

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**20-140**

**PHARMACOLOGY REVIEW(S)**



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	20-140
SERIAL NUMBER:	Major Amendment to NA
DATE RECEIVED BY CENTER:	6/29/07
PRODUCT:	LEVOLEUCOVORIN CALCIUM INJ.
INTENDED CLINICAL POPULATION:	Rescue after High-Dose Methotrexate Impaired Elimination or Inadvertent overdose
SPONSOR:	Spectrum Pharmaceuticals
DOCUMENTS REVIEWED:	None
REVIEW DIVISION:	Division of Drug Oncology Products (HFD-150)
PHARM/TOX REVIEWER:	Hans Rosenfeldt, Ph.D.
PHARM/TOX SUPERVISOR:	W. David McGuinn, Ph.D., D.A.B.T.
DIVISION DIRECTOR:	Robert Justice, M.D., MS
PROJECT MANAGER:	Paul Zimmerman. R. Ph

Date of review submission to Division File System (DFS):

### 2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

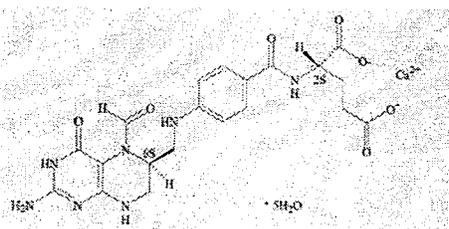
#### 2.6.1 INTRODUCTION AND DRUG HISTORY

**NDA number:** 20-140  
**Review number:** 3  
**Sequence number/date/type of submission:** Major Amendment to NA/  
 Yes  
**Sponsor and/or agent:** Spectrum Pharmaceuticals,  
 Irvine CA  
**Manufacturer for drug substance:** Merck Eprova AG  
**Reviewer name:** Hans Rosenfeldt  
**Division name:** Division of Drug Oncology Products  
**HFD #:** 150  
**Review completion date:** February 29, 2008  
**Drug:**

Trade name: At the time of this review the FDA and the sponsor had not reached agreement on a trade name  
 Generic name: levoleucovorin, l- leucovorin, folinic acid  
 Code name: CL 307,782; NSC 3590  
 Chemical name:

calcium (2R)-2-[[4-[[[(6S)-2-amino-5-formyl-4-oxo-1,6,7,8-tetrahydropteridin-6-yl]methylamino]benzoyl]amino]pentanedioate

CAS registry number: 80433-71-2  
 Molecular formula/molecular weight: C<sub>20</sub>H<sub>21</sub>CaN<sub>7</sub>O<sub>7</sub>  
 Structure:



**Relevant INDs/NDAs/DMFs:**

- IND \_\_\_\_\_
- IND \_\_\_\_\_
- NDA 8-107 (d,l-Leucovorin Injection and Solution)
- NDA 18-459 (d,l-Leucovorin 5 mg tablet)
- NDA 20-141 (Levoleucovorin \_\_\_\_\_)
- ANDA 71-962 (d,l-Leucovorin 10 mg tablet)
- ANDA 71-104 (d,l-Leucovorin 15 mg tablet)

b(4)

ANDA

b(4)

**Drug class:** Folate Analog

**Intended clinical population:**

L-leucovorin rescue is indicated after high-dose methotrexate therapy in osteosarcoma.

L-leucovorin is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdosage of folic acid antagonists.

**Clinical formulation:**

Component (Concentrate)	Grade	Function	Amt./Vial (mg)
Calcium levofolinate pentahydrate	EP	Drug Substance	10 mg/mL
Mannitol		_____	_____
Sodium hydroxide		pH Adjustment	_____
Hydrochloric acid		pH Adjustment	_____
_____		_____	_____
_____		_____	
Label Claim			50 mg/vial

b(4)

**Route of administration:** Intravenous Injection

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

The following recommendations to the sponsor's proposed labeling are given. The sponsor's proposed wording is followed by the recommendation with the rationale for the recommended changes. The word Trademark™ is substituted for the eventual trade name.

Highlights section of the label

\_\_\_\_\_

b(4)

2   Page(s) Withheld

       Trade Secret / Confidential (b4)

  x   Draft Labeling (b4)

       Draft Labeling (b5)

       Deliberative Process (b5)

Signatures (optional):

Reviewer Signature: Hans Rosenfeldt, Ph.D.

Supervisor Signature: W. David McGuinn, Ph.D., D.A.B.T. Concurrence Yes X No

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**This is a representation of an electronic record that was signed electronically and  
this page is the manifestation of the electronic signature.**  
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/s/

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Hans Rosenfeldt  
2/29/2008 05:24:22 PM  
PHARMACOLOGIST

William McGuinn  
2/29/2008 05:30:57 PM  
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES  
PUBLIC HEALTH SERVICE  
FOOD AND DRUG ADMINISTRATION  
CENTER FOR DRUG EVALUATION AND RESEARCH

## PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	20-140
SERIAL NUMBER:	Major Amendment to NA
DATE RECEIVED BY CENTER:	June 29, 2007
PRODUCT:	LEVOLEUCOVORIN CALCIUM INJ.
INTENDED CLINICAL POPULATION:	Rescue after High-Dose Methotrexate Impaired Elimination or Inadvertent overdose
SPONSOR:	Spectrum Pharmaceuticals
DOCUMENTS REVIEWED:	None
REVIEW DIVISION:	Division of Drug Oncology Products (HFD-150)
PHARM/TOX REVIEWER:	Hans Rosenfeldt, Ph.D.
PHARM/TOX SUPERVISOR:	W. David McGuinn, Ph.D., D.A.B.T.
DIVISION DIRECTOR:	Robert Justice, M.D.
PROJECT MANAGER:	Paul Zimmerman, R. Ph
Date of review submission to DFS:	February 26, 2008

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## **EXECUTIVE SUMMARY**

### **I. Recommendations**

#### **A. Recommendation on approvability**

- This application is approvable.

#### **B. Recommendation for nonclinical studies**

- Submitted nonclinical studies have been reviewed in detail by Dr. A.W. Coulter and are only summarized in this review. Please see attached review from Dr. Coulter.
- This application refers to teratology studies submitted under NDAs 8-107 and 18-459. These studies were performed with the tablet form of dl-leucovorin. Subsequent bioavailability studies show that the oral bioavailability of dl-leucovorin tablets at the doses given in the referred teratology studies is 5-10%. These teratology studies are therefore inadequate and cannot support a Pregnancy Category I, as proposed by the sponsor. The sponsor has agreed to accept a Pregnancy Category C. b(4)
- No new nonclinical studies are required for the approval of this NDA

#### **C. Recommendations on labeling**

- A separate review will be conducted

### **II. Summary of nonclinical findings**

#### **A. Brief overview of nonclinical findings**

The sponsor submitted toxicology studies in mice, rats, and dogs with the original submission of this NDA. Overall, these studies indicate that levoleucovorin is less toxic than dl-leucovorin. At high doses, levoleucovorin causes sedation, rapid breathing, tremors, convulsions and death in mice and rats. No mortalities were observed at doses given to dogs and the only clinical sign observed in this study was a slightly increased emesis in dogs treated with high-dose levoleucovorin or dl-leucovorin.

#### **B. Pharmacologic activity**

Levoleucovorin is converted to 5-methyltetrahydrofolate (5-MeTHF) *in vivo*. 5-MeTHF is readily converted to tetrahydrofolic acid (THF) by 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), where it can

participate in thymidylate synthesis and other metabolic processes as a single carbon donor. The conversion of levoleucovorin to THF bypasses the inhibition of dihydrofolate reductase by methotrexate and rescues DNA metabolism from the effects of this drug. Thus, the pharmacologic activity of levoleucovorin is consistent with the proposed indications of co-administration of levoleucovorin with high-dose methotrexate in the treatment of osteosarcoma and treatment of accidental methotrexate overdoses.

C. Nonclinical safety issues relevant to clinical use

- Nonclinical data submitted by the sponsor demonstrate that levoleucovorin is the pharmacologically active diastereoisomer of dl-leucovorin, and that levoleucovorin is about twice as potent as dl-leucovorin in ameliorating the toxic effects of methotrexate.
- A pharmacokinetic study of levoleucovorin and dl-leucovorin in dogs shows that the half-life of levoleucovorin is three times shorter than d-leucovorin. This pharmacokinetic difference reflects the fact that levoleucovorin is readily metabolized and renally excreted while d-leucovorin cannot be metabolized and can only be excreted renally.

**APPEARS THIS WAY ON ORIGINAL**

**2.6 PHARMACOLOGY/TOXICOLOGY REVIEW**

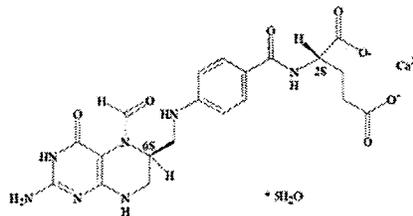
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**Review number:** 2  
**Sequence number/date/type of submission:** Major Amendment to NA  
 Yes  
**Sponsor and/or agent:** Spectrum Pharmaceuticals,  
 Irvine CA  
**Manufacturer for drug substance:** Merck Eprova AG  
**Reviewer name:** Hans Rosenfeldt, Ph. D.  
**Division name:** Division of Drug Oncology Products  
**HFD #:** 150  
**Review completion date:** December 12, 2007

**Drug:**  
 Trade name: At the time of this review the FDA and the sponsor had not reached agreement on a trade name  
 Generic name: levoleucovorin, l- leucovorin, folinic acid  
 Code name: CL 307,782; NSC 3590  
 Chemical name:

Calcium (2R)-2-[[4-[[[(6S)-2-amino-5-formyl-4-oxo-1,6,7,8-tetrahydropteridin-6-yl]methylamino]benzoyl]amino]pentanedioate

CAS registry number: 80433-71-2  
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**Relevant INDs/NDAs/DMFs:**

IND \_\_\_\_\_  
 IND \_\_\_\_\_  
 NDA 8-107 (d,l-Leucovorin Injection and Solution)  
 NDA 18-459 (d,l-Leucovorin 5 mg tablet)  
 NDA 20-141 (Levoleucovorin \_\_\_\_\_)  
 ANDA 71-962 (d,l-Leucovorin 10 mg tablet)  
 ANDA 71-104 (d,l-Leucovorin 15 mg tablet)

**b(4)**

ANDA

b(4)

**Drug class:** Folate Analog

**Intended clinical population:**

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Component (Concentrate)	Grade	Function	Amt./Vial (mg)
Calcium levofolinate pentahydrate	EP	Drug Substance	10 mg/mL
Mannitol			
Sodium hydroxide		pH Adjustment	
Hydrochloric acid		pH Adjustment	
Label Claim			50 mg/vial

b(4)

**Route of administration:** Intravenous Injection

**Disclaimer:** Tabular and graphical information are constructed by the reviewer unless cited otherwise.

**Studies reviewed within this submission:**

The current submission is a complete response to a Non-Approvable letter sent to the sponsor of this NDA (at the time Lederle Laboratories) dated December 13 1993. Ownership of the NDA was transferred to Merck Eprova, then to Tangent, Inc. and last to Spectrum Pharmaceuticals, Inc. The sponsor submitted no new nonclinical toxicology or pharmacology information in the current submission. Therefore, all toxicology and pharmacology studies submitted in support of this NDA have already been reviewed by the original Pharmacology/Toxicology reviewer, Dr. A.W. Coulter. Please see Dr. Coulter's attached Pharmacology/Toxicology review.

