CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-941

PHARMACOLOGY REVIEW

Review and Evaluation of Pharmacology and Toxicology Data

Key Words: **Antiviral; Herpes Labialis**

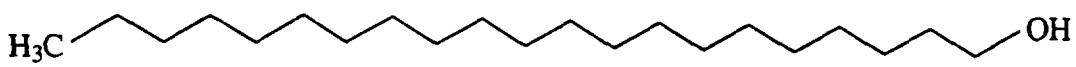
Reviewer: Lynnda Reid, Ph.D.
Division: Dermatologic and Dental Drug Products, HFD-540
Date: November 1, 1999

NDA No: NDA 20-941 - Addendum
Date: March 18, 1999

Information to Sponsor: Yes (x) No ( )

Sponsor: AVANIR Pharmaceuticals
9393 Towne Centre Drive, Suite 200
San Diego, CA 92121
(619) 558-0364

Drug: LIDAKOL®, 10% Cream
Code Name:
Generic Name: *n*-Docosanol, Behenyl Alcohol
Trade Name: Lidakol
Chemical Name(s):
CAS Number: 661-19-8.
Molecular Formula: 326.61
Molecular Weight: $C_{22}H_{46}O$
Structure:



Description: Waxy, white solid, insoluble in water.

Relevant IND and NDA Submissions: [redacted]

Drug Class: Anti-viral

Indication: Oral-Facial Herpes Simplex

Clinical Formulation:

Component	% w/w
n-Docosanol	10.0
Propylene Glycol,	[redacted]
Benzyl Alcohol,	
Sucrose Stearate (&) Sucrose Distearate	
Light Mineral Oil	
Purified Water,	

Route of Administration: Topical cream packaged in 1, 2, 5 and 15 gram tubes.

Proposed Clinical Protocol or Use: The upper estimate of the anticipated daily dose of n-docosanol for treatment of oral herpes is 0.5 to 1.0 mg/kg body weight (5 applications of 50 to 100 mg 10% n-docosanol cream per 50 kg body weight). The clinical endpoint is complete resolution of herpes lesions, and the prescribed maximum time of usage for a single episode is 10 days.

BACKGROUND

To be consistent with the requirement of drugs reviewed and approved in HFD-540 for chronic use, and due to the pharmacologic action of n-docosanol on cell membrane structures and its use on areas exposed to the sun, the Sponsor was asked to commit to dermal and photo-cocarcinogenicity studies during Phase 4 of development. At the post-NA meeting held on March 15, 1999, Avanir was informed that the only reason they were allowed to proceed with an NDA without addressing the issues of dermal carcinogenicity and photo-cocarcinogenicity potential prior to filing was in honor of the previous communications regarding carcinogenicity studies with HFD-530. They verbally acknowledged this request although a written commitment has not been received.

All other nonclinical issues communicated in the December 22, 1998 NA letter have been satisfied

CONCLUSION

From a Pharm/Tox perspective, NDA 20-941 is appovable provided the Sponsor gives a commitment to conduct Phase 4 dermal carcinogenicity and photo-cocarcinogenicity studies to assess the long-term effects of this drug product for a chronic indication.

/S/ 11-1-99

Lynnda Reid, Ph.D. Date
Pharmacologist/Toxicologist

cc:
NDA 20-941
HFD-540
HFD-540/Pharm/Reid
HFD-540/Pharm/Jacobs

For Concurrence Only:
HFD-540/DD/JWilkin /S/ 11/2/99 DFS
HFD-540/TL/AJacobs 11/2/99 for DFS

MAY 26 1998

Review and Evaluation of
Pharmacology and Toxicology Data
Division of Dermatologic and Dental Drug Products (HFD-540)

NDA 20-941 LIDAKOL®, 10% Cream

Drug: *n*-Docosanol, Behenyl Alcohol
Category: Anti-viral
Indication: Oral-Facial Herpes Simplex

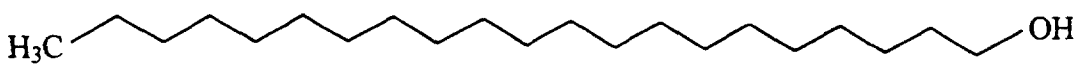
Sponsor: [redacted]

Number of Volumes: 16 (Vols. 2.1 and 1.3-1.17)
Date CDER Received: December 22, 1997
Date Assigned: January 9, 1998
Fileability Review Completed: January 15, 1998
Date 1st Draft Completed: May 13, 1998
Date Review Accepted by Supervisor: May 26, 1998

Chemical Names: *n*-Docosanol; Behenyl alcohol. CAS no. 661-19-8.

Physical and Chemical Characteristics:
Empirical Formula: $C_{22}H_{46}O$
Molecular Weight: 326.61
Description: Waxy, white solid, insoluble in water.

Structure:



Formulation and Route of Administration: Topical cream packaged in 1, 2, 5 and 15 gram tubes.

Clinical Formulation:

Component	% w/w
n-Docosanol	10.0
Propylene Glycol,	
Benzyl Alcohol,	
Sucrose Stearate (&) Sucrose Distearate	
Light Mineral Oil,	
Purified Water,	

Quantitative Composition of LIDAKOL Creams used in Nonclinical Studies:

Component (% w/w)	Formulation 1*	Formulation 3**
n-Docosanol	10	10
Propylene Glycol,		
Benzyl Alcohol,		
Sucrose Stearate (&) Sucrose Distearate		
Light Mineral Oil,		
Purified Water,		

* Formulation 1

** Formulation 3 is identical to the proposed clinical formulation and was used to make up the 10, 12 and 20% n-docosanol Creams used in the nonclinical studies

Review Index:

INTRODUCTION

LIDAKOL® 10% Cream is being developed to treat recurrent Oral-Facial Herpes Simplex. LIDAKOL is a highly lipophilic compound and, as such, may be effective in exerting inhibitory activity for viruses which are lipid-enveloped and utilize this property to gain entry into target cells via membrane fusion.

The upper estimate of the anticipated daily dose of n-docosanol for treatment of oral herpes is 0.5 to 1.0 mg/kg body weight (5 applications of 50 to 100 mg 10% n-docosanol cream per 50 kg body weight). The clinical endpoint is complete resolution of herpes lesions, and the prescribed maximum time of usage for a single episode is 10 days.

Associated IND Number: [redacted]

NONCLINICAL PHARMACOLOGY AND TOXICOLOGY STUDIES

Index of Submitted Nonclinical Studies:

No.	Pharmacodynamic Studies	Report No. (GLP *)	NDA Vol/Page	Rev. Page
1	1-Docosanol Inhibition of Enveloped Viruses: Mode of Action Studies - i. Effects of 1-Docosanol on Enveloped Viruses Entering Cells by Receptor-mediated Endocytosis. ii. Effects of Multiplicity of Infection (MOI) on the Antiviral Activity of 1-docosanol Against Selected Enveloped Viruses That Enter Cells by Receptor-mediated Endocytosis.	[redacted]	1.4/143 1.4/150	10
2	Studies on Mechanism of Viral Inhibitory Activity of LIDAKOL: a) Temporal-Relationship of Target Cell Treatment and Antiviral Activity of LIDAKOL. b) Binding and Uptake of LIDAKOL by Vero Cells. c) Study on LIDAKOL Effects on Viral Entry into Target Cells. d) Effect of LIDAKOL on HSV Receptors on Vero Cells. e) Effect of LIDAKOL on Replication of HSV. f) LIDAKOL Exhibits Preferential Inhibitory Activity for Lipid-Enveloped Viruses.	 LIDAK 105 LIDAK 106 LIDAK 107 LIDAK 108 LIDAK 109 LIDAK 110	 1.4/106 1.4/111 1.4/116 1.4/121 1.4/129 1.4/137	10
3	Verification of Binding Specificity of Radiolabeled HSVOI for Vero Cells.	LIDAK 112	1.4/126	10
4	Further Studies on the Temporal Relationship of Target Cell Treatment and Antiviral Activity of n-Docosanol.	LIDAK 120	1.4/196	10
5	The Anti-herpes Simplex Virus (HSV) Activity of n-Docosanol Includes Inhibition of the Viral Entry Process.	LIDAK 118	1.4/155	10
6	Anti-herpes Simplex Virus Activity of n-Docosanol Correlates with Intracellular Metabolic Conversion of the Drug.	LIDAK 119	1.4/184	10
7	Evaluation of LIDAKOL Suspensions in *in vitro* Herpes Simplex Virus.	LIDAK 102	1.4/046	10
8	Evaluation of LIDAKOL Suspension in *in vitro* Infectivity of Acyclovir-resistant HSV.	LIDAK 103	1.4/055	10
9	Effect of LIDAKOL on clinical isolates of HSV.	LIDAK 104	1.4/087	10

No.	*Pharmacodynamic Studies (Cont'd)*	*Report No. (GLP*)*	*NDA Vol/Page*	*Rev. Page*
10	Antiviral Effects of n-Docosanol Against Acyclovir-resistant Herpes Simplex Virus Type 1, Human and Murine Cytomegalovirus, Varicella-zoster Virus, Human Herpes Virus 6, Influenza A Virus, LP-BM5 Murine Retro virus, Vaccinia Virus, Adenovirus, and Reovirus.	LIDAK 117	1.4/061	11
11	Comparison of n-Docosanol Cream [redacted] Formulations with Acyclovir Ointment for Inhibitory Activity on Cutaneous Herpes Simplex Virus (HSV) Infections in Hairless and Hartley Guinea Pigs.	LIDAK 115	1.4/005	11
12	Evaluation of LIDAKOL Cream Activity in Cutaneous HSV-Induced Lesions in Guinea Pigs.	LIDAK 101	1.4/019	11
13	The Influence of Topical LIDAKOL on Herpes Virus-induced Cutaneous Lesions in Hairless Guinea Pigs.	LIDAK 113	1.4/024	11
14	Reevaluation of the Stearic Acid Containing Placebo and Characterization of the PEG Placebo in the HSV-2/Hairless Guinea Pig Model System.	LIDAK 116	1.4/028	11
15	[redacted] Safety Pharmacology: 1) Irwin Test in Mice Including Body Temperature Alterations 2) Spontaneous Motor Activity in Mice. 3) Barbiturate Induced Sleeping Time in Mice. 4) Proconvulsive Activity (Pentylenetetrazol Induced) in Mice. 5) Cardiovascular and Respiratory Parameters in the Anaesthetized Rat. 6) Charcoal Meal Transit in the Rat Small Intestine. 7) Urinary Output in Rats.	Toxicol * GOC/10/PH GOC/11/PH GOC/12/PH GOC/13/PH GOC/14/PH GOC/15/PH GOC/16/PH	 1.5/056 1.5/089 1.5/117 1.5/145 1.5/174 1.5/220 1.5/247	12
16	A General Pharmacology Study of n-Docosanol. [Addendum: Determination of Docosanoic Acid Using GC/NCI-MS.]	(b) (4) 48BL-20	1.5/003 1.5/048	14

No.	**ADME and Pharmacokinetic Studies**	Report No. (GLP*)	NDA Vol/Page	Rev. Page
17	The Absorption, Distribution, Metabolism and Excretion of n-[1-^{14}C] Docosanol 10% Cream (LIDAKOL™) Formulation 3 Following Oral Gavage to Rats.	LIDAK A100	1.15/096	16
18	The Absorption, Distribution, Metabolism and Excretion of n-[1-^{14}C] Docosanol 10% Cream (LIDAKOL™) Formulation 3 Following Oral Gavage to Rats.	LIDAK A101	1.15/101	16
19	The Absorption, Distribution, Metabolism and Excretion of n-[1-^{14}C] Docosanol 10% Cream (LIDAKOL™) Formulation 3 Following Oral Gavage to Rats.	LIDAK A102	1.15/118	16
20	Comparison of the Absorption of n-[1-^{14}C]Docosanol Formulated in LIDAK Cream Formulation 3, [redacted] Following Oral Gavage to Rats.	LIDAK A103	1.15/139	16
21	n-[^{14}C]Docosanol: Oral Absorption, Distribution, Metabolism and Excretion Study in the Rat.	[redacted]	1.15/154	18
22	Blood Levels of n-[^{14}C]Docosanol and Metabolites Following Dermal Application of LIDAKOL Cream Formulation 3 to Mice.	B101	1.15/002	19
23	Absorption and Pharmacokinetics of n-[1-^{14}C]Docosanol after Dermal Application to Rabbits (Preliminary & Definitive Phases).	[redacted]	1.15/013	20

No.	**Acute and Repeat Systemic Toxicology Studies**	Report No. (GLP*)	NDA Vol/Page	Rev. Page
24	Acute Oral Toxicity Study with LIDAKOL in Rats.	[redacted]255576*	1.6/035	22
[redacted]				
26	A 26-Week Daily Oral Toxicology Study of n-Docosanol Suspensions in Rats including Toxicokinetic Assessments.	LAK008*	1.7/133	23
[redacted]				
28	A 26-Week Oral Toxicity Study of n-Docosanol Suspension in Beagle Dogs Including Toxicokinetic Assessments.	LAK006*	1.10/002	26

No.	Acute and Repeat Topical Toxicology Studies	Report No. (GLP*)	NDA Vol/Page	Rev. Page
29	Acute Dermal Toxicity Study with LIDAKOL Cream in Rats.	[redacted]255587*	1.6/002	28
30	Primary Skin Irritation/Corrosion Study with LIDAKOL and LIDAKOL Placebo in the Rabbit (4-Hour Semi-Occlusive Application).	[redacted]087547*	1.6/071	28
31	Primary Skin Irritation Study with LIDAKOL Cream in Rabbits (4-Hour Semi-Occlusive Application).	[redacted]225598*	1.6/090	28
32	Primary Eye Irritation Study with LIDAKOL in Rabbits.	[redacted]255600*	1.11/159	29
33	Primary Eye Irritation Study with LIDAKOL Suspension in Rabbits.	[redacted]255622*	1.11/189	29
34	Screening for Eye Irritancy Potential using the Bovine Eye / Chorioallanoic Membrane (BECAM) Assay with LIDAKOL Cream.	[redacted]170054*	1.12/074	29
35	Acute Eye Irritation/Corrosion Study with LIDAKOL in the Rabbit.	[redacted]107505*	1.11/141	30
36	Contact Hypersensitivity to LIDAKOL Cream in Albino Guinea Pigs Maximization Test.	[redacted]255611*	1.11/024	30
37	Assessment of Contact Hypersensitivity to LIDAKOL in Albino Guinea Pig (Maximization Test).	[redacted]107516*	1.11/002	31
38	Phototoxicity Study of 10% n-Docosanol Cream (LIDAKOL) in the Guinea Pig.	LAK013*	1.11/067	31
39	Photosensitivity Study of 10% n-Docosanol Cream (LIDAKOL) in the Guinea Pig.	LAK014*	1.11/096	32
40	A 13-week Toxicity Study by Dermal Application of n-Docosanol Cream (LIDAKOL®) to CD-1 Mice Including Toxicokinetic Assessments.	LAK018*	1.6/120	33
41	Subacute 28-Day Repeated-Dose Dermal Toxicology Study on Intact and Abraded Skin in Rabbits.	[redacted]270382*	1.9/002	34
42	Subacute 28-Day Dermal Tolerance Study with n-Docosanol (LIDAKOL) by Daily 6 Hours Administrations to the Intact and Abraded Skin of Rabbits.	[redacted]107527*	1.8/268	36
43	A Penile Irritation Study in Rabbits with n-Docosanol 10% Cream and n-Docosanol 12% Cream.	SLS 3333.4*	1.12/002	37
44	A Vaginal Irritation Study in Rabbits with n-Docosanol 10% Cream and n-Docosanol 12% Cream.	SLS 3333.3*	1.11/437	38
45	Rabbit Vaginal Toxicology Study (28-Day) with Gas Chromatographic Analysis of Plasma From LIDAKOL Treated Rabbits. Analytical Report GC Analysis of 1-Docosanol in Rabbit Plasma.	PH 427-LK-001-91*	1.11/221 1.11/273 1.11/277	39

No.	Reproductive and Developmental Toxicology Studies	Report No. (GLP*)	NDA Vol/Page	Rev. Page
46	An Oral Dose Range-finding Fertility and Pre- and Post-natal Development Study of n-Docosanol Suspension in Rats.	LAK003*	1.12/164	40
47	An Oral Dose Range-finding Embryo-Fetal Development Study of n-Docosanol Suspension in Rats.	LAK004*	1.12/097	41
48	A Combined Fertility, General Reproductive Performance, and Embryo-Fetal Development Study of Orally Administered n-Docosanol Suspension in Rats.	LAK009*	1.12/258	42
49	A Pre- and Post-natal Development Study of Orally Administered n-Docosanol Suspension in Rats.	LAK011*	1.13/002	43
[redacted]				
51	An Oral Dose Range-finding Study of n-Docosanol Suspension in Rabbits.	LAK005*	1.13/254	45
52	A Dose Range-finding Embryo-Fetal Development Study of Orally Administered n-Docosanol Suspension in Rabbits.	LAK007*	1.14/002	46
53	An Embryo-Fetal Development Study of Orally Administered n-Docosanol Suspension in Rabbits.	LAK010*	1.14/056	46
54	A Fertility and General Reproduction Study in Rabbits with n-Docosanol 12% Cream.	[redacted]3333.2*	1.14/159	47

No.	Genotoxicity Studies	Report No. (GLP*)	NDA Vol/Page	Rev. Page
55	Salmonella typhimurium Reverse Mutation Assay with LIDAKOL.	[redacted]170010*	1.14/237	48
56	Gene Mutation Assay in Chinese Hamster V79 Cells in vitro with LIDAKOL.	[redacted]170021*	1.14/270	49
57	Chromosome Aberration Assay in Chinese Hamster V79 Cells in vitro with LIDAKOL.	[redacted]170032*	1.14/295	49
58	Micronucleus Assay in Bone Marrow Cells of the Mouse with LIDAKOL.	[redacted]170043*	1.14/340	49

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PHARMACOLOGY STUDY REVIEWS

Pharmacodynamic Study Reviews

A more complete review of the studies discussed in the Pharmacodynamic section of this review may be found in the Microbiology Review. Only a brief summary review of the findings from these studies is included in this review to describe 1) the proposed 'mechanism of action'; 2) the effect of n-docosanol on the Herpes Simplex virus *in vitro* and *in vivo*; and 3) safety pharmacology in rodents.

