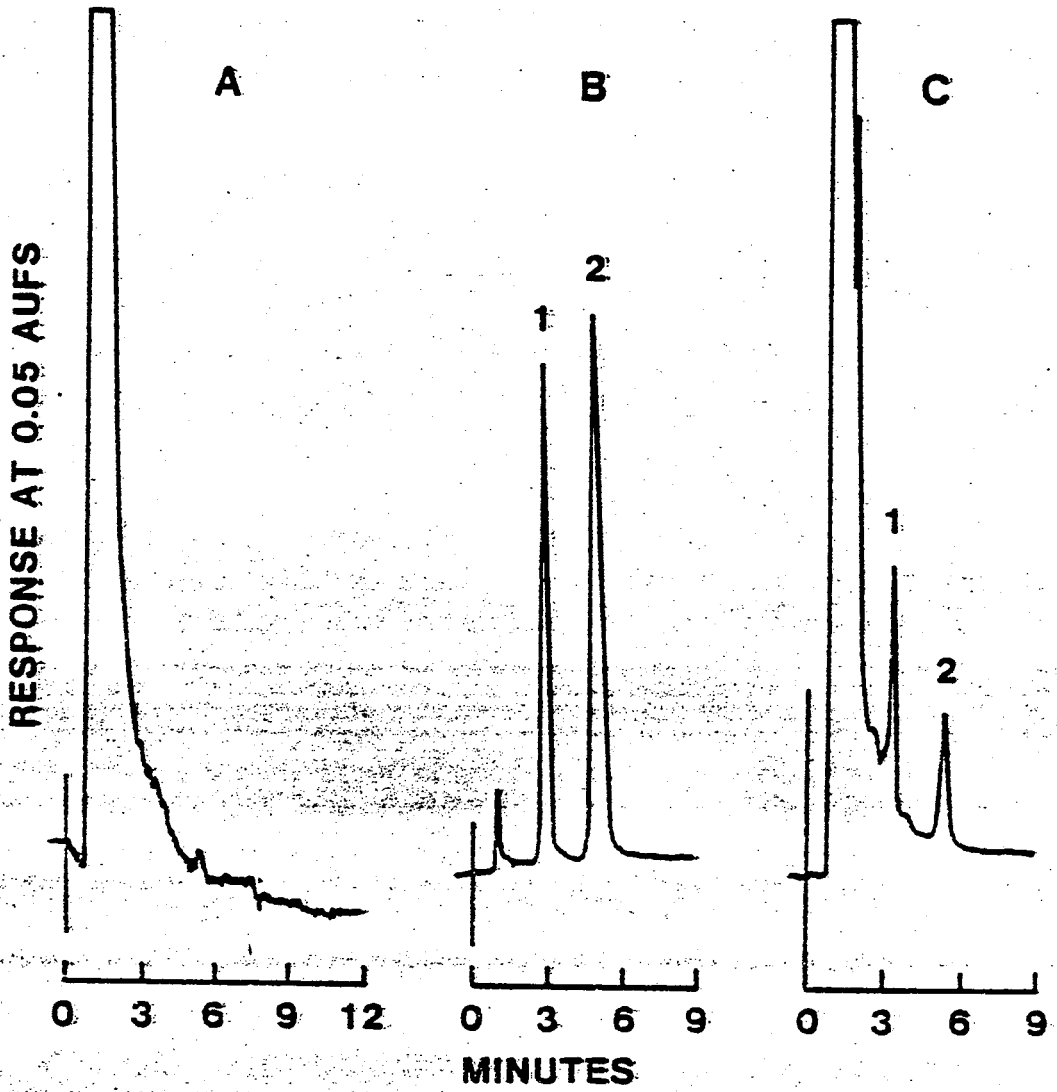


Figure 12. High performance liquid chromatogram of lincomycin.
Key: A, fermentation beer blank; B, standard mixture of lincomycin A and B; and C, lincomycin fermentation beer. Peaks 1 and 2 are lincomycin B and A, respectively.

Mobile phase: Weigh accurately ≈1.75 gm of K_2HPO_4 and ≈140 mg KH_2PO_4 into a 1-liter flask and dissolve in 720 ml of double distilled water (PH=8.2). Then add 280 ml UV grade acetonitrile. Allow to come to room temperature and degas by sonication or aspiration under vacuum.

Standard: Weigh accurately ≈5 mg of lincomycin HCl reference standard into a 100 ml volumetric flask. Dissolve and dilute to volume with double distilled water.

Sample preparation: None

Calculations: The concentrations of lincomycin A and B in the fermentation beer are calculated in the usual manner from the peak height/area responses of the sample against the standard.

4.34 Microbiological Method

The microbiological assay (24) is a general method which can be used for the bulk drug and pharmaceutical products. Since lincomycin is very widely used alone or in combination with other antibiotics and chemicals as a feed additive for swine and chicken, the microbiological assay has wider practical application for the analysis of such products. A larger number of such samples can be analyzed simultaneously with minimal clean-up operations, but the precision and accuracy is less than that of the GLC or HPLC procedures.

Media: Penassay Base Agar, Penassay Seed Agar, and Penassay Broth (Difco Laboratories) or Base Agar, Seed Agar, and Antibiotic Assay Broth (Baltimore Biological Laboratories).

Reagents: 0.1 M potassium phosphate buffer, pH 8.0.

Inoculum Preparation: Sarcina lutea UC-130 (ATCC 9341) is maintained on Penassay Seed Agar or suspended in sterile USP saline and stored in the gaseous phase of liquid nitrogen. Prepare colony isolation plates using Penassay Seed Agar. Incubate at 32-37°C for 24 to 48 hours or until discreet colonies may be selected. Transfer one colony to each of one or more slants of Penassay Seed Agar and incubate for 24 hours at 32-37°C. Using freshly grown slants, wash each slant with 3.0 ml sterile USP saline and pour into a Roux bottle containing Penassay Seed Agar. Incubate for 24 hours at 32-37°C. Wash each seeded Roux bottle with 50 ml sterile USP saline and glass beads. Pooling these washings, yields the culture suspension. This suspension should give about 25% light transmission when diluted 1:20 and read on a suitable photometric colorimeter at 580 mμ. This culture suspension

will last for three weeks if refrigerated (~4°C) or may be dispensed into ampoules and frozen in liquid nitrogen.

Assay Plates: Base layer - 21 ml base agar
Seed layer - 4 ml seed agar (melted and cooled to 48°C) to which has been added 1.0% of the inoculum.

Standard Preparation: Accurately weigh a suitable quantity of the standard material and dilute with 0.1 M potassium phosphate buffer, pH 8.0. The concentration of this solution should be 1 mg/ml. This stock solution may be used for one week if stored at ~4°C or it may be dispensed into vials and frozen in the gaseous phase of liquid nitrogen for an unlimited storage period. Final concentrations of 3.2 mcg/ml, 4.0 mcg/ml, 5.0 mcg/ml, 6.25 mcg/ml, and 7.8 mcg/ml are prepared with the pH 8.0 buffer for the assay. The reference point is 5.0 mcg/ml.

Assay according to the General Procedure - Microbiological Plate Assay for Antibiotics, using the standard curve design as described in detail in The Methods of Analysis, A.O.A.C. (25).

5. Metabolism and Pharmacokinetics

In preliminary reports on the absorption and excretion of lincomycin HCl in man and rats, Lewis and Meyer (26) reported the following observations: 1) lincomycin is solely absorbed from the small intestine, 2) about 35-40% of the administered oral dose is excreted in the feces after 12 hours, 3) the antibiotic is not degraded by stomach acidity, gastric enzymes or by bacterial action in the caecum or large intestine, 4) there is resorption of the circulating lincomycin into the small intestine (11% after 4 hours and 17% after 8 hrs) by way of the bile.

Vavra et al. (27) have reported on the absorption and excretion of lincomycin HCl in normal adult human volunteers after oral, intramuscular and intravenous routes of administration. Lincomycin given orally as a single 500 mg dose to 50 normal adults produced an average serum concentration that peaked at 4 hour at 3.4 ± 0.4 µg/ml and remained at or above 1.1 ± 0.1 µg/ml for at least 12 hours. In oral, multiple-dose studies (500 mg every 6 hours), lincomycin serum levels did not appear to build up with time. High serum levels of 5.7 ± 1.2 µg/ml were obtained within 4 hours after the first dose with subsequent nadir values ranging between 2.4 and 3.6 µg/ml for 174 hours, the entire duration of the study.

With single intramuscular doses of 100, 200 and 600 mg, the following respective peak levels were obtained within the first hour after dosing: 2.7, 3.8 and 11.6 µg/ml. In the case of the 600 mg dose, detectable amounts of lincomycin were present in sera from 18 of the 20 subjects as late as 24 hours after dosing. When 600 mg was administered every 8 hours, high concentrations of the antibiotic were present in the serum for 97 hours, the duration of the study.

The authors also reported serum levels after single and multiple 300 and 600 mg dose intravenous administration. With the 300 mg dose infused

every 12 hours for 74 hours, the high level was 9.5 µg/ml and the low level 1.6 µg/ml with essentially no accumulation of the antibiotic in the serum. However at 600 mg every 6 hours, the average high level was 17.5 µg/ml and the low level 8.2 µg/ml during a 74-hour period. The urinary excretion from the single and multiple oral dose serum level studies described above was 3 to 5% of the dose after 24 hours. Higher urine recoveries were seen after parenteral administration of the antibiotic.

Eberts et al. (28) studied the fate of tritium-labeled lincomycin in man and has postulated a kinetic model for its metabolism and excretion. ^{3}H-lincomycin HCl was administered to two panels of five subjects each. The oral dose was a single 500 mg capsule including 250 µCi of ^{3}H-lincomycin HCl. The I.M. dose was 2 ml of a 300 mg/ml solution containing 50 µCi of ^{3}H-lincomycin HCl. Their conclusions were:

(1). The mean peak-plasma level of 1.7 µg/ml (0.64-4.10) in the subjects was achieved within 2-4 hrs. In the I.M. group the mean level was 10.5 µg/ml (8.2-12.9) achieved within 0.5-1 hour.

(2). The mean recovery of the radioactive dose in the P.O. study was: urine, 8.6% (4.9-19.9); feces, 50.3% (13.6-78.9); total, 59.0% (18.5-84.4). In the I.M. study recovery was: urine, 55.3% (48.1-62.6); feces, 38.1% (36.6-40.3); total, 93.4% (86.3-99.5).

(3). When lincomycin was administered either P.O. or I.M., it was excreted via urine and feces as unchanged lincomycin plus an inactive metabolite(s). However, since the plasma disappearance rate exceeded the combined urinary and fecal excretion rates, an additional lincomycin compartment was suggested.

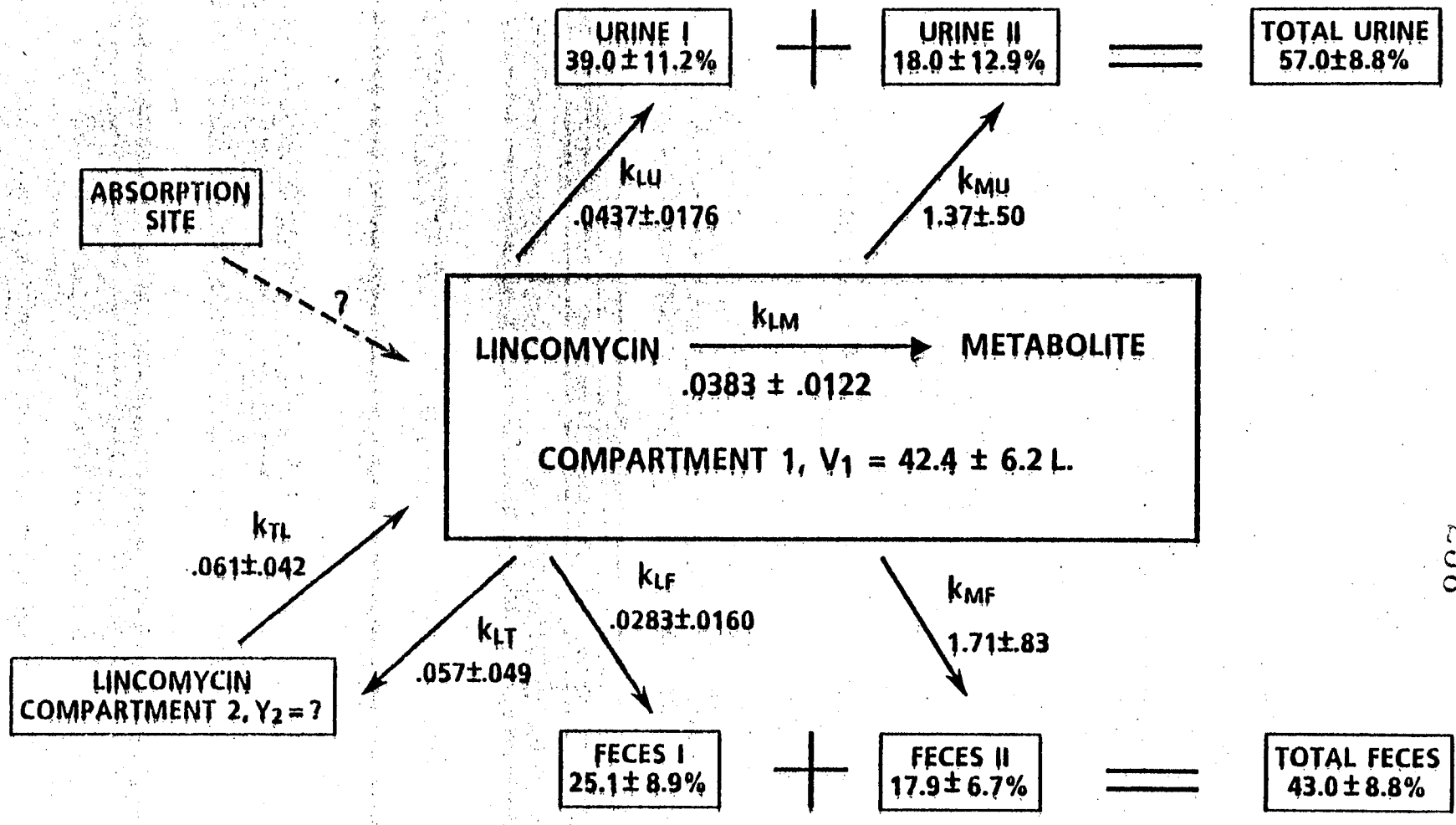
From these observations the kinetic model shown in Scheme III was developed utilizing the analog computer data simulator. It is proposed as the simplest model consistent with the experimental data.

(4). This complicated transport mechanism permitted calculation of only a minimal plasma half-life of 6.67 ± 1.77 hr. The primary volume of distribution, instantaneously equilibrated with plasma, was estimated to be 42.4 ± 6.2 L. The volume of the secondary compartment could not be estimated.

(5). The bulk of the I.M. dose was equilibrated instantaneously throughout the primary volume of distribution; however, a variable amount showed delayed absorption with an estimated maximal absorption half-time of 1.20 hr. Absorption of the P.O. dose appeared to be of an exponential-growth type and could not be described by simple first-order models. Paucity of data in this phase and the limited capacity of the analog computer precluded estimation of absorption half-time of the oral dose.

(6). It was calculated that from 7-32% of the oral dose was absorbed. The absorption-efficiency distribution was variable but appeared to

THE RATE AND EXTENT OF TRANSPORT OF LINCOMYCIN



Scheme III.

center around 7% and averaged about 10% with tailing to higher values. Thus, the results of this study were comparable to the results of earlier clinical studies.

(7). Although the percent of the absorbed dose excreted in urine (51-66%) vs. feces (34-49%) was relatively constant, the amount of lincomycin vs. metabolite in either urine or feces was highly variable from subject to subject.

In a subsequent report on the characterization of the urinary excretion products in dog and man, Eberts and Meeks (29) have made the following conclusions; the primary urinary excretory product of lincomycin administered orally or intramuscularly to dog or man is unmetabolized drug. In the dog, this amounted to 74% (P.O.) and 80-85% (I.M.) of the fraction of the dose found in urine, and 11% (P.O.) and 33-45% (I.M.) of the administered dose. Comparable figures for man were 56% (P.O.) and 83% (I.M.) based on urinary excretion, and 8% (P.O.) and 49% (I.M.) based on the administered dose. Although none of the metabolites were fully characterized, they possessed little or no bioactivity.

The above findings were confirmed by Daniels and Van Eyk (30) in a dog metabolism study using ^{14}C-lincomycin HCl. However, they had evidence to suspect that lincomycin sulfoxide and N-demethyl lincomycin to be minor metabolites (<3% of the dose).