In this review, I apply Dr. Coulter's scientific decisions to current regulatory standards regarding the labeling of levoleucovorin and adapt his findings to the current review format. However, certain regulatory decisions made by Drs. A.W. Coulter and J. DeGeorge in 1991 and 1993 with regard to the requirement for reproductive toxicology,

genotoxicity, and carcinogenicity assessments of levoleucovorin when they designated the levoleucovorin pharmacology/toxicology program as “approvable” are binding and cannot be updated to a modern format. Therefore, no new reproductive toxicology studies can be required for approval, even if the studies submitted with the NDA are considered inadequate. Moreover, no genotoxicity or carcinogenicity studies can be required for the approval of this application even though none were submitted with this application.

**APPEARS THIS WAY ON ORIGINAL**

## Studies reviewed previously by A.W. Coulter:

Nonclinical Pharmacology and Toxicology	
Study	Volume
Matherly L, Barlowe C, Goldman I. Antifolate polyglutamylated and competitive drug displacement at dihydrofolate reductase as important elements in leucovorin rescue in L1210 cells. <i>Cancer Res.</i> 1986; 46: 588-593.	8
Rustum YM, Trave F, Zakrzewski SF, Petrelli N, Herrera L, Mittelman A, Arbruck SG, Creaven PJ. Biochemical and pharmacologic basis for potentiation of 5-fluorouracil action by leucovorin. <i>NCI Monographs.</i> 1987;5:165-170.	8
Zittoun J. Evaluation of the two isomers of leucovorin, land d form. Comparison with the racemic form, d,l leucovorin. <i>Hopital Henri Mondor.</i> March 1990.	8
Sato JK, Newman EM, Moran RG. Preparation of (6R)-tetrahydrofolic acid and (6R)-5-formyltetrahydrofolic acid of high stereochemical purity. <i>Analyt Biochem.</i> 1986;154:516-524.	8
Bertrand R, Jolivet J. The natural and unnatural diastereomers of leucovorin: aspects of their cellular pharmacology. In: <i>The Expanding Role of Folates and Fluoropyrimidines in Cancer Chemotherapy, an International Symposium.</i> Roswell Park Memorial Institute, Buffalo, N.Y.; April 28-29, 1988; 13-24.	8
Choi K, Schilsky R. Resolution of the stereoisomers of leucovorin and 5 methyltetrahydrofolate by chiral high performance liquid chromatography. <i>Analyt Biochem.</i> 1988; 168: 398-404.	8
Schilsky RL, Choi KE, Liebner MA, Vokes EE, Guaspari A. Cellular and clinical pharmacology of the stereoisomers of leucovorin. <i>Invest New Drugs.</i> 1989;7:382 Abstract.	8
Sirotnak FM, Chello PL, Moccio OM, et al. Stereospecificity at carbon 6 of formyltetrahydrofolate as a competitive inhibitor of transport and cytotoxicity of methotrexate <i>in vitro.</i> <i>Biochem Pharmacol.</i> 1979;28:2993-2997.	8
Choi KE, Schilsky RL, McGrath SC, Van Kast CA. Purification and biological activity of the stereoisomers of leucovorin (LV). <i>Proc Am Assoc Cancer Res.</i> 1987;28:275 Abstract.	8
Hakala MT, Recht P, Zakrzewski SF. Effect of stereoisomers of folinic acid (CF) on intracellular stability of thymidylate synthetase (TS)-FdUMP-complex. <i>Proc Am Assoc Cancer Res.</i> 1985;26:245 Abstract.	8
Temple C Jr, Rose JD, Laster WR, Montgomery JA. Reversal of methotrexate toxicity in mice by a calcium salt of citrovorum factor and related compounds. <i>Cancer Treat Rep.</i> 1981; 65:1117- 1119.	8
Burden EJ. Acute gavage toxicity study of calcium l-leucovorin in mice (SN 88299). Final report. T.E. 23:753-780 (1988)	8
Burden EJ. Acute gavage toxicity study of calcium l-leucovorin in mice (SN 88316). Final report. T.E. 23:726-752 (1988).	8
Burden EJ, Lindemann E. Calcium leucovorin: Comparison of the acute intravenous toxicity of the racemate (CL 6687) and the L-isomer (CL 307,782) in mice. T.E. 23:581-628 (1988). Amendment: 696-697.	8
Burden EJ. Acute gavage toxicity study of calcium l- and d,l-leucovorin in rats (SN 88318). Final Report. T.E: 23: 698-725 (1988).	8
Burden EJ. Calcium L-leucovorin (CL 307,782): Acute intravenous toxicity in rats (SN 88300). Final Report. T.E. 23: 781-820 (1988). Amendment: 1-9 (1990).	9
Marini PP, Vella A. Four week toxicity study of l-leucovorin given by intravenous route to beagle dogs. PTR XLIX: 94-326, 1990.	9
Burden EJ. Calcium L-leucovorin (CL 307,782) ~ Rescue therapy study of mice dosed by gavage with toxic doses of methotrexate (CL 14,377). Final Report (SN 88215). T.E. 23:821-896 (1988).	9
Burden EJ. A two week intraperitoneal study of l-leucovorin, d-leucovorin, or d, l-leucovorin in mice in combination with oral methotrexate (Study 89226). 1990: 1-92.	9
Monnot G. Folinic acid - Study of the possible potentialization by calcium folinate-racemic	9

and levogyrous forms, on the mortality provoked by 5-Fluorouracil in the mouse by the intraperitoneal route. Hazleton France. Report 805953, 1989.	
Monnot G. Folinic acid -Study of the possible increase of the mortality provoked by 5-Fluorouracil with concomitant administration of calcium folinate levogyrous in the mouse by intraperitoneal route. Hazleton France. Report 301128-0, 1990.	9
Burden EJ. A two week intraperitoneal study of l-leucovorin, d-leucovorin, or d, l-leucovorin in rats in combination with intraperitoneal 5-Fluorouracil. Study 89130, 1990;1-296.	9
Straw J, Covey J, Szapary D. Differences in the pharmacokinetics of the diastereoisomers of citrovorum factor in dogs. <i>Cancer Res.</i> 1981;41:3936-3939.	9

**Studies not reviewed within this submission or NDA .....: None**

## 2.6.2 PHARMACOLOGY

### 2.6.2.1 Brief summary

Levoleucovorin (l-leucovorin) is the active diastereoisomer of "leucovorin" a racemic 50:50 mixture of 6S, L-folinic acid and 6R, L-folinic acid that was approved in 1952 (NDA 08-107). 6S, L-folinic acid can function as an antidote to dihydrofolate reductase inhibition by methotrexate. Levoleucovorin consists of 6S, L-folinic acid purified to >99.7% racemic purity and is twice as potent as racemic leucovorin in mitigating methotrexate toxicity.

### 2.6.2.2 Primary pharmacodynamics

#### Mechanism of action:

Levoleucovorin (l-leucovorin, L-folinic acid) is converted to 5-methyltetrahydrofolate (5-MeTHF) through intermediate steps (see Figure 1). 5-MeTHF is then readily converted to tetrahydrofolic acid (THF) by 5-methyltetrahydrofolate-homocysteine methyltransferase (MTR), where it can participate in pyrimidine synthesis and other metabolic processes. The conversion of L-folinic acid to THF bypasses the inhibition of dihydrofolate reductase by methotrexate and rescues DNA metabolism from the effects of this drug.

**APPEARS THIS WAY ON ORIGINAL**

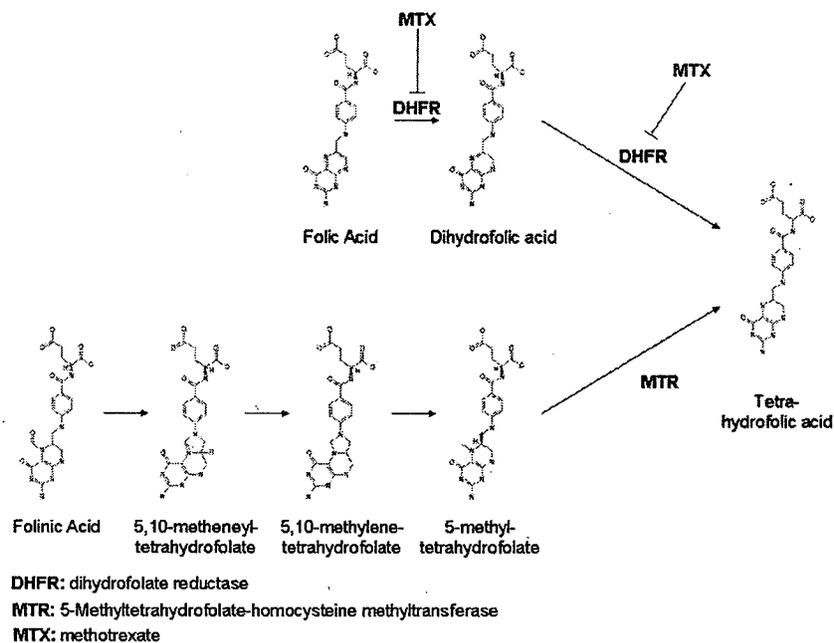


Figure 1: Folic acid and L-folinic acid (Levoleucovorin, 1-leucovorin) metabolism results in the creation of tetrahydrofolic acid by different pathways. Folinic acid metabolism to tetrahydrofolic acid is not sensitive to methotrexate inhibition of dihydrofolate reductase.