For *in vitro* pharmacodynamic studies, n-docosanol was with polyethylene oxide-polypropylene oxide block copolymers, [redacted] The resulting suspensions consisted of uniformly distributed globular particles ranging from [redacted] microns with an average size of 1.0 microns. Vehicle controls for *in vivo* studies utilized the same inactive components and substituted either water or stearic acid for the n-docosanol.

Effect of n-Docosanol *in vitro* on Herpes Simples Virus:

Study 1 - 1-Docosanol Inhibition of Enveloped Viruses: Mode of Action Studies - Conducted by the [redacted]

I. Effects of 1-Docosanol on Enveloped Viruses Entering Cells by Receptor-mediated Endocytosis.

II. Effects of Multiplicity of Infection (MOI) on the Antiviral Activity of 1-Docosanol Against Selected Enveloped Viruses That Enter Cells by Receptor-mediated Endocytosis.

Study 2 - Studies on Mechanisms of Viral Inhibitory Activity of LIDAKOL:

a) Temporal Relationship of Target Cell Treatment and Antiviral Activity of LIDAKOL. [Lidak Study 105]

b) Binding and Uptake of LIDAKOL by Vero Cells. [Lidak Study 106]

c) Study on LIDAKOL Effects on Viral Entry into Target Cells. [Lidak Study 107]

d) Effect of LIDAKOL on HSV Receptors on Vero Cells. [Lidak Study 108]

e) Effect of LIDAKOL on Replication of HSV. [Lidak Study 109]

f) LIDAKOL Exhibits Preferential Inhibitory Activity for Lipid-Enveloped Viruses. [Lidak Study 110]

Study 3 - Verification of Binding Specificity of Radiolabeled HSVOI for Vero Cells. [Lidak Study 112]

Study 4 - Further Studies on the Temporal Relationship of Target Cell Treatment and Antiviral Activity of n-Docosanol. [Lidak Study 120]

Mechanism of Action: In the studies listed above, n-docosanol, the active ingredient in LIDAKOL, demonstrated inhibition of viral replication for several lipid enveloped viruses. Using radiolabeled n-docosanol, *in vitro* studies demonstrated that uptake and metabolism of n-docosanol were necessary for anti-viral activity. Following bioactivation, n-docosanol reportedly blocks fusion of lipid-enveloped viruses, e.g. HSV-1 and HSV-2, with cell membranes, thus inhibiting cellular entry, nuclear localization, and subsequent viral replication. The exact mechanism behind this inhibition is unknown, but it has been hypothesized that biophysical changes in target cell membranes, e.g. changes in membrane fluidity, may induce cellular resistance to fusion with these viruses.

Study 5 - Evaluation of LIDAKOL Suspensions in *in vitro* Herpes Simplex Virus. [Lidak Study 102]

Study 6 - Evaluation of LIDAKOL Suspension in *in vitro* Infectivity of Acyclovir-resistant HSV. [Lidak Study 103]

Study 7 - Effect of LIDAKOL on clinical isolates of HSV. [Lidak Study 104]

Study 8 - Antiviral Effects of n-Docosanol Against Acyclovir-resistant Herpes Simplex Virus Type 1, Human and Murine Cytomegalovirus, Varicella-zoster Virus, Human Herpes Virus 6, Influenza A Virus, LP-BM5 Murine Retrovirus, Vaccinia Virus, Adenovirus, and Reovirus. [Lidak 117]

Study 9 - The Anti-herpes Simplex Virus (HSV) Activity of n-Docosanol Includes Inhibition of the Viral Entry Process. [Lidak Study 118]

Study 10 - Anti-herpes Simplex Virus Activity of n-Docosanol Correlates with Intracellular Metabolic Conversion of the Drug. [Lidak Study 119]

Summary of in vitro Pharmacology Study Results: As a result of the failure of viral material to move to the nucleus, there is a significant inhibition of 1) detectable HSV core and envelope proteins; 2) the number of cells (↓ by 68%) expressing the immediate early protein, ICP-4; and 3) viral production as judged in secondary plaque assays. Optimal activity *in vitro* requires incubation of cells with n-docosanol for several hours prior to HSV exposure.

In cells incubated with 7.5 mM radiolabeled n-docosanol prior to HSV inoculation, there was a 73% decrease in radioactivity in isolated nuclei as compared to untreated and control treated cells. This closely corresponds to the decrease in HSV plaque-formation generally observed with 18 mM n-docosanol. The ID50 (50% inhibitory dose) is approximately 12 mM.

The drug was found to be equally effective against wild type, clinical isolates and acyclovir resistant mutants of HSV. Other viruses which have been shown to be inhibited by n-docosanol include Varicella zoster virus, Herpes virus 6, Respiratory Syncytial Virus, Cytomegalovirus, Influenza A, HIV-1, Semliki Forest Virus and LP-BM-5 (Murine) Virus. Resistant viruses include Reovirus, Adenovirus (Type I), Poliovirus, Vaccinia Virus, and Vesicular stomatitis Virus.

In summary, n-docosanol *in vitro* -

- has no direct viricidal activity or loss of infectivity;
- does not interfere with binding of herpes virus to HSV-specific receptors;
- significantly inhibits cell wall translocation (viron-associated regulatory protein - VP16 transactivator); and
- significantly inhibits viral localization to cell nuclei.

Effect of n-Docosanol *in vivo* on Herpes Simples Virus:

Study 11 - Comparison of n-Docosanol Cream [redacted] Formulations with Acyclovir Ointment for Inhibitory Activity on Cutaneous Herpes Simplex Virus (HSV) Infections in Hairless and Hartley Guinea Pigs. [Lidak Study 115]

Study 12 - Evaluation of LIDAKOL Cream Activity in Cutaneous HSV-Induced Lesions in Guinea Pigs. [Lidak Study 101]

Study 13 - The Influence of Topical LIDAKOL on Herpes Virus-induced Cutaneous Lesions in Hairless Guinea Pigs. [Lidak Study 113]

Study 14 - Reevaluation of the Stearic Acid Containing Placebo and Characterization of the PEG. [Lidak Study 116]

Summary of in vivo Pharmacology Study Results: Efficacy of n-docosanol 10% cream against HSV-1 and HSV-2 induced cutaneous lesions was examined in hairless and Hartley guinea pigs. Adult guinea pigs were inoculated on the back (6-8 sites/animal) with 1 x 10^6 PFU/site using cutaneous puncture with a tattoo pen set at a depth of 2 mm. Treatment was initiated either 2 or 48 hours post inoculation with n-docosanol 10% (formulation 1), n-docosanol 10% in [redacted] 5% Acyclovir Ointment (positive control), or the appropriate vehicle control. Approximately 200 µl was applied with a glass rod with gentle circular rubbing t.i.d. When applied 2 hours after inoculation, n-docosanol 10% was shown to inhibited vesicle formulation. When first applied 48 hours after inoculation to established vesicles, n-docosanol appeared to significantly hasten disease resolution.

Safety Pharmacology Studies:

Study 15 - Safety Pharmacology: Conducted by [redacted] under GLP conditions, study dates: 12/6/95 through 1/19/96.

(1) Irwin Test in Mice Including Body Temperature Alterations. [Toxicol GOC/10/PH]
(2) Spontaneous Motor Activity in Mice. [Toxicol GOC/11/PH]

Study Designs: CD-1 male mice (6/group, ages 6-8 weeks) were treated with 0, 20, 200 or 2000 mg/kg n-docosanol suspended in [redacted] in water. Doses were administered by oral gavage at volumes of 20 ml/kg. Animals were evaluated for pharmacologic effects at 2 hours post n-docosanol dosing. Chlorpromazine (10 mg/kg) served at the positive control for suppression of spontaneous motor activity.

Summary of Study Results: Oral administration of doses up to 2000 mg/kg n-docosanol had no significant systemic pharmacologic effects on clinical behavior, body temperature, or spontaneous motor activity.

(3) Barbiturate Induced Sleeping Time in Mice. [Toxicol GOC/12/PH]
(4) Proconvulsive Activity (Pentylenetetrazol Induced) in Mice. [Toxicol GOC/13/PH]

Study Designs: CD-1 male mice (6/group, ages 6-8 weeks) were treated with 0, 20, 200 or 2000 mg/kg n-docosanol suspended in [redacted] in water. Doses were administered by oral gavage at volumes of 20 ml/kg. Two (2) hours following oral dosing, animals were dosed i.p. with either hexobarbitone (80 mg/kg) to evaluate barbiturate induced sleeping time, or pentylenetetrazol (30 mg/kg) to evaluate any proconvulsive activity of n-docosanol. Chlorpromazine (10 mg/kg) served as the positive control for time to loss of righting reflex and induced sleeping time, while caffeine (150 or 250 mg/kg) served as the positive control for proconvulsive activity.

Summary of Study Results: Oral administration of doses up to 2000 mg/kg n-docosanol had no systemic pharmacologic effects on hexobarbitone induced time to loss of righting reflex or sleeping

time, and proconvulsive activity in pentylenetetrazol treated mice. Positive controls reacted as expected.

(5) Cardiovascular and Respiratory Parameters in the Anaesthetized Rat. [Toxicol GOC/14/PH]

Study Designs: This study was designed to assess the effect of administration of n-docosanol on arterial blood pressure, heart rate, ECG and respiration and to determine the effect on the responses to acetylcholine and noradrenaline, in the anaesthetized rat. Crl:CD(SD)BR(VAF+) rats (6/group) were treated with 0, 250, 500 or 2000 mg/kg n-docosanol suspended [redacted] in water. Doses were administered by oral gavage at volumes of 20 ml/kg. Forty-five minutes following oral dosing, animals were anaesthetized with an intraperitoneal injection of 30 mg/kg sodium pentobarbitone and 1 g/kg urethane. Surgical preparation of the animals was performed for measurement of blood pressure (systolic, diastolic, mean), heart rate, ECG (QRS amplitude, PR interval) and respiration (rate and flow). Approximately 90-120 minutes after administration of the vehicle or n-docosanol the peak response of each parameter was measured before and after intravenous administration of noradrenaline (NA), and then similarly before and after intravenous administration of acetylcholine (ACh) for a period of 50 minutes.

Summary of Study Results: There were no significant direct dose-related effects of n-docosanol against any measured parameter: blood pressure, heart rate, ECG and respiration.

(6) Charcoal Meal Transit in the Rat Small Intestine. [Toxicol GOC/15/PH]

Study Design: This study was designed to identify any activity of n-docosanol on the gastrointestinal tract using charcoal mean transit in the rat small intestine. Crl:CD(SD)BR (VAF+) rats (6/group) were treated with 0, 250, 500 or 2000 mg/kg n-docosanol suspended [redacted] in water. The positive control/standard was 50 mg/kg morphine. Doses were administered by oral gavage at volumes of 20 ml/kg to fasted animals. Two hours after dosing, 1 ml of a charcoal meal was administered orally to each animal. Animals were sacrificed 30 minutes later and the small intestines were removed. The distance the charcoal meal had traveled and the total length of small intestine were measured.

Summary of Study Results: As expected, administration of the standard, 50 mg/kg morphine, significantly ($p<0.01$) reduced the movement of the charcoal meal along the small intestine. Administration of n-docosanol had no effect on the mean transit times.

(7) Urinary Output in Rats. [Toxicol GOC/16/PH]

Study Design: This study was designed to assess the effect of n-docosanol on renal function by analysis of urine output. Crl:CD(SD)BR (VAF+) rats (6/group) were treated by oral gavage (10 ml/kg) with 0, 250, 500 or 2000 mg/kg n-docosanol suspended [redacted] in water. The positive control/standard was 20 mg/kg furosemide. Doses were administered 30 minutes after

administration of 20 ml/kg saline (food and water were withheld for two hours prior to saline dosing and for 6 hours after dosing). Urine was collected at 3, 6 and 23 hours after dosing, the volume recorded and samples analyzed for sodium (Na), potassium (K), chloride (Cl) and inorganic phosphate (Pi).

Summary of Study Results: As expected, furosemide caused significant ($p>0.001$) increases in urine volume and concentrations of Na, K and Cl 3 hours post dose and significant ($p>0.001$) decreases in Na and Cl 23 hours post dose. There were no changes in urinary output parameters in the n-docosanol treated rats during the 23 hours observed.

Study 16 - A General Pharmacology Study of n-Docosanol. [Addendum: Determination of Docosanoic Acid Using GC/NCI-MS.] Study Report no. 48BL-20. Conducted by [redacted] in accordance with Japanese Guidelines for General Pharmacology Studies.

Study Design: This general pharmacology study was designed to study the effect of n-docosanol on the central nervous system, autonomic system, respiratory and cardiovascular system, digestive system, renal system, and local anesthetic activity (corneal and cutaneous reflexes). n-Docosanol was administered to male ICR mice (21-31 g), male Sprague-Dawley rats (100 to 175 g), Beagle dogs (7-10 months, 9.0-9.3 kg), and male Hartley guinea pigs (6-8 weeks, 372-569 g) as follows: orally to male mice and rats at doses of 100, 300 and 1000 mg/kg; intravenously to dogs of either sex at doses of 0.1, 0.3, 1.0 and 3.0 mg/kg; and topically (ocular & dermal) to guinea pigs at 0.3, 1.0 and 3.0 % cream. In addition, an *in vitro* study with isolated guinea pig ileum was conducted with final concentrations of n-docosanol of 10^{-6}, 10^{-5} and 10^{-4} M added to the bathing fluid. n-Docosanol was suspended in aqueous [redacted] for oral administration, F-68 saline for i.v. injection, and in Tyrodes's solution for *in vitro* use. Control and inducing substances included hexobarbitol, phenobarbital, histamine, pentobarbital, charcoal, acetylcholine, diazepam, chlorpromazine, caffeine, atropine, lidocaine, pentylenetetrazole, aminopyrine, furosemide, and acetic acid.

Summary of Study Results: n-Docosanol at oral doses of $\leq$1000 mg/kg produced no significant effect on general or clinical behavior, locomotor activity, thiopental-induced sleeping time, synergistic or antagonistic convulsant activity, or intestinal charcoal transport in male mice (10-20/group). n-Docosanol had no significant effect on normal body temperature or urinary volume or electrolyte excretion in male rats (8/group).

Following i.v. administration to dogs (3), n-docosanol produced little or no effect on the respiratory rate, blood pressure, heart rate and ECG in anesthetized animals at doses $\leq$3 mg/kg.

Topical and ocular applications of $\leq$3.0 % n-docosanol did not demonstrate any local anesthetic activity in male guinea pigs (5-15/group). In the *in vitro* study, n-docosanol did not show any significant influence on the spontaneous movements of isolated guinea pig ileum (5) at $\leq 10^{-4}$ M, and it had little or no effect on acetylcholine-, histamine- or barium-induced contractions.

All positive controls responded appropriately.

Addendum: **Determination of Docosanoic Acid Using GC/NCI-MS**

This study was performed to examine the relationship between plasma concentrations of n-docosanol and it's major metabolite docosanoic acid. Male Sprague-Dawley rats (1/dose, ages 6-7 weeks, weighing 202.6 to 219.1 g) were administered doses of 30, 100, 300 and 1000 mg/10ml/kg n-docosanol suspended in aqueous solutions of [redacted] prepared by both [redacted] pharmaceuticals. Blood samples were collected in heparinized capillary tubes from a tail vain at 0.5, 1, 2, 4 and 8 hrs. Following centrifugation, 50 µl of plasma was diluted 1:1 with [redacted] water and stored at -20°C. For analysis, 100 µl diluted rat plasma were mixed with 200 µl of 0.1 M phosphoric acid and 50 µl of internal standard solution (eicosanoic acid 100 ng/ml ethanol) and the acids were extracted and then derivatized to corresponding PFB-esters with Na_2SO_4 and K_2CO_3 (1:1, Ca. 10 mg). After the esterfication, the reaction mixture was diluted with 2000 µl of n-hexane and centrifuged. The residue was redissolved in 100 µl of n-hexane and 1 µl of the resulting solution was subjected to GC/NCI-MS. GC separations were carried out with a RTX-1 chemically bonded fused silica capillary column with methane as the GC carrier gas. Column head pressure was held at 0.7 kg/cm2 and the flow rate was 1 ml/min. Column temperature, injection-port temperature and ion-source temperature were kept at 170, 280 and 200°C, respectively. The ionization energy and the trap current were maintained at 70 eV and 350 µA, respectively.

Summary of Study Results: n-Docosanoic acid concentrations found in rat plasma after a single oral administration of n-docosanol suspensions in aqueous [redacted] prepared by either [redacted] Pharmaceuticals are presented in Table PK-1a and PK-1b, respectively.

Table PK-1a: Determination of n-Docosanoic acid in rat plasma following a single oral administration of a n-docosanol suspension in aqueous [redacted] from solutions prepared by [redacted]

Time	Concentration of Docosanoic Acid (ng/ml)			
(hr)	30 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	29.9	38.6	55.7	69.1
1	140.1	202.9	213.0	236.6
2	161.6	230.8	315.4	309.5
4	98.4	124.3	141.3	146.2
8	46.3	36.3	45.6	45.0

Table PK-1b: Determination of n-Docosanoic acid in rat plasma following a single oral administration of a n-docosanol suspension in aqueous [redacted] from solutions prepared by [redacted] Pharmaceuticals.