6. References

1. Mason, D. J., Dietz, A., and DeBoer, C., Antimicrobial Agents and Chemotherapy, 1962, 554-559.

2. Meulman, P. A. Personal communication, The Upjohn Company, Kalamazoo, Michigan.

3. Slomp, G., and MacKeller, F. A., J. Amer. Chem. Soc., 89, 2454 (1967).

4. Mizsak, S., Slomp, G., Neszmelyi, A., Gero, S. D., and Lukacs, G., Tetrahedron Letters, 8, 721 (1977).

5. Slomp, G., and Mizsak, S. Personal communication, The Upjohn Company, Kalamazoo, Michigan.

6. Boal, J. Personal communication, The Upjohn Company, Kalamazoo, Michigan.

7. Daniels, E. G. Personal communication, The Upjohn Company, Kalamazoo, Michigan.

8. Struck, W. A. Internal communication, The Upjohn Company, Kalamazoo, Michigan 49001.

9. Bergren, M. S. Personal communication, The Upjohn Company, Kalamazoo, Michigan.

10. Knuth, M. L. and Zipple, K. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

11. Forist, A. A. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

12. Marsh, J. R., and Weiss, P. J., J. Assoc. Offic. Anal. Chem., 50, 457 (1967).

13. Koshy, K. T. and Knuth, D. W. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

14. Herr, H. R., and Slomp, G., J. Amer. Chem. Soc., 89, 2444 (1967).

15. Schroeder, W., Bannister, B., and Hoeksma, H., Ibid, 89, 2448 (1967).

16. Slomp, G., and MacKeller, F. A., Ibid, 89, 2454 (1967).

17. Magerlein, B. J., Birkenmeyer, R. D., Herr, H. R. and Kagan, F., Ibid, 89, 2459 (1967).

18. Forist, A. A., Royer, M. E. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

19. Forist, A. A., Brown, L. W., and Royer, M. E., J. Pharm. Sci. 54, 476 (1965).

20. Oestering, T. O., Ibid, 59, 63 (1970).

21. Prescott, G. C., Ibid, 55, 423 (1966).

22. Control Laboratory Procedure No. 135, January 14, 1977, The Upjohn Company, Kalamazoo, Michigan.

23. Kuo, M. S., and Kreling, J. R. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

24. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

25. Methods of Analysis, A.O.A.C., 1980, 13th Edition 42.196-42.201.

26. Lewis, C., and Meyer, C. E. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

27. Vavra, J. J., Sokolski, W. T. and Lawson, J. R., Antimicrobial Agents Chemotherapy, 176 (1963).

28. Eberts, F. S., Jr., Baker, R. H., Jr., Meeks, R. C., and Vliek, R. W. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

29. Eberts, F. S., and Meeks, R. C. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

30. Daniels, E. G., and Van Eyk, R. L. Internal communication, The Upjohn Company, Kalamazoo, Michigan.

Table I. Infrared Band Assignments for Lincomycin Hydrochloride, Monohydrate

Wave numbers (cm^{-1})	Structural Feature	Assignment
3529, 3489, 3453, 3380, 3339, 3290, 3228, 3199, 3076 3046, 3023	Alcohols and secondary amide	O-H stretch and N-H stretch
2751 broad	Amine salt	N-H stretch
1658	Secondary amide	C=O stretch
1567	Secondary amide	Amide II
1107, 1092, 1077 1042	Alcohols and cyclic ether	C-O stretch

Table II. Proton NMR Spectral Assignments for Lincomycin Hydrochloride

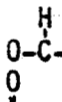
Group	Shape	Chemical Shift
$CH_3-CH_2-CH_2-$	distorted triplet	0.87
$CH_3-CHOH-$	doublet (J=6.1)	1.04
$CH_3-CH_2-CH_2$	broad	1.35
$S-CH_3$	Singlet	1.99
N^+-CH_3	Singlet	2.82
	doublet (J=5.4)	5.17
	doublet (J=8.4)	8.52
HCl	broad singlet	9.7

Table III. ^{13}C Chemical Shifts of Lincomycin Hydrochloride at 25.16 MHz in D_2O

Carbon	Chemical Shifts	Carbon	Chemical Shifts
1	89.2[a]	2'	69.5
2	68.8	3'	36.4
3	71.4	4'	37.6
4	69.5	5'	62.4
5	70.5	α CH_2	35.0
6	54.9	β CH_2	21.5
7	67.4	ν CH_3	14.3
8	17.2	N CH_3	41.8
SCH_3	14.2	Carbonyl	170.1

[a]All chemical shifts are given in parts per million relative to tetramethylsilane. 1,4-Dioxane was used as the internal standard, the shifts were converted to TMS by the relationship $\delta_C(CH_3)_4Si = \delta_C$(p-dioxane) + 67.4 ppm

Table IV. Powder X-Ray Diffraction Data of Lincomycin Hydrochloride Polymorphs.

Form I			Form II		
2θ	d-spacing(Å)	Intensity*	2θ	d-spacing(Å)	Intensity*
5.55	15.92	1	6.30	14.03	2
6.40	13.81		8.50	10.40	
6.90	12.81	4	9.45	9.36	5
7.45	11.87		10.35	8.55	
11.15	7.94	2	12.75	6.94	4
13.00	6.81		14.15	6.26	1
13.95	6.35	5	15.00	5.91	
14.40	6.15		15.70(w)	5.64	
17.00	5.22	3	15.95(w)	5.56	
17.85	4.97		16.80	5.28	
18.40(b)	4.82		17.25	5.14	
19.40	4.58		17.85	4.97	
20.15	4.41		18.25(w)	4.86	
21.05	4.22		19.15	4.63	3
21.95(sh)	4.05		19.80	4.48	
22.35	3.98		21.00	4.23	
23.15	3.84		21.55	4.12	
23.95	3.72		22.00	4.04	
25.80	3.45		22.85	3.89	
27.10	3.29		24.35	3.65	
			25.40	3.51	
			25.80	3.45	
			26.35	3.38	
			27.75	3.21	
			29.20	3.06	
			29.60	3.02	
			30.80	2.90	

Note: b = broad
w = weak
sh = shoulder

*Five strongest peaks (1 = the most intense peak)

$$\text{d-spacing Å} = \left(\frac{n\lambda}{2 \sin \theta}\right)$$

Table V. Solubilities of Lincomycin Hydrochloride in Common Organic Solvents (From Ref. 12)

Solvent	Solubility (mg/ml)*
Methanol	>20
Ethanol	>20
Isopropanol	4.83
Isoamyl alcohol	1.06
Cyclohexane	0.02
Benzene	0.08
Petroleum ether	0.01
Isooctane	0.02
Carbon tetrachloride	0.02
Ethyl acetate	0.03
Isoamyl acetate	0.05
Acetone	0.07
Methyl ethyl ketone	0.03
Diethyl ether	0.01
Ethylene chloride	0.01
1,4-Dioxane	1.37
Chloroform	0.06
Carbon disulfide	0.03
Pyridine	>20
Formamide	>20
Ethylene glycol	>20
Propylene glycol	>20
Dimethyl sulfoxide	>20

*The experimental design was such that if all the material appeared to be in solution, the solubility was considered to be greater than 20 mg/ml.

Table VI. Partition Coefficient of Lincomycin Hydrochloride Between Water and a Few Organic Solvents.

Solvent Pair	P.C. $C_{organic}/C_{water}$
Butanol:water (pH 10)[1]	2.5
CH_2Cl_2:water (pH 9.9)[1]	0.38
Butyl acetate:water (pH 9.6)[1]	0.19
Methyl ethyl ketone:water (pH 9.6)[1]	0.77
n-octanol:water (pH 2)[2]	0.0031
n-octanol:water (pH 7)[2]	2.55
n-octanol:water (pH 9, Borate)[2]	0.20
n-octanol:water (pH 9, THAM (tris))[2]	2.98

[1] From reference 11
[2] From reference 13

Table VII. Paper Chromatographic Systems for Lincomycin[1]

Solvent System	Development Time, Hours	RF Values
1) 1-butanol-water (84:16)	16	0.42
2) 1-butanol-water (84:16) plus 0.25% p-toluenesulfonic acid	16	0.42
3) 1-butanol-acetic acid-water (2:1:1)	16	0.70
4) 1-butanol-water (84:16) plus 2% piperidine	16	0.72
5) 1-butanol-water (4:96)	5	0.90
6) 1-butanol-water (4:96) plus 0.25% p-toluenesulfonic acid	5	0.90

[1] Developed on Whatman No. 1 filter paper (Whatman Inc., Clifton, New Jersey)

Table VIII. Thin Layer Chromatographic Behavior of Lincomycin Base on Silica Gel[1] Plates

Solvent	RF Values
Hexane (1.9)[2]	0
1,4-Dioxane (2.2)	0.62
Benzene (2.3)	0
Toluene (2.4)	0
Ethyl ether (4.3)	0
Chloroform (4.8)	0
Ethyl acetate (6.0)	0.05 (Tailing)
Methylene chloride (9.1)	0
Cyclohexanone (18.3)	0.2 (Severe tailing)
Acetone (21)	0.4 (Tailing)
Dimethyl formamide	0.93
Acetonitrile (38.8)	0.13 (Tailing)
Ethanol (95%)	0.65
Methanol (32.6)	0.68
Methanol, ethyl acetate, water 15:30:0.9	0.58
Chloroform, methanol, water 30:15:0.7	0.73
Chloroform, methanol, ammonium hydroxide (17%) 4:5:2	0.86
Isopropanol, water, ammonium hydroxide (17%) 8:1:1	0.66

[1]Silica Gel GF_{254}, 250 μm, Analtech Inc., Newark, Delaware
[2]Numbers in parentheses are the dielectric constant of the solvent.

AGRICULTURAL RESEARCH AND
DEVELOPMENT LABORATORIES,
THE UPJOHN COMPANY

TECHNICAL REPORT NO. 524-9760-83-001

PATHOLOGY/TOXICOLOGY NO. _____

TECHNICAL REPORT

TRIAL OR STUDY NO. _____

DATE: April 6, 1983

TITLE: Determination of the Octanol-Water Partition Coefficient of Lincomycin HCl at pH 2, 7 and 9

AUTHOR: D. W. Knuth and K. T. Koshy DK

ABSTRACT: The n-octanol/water partition coefficient (K_{ow}) can be used as an estimate of the tendency of a compound to bio-concentrate in living cells, and has practical implications for aqueous/organic solvent extractions. A procedure was designed to determine the K_{ow} for the hydrochloride salt of lincomycin (Lincomycin HCl), and to comply with the major criteria described in the Federal Register, Vol. 45, #227, (21 November 1980), §772.122-4. The K_{ow} value was buffer dependent at pH 9. The mean values obtained at the different pH values are:

	pH 2 = 0.003	$\log K_{ow} = -2.52$
	pH 7 = 2.55	$\log K_{ow} = 0.406$
(borate)	pH 9 = 0.201	$\log K_{ow} = -0.696$
(Tris)	pH 9 = 2.98	$\log K_{ow} = 0.474$

These values indicate that the majority of the lincomycin remains in the aqueous buffer phase.

en

Upjohn

MEMO

80-126 8/83

TO List

FROM D. W. Knuth DK

SUBJECT Corrections for Technical Report #524-9760-83-001, dated April 6, 1983

272

DATE April 9, 1984

COPIES TO

KAAsh
REBloss
RAEvans
CJFarho
BJGreve
WDHowser
JHLuchsinger
CCMiller
AWNeff
EFNicks
MLOgilvie
GHSwenson
GSwank
BSO (2)
An.Hlth.Cent.
Files (3)

The following corrections are necessary in Technical Report No. 524-9760-83-001 entitled, "Determination of the Octanol-Water Partition Coefficient of Lincomycin HCl at pH 2, 7 and 9," dated April 6, 1983:

Page 2, Line 6, under MATERIALS AND METHODS

specific activity = 2.468 μCi/g, 5480 dpm/g

should read:

specific activity = 2.468 μCi/mg, 5480 dpm/μg

Please place corrected page in body of the report. Retain the incorrect (replaced) page in a section at the end of the report.

en

INTRODUCTION

The n-octanol/water partition coefficient of Lincomycin HCl was determined to satisfy a specific request by Dr. L. W. Luther, Department of Health and Human Services, Bureau of Veterinary Medicine, to Dr. C. C. Miller, Research Manager and FDA Liaison, The Upjohn Company, in a letter dated 28 September 1981. This work should satisfy part of the deficiencies noted by the government in the original Environmental Impact Analysis Report filed for this compound (NADA #97-505-C73). The n-octanol/water partition coefficient (K_{ow}) is used as an estimate of the tendency of an organic chemical to bio-concentrate in living cells. The Federal Register, Vol. 45, #227 (21 November 1980), §772.122-4 lists specific requirements for the design of a procedure to determine these values. The procedure described herein meets the major criteria established by the government guidelines. Available equipment necessitated some deviation from specific requirements; these are described fully, and a justification for each is given. It is unlikely that any significant change in the K_{ow} value resulted from these modifications.

MATERIALS AND METHODS

A. Reagents

Lincomycin HCl monohydrate: Control Laboratory, The Upjohn Company, Issue C

Labelled ^{14}C-lincomycin: lot #15830-WTS-154A (REH-XLV-65)
purity = 98.7 %
specific activity = 2.468 µCi/mg, 5480 dpm/µg

n-Octanol: Certified 1-Octanol, Class IIIA, Lot #720754
Fisher Scientific Co., Fairlawn, NJ

Water: Upjohn distilled water, double-distilled in glass using in-house deionized water

Potassium Chloride: granular AR; Mallinckrodt Co., Inc., Paris, KY

Boric Acid: granular AR; Mallinckrodt Co., Inc., Paris, KY

Potassium Phosphate, monobasic: crystal AR; J. T. Baker Co., Inc., Phillipsburg, NJ

Tris(hydroxymethyl)aminomethane: Certified Primary Standard,
aka: Tris, THAM Fisher Scientific Co., Fair Lawn, NJ

Sodium Hydroxide: 1.0 N Acculute; Anachemia Chemicals, Inc., Champlain, NY

Hydrochloric Acid: 1.0 N Acculute; Anachemia Chemicals, Inc., Champlain, NY

Scintillant: ACS (Aqueous Counting Scintillant); Amersham Corp., Arlington Heights, IL

Internal Counting Standard: Toluene-^{14}C, dated 17 October 1979, listed radioactivity = $5.27 \times 10^5 \mp 3.2\%$ dpm/g Packard Instrument Co., Inc., Downers Grove, IL

B. Instrumentation

1. SHAKERS: a) Burrell Model #DD; Burrell Corp., Pittsburgh, PA
   b) NBS Gyrotory Model #G-10; New Brunswick Scientific Co., New Brunswick, NJ

2. pH METER: Beckman model Zeromatic IV; Beckman Instruments, Inc., Irvine, CA

3. CENTRIFUGE: Sorvall model #RC-5 refrigerated centrifuge; Sorvall Division, E. I. DuPont de Nemours & Co., Inc., Newtown, CT

4. ROTARY EVAPORATOR: Rinco, with heated (37°C) water bath; Servo-Instruments Corp., Spring Valley, IL

5. SCINTILLATION COUNTER: Packard Tri-Carb Model #3375; Packard Instrument Co., Inc., Downers Grove, IL

C. Preparation of Buffered Aqueous Phases

Clark and Lubs buffers used in these trials are described in "The United States Pharmacopeia", XVII edition, p. 913-914. In addition, a Tris buffer was prepared to check the dependence of Kow on buffer type at pH 9. The effect of changing ionic strength for a given buffer and pH level was not investigated. All buffer salts were used "as is" with no washing prior to use.

1. pH 2.00 Hydrochloric Acid Buffer

The pH of the distilled water was 6.1 at room temperature (23°C). A 0.2 M KCl solution was prepared by weighing 14.9110 g of the salt into a 1 L glass-stoppered volumetric flask, and dissolving in distilled water. After equilibration, the volume was adjusted to the mark with additional water. A 1.0 N HCl solution was prepared from the Acculute kit in a similar fashion. The final buffer was prepared by transferring 250 ml of the KCl solution and 13.0 ml of the HCl solution to a 1 L volumetric flask using volumetric pipettes. After adding the bulk of the water necessary and equilibrating, the volume was adjusted to the mark, and the solution transferred to a stoppered Erlenmeyer flask for storage. The measured pH was 2.02 at room temperature. The ionic strength μ was calculated in the manner of Castellan[1] to be 0.063.

2. pH 7.00 Phosphate Buffer

A 0.2 M KH_2PO_4 solution and a 1.0 N NaOH solution were prepared using 27.2174 g of the salt in the fashion described for the pH 2.00 buffer above. Exactly 250 ml of the KH_2PO_4 solution and 29.1 ml of the NaOH solution were used to prepare 1.0 L of the buffer; which was stored in a rubber-stoppered Erlenmeyer flask. The measured pH was 7.03 at room temperature. The calculated ionic strength μ was 0.079.

3. pH 9.00 Borate Buffer

A 0.2 M H_3BO_3/KCL solution was prepared using 12.3573 g and 14.9111 g of the salts, respectively, in the fashion described for the pH 2.00 buffer above. The 1.0 N NaOH solution prepared for the pH 7.00 buffer was also used. Exactly 250 ml of the H_3BO_3/KCl solution and 20.8 ml of the NaOH solution were used to prepare 1.0 L of the buffer, which was stored in a rubber-stoppered Erlenmeyer flask. The measured pH was 9.01 at room temperature. The calculated ionic strength μ was 0.121.

4. pH 9.00 Tris buffer

A 0.05 M Tris buffer was prepared by accurately weighing 6.057 g THAM, dissolving and diluting almost to volume, then adjusting the pH with 2.0 N HCl. The measured pH was 9.00 at room temperature. The calculated ionic strength μ was 0.050.

D. Phase Preparation

The n-octanol ("phase A" or "lipid") and buffer ("phase B" or "aqueous") were co-saturated with one another by transferring about 450 ml of each to a glass-stoppered 1 L round bottom flask, which was then sealed with tape and aluminum foil. The flasks were shaken so that the interface was continuously and vigorously disrupted for 16 hours at 23° ∓ 0.5°C on the NBS gyrorotatory shaker. The phases then stood at room temperature until further use.

E. Preparation of Solute in phase B

Stock solutions of Lincomycin HCl were prepared at three concentrations in each buffer by sequential dilution. The ratio of lincomycin base to Lincomycin HCl monohydrate is 100:113.5. The stock solutions for each buffer were prepared so that each would contain approximately 10 mg/ml lincomycin base. Further dilutions with additional buffer yielded 1 mg/ml and 0.1 mg/ml solutions.

An aqueous 1 μg/μl solution of the ^{14}C-lincomycin was provided by Dr. R. E. Hornish of the Upjohn Company. Exactly 45 μl or 50 μl was added to each solution above using a Hamilton 100 μl syringe before final dilution; the exact sequence of preparation is shown in Scheme 1. The amount used provided 2740 dpm/ml in each buffer, or 54800 dpm total in the 20 ml aliquot

used to partition. Triplicate aliquots were withdrawn from each buffer stock solution as described in section H, and their values used to determine recoveries of the ^{14}C label.

F. Partitioning

Separate 50 ml round bottom flasks were pre-rinsed with the desired phase B, then dried. Exactly 20 ml each of phase A and the desired solute-containing phase B were then transferred to each using volumetric pipettes. Triplicate sub-samples were prepared at each concentration for each buffer. The flasks were tightly glass-stoppered, sealed with tape, and shaken on the Burrell shaker with continuous disruption of the interface for 16 hours at ambient temperature. All were allowed to stand until a clean interface formed (<1 minute) before preparing them for centrifugation.

G. Centrifugation

The solutions from sections E and F were separated into their respective phases, then transferred to 15 ml snap-top polyethylene centrifuge tubes and spun 20 minutes at 22°C. Using the Sorvall centrifuge with a 4.34" radius head at 9000 rpm provided 9990 g's to the samples. Residual amounts of the opposing phases were removed by aspiration. Disposable pipettes were used to transfer each centrifuged phase to glass-stoppered containers for storage.