#### Drug activity related to proposed indication:

The proposed indication for rescue after high-dose methotrexate therapy is consistent with the mechanism of action of levoleucovorin.

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

Please see attached review by A.W. Coulter.

### 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

#### 2.6.4.1 Brief summary

The pharmacokinetic profiles of levoleucovorin and d-leucovorin have been determined in a single study with four dogs (Straw et al, 1981). This study showed that the half-life of levoleucovorin was three time shorter than that of d-leucovorin (47 min vs. 143 min) as levoleucovorin was readily converted to 5-MeTHF and metabolized. The inactive stereoisomer, d-leucovorin was by contrast slowly excreted in the urine.

### 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Please see attached review by A.W. Coulter.

## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

The sponsor submitted toxicology studies in mice, rats, and dogs with the original submission of this NDA and NDA No. 20-141 (the oral tablet). Dr. A. W. Coulter reviewed these studies in 1991; I have summarized the results of his review herein

Only one repeat-dose toxicity study of levoleucovorin alone is provided (Study #344 done in dogs); all other studies were either single dose studies, or were done in combination with 5-FU or methotrexate. Overall, these studies indicate that levoleucovorin is less toxic than dl-leucovorin. For example, the LD<sub>50</sub> for levoleucovorin given by intravenous injection is 575 mg/kg in mice and 378 mg/kg in rats. These toxic doses are lower on a per L-folinic acid molecule basis than those for dl-leucovorin (Table 1). This suggests that d-folinic acid is more toxic than the levo isomer.

Species	LD <sub>50</sub> Levoleucovorin (mg/kg)			LD <sub>50</sub> dl-leucovorin (mg/kg)		
	L-folinic Acid	D-folinic Acid	Total folinic Acid	L-folinic Acid	D-folinic Acid	Total folinic Acid
Mouse	575	--	575	370	370	740
Rat	378	--	378	111	111	221

Table 1: L-folinic acid tolerance in rats and mice decreases when L-folinic acid is co-administered with D-folinic acid.

At high doses, levoleucovorin caused neurological symptoms including sedation, rapid breathing, tremors, convulsions and death in mice and rats. No mortalities were observed at the highest doses given to dogs and no clinical signs were reported. In dogs, hematological toxicities included decreases in white blood cells, neutrophils and an increase in APTT. Histopathology results from the dog study also show that end-organ toxicities occurred in the liver, spleen, and GI tract. Hematology and histopathology after dosing with levoleucovorin by itself was not assessed in rats and mice.

In addition to toxicology studies done with levoleucovorin alone, the sponsor included studies that tested the ability of levoleucovorin to mitigate the toxicity of methotrexate (MTX) or increase the toxicity of 5-Fluorouracil (5-FU). Studies showed that levoleucovorin is about twice as potent as dl-leucovorin in its ability to rescue mice from lethal doses of MTX. The toxicities seen in these studies were those anticipated with MTX overdose including GI-tract toxicity, weight loss, hypothermia, and decreased platelet counts. These non-clinical pharmacological results support the approval of this NDA for the sponsors proposed indication, i.e. rescue from high dose MTX. Studies with 5-FU were more equivocal, with one study suggesting that levoleucovorin increases 5-FU toxicity, and another suggesting that it does not. The non-clinical information submitted does not support the use of levoleucovorin as a substitute for leucovorin in 5-FU containing cancer chemotherapy regimens.

#### General toxicology:

Please see attached review by A.W. Coulter.

Genetic toxicology:

No genotoxicity studies were submitted in the original NDA application.

Carcinogenicity:

No carcinogenicity studies were submitted in the original NDA application.

Reproductive toxicology:

Please see attached review by A.W. Coulter.

Special toxicology:

Please see attached review by A.W. Coulter for details regarding levoleucovorin rescue methotrexate toxicity and enhancement of 5-Fluorouracil toxicity.

**2.6.7 TOXICOLOGY TABULATED SUMMARY**

(Summarized from review by A.W. Coulter)

Report Number	Test System	Dose and Route	Findings
88299 and 88316	Male CD-1 mouse (n=10)	5000 mg/kg levoleucovorin 4340 mg/kg dl-leucovorin Oral gavage; single dose	1. GLP Study 2. No deaths after 15 days observation 3. No differences in body weight 4. No necropsy findings
88205 and 88207	CD-1 mouse 5/sex/ group	0.5% methylcellulose control 500, 600, 700 mg/kg levoleucovorin 500, 750, 1000 mg/kg dl-leucovorin Intravenous, slow bolus; single dose	1. LD <sub>50</sub> a. levoleucovorin: 575 mg/kg b. dl-leucovorin: 740 mg/kg (approximately equivalent to 370 mg/kg levoleucovorin) 2. Mortality observed at 24 h 3. Similar toxic signs for both compounds: a. sedation b. rapid breathing c. tremors d. convulsions e. death
88318 and 88300	Male SD Rat	0.5% methylcellulose control 2500 mg/kg levoleucovorin 5000 mg/kg dl-leucovorin Oral gavage; single dose	1. Oral Gavage a. no deaths b. inactivity after dosing c. no body weight changes d. no gross necropsy findings

		<p>Saline control                      150-300 mg/kg l-leucovorin                      300 mg/kg dl-leucovorin                      Intravenous; single dose</p>	<p>2.Int ravenous Administration                      a. LD<sub>50</sub>:  <ul style="list-style-type: none"> <li>• levoleucovorin: 378 mg/kg</li> <li>• dl-leucovorin: 221 mg/kg</li> </ul>                     b. Toxic signs:  <ul style="list-style-type: none"> <li>• Inactivity</li> <li>• Labored breathing</li> <li>• Convulsions</li> <li>• All deaths day occurred day of dosing</li> </ul> </p>
344	<p>Beagle dog                      3/sex/                      group</p>	<p>Saline control,                      15, 30, 60 mg/kg levoleucovorin                      129.2 mg/kg dl-leucovorin                      Intravenous; daily for 28 days</p>	<p>1.GL P Study                      2.No mortalities                      3.E mesis at 60 mg/kg levoleucovorin and 129.2 mg/kg dl-leucovorin                      4.All groups, including saline control lost body weight                      5.lev oleucovorin: decreases in MCV, WBC, absolute neutrophils                      6.lev oleucovorin: increase in APTT                      7.lev oleucovorin: decreases in LDH, gamma globulin                      8.No changes in mean arterial BP                      9.lev oleucovorin: increased prostate weight                      10. Histopathology (levoleucovorin)                      a. Liver congestion                      b. Stomach congestion                      c. Injection site swelling                      d. Spleen congestion                      e. Lymph nodes: perilymphadenitis</p>
88215-F	<p>Cr1:CO                      BS:CD1                      (ICR)                      BR                      mouse                      5/sex/                      group</p>	<p>Control (water),                      200, 400 mg/kg methotrexate                      100, 200 mg/kg levoleucovorin                      Oral gavage; all methotrexate and levoleucovorin doses are daily for five days</p>	<p>1.GL P Study                      2.All methotrexate-treated mice that did not receive levoleucovorin died by day 10                      3.No deaths in levoleucovorin-treated groups                      4.Food consumption decreased in all groups except control                      5.All methotrexate treated groups: decreases in platelets, Hematocrit, hemoglobin, RBC's and WBC's</p>

<p>89226</p>	<p>Crl:CO B:CD1 male mice 10/ group</p>	<p>200 mg/kg methotrexate 3, 10, 30, 100 mg/kg levoleucovorin 100 mg/kg d-leucovorin 6, 20, 60, 154 mg/kg dl-leucovorin Methotrexate p.o.; Leucovorin IP; all methotrexate, levoleucovorin, and dl-leucovorin doses are daily x 5</p>	<ol style="list-style-type: none"> <li>1. GL P Study</li> <li>2. All methotrexate-treated mice that did not receive leucovorin (dl or l) died by day 12</li> <li>3. 8/10 mice that received d-leucovorin died by day 12</li> <li>4. Some mortality occurred in leucovorin-treated mice (dl or l); 2-4 animals/group</li> <li>5. No statistically significant difference in mortality between groups receiving similar doses of l- and dl-leucovorin</li> <li>6. Clinical signs (occurring mostly in mice that received either no leucovorin or d-leucovorin):             <ol style="list-style-type: none"> <li>a. hunched posture</li> <li>b. inactivity</li> <li>c. unkempt/sickly appearance</li> <li>d. soft feces</li> <li>e. hypothermia</li> <li>f. Significant body weight in all methotrexate-treated groups, but more in mice that received either no leucovorin or d-leucovorin</li> <li>g. Platelets decreased in all methotrexate-treated groups except high dose l and dl-leucovorin groups</li> <li>h. Gross pathology:                 <ul style="list-style-type: none"> <li>• Distended large intestine in methotrexate alone, low dose levoleucovorin and d-leucovorin, and in all animals that were found dead</li> <li>• Hydronephrosis increased in mid and high-dose dl-leucovorin</li> <li>• Splenomegaly occurred in all leucovorin groups</li> </ul> </li> </ol> </li> </ol>
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			(l, d, and dl) except for high-dose levoleucovorin
404/004	Male B6D2F1 mice 8/group	90 mg/kg 5-FU 200, 400 mg/kg levoleucovorin 400 mg/kg dl-leucovorin IP; Days 1, 5, 9	1.GL P Study 2.Clinical signs: piloerection, tremors 3.Mortality increased from 1/8 animals in 5-FU alone group to 4/8 in high-dose levoleucovorin given in combination with 5-FU. 4.No mortality in 5-FU/dl-leucovorin group
404/005	B6D2F1 mice 20/sex/group	90 mg/kg 5-FU 100, 200, 400 mg/kg levoleucovorin IP; Days 1, 5, 9	1.GL P Study 2.No mortality with high-dose levoleucovorin alone 3.In males mortality increased from 10% for 5-FU alone to 20, 30, and 70% as levoleucovorin dose was increased from low to mid to high dose. 4.In females mortality increased from 0% for 5-FU alone to 5% for both mid and high levoleucovorin doses 5.Clinical signs: prostration and piloerection
89130	Male SD Rat	10 mg/kg 5-FU 1, 10, 100, 300 mg/kg levoleucovorin 100 d-leucovorin 1, 10, 100, 300 mg/kg dl-leucovorin IP; Daily x 14	1.GL P Study 2.Weight loss in groups given 5-FU in combination with either high-dose levoleucovorin or dl-leucovorin 3.no potentiation of 5-FU-induced mortality for either l-or dl-leucovorin

**OVERALL CONCLUSIONS AND RECOMMENDATIONS**

Conclusions:

The details of the pharmacology and toxicology program submitted with the original submission of this NDA are covered in an attached review by A.W. Coulter. In this review, Dr. Coulter recommends the approval of this NDA and his signature still stands as the primary reviewer evaluation.