Time	Concentration of Docosanoic Acid (ng/ml)			
(hr)	30 mg/kg	100 mg/kg	300 mg/kg	1000 mg/kg
0.5	95.9	67.1	24.9	29.9
1	179.5	219.2	184.6	165.8
2	152.3	283.6	257.8	263.8
4	86.6	220.7	206.0	202.5
8	46.5	97.3	134.2	144.5

Review Notes: *From this data, it appears that n-docosanol is readily metabolized following absorption with absorption and/or metabolism of n-docosanol peaking between 1 and 4 hours. As observed in the previous studies looking at n-docosanol plasma levels, the relationship between the administered dose and the appearance of n-docosanoic acid in the plasma is non-linear and dose-dependent. There are no significant differences in the plotted area under the curve (AUC) following administration of the 300 and 1000 mg/kg doses for either preparation, however, exposure, as measured by AUC, does appear to be prolonged following administration of the [redacted] preparation. AUC calculations were not submitted and samples were only measured for 8 hours following administration. The Sponsor will be asked to provided the calculated AUC_{0-8} and any available information on n-docosanol plasma levels in these animals.*

Pharmacokinetic Study Reviews

In vitro Pharmacokinetic Studies: To investigate the uptake, distribution, and metabolism of n-docosanol by cultured cells, target cells were incubated with n-[^{14}C]docosanol. Most of the radiolabel (73%) was recovered in membranous fractions and <1% was associated with a nuclear fraction. Analysis by chemical (Vitride) reduction suggested that a significant portion of n-docosanol is oxidized to n-docosanoic acid and then incorporated as a acyl group on polar lipids. Up to 60% of the cell-associated radiolabel was incorporated into phospholipids that co-purified with phosphatidylcholine and phosphatidylethanolamine. The rate and extent of metabolic conversion of n-docosanol varied with the cell type and surfactant used to suspend the compound. The anti-HSV activity was quantitatively proportional to the amount of metabolism observed.

In vitro penetration of n-[1-^{14}C]docosanol formulations [Study LP-17339] was compared using human cadaver skin (Table PK-2). Less than 2.0 % of the radio-label from the proposed formulation (Formulation 3) penetrated the skin and less than 0.01 % was found in the reservoir fluid.

Table PK-2: 24 Hour Cumulative Mean ± SD Penetration [µg n-Docosanol (% dose)].

Sample	Formulation 1		Formulation 3	
Stratum Corneum	15.12 ± 4.770	(0.500 ± 0.160)	34.47 ± 32.98	(1.150 ± 1.100)
Epidermis	13.02 ± 6.810	(0.004 ± 0.002)	21.92 ± 18.63	(0.730 ± 0.620)
Dermis	0.630 ± 0.520	(0.020 ± 0.020)	1.280 ± 0.560	(0.040 ± 0.020)
Reservoir	0.111 ± 0.052	(0.004 ± 0.002)	0.083 ± 0.028	(0.003 ± 0.001)

Acute Oral ADME Studies:

Study 17 - The Absorption, Distribution, Metabolism and Excretion of n-[^{14}C]Docosanol 10% Cream (LIDAKOL™) Formulation 3 Following Oral Gavage to Rats. [Lidak Study A100, *in life:* 7/7/93 to 8/4/93.]

Study 18 - The Absorption, Distribution, Metabolism and Excretion of n-[^{14}C]Docosanol 10% Cream (LIDAKOL™) Formulation 3 Following Oral Gavage to Rats. [Lidak Study A101, *in life:* 10/23/93]

Study 19 - The Absorption, Distribution, Metabolism and Excretion of n-[^{14}C]Docosanol 10% Cream (LIDAKOL™) Formulation 3 Following Oral Gavage to Rats. [Lidak Study A102, *in life:* 1/27/94]

Study 20 - Comparison of the Absorption of n-[^{14}C]Docosanol Formulated in LIDAK Cream Formulation 3, [redacted] Following Oral Gavage to Rats. [Lidak Study A103, *in life:* 10/3/94]

Study Design: The oral ADME studies (17-20) were designed and conducted by LIDAK Pharmaceuticals, 11077 North Torrey Pines Road, La Jolla, CA, to investigate the absorption,

metabolism, and excretion of n-[1-^{14}C]docosanol and to provide data to evaluate the extent of exposure to n-docosanol from accidental oral ingestion following application to the lips. n-Docosanol (1-50 mg/rat) formulated at concentrations of 10% in cream (Formulation 3), [redacted] was administer to female [redacted] rats (~8 weeks in age, 1-3 rats/time point). Animals were fasted overnight prior to treatment and for 6 hours after treatment (water was available throughout the study period). Plasma, RBC fractions, tissues, urine and feces were analyzed at various time points between 0.2 hours and 32 days post-dosing for total radioactivity and metabolic products of n-docosanol. The percent of intact n-docosanol to metabolic products in plasma and tissues was determined by thin layer chromatography (TLC) following lipid extraction.

Summary of Study Results:

Absorption: The rate and amount of n-docosanol absorbed into the body through the gastrointestinal tract appeared to be similar between doses formulated in cream [redacted] Saturation of n-docosanol uptake was not observed and increasing plasma radioactivity was associated with increasing dosages of the drug over a 24 hour time period. Higher plasma levels were observed following oral administration of n-docosanol suspended [redacted] however, the observed kinetics of appearance and decline of plasma radioactivity was similar for all three formulations (Table PK-3).

Table PK-3: Comparison of systemic exposure (AUC_{0-24}) resulting from oral gavage of n-[1-^{14}C] docosanol 10% cream formulations to adult female rats weighing 194 ± 3.3 g.

Dose	Cream Formulation 3	1% Tween 80	Pluronic F-68
3 mg/kg	2.6 µg-eq.hr/ml	4.8 µg-eq.hr/ml	17.7 µg-eq.hr/ml
300 mg/kg	80.6	52.0	149.0

Distribution: Radioactivity was detected in the plasma within 0.5 hours, with significant levels detected at 1 hour and peak concentrations occurring between 6 and 12 hours following a single oral dose. Radioactivity was detected in all tissues examined within 1 day of dosing: intestine, stomach, gastrointestinal contents, liver, spleen, lung, heart, kidney, muscle, brain, brown fat and white fat. On day 1, the liver, spleen and brown fat contained the highest levels of radioactivity (Table PK-4). Within 24 hours post-dosing. over 90 % of the radioactivity in the liver was determined to be in the form of polar lipid metabolites. The half-life of n-docosanol derived radioactivity in the liver was 4-5 days. Similar rates of clearance were observed from the spleen. By day 32 post-gavage, most of the tissue-associated radioactivity had been eliminated, with only about 1% of the original dose localized in brown fat (primarily incorporated as triglycerides) and brain lipids.

Metabolism: Plasma samples from the 3, 6 and 12 hour time points were extracted and analyzed by TLC. At 3 hours n-docosanol accounted for 11% of the radioactivity, n-docosanoic acid 66%, and 23% remained at the origin as polar phosphatides. By 6 hours, only traces of n-docosanol could be detected and the n-docosanoic acid and polar phosphatides accounted for approximately 25% and 75% of the radioactivity, respectively. Metabolic conversion appeared nearly complete by 12 hours post-gavage when only slight traces of n-docosanol and n-docosanoic acid could be detected and the radioactivity migrated as polar phosphatides. The metabolic pathway appears similar to other fatty

alcohols: oxidation to fatty acids followed by esterfication to a wide variety of lipids, glycerides and phosphoglycerides which are then appear to be universally distributed in tissues.

Excretion: Approximately 29 to 90.6% of the administered radioactivity was detected in the feces at 24 hours and 76 to 93.2% at 72 hours post-dosing. Total recovery of radioactivity between feces and cage washings (primarily fecal and urinary products) accounted for 29.5-90.8% and 76.9-99.4% of administered dose at 24 and 72 hours, respectively. The Sponsor has estimated that 75-90% of the radioactivity in the feces is attributable to unabsorbed product.

Table PK-4: Tissue radioactivity levels following a single gavage administration of n-[1-^{14}C]docosanol 10% cream to adult female rats weighing 165-175 g.

Tissue	Day 1	Day 32
Liver	1.23 % dose/g tissue	0.010 % dose/g tissue
Spleen	0.52	0.013
White Fat	0.19	0.047
Brown Fat	0.57	0.083
Muscle	0.04	0.007
Brain	0.02	0.011
Heart	0.22	0.021
Lung	0.23	0.017
Kidney	0.18	0.016
Stomach	0.33	0.017
Intestine	0.49	0.031
Total Tissue	**13.3 % of total dose**	**1.07 % of total dose**
Total Tissue plus GI contents	**18.9 % of total dose**	**1.10 % of total dose**

Study 21 - n-[^{14}C]Docosanol: Oral Absorption, Distribution, Metabolism and Excretion Study in the Rat. Study Report No. LAK012. *In life:* 9/26/94 - 3/28/95, Conducted by [redacted] in accordance with internationally recognized Good Laboratory Practices.

Study Design: This study was designed to 1) determine the rate and extent of absorption by comparing urinary excretion of radioactivity after oral and intravenous administration; 2) investigate the time course of radioactivity in the blood; 3) examine the qualitative tissue distribution (including pregnant animals); and 4) determine the metabolite profile in urine, feces, and plasma following a single dose of n-[13-^{14}C]docosanol. Rats were administered 10 mg/kg labeled n-docosanol in [redacted] by oral gavage (18 males, 24 females, 20 non-pregnant and 4 pregnant) or 1 mg/kg labeled n-docosanol [redacted] by intravenous injection (6 males, 4 females). For autoradiography studies, pregnant females were dosed on day 18 of gestation.

Summary of Study Results: After i.v. doses, approximately 50% of the radioactivity was excreted in the expired air (presumably as $^{14}CO_2$), 2% in the urine, 1% in the feces, and 27% was present in the tissues at the time of sacrifice 168 hours postdosing (Table PK-5a). Following oral dosing over a period of 168 hours, approximately 79% of the radioactivity was found in the feces, 10% was

radioactivity recovered from tissue, plasma and urine at sampling time points is presented in Table PK-6. Systemic absorption of n-docosanol, <0.0003% of the applied dose, appears to be limited.

Table PK-6: Total ^{14}C counts (DPM) following dermal application of n-[1-^{14}C]docosanol to mice.

	265 mg/kg (4.47 X 10^9 DPM/kg) n-[1-^{14}C]Docosanol			213 mg/kg (5.14 X 10^9 DPM/kg) n-[1-^{14}C]Docosanol		
Time (hrs)	Cage Wipes (DPM-Bkgd)	Plasma (DPM-Bkgd/100 µl)	Plasma (ng.Equiv/ml)	Cage Wipes (DPM-Bkgd)	Plasma (DPM-Bkgd/100 µl)	Plasma (ng.Equiv/ml)
0.5	2 - 4	1 - 13	0.6 - 7.6	ND	ND	ND
1.0	0 - 5	3 - 12	1.8 - 7.0	ND	ND	ND
2.0	3 - 192	3 - 5	1.8 - 2.9	ND	ND	ND
4.0	11 - 292	4 - 8	2.3 - 4.7	ND	ND	ND
8.0	2 - 17	4 - 10	2.3 - 5.9	ND	ND	ND
24.0	6 - 13	1 - 8	0.6 - 4.7	17 - 135	54 - 60	22 - 24
48.0	ND	ND	ND	4 - 16	43 - 57	17 - 23
72.0*	33 & 65	10 & 11	5.9 & 6.5	19 & 53	42 & 50	17 & 20

* At 72 hours, only 2 animals were samples. All other time points represent a n of 3. Limit of detection - 10 ng/ml.. ND = No Data.

Study 23 - Absorption and Pharmacokinetics n-[^{14}C]Docosanol after Dermal Application to Rabbits (Preliminary & Definitive Phases). Study report no. 6634-100, *In life:* 5/18 to 6/21/95, Conducted by [redacted] in compliance with Good Laboratory Practices (21 CFR 58).

Study Design: A single dermal dose of n-[1-^{14}C]docosanol was administered to 12 male rabbits at a dose of 25 mg/kg over an area of approximately 43 cm^2 of nonabraded and abraded skin. Doses were removed 24 hours after application by rinsing. Rinse samples were retained for radioactivity analysis. Blood, skin, urine, feces and expired air were collected at various time points and assayed for radioactivity.

Summary of Study Results: Only a small percentage of the radiolabeled dose was excreted in the urine, feces, CO_2, and organic volatiles. Recovery of the radioactivity in the cage wash, cage wipe, urine, feces and expired air averaged 0.178% and 0.086% of the applied dose for animals with nonabraded and abraded skin, respectively. Cmax values were minimal and ranged from 0.004 µg eq/g to 0.011 µg eq/g (Table PK-7). Most of the applied radioactivity was recovered in the skin wash (>93%) (Table PK-8). Low but quantifiable levels of radioactivity were recovered as $^{14}CO_2$.

Table PK-7: Pharmacokinetic parameters for n-[1-^{14}C]docosanol-derived radioactivity in blood and plasma of rabbits receiving a single topical dose (25 mg/kg).

Parameter	Nonabraded Skin		Abraded Skin	
	Blood	Plasma	Blood	Plasma
C_{max} (µg.eq/g)	0.011	0.007	0.009	0.004
T_{max}	32	88	40	56
AUC_{0-last} (µg eq.hr/g)	1.28	1.02	1.04	0.649

Table PK-8: Percent of radioactivity observed at 168 hours post-dose for male rabbits following a single dermal application of n-[1-^{14}C]docosanol (25 mg/kg).

Sample	Nonabraded Skin	Abraded Skin
Occlusive Cover *	0.546 ± 0.762	0.083 ± 0.119
Enclosure Rinse	0.303 ± 0.114	0.566 ± 0.448
Cage Wash	0.008 ± 0.005	0.002 ± 0.002
Cage Wipe	0.010 ± 0.007	0.002 ± 0.001
Skin Wash *	93.69 ± 8.155	94.36 ± 2.052
Skin Test Site	1.713 ± 0.296	1.151 ± 0.261
Plasma	NQ	NQ
Urine	0.018 ± 0.003	0.018 ± 0.004
Feces	0.002 ± 0.003	NQ
CO2	0.136 ± 0.074	0.057 ± 0.034
Volatiles	0.004 ± 0.003	0.008 ± 0.007
Total Recovery	96.44 ± 9.321	96.24 ± 2.038
Total Absorbed **	**1.898 ± 0.359**	**1.231 ± 0.304**

* Collected at 24 hours post-dosing.

** Total radioactivity minus radioactivity from occlusive cover, enclosure rinse & skin rinse.

There were no significant differences between abraded and nonabraded skin. Absorption of the test material was minimal with most of the test material remaining on the surface of the skin.

TOXICOLOGY STUDY REVIEWS

Unless otherwise stated animals used in toxicology studies were all acclimatized prior to study initiation and randomized to treatment groups. Food and water were analyzed for impurities and were freely available unless otherwise stated.

Aqueous suspensions of n-docosanol for oral administration were prepared from a 20 % n-docosanol stock suspension in [redacted] prepared weekly or as needed. This suspension was used for high group dosing, while lower concentrations were prepared on the day of use by dilution of the 20 % suspension with [redacted] Quality control of dosing solutions was performed by [redacted] and unless otherwise stated, were found within protocol limits.

Acute and Repeat Systemic Toxicology Study Reviews (GLP)

Study 24 - Acute Oral Toxicity Study with LIDAKOL in Rats. Study report no. [redacted] 255576. *In life:* 10-27 to 11/10/89, conducted by [redacted] in accordance with OECD GLP guidelines. n-Docosanol batch no. 6232.

Summary Results: Administration of 2000 mg/kg n-docosanol suspended in olive oil to Wistar rats (5 animals/sex) by oral gavage did not result in any moralities or drug related clinical signs of toxicity during a 15 day post-dosing observation period. There were no macroscopic findings observed at necropsy.

[redacted]

[redacted]

Study 26 - A 26-Week Daily Oral Toxicology Study of n-Docosanol Suspensions in Rats including Toxicokinetic Assessments. Study report no. 94/LAK008/0963. *In life:* 12/14/94 to 6/19/95, conducted at [redacted] in compliance with OEDC GLP guidelines.

Study Design: CD rats (20/sex/group, ages 28-35 days) were treated by oral gavage with 0, 10, 100 and 1000 mg/kg/day n-docosanol suspended in [redacted] aqueous solutions for 26 weeks at a constant volume-dosage of 5 ml/kg. Animals were evaluated for the following: clinical signs of toxicity; changes in bodyweight and food intake; ophthalmoscopy (weeks 12 and 25); hematology and clinical (serum and urine) chemistry (weeks 13 and 26); and organ weights, gross necropsy, and microscopic tissue changes at study termination. All gross lesions and the following tissues were evaluated microscopically:

-adrenals *
-aorta (thoracic)
-brain *
-cecum
-colon
-duodenum
-epididymides
-esophagus
-eyes and optic nerves
-femur w/marrow
-heart *
-ileum
-jejunum
-kidneys *
-liver *
-lungs w/mainstem bronchi *
-lymph nodes (mandibular and mesenteric)
-mammary gland
-ovaries *
-pancreas
-pituitary *
-prostate *
-rectum
-salivary glands (submandibular)
-sciatic nerve (left)
-seminal vesicles
-skeletal muscle (thigh)
-spinal cord
-spleen *
-sternum
-stomach
-testes *
-thymus *
-thyroid and parathyroid *
-trachea
-urinary bladder
-uterus w/cervix *

* analysis included organ weight. Tissues preserved but not examined included the harderian glands, mammary gland (cranial), sciatic nerve (right) and tongue.

Samples for toxicokinetic evaluations were collected from the retro-orbital sinus at 0.5 1, 2, 4, 8, and 24 hours after dosing on day 1 and at the end of study weeks 13, and 26 from satellite animals assigned to treatment groups (10/sex/treatment group) and the vehicle control group (6/sex). Three treated animals or 2 control animals per sex/group/time point were preselected by numerical ordering and sampled at either 0.5 and 4 hours, 1 and 8 hours, or 2 and 24 hours. All satellite animals were discarded, without necropsy, after the completion of sampling in week 26 of treatment.

Statistical significance was defined as $p<0.05$. Statistical analyses were performed as follows:

Clinical laboratory results - Student's t-test
Organ weights and bodyweight - Bartlett's test, Behren-Fisher test and Dunnett's test.
Macroscopic and microscopic pathology - Fisher's Exact test.

Summary of Study Results: With two exceptions, there were no significant differences between groups in bodyweight, food intake, blood and urine profiles, and organ weights. No ophthalmic, macroscopic or microscopic pathological abnormalities were observed.

One female (#49), dosed at 100 mg/kg/day, died in week 25. At necropsy, the animal presented with moderate lung congestion and slight dilation of tracheal glands. The cause of death was thought to be the result of accidental aspiration of test material, unrelated to toxicity as a result of exposure to n-docosanol.

One male (#80), dosed at 1000 mg/kg/day presented with several abnormal clinical pathology parameters including increased (2 fold) kidney weights; slight anemia; high plasma urea, creatinine, cholesterol, phosphorus and triglyceride concentrations. However, since this was an isolated incidence, its relationship to n-docosanol was considered unlikely.