H. Analysis of ^{14}C-lincomycin in post-partition phases

The centrifuged phases from section G were sampled directly using 2 ml volumetric pipettes. The exterior of the pipette was wiped dry, and the sample transferred to a 20 ml screw-top scintillation vial. A single 2 ml aliquot was counted from each phase for each buffer concentration. Exactly 15 ml ACS counting scintillant was added using a Repipet pumping dispenser, and the samples counted three times for five minutes in the Packard Tri-Carb counter using the ^{14}C channel. The samples were then fortified with 50 µl of the internal counting standard and re-counted twice.

J. Procedural Deviations

Deviations from the recommended guidelines are described below, listed according to the appropriate sub-section of §772.122-4 in the Federal Register.

Item # Location, Title, Description

I. (d) Conditions (1) Special laboratory equipment (i) A thermostatic bath...

No thermostatic bath, chamber, or room was used to provide temperature control. This was felt to be an unnecessary and inconvenient requirement for this chemical.

2. (d) Conditions (1) Special laboratory equipment (ii) An ultracentrifuge...

   No ultracentrifuge was readily available, so the Sorvall model with spin speeds to 20K rpm was used instead. There were no apparent emulsions formed between the buffer and the n-octanol phases, so that the centrifuging would have only served to separate micro-emulsions which may or may not have been present.

3. (d) Conditions (1) Special laboratory equipment (iii) Stainless steel or glass centrifuge tubes...

   No such tubes were on hand for the centrifuge described under item 2 above. Polyethylene tubes were substituted.

4. (d) Conditions (2) Temperature control

   The procedure was conducted at the ambient temperature of 23.0° ∓ 1.0° C. The logistics involved in maintaining the recommended temperature of 25° C would have been prohibitive, and probably would have exerted an insignificant effect on the final Kow values.

5. (d) Conditions (4) Concentration of solute
   (10) Speciation effects (i)
   (2) Procedure (vii)

   The federal guidelines are contradictory in these sections; one paragraph requires the solute concentrations tested to be $C < 0.01$ M, $C_1 = 0.01C$; another has $C_1 = 0.1C$. The 10 mg/ml concentration exceeds the upper limit by a factor of 2.5. The results suggest that the concentrations examined were appropriately scaled.

6. (d) Conditions (9) Equilibration vessel

   Small round bottom flasks were used instead of centrifuge tubes. All contained <10 ml air to minimize its effect on partitioning, which should be insignificant for this non-volatile chemical.

7. (g) Test procedures (1) Reagents and solutions (ii) Buffer solutions

   Interest in the behaviour of lincomycin in a more acidic medium prompted the selection of pH 2 for investigation rather than pH 5. The Clark and Lubs buffers used are those recommended in a separate federal publication governing related work: "Guidelines for Registering Pesticides in the United States, subpart N, Chemistry Requirements; Environmental Fate", draft dated 3 October 1980, §163.161-1, specifically the reference to the work of S. F. Krzeminski, et al, J. Agr. Food Chem., 23, 1060-1068 (1975).

The recommended borate buffer at pH 9 is a good complexing agent for sugars and compounds with multiple hydroxy groups, so a Tris buffer was selected as a means of double-checking the results obtained.

8. (g) Test procedures (1) Reagents and solutions (iv) Preparation of a test solution

The availability of the radiolabel in an aqueous solution, the reasonable stability of lincomycin in water and the ease of handling prompted the preparation of the solute in the aqueous phase rather than the n-octanol phase.

K. Calculations

The post-partition concentration of lincomycin in each phase was computed from the raw scintillation data using a computer program described in Technical Report #060-78-9760-002, J. L. Nappier, 10 March 1978, using an IBM 370 mainframe computer. A Texas Instruments TI-55 hand calculator was used to generate mean values and calculate both K_{ow} and log K_{ow}.

The n-octanol/water partition coefficient (K_{ow}) was calculated by dividing the number of dpm in post-partition phase A by the number of dpm in post-partition phase B, and generating mean and standard deviation values from these results.

RESULTS AND DISCUSSION

The K_{ow} and log K_{ow} values for each pH are presented in Tables 1-4. Assuming minimal partitioning, a 50 dpm variance in the phase B aliquot would represent a 1 % error. The mean values presented do not include the results obtained at 10 mg/ml. This concentration is well below the established solubility of lincomycin in water[2], but exceeds the directed C < 0.01M guideline. The K_{ow} values at 10 mg/ml would lie within the 95 % confidence interval for the results (95 % C.L. = $(2.306 \times (SD/n^{1/2}))$, indicating that the behavior of the solute does not differ drastically for slightly higher concentrations.

All of the values are relatively low (log K_{ow} < 1). This implies that any attempt to extract lincomycin into an organic solvent such as n-octanol may require several successive partitions in order to transfer a majority of the chemical to the desired solvent. It also suggests that lincomycin would be excreted from animals before it could accumulate in fatty tissues.

The ability of the pH 9 borate buffer to suppress lincomycin partitioning can be seen by comparison of its value with that for the Tris buffer. Under increasingly basic conditions, the partition coefficient of lincomycin is expected to increase, a prediction which the borate buffer result appears to negate. The Tris buffer result shows the probable complexation of the sugar moiety of lincomycin by the borate ion to produce this misleading indication.

The mean results are:	pH 2 = 0.003	log K_{ow} = -2.52
	pH 7 = 2.55	log K_{ow} = 0.406
(borate)	pH 9 = 0.201	log K_{ow} = -0.696
(Tris)	pH 9 = 2.98	log K_{ow} = 0.474

REFERENCES

1. Castellan, G. W., "Physical Chemistry," 2nd Edition, Addison-Wesley Co., 1971.

2. Forist, A. A., Internal Communications, The Upjohn Company, Kalamazoo, MI.

3. Research Notebook Reference: KTK.12110.121-131
DWK.I.1-87

Table 1: K_{ow} at pH 2

Lincomycin Concentration[1]	Sample #	N=	Mean Total dpm/20 ml N-Octanol Phase A	Mean Total dpm/20 ml Buffer Phase B	Total dpm Recovered	% Recovery[3]	K_{ow}	Log K_{ow}
10 mg/ml	NP[2]	3	---	57324	---	---	---	---
	1	3	163	56185	56348	98.3	0.003	-2.52
	2	3	197	55967	56164	98.0	0.004	-2.40
	3	3	188	56692	56880	99.2	0.003	-2.52
	Mean 1,2,3	9	183	56281	56464	98.5 ∓ 0.65 CV=0.66	0.003 ∓0.0003 CV=9.45	-2.48
1 mg/ml	NP[2]	3	---	55812	---	---	---	---
	1	3	184	57109	57293	102.7	0.003	-2.52
	2	3	181	57586	57767	103.5	0.003	-2.52
	3	3	175	58055	58230	104.3	0.003	-2.52
	Mean 1,2,3	9	180	57583	57763	103.5 ∓ 0.84 CV=0.81	0.003 ∓0.0001 CV=3.23	-2.52
0.1 mg/ml	NP[2]	3	---	57746	---	---	---	---
	1	3	194	56693	56887	98.5	0.003	-2.52
	2	3	195	61371	61566	106.6	0.003	-2.52
	3	3	157	57422	57579	99.7	0.003	-2.52
	Mean 1,2,3	9	182	58495	58677	101.6 ∓ 4.37 CV=4.30	0.003 ∓0.0004 CV=11.63	-2.52
					OVERALL MEAN[4]:		0.003 ∓0.0002 CV=7.63	-2.52

[1]Approximate values
[2]Non-partitioned buffer aliquots
[3]Based on value from non-partitioned sample
[4]Using only the values from 1 mg/ml and 0.1 mg/ml

281

Table 2: K_{ow} at pH 7

Lincomycin Concentration[1]	Sample #	N=	Mean Total dpm/20 ml N-Octanol Phase A	Mean Total dpm/20 ml Buffer Phase B	Total dpm Recovered	% Recovery[3]	K_{ow}	Log K_{ow}
10 mg/ml	NP[2]	3	---	58867	---	---	---	---
	1	3	40276	16793	57069	96.9	2.40	0.380
	2	3	39458	17275	56733	96.4	2.28	0.359
	3	3	39379	17243	56622	96.2	2.28	0.359
	Mean 1,2,3	9	39704	17104	56808	96.5 ∓ 0.40 CV=0.41	2.32 ∓ 0.066 CV=2.85	0.366
1 mg/ml	NP[2]	3	---	59369	---	---	---	---
	1	3	40752	15968	56720	95.5	2.55	0.407
	2	3	41069	16077	57146	96.3	2.55	0.407
	3	3	41013	16082	57095	96.2	2.55	0.407
	Mean 1,2,3	9	40945	16042	56987	96.0 ∓ 0.39 CV=0.41	2.55 ∓ 0.002 CV=0.08	0.407
0.1 mg/ml	NP[2]	3	---	56265	---	---	---	---
	1	3	40199	15567	55766	99.1	2.58	0.412
	2	3	40434	16088	56522	100.5	2.51	0.400
	3	3	40536	16031	56567	100.5	2.53	0.403
	Mean 1,2,3	9	40390	15895	56285	100.0 ∓ 0.80 CV=0.80	2.54 ∓ 0.036 CV=1.43	0.405
						OVERALL MEAN[4]:	2.55 ∓ 0.024 CV=0.93	0.406

[1]Approximate values
[2]Non-partitioned buffer aliquots
[3]Based on value from non-partitioned sample
[4]Using only the values from 1 mg/ml and 0.1 mg/ml

Table 3: K_{ow} at pH 9, Borate Buffer

Lincomycin Concentration[1]	Sample #	N=	Mean Total dpm/20 ml N-Octanol Phase A	Mean Total dpm/20 ml Buffer Phase B	Total dpm Recovered	% Recovery[3]	K_{ow}	Log K_{ow}
10 mg/ml	NP[2]	3	---	57625	---	---	---	---
	1	3	7091	50040	57131	99.1	0.142	-0.849
	2	3	7222	50015	57237	99.3	0.144	-0.840
	3	3	7383	49707	57090	99.1	0.149	-0.828
	Mean 1,2,3	9	7232	49921	57153	99.2 ∓ 0.13 CV=0.13	0.145 ∓0.003 CV=2.37	-0.839
1 mg/ml	NP[2]	3	---	56793	---	---	---	---
	1	3	9071	47730	56801	100.0	0.190	-0.721
	2	3	9489	47740	57229	100.8	0.199	-0.702
	3	3	9820	47324	57144	100.6	0.208	-0.683
	Mean 1,2,3	9	9460	47598	57058	100.4 ∓ 0.40 CV=0.40	0.199 ∓0.009 CV=4.39	-0.702
0.1 mg/ml	NP[2]	3	---	56660	---	---	---	---
	1	3	9765	46528	56293	99.4	0.210	-0.678
	2	3	9648	47374	57022	100.6	0.204	-0.691
	3	3	9444	47574	57018	100.6	0.199	-0.702
	Mean 1,2,3	9	9619	47159	56778	100.2 ∓ 0.74 CV=0.74	0.204 ∓0.006 CV=2.79	-0.690
					OVERALL MEAN[4]:		0.201 ∓0.007 CV=3.57	-0.696

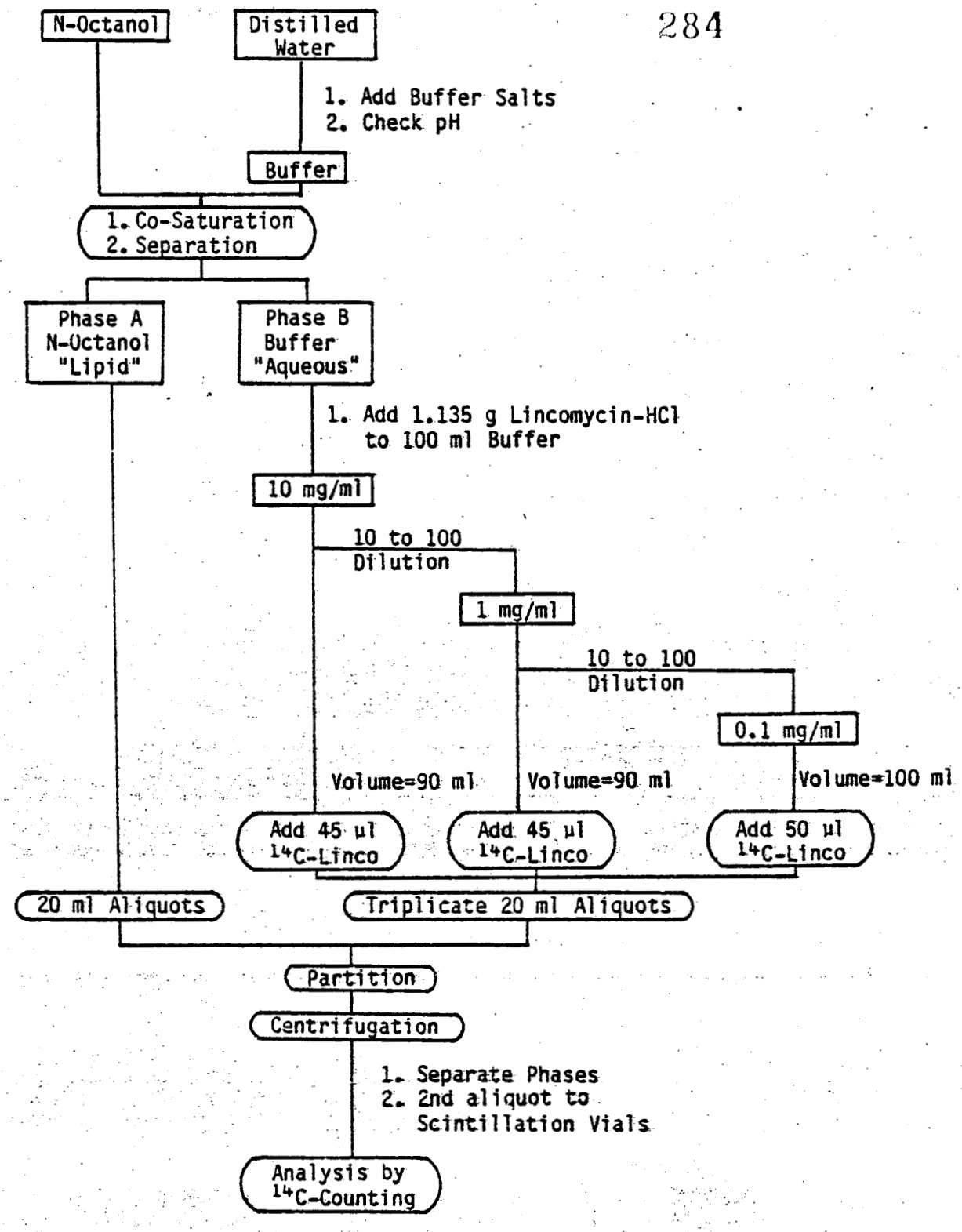
[1] Approximate values
[2] Non-partitioned buffer aliquots
[3] Based on vlaue from non-partitioned sample
[4] Using only the values from 1 mg/ml and 0.1 mg/ml

Table 4: K_{ow} at pH 9, Tris Buffer

Lincomycin Concentration[1]	Sample #	N=	Mean Total dpm/20 ml N-Octanol Phase A	Mean Total dpm/20 ml Buffer Phase B	Total dpm Recovered	% Recovery[3]	K_{ow}	Log K_{ow}
10 mg/ml	NP[2]	3	---	57616	---	---	---	---
	1	3	39772	17306	57078	99.1	2.30	0.361
	2	3	38878	17845	56723	98.5	2.18	0.338
	3	3	39223	17901	57124	99.1	2.19	0.341
	Mean 1,2,3	9	39291	17684	56975	98.9 ∓ 0.38 CV=0.39	2.22 ∓0.066 CV=2.96	0.347
1 mg/ml	NP[2]	3	---	62008	---	---	---	---
	1	3	42373	14795	57168	92.2	2.86	0.457
	2	3	41923	14897	56820	91.6	2.81	0.449
	3	3	43953	15077	59030	95.2	2.92	0.465
	Mean 1,2,3	9	42750	14923	57673	93.0 ∓ 1.92 CV=2.06	2.86 ∓0.051 CV=1.76	0.457
0.1 mg/ml	NP[2]	3	---	54339	---	---	---	---
	1	3	50233[5]	16591[5]	66824[5]	123.0[5]	3.03	0.481
	2	3	41090	13172	54262	99.9	3.12	0.494
	3	3	41527	13193	54720	100.7	3.15	0.498
	Mean 1,2,3	9	41309	13183	54491	100.3 ∓ 0.60 CV=0.59	3.10 ∓0.063 CV=2.02	0.491
					OVERALL MEAN[4]:		2.98 ∓0.138 CV=4.62	0.474

[1]Approximate Values
[2]Non-partitioned buffer aliquots
[3]Based on value from non-partitioned sample
[4]Using only the values from 1 mg/ml and 0.1 mg/ml
[5]Excluded from mean calculations

284



Scheme 1: Diagram of Sample Preparation

INTRODUCTION

The n-octanol/water partition coefficient of Lincomycin HCl was determined to satisfy a specific request by Dr. L. W. Luther, Department of Health and Human Services, Bureau of Veterinary Medicine, to Dr. C. C. Miller, Research Manager and FDA Liaison, The Upjohn Company, in a letter dated 28 September 1981. This work should satisfy part of the deficiencies noted by the government in the original Environmental Impact Analysis Report filed for this compound (NADA #97-505-C73). The n-octanol/water partition coefficient (K_{ow}) is used as an estimate of the tendency of an organic chemical to bio-concentrate in living cells. The Federal Register, Vol. 45, #227 (21 November 1980), §772.122-4 lists specific requirements for the design of a procedure to determine these values. The procedure described herein meets the major criteria established by the government guidelines. Available equipment necessitated some deviation from specific requirements; these are described fully, and a justification for each is given. It is unlikely that any significant change in the K_{ow} value resulted from these modifications.