A few key points from Dr. Coulter's review need to be emphasized:

1. The data submitted by the sponsor demonstrates that levoleucovorin is the pharmacologically active diastereoisomer of dl-leucovorin.
2. levoleucovorin is less toxic than dl-leucovorin while retaining the ability to rescue animals from methotrexate toxicity consistent with the sponsor's proposed indication.
3. Toxic signs from high levoleucovorin doses were neurological in nature, including sedation, rapid breathing, tremors, and convulsions in mice and inactivity, prostration, labored breathing, and convulsions in rats. These toxicities may derive from a mechanism distinct from levoleucovorin's primary pharmacological mechanism of action.
4. A pharmacokinetic study of levoleucovorin and dl-leucovorin in dogs shows that the half-life of levoleucovorin is three times shorter than d-leucovorin. This is probably due to rapid intracellular distribution and not elimination.

No genotoxicity or carcinogenicity studies appear to have been submitted with this NDA. To verify this omission, the original action package for this NDA and volume 1 of NDA 20-141 (levoleucovorin tablets) were digitized to PDF format and searched for the following terms:

1. Genotoxicity
2. Genotox
3. Geno
4. Ames
5. Lymphoma
6. Micronucleus

These searches revealed no information relevant to genotoxicity.

In addition, a search for the term "carcinogenicity" revealed that this subject was discussed in a Memorandum from Dr. Sally A. Look dated January 11, 1991 that is included in the first volume of NDA 20-141. However, the levoleucovorin labels that are included in both the action package of NDA 20-140 and the first volume of NDA 20-140 state that no carcinogenicity studies have been done for levoleucovorin.

Unresolved toxicology issues (if any): None

#### Recommendations:

A literature review indicates that there are no reports of genotoxicity or carcinogenicity resulting from either l- or dl-leucovorin administration in either animals or humans in the

two decades that leucovorin has been used in the United States and Europe. Therefore, no new genotoxicity or carcinogenicity studies are indicated.

In his review, Dr. Coulter states that the Division agreed to accept Segment II studies that were carried out with dl-leucovorin. However, Dr. Coulter recommends that levoleucovorin be classified a Pregnancy Category C drug under the classification system used at that time.

Suggested labeling:

The proposed indication for rescue after high-dose methotrexate therapy is consistent with the drug's mechanism of action. However, the following changes are recommended:

1. Pending data on leucovorin bioavailability in the submitted Segment II teratology studies, the Pregnancy Category classification of levoleucovorin should be "C". The sponsor has agreed to this labeling requirement.
2. No genotoxicity or carcinogenicity data were submitted with this NDA. The label should clearly state that no genotoxicity or carcinogenicity studies have been performed with levoleucovorin. The sponsor has agreed to this labeling requirement.
3. The sponsor should provide a clear description of high-dose levoleucovorin toxicity in the animal toxicology and/or pharmacology portion of the label. The sponsor has agreed to this labeling requirement.

I will discuss these changes to the label in detail in the separate labeling review.

External Comments Conveyed to Sponsor:

None

Signatures (optional):

Reviewer Signature: Hans Rosenfeldt, Ph.D.

Supervisor Signature: W. David McGuinn, Ph.D., D.A.B.T.

I concur with Dr. Rosenfeldt's conclusions and recommendations - *WDM*

**APPENDIX/ATTACHMENTS:**

- I. ORIGINAL REVIEW BY DR. A.W. COULTER OF NDA 20-140
- II. ORIGINAL REVIEW BY DR. A.W. COULTER OF  
TERATOLOGY STUDIES IN SUPPORT OF NDA 20-140 AND  
NDA 20-141

I. ORIGINAL REVIEW BY DR. A.W. COULTER OF NDA 20-140

PHARMACOLOGY AND TOXICOLOGY REVIEW OF  
ND [REDACTED] (Injectable)  
NDA 20-141 (Tablet)

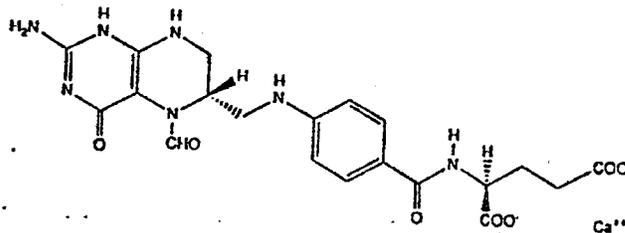
AUG 5 1991

ORIGINAL REVIEW

REVIEWER: A. W. Coulter, Ph.D.  
DATE OF SUBMISSION: DECEMBER 14, 1990  
DATE RECEIVED IN CDER: DECEMBER 18, 1990  
DATE REVIEW COMPLETED: July 18, 1991

SPONSOR: Lederle Laboratories  
Pearl River, NY 10965

DRUG: 1-Leuovorin Calcium (proposed) Tablets  
ISOVORIN (proprietary name)  
CL 307,782\*  
(6S)-5-formyl-5,6,7,8-tetrahydrofolic acid, Ca salt  
NSC 3590

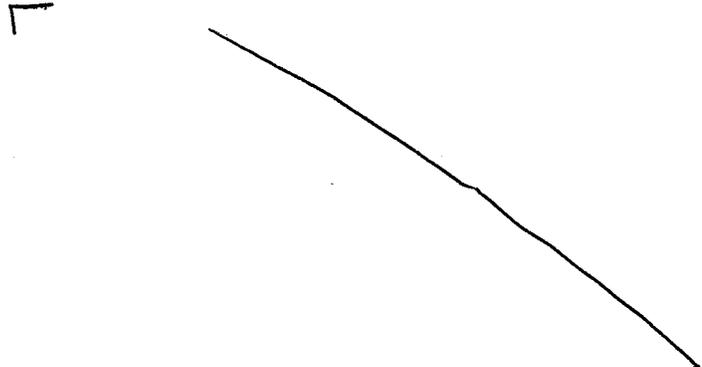


(S)-N-[4-[[2-amino-5-formyl-1,4,5,6,7,8-hexahydro-4-oxo-6-pteridiny]methyl]benzoyl]-L-glutamate, calcium salt

$C_{20}H_{21}CaN_7O_7$   
Mol. Wt. 511.51 (Ca salt)  
Mol. Wt. 473.45 (free acid)  
[ $\alpha$ ]<sub>D</sub><sup>20</sup> = -11 ± 4 (H<sub>2</sub>O)

\* The American Cyanamid Co. code number for 1-leuovorin Ca is CL 307,782, for d,l-leuovorin Ca, CL 6687, and for d-leuovorin Ca, CL 315,220. In this review leuovorin will also be indicated as LV.

TABLET DOSAGE STRENGTH



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The intravenous dosage form (NDA 20-140) of 1-LV is a sterile, powder, formulated with mannitol and supplied in 50, and mg quantities in glass vials.

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CATEGORY: Antidote for folic acid antagonists (rescue after high-dose methotrexate therapy)

PROPOSED MARKETING INDICATION:

"ISOVORIN rescue is indicated after high-dose methotrexate therapy in osteosarcoma. ISOVORIN is also indicated to diminish the toxicity and counteract the effects of impaired methotrexate elimination and of inadvertent overdoses of folic acid antagonists."

RELATED DRUGS/INDs/NDAs:

Leucovorin:

NDA 8-107 (d,l-Leucovorin Injection and Solution)

NDA 18-459 (5 mg tablet)

ANDA 71-962 (10 mg tablet)

ANDA 71-104 (15 mg tablet)

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PRECLINICAL STUDIES:

The preclinical studies are contained in Volumes 8 and 9 of this submission, in NDA 8-107, and in

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Additional data in the NDA comes from literature publications searched by the sponsor from CAS (1967 to Nov. 1990), CANCERLIT (1970 to Nov. 1990), RINGDOC (1983 to Nov. 1990), and EMBASE (1974 to Nov. 1990). New preclinical studies in this submission include the following:

- a) Acute gavage toxicity study of Ca 1-LV in mice
- b) Acute gavage toxicity study of Ca 1- and d,1-LV in rats
- c) Comparison of the acute intravenous toxicity of the racemate and the 1-isomer
- d) Acute intravenous toxicity study in rats
- e) Four-week intravenous toxicity study of 1-LV in dogs
- f) PK data in the dog

#### PHARMACOLOGY:

The pharmacology section of the application consists of eleven reprints or abstracts taken from the literature.

Matherly, Barlowe, and Goldman (Report 1) correlated the growth inhibition of L1210 cells in the presence of LV with the accumulation of dihydrofolate. Control levels of dihydrofolate were formed from radiolabeled cofactor, consistent with sustained dihydrofolate reductase activity. LV was able to protect cells from methotrexate toxicity by increasing the cellular pool of dihydrofolate.

Rustrum et al. (Report 2) showed that 5-FU cytotoxicity could be modulated by deoxythymidine in in vitro studies but failed to produce any therapeutic advantage in patients with advanced colorectal cancer. The modulation of 5-FU bioactivation by coadministration of high-dose LV resulted in enhanced therapeutic response. The binding of 5,10-methylenetetrahydrofolate, derived from LV, forms a stable complex with thymidylate synthase.