The no-effect level determined in this study was 1000 mg/kg/day.

[redacted]

Dose Verification: Analysis of 20% n-docosanol study suspensions by [redacted] resulted in levels ranging between [redacted]% w/w n-docosanol during the study period.

Review Comment: *Three plasma samples from control animals also had measurable n-docosanol levels: 1 male at 13 weeks of 16 ng/ml, and 1 male and 1 female at 26 weeks of 10 and 18 ng/ml, respectively. These levels are close to the limit of detection and may represent background, contamination, or errors in labeling or recording samples. Due to the negligibility of these readings and the absence of adverse effects at the highest doses administered, these results do not sufficiently impact*

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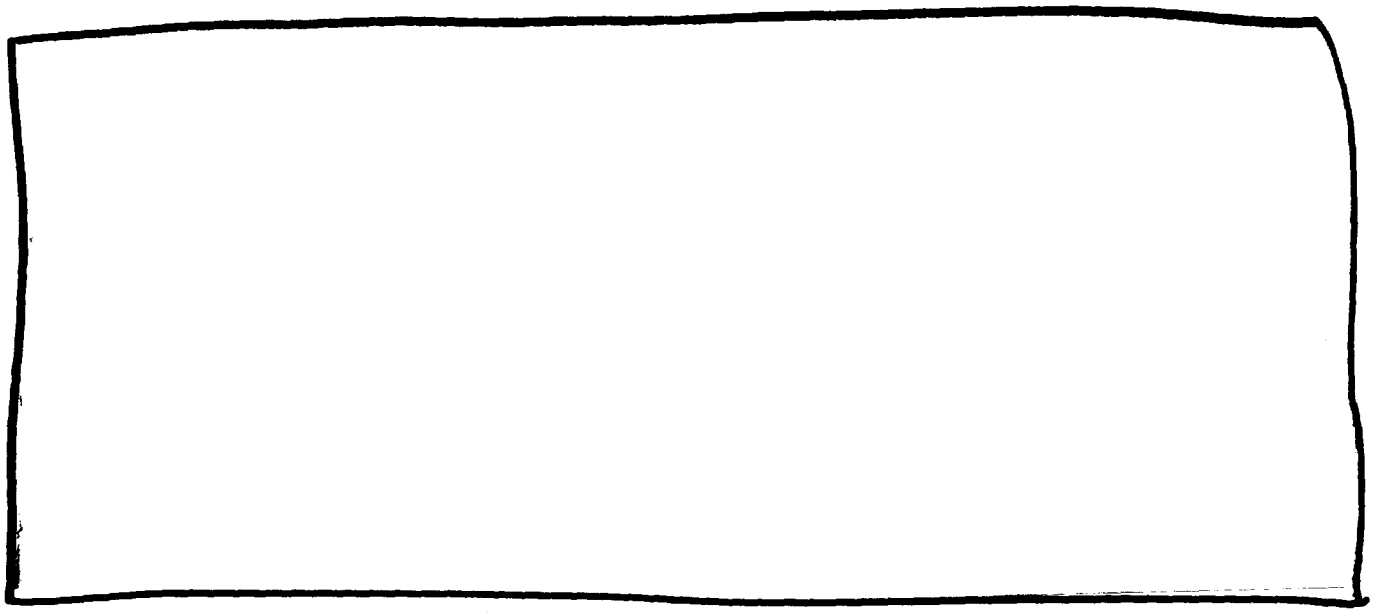

Study 28 - A 26-Week Oral Toxicity Study of n-Docosanol Suspension in Beagle Dogs Including Toxicokinetic Assessments. Study report no. 95/LAK006/0406. *In life:* 7/27/94 to 2/6/95, conducted at [redacted] with toxicokinetic sampie analyses performed by [redacted] in compliance with OEDC GLP guidelines. n-Docosanol batch no. 45228.

Study Design: Beagle dogs (4/sex/group, ages 19-23 weeks, 6.6 to 9.6 kg) were treated by oral gavage with 0, 20, 200 and 2000 mg/kg/day n-docosanol suspended in 1% Tween 80 aqueous solutions for 27 weeks. Animals were evaluated for clinical signs of toxicity; changes in bodyweight, food intake, ophthalmoscopy (weeks 11 and 24), hematology, plasma and urine chemistries, and organ weights, and gross and microscopic tissue changes. Samples for toxicokinetic evaluations were collected at 2, 4, 8, 12, 16 and 24 hours after dosing on day 1 and at the end of study weeks 13, and 26. Tissues preserved for histopathology include the following:

-adrenals *
-aorta
-brain *
-cecum
-colon
-duodenum
-epididymides
-esophagus
-eyes and optic nerves
-heart *
-ileum
-jejunum
-kidneys *
-liver *
-lungs w/mainstem bronchi *
-lymph nodes - axillary, mandibular and mesenteric
-mammary gland
-ovaries *
-pancreas
-pituitary *
-prostate w/urethra*
-rectum
-salivary glands (submandibular)
-sciatic nerve (left)
-skeletal muscle (thigh)
-skin - test site and untreated
-spinal cord
-spleen *
-sternum with marrow
-stomach
-testes *
-thymus *
-thyroid and parathyroid *
-trachea
-urinary bladder
-uterus w/cervix *

* included organ weight. Tissues preserved, but not examined included the bronchi, femur w/joint, salivary gland (submandibular), sciatic nerve, skin and tongue.

Summary of Study Results: Signs of reaction to treatment were limited to observation of pale feces in the animals treated with 2000 mg/kg/day (the result of unabsorbed test material in the feces). There were no significant differences between groups in bodyweight, food intake, blood and urine

profiles, and organ weights. No ophthalmic, macroscopic or microscopic pathological abnormalities were observed. The no effect level (NOEL) determined in this study was 2000 mg/kg/day.

Toxicokinetic Results: Dose related n-docosanol concentrations were detected in the plasma of all treated dogs but not in controls. As observed in rats, plasma concentrations of n-docosanol in dogs, characterized by C_{max} and AUC_{0-24}, were less than the proportionate dose increment and appeared tc be characterized by non-linear (dose-dependent) kinetics (Table TK-2). Inter-individual variation in plasma concentrations was high (coefficient of variation generally being greater than 50%, and in the range of [redacted] The time to maximum n-docosanol plasma levels (T_{max}) was also highly variable, with both intra- and inter-animals times ranging between 2 and 12 hours. The maximum n-docosanol plasma level (C_{max}) was 3 µg/ml in animals dosed at 2000 mg/kg/day.

Table TK-2: The mean maximum plasma concentration (C_{max}) of n-docosanol and the mean areas under the plasma n-docosanol concentration time curves estimated up to 24 hours post dose (AUC_{0-24}) on Day 1 and during Weeks 13 and 26 in Beagle dogs are summarized below, with standard deviations in parentheses.

A: C_{max} (ng/ml)

Dose	Day 1		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females
20	74 (50)	26 (10)	159 (91)	151 (44)	137 (53)	310 (205)
200	473 (244)	207 (93)	1841 (601)	1186 (854)	757 (388)	1289 (1020)
2000	1308 (326)	1469 (463)	2140 (272)	2860 (1077)	1094 (814)	2255 (718)

B: AUC_{0-24} (ng.h/ml)

Dose	Day 1		Week 13		Week 26	
(mg/kg/day)	Males	Females	Males	Females	Males	Females
20	610 (473)	177 (115)	1550 (1377)	1377 (449)	848 (434)	2031 (1073)
200	4419 (1856)	1781 (1166)	14370 (3755)	8425 (6533)	7435 (3575)	7799 (5492)
2000	11830 (3238)	15080 (7425)	22430 (2275)	27830 (8361)	12340 (9028)	24400 (8043)

Plasma concentrations of n-docosanol at 24 hours post-dose were generally below the limit of detection (<10 ng/ml) following administration of 20 mg/kg/day. At 200 and 2000 mg/kg/day, measurable levels were detected at most sampling times, especially following multiple days of dosing. There were no statistically significant differences in toxicokinetics between male and females.

Dose Verification: Analysis of 20% n-docosanol study suspensions by [redacted] resulted in levels ranging between [redacted] w/w n-docosanol during the study period.

Acute and Repeat Topical Toxicology Study Reviews

Study 29 - Acute Dermal Toxicity Study with LIDAKOL Cream in Rats. Study report no. [redacted] 255587, . *In life:* 10/25 to 11/8/89, conducted by [redacted] in accordance with OECD GLP guidelines.

Summary Results: Administration of 2000 mg/kg n-docosanol topically to Wistar rats (5 animals/sex) under occlusive dressings (20 % n-docosanol cream) did not result in any mortalities or drug related clinical signs of toxicity during a 15 day post-dosing observation period. Slight scaling was observed between days 3 and 7. There were no macroscopic findings observed at necropsy.

Study 30 - Primary Skin Irritation/Corrosion Study with LIDAKOL and LIDAKOL Placebo in the Rabbit (4-Hour Semi-Occlusive Application). Study report no. [redacted] 087547, *In life:* 11/17 to 11/20/92, Conducted by [redacted] in accordance with GLP guidelines (OECD and 21 CFR 58).

Study Design: Male and female New Zealand White rabbits (3/study, aged 14-15 weeks) were dosed with 50 mg n-docosanol (0.5 grams of 10 % LIDAKOL cream - formulation 3) to a 6 cm^2 area of intact shaved skin. The area was covered with a semi-occlusive dressing for 4 hours, then flushed with water. Placebo cream was applied to the opposite flank. Skin reaction was assessed for 7 days post-dosing on a grading scale of 0 to 8 where 0=non-irritating, 0.1-2.0 = mildly irritating, 2.1-5.0 = moderately irritating, and 5.1-8.0 = severely irritating.

Summary of Study Results: Application of the 10% n-docosanol cream resulted in very slight erythema with no edema to well defined erythema with slight edema. Reactions resolved within 24 to 48 hours after exposure. No staining of treated skin was observed. Comparable skin irritation was observed with the topical placebo applied to the opposite flank. Both n-docosanol 10% cream and the placebo were assigned primary irritation indices of 0.2 (mildly irritating) when applied to intact rabbit skin. No signs of systemic toxicity were observed during the study period.

Study 31 - Primary Skin Irritation Study with LIDAKOL Cream in Rabbits (4-Hour Semi-Occlusive Application). Study report no. [redacted] 225598, *In life:* 10/24 to 10/31/89, Conducted by [redacted] in accordance with OECD GLP guidelines.

Study Design: This study was designed similarly to Study 30, except a 20% n-docosanol cream formulation was used. Male and female New Zealand White rabbits (3/study, aged 14-15 weeks) were dosed with 50 mg n-docosanol (0.5 grams of 20 % LIDAKOL cream - formulation 3) to a 6 cm^2 area of intact shaved skin. There were no placebo or control animals used in this study. The area was covered with a semi-occlusive dressing for 4 hours, then flushed with water. Skin reaction was assessed for 7 days post-dosing on a grading scale of 0 to 8 where 0 = non-irritating, 0.1-2.0 = mildly irritating, 2.1-5.0 = moderately irritating, and 5.1-8.0 = severely irritating.

Summary of Study Results: Slight scaling was observed between days 3 and 7 in all animals treated with the 20 % n-docosanol cream. Local signs consisted slight erythema. No staining of treated skin was observed.

Study 32 - Primary Eye Irritation Study in with LIDAKOL in Rabbits. Study report no. 255600, *In life:* 10/24 to 10/27/89, conducted by in compliance with OECD GLP guidelines. LIDAKOL batch no. 7-143-6/28/89.

Study Design: Three New Zealand White rabbits (1 male and 2 females, ages 14 and 15 weeks, respectively) were administered a single dose (100 mg) of n-docosanol 10 % cream (formulation 1) in the conjunctival sac.

Summary of Study Results: n-Docosanol cream showed a primary irritation score of 0.25, reflecting a conjunctival redness grade of 1 (out of a maximum of 3) at the end of 1 hour observation. All other eye irritation scores were 0. There were no acute clinical symptoms, staining of the cornea or conjunctiva, or corrosion of the cornea at any time during the course of the study.

Study 33 - Primary Eye Irritation Study in with LIDAKOL™ Suspension in Rabbits. Study report no. 255622, *In life:* 10/24 to 10/27/89, conducted by in compliance with OECD GLP guidelines. LIDAKOL batch no. 7-143-6/28/89.

Study Design: This study was conducted as described for Study 255600 (#34 above), except that a 20 % n-docosanol suspension (100 mg) was instilled into the conjunctival sac of only 1 male rabbit (age 14 weeks).

Summary of Study Results: Findings were consistent with the preceding study, with a primary irritation score of 0.25 and no adverse reactions observed except for a conjunctival redness of grade 1 observed 1 hour post administration.

Study 34 - Screening for THE Eye Irritancy Potential Using the Bovine Eye / Chorioallanoic Membrane (BECAM) Assay with LIDAKOL Cream. Study report no. 170054. *In life:* 11/8 to 11/9/89, conducted by in compliance with OECD GLP guidelines. LIDAKOL batch no. 7-143-6/28/89.

This study was previously submitted under submission N001, dated 7/16/91, and reviewed by Dr. Lauren Black, HFD-530, review dated 8/26/91. Her review has been incorporated below:

The bovine eye assay is an in vitro assay to detect corneal damage resulting from the application of test compounds. Eyes were obtained from cows within 15 min of their slaughter; eyes with evidence of prior corneal damage were excluded from the assay. Eight eyes were placed in a plastic egg tray (above the water level) in a bath maintained at 37° degrees with a humid atmosphere. One negative control (saline

treated) and 2 positive control eyes (toluene and acetone) were used. Test article was applied for 30 seconds to the cornea of 5 eyes, followed by a saline rinse. 10 minutes later, damage was scored with reference to the following parameters: opacity (0-4), epithelial detachment (0, 2, 3, or 4), and epithelial integrity (0, 0.5, 1, or 1.5). Scores for the different types of damage were summed and average for the 5 treated eyes. An average score of 1.1 was seen for LIDAKOL, in line with other "slight irritants".

For the chorioallantoic membrane assay, the membrane was exposed in day 10 fertilized chicken eggs. Twelve eggs were used - 6, for test article; 2 eggs for negative control (saline), and 4 eggs for positive controls (sodium hydroxide and 1% SDS, 2 eggs each). A tenth of a gram of LIDAKOL cream was placed in contact with the surface of the membrane for 20 seconds, then rinsed. The capillary system and albumen were scored for hemorrhage, coagulation, and lysis. Scores were averaged and combined to yield a scale from 0-21. The positive control yielded scores of 11-19; the test article, 6.

The results of this assay showed LIDAKOL to be moderately irritating.

Study 35 - Acute Eye Irritation/Corrosion Study with LIDAKOL in the Rabbit. Study report no. [redacted] 107505. *In life:* 11/16 to 11/19/93, conducted by [redacted] in compliance with OECD GLP guidelines. LIDAKOL batch no. 153 (exp. date 6/1/94).

Study Design: The purpose of this study was to assess the possible irritation or corrosion potential when a single dose of LIDAKOL 10 % Cream was placed in the conjunctival sac of an albino rabbit eye (ages ~14 weeks). A single doses of 100 mg of n-docosanol 10 % cream was instilled into one eye each of 3 male rabbits. After 24 hours, both eyes were gently flushed with a solution of 2% fluorescein in water. The 2 % fluorescein allowed quantitative determination of corneal epithelial damage. Any bright green stained area, indicating epithelial damage, was estimated as a percentage of the total corneal area. The eyes of each animal were examined approximately 1, 24, 48 and 72 hours after instillation.

Summary of Study Results: Installation of LIDAKOL 10 % Cream resulted in slight irritation of the conjunctival tissues (conjunctival redness and chemosis, severity score = 1) at 1 hr in all three animals, which resolved within 48 hours. No signs of corneal epithelial damage or systemic toxicity were reported.

Study 36 - Contact Hypersensitivity to LIDAKOL Cream in Albino Guinea Pigs Maximization Test. Study report no. [redacted] 255611, *In life:* 12/12/89 to 1/5/90, conducted by [redacted] in compliance with OECD GLP guidelines.

Study Design: Himalayan spotted female guinea pigs (20 test and 10 control) received intradermal injections of Freund's complete adjuvant (CFA), n-docosanol 20 % (diluted to 5 % with ethanol), n-docosanol 20 % cream (diluted to 5 % with ethanol) with CFA, and vehicle at different sites. Intradermal injections (3 pairs/animal) were made at the border of a 4x6 cm area of shaved skin on each animal. Control groups were treated identically, with the omission of the test article, and substitution of ethanol. Six days after injections, 10 % SDS was applied to the shaved area to

enhance the appearance of any sensitization reactions. The next day, saturated filter patches of undiluted LIDAKOL were applied to the shaved area. An occlusive bandage was secured over the patch for 48 hours. Following this epidermal application, the area was washed and scored for erythema and edema immediately, 24, and 48 hours later. Two weeks following the epidermal application, an area on the right and left flanks of each pig was clipped, and filter patches saturated with LIDAKOL were applied to the right side, while ethanol soaked patches were secured to the left. Twenty-four hr later, the patches were removed, and the area washed and scored for reaction immediately, 24 and 48 hours later. Allergic reaction was scored "positive" if the challenge site was visibly reddened. In addition to skin reactions, mortality, body weights, and clinical symptoms were evaluated; no necropsy was performed. Formaldehyde (HCHO) was used as the positive control.

Summary of Study Results: No systemic symptoms were noted, nor were body weight gains affected by treatment with LIDAKOL. In this 25-day test, no differences attributable to test article were noted, with the exception of staining and fissures in the treated groups at the site of epidermal induction on day 11-15. No erythema was noted following challenge with LIDAKOL, indicating LIDAKOL is not sensitizing in this animal model.

Comment: Local symptoms at the injection sites were rather severe in both drug-treated and control groups. These included erythema and edema, days 2-6; necrosis, days 7-11; desiccation from days 11-16; and exfoliation, day 17-25.

Study 37 - Assessment of Contact Hypersensitivity to LIDAKOL in the Albino Guinea Pig (Maximization Test). Study report no. [redacted]107516, *In life:* 10/9/ to 12/4/93, conducted by [redacted] in compliance with OECD GLP guidelines.