MATERIALS AND METHODS

A. Reagents

Lincomycin HCl monohydrate: Control Laboratory, The Upjohn Company, Issue C

Labelled ^{14}C-lincomycin: lot #15830-WTS-154A (REH-XLV-65)
purity = 98.7 %
specific activity = 2.468 µCi/g, 5480 dpm/g

n-Octanol: Certified 1-Octanol, Class IIIA, Lot #720754
Fisher Scientific Co., Fairlawn, NJ

Water: Upjohn distilled water, double-distilled in glass using in-house deionized water

Potassium Chloride: granular AR; Mallinckrodt Co., Inc., Paris, KY

Boric Acid: granular AR; Mallinckrodt Co., Inc., Paris, KY

Potassium Phosphate, monobasic: crystal AR; J. T. Baker Co., Inc., Phillipsburg, NJ

Tris(hydroxymethyl)aminomethane: Certified Primary Standard,
aka: Tris, THAM Fisher Scientific Co., Fair Lawn, NJ

Sodium Hydroxide: 1.0 N Acculute; Anachemia Chemicals, Inc., Champlain, NY

VAPOR PRESSURE OF LINCOMYCIN HYDROCHLORIDE

K. T. K

K. Thomas Koshy
Biochemistry and Residue Analysis
Agricultural Division
The Upjohn Company
Kalamazoo, Michigan 49001

October 14, 1982

Lincomycin hydrochloride is the salt of a highly polar molecule. Therefore it is not expected to have any significant vapor pressure at ambient temperature. This is substantiated by the loss on drying at reduced pressures (0.1 to 0.3 torr) at room temperatures. The Physical and Analytical Chemistry Laboratory of The Upjohn Company has data on the loss of drying on 42 production lots of lincomycin hydrochloride manufactured from 1962-1969. The weight losses ranged from 0 to 2.08% with a mean weight loss of 0.39%. This small loss indicates that the compound is essentially non-volatile and that the water of crystallization (3.9%) is not lost during the drying process. This assumption is further supported by a comparison of the loss of drying of lincomycin hydrochloride with two compounds commonly used to calibrate standard vapor pressure determination equipment (vapor pressure balance and gas saturation methods). Under almost identical conditions crystalline naphthalene with a vapor pressure of 0.07 torr at 20°C (1) and crystalline benzophenone with a vapor pressure of 5×10^{-4} torr at 23.1°C (2) lost 42.5 and 8% of its weight respectively. Lincomycin hydrochloride which had a mean weight loss of 0.39%, therefore, has very low vapor pressure, if any, at ambient temperature (21-22°C) and is certainly lower than 5×10^{-4} torr.

---

(1) T. H. Swan and E. Mack, Jr., J.A.C.S., 47, 2112 (1925).

(2) G. W. Thompson, Technique of Organic Chemistry, Vol. 1, Physical Methods, Part 1, p. 464.

AGRICULTURAL RESEARCH AND
DEVELOPMENT LABORATORIES,
THE UPJOHN COMPANY

TECHNICAL REPORT NO. 524-9760-83-002

PATHOLOGY/TOXICOLOGY NO. _____

TECHNICAL REPORT

TRIAL OR STUDY NO. _____

DATE: March 21, 1983

TITLE:

Sorption/Desorption of U-10,149A (Lincomycin) in Three Soil Types at 0.2, 1.0, 5.0 and 25.0 mg/Liter

AUTHOR:

DBJ

D. B. Johnson and B. L. Cox

ABSTRACT:

The sorption-desorption of lincomycin in three soils was examined to qualitatively assess the leaching characteristics of the antibiotic. Approximately 30 to 50 percent of the ^{14}C lincomycin was sorbed by all three soils from the aqueous calcium chloride solutions. Six hours were required for the lincomycin to reach soil/water equilibrium in all three soils. However, after washing the treated soils, approximately 40 to 60 percent of sorbed ^{14}C lincomycin was desorbed. Further, the Koc coefficients ranged from 0.12 to 1.59 for the three soils. These very low Koc values were indicative of a compound which is not appreciably sorbed to soil. Therefore, lincomycin would be expected to leach from all three soils tested.

msj

I. Introduction

The FDA requires sorption-desorption data (1) for the evaluation of the migratory tendency of chemicals into water, soil, or sediment compartments of the environment. Swine, fed lincomycin treated feed for dysentery, excret intact lincomycin in their urine and feces. A qualitative estimate of the leaching potential of the excreted lincomycin may be determined from solubility and sorption-desorption data. Therefore, this report describes the sorption-desorption of lincomycin in three soil types.

II. Experimental

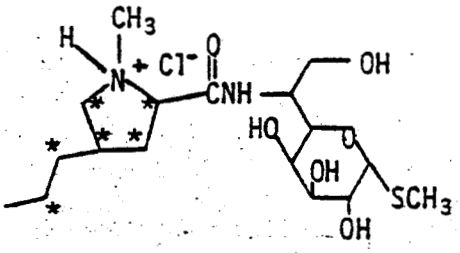
1. Materials and Methods

a. Materials - All of the solvents were of analytical grade and were purchased from the Burdick and Jackson Co., as was the Diotol liquid scintillation counting fluid. The $^{14}CO_2$ absorbing liquid scintillation cocktail was prepared by thoroughly mixing Permafluor (2L) and Carbosorb (1L), Packard Instrument Co., and storing the mixture at 10°C in a tightly closed bottle. Toluene-^{14}C internal standard was also obtained from Packard Instrument Co. Silica gel thin layer plates and x-ray film (AA-5) were purchased from Analtech and Kodak Co., respectively. A mixture of ^{14}C-histidine (New England Nuclear) and blue recorder ink was used as a reference spot in the autoradiochromatographic analysis. The 250 ml polyethylene centrifuge bottles were purchased from the Corning Co. The anhydrous calcium chloride was obtained from Sargent-Welch and the hydrochloric acid and sodium hydroxide from the Mallinckrodt Chemical Co.

b. Methods - All of the soluble ^{14}C samples were analyzed by liquid scintillation counting (LSC) on a Packard Tri-Carb Model 3255 scintillation spectrometer with ^{14}C toluene as the internal standard. The soils from the mass balance determination were analyzed for bound residue with a Beckman Biological Oxidizer. The soil samples were mixed with mannitol (~200 mg), combusted, and the $^{14}CO_2$ was trapped in $^{14}CO_2$ absorbing liquid scintillation cocktail and counted by LSC. An ethanol standard of ^{14}C lincomycin was used to determine the combustion unit efficiency by comparing the recovery of combusted triplicate (50 µL) aliquots with triplicate (50 µL) aliquots that were counted directly. Sample weights were determined on Mettler Model P1200N and A30-12 balances and pH determinations were obtained on a Beckman Zeromatic Model SS-3. TLC plates were scraped with an Analabs, Inc., Multi-vial TLC Scraper. All solvents were evaporated on a Rinco Rotary Evaporation Unit in a water bath at 40-45°C. A Burrells Wrist Action Shaker was used to shake the aqueous soil mixtures. The aqueous soil mixtures were centrifuged in an International Six Cup Centrifuge at 1800 rpm to clarify the aqueous phase.

2. Acquisition and Purification of Labeled and Unlabeled Lincomycin

a. Labeled ^{14}C Lincomycin - The labeled ^{14}C lincomycin (Lot No.



* ⟶ ^{14}C positions

M.W. 428.5 M.F. $C_{17}H_{33}N_2O_6SCl$

1583-WTS-154A) was obtained from R. S. Hsi, Drug Metabolism Research, Unit 7256, The Upjohn Co., Kalamazoo, Michigan. Ammonium hydroxide was added to the labeled lincomycin in methanol to generate the free base. The free base was applied to a 2.0 mm silica gel plate and the plate was developed (100 mm) in ethyl acetate, methanol, water (30:15:1). The ^{14}C lincomycin band was located by autoradiography, scraped, and eluted from the silica gel with methanol (5 x 5.0 mL). The purified ^{14}C lincomycin was 96 to 97% radiochemically pure by histogram analysis (Appendix, p. 1,2). A total of 24 mg of ^{14}C lincomycin (Specific Activity = 25 µCi/mg) was obtained from the purification. The radiochemical purity of the ^{14}C lincomycin remained at 96 to 97% throughout the experiment.

b. Non-Labeled Lincomycin - The non-labeled lincomycin used in the studies was an Upjohn Reference Standard (Issue C-Lot No. 248 PLX) from Unit 7843, Kalamazoo, Michigan. The lincomycin hydrochloride monohydrate was 95.8% lincomycin hydrochloride and 4% water with 0.2% organic impurities. The potency of the lincomycin was 879 µg/mg and the water solubility was greater than 500 mg/mL, but less than 1000 mg/mL.

3. Preparation of ^{14}C-Lincomycin Aqueous $CaCl_2$ Solutions

a. Aqueous $CaCl_2$ Solution Preparation - The 0.01 M $CaCl_2$ solutions were prepared by dissolving 1.11 grams of anhydrous $CaCl_2$ in one liter of boiled deionized water. The pH of the solution was adjusted to 7.0 and analyzed by LSC for ^{14}C residues prior to the addition of the ^{14}C lincomycin (Appendix, p. 3). Aliquots (0.1 mL) of the solution in diotol (15 mL) were used as counting blanks in the preliminary screening and advanced tests.

b. Preparation of ^{14}C-Lincomycin Solutions - The purified ^{14}C lincomycin (24.0 mg) was dissolved in methanol (12.0 mL) to give a 2.0 mg/mL concentration (Stock Solution 1). Also, unlabeled lincomycin (449 mg) was dissolved in methanol (50.0 mL) to yield a 8.0 mg/mL concentration (Stock Solution 2). The following ^{14}C lincomycin solutions were prepared from the above stock solutions.

Solution A (25.0 mg/L) - Aliquots of Stock Solution 1 (0.1 mL) and Stock Solution 2 (3.1 mL) were added to a one liter graduated cylinder and the methanol was removed in a nitrogen stream. After the methanol was removed, the lincomycin was q.s. to the one liter volume with 0.01M $CaCl_2$ solution.

Solution B (5.0 mg/L) - Identical procedures for the preparation of Solution A were used to prepare Solution B, except for the amount of Stock Solution 2 (0.6 mL) that was added to produce the 5.0 mg/L Solution B.

Solution C (1.0 mg/L) - Identical procedures for the preparation of Solution A were used to prepare Solution C, except for the amount of Stock Solution 2 (0.1 mL) that was added to produce the 1.0 mg/L Solution B.

Solution D (0.2 mg/L) - The Solution D was prepared in the same manner as Solution A, except that only Stock Solution 1 (0.1 mL) was used to give a 0.2 mg/L concentration of ^{14}C lincomycin.

4. Soil Acquisition, Analysis, and Preparation

a. Soil Acquisition - Sandy clay loam, clay, and clay loam soils were obtained from cooperators in Texas, Illinois, and Michigan, respectively. The soils were analyzed (Figures 1-3) by the Michigan State Soil Laboratories and they met the requirements of pH and % organic matter set by the FDA guidelines for the study of sorption-desorption (1).

b. Soil Preparation - One kilogram of each soil was sieved through a #10 mesh screen (2 mm) to remove pebbles and other debris. The soil moisture was 8.0, 25.0, and 12.0 percent for the Texas sandy clay loam, Illinois clay, and Michigan clay loam soils, respectively. Twenty-five grams of each soil was weighed and extracted with methanol and the methanol extracts were subjected to a microbiological assay for lincomycin (2). The microbiological assay was negative for the three soils. Also, soil samples were combusted and analyzed for ^{14}C by LSC (Appendix, p. 4).

5. Preliminary Test for Lincomycin in the Illinois Soil

a. Test Procedure - Three aliquots (25 g dry weight) of Illinois soil were mixed with 0.01M $CaCl_2$ solution (130 mL) in 250 mL centrifuge bottles and continuously shaken for 16 hours. After shaking, the mixtures were centrifuged (1800 rpm) for 10 min. and the clear aqueous solutions were decanted into mixing cylinders (200 mL). Each mixing cylinder contained 13 µL of Stock Solution 1 (0.026 mg ^{14}C lincomycin) and 0.4 mL of Stock Solution 2 (3.2 mg lincomycin) to give a 25.0 ppm concentration of lincomycin in the $CaCl_2$ solutions. The methanol was removed under N_2 from the stock solutions in the cylinders before the addition of the $CaCl_2$ solutions. Also, each cylinder was brought to the 130 mL mark with (~10 mL) fresh $CaCl_2$ solution.

b. Measurement - Triplicate aliquots (0.1 mL) of the $CaCl_2$ solutions were analyzed by liquid scintillation counting (LSC), and interfaced with a CMS computer program (3). The results were computed in average DPM of ^{14}C lincomycin/mL of $CaCl_2$ solution (Appendix, p. 5,6). Percent recoveries were determined by comparison of the fortified extracted soil with a 25.0 ppm standard of ^{14}C lincomycin in fresh $CaCl_2$ solution (130 mL). The mean and standard deviation of the three replicates was determined (Table 1). The results for all of the test procedures are given relative to the concentration in the aqueous phase.

6. Screening Test for Lincomycin in the Three Soils

a. Test Procedure - The screening test for sorption-desorption of lincomycin was run at a 25 mg/L (25 ppm) concentration in all three of the soils tested. Thus, Solution A (130 mL) was added to three replicate 25.0 g (dry weight) samples of the Illinois, Texas, and Michigan soils in 250 mL centrifuge bottles. The mixtures were continuously shaken for 16 hrs, then centrifuged and the supernates were decanted and labeled sorption step.

The desorption phase was accomplished by adding aliquots (130 mL) of fresh aqueous 0.01M $CaCl_2$ solutions to each of the above replicate soils. The soils were resuspended in the solution and shaken continuously for 16 hrs. After shaking, the mixtures were centrifuged and the supernates were decanted and identified as the first wash. The above process was repeated with fresh $CaCl_2$ solution (130 mL) and the resulting supernates were labeled the second wash.

b. Measurement - Triplicate aliquots (0.1 mL) of the sorption step and both the first and second washes in the desorption step were analyzed by LSC with CMS computation (Appendix, p. 7-13). The mean and standard deviation of the three replicates was determined (Tables 2-4).

7. Advanced Tests: Kinetic, Isotherm, and Mass Balance

a. Kinetics Test - The kinetics test was run in duplicate for each test soil (Illinois, Texas, and Michigan). Thus, aliquots (25 g dry weight) of the soils were shaken with solution B (130 mL, 5.0 mg/L of ^{14}C lincomycin). The mixtures were continuously shaken, except during sampling times at 2, 4, 6, 8, and 24 hrs. The mixtures were sampled by centrifugation and removal of triplicate aliquots (0.1 mL) of the supernate. The aliquots were analyzed by LSC and CMS computation (Appendix, p. 14-17).

b. Isotherm Test - Triplicate soil samples (25 g dry weight) from Illinois, Texas, and Michigan were shaken for six hrs with 0.01M $CaCl_2$ solutions containing ^{14}C lincomycin. Three samples of each soil type were shaken with solution containing ^{14}C lincomycin concentrations of 25 mg/L (Solution A), 5.0 mg/L (Solution B), 1.0 mg/L (Solution C), and 0.2 mg/L (Solution D). Each sample was

shaken for 6 hrs, centrifuged, and triplicate aliquots (0.1 mL) of the supernate was taken for analysis. The aliquots were analyzed by LSC and CMS computation (Appendix, p. 18-23).

c. Mass Balance Determinations - The soils, from the Solution B isotherm test, were each shaken for 10 min. with methanol (50 mL). After decantation of the methanol, the above process was repeated twice and the combined methanol extracts were evaporated in vacuo. The residue was dissolved in methanol (3.0 mL) and triplicate aliquots (10 µL) were assayed by LSC and CMS computation (Appendix, p. 18-23). The extracted soils were dried under vacuum overnight and equal weights of the three replicates were combined for each soil. Further, the soil replicates were thoroughly mixed before three aliquots (~250 mg) were taken for combustion analysis. The combustions were analyzed by LSC and CMS computation (Appendix, p. 24).

The $CaCl_2$ solutions from the Solution B isotherm test were adjusted to pH 10 and extracted with $CHCl_3$ (3 x 50 mL). The extracts were dried in vacuo, reconstituted with methanol (5.0 mL), and triplicate aliquots (10 µL) were analyzed by LSC and CMS computation (Appendix, p. 18-23).

8. Identification of Lincomycin in Aqueous and Soil Extracts

a. Aqueous Extracts - The 0.01M $CaCl_2$ solutions from the preliminary, screening, kinetics, and isotherm test (5.0 mg/L) were adjusted to pH 9-10, extracted with chloroform (3 x 50 mL), and the chloroform was removed in vacuo. Methanol (5 mL) was added to dissolve the residue and three (10 µL) aliquots were examined by LSC and CMS computation (Appendix, p. 5-23). TLC plates were spotted with the methanol solutions and developed in ethyl acetate, methanol, water (30:15:1). The developed plates were analyzed by TLC-autoradiography (Appendix, p. 25, 32, 39, 46, 51, 56) and histography (Appendix, p. 26-31, 33-38, 40-45, 47, 48, 52, 53, 57, 58).

b. Soil Extracts - The methanol soil extracts from the mass balance study were spotted on TLC plates and developed in the same solvent system as the aqueous extracts. The TLC plates were also analyzed by autoradiography (Appendix, p. 46, 51, 56) and histography (Appendix, p. 49, 50, 54, 55, 59, 60).

III. Results and Discussion

1. Preliminary Test - The preliminary test was used to determine if the amount of lincomycin could be quantitated from aqueous solutions. Since ^{14}C lincomycin was used in the study, the concentration of lincomycin could be determined directly from the aqueous solutions by LSC and CMS computation (Table 1). The percent overall mean ± S.D. for the three replicates was 101.4 ± 1.0. Thus, the concentration (mg/L) of ^{14}C lincomycin in the aqueous solutions could be accurately determined by these procedures.

2. Screening Test - The screening test was used to provide a semi-quantitative measure of sorption and desorption of the test chemical. Further, the information was used to determine the necessity for advanced testing. The sorption part of the test was run at a 25 mg/L concentration of ^{14}C lincomycin for all three soils. The results (Table 2) show that only half (51.5%) of the ^{14}C lincomycin was sorbed to the Illinois soil, while less than half was sorbed to the Texas (37.3%) and Michigan (32.9%) soils.

   The desorption part of the screening test was used to assess whether the test chemical could be leached from the soil. The results for the first and second aqueous $CaCl_2$ washes of the desorption step are given in Tables 3 and 4, respectively. A considerable amount of the ^{14}C lincomycin sorbed in the sorption step was desorbed after two washes (Table 5). The total percentage of the sorbed ^{14}C lincomycin that was desorbed was 61.7, 54.6, and 41.3 percent for the Illinois, Texas, and Michigan soils, respectively.

   From the above data and the organic carbon content of the soil, it was possible to calculate the Kd and Koc coefficients for the three soils used in this study (Table 6). The Koc values are predictive of the amount of sorption of a test substance. Further, the Koc value can be compared to Koc values of other chemicals to assess the test chemical's sorption characteristics. The range of Koc values for lincomycin was 0.50 to 1.59 which are very low compared to representative values for other compounds (4). Therefore, lincomycin would be expected to exhibit very weak sorption properties which are characteristic of polar compounds with high water solubilities.