The evaluation of the efficacy of the two isomers of LV and dl-LV were compared in folate deficient cells. 1-LV could renew thymidylate biosynthesis at concentrations of  $5 \times 10^{-9}$  M to  $10^{-8}$  M. No effect occurred at d-LV concentrations up to  $10^{-8}$  M. With dl-LV, the effective concentration was  $2.5 \times 10^{-9}$  M. LV rescue of methotrexate toxicity was studied in CCRF-CEM leukemia cells and in HL 60 cells (derived from a promyelocytic leukemia). With methotrexate concentrations of  $10^{-7}$  to  $10^{-5}$  M, deoxyuridine levels could be reversed to their normal range with 1 or dl-LV concentrations 100 times higher than methotrexate. Only at 1000 times higher did d-LV have any effect. Zittoun (Report 3) also showed cytotoxicity of 5-FU

increased in the CCRF-CEM tumor cells after exposure to 10 to 10<sup>-6</sup> M dl or l-LV for 2 hours. The d isomer had no effect.

Studies (Sato et al., Report 4) in the microorganism *Saccharomyces cerevisiae* showed that d-LV could support growth only at a 600 fold increase in concentration above l-LV concentrations. The d isomer was able to promote growth of folate depleted colon cancer cells, but only at 100 times greater concentration than the l isomer. Membrane transport inhibition of methotrexate was 20 fold less effective with d-LV than with l-LV in L1210, S180, and Ehrlich cells. d-LV was also 100 times less effective than l-LV in preventing L1210 cell growth inhibition due to methotrexate.

Bertrand and Jolivet (Report 5) looked at the pharmacology of d- and l-LV. Following the IV administration of dl-LV to patients, the l isomer disappeared with a plasma t<sub>1/2</sub> of 30 minutes. The d isomer had a plasma t<sub>1/2</sub> of 7.5 hours. Cell uptake of d-LV was 45 fold less than the l-LV in an anion free buffer which optimized transport. The d isomer was a potent inhibitor of the uptake of several folates. At 10 μM, d-LV could not support the growth of folate-depleted human leukemic CCRF-CEM cells.

Choi et al. reported on an HPLC method of separating the stereoisomers of LV using bovine serum albumin bonded to silica as the chiral stationary phase. The purity of the obtained isomers was said to be greater than 99%. (Reports 6 and 7). The effect of the purified compounds was evaluated using CEM cells and methotrexate (Report 9).

The d isomer of LV was found to be 20-fold less effective as a competitive inhibitor of methotrexate influx into Ehrlich cells than l-LV. The d isomer was 100-fold less effective and the dl mixture was 2-fold less effective than l-LV in preventing inhibition of L1210 cell growth by methotrexate (Sirotnak et al., Report 8).

Hakala et al. (abstract, Report 10) reported on the uptake and effect of dl-LV or l-LV in human Hep-2 cells in vitro. The presence of d-LV did not potentiate 5-FU activity.

The isolation of citrovorum factor (l-LV) from the racemic mixture (leucovorin) was described by Temple et al. (Report 11). They also demonstrated the reversal of methotrexate toxicity in mice by Ca citrovorum factor. BDF1 mice (6/group) were given a single dose of 1000 mg/Kg (3000 mg/sq M) of methotrexate. Ca leucovorin (dl-LV) and citrovorum factor (l-LV) were administered 6 hours after methotrexate

administration. Their results are indicated in the following table:

Compound	Dose (mg/aqM)	% Survivors	
		Males	Females
None	-	0	0
Ca leucovorin	200	50	83
	100	0	67
	50	33	17
	25	17	0
	12.5	0	0
Citrovorum factor	200	100	100
	100	100	83
	50	50	33
	25	17	67
	12.5	67	17

TOXICOLOGY:

ACUTE TOXICITY:

MOUSE: STUDY NO. 88299 AND 88316, Vol 1.8

The drug was suspended in 0.5% methylcellulose to give concentrations of 250 mg/mL l-LV and 217 mg/mL dl-LV. Ten males were dosed at 2.0 mL/100 g body weight. Animals were observed daily for 15 days. Gross necropsies were performed on all animals sacrificed at study termination. A GLP statement was present and signed.

Strain	Route	Isomer	Dose (mg/Kg)	Sex	LD50 (mg/Kg)
CD-1	gavage	l	5,000	10 M	no deaths
CD-1	gavage	dl	4,340	10 M	no deaths

All mice survived the 15 day observation period. Clinical signs were squinting and inactivity. There were no significant differences in body weights. No postmortem findings were reported. Mice were also administered 2500 mg l-LV and 5000 mg dl-LV. Both of these groups gave identical results.

STUDY NO. 88205 and 88207, Vol 1.8

<u>Strain</u>	<u>Route</u>	<u>Isomer</u>	<u>Dose</u> (mg/Kg)	<u>Sex</u>	<u>LD50</u> (mg/Kg)
CD-1	IV	l	500-700	M,F	575
CD-1	IV	dl	500-1000	M,F	740

The compounds were dissolved in sterile water and administered as a slow bolus at 1 mL/100 g body weight. Drug purity was 96.9% for anhydrous l-LV and 98% for anhydrous dl-LV. Toxic signs were similar from both compounds and included sedation, rapid breathing, tremors, convulsions, and death. Mortality observed 24 hours after dosing is indicated below.

<u>Group</u>	<u>Treatment</u>	<u>Dose</u> mg/Kg	<u>Males</u>	<u>Females</u>
1	water	-	0/5	0/5
2	l	500	0/5	2/5
3	l	600	4/5	3/5
4	l	600	2/5	3/5
5	l	700	4/5*	5/5
6	dl	500	0/5	1/5
7	dl	750	1/5	3/5
8	dl	750	3/5	3/5
9	dl	1000	4/5	5/5

\* an additional mouse died Day 7.

RAT: STUDY NO. 88318 and 88300, Vol 1.8

The compounds were dissolved in 0.5% methylcellulose and administered to CD rats at 2 mL/100 g body weight. All rats survived the 15 day observation period.

<u>Strain</u>	<u>Route</u>	<u>Compound</u>	<u>Dose</u> (mg/Kg)	<u>Sex</u>	<u>LD50</u> (mg/Kg)
SD	gavage	l	2500	M	no deaths
SD	gavage	dl	5000	M	no deaths
SD	IV	l	150-500	M	378 (285-507)
SD	IV	dl	300	M	221 (216-230)

Gavage: Inactivity developed after dosing. No significant changes occurred in body weight and no gross postmortem findings were reported. GLP statement was present and signed.

IV: Toxic signs which developed immediately after dosing were inactivity, labored breathing, and convulsions. All deaths appeared the day of dosing.

SUBACUTE/CHRONIC:DOG: 4-WEEK TOXICITY STUDY:

Study No. 344, Vol 1.8

Compound: CL 307,782 (l-LV Ca, purity-98.9% anhydrous)

CL 6687 (dl-LV Ca, purity not indicated)

Formulation: Solution in 1.35% NaCl in distilled water at  
15 mg/mL. The concentration of dl-leucoverin was  
32.3 mg/mL.

Route: Right and left cephalic vein

Dose Levels: VC, 15, 30 60 mg/Kg/day l-LV and 129.2 mg/Kg/day  
dl-LV (Groups 1, 2, 3, 4, 5, respectively)

Strain: Beagle, 17 months old, 9.85-14.56 Kg body weight

Number: 3/sex/dose

Control Treatment: 4.0 mL/Kg of 1.35% NaCl in water

Study Site/Date: \_\_\_\_\_

Nov. 1989-Mar 1990

GLP/QAU Statements: Both present and signed

Solutions were prepared daily and doses adjusted weekly, based on body weight. Doses were divided and administered five hours apart. The volumes administered were 4, 1, 2, 4, and 4 mL/Kg/day for the five groups, respectively. The infusion rate was 5 mL/minute. The animals were weighed predose and weekly. Food consumption was determined daily. A physical examination was performed predose and during the second and fourth week of the study. EKG, BP, and ophthalmoscopic examinations were also carried out. Hematology, serum chemistry, and urinalysis were evaluated pretest and at the end of the study. Gross and histopathology were done on all animals.

## Results

Emesis was observed more frequently in the two high dose groups (60 and 129 mg/Kg) during the first 3 weeks. No dogs died during the treatment period. All groups showed a body weight decrease, which tended to be greater in females and controls.

MCV showed a dose-related decrease in Gs 2, 3, and 4 (significant in G4M (5x)). WBC and absolute neutrophils were significantly decreased (16% and 26%) in G3M, while APTT increased (p=0.05, 7.6%).

Significant blood chemistry changes that occurred were

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total protein (decrease in G2M, 10%), CPK (decrease in G2 and 3M, 30% and 23% respectively), LDH (decrease in G3M, 45%), percent albumin (decrease in G3F), percent gamma globulin (dose-related decrease in G3M (23%), G4M (32%), and G5M (16%).

Acidic urine, accompanied by calcium oxalate crystals, was present in some of animals in all groups, including the controls, at the end of the study.

There were no mean changes in arterial BP in any group, and EKGs were said to reveal no abnormal conditions; however, no EKG data was in the report.

Absolute lung weight (decrease, G3M) and prostate weight (increase, G4) were significantly different from the controls. Significant changes occurring in relative weights include uterus (decrease, G3), spleen (increase, G2F), and prostate (increase, G4, dose-related for the 1-LV). The mean absolute uterine weight of the dl group (G5) was 14.397 g, which was due to two animals with very high weights. No adverse eye findings were observed during the study.

Nodules were observed on the lungs of one or two treated females in each group and enlarged ovaries were found in 2/3 in G5. Possible treatment related histopathologic findings were:

Lung: inflammatory cell accumulation; 1M, 1F G5  
 Liver: congestion; F G2 (2/3), G3 (3/3), G4 (1/3),  
 G5 (3/3)  
 Stomach: congestion; G4 (1/3M, 1/3F), G5 (1/3F)  
 Small intestine: congestion; 2/3F G5  
 Injection site: swelling; G4F and G5M  
 endothelial proliferation; G2, 4, 5  
 edema; G2, 3, 4 M and/or F  
 Spleen: congestion; G3, 4, 5 M and/or F  
 pigment; G3, 4, 5 M and/or F  
 Lymph nodes: perilymphadenitis; G3 (2/3F) and G5 (1/3 F)

RESCUE STUDIES WITH 1-LEUCOVORIN Ca:

MOUSE: CALCIUM L-LEUCOVORIN RESCUE THERAPY WITH TOXIC DOSES  
 OF METHOTREXATE:

Study No. 88215-F, Vol 1.9  
 Formulation: Solutions in Sterile Water for Injection  
 Route: Gavage, 10 or 20 mg/mL Ca 1-LV (CL 307,782)

## Dose Levels:

Group	Methotrexate (mg/Kg/day x 5)	Ca l-leuovorin (mg/Kg/day x 5)
1	water	water
2	200	water
3	200	200
4	200	100
5	400	water
6	400	200
7	400	100
8	water	200

Strain: Crl:COBS:CD1(ICR) BR, 30-45 g body weight, 14 weeks old

Number: 5/sex/group

Study Site/Date:

GLP/QAU: Both present and signed.