Study Design: Guinea pigs were injected intradermally with a 25 % concentration of n-docosanol 10 % cream diluted in distilled water (20 animals) or physiological saline (10 animals). One week later, the animals were induced with an epidermal exposure to undiluted n-docosanol cream. Two weeks later, animals were challenged with n-docosanol 10% cream at concentrations of 25 %, 50 % and 100 % and with distilled water. Formaldehyde (0.05 to 0.2 %) was used as the positive control.

Summary of Study Results: 48 Hours after the occluded epidermal induction period, 8/20 animals presented with slight (1) to well defined (2) erythema, accompanied by slight edema in 3 of these animals. There were no positive reactions in any of the animals, at any dose in response to rechallenge. There were no signs of systemic toxicity observed in any of the animals and weight gain between control and treated animals was similar over the study period.

Study 38 - Phototoxicity Study of 10 % n-Docosanol Cream (LIDAKOL) in the Guinea Pig. Study report no. 95/LAK013/0038, *In life:* 12/7 to 12/14/94, conducted by [redacted] in compliance with GLP guidelines (OECD and 21 CFR 58). LIDAKOL and placebo batch nos. 153-93L and 152-93L, respectively.

Study Design: The potential of 10 % n-docosanol cream (LIDAKOL) to cause phototoxicity was investigated in Dunkin-Hartley guinea pigs (3/sex, 5-6 weeks of age). The backs of the animals (~6 cm^2) were clipped, depilated and stripped, and test material (0.5 ml) was applied to anterior and posterior sites (1.5 cm^2 each). Thirty minutes later, the posterior site was covered with [redacted] while the anterior site was irradiated with UVA light. Animals were evaluated at 1, 24, 48 hours and 7 days after treatment. Results were compared to concurrent control animals (3/sex/group) receiving either placebo cream or 0.01 % 8-methoxyposralen. The source of the irradiation was an array of [redacted] fluorescent tubes, monitored by a [redacted] (This system emitted primarily UVA radiation with small quantities of UVB.)

Summary of Study Results: There was no response for either the UVA-irradiated or the non-irradiated n-docosanol treated sites. There was slight to moderate erythema for both irradiated and non-irradiated placebo controls; exfoliation was apparent in 4/6 animals on day 8. Animals treated with [redacted] exhibited slight to moderate erythema and/or edema at 24 and 48 hours; by day 8, slight erythema, edema, eschar, exfoliation and a single case of scar tissue were apparent.

Study 39 - Photosensitivity Study of 10 % n-Docosanol (LIDAKOL) Cream in the Guinea Pig. Study report no. 95/LAK014/0260, *In life:* 1/16 to 2/23/95, conducted by [redacted] in compliance with GLP guidelines (OECD and 21 CFR 58). LIDAKOL batch no. 153-93L.

Study Design: [redacted]

Summary of Study Results: Test group animals challenged with 10 % n-Docosanol Cream showed no response at any of the non-irradiated or irradiated sites. After the fourth induction (day 8) there was a significant dermal reaction to TCSA which included exfoliation and a low incidence of eschar. Upon challenge with TCSA, 9/10 animals displayed slight erythema at the irradiated site. As expected, no reaction was observed at the TCSA non-irradiated sites. Slight to moderate erythema was observed in 5/10 of the negative control animals at the non-irradiated and/or irradiated challenge

sites treated with 10 % n-docosanol cream. The cause of this irritation is unknown, since all the test animals treated with 10 % n-docosanol cream throughout the study period were negative.

Study 40 - A 13-Week toxicity study by Dermal Application of n-Docosanol Cream (LIDAKOL®) to CD-1 Mice Including Toxicokinetic Assessments. Study Report no. 95/LAK018/0864, *In life:* 2/8 to 5/11/95, conducted by [redacted] with toxicokinetic sample analyses performed by [redacted] in compliance with OECD GLP guidelines. Test substance batch nos.: n-Docosanol 10 % Cream - GL017F-94L; Vehicle Control Cream - 318-09-95A.

Study Design: CD-1 mice (10/sex/toxicology group, 35-42 days of age) were treated topically with a cream containing 0.4, 2.0, and 10 % n-docosanol, vehicle cream, or water. The 0.4 and 2.0 % creams were made by diluting 10 % n-docosanol cream with water. Approximately 100 µl/day of cream or water was applied by syringe to the clipped back of the animals, estimated to be at least 10% of the total body surface area, and then spread evenly over the clipped area. Treatment sites were non-occluded. Before each administration, test sites were washed to remove any residual compound and examined for signs of irritancy. In addition, the following parameters were evaluated over the course of the study: mortality, clinical behavior (twice daily), bodyweight (weekly), food consumption (weekly), clinical hematology and chemistry (week 14), ophthalmoscopy (week 12), organ weights, and macroscopic and microscopic morphology. The following tissues were collected for histopathology (* included organ weight):

-adrenals *
-aorta
-brain *
-cecum
-colon
-duodenum
-epididymides
-esophagus
-eyes and optic nerves
-femoral bone including joint and marrow
-gall bladder
-harderian glands
-heart *
-ileum
-jejunum
-kidneys *
-liver *
-lungs w/mainstem bronchi *
-lymph nodes - axillary, mandibular and mesenteric
-mammary gland
-ovaries *
-pancreas
-pituitary *
-prostate *
-rectum
-salivary glands (submandibular)
-sciatic nerve (left)
-seminal vesicles
-skeletal muscle (thigh)
-skin - test site and untreated
-spinal cord
-spleen *
-sternum
-stomach
-testes *
-thymus *
-thyroid and parathyroid *
-tongue
-trachea
-urinary bladder
-uterus w/cervix *
-vagina

Satellite animals (16/sex/group) were dosed for toxicokinetic analysis on day 1. Blood samples were obtained from 2 animals/sex at 0, 0.5, 1, 2, 4, 6, 8, and 24 hours post-dosing. Each animals was sampled once and killed without recovery from anesthesia without necropsy. Following the final treatment in week 13, samples were collected from 8 animals/sex/treatment group as follows: each animal was sampled twice, once at 0 and 4 hours, 0.5 and 6 hours, 1 and 8 hours, or 2 and 24 hours post-dosing then sacrificed without necropsy.

Summary of Study Results: There were no deaths or clinical signs of toxicity which appeared to be dose-related. Bodyweight gain, food consumption, food conversion efficiency, organ weights, hematology, blood chemistry and the composition of the urine and bone marrow were unaffected by treatment. There were no ophthalmic, macroscopic or microscopic findings which were attributed to treatment with n-docosanol. 10 % n-Docosanol Cream was considered the NOEL in mice treated daily for 13 weeks on approximately 10 % of their total body surface area.

The toxicokinetic data verified systemic exposures, however, because the sites were non-occluded it is not possible to determine how much material was absorbed through the skin or through the gut following oral ingestion. Maximum mean plasma concentrations (Cmax) of n-docosanol ranged from 15 ng/ml following exposure to the 0.4 % cream to 250 ng/ml in animals dosed with the 10 % cream (Table TK-3). Tmax occurred between 1.0 to 4.0 hours following topical administration. The terminal rate constants and the terminal half-lives could not be determined from the data collected in this study.

Table TK-3: Selected toxicokinetic data following a single dose (day 1) and daily dosing for 13 weeks (~ day 91) of n-docosanol in CD-1 mice.

	Day 1			Day 91		
C_{max} (ng/ml)	0.4%	2.0%	10%	0.4%	2.0%	10%
Males	20	27	225	-	-	205
Females	15	57	250	-	-	232

AUC_{0-24} (ng.h/ml)	0.4%	2.0%	10%	0.4%	2.0%	10%
Males	-	-	739	-	-	1390
Females	-	402	1140	-	-	1660

Concentrations of n-Docosanol below the limit of quantification (BLQ = <10 ng/ml) were entered as zero for calculation of means. If 50% of individual values (i.e. one of two values at each time point) were BLQ, the means were not calculated.

Study 41 - Subacute 28-Day Repeated-Dose Dermal Toxicology Study on Abraded Skin in Rabbits. Study report no. [redacted] 270382, *In life:* 7/25 to 9/10/90, conducted by [redacted] and [redacted] in compliance with GLP guidelines (OECD and 21 CFR 58). LIDAKOL batch nos. 211-112 and 214-11; and Placebo batch no. 214-12.

A draft report of this study was previously submitted under [redacted] submission N001, dated 7/16/91, and reviewed by Dr. Lauren Black, HFD-530, review dated 8/26/91. Her comments have been incorporated into this review.

Study Design: LIDAKOL 10 % Cream was applied to the abraded skin of New Zealand White rabbits for 28 consecutive days, followed by a 14-day recovery period. Animals, aged 12-15 weeks and weighing between 2.0 to 2.8 kg, were dosed with 0 (placebo cream, 10/sex), 50 mg (5/sex), 200 mg (5/sex), or 1000 mg/kg (10/sex) cream. Application sites were abraded at the beginning of the study and once weekly thereafter using a [redacted] which makes minor incisions through the stratum corneum but not into the dermis or at a depth which results in bleeding. The cream was applied in a uniform film over a shaved, abraded area of 2.5 cm^2 and covered with a semi-occlusive bandage for 6 hours/day. After 6 hours, the skin was washed gently with warm water and dried. Half the rabbits in each of the control and high dose groups (5/sex/group) were sacrificed on day 28; the remaining animals were observed for an additional 14-day recovery period prior to sacrifice. The following measures and evaluations were made: mortality, clinical signs, irritation of

the application site, food consumption, body weights, opthalmic exams, hematology, clinical chemistry (fasted at 4 & 6 weeks), organ weights, macroscopic necropsy observations, and histopathology. Organ weights were recorded for the brain, heart, liver, kidneys, adrenals, spleen and gonads. The following tissue samples were collected from all animals and fixed in 4% buffered neutral formaldehyde:

-adrenals
-aorta
-brain
-cecum
-colon
-duodenum
-epididymides
-esophagus
-eyes & optic nerves
-female mammary gland
-femur with joint
-harderian glands
-heart
-ileum
-jejunum
-kidneys
-larynx
-lachrymal gland
-liver
-lungs infused w/formalin
-lymph nodes (mandibular & mesenteric)
-nasopharynx
-ovaries
-pancreas
-pituitary
-prostate
-rectum
-salivary glands (mandibular & sublingual)
-sciatic nerve (left)
-seminal vesicles
-skeletal muscle
-skin (treated & untreated)
-spinal cord (cervical, midthoracic & lumbar)
-spleen
-sternum w/marrow
-stomach
-testes
-thymus
-thyroid & parathyroid
-tongue
-trachea
-urinary bladder
-uterus w/cervix
-vagina

Histopathology evaluations were only performed on the following tissues: adrenals, heart, kidneys, and liver from all control and high-dose animals terminated at both 4 and 6 weeks; treated and untreated skin from all animals; and gross lesions from all animals.

Review Comment: *Histopathology was performed on fewer than the recommended number of tissues and should have at least include gonads, brain, lung, and spleen. In addition, although gross lesions were examined histologically, the same tissues were not examined in other animals in either the same dosing group or in other groups to confirm that these lesions were incidental and were not microscopically present in other animals at lower or higher doses.*

Summary of Study Results: There were no mortalities, significant changes in food consumption or weight gain patterns, changes in opthalmic parameters, or clinical signs of toxicity in any of the groups during the treatment period. There was a higher incidence in local irritation effects in the controls and high-dose animals (slight erythema in ~50 % of the animals). Controls presented with minimal to slight general and focal erythema in up to 85 % of the males and <50 % of the females. About 50% of the animals also presented with edema. Both erythema and edema had subsided completely by the end of the 2-week recovery period, however, ~15-25 % of the control animals developed scaling during this time. There were no dermal effects noted in males dosed at 50 mg/kg and only slight erythema in <25 % of females during week 1. Males and females dosed with 200 mg/kg developed slight, transient erythema during weeks 1 and 3, respectively, of treatment. At 1000 mg/kg, slight erythema was evident in males primarily during week 1 (~50 %) of treatment and week 1 of recovery (<15 %); in females, minimal to slight erythema and edema were observed in <25 % of females beginning in week 2 and persisting through week 1 of the recovery period. Scaling was also noted in females during the recovery period, but was resolved by the end of the 2nd week.

Organ weights, either absolute or relative (brain and body weight ratios) were similar in all groups, with one exception, a decrease in liver weights at day 28 in all drug treated males. This effect appeared to be dose related, and resulted in a 13 % reduction in liver weight in the high-dose group relative to control. There was also one male in the high dose group which had a significant increase in AST and ALT, resulting in an increased group mean when compared to the control group. These

increases persisted through the recovery period, however, the significance of these increases in this animal is unknown. There were no changes in any of the hematologic parameters measured. One high-dose female showed a dark red discoloration under the application site, but this response was atypical of any other animal of this treatment group and did not correlated with any histopathological finding. At the end of the recovery period, females in the high-dose group had a slight drop in cholesterol and phosholipid concentration in blood. This drop was not statistically significant and is of questionable clinical relevance. There were no apparent drug-related histopathological changes or other signs of toxicologic effects from topical LIDAKOL application evident in this study following 4 weeks of treatment or the 2 week post-treatment recovery period.

Study 42 - Subacute 28-Day Dermal Tolerance Study with n-Docosanol (LIDAKOL) by Daily 6 Hours Administrations to the Intact and Abraded Skin of Rabbits. Study report no. [redacted] 107527. *In life*: 11/9 to 12/10/93, conducted by (b) (4) [redacted] with toxicokinetic sample analyses performed by [redacted] in compliance with OECD GLP guidelines. LIDAKOL Lot no. 153 (formulation 3, exp. date 6/1/94); placebo lot no. 152 (exp. date 6/1/94).

Study Design: A 5 day pilot study was performed between 10/25 and 10/30/93 with New Zealand White Rabbits (2/sex/group) to provide a basis for the selection of a treatment level for the 28 day topical study. As a result, a dose level of 1 g/kg/day was selected as the maximum amount of cream that could be applied to the skin of rabbits. 100 mg n-Docosanol (LIDAKOL 10 % Cream) was selected by the sponsor as the required active concentration.

Either test or placebo cream was applied to a shaved area of intact or abraded skin (3 rabbits/sex/group, age ~12 weeks, 2.0-3.0 kg) comprising approximately 10 % of the total body surface area (~120-140 cm^2). On day 1, the skin was abraded by making parallel caudalward scratches over the length of the exposure area with a sharp needle. This procedure was repeated during the study as soon as the scratches or lesions related to the scratches had visually disappeared. Test material was applied to a Metalline patch and left in contact with the skin 6 hours. After removal of the patch, residual test material was removed using tap-water and dry tissues. Animals were monitored for daily mortality, clinical signs of toxicity, and skin irritation; weekly for body weight; and clinical laboratory evaluations, necropsy and histology were performed at the end of the study. Blood samples for toxicokinetic evaluation were collected prior to treatment on study days 1, 7 and 28.

Organ weights were recorded for the adrenal glands, heart, kidneys, liver, spleen and testes. The following tissue samples were collected from all animals and fixed in 4 % buffered neutral formaldehyde:

-adrenals
-aorta
-brain
-cecum
-cervix
-clitoral gland
-colon
-duodenum
-epididymides
-esophagus
-eyes & optic nerves
-female mammary gland
-femur with joint
-gall bladder
-harderian glands
-heart
-ileum
-jejunum
-kidneys
-larynx
-lachrymal gland (exorbital)
-liver
-lung
-lymph nodes (mandibular & mesenteric)
-nasopharynx
-ovaries
-pancreas
-pituitary
-preputial gland
-prostate
-rectum
-salivary glands (mandibular & sublingual)
-sciatic nerve
-seminal vesicles
-skeletal muscle
-skin (treated & untreated)
-spinal cord (cervical,

midthoracic & lumbar)	-stomach	-thyroid & parathyroid	-urinary bladder
-spleen	-testes	-tongue	-uterus w/cervix
-sternum w/bone marrow	-thymus	-trachea	-vagina

Histopathology evaluations were only performed on the following tissues: adrenals, heart, kidneys, liver, spleen , treated and untreated skin area, testes and all gross lesions from all animals.

Summary of Study Results: There were no mortalities, observed clinical signs of toxicity (other than dermal irritation) or significant changes in weight gain during the study period. All animals, with the exception of 1 male and 1 female treated with LIDAKOL, showed minimal to slight erythema which was localized to the treated skin area. This erythema was accompanied by incidental edema and scabbing only in abraded areas. In general, dermal irritation was slightly more pronounced in placebo vs n-docosanol treated animals; and in abraded vs unabraded skin. Although concurrent untreated controls were not evaluated in this study, clinical laboratory results were compared to historical "normal" control data for New Zealand White rabbits of the same age. There were no significant differences in any hematologic (including PT and PTT) or serum chemistry parameter evaluated when compared between either placebo vs n-docosanol animals or treated animals vs historical controls. Lesions observed during necropsy, variations in organ weights, and histology findings were not considered to be treatment related. The small number of changes recorded in treated animals were within the range commonly seen for rabbits of this age and strain.

Toxicokinetics Evaluation: Plasma concentrations of n-docosanol ranged between <10 ng/ml (limit of detection) to 40.1 ng/ml.

Review Comments: *Unfortunately, measurable levels of n-docosanol were found in some of the animals from all dosing groups, including placebo, on each of the days tested. It must therefore be concluded that either 1) no true placebo was used in this study; 2) the animal area was contaminated with n-docosanol cream and all animals were exposed through ingestion; or 3) the method used for quanitation of n-docosanol in plasma is flawed. Samples from the above lots should be reanalyzed in order to validate the dermal portion of this study. All toxicokinetic results should be considered invalid and the results from this study suspect.*

Study 43 - A Penile Irritation Study in Rabbits with n-Docosanol 10 % Cream and n-Docosanol 12 % Cream. Study report no. SLS 3333.4, *In life:* 3/23 to 4/1/95, conducted by [redacted] in compliance with GLP guidelines (21 CFR 58). Batch nos. - 10% n-Docosanol Cream lot 223 (exp. date 11/7/96); 12% n-Docosanol Cream lot LP-149A (exp. date 3/3/96); 0.9% Sodium Chloride for Injection, USP lot 51259 (exp. date 10/96); and Gynol II with Nonoxynol-9 lot 24M710 (exp. date 10/97).