3. Advanced Tests.

   a. Kinetics Test - The kinetics test was used to determine the time necessary to establish equilibrium between the lincomycin, soil, and water partition. Aqueous samples were taken at 0, 2, 4, 6, 8, and 24 hrs and analyzed for ^{14}C activity. The concentration of lincomycin in the water reached equilibrium after 6 hrs and remained constant through the 24 hr sampling period. Further, the 6 hr time required to reach equilibrium was identical for all three soils (Figures 4-6). However, the concentration of sorbed lincomycin was different in each of the three soils. The equilibrium concentrations were 2.6, 1.8, and 1.6 mg/L for the Illinois, Texas, and Michigan soils, respectively. The equilibration time (6 hrs) was used in the following isotherm determination.

   b. Isotherm Determination - The isotherm determination provides a quantitative measure of sorption and desorption of lincomycin. Each soil was equilibrated for 6 hrs with 25, 5, 1, and 0.2 mg/L of ^{14}C lincomycin in 0.01M $CaCl_2$ solution. The concentrations of lincomycin in the $CaCl_2$ solution and soil were determined (Tables 7-9) and the x/m and Ce values were calculated (Tables 10-12). Further, the log x/m and log Ce values were calculated for each replicate.

Graphs were prepared using both x/m vs. Ce (Figures 7-9) and log x/m vs. log Ce (Figures 10-12). The replicates for both the x/m vs. Ce and log x/m vs. log Ce were nearly identical, and therefore, were plotted as the mean of the three replicates. The absorption constant K and constant 1/n, as well as, the Koc constant were calculated from the log x/m vs log Ce plots (Table 14). The isotherm Koc values are 2 to 6 times smaller than the Koc values determined in the screening test. These very low Koc values are a further indication of lincomycin's poor soil sorption characteristics.

c. Mass Balance. Both the soils and $CaCl_2$ solutions used in the Solution B isotherm test were analyzed for ^{14}C lincomycin residues. The $CaCl_2$ solution was analyzed directly for ^{14}C lincomycin by LSC and CMS computation (Table 15). Also, the solution was extracted with chloroform and the extracts were analyzed as previously described. The results show that 63.1 to 71.5% of the ^{14}C activity was extracted with chloroform. These results are consistent with the chloroform extraction of fortified controls, where approximately 60% of the ^{14}C lincomycin was extracted from the $CaCl_2$ solutions. Evidently, the lincomycin is not completely extracted due to its high water solubility (>500 mg/mL).

The $CaCl_2$ solutions from the preliminary, screening, and kinetics (5.0 mg/L) determinations were also extracted with chloroform. The chloroform extracts were examined by TLC-autoradiography and histogram analysis (Appendix, p. 46-60). The purity of the extracted ^{14}C lincomycin was determined from the histograms (Table 16). Although the lincomycin observed purity ranged from 73.1 to 97.6%, most of the values were above 90%. Therefore, analysis of the $CaCl_2$ solutions indicates that little degradation of the ^{14}C lincomycin took place in solution during the soil studies. However, the methanol extracts of the soil from the 5.0 mg/L isotherm experiment contained a ^{14}C compound that was more polar than lincomycin (Appendix, p. 46, 51, 56). The ^{14}C metabolite comprised 83.7, 71.5, and 54.0 percent of the ^{14}C activity in the methanol extracts of the Illinois, Texas, and Michigan soils, respectively. Further, the ^{14}C metabolite accounted for 16.4, 16.2, and 5.2 percent of the initial ^{14}C lincomycin added to the Illinois, Texas, and Michigan soils, respectively. Therefore, some of the ^{14}C lincomycin that was sorbed to the soil was further metabolized by the soil and the metabolic product was not released into the aqueous media.

IV. Conclusion

The previously described experiments have demonstrated that lincomycin was not readily sorbed to soil. Further, approximately 38 to 59 percent of the sorbed ^{14}C lincomycin is washed out. Also, the Koc data coupled with the high water solubility of lincomycin indicated that the antibiotic will readily leach from soil. However, preliminary evidence indicates that lincomycin may be converted to a metabolite in soil which is not readily leached from the soil.

Study Director — April 17, 1983 Date

524-9760-83-002

298

DATA SHEET

Study Sponsor: C. C. Miller, Research Manager, Performance Enhancement and FDA Liaison, Unit 9650, The Upjohn Co., Kalamazoo, Michigan.

Location of Study: Biochemistry and Residue Analysis (BRA), Unit 9760, Bldg. 209-5, The Upjohn Co.

Location of Raw Data: BRA, Unit 9760, Bldg. 209-5.

Location of the Final Report: Units 9650 and 9760, The Upjohn Co., Kalamazoo, Michigan.

Study Personnel

D. B. Johnson: Study Director, Scientist II, BRA, Unit 9760, Bldg. 209-5.

B. L. Cox: Chemical Assistant II, BRA, Conduct of the Study and Report Preparation, Unit 9760, Bldg. 209-5.

Date of Study Initiation: October 4, 1982.

Date of Study Completion: December 15, 1982.

Purity and Stability of the Test Substance: As stated on page 3.

299

REFERENCES

1. Food and Drug Administration Guidelines 10:08, August 16, 1982; Organization for Economic Co-Operation and Development (OECD). 1981. OECD Guidelines for Testing Chemicals, Section 1, No. 106, p. 1-23.

2. Barbiers, A. and Neff, A. W., JAOAC, 59, 849 (1976).

3. "PTSCINT 4: A New Version of the Scintillation Program," J. L. Nappier, Tech. Report 060-78-9760-002, March 10, 1978.

4. Hamaker, J. W. and Thompson, J. M., 1972. Adsorption, p. 49-144. In C.A.I. Goring and Hamaker, eds. Organic Chemicals in the Soil Environment, Vol. I, Marcel Dekker, New York.

Table 1. Preliminary Study 25 mg/mL Concentration

Recovery of ^{14}C Lincomycin[1] in 0.01M $CaCl_2$ Solutions from Illinois Soil

Replicates	Average DPM/mL[2]	% Recovery
1	8895	102.5
2	8791	101.3
3	8712	100.4
Overall % Mean and Standard Deviation		101.4 ± 1.0

[1]Average ^{14}C lincomycin value of 8680 DPM/mL used to calculate recovery (see Appendix, p. 5).

[2]Values are the average of triplicate analysis, corrected for volume change (see Appendix, p. 5).

Table 2. Sorption of ^{14}C Lincomycin[1] in Soils from Aqueous Calcium Chloride Solution (Screening Test).[2,3]

Soils	Avg % Non-Sorbed	Avg mg/L Non-Sorbed (Ce)	Avg % Sorbed	Avg mg/L Sorbed (x)
Ill-1	51.9	12.98	48.1	12.02
Ill-2	46.9	11.73	53.1	13.27
Ill-3	46.7	11.68	53.3	13.32
Mean ± SD	48.5 ± 2.9	12.13 ± 0.74	51.5 ± 2.9	12.87 ± 0.74
Tex-1	63.3	15.82	36.6	9.18
Tex-2	61.8	15.45	38.2	9.55
Tex-3	62.9	15.72	37.1	9.28
Mean ± SD	62.7 ± 0.8	15.66 ± 0.19	37.3 ± 0.8	9.34 ± 0.19
Mich-1	67.8	16.95	32.2	8.05
Mich-2	66.5	16.63	33.5	8.37
Mich-3	66.9	16.73	33.1	8.27
Mean ± SD	67.1 ± 0.7	16.77 ± 0.16	32.9 ± 0.7	8.23 ± 0.16

[1]Concentration of lincomycin was 25 mg/L (11524 DPM/mL) for the three soils tested (see Appendix, pg. 7).

[2]Equilibrated for 16 hrs.

[3]All figures are corrected for the volume change, therefore, Avg. %

$$\text{Non-sorbed} = \frac{\text{DPM in } CaCl_2 \text{ Sol'n (V)}}{\text{Initial DPM (Vo)}} \times 100$$ where Vo is the volume (130 mL) of $CaCl_2$ solution added to the soil and V is the volume (120 mL) after mixing with the soil.

Table 3. Desorption (1st Wash) of ^{14}C Lincomycin in Soils in Aqueous Calcium Chloride Solution[1,2]

Soils	Avg % Desorbed	Avg mg/L Desorbed	Avg % Remaining Bound	Avg mg/L Remaining Bound
Ill-1	49.9	6.00	50.1	6.02
Ill-2	40.7	5.40	59.3	7.87
Ill-3	39.6	5.27	60.4	8.05
Mean ± SD	43.4 ± 5.6	5.56 ± .39	56.6 ± 5.6	7.31 ± 1.1
Tex-1	44.3	4.07	55.7	5.11
Tex-2	43.5	4.15	56.5	5.40
Tex-3	42.1	3.91	57.9	5.37
Mean ± SD	43.3 ± 1.1	4.04 ± .12	56.7 ± 1.1	5.30 ± .16
Mich-1	34.6	2.78	65.4	5.26
Mich-2	34.2	2.86	65.8	5.51
Mich-3	35.3	2.92	64.7	5.35
Mean ±SD	34.7 ± 0.56	2.85 ± 0.07	65.3 ± 0.56	5.37 ± 0.13

[1] $\% \text{ Desorbed} = \frac{C_1}{Ci - Ce} \times 100$

where C_1 = concentration (DPM/mL) of lincomycin in first wash

Ci = initial concentration (DPM/mL) of lincomycin

Ce = concentration (DPM/mL) of lincomycin in solution of the sorption step

[2] See Appendix, p. 10.

Table 4. Desorption (2nd Wash) of ^{14}C Lincomycin in Soils in Aqueous Calcium Chloride Solution[1]

Soils	Avg % Desorbed	Avg mg/L Desorbed	Avg % Remaining Bound	Avg mg/L Remaining Bound
Ill-1	41.4	2.49	58.6	3.53
Ill-2	29.9	2.35	70.1	5.52
Ill-3	27.6	2.22	72.4	5.83
Mean ± SD	33.0 ± 7.4	2.35 ± 0.14	67.0 ± 7.4	4.96 ± 1.2
Tex-1	20.4	1.04	79.6	4.07
Tex-2	20.2	1.09	79.8	4.31
Tex-3	19.4	1.04	80.6	4.33
Mean ± SD	20.0 ± 0.53	1.06 ± 0.03	80.0 ± 0.53	4.24 ± 0.14
Mich-1	9.6	0.50	90.4	4.76
Mich-2	10.0	0.55	90.0	4.96
Mich-3	11.0	0.59	89.0	4.76
	10.2 ± 0.72	.55 ± 0.04	89.8 ± 0.72	4.82 ± 0.12

[1]% Desorbed (2nd wash) = $C_2/(Ci-Ce-C_1) \times 100$

where: C_2 = Concentration (DPM/mL) of lincomycin in second wash

Ci = Initial concentration (DPM/mL) of lincomycin

Ce = Concentration (DPM/mL) of lincomycin in solution of the sorption step

C_1 = Concentration (DPM/mL) of lincomycin in the first wash

[2]See Appendix, p. 12.

Table 5. Desorption of Lincomycin in Soil

Soils	$C_1 + C_2$	Ci - Ce	% Desorbed[1]	% Bound to Soil
Ill-1	8.49	12.02	70.6	29.4
Ill-2	7.75	13.27	58.4	41.6
Ill-3	7.49	13.32	56.2	43.8
Mean ± SD	7.91 ± 0.52	12.87 ± 0.74	61.7 ± 7.8	38.2 ± 7.8
Tex-1	5.11	9.18	55.7	44.3
Tex-2	5.24	9.55	54.9	45.1
Tex-3	4.95	9.28	53.3	46.7
Mean ± SD	5.10 ± 0.14	9.34 ± 0.21	54.6 ± 1.2	45.4 ± 1.2
Mich-1	3.28	8.05	40.7	59.3
Mich-2	3.41	8.37	40.7	59.3
Mich-3	3.51	8.27	42.4	57.6
Mean ± SD	3.40 ± 0.12	8.23 ± 0.16	41.3 ± 1.0	58.7 ± 1.0

[1]% Desorbed = $(C_1 + C_2)/(Ci - Ce) \times 100$

where:

C_1 = Concentration (DPM/mL) of lincomycin in first wash

C_2 = Concentration (DPM/mL) of lincomycin in second wash

Ci = Initial concentration (DPM/mL) of lincomycin

Ce = Concentration (DPM/mL) of lincomycin in solution in the sorption step

Table 6. Kd and Koc Coefficients of Lincomycin from the Screening Test Data

Soil	X	Ce	Kd x 10^{-2}	Koc
Ill-1	12.02	12.98	3.70	0.44
Ill-2	13.27	11.73	4.52	0.53
Ill-3	13.32	11.68	4.56	0.54
Mean ± SD	12.87 ± 0.74	12.13 ± 0.74	4.26 ± 0.48	0.50 ± 0.06
Tex-1	9.18	15.82	2.32	1.55
Tex-2	9.55	15.45	2.47	1.65
Tex-3	9.28	15.72	2.36	1.57
Mean ± SD	9.34 ± 0.19	15.66 ± 0.19	2.38 ± 0.08	1.59 ± 0.05
Mich-1	8.05	16.95	1.90	0.70
Mich-2	8.37	16.63	2.01	0.74
Mich-3	8.27	16.73	1.98	0.73
Mean ± SD	8.23 ± 0.16	16.77 ± 0.16	1.96 ± 0.06	0.72 ± 0.02

$Kd = \frac{x/m}{Ce}$ where x is the concentration (mg/L) of sorbed lincomycin, m is the dry weight of the soil (25.0 g), and Ce is the concentration (mg/L) of lincomycin in solution.

$Koc = \frac{Kd}{\%\ \text{organic carbon}} \times 100$ where:

Ill. % organic carbon = 8.5
Tex. % organic carbon = 1.5
Mich. % organic carbon = 2.7

Table 7. Illinois Soil Isotherm Test[1] 306

Soils	Ce[2] DPM/mL	% Ce	Non-Sorbed mg/L	Sorbed mg/L
	25.0 mg/L[3]			
Ill-1	6,464	63.9	15.97	9.03
Ill-2	5,874	58.0	14.51	10.49
Ill-3	5,955	58.8	14.71	10.29
Mean ± SD	6,098 ± 320	60.2 ± 3.2	15.06 ± 0.80	9.94 ± .79
	5.0 mg/L[4]			
Ill-1	5,563	55.2	2.76	2.24
Ill-2	5,755	57.1	2.86	2.14
Ill-3	5,557	55.2	2.76	2.24
Mean ± SD	5,625 ± 112	55.8 ± 1.1	2.79 ± 0.06	2.21 ± 0.06
	1.0 mg/L[5]			
Ill-1	5,307	51.3	0.51	0.49
Ill-2	5,298	51.2	0.51	0.49
Ill-3	5,050	48.8	0.49	0.51
Mean ± SD	5,218 ± 146	50.4 ± 1.4	0.50 ± 0.01	0.50 ± 0.01
	0.2 mg/L[6]			
Ill-1	5,267	45.3	0.09	0.11
Ill-2	5,337	45.9	0.09	0.11
Ill-3	5,270	45.3	0.09	0.11
Mean ± SD	5,291 ± 40	45.5 ± 0.35	0.09 ± 0.0	0.11 ± 0.0

[1]Equilibrium was reached after 6 hrs.

[2]Ce = Concentration (DPM/mL) of lincomycin in solution, corrected for the volume change.

[3]Initial concentration = 10,121 DPM/mL.

[4]Initial concentration = 10,074 DPM/mL.

[5]Initial concentration = 10,346 DPM/mL.

[6]Initial concentration = 11,634 DPM/mL.

Table 8. Texas Soil Isotherm Test[1]

307

Soils	Ce[2] DPM/mL	% Ce	Non-Sorbed mg/L	Sorbed mg/L
	25.0 mg/L[3]			
Tex-1	7,591	75.0	18.75	6.25
Tex-2	7,634	75.4	18.86	6.14
Tex-3	7,826	77.3	19.33	5.67
Mean ± SD	7,684 ± 125	75.9 ± 1.2	18.98 ± 0.31	6.02 ± 0.31
	5.0 mg/L[4]			
Tex-1	7,632	75.8	3.79	1.21
Tex-2	7,814	77.6	3.78	1.22
Tex-3	7,864	78.1	3.90	1.10
Mean ± SD	7,770 ± 122	77.2 ± 1.2	3.82 ± 0.07	1.18 ± 0.07
	1.0 mg/L[5]			
Tex-1	7,455	72.0	0.72	0.28
Tex-2	7,401	71.5	0.72	0.28
Tex-3	7,286	70.4	0.70	0.30
Mean ± SD	7,381 ± 87	71.3 ± 0.8	0.71 ± 0.01	0.29 ± 0.01
	0.2 mg/L[6]			
Tex-1	7,216	62.0	0.12	0.08
Tex-2	7,104	61.1	0.12	0.08
Tex-3	7,124	61.2	0.12	0.08
	7,148 ± 60	61.4 ± 0.5	0.12 ± 0.0	0.08 ± 0.0

[1]Equilibrium was reached after 6 hrs.

[2]Ce = Concentration (DPM/mL) of lincomycin in solution, corrected for the volume change.

[3]Initial concentration = 10,121 DPM/mL.

[4]Initial concentration = 10,074 DPM/mL.

[5]Initial concentration = 10,346 DPM/mL.

[6]Initial concentration = 11,634 DPM/mL.

Table 9. Michigan Soil Isotherm Test[1]

308

Soils	Ce[2] DPM/mL	% Ce	Non-Sorbed mg/L	Sorbed mg/L
	25.0 mg/L[3]			
Mich-1	8,838	88.8	22.20	2.80
Mich-2	8,530	85.7	21.42	3.58
Mich-3	8,180	82.2	20.55	4.45
Mean ± SD	8,516 ± 329	85.6 ± 3.3	21.39 ± 0.82	2.72 ± 0.86
	5.0 mg/L[4]			
Mich-1	8,212	77.7	3.88	1.12
Mich-2	8,207	77.6	3.88	1.12
Mich-3	8,028	75.9	3.80	1.20
Mean ± SD	8,149 ± 105	77.1 ± 1.0	3.85 ± 0.05	1.15 ± 0.05
	1.0 mg/L[5]			
Mich-1	7,898	78.2	0.78	0.22
Mich-2	7,709	76.4	0.76	0.24
Mich-3	7,782	77.1	0.77	0.23
Mean ± SD	7,796 ± 95	77.2 ± 0.9	0.77 ± 0.01	0.23 ± 0.01
	0.2 mg/L[6]			
Mich-1	7,878	76.9	0.15	0.05
Mich-2	8,008	78.2	0.16	0.04
Mich-3	8,102	79.1	0.16	0.04
Mean ± SD	7,996 ± 112	78.1 ± 1.1	0.16 ± 0.01	0.04 ± 0.01

[1]Equilibrium was reached after 6 hrs.