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This study was done to confirm the ability of Ca l-LV to reverse the toxicity of methotrexate. Mice were dosed daily by gavage for 5 days with methotrexate, followed 4 to 5 hours later with water or l-LV Ca. Body weight, food consumption, and physical conditions were recorded Days -7, 0, 7, and 14. Hematology was determined Days 5 and 14.

## Results

No deaths in Ga 1, 3, 4, 6, 7, or 8. All mice treated with methotrexate (Ga 2 and 5) and not rescued with l-LV died by day 10. Body weight was reduced in all groups on D7 and was significant in Ga 2 and 5. Food consumption decreased significantly in all groups except the control, and by D14 had returned to normal in all groups except G7 females. Hematologic parameters that decreased significantly were the platelets in G2 through G7 (33%-66% in M and F D5-rebound was seen D14 in Ga 3, 6, and 7), Hct (11%-23% Ga 4, 6, 7, M and F), Hb (11%-21% G7 M and F), RBCs (15%-23% in Ga 3, 4, 6, and 7) and WBCs in F (39%-52% D5). Recovery occurred in most parameters by D14. Of the above hematological changes, the platelets showed the greatest change and occurred in all groups that received methotrexate.

MOUSE: TWO-WEEK IP STUDY OF LV IN COMBINATION WITH  
ORAL METHOTREXATE.

Study No 89226, Vol 1.9

Compounds: dl-, d-, and l-LV Ca, and methotrexate

Formulations: Solutions in Sterile Water for Injection

## Dose Levels:

<u>Group</u>	<u>Methotrexate PO</u> <u>mg/kg/day x 5</u>	<u>Ca Leucovorin IP</u> <u>mg/Kg/day x 5</u>
1	water	water
2	200	water
3	200	3 l
4	200	10 l
5	200	30 l
6	200	100 l
7	200	100 d
8	200	6 dl
9	200	20 dl
10	200	60 dl
11	200	154 dl

Strain: Crl:COBS:CD1, 47 days old, 27 to 36 g body weight

Number: 10 M/group

Study Site/Date:

Glp/QAU: Both present and signed

b(4)

All mice received two treatments daily for five consecutive days. The first treatment was with 200 mg/Kg methotrexate. The second treatment was with LV administered approximately 4 to 6 hours after the first treatment. Body weight, food consumption, and hematology were evaluated. Gross examination was done on all animals. G11 received 154 mg/Kg rather than the intended 200 mg/Kg Ca dl-LV due to an error in preparing the solution. The animals were observed 10-12 days before necropsy.

## Results

Mortality: 10 G2, 2 G3, 3 G4, 2 G5, 8 G7, 4 G10. No statistically significant difference occurred in mortality in groups receiving comparable doses of l-LV and dl-LV.

Clinical signs: hunched posture, inactivity, unkempt/sickly appearance, soft feces, and hypothermia. These signs were observed mostly in Gs 2 and 7, although many of the signs occurred in Gs 3, 4, 5, 7, and 8. No clinical signs were reported in the other groups.

Significant decreases occurred in body weight in G2 (34%), G3 (12%), G4 (9.8%), G7 (25%), G8 (16%), and G9 (7%). Platelet counts decreased significantly (42%-55%) in all groups except 1, 6, and 11. G6 had only mild changes in Hct, Hb, RBCs, MCV, MCH. Significant increases occurred in G2 in Hb ( $p < 0.05$ ), RBC ( $p < 0.05$ ), MCV ( $p < 0.05$ ), MCHC ( $p < 0.001$ ), platelets ( $p < 0.001$ ), and WBCs ( $p < 0.001$ ), all on D5.

At pathologic examination, distended large intestines were seen in Gs 2, 3, and 7, and in animals found dead during the study. Hydronephrosis appeared to increase in G10 and G11. The contents of small intestines were discolored or intestines were distended with water (Gs 2, 3, 7, 8). Splenomegaly occurred in Gs 3, 4, 7, 8, 9, and 10. Small testes were seen in one mouse which died early in G2. No histopathologic examinations were conducted in this study.

#### Conclusion

Ca 1-LV administered 4 hours after a lethal dose of methotrexate (200 mg/Kg/D x5) was able to rescue mice. Ca dl-LV at greater than 20 mg/Kg also prevented lethality. It appeared that Ca d-LV also offered some protection, as one of the 100 mg/Kg d-LV treated mice survived.

#### MOUSE: THREE-WEEK IP STUDY WITH dl-LV, l-LV, AND 5-FU

Study No: 404/004, Vol 1.9  
 Compound: Ca dl-LV (95.5%) and Ca l-LV (98.5%)  
 Formulations: Solutions in 0.9% saline  
 Route: IP  
 Dose Levels:

Group	5-FU (mg/Kg)	Ca dl-LV (mg/Kg)	Ca l-LV (mg/Kg)
1	90	0	0
2	90	400	0
3	90	0	400
4	90	0	200

Strain: B6D2F1, 6 weeks old, 19.4 to 28.7 g body weight  
 Number: 8 males/group  
 Study Site: \_\_\_\_\_  
 GLP/OAU: Both present and signed

b(4)

This study was conducted to evaluate possible potentiation of mortality of 5-FU by dl-LV or l-LV administration. Each of the LV groups was dosed with 90 mg/Kg 5-FU IP on Ds 1, 5, and 9. The animals were observed for 21 days from the start of the study. Mice were examined twice daily. Body weight was determined several times during the study.

#### Results

Clinical signs: piloerection and tremors. Body weight loss: reduced (-2 to -6.7%) in the drug treated groups after

each treatment, with slight recovery before the next drug administration. Mortality is indicated in the following table:

	LV (mg/Kg)	5-FU (mg/Kg)	Mortality
	0	90	1/8
Ca 1-LV	200	90	1/8
	400	90	4/8
Ca d1-LV	400	90	0/8

The results indicate potentiation of 5-FU toxicity (mortality and weight loss) with increase in the dosage of 1-LV.

MOUSE: SEVEN-WEEK IP STUDY WITH 5-FU.  
Study No. 404/005, Vol 1.9

This study was done to further evaluate the ability of Ca 1-LV to potentiate the toxicity of 5-FU when administered concurrently. The five groups (20/sex) of B6D2F1 mice were administered drugs on Days 1, 5, and 9. Mortality, body weight, and gross lesions were recorded. The mortality is indicated below.

Group	5-FU (mg/Kg)	Ca 1-LV (mg/Kg)*	Mortality %	
			M (days)	F (days)
1	90	0	10 (17,21)	0
2	90	100	20 (16-21)	0
3	90	200	30 (16-20)	5 (17)
4	90	400	70 (12-19)	5 (24)
5	0	400	0	0

\* Doses expressed as folinic acid

Prostration and piloerection appeared, in general, one or two days prior to death. These signs also occurred in some of the surviving animals. Body weight loss occurred after dosing in several of the groups. No treatment related abnormalities were seen at necropsy. The study was done by Hazelton-France under GLP regulations.

RAT: TWO-WEEK IP TOXICITY STUDY WITH 5-FU, d1-, 1-, AND d-LV

Study No. 89130, Vol. 1.9

Drug: 1-LV Ca (CL 307,782), (>96.9 x anhydrous)  
d-LV Ca (CL 315,220), (>99.6x anhydrous)

dl-LV Ca USP (CL 6,687), (98.0% anhydrous)  
 5-FU USP (CL 45,076)

Formulations: Solutions in sterile water

Route: IP

Dose:

Group	Vehicle	Treatment (mg/Kg/day)			
		5-FU	l-LV	d-LV	dl-LV*
1	water				
2		10			
3		10	1		
4		10	10		
5		10	100		
6		10	300		
7		10		100	
8		10			1
9		10			10
10		10			100
11		10			300

\* Doses expressed as content of l-LV

Strain: Cr1:CD (SD) BR, 38 days old, 154-193 g body weight

Number: 10 M/group

Study Site/Date:

GLP/QAU: Both present and signed

b(4)

This study was done to determine the effect of the enantiomers of LV and dl-LV on the toxicity of 5-FU. All groups except the VC were administered 10 mg/Kg of 5-FU on Days 0-4. Four hours later LV was administered qD x14. An earlier range-finding study indicated 10 mg/Kg 5-FU would result in myelosuppression. The rats were observed twice daily. Physical examinations, body weight, and food consumption were measured weekly. Hematologic parameters were determined D5 and D14, and select organs were weighed and examined microscopically.

#### Results

Some of the animals developed thickened skin at the injection site. No premature deaths occurred in any group. Mean body weights for G6 and G11 were significantly ( $p < 0.05$ ) decreased D7 and D14. Body weight decreased D7 in G4, 6, 10, and 11. Food consumption was a bit low in G8 on D7. WBCs were significantly decreased (17%-47%) D5 in all but the control and G7 (some decrease, but not significant). Platelets were increased ( $p < 0.05$ , 23%-39%) in G5, 10, and 11 on D5 and in G10 and 11 on D14 (26%-42%). Other significant ( $p < 0.05$ ) changes occurred in G5, 7, 10, and 11 and include Hct, Hb,

MCV, MCH, and MCHC. RBCs decreased in G5 on D14 ( $p < 0.5$ ). At necropsy a dose related grey or chalky white material was observed in abdominal organs, testes, urinary bladders, and at the injection site and was accompanied by granulomatous reactions in Gs 5, 6, 7, 10, and 11. This material was assumed to be calcium from the LV. No relative liver weight changes appeared. GI mucosal congestion was seen in groups administered 5-FU. Hepatocellular vacuolation was seen in a few animals in Gs 2 and 3.

These results did not indicate potentiation of 5-FU toxicity by l-LV or dl-LV, based on mortality; however, body weights of Gs 6 and 11 (300 mg/Kg of l- or dl-LV) were decreased relative to the control.