Study Design: This study was performed to assess the potential irritant and corrosive effects of n-Docosanol 10 % Cream and n-Docosanol 12 % Cream in the penile tissue of New Zealand White rabbits (age 7 months, 3.2-3.7 kg) when administered daily for 10 consecutive days. Four groups of male rabbits (10/group) were treated with ether 0.9 % NaCl solution, n-docosanol 10 % cream, n-docosanol 12 % cream, or a 1:1 (v/v) mixture of Gynol II with Nonoxynol-9 and n-docosanol 12 % cream. Using a 1 ml syringe, each rabbit was dosed once daily with 0.2 ml of the appropriate test or control article applied to the penis and surrounding prepuce. Collars were placed on each animal following the first and remained in place throughout the study period. Animals were examined daily

for signs of clinical toxicity and scored for penile irritation according to Draize; body weights were recorded upon receipt of the animals and on days -1 and 10 of the study; and histopathology was performed on the penis and surrounding prepuce.

Summary of Study Results: There were no significant clinical abnormalities during the study or changes in body weight over the course of the study. Very slight erythema (grade 1) was observed more frequently in the animals treated with n-Docosanol 12 % Cream. Microscopic examination of the penile mucosa and penile urethra revealed no evidence of drug related irritation. Occasional lympocytic and/or polymorphonuclear submucosal or intra-epithelial infiltrates and/or focal areas of recent submucosal hemorrhage were observed, they occurred with approximately equal frequency in control rabbits as in treated animals and were judged to have been unassociated with treatment.

Study 44 - A Vaginal Irritation Study in Rabbits with n-Docosanol 10 % Cream and n-Docosanol 12 % Cream. Study report no. [redacted]3333.3. *In life:* 3/23 to 3/31/95, conducted by [redacted] in compliance with GLP guidelines (21 CFR 58). Batch nos. - 10% n-Docosanol Cream lot 223 (exp. date 11/7/96); 12% n-Docosanol Cream lot LP-149A (exp. date 3/3/96); 0.9% Sodium Chloride for Injection, USP lot 51259 (exp. date 10/96); and Gynol II with Nonoxynol-9 lot 24M710 (exp. date 10/97).

Study Design: This study was performed to assess the potential irritant and/or corrosive effects of n-Docosanol 10 % Cream and n-Docosanol 12 % Cream in the vaginal tissue of virgin New Zealand White rabbits (age 4 months, 2.5-3.1 kg) when administered daily for 10 consecutive days. Five groups of female rabbits (10/group) were treated with ether 0.9 % NaCl solution, Gynol II with Nonoxynol-9, n-Docosanol 10 % Cream, n-Docosanol 1% Cream, or a 1:1 (v/v) mixture of Gynol II with Nonoxynol-9 and n-Docosanol 12 % Cream. Using a 1 ml syringe, each rabbit was dosed once daily with 1 ml of the appropriate test expressed directly into the vaginal vault. Animals were examined twice daily for signs of clinical toxicity; body weights were recorded upon receipt of the animals and on days -1 and 10 of the study; and the uterus, ovaries, vagina and cervix were removed and examined macroscopically. Organ weights were recorded for the uterus and ovaries and the vagina and cervix were opened longitudinally and evaluated for irritation using Draize scoring. Histopathology was performed on the 3 approximate 1 cm x 0.2 cm sections of the fixed vaginal tissue (one section each from the cranial, medial and caudal areas) and any other gross lesions noted at necropsy.

Summary of Study Results: All females survived to scheduled euthanasia. There were no clinical signs of toxicity in any of the groups or differences in weight gain between groups. Uterine and ovarian organ weights were similar between groups. Macroscopic evaluation of the vaginal tissue for irritation resulted in scores ranging from very slight (grade 1) to moderate/severe (grade 3) erythema and edema. These responses generally occurred in all groups but there was an apparent dose related increase in overall scores in the animals treated with n-Docosanol Cream. Histopathological evaluation of the vaginal tissue resulted in composite irritation scores of 1.2 for the 0.9 % NaCl solution; 2.3 and 2.7 for the n-Docosanol 10 % and 12 % Creams, respectively; and 6.1 and 6.0 for the Gynol II with Nonoxynol-9 and the 1:1 mixture of Gynol II with Nonoxynol-9 and n-Docosanol 12 % Cream, respectively. Scores between 1 and 8 are deemed acceptable levels for vaginal irritation (1-4 minimal, 5-8 mild, 9-11 moderate, and 12-16 marked irritation).

Study 45 - Rabbit Vaginal Toxicology Study (28-Day) with Gas Chromatographic Analysis of Plasma From LIDAKOL Treated Rabbits. Study report no. [redacted]427-LK-001-91. *In life:* 9/12 to 10/8/91, conducted by [redacted] in compliance with GLP guidelines (21 CFR 58). LIDAKOL 20% Cream - Lot no. 254L-39.

Study Design: This study was designed to evaluate the toxicity of n-Docosanol 20% Cream when administered to the vaginal mucous membranes in New Zealand White female rabbits. n-Docosanol 20% Cream was applied twice daily to both vulvar lips of 5 female rabbits at a dose of 0.25 ml/lip (1.0 ml/day/animal) for 28 days. Deionized water, at the same dose volume, was applied to 2 control animals. Rabbits were weighed on the first day of dosing and at termination. During the treatments, signs of irritation and discharge were evaluated prior to each dose. Clinical observations were recorded daily and blood samples were collected on days 1, 14 and 28 at 1, 4 and 8 hours after the morning dose for toxicokinetic analysis. Approximately 24 hours after the last dose, the rabbits were sacrificed and a gross necropsy was performed. Treated and untreated sites were examined histologically and scored for irritation.

Summary of Study Results: There was one death (1/5) on day 16 in the treated animals. This animal evinced decreased activity and reduced food consumption and fecal output on day 15, and at necropsy presented with distended and fluid filled intestines, ascites and distended cecum. These observations were not believed to be drug related. There were no other clinical signs of toxicity observed in any of the other control or treated animals during the study period. Macroscopic evaluation of treatment sites did not revealed noticeable signs of irritation or discharge in control or treated animals. Histopathological evaluation included examination of the epithelium, leukocyes, congestion and edema of the distal vagina. Minimal irritation of distal vagina was observed in both control animals and in 3/4 n-docosanol treated animals; mild irritation was observed in the 4th animal. The mean irritation scores for control and treated animals were 2.5 and 4.0, respectively.

Analytical Report GC Analysis of 1-Docosanol in Rabbit Plasma: Systemic exposure following topical application to the vulvar lips of female rabbits was minimal. Rabbit plasma samples were analyzed by [redacted] Levels of n-docosanol in the plasma were generally below the limit of quanitation (10 ng/ml). Each dosed animal had at least one sample with n-docosanol levels >10 ng/ml. Readings ranged between 11.0 to 13.4 ng/ml at 4 hrs on day 1; 10.7 to 20.9 ng/ml at 8 and 4 hrs, respectively, on day 14; and a single reading of 12.8 ng/ml at 4 hrs on day 28. The plasma concentrations which did register above the level of detection represent a combination of absorbed material from the vaginal tissues as well as from the gut following oral ingestion during grooming.

Reproductive and Developmental Toxicology Study Reviews

Study 46 - An Oral Dose Range-finding Fertility and Pre- and Post-natal Development Study of n-Docosanol Suspension in Rats. [redacted] study report no. 94/LAK003/0898. *In life:* 5/31 to 7/25/94, conducted at [redacted] in compliance with GLP guidances OEDC and 21 CFR 58. n-Docosanol batch no. 6232.

Study Design: Stock n-docosanol solutions were prepared as a 20 % aqueous suspension in [redacted] batches as needed. Adult virgin male and female Sprague Dawley CD rats (6/sex/group, ages 10-11 weeks, weighing 315-370 g and 216-250 g, respectively) were treated with by oral gavage with 500, 1000 and 2000 mg/kg/day n-docosanol or with vehicle beginning 15 days prior to pairing and throughout pairing. Males continued treatment until termination and females were treated throughout gestation and lactation until termination on day 4 of lactation. During treatment, all animals were monitored for clinical signs of toxicity, bodyweight, food and water consumption and female estrous cycling. Females were permitted to deliver their young naturally and rear their own offspring until day 4 of lactation. Parturition and duration of gestation were recorded. Terminal litter observations included number of offspring, fetal bodyweight, sex ratio and health of offspring. Males were terminated after successful parturition by the females, while the females and offspring were euthanized on day 4 of lactation. All animals were examined externally and internally for macroscopic abnormalities.

Review Note: *An error was made in preparing the 20% solution (w/v was used rather than w/w) which resulted in a 7% lower dose than called for in the protocol. This error was consistent and resulted in proportionately smaller doses for all dosing levels, e.g. doses of 465, 930 and 1860 mg/kg/day instead of 500, 1000 and 2000 mg/kg/day, respectively.*

Summary of Study Results: The general condition of all animals appeared to be unaffected by treatment. One female dosed at 1000 mg/kg/day was found dead after four days of treatment. Necropsy findings suggested that the death may have been associated with the dosing procedure and was unrelated to n-docosanol toxicity. There was a slight, nonsignificant, dose-related decrease in overall bodyweight gain in treated males when compared to controls (15 % at 1860 mg/kg/day). There were no significant difference in estrous cycles, mating performance and fertility, gestation length, parturition and fertility in treated animals. Two pairings in the 1930 mg/kg/day group failed to achieve pregnancy, however, in the absence of any effects in the 1860 mg/kg/day group, these results were not considered a result of n-docosanol treatment. The general condition of offspring, litter size, survival, and sex ratio were similar in all groups. Absolute body weights of offspring from animals treated at 1860 mg/kg were slightly lower than concurrent controls, however, body weight gains to day 4 were unaffected. There were no gross treatment-related findings noted at necropsy of both the F0 and F1 generations.

Based on the study results, 2000 mg/kg/day was considered suitable as the high dose for the main pre- and post-natal study in rats.

Study 47 - An Oral Dose Range-finding Embryo-Fetal Development Study of n-Docosanol Suspension in Rats. [redacted] study report no. 94/LAK004/0675. *In life:* 5/23 to 6/15/94, conducted at [redacted] in compliance with GLP guidances OEDC and 21 CFR 58. n-Docosanol batch no. 6232.

Study Design: Stock n-docosanol solutions were prepared twice weekly as a 20 % aqueous suspension in [redacted]. Presumed pregnant Sprague Dawley CD rats (6/group, ages 10-11 weeks, weighing 216-251 g) were dosed by oral gavage from day 6 to day 15 inclusive of gestation with either vehicle or n-docosanol suspension of 500, 1000 or 2000 mg/kg/day. During treatment, animals were monitored for clinical signs of toxicity, maternal bodyweight, food and water consumption. All females were euthanized on day 20 of gestation and examined macroscopically for any signs of toxicity. Terminal evaluation for embryo-fetal development included quantification of the numbers of corpora lutea in each ovary, implantation sites, resorption sites, fetuses in each uterine horn; weight and sex of individual fetuses; individual placental weights; and examination of all fetuses for external abnormalities and approximately 50 % of fetuses for gross internal abnormalities.

Review Note: *An error was made in preparing the 20% solution (w/v was used rather than w/w) which resulted in a 7% lower dose than called for in the protocol. This error was consistent and resulted in proportionately smaller doses for all dosing levels, e.g. doses of 465, 930 and 1860 mg/kg/day instead of 500, 1000 and 2000 mg/kg/day, respectively.*

Summary of Study Results: The general condition, weight gain, food and water consumption and necropsy results of all treated animals were similar to the controls and showed no adverse effects of treatment. One control female was *killed in extremis* on day 16 of gestation, exhibiting hunched posture and piloeretion in association with bodyweight loss of 35 g over 48 hours. Necropsy revealed 14 late resorptions *in utero* and the vagina contained red mucoid material.

Litter responses, as assessed by numbers of corpora lutea, implantations and viable fetuses, resorption sites, and placental weights were similar in all groups and showed no adverse effects of maternal treatment with n-docosanol. The mean fetal weights from dams treated with 2000 mg/kg/day were slightly lower than the concurrent controls but were not statistically significant and fell within the historical control ranges. However, the mean number of viable young (15.3) from this group was greater than the other groups (control = 13.8), possibly accounting for the slightly lower mean fetal weight. Bilateral increased renal pelvic cavitation and hyroureter was observed in approximately 18% of the fetuses, affecting 3/6 litters, from dams in the 500 mg/kg/day group. However, since this effect was not observed in fetuses from either of the two higher dose groups it was considered unlikely to be related to maternal treatment with n-docosanol.

Based on the results of this study, a high dosage of 2000 mg/kg/day was considered appropriate for the main combined fertility and teratology study in rats.

Study 48 - A Combined Fertility, General Reproductive Performance, and Embryo-Fetal Development Study of Orally Administered n-Docosanol Suspension in Rats. [redacted] study report no. 95/LAK009/0615. *In life:* 12/13/94 to 4/5/95, conducted at [redacted] in compliance with GLP guidelines OEDC and 21 CFR 58 and in accordance with the ICH guidelines. Fetal pathology was performed by [redacted] n-Docosanol batch no. 52201.

Study Design: The influence of n-docosanol on reproductive function, fertility and embryo-fetal development was assessed in groups of sexually mature male and female Sprague Dawley CD rats. Groups of 22 rats/sex/group (males: ages 10-11 weeks, weighing 208-262 g & females ages 6-7 weeks, weighing 193-240 g) were administered 0, 10, 100, or 1000 mg/kg/day n-docosanol in [redacted] aqueous suspensions. The males were dosed for 71 days before pairing, throughout pairing, and until termination following necropsy of the females. The females were dosed daily from 15 days prior to pairing through day 17 after mating. Females were euthanized on day 20 after mating for examination of their ovaries and uterine contents. Necropsies in both sexes involved macroscopic inspections.

Summary of Study Results:

Adult Animals: One male, dosed at 1000 mg/kg/day, was euthanized for humane reasons during week 6 of treatment after exhibiting abdominal distention, pallor, ptosis, irregular respiration and weight loss. Necropsy findings included watery blood, enlargement of the liver with the lobular pattern accentuated, an enlarged pale spleen and reduced gastro-intestinal tract content. Since this was the only death in the study and no toxicity was observed in other animals, investigators did not considered it to be drug-related. There were no other significant differences between treated animals and controls or between treatment groups in clinical behavior and appearance; bodyweight gain, food and water consumption; female estrous cycling; sperm morphology, motility and numbers; mating behavior and fertility; and gross appearance of reproductive organs (and relative weights) and internal tissues at necropsy.

Litter Parameters: Litter parameters (number of corpora lutea in each ovary, number of implantation sites, number of early and late resorption sites, and the number, sex ratio and distribution of fetuses in each uterine horn), fetal survival, and fetal growth were all comparable between treatment groups, concurrent controls, and/or historical control ranges. With the exception of 3 fetuses, morphological development (including visceral and skeletal) in fetuses appeared normal. The 3 fetuses had notable findings: one fetus from the 100 mg/kg dosing group (dam 154) had small eye orbits consistent with agenesis of the eyes; one fetus from the 1000 mg/kg dosing group (dam 175) had a retro-esophageal right subclavian artery with an associated misshapen thymus gland; and another fetus from the 1000 mg/kg dosing group (dam 176) had cleft and imcomplete basisphenoid and basioccipital bones. These incidences were isolated, did not appear to be dose-related, and/or were within the incidence rates reported for the historical controls. Under the condition of this study, n-docosanol was not considered to adversely affect reproduction or to be teratogenic.

Review Note: Quality control of dosing solutions revealed that the dose solutions for the 10 and 100 mg groups at weeks 5 and 9 were lower than expected. However, all high dose solutions (1000 mg) were within study limits. Therefore the importance of this deviation in protocol is considered inconsequential to the study results.

Study 49 - A Pre- and Post-natal Development Study of Orally Administered n-Docosanol Suspension in Rats. Study report no. 95/LAK011/0841. *In life:* 12/20/94 to 4/24/95, conducted at [redacted] in compliance with GLP guidelines OEDC and 21 CFR 58 and in accordance with the ICH guidelines. n-Docosanol batch no. 52201.

Study Design: The objective of this study was to assess the effects of repeated oral administration of n-docosanol on pre- and post-natal development in the Sprague Dawley rat. Four groups (22 females/group) of presumed pregnant F0 female rats (ages 10-11 weeks, weighing 243-283 g) were dosed with 0, 10, 100 or 1000 mg/kg/day n-docosanol [redacted] from day 7 after mating to day 20 of lactation. All females were allowed to give birth naturally and rear their offspring to weaning at day 21 of lactation. F0 females were monitored pre- and post-natally for clinical signs of toxicity, bodyweight gain, food and water consumption, and partuition and length of gestation. Litters were monitored number of live and stillborn births, body weight, and sex ratios. Development of culled (8 pups/dam) F1 offspring was evaluated by monitoring clinical signs and behavior, mortality, physical development (pinna unfolding, hair growth, tooth eruption, eye opening, vaginal opening, balanopreputial separation), auditory and visual function, activity, learning ability, and neuromuscular function (traversing flat and round rods, rotarod treadmill, mid-air righting reflex; fore- and hind-limb wire-hanging, and grid-gripping ability). At approximately 5 weeks of age, 20 males and 20 female F1 rats from each group were evaluated for physical and sexual maturation and reproductive performance. F1 females were killed on day 14 after mating for examination of ovaries and uterine contents. Gross necropsies were performed on all F0 females, F1 offspring, and F2 fetuses.

Summary of Study Results:

F0 Females: One control animal was euthanized on day 4 of lactation following the death of her offspring. Macroscopic examination revealed that the mammary tissue was pale and inactive. Another female in the 100 mg/kg dose group was found dead on day 5 of lactation having shown no clinical signs of illness. Necropsy revealed cyanosis of the extremities, red/yellow fluid and pale amorphous tissue in the thoracic cavity, pulmonary congestion and reduced, dehydrated cecal contents. The cause of death was considered unrelated to treatment. There were no changes in body weight, food and water consumption, gestation length, parturition, or lactation in n-docosanol treated animals.

F1 Offspring: The general condition of offspring was similar in all groups with no apparent adverse effects of maternal treatment with n-docosanol. Litter sizes, sex ratios, birth weights and survival rates were similar to controls. There were no dose-related differences in weight gain, physical development, auditory and visual responses, learning ability, or neuromuscular function. A small number of males and females in the treated groups failed to mate or failed to achieve pregnancy. However, the mating rate, conception rate and fertility index values were within, or close to, the historical control ranges.