[2]Ce = Concentration (mg/L) of Tincomycin in solution, corrected for the volume change.

[3]Initial concentration = 9,555 DPM/mL.

[4]Initial concentration = 10,572 DPM/mL.

[5]Initial concentration = 10,096 DPM/mL.

[6]Initial concentration = 10,239 DPM/mL.

Table 10. Illinois Soil Isotherm for Lincomycin

Soils	x/m (10^{-4})	Ce (10^{-2})	Log x/m	Log Ce
	0.025 g/L			
Ill-1	3.61	1.60	-3.44	-1.80
Ill-2	4.20	1.45	-3.38	-1.84
Ill-3	4.12	1.47	-3.38	-1.83
Mean ± SD	3.98 ± 0.32	1.51 ± 0.08	-3.40 ± 0.03	-1.82 ± 0.02
	0.005 g/L			
Ill-1	0.90	0.28	-4.04	-2.55
Ill-2	0.86	0.29	-4.06	-2.54
Ill-3	0.90	0.28	-4.04	-2.55
Mean ± SD	0.89 ± 0.02	0.28 ± 0.01	-4.05 ± 0.01	-2.55 ± 0.01
	0.001 g/L			
Ill-1	0.20	0.05	-4.70	-3.30
Ill-2	0.20	0.05	-4.70	-3.30
Ill-3	0.20	0.05	-4.70	-3.30
Mean ± SD	0.020 ± 0.0	0.05 ± 0.0	-4.70 ± 0.0	-3.30 ± 0.0
	2 x 10^{-4} g/L			
Ill-1	0.04	0.01	-5.40	-4.00
Ill-2	0.04	0.01	-5.40	-4.00
Ill-3	0.04	0.01	-5.40	-4.00
Mean ± SD	0.04 ± 0.0	0.01 ± 0.0	-5.40 ± 0.0	-4.00 ± 0.0

where:

x is the amount (g/L) of lincomycin sorbed

m is the dry weight of the soil (25 g)

Ce is the amount of lincomycin (g/L) in the $CaCl_2$ solution

Table 11. Texas Soil Isotherm for Lincomycin

310

Soils	x/m (10^{-4})	Ce (10^{-2})	Log x/m	Log Ce
	0.025 g/L			
Tex-1	2.50	1.88	-3.60	-1.72
Tex-2	2.46	1.89	-3.61	-1.72
Tex-3	2.27	1.93	-3.64	-1.71
Mean ± SD	2.41 ± 0.12	1.90 ± 0.03	-3.62 ± 0.02	-1.72 ± 0.01
	0.005 g/L			
Tex-1	0.48	0.38	-4.32	-2.42
Tex-2	0.49	0.38	-4.31	-2.42
Tex-3	0.44	0.39	-4.36	-2.41
Mean ± SD	0.47 ± 0.03	0.38 ± 0.01	-4.33 ± 0.03	-2.42 ± 0.01
	0.001 g/L			
Tex-1	0.11	0.07	-4.96	-3.15
Tex-2	0.11	0.07	-4.96	-3.15
Tex-3	0.12	0.07	-4.92	-3.15
Mean ± SD	0.11 ± 0.01	0.07 ± 0.0	-4.95 ± 0.02	-3.15 ± 0.0
	2×10^{-4} g/L			
Tex-1	0.03	0.01	-5.50	-3.92
Tex-2	0.03	0.01	-5.50	-3.92
Tex-3	0.03	0.01	-5.50	-3.92
Mean ± SD	0.03 ± 0.0	0.01 ± 0.0	-5.50 ± 0.0	-3.92 ± 0.0

where:

x is the amount (g/L) of lincomycin sorbed

m is the dry weight of the soil (25 g)

Ce is the amount of lincomycin (g/L) in the $CaCl_2$ solution

Table 12. Michigan Soil Isotherm for Lincomycin

Soils	x/m (10^{-4})	Ce (10^{-2})	Log x/m	Log Ce
0.025 g/L				
Mich-1	0.75	2.31	-4.12	-1.64
Mich-2	1.07	2.23	-3.97	-1.65
Mich-3	1.44	2.14	-3.84	-1.67
Mean ± SD	1.09 ± 0.34	2.23 ± 0.08	-3.98 ± 0.14	1.65 ± 0.02
0.005 g/L				
Mich-1	0.45	0.39	-4.35	-2.41
Mich-2	0.45	0.39	-4.35	-2.41
Mich-3	0.48	0.38	-4.42	-2.42
Mean ± SD	0.46 ± 0.02	0.39 ± 0.01	-4.37 ± 0.04	-2.41 ± 0.01
0.001 g/L				
Mich-1	0.09	0.08	-5.04	-3.10
Mich-2	0.10	0.08	-5.00	-3.10
Mich-3	0.10	0.08	-5.00	-3.10
Mean ± SD	0.10 ± 0.0	0.08 ± 0.0	-5.01 ± 0.02	-3.10 ± 0.0
2×10^{-4} g/L				
Mich-1	0.02	0.02	-5.70	-3.70
Mich-2	0.02	0.02	-5.70	-3.70
Mich-3	0.02	0.02	-5.70	-3.70
Mean ± SD	0.02 ± 0.0	0.02 ± 0.0	-5.70 ± 0.0	-3.70 ± 0.0

where:

x is the amount (g/L) of lincomycin sorbed

m is the dry weight of the soil (25 g)

Ce is the amount of lincomycin (g/L) in the $CaCl_2$ solution

Table 13. Summary of the Soil Isotherms for ^{14}C Lincomycin

Soils	x/m (10^{-4})	Ce (10^{-2})	Log x/m	Log Ce
	0.025 g/L			
Ill	3.98	1.51	-3.40	-1.82
Tex	2.41	1.90	-3.62	-1.72
Mich	1.09	2.23	-3.98	-1.65
	0.005 g/L			
Ill	0.89	0.28	-4.05	-2.55
Tex	0.47	0.38	-4.33	-2.42
Mich	0.46	0.39	-4.37	-2.41
	0.001 g/L			
Ill	0.20	0.05	-4.70	-3.30
Tex	0.11	0.07	-4.95	-3.15
Mich	0.10	0.08	-5.01	-3.10
	2×10^{-4} g/L			
Ill	0.04	0.01	-5.40	-4.00
Tex	0.03	0.01	-5.50	-3.92
Mich	0.02	0.02	-5.70	-3.70

where:

x is the amount (g/L) of lincomycin sorbed

m is the dry weight of the soil (25 g)

Ce is the amount of lincomycin (g/L) in the $CaCl_2$ solution

Table 14. Freundlich Isotherm and Linear Regression Values

Soil	Slope	Intercept	1/n	K	Koc	Correlation Coefficient
Illinois	0.912	-1.727	0.912	0.0187	0.22	0.9995
Texas	0.852	-2.211	0.852	0.0062	0.41	0.9969
Michigan	0.841	-2.483	0.841	0.0033	0.12	0.9855

Table 15. Mass Balance of ^{14}C Lincomycin in Soil at 5.0 mg/L Concentration[1]

Soil	$CaCl_2$ Solution Avg mg	$CHCl_3$ Extract Avg mg	CH_3OH Extract Avg mg	Combustion Avg mg	Total mg	% Accountabilit
Ill-1	2.76	1.76	0.99	1.75	5.50 (4.50)	110.0 (90.0)
Ill-2	2.86	1.80	0.98	1.75	5.59 (4.52)	111.8 (90.4)
Ill-3	2.76	1.71	0.97	1.75	5.48 (4.43)	109.6 (88.6)
Mean ± SD	2.79 ± 0.06	1.76 ± 0.04	0.98 ± 0.01	1.75 ± 0.0	5.52 ± 0.06 (4.48 ± 0.05)	110.5 ± 1.2 (89.7 ± 0.94
Tex-1	3.79	2.68	1.14	0.30	5.23 (4.12)	104.6 (82.4)
Tex-2	3.78	2.78	1.15	0.30	5.23 (4.23)	104.6 (84.6)
Tex-3	3.90	2.72	1.10	0.30	5.30 (4.12)	106.0 (82.4)
Mean ± SD	3.82 ± 0.07	2.73 ± 0.05	1.13 ± 0.03	0.30 ± 0.0	5.25 ± 0.04 (4.15 ± 0.06)	105.0 ± 0.8 (83.1 ± 1.27
Mich-1	3.88	2.76	0.46	0.64	4.98 (3.86)	99.6 (77.2)
Mich-2	3.88	2.74	0.48	0.64	5.00 (3.86)	100.0 (77.2)
Mich-3	3.80	2.71	0.49	0.64	4.93 (3.84)	98.6 (76.8)
Mean ± SD	3.85 ± 0.05	2.74 ± 0.02	0.48 ± 0.02	0.64 ± 0.0	4.97 ± 0.04 (3.85 ± 0.01)	99.4 ± 0.0 (77.1 ± 0.0)

[1]The total mg and % accountability figures in parentheses were calculated from the $CHCl_3$ extract figures, while the $CaCl_2$ solution figures were used to calculate total mg and % accountability figures that are not in parentheses.

Table 16. Histogram Analysis of the ^{14}C Lincomycin $CaCl_2$ Solutions[1]

Soil	Experiment	% Purity[2,3]
Illinois	Preliminary	92.3
Illinois	Screening	86.6
Texas	"	97.6
Michigan	"	96.9
Illinois	Kinetics	92.2
Texas	"	93.6
Michigan	"	92.5
Illinois	Isotherm	90.4
Texas	"	93.7
Michigan	"	73.1

[1]Extracted with $CHCl_3$.

[2]% Purity of the ^{14}C lincomycin in the extract.

[3]Histograms in the Appendix, p. 26-31, 33-38, 40-45, 47-50, 52-55, 57-60.

Figure 1. Texas Soil

316

Upjohn Representaive: P.H. Parham

Sample Site: Donna, Texas

Date Collected: 11/12/80

Crop History: Milo in 1979 & 1980, Cotton 1978

Chemical History: None in 1979 & 1980, Methyl parathion, Toxaphene and Pydrin in 1978.

Sampling Procedure: 100 pounds of freshly disked topsoil

Soil Profile (Determined by MSU):

Soil pH 7.7

Pounds per acre of:

phosphorus	65
potassium	792
calcium	5120
magnesium	640

Calculated CEC	16
% Organic Matter	1.52

% of Total Exchangeable Bases:

potassium	6.2
calcium	77.7
magnesium	16.2

Soil Classification: Sandy Clay Loam

Particle Size:

% Sand	% Silt	% Clay
55.12	15.44	29.44

Bulk Density: 76.6 pounds/cu ft.

1/3 Bar Moisture Capacity (% by Weight): 18.4

Figure 2. Illinois Soil

317

Upjohn Representative: Todd Cutting

Sample Site: Pontiac, Illinois

Date Collected: 12/18/80

Crop History: Turf

Chemical History: None

Sampling Procedure: 100 lbs from top 6 inches

Soil Profile (Determined by MSU):

Soil pH 7.3

Pounds per acre of:

phosphorus	62
potassium	792
calcium	8000
magnesium	800

Calculated CEC 24
% Organic Matter 8.5

% of Total Exchangeable Bases:

potassium	4.2
calcium	82.1
magnesium	13.7

Soil Classification: Clay

Particle Size:

% Sand	% Silt	% Clay
29.68	27.44	42.88

Bulk Density: 69.0 pounds/cu ft.

1/3 Bar Moisture Capacity (% by Weight): 37.1

Figure 3. Michigan Soil

318

Upjohn Representative: B.L. Lee

Sample Site: Kalamazoo, MI

Date Collected: 11/19/80

Crop History: Wheat 1977, 1978, 1979, 1980

Chemical History: No pesticides

Sampling Procedure: 2 random samples from top 6 inches

Soil Profile (Determined by MSU):

Soil pH 6.3

Pounds per acre of:

phosphorus	156
potassium	408
calcium	1813
magnesium	331

Calculated CEC 8
% Organic Matter 2.72

% of Total Exchangeable Bases:

potassium	8.1
calcium	70.4
magnesium	21.4

Soil Classification: Clay Loam

Particle Size:

% Sand	% Silt	% Clay
39.68	33.44	26.88

Bulk Density: 75.3 pounds/cu ft

1/3 Bar Moisture Capacity (% by Weight): 20.7

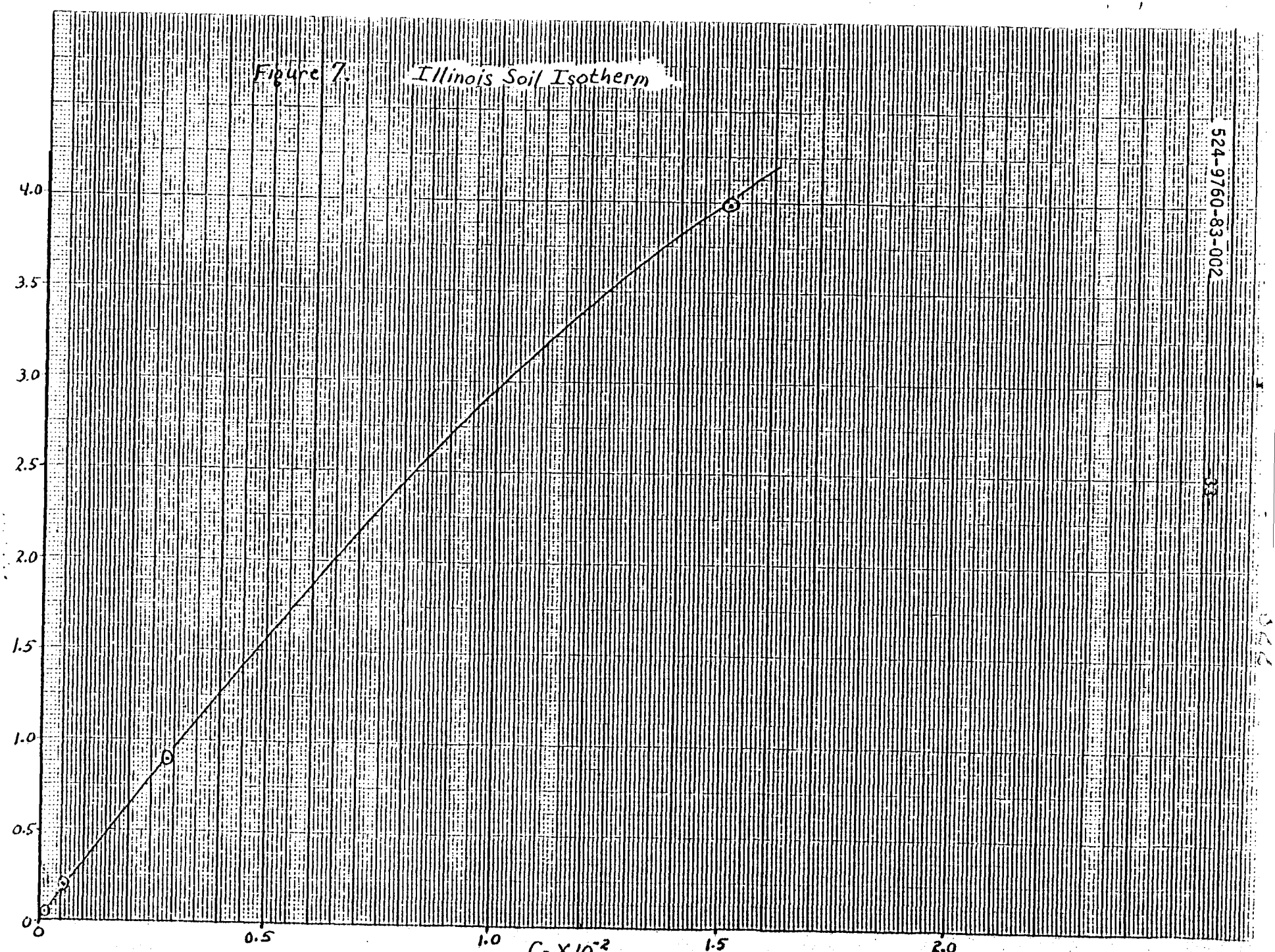


Figure 7.
Illinois Soil Isotherm
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0
0
0.5
1.0
1.5
2.0
Ce X 10⁻²
524-9760-83-002