#### PHARMACOKINETICS:

The pharmacokinetics of l-LV and d-LV (calculated as the difference between the concentration of dl-LV and l-LV) were determined in four mongrel dogs. Tritiated l-LV was infused at 20 ug/min/Kg to achieve plasma levels of L-LV in the range that would rescue from  $10^{-6}$  to  $10^{-7}$  M methotrexate plasma levels. The PK parameters are indicated below.

	<u>Vd</u> (ml/Kg)	<u>Plasma</u> <u>Clearance</u> (ml/min/Kg)	<u>Renal</u> <u>Clearance</u> (ml/min/Kg)	<u>C<sub>R</sub>/C<sub>inuline</sub></u> <u>Ratio</u>	<u>t<sub>1/2B</sub></u> (min)
l-LV	567+35	7.94+0.28	2.57+0.61	0.88+0.10	47+3.5
d-LV	597+170	3.23+0.56	2.67+0.68	0.89+0.06	143+15

l-LV is rapidly converted to the active metabolite, 5-MeTHF and cleared from the plasma. On the other hand, the inactive isomer, d-LV, is slowly cleared from the body via the urine, which is the major route of elimination. At four hours, the concentration of d-LV was 23x greater than the l-LV concentration. Both isomers have biphasic plasma decay curves. Plasma levels that were observed after infusion of 2, 20, or 100 ug/min/Kg were linear and would indicate that over a 50 fold concentration range no saturation was involved.

#### SUMMARY AND EVALUATION:

Leucovorin Calcium (LV) has two chiral centers—one is at the C6 carbon of the pteridiny ring, and the other is the alpha carbon of the glutamate moiety. The absolute configuration at the alpha carbon is L, the usual nomenclature for the natural amino acids. The presently marketed Leucovorin Calcium is a 50:50 mixture of 6S,L and 6R,L, designated as the

racemic drug (6RS-L-form) and marketed under NDAs 8-107 and 18-459. In this application, the presently marketed drug is designated as dl-leucovorin.

The biologically active diastereomer of leucovorin has been shown to have the 6S absolute configuration. The 6R isomer apparently has no activity and may contribute in part to the toxicity of leucovorin, or could inhibit or partially inhibit rescue by the active drug. Lederle has removed the inactive isomer and developed the active 6S,L compound to replace the presently marketed drug. In this application the 6S,L configuration is referred to as l-Leucovorin. Both l-Leucovorin Tablets (NDA 20-141) and l-Leucovorin Injection (NDA 20-140) will be marketed for rescue therapy after high dose methotrexate administration.

Preclinical studies were conducted in mice, rats, and dogs to determine and compare toxicity of l-Leucovorin to that of d-LV or dl-LV. Additional studies were conducted to evaluate rescue from toxic doses of methotrexate and to determine possible potentiation of 5-FU toxicity by l-LV.

In single dose experiments, lethality was not observed in mice administered oral dosed up to 5000 mg/Kg (15000 mg/sqM) l-LV or in rats dosed up to 2500 mg/Kg (15000 mg/sqM) orally. The intravenous 14 day LD50 values in male mice and rats were:

	CD-1	CD(SD)
	<u>Mouse</u>	<u>RAT</u>
Ca l-LV (mg/Kg)	575	378
Ca dl-LV (mg/Kg)	370	221

Toxic signs were sedation, rapid breathing, tremors, and convulsions in mice, and inactivation, prostration, labored breathing, and convulsions in rats. In both species, toxicity was greater with dl-LV.

A four-week intravenous toxicity study in the dog compared Ca l-LV (15, 30, 60 mg/Kg/day) to Ca dl-LV (129.2 mg/Kg/day, equal to two times the high dose of the l isomer). No mortality occurred with either isomer. Although there were significant changes in some hematology and serum chemistry parameters, none of the changes appeared to be drug related. Possible drug related histopathologic findings were congestion in the stomach, liver, small intestine, and spleen, and pigmentation in the spleen.

Two studies were done in mice to evaluate rescue from toxic doses of methotrexate. One was a five day gavage study;

the other was a two-week intraperitoneal study. In the five-day gavage study, methotrexate was administered at 200 or 400 mg/Kg/day, followed 4-5 hours later by 100 or 200 mg/Kg/day of Ca l-LV. The mice that were dosed only with methotrexate all died by Day 10; whereas, those that were dosed with both drugs all survived. There were no deaths in the controls or in those dosed only with l-LV. Body weight decreased in all drug groups, and a significant reduction in platelets (33%-66%) on D5 with recovery or rebound recovery by D14 was the most pronounced hematological change. The second study was designated a two week study, although dosing was only for five consecutive days. Methotrexate was administered at 200 mg/Kg orally, followed by IP administration of l-LV (3, 10, 30 mg/Kg), d-LV (100 mg/Kg), or dl-LV (6, 20, 60, 154 mg/Kg). All mice treated only with methotrexate died. Those groups rescued with l-LV or dl-LV had 60%-100% survival. There was no statistically significant difference in lethality between the l-LV or the dl-LV treated groups. Perhaps some slight protection occurred with the d-LV.

Studies to evaluate the possible potentiation of 5-FU toxicity with accompanying doses of LV were run in mice and rats. In a three-week IP mouse study, 90 mg/kg 5-FU was administered on Days 1, 5, and 9. Ca dl-LV (400 mg/Kg) or Ca l-LV (200 or 400 mg/Kg) were administered on the same days. Mortality occurred in those groups treated with Ca l-LV plus 5-FU and in the group treated only with 5-FU. Mortality was greater in the high dose l-LV plus 5-FU group than in the 5-FU control group. There was no mortality in the dl-LV plus 5-FU group. In the second study, mice were dosed IP for seven weeks with 90 mg/Kg 5-FU and 0, 100, 200, or 400 mg/Kg l-LV on Days 1, 5, and 9. The results showed a clear dose-related increase in mortality, with more deaths appearing in males. There was no mortality in the group treated only with 400 mg/Kg l-LV. A third IP two-week study was conducted in rats. Drug groups received 10 mg/Kg 5-FU plus l-LV (1, 10, 100, 300 mg/Kg), d-LV (100 mg/Kg), or dl-LV (1, 10, 100, 300 mg/Kg). Both drugs were administered on Days 0-4. No mortality occurred in any group. Toxicity was limited to body weight loss of 8.5% to 13%, decrease in WBCs (17%-47%), and increase in platelets (23%-39%). Mineral deposits, assumed to be calcium, accompanied by granulomatous reactions were seen in some abdominal organs.

The pharmacokinetics in dogs demonstrated the active isomer to be more rapidly metabolized and cleared from the plasma than the inactive isomer, but renal clearance and volume of distribution were similar for both isomers. The plasma half-life was three times greater for the inactive isomer.

In conclusion, the sponsor has submitted data which demonstrate 1-LV to be the pharmacologically active diastereoisomer of dl-LV. The active isomer is less toxic than dl-LV, can rescue animals from methotrexate toxicity, and has a potentiating effect on 5-FU toxicity.

RECOMMENDATIONS:

The Division has agreed to accept Segment II studies that were carried out with dl-LV, provided the sponsor can show there was good bioavailability in those studies. If it turns out that oral absorption is low, the sponsor will be required to conduct Segment II studies with 1-LV by the IV route. It is recommended that the Pregnancy statement be modified to read:

"Pregnancy Category C. Animal reproduction studies have not been conducted with 1-leucovorin. It is also not known whether 1-leucovorin can cause fetal harm when administered to a pregnant woman or can affect reproduction capacity. Reproduction studies have been performed in rats and rabbits with dl-leucovorin, containing 1-leucovorin at doses up to 150 mg/Kg. No evidence of harm to the fetus was observed; however, bioavailability of leucovorin in these studies was not determined. 1-Leucovorin should be given to a pregnant woman only if clearly needed."

  
Almon W. Coulter, Ph.D.

cc:  
NDA 20-140  
NDA 20-141  
HFD-150/Division Files  
    /ACoulter  
    /RJustice  
HFD-151/CSO PZimmerman  
HFD-340  
HFD-502/JWeissinger  
R/D Init by JDeGeorge  
F/T ACoulter

*Jfo 8/5/91*

II. ORIGINAL REVIEW BY DR. A.W. COULTER OF  
TERATOLOGY STUDIES IN SUPPORT OF NDA 20-140 AND  
NDA 20-141

**APPEARS THIS WAY ON ORIGINAL**

AUG 5 1991

PHARMACOLOGY AND TOXICOLOGY REVIEW OF  
NDA 20-140  
NDA 20-141 | - \

Reviewer: A. W. Coulter, Ph.D.  
Date of Submission: April 30, 1991  
Date Review Completed: May 22, 1991

SPONSOR: Lederle Laboratories  
Pearl River, NY 10965

DRUG: 1-Leucovorin Calcium Injectable (NDA 20-140) and  
1-Leucovorin Calcium Tablets (NDA 20-141)

Lederle was informed on April 12, 1991 that teratogenesis studies (Segment II) would be required for the 1-isomer of leucovorin. The CDER Stereoisomer Committee Guidelines, in draft form at this date, are recommending reproduction studies be conducted on all single stereoisomers developed from an approved racemic mixture. In discussions with Lederle it was agreed that they could submit for evaluation their rationale for using the Segment II studies that were submitted to their approved NDA 8-107 (dl-Leucovorin Injection) and NDA 18-459 (dl-Leucovorin Tablet).

This two volume submission contains Lederle's response (six reports) to support a waiver from conducting Segment II studies on the 1-isomer of leucovorin. Reports 1 through 4 are resubmissions of their preliminary and final Segment II studies in the rat and rabbit conducted with the racemic mixture of leucovorin. Report 5 assesses the modifying effect of leucovorin (folinic acid) on methotrexate induced embryotoxicity in rats, and Report 6 is a reprint, "Amelioration by Leucovorin of Methotrexate Developmental Toxicity in Rabbits," Teratology 43, 201-215, 1991.

REPORT 1:

This preliminary teratogenesis study was conducted in OFA rats to determine the dose of folinic acid Ca to be used in the final Segment II study. Females (8/group) received VC, 30, 100, or 300 mg/Kg Ca folinate (84% purity) by gavage Day 6

through Day 15 of gestation. The vehicle was 1% carboxy-methylcellulose (CMC). The study was done under GLP and QA by b(4)

The results indicated no maternal or fetal deaths, and no clinical signs were produced at these doses. Body weight gain of drug treated dams did not vary by 1-3%. Food consumption was comparable in each group. The number of term pregnancies was 6, 6, 7, and 5, with 63, 73, 94, and 66 live fetuses in groups 1 through 4, respectively. There was one abortion each in groups 2 and 4. No fetal deaths or embryotoxicity occurred. The mean number of corpora lutea, implantations, resorptions, and live fetuses were similar in all groups. No major external anomalies were reported. There was no evidence of teratogenicity in this study.