Necropsies of animals which died prior to termination, F1 animals terminated at the time of weaning and mated F1 males and females terminated on day 14 of gestation, revealed no adverse effects which were linked to n-docosanol treatment. The numbers of corpora lutea, implantations, viable young and resorptions for F1 females were comparable to both concurrent controls and historical values.

Review Note: *Quality control of dosing solutions revealed that the dose solutions for the 10 and 100 mg groups at weeks 5 and 9 were lower than expected. However, all high dose solutions (1000 mg) were within study limits. Therefore the importance of this deviation in protocol is considered inconsequential to the study results.*

[redacted]

Study 51 - An Oral Dose Range-finding Study of n-Docosanol Suspension in Rabbits. Study report no. 94/LAK005/0588. *In life:* 5/16 to 6/14/94, conducted at [redacted] in compliance with GLP guidelines OEDC and 21 CFR 58. n-docosanol batch no. 6232.

Study Design: Rising doses of n-docosanol suspension were administered by oral gavage to 2 non-pregnant New Zealand White rabbits (group 1, ages 18-26 weeks, weighing 4.11 to 4.38 kg) over 8 days, commencing at an initial dose of 250 mg/kg/day until a maximum dose of 2000 mg/kg/day was achieved (2 day increments of 250→500→1000→2000). Two females (group 2) were then inseminated and received n-docosanol at a dose of 2000 mg/kg/day for 7 consecutive days from day 6 to day 12 of gestation. Animals were monitored for clinical signs of toxicity and body weight changes, and ~24 hours following completion of treatment all animals were euthanized and examined macroscopically for adverse reactions to treatment.

Review Note: *An error was made in preparing the 20% solution (w/v was used rather than w/w) which resulted in a 7% lower dose than called for in the protocol. This error was consistent and resulted in proportionately smaller doses for all dosing levels, e.g. doses of 232.5, 465, 930 and 1860 mg/kg/day, respectively.*

Summary of Study Results: The body weight gains and general condition of pregnant and nonpregnant female rabbits were unaffected by treatment with n-docosanol. No macroscopic changes were seen at necropsy. Implantations appeared normal by gross observation. It was concluded that 2000 mg/kg/day was appropriate for use in the dose range-finding embryo-fetal study in rabbits.

Study 52 -A Dose Range-finding Embryo-Fetal Development Study of Orally Administered n-Docosanol Suspension in Rabbits. Study report no. 94/LAK007/1115. *In life:* 7/5 to 7/28/94, conducted at [redacted] in compliance with GLP guidelines OEDC and 21 CFR 58. n-Docosanol batch no.6232.

Study Design: The objective of this preliminary investigation was to examine the effects of repeated oral administration of n-docosanol during the organogenesis phase of gestation and on the progress and outcome of pregnancy in New Zealand White rabbits; and to establish suitable dosages for use in the main embryo-fetal development study (LAK010). Rabbits (4/group, ages 19-27 weeks, weighing 3.49-4.83 kg) were treated by oral gavage from gestation day 6 to 29, inclusively, at n-docosanol dose levels of 0, 500, 1000 or 2000 mg/kg/day. Animals were monitored for clinical signs of toxicity, bodyweight gain, and food and water consumption. The animals were euthanized on day 29 of gestation and gross necropsies performed. Ovaries and uterine contents were examined for the number of corpora lutea, number of implantation sites, number of resorption sites, number and distribution of fetuses, and fetal and placental weights.

Review Note: *An error was made in preparing the 20% solution (w/v was used rather than w/w) which resulted in a 7% lower dose than called for in the protocol. This error was consistent and resulted in proportionately smaller doses for all dosing levels, e.g. doses of 465, 930 and 1860 mg/kg/day, respectively.*

Summary of Study Results: One control female died as a result of the dosing procedure. Other than pale feces observed in animals receiving 1860 mg/kg/day (probably related to unabsorbed drug), the general condition of the dams and offspring were unaffected by treatment with n-docosanol. The group mean numbers of corpora lutea, implantations and viable young in high dose females (2000 mg/kg/day) appeared low in comparison with the controls. This was considered to be due to one animal which had unilateral implantation. Exclusion of this animal from the group means gave values similar to those of the control.

It was concluded that 2000 mg/kg/day would be a suitable dose as the highest dosage for use in a main teratology study in the rabbit.

Study 53 - An Embryo-Fetal Development Study of Orally Administered n-Docosanol Suspension in Rabbits. Study report no. 95/LAK010/0760. *In life:* 5/9 to 6/14/94, conducted at [redacted] in compliance with GLP guidelines OEDC and 21 CFR 58. n-Docosanol batch no. 52201.

Study Design: The effects of n-docosanol on the progress and outcome of pregnancy were assessed in sexually mature New Zealand White female rabbits (22/group, ages 19-27 weeks, weighing 3.29-4.98 kg). Rabbits were treated daily by oral gavage with aqueous solutions of [redacted] containing 0, 125, 500 or 2000 mg/kg n-docosanol from day 6 through day 29 of gestation. Animals were monitored for clinical signs of toxicity, bodyweight gain, and food and water consumption; and gross necropsies were performed after termination on day 29 of gestation. Ovaries and uterine contents were examined for the number of corpora lutea, number of implantation sites, number of resorption sites, number and distribution of fetuses, and fetal and placental weights.

Summary of Study Results: One control female delivered prematurely on day 29 of gestation after exhibiting marked weight loss and low food and water intake at the end of the gestation period. Seven live and ten dead fetuses were found; at least two of the dead fetuses were considered to have been alive at birth. There were no other effects on the overall condition of the other animals, with the exception of pale feces observed in the majority of females receiving 2000 mg/kg n-docosanol/day. Weight gain, food consumption, necropsy results and litter parameters were comparable between groups: 3 females (1 control, 1 dosed with 125 mg/kg and 1 dosed with 500 mg/kg) showed early total litter loss. Fetal anomalies (visceral and skeletal) observed in treated litters at necropsy were similar in both type and frequency to the concurrent control and/or the historical control data from the laboratory and were not dose-related. Therefore, the no observed adverse effect level (NOAEL) in this study was considered to be 2000 mg/kg/day.

Study 54 - A Fertility and General Reproduction Study in Rabbits with n-Docosanol 12% Cream. Study report no. SLS 3333.2. *In life:* 3/23 to 4/3/95, conducted at [redacted] in compliance with GLP guidelines 21 CFR 58. 12% n-Docosanol Cream lot no. LP-149A; K-Y® Jelly (control material) lot no. 2574L, exp. date 8/97.

Study Design: This study was performed to determine and evaluate the toxic potential of n-docosanol when administered intra-vaginally as a 12% cream (formulation 3) on the reproductive capabilities of female New Zealand White rabbits. Virgin rabbits (aged ~5.5 months, weighing 2.8-3.7 kg) were treated with a single dose, placed into the vaginal vault, of 1 ml of either n-docosanol 12% cream or control (K-Y Jelly®) prior to mating. Approximately 5 minutes following treatment, females were allowed to mate naturally with proven breeder males. Does were allowed to stay with the buck for approximately 30 minutes after mating and then treated with 100 I.U. of human chorionic gonadotropin, via the marginal ear vein. If copulation did not occur within approximately 20 minutes, the doe was placed in the cage of another buck and the mating process repeated. The first 16 females in each group that were treated and successfully mated were utilized for the study. Parameters evaluated during the study included clinical observations, body weights, and food consumption. All females were euthanized on gestation day 10 and dams and fetuses were subjected to gross necropsy. The ovaries and uterus were removed and the number of corpora lutea, number of implantation sites, number of resorption sites, number and distribution of fetuses, and fetal and placental weights were recorded.

Summary of Study Results: Vaginal administration of n-docosanol 12% cream prior to mating did not appear to have a significant effect on either the copulation index or the fertility of rabbits. No maternal toxicity as assessed by clinical observations, weight gain and changes in food consumption was observed during the study. There were no significant differences in the mean number of corpora lutea, early resorptions or post-implantation sites between the two treatment groups. However, there was a significant difference in the number of viable fetuses and implantation sites in the n-docosanol group compared to the K-Y Jelly group due to a statistically significant increase in the mean pre-implantation loss in the n-docosanol group (see Table below). Among the 11/16 gravid females in this group, 2 does had 1 implant each; 1 doe had 2 implants; and another doe had four implants while the number of corpora lutea in these females was comparable to the animals in the control group. These results indicated that these females ovulated normally but only a few of the ova were

fertilized, or if fertilization did occur, only a few of the fertilized eggs were successfully implanted, or a combination of both occurred. Since a majority of the does in this group did have implants and live fetuses comparable to the controls, it could not be determined whether the n-docosanol treatment may have had an effect on the sperm or the fertilized eggs which resulted in this increase in pre-implantation loss observed in 4 does.

Parameter	K-Y Jelly	12 % n-Docosanol
Copulation Index	100 %	80.0 %
Fertility Index	87.5 %	68.8 %
Mean numbers of -		
Corpora Lutea	10.1	11.4
Implantation Sites	7.6	5.5
Pre-implantation Loss	2.5	5.8 *
Viable Fetuses	7.4	5.5
Early Resorptions	0.2	0.1
Post-implantation Loss	0.2	0.1

* p = <0.05 (two-tailed nonparametric Mann-Whitney U test)

Certificate of Analysis: n-Docosanol 12% Cream was analyzed by [redacted] and was found to comply with protocol specifications (10.8-13.2% w/w n-docosanol).

Genotoxicity Study Reviews (GLP)

The following genotoxicity studies were performed by [redacted] in compliance with OECD GLP guidelines. All assays were conducted with n-docosanol, batch no. BRL C22 - 6/28/89; test material for the *in vitro* assay was dissolved in ethanol and for the *in vivo* assay in polyethylene glycol (PEG).

These studies were previously submitted under [redacted] submission N001, dated 7/16/91, and reviewed by Dr. Lauren Black, HFD-530, review dated 8/26/91.

Study 55 - *Salmonella typhimurium* Mutation Assay with LIDAKOL. Report no. [redacted]170010, *In life:* 11/14 to 12/4/89.

Study Design: This study was designed to elucidate the potential for n-docosanol to induce gene mutations in Salmonella Typhimurium, tester strains TA 1535 (base pair), 1537, 1538, 98, and 100 (base pair). The n-docosanol was evaluated at 10 - 1000 µg/ml, with and without the S9 liver microsomal fraction (derived from rat liver). All data points were performed in triplicate and the experiment was performed twice. Controls consisted of ethanol, sodium azide, 2-aminoanthracene, or 4-nitro-o-phenylene-diamine.

Summary of Study Results: n-Docosanol did not induce point mutations by base pair changes or frame shifts in the strains used. No increase in mutation rate (number of revertants) was measured. Positive controls performed as expected.

Study 56 - Gene Mutation Assay in Chinese Hamster V79 Cells *in vitro* with LIDAKOL. Report no. [redacted]170021, *In life:* 10/20 to 12/18/89.

Study Design: This study was performed to evaluate the potential of n-docosanol to induce gene (point) mutations at the HGPRT locus in V79 cells of the Chinese hamster *in vitro*. Two independent experiments were performed in the presence and absence of S9 microsomal liver fraction, and tested 2 - 20 µg/ml (limit of solubility) of n-docosanol. A positive result was scored if the test article triples the spontaneous mutation frequency. Control substances were ethanol, ethylmethanesulfonate (1 mg/ml), and 7,12-dimethylbenz(a)anthracene (15.4 µg/ml). Two independent experiments were performed with positive controls performing as expected.

Summary of Study Results: There were no signs of cytotoxicity or changes in the gene mutation rate at the HGPRT locus with any of the doses.

Study 57 - Chromosome Aberration Assay in Chinese Hamster V79 Cells in vitro with LIDAKOL. Report no. [redacted]170032, *In life:* 1/29 to 3/8/90.

Study Design: The ability of n-docosanol (in ethanol) to induce structural chromosome aberrations in Chinese hamster V79 cells was evaluated *in vitro* over a dose range of 0.6 - 20.0 µg/ml, with and without S9 metabolic activation. Metaphase spreads were prepared and scored for structural chromosome aberrations following 7, 18, and 28 hours of incubation with n-docosanol. Controls consisted of ethanol, ethylmethanesulfonate (0.72 mg/ml), and cyclophosphamide (1.4 µg/ml).

Summary of Study Results: Treatment with 20 µg/ml n-docosanol did not reduce the plating efficiency of the V79 cells or the mitotic index. There were no relevant increases in cells with structural aberrations after treatment with n-docosanol at any fixation interval, either in the presence or absence of liver S9 mix. Positive controls performed as expected.

Study 58 - Micronucleus Assay in Bone Marrow Cells of the Mouse with LIDAKOL. Report no. [redacted]170043, *In life:* 11/20/89 to 3/28/90.

Study Design: This study evaluated the potential for n-docosanol to induce micronuclei in polychromatic erythrocytes (PCE) in the bone marrow of the NMRI mouse. Fasted NMRI mice were treated with 50, 150, and 500 mg/kg n-docosanol administered by oral gavage at a volume of 10 ml/kg n-docosanol suspended in PEG. Five (5) mice/sex/group were sacrificed at intervals of at 24, 48, and 72 hours following treatment and femoral bone marrow cells harvested. To describe a cytotoxic effect, the ratio between polychromatic and normochromatic erythrocytes was determined at each sampling point and expressed as normochromatic erythrocytes/1000 polychromatic

erythrocytes (NCE/PCE). To describe a genotoxic effect, the number of cells with micronuclei were counted/1000 polychromatic cells. Slide analysis was performed with coded slides. Controls consisted of PEG and cyclophosphamide (40 mg/kg).

Summary of Study Results: Increased frequency of micronuclei was not detected at any dose level or interval examined following treatment with the n-docosanol. There were no significant differences in group means for NCE/PCE ratios between groups, although the ratios did decrease slightly over time (e.g. ↓NCE, ↑PCE). Controls performed as expected.

Review Note: *The appearance of "slight toxic reactions" e.g. "eyelid closure and apathy" was used for proof of absorption. PK data was not submitted to confirm systemic exposure.*

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HUMAN PHARMACOKINETIC DATA

[The data presented below was taken from the Sponsor's Summary Data. The Biopharmacology Review should be referred to for definitive human pharmacokinetic data.]

In a preliminary study [Study IRAD 040-003], LIDAKOL (300 mg, Formulation 1) was applied to the underside of the right forearm 4 times/day for 7 days. n-Docosanol concentrations were below the limit of quantification (<3 µg/ml) in all blood samples collected. Similar results were observed when LIDAKOL cream (90 mg/dose) was administered 5 times/day for 2 days to otherwise healthy patients experiencing a recurrence of herpes labialis: n-Docosanol concentrations were below the limit of quantification (<10 ng/ml) in all blood samples collected except one which was measured at 10 ng/ml [Clinical Study 95-LID-01].

Plasma levels of n-docosanol were detectable following oral administration of single doses of 1 and 5 g/kg n-docosanol solutions to healthy male subjects [n=5, Clinical Study 94-LID-02]. Selected pharmacokinetic parameters are presented below:

Parameter	1 g Dose		5 g Dose	
	Mean	Range	Mean	Range
Cmax (ng/ml)	19 ± 18		124 ± 120	
Tmax (hr)	8		6	
AUC, 0-48 hr (ng.h/ml)	172 ± 211		713 ± 829	
Cl_{po}/F (L/min)	33 ± 397		410 ± 609	

Elimination of n-docosanol in healthy male subjects was also examined using a single oral dose of ^{14}C-labeled n-docosanol in [Clinical Study 95-LID-01]. The primary route of elimination was in the feces, presumably as unabsorbed material.

% of Actual Dose (n=6) Found in -	Mean	Range
Urine	0.028 ± 0.0490	
Feces	103.73 ± 3.960	
Air	0.907 ± 0.400	
Total	104.68 ± 3.990	

Taking into account both oral and dermal absorption of n-docosanol following applications to the lips, the Sponsor has estimated the maximum human plasma concentrations, following multiple therapeutic topical doses to the lips, to be approximately 0.3 to 0.4 ng/ml.

SUMMARY AND DISCUSSION

n-Docosanol is a long chain alcohol which is intended for use as the active ingredient in LIDAKOL® 10% Cream. LIDAKOL® is being developed for the treatment of recurrent oral-facial herpes simplex lesions. n-Docosanol does not appear to have direct anti-viral activity. The proposed pharmacodynamic basis of action appears to be as an inhibitor of replication in lipid-enveloped viruses. Following bioactivation, n-docosanol appears to block fusion of lipid-enveloped viruses with cell membranes, thus inhibiting cellular entry, nuclear localization, and subsequent viral replication. *In vitro* studies found it to be equally effective against wild type, clinical isolates and acyclovir resistant mutants of the Herpes Simplex Virus (HSV).

The upper estimate of the anticipated daily dose of n-docosanol for treatment of oral herpes is 0.5 to 1.0 mg/kg body weight (5 applications of 50 to 100 mg 10% n-docosanol cream per 50 kg body weight). In the treatment of herpes labialis, systemic exposures to n-docosanol are likely to occur through dermal absorption as well oral ingestion. The Sponsor has estimated the total human systemic dose following multiple therapeutic topical doses to be approximately 0.3 to 0.4 ng/ml.

Safety pharmacology studies in mice and rats administered oral doses of $\leq$1000 mg/kg n-docosanol demonstrated no significant effects on any of the following parameters tested: general or clinical behavior, locomotor activity, thiopental-induced sleeping time, synergistic or antagonistic convulsant activity, or intestinal charcoal transport (*male mice*); and normal body temperature or urinary volume or electrolyte excretion (*male rats*). Following i.v. administration to anesthetized dogs, n-docosanol produced little or no effect on the respiratory rate, blood pressure, heart rate and ECG at doses of $\leq$3 mg/kg. Topical and ocular applications of $\leq$3.0 % n-docosanol in male guinea pigs did not demonstrate any local anesthetic activity. *In vitro*, n-docosanol did not show any significant influence on the spontaneous movements of isolated guinea pig ileum (5) or on acetylcholine-, histamine- and barium-induced contractions.