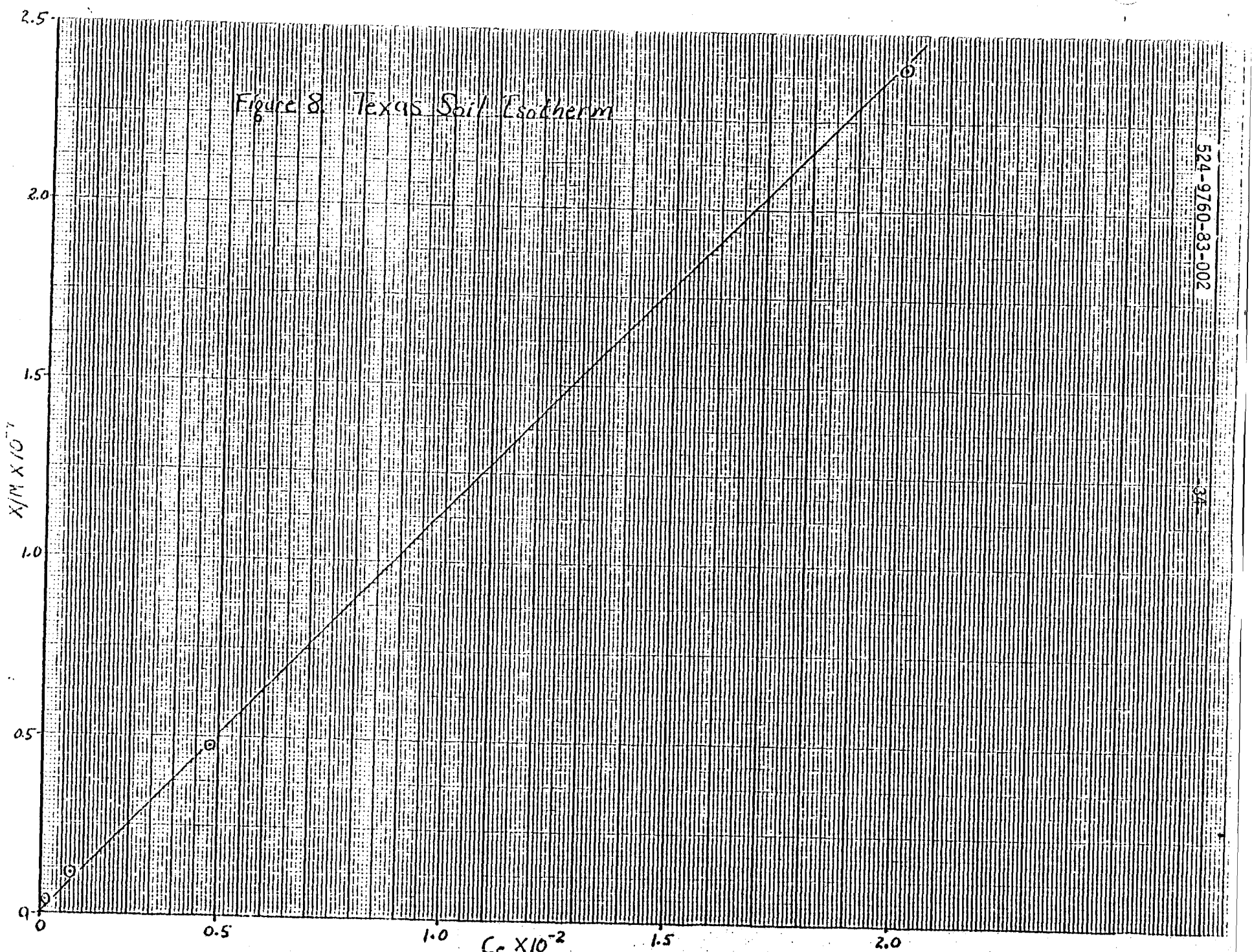


Figure 8. Texas Soil Isotherm
2.5
2.0
1.5
1.0
0.5
0
X/M X 10⁻¹
0.5
1.0
1.5
2.0
Ce X10⁻²
524-9760-83-002
-34-

Figure 9. Michigan Soil Isotherm

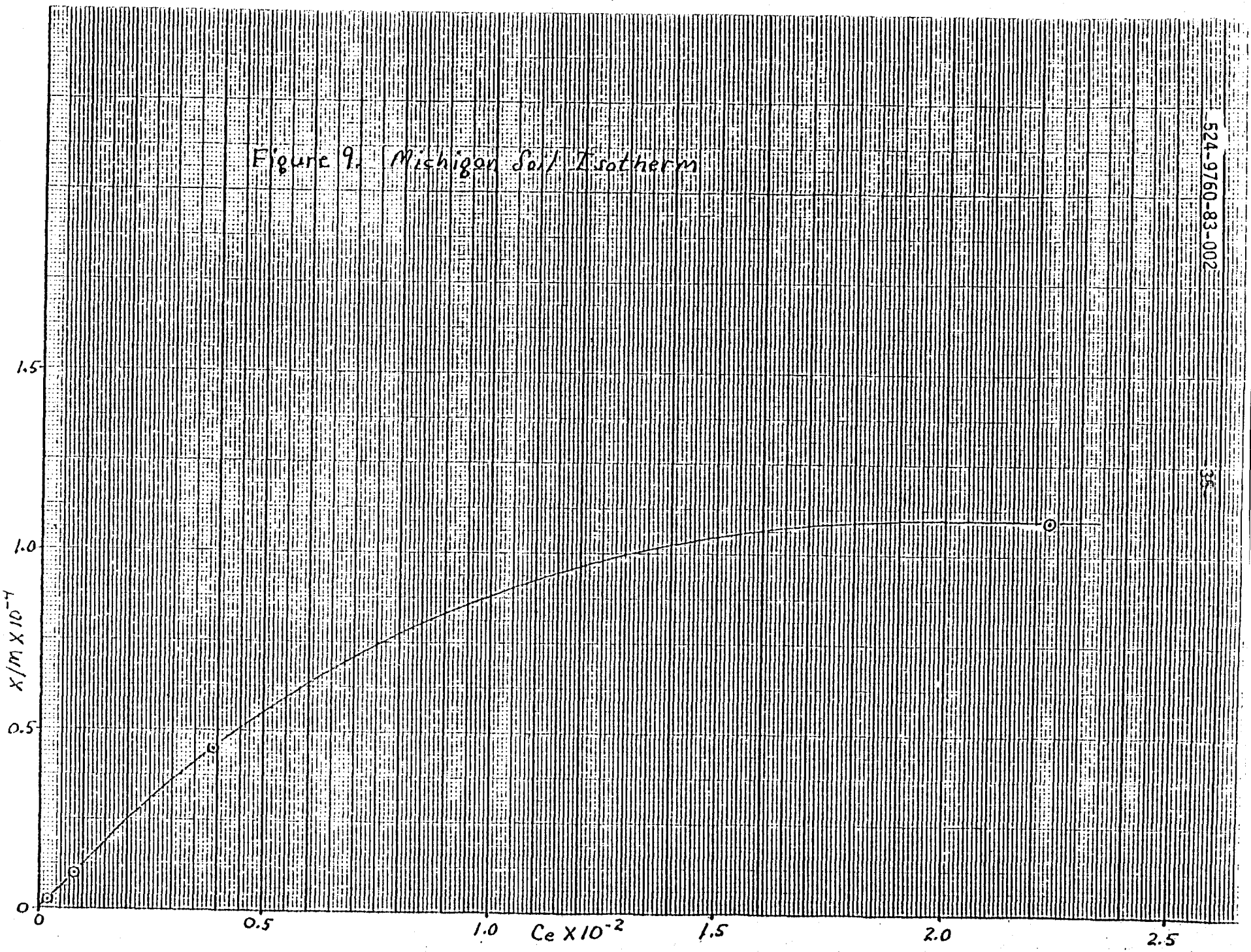


Figure 10. Illinois Soil Isotherm

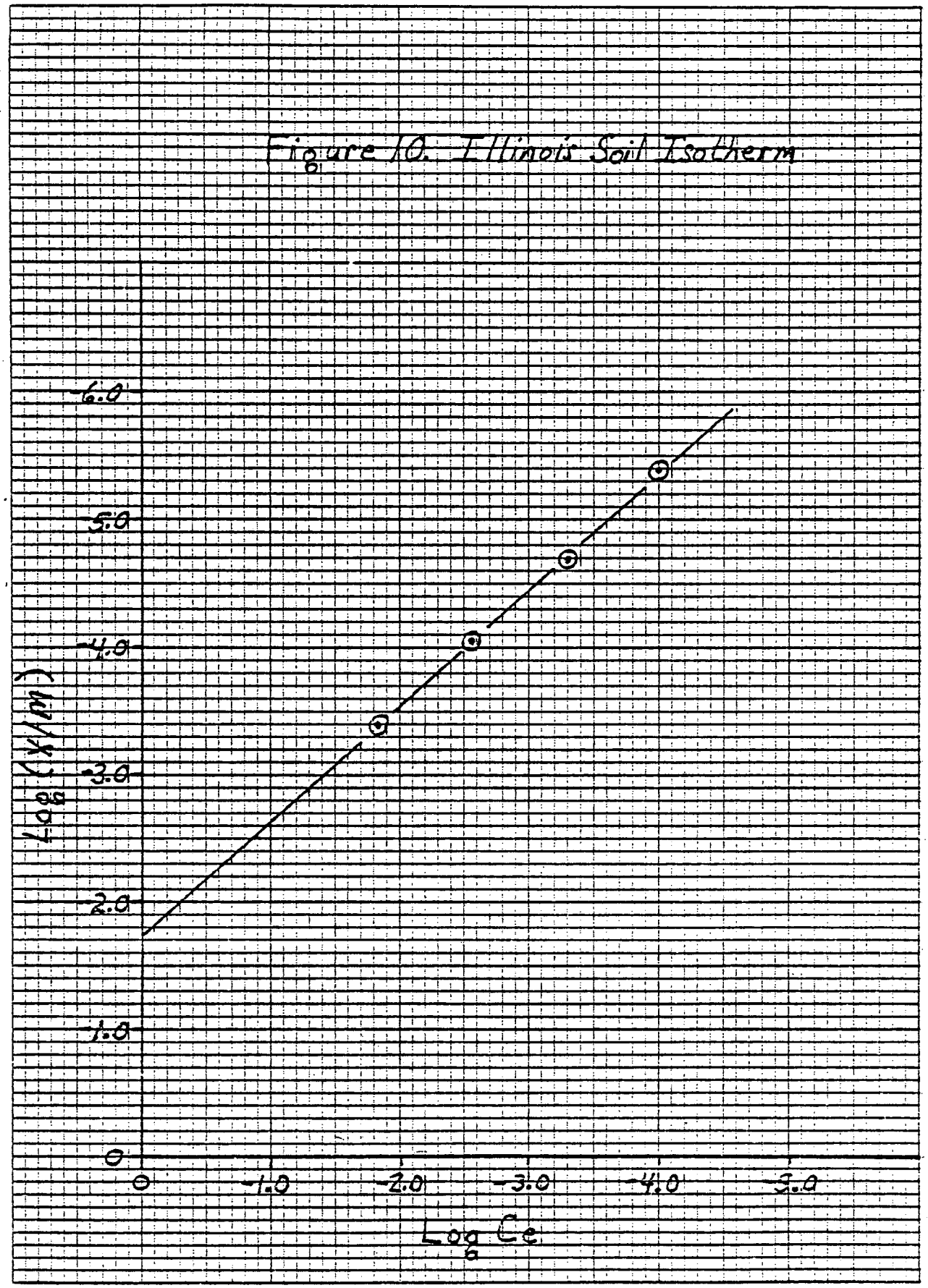
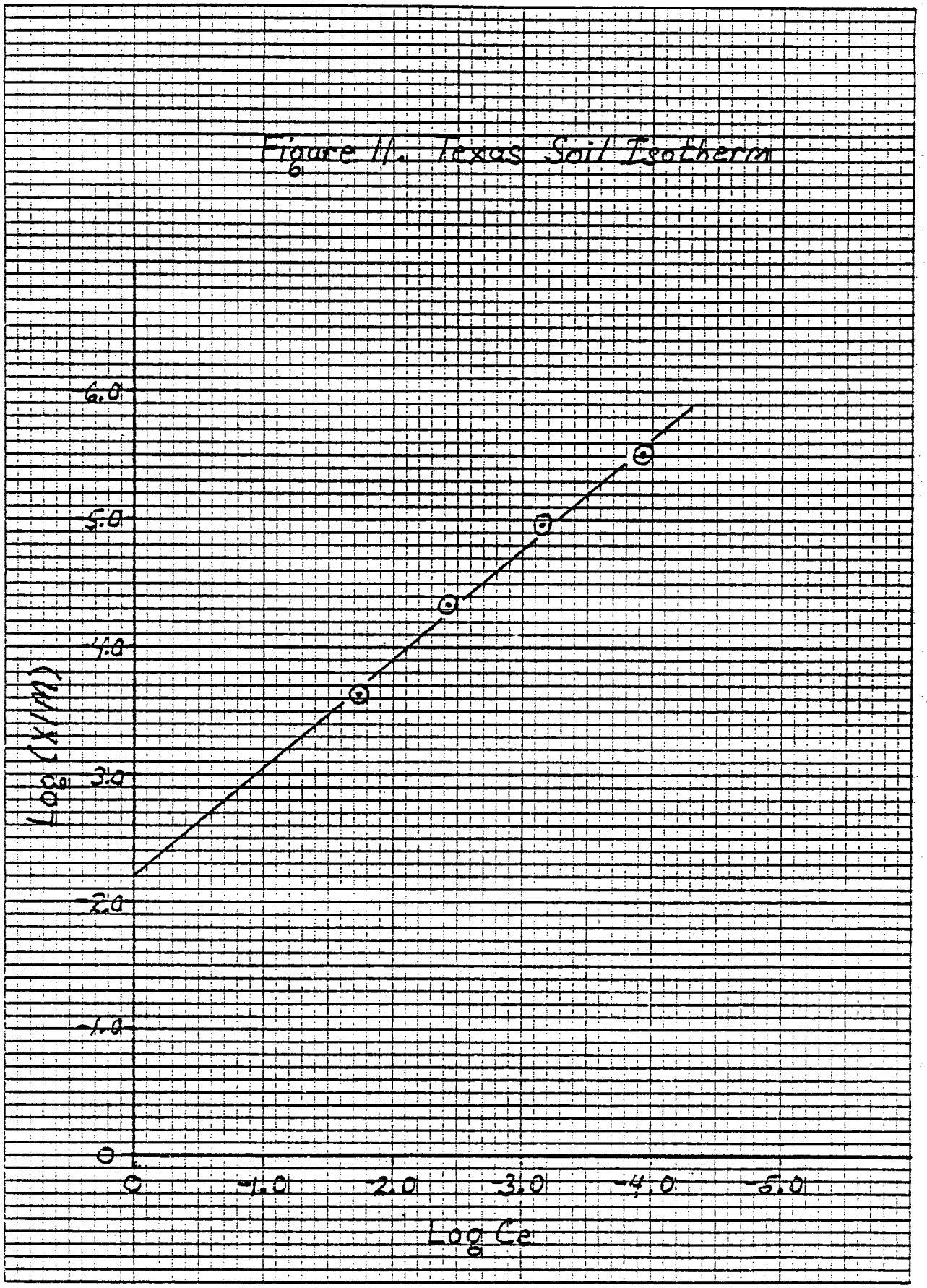


Figure 11. Texas Soil Isotherm



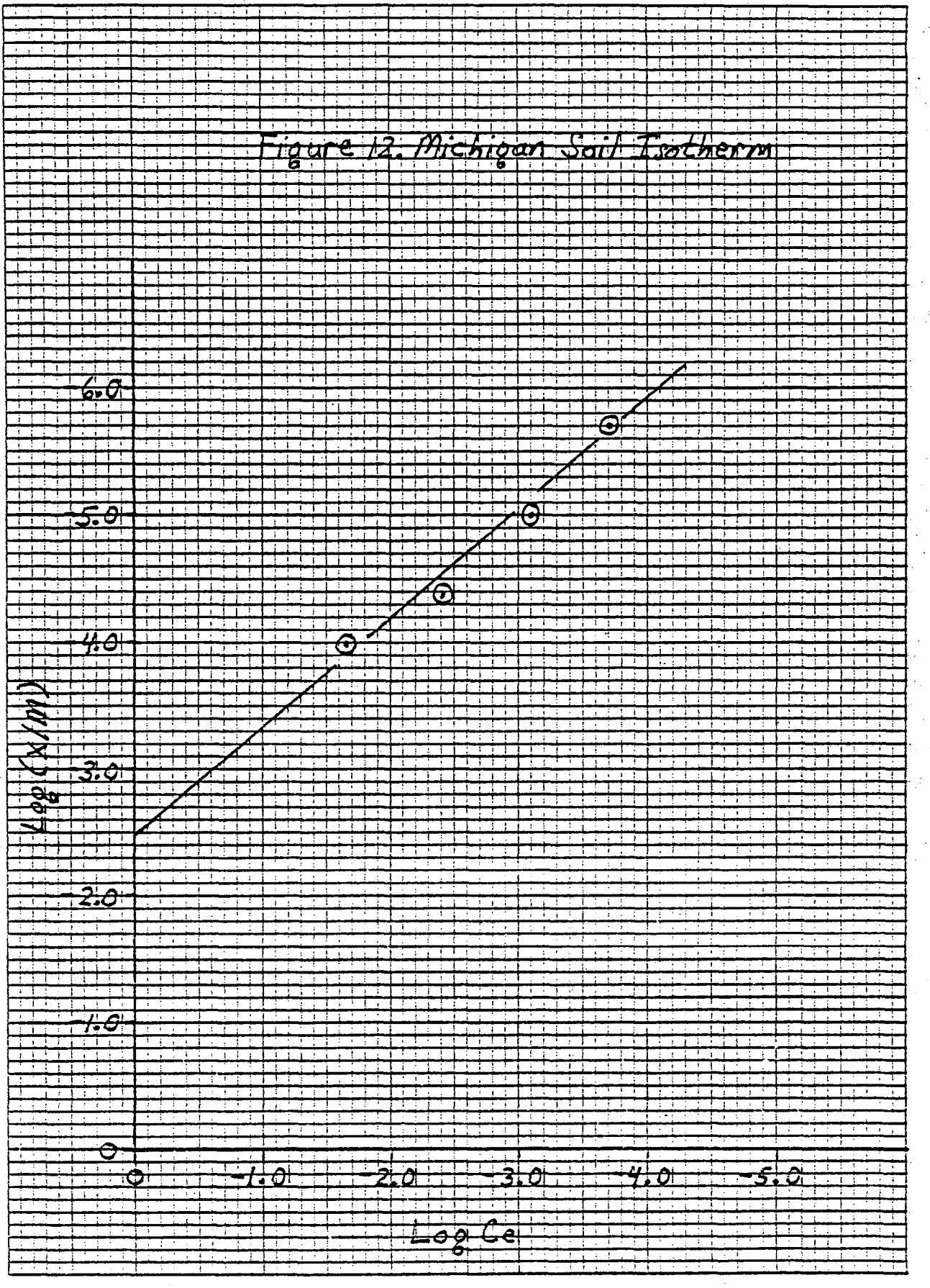


Figure 12. Michigan Soil Isotherm

PROPOSED DEGRADATION OF LINCOMYCIN IN SOIL

R. E. Hornish

The Upjohn Co., Kalamazoo, Michigan

390

March 23, 1983

The scientific literature on the fate of antibiotics in soil is not abundant, particularly with regard to the identification of metabolites or degradation products. The simple degradation of antibiotics in soil, as determined by quantitatively measuring the loss of bioactivity, without regard to products, has been studied for a number of antibiotics (1). The half life of lincomycin activity in a clay loam soil fortified at a level of 10 ppm with lincomycin was found to be about 20 days (2). However, the metabolic fate in soil of lincomycin or any other antibiotic or substance of similar structure has not been studied. Predicting the degradation or metabolic pathway of linco in soil is speculative at best. So much depends on the condition of the soil: moisture content, acidity, clay content, organic matter content, types and concentrations of microbes, etc. Perhaps the single most important aspect would be the route of deposition, e.g., as a water solution, or in animal droppings (with a high organic matter content and the presence of various organisms), or in animal urine (with a lower organic content, but still containing microorganisms, organic salts, enzymes, etc.).