#### REPORT 2:

This is the final Segment II study in the rat. It was reviewed by Dr. Alan Taylor for NDA 8-107. He concludes--"The administration of oral leucovorin calcium to pregnant rats at doses up to 1800 mg/eq M/day during the period of organogenesis caused no significant embryotoxicity or teratogenicity. A small increase in early resorptions was seen at high dose (1800 mg/eq M/day) relative to controls."

I am in agreement with Dr Taylor's evaluation of the study. There were no clinical signs or maternal deaths in the study. Weight gain of the dams was comparable in each group over the duration of the study (treated dams showed a slight increase). At necropsy, one dam in the low and mid group had blood in one of the uterine horns, and one high dose dam was found with a hyaline cyst on the ovary. There were four stillborn fetuses in one high dose dam. A slight increase in the ratio of resorptions/implantations was seen in the high dose compared to the control (7.7:10.1), and the live fetuses/pregnant dam was slightly reduced (13.2:12.2). The mean litter weight was lower in the high dose compared to the control (51.66:48.63). Major anomalies exhibited in fetuses in groups 1-4 were 0 (0%), 2 (0.7%), 3 (0.9%), and 2 (0.7%). Minor anomalies were similar between groups.

#### REPORT 3:

The preliminary rabbit teratogenesis study is described in this report. The study was conducted by \_\_\_\_\_ under GLP and QAU procedures. Six pregnant NZ rabbits/group b(4)

were administered VC, 30, 100, and 300 mg/Kg of dl-folinic acid Ca salt (84% purity) by gavage from Day 6 to Day 18 of gestation. The vehicle was 1% CMC.

There were 4, 6, 6, and 4 pregnant females in groups 1-4, respectively. A total of 38, 43, 47, and 37 fetuses were obtained and examined. Two pregnant does were found dead--one in the control and one in group 4. No clinical signs were observed. Weight gain and food consumption were comparable. The mean number of corpora lutea, implantations, resorptions, and live and stillborn fetuses were also comparable throughout the groups. Mean litter weights were reduced 25% in the low and 11% in the mid dose groups. Mean fetal weights were comparable. Hyaline cysts were found on two fetuses in group 2, one fetus in group 3, and one fetus in the high dose. External examination of the fetuses did not reveal major abnormalities. Thus, under the conditions of this study no teratogenic potential was observed for dl-leucovorin Ca.

#### REPORT 4:

This report contains the final Segment II study in rabbits. The study was done under GLP and OAU requirements by                      Pregnant NZ rabbits (18/group) were administered dl-Ca folinate by gavage (VC, 30, 100, 300 mg/Kg) on Day 6 through Day 19 of gestation. The vehicle was 1% CMC.

b(4)

Five animals were found dead during the study, one in group 2, two in group 3, and two in group 4. Mortality was attributed to improper dosing (2), purulent pleurisy (1), purulent pneumonia (1)--one death could not be explained. No treatment-related clinical signs were observed. The percent weight gain was slightly lower in the drug treated groups. Food consumption was comparable in the groups.

At necropsy on Day 29, ovarian hyaline cysts were found in a few does in all groups. The number of pregnant females was 18, 15, 16, and 15 in the VC, low, mid, and high dose, respectively. One low and mid dose doe aborted. The number of corpora lutea and implantations per gestating females at term were similar in all groups. Resorptions per gestating female were slightly increased in the drug treated groups, compared to the control. Two stillborn fetuses occurred in the VC group. The mean weight of fetuses and the number of female and male fetuses were comparable. Major anomalies occurred in only one mid dose fetus (abdominal celosoma and multiple malformations of the head). Minor internal and skeletal anomalies were similar between groups.

The results revealed no teratogenic potential for dl-leucovorin at doses of 300 mg/Kg (3300 mg/sq M), when conducted under the conditions of this study. Based on the 84% purity of the drug, the high dose would be around 2770 mg/sq M. A slight increase in resorptions in one high dose litter may indicate a hint of fetal toxicity.

Dr. Taylor also evaluated this study. He concluded-- "The administration of oral leucovorin Ca to pregnant rabbits at doses up to 3540 mg/sq M/day during the period of organogenesis caused no significant embryotoxicity or teratogenicity. An apparent increase in late resorptions reflected the large increase in a single litter. Therefore, this is not considered a drug-related finding."

#### REPORT 5:

Modifying Effect of Leucovorin (Folinic Acid) on  
Methotrexate-Induced Embryotoxicity in Rats:  
Report # 8335 (E)

This study was done at the request of the Japanese Regulatory Agency. It evaluated the mitigating effect of dl-leucovorin on the embryotoxicity and teratogenicity of methotrexate in rats.

#### Study Design

Report: #8335 (E)

Compounds: Methotrexate (MTX), 102% purity as is.  
Leucovorin (LV), 100% purity as is.

Formulation: Solutions in physiological saline

Route: Intraperitoneal (5 mL/Kg)

Dose Levels:

Group	MTX (mg/Kg)	LV (mg/Kg)
1 Vehicle	0	0
2 MTX	0.3	0
3 MTX	3.0	0
4 LV	0	1
5 LV	0	10
6 MTX+LV	0.3	1
7 MTX+LV	3.0	10

Duration of Treatment: Day 9 of gestation only

Strain: Crj:CD (SD), 12 weeks old at copulation

Number: 10 to 15 in the various groups

Control Treatment: Physiological saline

Study Site/Date: Lederle (Japan), Tokyo/ Nov 82 to May 83  
GLP/OAU: OAU statement present and signed-no GLP statement

Dose levels and interval of treatment between MTX and LV were based on preliminary studies and on results of Dr. James Wilson at Children's Hospital Medical Center in Cincinnati, who also conducted similar studies with similar results. Females were paired 1:1 until mating was confirmed by the presence of a copulatory plug. LV was administered at various time intervals from 0 to 81 hours after MTX administration. Body weight was determined on Days 0, 9, and 20 of gestation. All females were sacrificed on Day 20.

#### Results

No abnormal findings were reported in the dams. Maternal body weights of all groups were similar (no significant difference at  $p < 0.05$ ) on Days 0 and 9; however, significant decreases ( $0.05 > p < 0.001$ ) occurred in the MTX treated groups on Day 20, with the exception of the group treated simultaneously with 10 mg/Kg LV.

The single 3 mg/Kg MTX dose resulted in lethality to all litters (14/14 resorbed). At 0.3 mg/Kg MTX, 8/11 resorptions occurred. The high dose LV group had no resorptions. Embryoletality was reduced in the groups in which LV was administered simultaneously with MTX. Delaying the time interval of LV administration also resulted in increasing the embryoletality. Resorptions increased with increasing doses of MTX and with prolonging the time of administration of LV.

No external malformations were observed in the groups dosed only with LV. External malformations occurring in fetuses treated with 0.3 or 3 mg/Kg MTX plus 1 or 10 mg/Kg LV were cleft palate, crooked tail or short tail, club foot, abdominal hernia, exencephaly, and microphthalmia. Skeletal variations not related to drug dosing were short or lack of 13th rib, deformed ribs, and syndactyly. Delayed ossification was increased by increasing the time interval between MTX and LV administration. Also related to the delay of LV administration was an observed increase in the number of fetuses with dilated cerebral ventricles.

The results of an additional rat experiment confirmed the mitigative action of LV (30 and 100 mg/Kg) on 3 mg/Kg MTX induced decrease in survival rate, growth retardation, number of malformations, and dilation of cerebral ventricles. The mitigative effect of LV was again reduced with an increase in the lag time between LV administration.

Wilson et al. measured MTX concentrations in maternal plasma and rat fetal tissue. Maternal MTX plasma levels decreased rapidly but increased to a maximum at 2 and 4 hours in fetal tissue and remained higher than maternal plasma levels eight hours later. No reports apparently have looked at the fetal level or pharmacodynamics of LV.

Wilson, J.G., Scott, W.J., Ritter, E.J., and Frandkin, R., Distribution and Embryotoxicity of Methotrexate in Pregnant Rats and Rhesus Monkeys. *Teratology* 19, 71-80, 1979.

#### REPORT 6:

The final report is a reprint from *Teratology* 43, 201-215 (1991), Amelioration by Leucovorin of Methotrexate Developmental Toxicity in Rabbits, J. M. DeSesso and G. C. Goeringer.

The reprint describes the results of administering LV (75 mg/Kg iv) up to 24 hours after administration of MTX (19.2 mg/Kg iv) on Day 12 of gestation to pregnant rabbits. LV was administered at either 30 minutes, 1, 2, 3, 4, 5, 6, 8, 16, 20, or 24 hours after MTX. The three control groups received 19.2 mg MTX/Kg, physiological saline, and 75 mg LV/kg on Day 12.

LV was found to produce no adverse effects on fetuses, under the above conditions. Administration of LV 24 hours after MTX resulted in fewer malformed fetuses. The results reinforce what was described in Report 5.

#### EVALUATION:

In none of the above studies was there the appearance of a teratogenic potential for dl-leucovorin. No data was presented regarding the bioavailability of leucovorin; hence, it is not known what the blood or plasma levels of l-leucovorin were in these Segment II studies.

#### RECOMMENDATIONS:

Additional studies will be required to demonstrate bioavailability of leucovorin in the oral Segment II studies, and, if oral absorption is low, the firm should conduct intravenous Segment II studies with l-leucovorin. The labeling

for l-leucovorin should indicate that teratogenic studies have been conducted with the dl-leucovorin but not with l-leucovorin.

*Almon W. Coulter*

Almon W. Coulter, Ph.D.

cc:

NDA 20-140

NDA 20-141

HFD-150/Division Files

/ACoulter

HFD-151/CSO PZimmerman

R/D init by JDeGeorge

F/T ACoulter

*JDeGeorge 8/5/91*

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/s/

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2/26/2008 06:52:32 PM  
PHARMACOLOGIST

William McGuinn  
2/27/2008 04:38:03 PM  
PHARMACOLOGIST