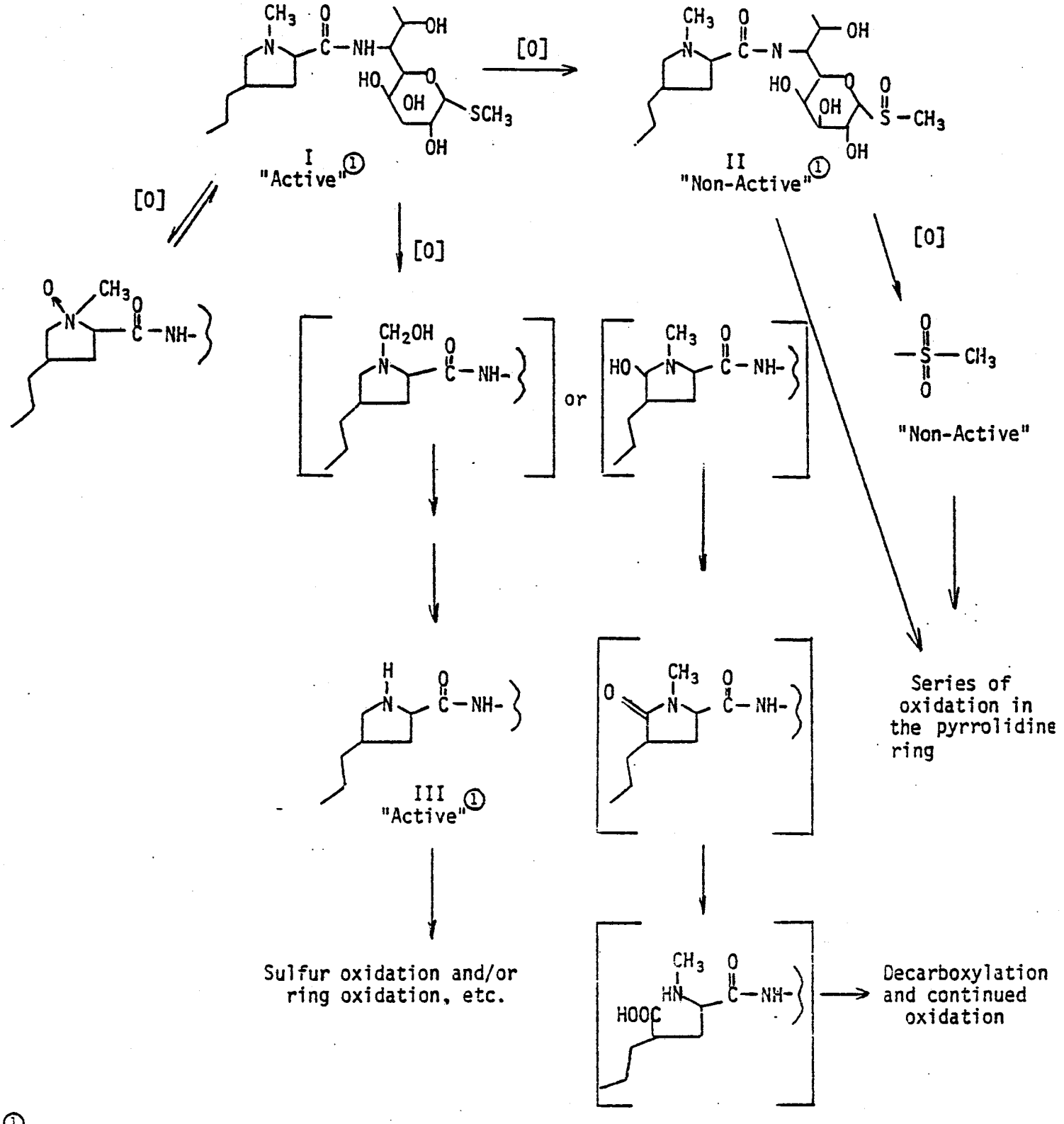
The major routes of lincomycin metabolism in animals are by oxidative pathways. Thus, linco-sulfoxide (II) and N-demethyllinco (III) are initial oxidative metabolites, leading eventually to smaller molecules by decarboxylations, or perhaps to conjugated or protein bound products. Oxidation in soils would likely occur since there are microbial systems under aerobic conditions to readily carry out these transformations. Hydrolysis of the amide linkage or of the methylmercapto functionality, although easy to propose, has not been observed in any of the animal studies to-date. This may well happen in the later stages of metabolism, but doesn't seem likely in the early stages in view of current evidence.

Lincomycin is a basic antibiotic, pKa' = 7.6. Linco itself as well as some of the basic metabolites might be expected to readily bind to clay components (3). Negative charges on the clay should ionically bind the positive charge of the protonated molecules. Humic acids and other soil components would do this quite effectively. However, Johnson and Cox have demonstrated that such binding of lincomycin itself is extremely weak: They found lincomycin to be easily leached from soils with varying clay content and a pH range of 6.0 to 8.0 (4).

References

1. G. Jagnow, Landwirtsch. Forsch., Sonderh., 1977, 34, 227.
2. G. L. Stahl and M. J. DeGeeter. Lincomycin Degradation in the Ecosystem. Research Report 524-9660-012, October 11, 1974.
3. D. Gottlieb, J. Antibiot., 1976, 29, 987, and references therein.
4. Johnson, D. B., and Cox, B. L., Sorption/Desorption of U-10,149A (Lincomycin) in Soils at 0.2, 1.0, 5.0, and 25.0 mg/Liter. Upjohn Tech. Rep. No. 524-9760-83-002, March 21, 1983.

PROPOSED LINCOMYCIN DEGRADATION IN SOIL



① by the traditional microbiological screens for measuring biological activity.

AGRICULTURAL RESEARCH AND
DEVELOPMENT LABORATORIES,
THE UPJOHN COMPANY

TECHNICAL REPORT NO. 524-9760-83-004

PATHOLOGY/TOXICOLOGY NO. _____

TRIAL OR STUDY NO. _____

DATE: April 26, 1983

TECHNICAL REPORT

TITLE:

Minimum Inhibitory Concentration (MIC) In Vitro for Lincomycin (U-10,149A) Against Organisms Commonly Found in The Environment

AUTHOR:

ARB
A. R. Barbiers

ABSTRACT:

The minimum inhibitory concentration (MIC) for lincomycin was determined in vitro against pure cultures of beneficial bacteria, fungi, and blue-green algae normally found in the environment. The MIC's were determined by the use of the agar plate dilution technique commonly used to test the susceptibility of pathogenic organisms to antimicrobial agents. The MIC's for each organism are listed below.

Table 1. Minimum Inhibitory Concentration In Vitro for Lincomycin Against Tested Organisms

Tested Organism	MIC mcg/ml
Aspergillus carbonarius, UC-1511	>1000.0
Chatomium cochliodes, UC-7217	>1000.0
Fusarium roseum, UC-7170	>1000.0
Penicillium notatum, UC-1296	>1000.0
Trichoderma viride, UC-4021	>1000.0
Streptomyces albus, UC-2043	>1000.0
Psuedomonas fluorescens, UC-3049	>1000.0
Clostridium butyricum, UC-9385	1.56
Clostridium perfringens, UC-247	0.78
Clostridium perfringens, UC-6509	0.78
Cellulomonas sp., UC-6274	16.0
Arthrobacter globiformis, UC-3604	16.0
Flavobacterium heparinum, UC-6284	80.0
Cytophaga johnsonae, UC-9386	40.0
Bacillus subtillis (Difco)	12.0
Bacillus cereus (Difco)	12.0
Azobacter vinelandii, UC-3144	500.0
Nostoc sp., ATCC 27895	>1000.0

Ref. LII-ARB-36-47

msj

INTRODUCTION

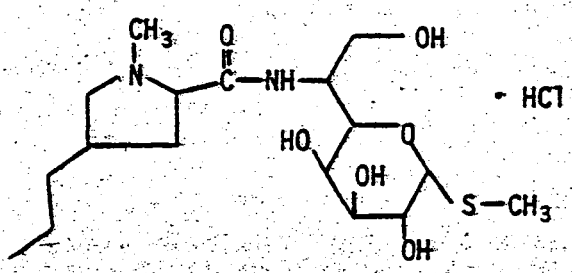
The main objective of this test was to determine the lowest concentration of lincomycin that will inhibit the growth of test microbial strains or species. Widespread microbial growth inhibition may result in ecosystem-level effects, which may include, depending on the organisms inhibited, reduction in plant growth or quality through nutritional disturbances (i.e., interruption of nutrient cycling) and interference with the natural degradative functions of microorganisms which play a dominant role in transformations of biotic and xenobiotic wastes. Microorganisms serve many important functions associated with the major biogeochemical cycles, e.g., carbon, nitrogen, and sulfur.

MATERIALS AND METHODS

A. Test Substance

1. Lincomycin Hydrochloride (monohydrate)

2. Empirical Formula: $C_{18}H_{34}N_2O_6S.HCl$ $(1/2\ H_2O)$



3. Manufacturer – The Upjohn Company

4. Lot – Upjohn Company Reference Standard
Issue C
879 mcg/mg on the "as is" basis activity

5. Water solubility 500 to 1000 mg/ml

B. All cultures were obtained through the Upjohn Culture Collections (UC), number of and source of cultures, culture methods given under listing of organisms.

C. Name and Address of Laboratory Conducting the Test.

Biochemistry and Residue Analysis
Agricultural Division
The Upjohn Company
Kalamazoo, Michigan 49001

Person Responsible for Carrying Out the Test.

Arthur R. Barbiers
Research Scientist

D. Dates of Testing.

Dates are given under individual organisms.

E. Description of the Test Material.

1. An agar-containing medium appropriate to the test organisms was used (See under individual organism) and dispensed in 18 ml amounts in 150 x 25 mm screw-cap tubes.

The lincomycin stock solution was prepared by dissolving 1.138 gms of the lincomycin standard, dissolving in distilled water and volume brought to 100 mls with distilled water. The stock solution was sterile filtered through a 0.2 micron filter. Final concentration was 10 mg/ml of activity. Working test solutions were prepared by dilution in sterile distilled water.

The agar was melted, cooled to 48°C and held at this temperature in a water bath. Two ml of the working test solution was added to the 18 ml agar pour tube, mixed by inversion 2 or 3 times, then poured into 100 mm round plastic petri dishes. The plates were allowed to solidify and dry before inoculation. For preparation of inoculum, see procedure for each of the individual organisms listed below. The plates were marked with circles for each test organism and .001 ml placed on the plates in these marked circles.

Plates were incubated at proper temperature aerobically or anaerobically depending on the growth requirements of the test organism. Plates were incubated until colony growth was well developed on control plates. All tests were carried out in duplicate.

The end point (MIC) was the least concentration of lincomycin that completely inhibited growth. A barely visible haze of growth or a single colony was disregarded.

F. Actual Tests for Each Organism.

Aspergillus carbonarius (formerly niger), UC-1511 (ATCC 10535)
Chatomium cochliodes, UC 7217 (ATCC 10195)
Fusarium roseum, UC 7170 (ATCC 20352)

The organisms were maintained on Potato Dextrose Agar (Difco) plus 0.5% yeast extract slants. Slants were scraped twice with a loop and transferred into 5 ml sterile distilled water. Test medium was PDY agar. Plates were inoculated on 11-19-82 and read on 11/22/82. Incubation temperature was 24°C ± 1°. Lincomycin levels tested were 0, 100, 500, and 1000 mcg/ml.

Results - no inhibition at 1000 mcg/ml for any of these organisms.

Penicillium notatum, UC-1296 (NRRL-1249-B21)
Trichoderma viride, UC-4021 (NRRL 1762)

These organisms were tested the same as previous organisms.

Streptomyces albus, UC-2043 (ATCC 3004).

Grown in yeast extract broth (Difco). Glass beads added and culture shaken to break up growth. Diluted 1-5 in similar broth. Test medium was PDY agar. Test concentrations were 0, 100, 500, and 1000 mcg/ml. For this group of organisms, plates were inoculated 11-17-82 and read 12-2-82. Incubation temperature was 24°C ± 1°.

Results - no inhibition at 1000 mcg/ml for any of the organisms.

Psuedomonas fluorescens, UC-3049 (ATCC 11172)

The organism was maintained on slants of Trypticase Soy Agar (TSA-BBL). Inoculated into Trypticase Soy Broth (TSB-BBL) and incubated overnight at 26°C ± 1°. The inoculum was prepared by diluting the overnight culture 1-10000 in TSB. Test medium was TSA. Test concentrations were 0, 100, 500, and 1000 mcg/ml. Plates incubated overnight at 26°C ± 1°. Plates were inoculated 12-7-82 and read 12-8-82.

Results - no inhibition at 1000 mcg/ml

Clostridium butyricum, UC-9385 (ATCC 19398)
Cl. perfringens, UC-247
Cl. perfringens, UC-6509

The MIC for these organisms were determined by Unit 7254. See attached memo. The MIC for Cl. butyricum was 1.56 mcg/ml and for the other 2 species was 0.78 mcg.ml.

Cellulomonas sp., UC-6274 (ATCC 21399)
Arthrobacter globiformis, UC-3604 (NRRL B-2880)

The organisms were maintained on TSA slants. Inoculated into TSB and incubated for 48 hrs at 26°C ± 1°. The broth culture for Cellulomonas was diluted 1-10000 and for Arthrobacter was diluted 1-500 in TSB for the inoculum. Test medium was TSA. Test concentrations were 0, 2, 4, 8, 16, 32, and 64 mcg/ml of Tincomycin. Plates were incubated for 48 hrs at 26°C ± 1°. Broth inoculated 1-3-83, plates inoculated 1-5-83, and read on 1-7-83.

MIC for Cellulomonas was 16 mcg/ml and for Arthrobacter it was 16 mcg/ml.

Flavobacterium heparinum, UC-6284 (ATCC 13125)
Cytophaga johnsonae, UC-9386 (ATCC 29589)

The organisms were maintained on TSA slants. Inoculated into TSB and incubated for 48 hrs at 26°C ± 1°. The broth cultures were diluted 1-500 in TSB for the inoculum. Test medium was TSA. Lincomycin concentrations tested were 0, 20, 40, 80, 160, and 320 mcg/ml. Plates were incubated for 48 hrs at 26°C ± 1°. Broth inoculated 1-10-83, plates inoculated 1-12-82, and read 1-14-83.

MIC for Flavobacterium was 40 mcg/ml and for Cytophaga was 80 mcg/ml.

Bacillus subtilis (Difco, ATCC 6633) and B. cereus (Difco, ATCC 11778).

TSB was inoculated from spore suspensions of the organisms and incubated overnight at 35°C ± 1°. The inocula were prepared by diluting the overnight cultures 1-10000 in TSA. Test medium was TSA. Test concentrations were 0, 1.5, 3.0, 6.0, 12.0, 24.0, and 48.0 mcg/ml. Plates were incubated overnight at 35°C ± 1°. Broth was inoculated 1-18-83, plates inoculated 1-19-83, and read 1-20-83.

The MIC for both strains of Bacillus was 12.0 mcg/ml.

Azobacter vinelandii, UC-3144

The following medium was used:

Mannitol, 15 gms
K_2HPO_4, 0.2 gms
$MgSO_4 \cdot 7H_2O$, 0.2 gms
$CaCl_2$, 0.02 gms
$FeCl_2$ (10% aq. sol.), 0.05 ml
Tap water to 1000.0
pH adj. to 7.2
For slants and plates agar, 15.0 gms

The test organism was maintained on slants of the medium. The broth medium was inoculated from the slant and incubated for 3 days at 26°C ± 1°. The inoculum was prepared by diluting the 3 day old culture 1-1000 in the broth medium. Test medium was the medium with agar. Test concentrations of lincomycin were 0, 10, 25, 50, 100, 500, and 1000 mcg/ml. Plates were incubated for 48 hrs at 26°C ± 1°. Broth was inoculated 1-21-83, plates inoculated 1-24-83, and plates read 1-26-83.

The MIC for this test organism was 500 mcg/ml.

Nostoc sp., ATCC 27895

Bristol's Modified Sodium Nitrate Solution Medium

KH_2PO_4, 0.50 gm
$NaNO_3$, 0.50 gm
$MgSO_4 \cdot 7H_2O$, 0.15 gm

$CaCl_2.2H_2O$, 0.03 gm
NaCl, 0.05 gm
$FeCl_3.6H_2O$, 0.01 gm
Tap water to 1000 ml
For agar medium - agar 20 gm

The test organism was inoculated into 50 ml of medium and incubated for 2 weeks at 22°C with constant fluorescent light. The growth was homogenized in a Virtis Homogenizer for 10 min. and then diluted with an equal volume of medium. Test medium was medium with agar. Test concentrations of lincomycin were 0, 50, 100, 500, and 1000 mcg/ml. Plates were incubated for one week at 22°C with constant fluorescent light and rechecked at 2 weeks. Inoculum was started 1-31-83, plates inoculated 2-14-83, read 2-21-83 and 2-28-83.

The MIC for the test organism was >1000 mcg/ml.

RESULTS AND DISCUSSION

As a part of the FDA requirements, the minimum inhibitory concentration (MIC) for lincomycin was determined in vitro by the agar plate dilution technique against beneficial microorganisms normally found in the environment. The test organisms included free-living nitrogen fixing organisms and soil organisms affecting various substrates such as cellulose, etc. The results for the MIC's against the organisms listed as required by FDA are shown in Table 1.

Table 1

Minimum Inhibitory Concentration *In Vitro* for Lincomycin Against Tested Organisms

Tested Organism	MIC mcg/ml
Aspergillus carbonarius, UC-1511	>1000.0
Chatomium cochliodes, UC-7217	>1000.0
Fusarium roseum, UC-7170	>1000.0
Penicillium notatum, UC-1296	>1000.0
Trichoderma viride, UC-4021	>1000.0
Streptomyces albus, UC-2043	>1000.0
Psuedomonas fluorescens, UC-3049	>1000.0
Clostridium butyricum, UC-9385	1.56
Clostridium perfringens, UC-247	0.78
Clostridium perfringens, UC-6509	0.78
Cellulomonas sp., UC-6274	16.0
Arthrobacter globiformis, UC-3604	16.0
Flavobacterium heparinum, UC-6284	80.0
Cytophaga johnsonae, UC-9386	40.0
Bacillus subtillis (Difco)	12.0
Bacillus cereus (Difco)	12.0
Azobacter vinelandii, UC-3144	500.0
Nostoc sp., ATCC 27895	>1000.0

Ref. LII-ARB-36-47