In an *in vitro* penetration study with n-[1-^{14}C]docosanol with the proposed 10 % cream formulation, < 2.0 % of the radio-label was found in the human cadaver skin and < 0.01 % was found in the reservoir fluid. Limited absorption was also observed *in vivo* in both mice and rabbits: < 0.001 % of the radiolabel was found in the plasma of mice, and ~ 2 % of the administered radiolabel was recovered from the skin, plasma and waste products of rabbits. No significant differences were observed in absorption following application to intact and abraded rabbit skin.

Following oral administration, systemic exposures to n-docosanol appeared similar across species and were characterized by non-linear (dose-dependent) kinetics, i.e. increasing the dose of n-docosanol resulted in a disproportionately lower systemic exposure than would have been predicted from a linear relationship. Total absorbed dose following oral gavage with radio-labeled n-docosanol was estimated to be approximately 15 to 20 % . Radioactivity was detected in the plasma within 0.5 hours, with significant levels detected at 1 hour. Peak concentration (C_{max}) varied considerably, 0.5 to 12 hours, but occurred in most animals between 1 and 4 hours post administration. Radioactivity was detected in all tissues examined within 1 day of dosing with the highest levels found in the liver, spleen and brown fat. The half-life of n-docosanol derived radioactivity in the liver was 4-5 days.

By day 1, over 90 % of the radioactivity in the liver was determined to be in the form of polar lipid metabolites. Similar rates of clearance were observed from the spleen. By day 32 post-gavage, most of the tissue-associated radioactivity had been eliminated, with only about 1 % of the original dose localized in brown fat (primarily incorporated as triglycerides) and brain lipids. Metabolism appears to be rapid (nearly complete by 24 hours post-administration) and the pathway appears similar to other fatty alcohols: oxidation to fatty acids (primarily n-docosanoic acid) followed by esterfication to a wide variety of lipids, glycerides and phosphoglycerides which are then appear to be universally distributed in tissues. Following i.v. administration of radiolabeled n-docosanol, approximately 50 % of the radioactivity was excreted in the expired air (presumably as $^{14}CO_2$), 2 % in the urine, 1 % in the feces, and 27 % was present in the tissues 168 hours post-dosing.

Oral doses of 1000 mg/kg/day in rats and 2000 mg/kg/day in dogs for 26 weeks resulted in no discernible toxicity associated n-docosanol treatment. Mean maximum n-docosanol concentrations in plasma (C_{max}) and mean AUC_{0-24} values are presented below.

A: Rats (1000 mg/kg/day)

	Males	Females
C_{max} (ng/ml)	626	529
AUC_{0-24} (ng.h/ml)	3196	2255

B: Dogs (2000 mg/kg/day)

	Males	Females
C_{max} (ng/ml)	1094	2255
AUC_{0-24} (ng.h/ml)	12340	24400

In rabbits, both LIDAKOL 10 % cream and vehicle cream were assigned primary irritation scores of 0.2 when applied to intact rabbit skin, 0.0 when applied to the penis, 2.3 when applied intravaginally (mildly irritating = 0.1-2.0). Increasing the n-docosanol concentration of the cream formulations increased the irritation scores: LIDAKOL 20 % cream resulted in a primary dermal irritation score of 1.11 and LIDAKOL 12 % Cream resulted in an intravaginal irritation score of 2.7. When placed in the rabbit conjunctival sac, LIDAKOL 10 and 20 % creams were both given a primary eye irritation score of 0.25, reflecting conjunctival redness (grade 1) at the end of 1 hour. LIDAKOL 10% cream failed to induce either contact hypersensitivity, photosensitivity or phototoxicity when tested in albino guinea pigs.

Subacute dermal applications (4 weeks) of LIDAKOL 10 % Cream in rabbits at dose levels of up to 1000 mg/kg for 6 hours/day (occluded) on intact and abraded skin or injected directly into the vaginal vault, and subchronic topical applications (13 weeks) in mice at 100 µl (~10 % total body surface area) did not produced any histologic evidence of local or systemic toxicity. Plasma concentrations were generally below the limit of detection in the dermal and vaginal rabbit studies. In mice, where systemic exposure occurred through both dermal and oral absorption, the mean C_{max} concentrations were 205 and 232 ng/ml, and the mean AUC_{0-24} values were 1390 and 1660 ng.h/ml for males and females, respectively.

Reproductive and developmental studies were performed in rats and rabbits with oral doses up to 2000 mg/kg/day. In the rabbit, toxicokinetic analysis on day 14 of gestation revealed C_{max} levels of 142, 46 and 119 ng/ml with AUC_{0-24} values of 945, 667 and 1670 ng.h/ml at doses of 500, 1000 and 2000 mg/kg, respectively. There were no significant effects of n-docosanol treatment in F0 males and females as assessed by clinical behavior and appearance; bodyweight gain; food and water consumption; female estrous cycling; sperm morphology (motility and numbers); mating behavior and fertility index; gestation length, parturition, and lactation; and gross appearance of reproductive

organs (and relative weights) and internal tissues at necropsy. Litter responses, as assessed by numbers of corpora lutea, implantations and viable fetuses, resorption sites, fetal survival, fetal growth, and placental weights were similar in all groups and showed no adverse effects of parental treatment with n-docosanol. Fetal anomalies (visceral and skeletal) observed in treated litters at necropsy were similar in both type and frequency to the concurrent control and/or the historical control data. The general condition of F1 offspring (rats) was similar in all groups with no apparent adverse effects of maternal treatment with n-docosanol. Litter sizes, sex ratios, birth weights and survival rates were similar to controls. There were no dose-related differences in weight gain, physical development, auditory and visual responses, learning ability, neuromuscular function, or reproductive ability. Under the conditions of these study, oral administration of 2000 mg/kg/day n-docosanol was not considered to adversely affect reproduction or to be teratogenic in rats and rabbits.

Vaginal administration of n-docosanol 12 % cream prior to mating did not appear to have a significant effect on either the copulation index or the fertility of rabbits. No maternal toxicity was observed during the study and here were no significant differences in the mean number of corpora lutea, early resorptions or post-implantation sites between the two treatment groups. However, there was a significant difference in the number of viable fetuses and implantation sites in the n-docosanol group compared to the K-Y Jelly group due to a statistically significant increase in the mean pre-implantation loss in the n-docosanol group. However, since a majority of the does in this group did have implants and live fetuses comparable to the controls, it could not be determined if oral administration of n-docosanol may have had an effect on the sperm and/or the fertilized eggs. Further testing would be necessary to resolve this question if the Sponsor were to pursue a vaginally administered product.

Several of the toxicology studies were compromised due to measurable plasma levels of n-docosanol reported in control animals (Study #25 ([redacted]94/LAK002/0706), #26 ([redacted] 94/LAK008/0963), #42 ([redacted]107527) and #50 ([redacted]96/LAK015/0839). For the most part these levels were just above or close to background, however, in studies 42 and 50, control plasma levels were close to the levels found in dosed animals following a single day of dosing. Lidak should address this issue and an inspection of these study sites may be indicated.

Genotoxicity was assessed using the Ames Bacterial Mutation Assay, HPRT Gene Mutation Assay in Chinese Hamster Cells, Chromosome Aberration Assay in Chinese Hamster Cells, and the *in vivo* Micronucleus Assay in Mouse Bone Marrow Cells. Under the conditions used in these studies, there was no indication that n-docosanol had either mutagenic or genotoxic potential.

Carcinogenicity studies were not performed. During a tele-con held on January 123, 1995 between Lidak and Drs. Lauren Black and James Farrelly, HFD-530, Lidak was informed that carcinogenicity studies would not be necessary. However, to be consistent with the requirement of drugs reviewed and approved in HFD-540, I propose, that if LIDAKOL is approvable, Lidak be asked to commit to dermal and photo-cocarcinogenicity studies during Phase 4 of development. This drug product will be used in an area exposed to the sun and for an indication which will necessitate intermittent chronic use (greater than 6 months over ten years). Lidak has argued that his product is already used in cosmetics, particularly lipsticks, however, it has never been previously used as the active ingredient in a drug product or tested for carcinogenicity potential.

AUC levels achieved following oral dosing in animal studies were 2255-3196 ng.h/ml for the rat (1000 mg/kg/day), 1670 for the pregnant rabbit (2000 mg/kg/day), and 12,340-24,400 ng.h/ml for the dog (2000 mg/kg/day). In addition, AUC values in mice, where systemic exposure occurred through both dermal and oral absorption following application of 10% n-docosanol over approximately 10% of the total body surface, the mean AUC values were 1390-1660 ng.h/ml. AUC levels of 503 and 892 ng.h/ml were achieved in healthy human males following a single oral dose of 1000 or 5000 mg n-docosanol, respectively, with no observed adverse effects. Topical application of LIDAKOL 10% Cream to patients with recurrent oral-facial herpes lesions did not result in measurable plasma levels from which to calculate an AUC. However, the Sponsor has estimated the human systemic exposure (maximum plasma concentration) following multiple therapeutic topical doses to be approximately 0.3 to 0.4 ng/ml. The plasma levels of n-docosanol achieved in the animal studies are greater than 1000 fold that expected in humans. Although toxic levels were not achieved in the animal studies, the doses appear adequate to evaluate the potential of n-docosanol to cause adverse effects in humans under the conditions of use described in this NDA.

Proposed Labeling

[redacted]

4 pages of revised draft labeling have been redacted from this portion of the document.

/S/ 5-26-98

Lynnda Reid, Ph.D. Date
Pharmacologist/Toxicologist

cc:
NDA 20-941
HFD-540
HFD-540/Pharm/Reid
HFD-540/Pharm/Jacobs
HFD-540/CSO/White
HFD-540/MO/Okun
HFD-540/Chem/Hathaway
HFD-530/Micro/Biswal
HFD-880/Biopharm/Bashaw
HFD-345/Viswanathan

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n-Docosanol 10% Cream
NDA 20-941
Lidakol® 10% Cream
Reviewer: E.D. Bashaw, Pharm.D.
APW

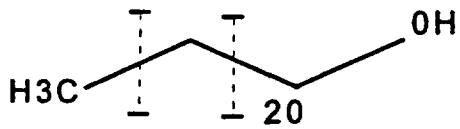
Lidak Pharmaceuticals
La Jolla, CA 92037

Submission Date:
Dec. 23, 1997

Review of an NDA

I. Background

Lidakol® (n-docosanol, behenyl alcohol) is a naturally occurring fatty alcohol. It is a 22-carbon straight chain saturated alcohol with a formula of $C_{22}H_{46}0$ and a molecular weight of 326.61. It is a white solid and is soluble in chloroform, slightly soluble in ethanol and insoluble in water. It has the following general structure:



n-Docosanol is a broad spectrum anti-viral agent having topical activity against lipid-enveloped viruses including herpes simplex virus 1 & 2 (in vitro and in vivo), and varicella zoster, cytomegalovirus, human herpes virus 6, influenza A, and the human immunodeficiency virus (HIV-1) in vitro. It is being developed as a topical anti-herpes simplex virus (HSV) agent for the management of recurrent outbreaks of fever blisters/cold sores. It's mechanism of action is related to its ability to block one or more of the steps of viral entry into a cell. In vitro it has been shown to block the fusion of the viral envelope with the cellular membrane of normal cells. By blocking viral entry to the cell it effectively inhibits viral replication. It is this mechanism of action that explains it's wide spectrum of activity against lipid-enveloped viruses that utilize fusion as the sole or major mechanism of entry into the cell. This mechanism of action also makes the development of resistant strains of the virus highly unlikely as it exerts its action at the mammalian cell wall and not against the viral sub-unit itself. In this product it is formulated as a 10% cream for topical administration.

II. Recommendation

In this NDA the applicant has submitted the results of one in vitro and three in vivo pharmacokinetic studies. The in vivo studies included oral administration of a radiolabeled dose of n-docosanol, oral and topical administration of n-docosanol in healthy males, and topical administration in patients with oral facial HSV infection. The results of these studies indicate that n-docosanol is minimally absorbed with only 1 sample out of 208 samples having detectable levels in the plasma after dosing with the to-be-marketed formulation. From a biopharmaceutic standpoint the sponsor has adequately addressed the issues of systemic absorption/bioavailability and the application is acceptable from a Clinical Pharmacology/Biopharmaceutic standpoint.

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III. NDA Overview

As noted in the background section, this NDA consists of three in vivo and one in vitro study. Only 1 of the in vivo studies examined the issue of systemic absorption of n-docosanol in patients with oral-facial HSV infection. The other two studies, conducted in healthy adults, deal with the disposition and metabolic fate of n-docosanol following topical and oral ingestion of both cold and radiolabled doses.

Formulation

In this NDA the active ingredient, n-docosanol, is formulated as a white, non-staining, 10% cream. Reproduced below is its quantitative and qualitative formulation.

Ingredient	Percent	Formula
n-Docosanol	10%	
Sucrose Stearate and Sucrose Distearate		
Light Mineral Oil,		
Propylene Glycol,		
Benzyl Alcohol,		
Purified Water,		

As Lidakol® the cream will be available in the market as a 2, 5, and 15gm tube.

1 page(s) have been removed because it contains trade secret and/or confidential information that is not disclosable.

[redacted]

V. General Pharmacokinetics (In Vivo)

Study #95-LID-01

"Excretion Balance, Pharmacokinetic and Metabolism Study after a Single Oral Dose of 14C-Labeled n-Docosanol in Healthy Male Volunteers"

Objective: The objective of this study was to determine the systemic absorption of n-docosanol following the administration of a radiolabeled dose of docosanol to healthy male subjects.

Investigator: J.J. van Lier, M.D.

Study Site: [redacted]

Treatments: 1000mg n-docosanol [redacted]
2 patients received 5 µCi of ^{14}C labeled docosanol
4 patients received 15 µCi of ^{14}C labeled docosanol

Methods

This study was initiated to investigate the systemic availability of docosanol and to identify routes and possible mechanisms of drug elimination. A total of six healthy adult males were enrolled and completed all phases of this trial.

Demographics of Enrolled Subjects

N=10	Mean (%CV)	Range
Age (yrs)	25 (36%)	42-19
Weight (kg)	72 (14%)	83.6-59.1
Height (cm)	185 (4.3%)	195-175

The study was split into two phases using different levels of radioactivity. Initially two subjects were dosed with 5 μCi of ^{14}C labeled docosanol and the disposition of radioactivity was monitored. Due to the low levels of systemic radioactivity detected it was decided to dose the remaining four subjects with higher doses of radioactivity (15 μCi) so that it might be possible to quantify systemic absorption.

Despite the two phases of the trial all subjects followed the same dosing and sampling protocol. Following a 10 hour fast all subjects were given their dose of docosanol (5g of a 20% dispersion) in [redacted] Following dosing, extensive blood sampling was initiated according to the following schedule:

For total radioactivity (plasma)
10ml blood samples were collected and separated pre-dose and at 0.5, 1, 2, 3, 4, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120, 144, and 168 hours post dose

For total radioactivity (whole blood)
1ml blood samples were collected pre-dose and at 1, 2, 3, 4, 12, 24, 48, and 168hrs post-dose.

Metabolic profiling
20ml blood samples were collected at 4 and 24 hours post-dose for profiling purposes.

In addition to the blood and plasma, urine was collected following a pre-dosing void over the following intervals: 0-4, 4-8, 8-12, 12-24, 24-36, 36-48, 48-72, 72-96, 96-120, 120-144, 144-168hrs. All feces were also collected over the 168hr period following dosing.

Expired CO_2 was also analyzed for ^{14}C pre-dose and at 2, 4, 8, 12, 24, and 48hrs post-dosing.

Results
Reproduced below is a summary table of the cumulative excretion data collected in this study. In general total excretion of drug was completed by 96hrs post-dose.

Amount Excreted (Ae) as % of labeled dose

N=6	Ae urine	Ae feces	Ae air	Ae total
Mean (%CV)	0.028 (175%)	103.73 (3.8%)	0.907 (44%)	104.68 (3.8%)
range	[redacted]			

The results of this study clearly indicate that there is a low degree of systemic absorption of docosanol following oral administration. Metabolic profiling of the feces revealed that approximately 80% of the excreted compound was docosanol and 8% was docosanoic acid (the corresponding carboxylic acid to the alcohol). The remainder was unidentified polar and non-polar compounds. Following hydrolysis these ratios changed to 68% for docosanol and 28% for docosanoic acid, suggesting that these previously unidentified metabolites were glucuronides or other conjugates of both docosanol and docosanoic acid.

A similar attempt at metabolic profiling of the urine and plasma results were unsuccessful as the amounts of detectable species present in these samples were too low to reliably quantify. As the fractional recovery of radioactivity clearly indicates that fecal excretion is the primary route of elimination, it is unlikely that either matrix has significant amounts of any additional unidentified metabolites.

Study Conclusions

The results of this study indicate that docosanol has a low degree of absorption following oral administration and that it is almost completely recovered as either unchanged drug or as the corresponding organic acid (to the alcohol). While small amounts of ^{14}C were detected in the expired air and in the plasma, these amounts account for <1% of the total dose of 1gm.

Study #94-LID-01

"A Combination of a Single-Dose Topical Study with n-Docosanol and a Single Rising-Dose Oral Study with n-Docosanol in Healthy Male Volunteers"

Objective: The objective of this study was to determine the systemic absorption of n-docosanol as a 10% cream to two oral doses of docosanol in healthy adult males.

Investigator: I.J. Terpstra, M.D.

Study Site: [redacted]

Treatments: Phase 1-10g of 10% docosanol topical cream (1g total dose)
Phase 2-5ml of 20% docosanol [redacted] dispersion (1g total dose)
Phase 3-25ml of 20% docosanol [redacted] dispersion (5g total dose)

Methods

This study was designed as non-randomized three-period crossover comparative bioavailability study. A total of six healthy males were enrolled in this study and all subjects completed the study (although one subject was excluded from phase 2 due to the flu). The demographics for these subjects is reproduced below:

Demographics of Enrolled Subjects

N=6	Mean	Range
Age (yrs)	23	25-22
Weight (kg)	82	87-77

Upon enrollment in the trial the subjects were required to spend three 3 day periods in the clinical study unit. At 9am on the morning of the second day the subjects were dosed with the appropriate formulation/dose for that period. As noted earlier this was NOT a randomized trial. All subjects received all phases in a 1-2-3 order with a 1 week washout period between phases.

For Phase 1 the subjects had 10g of cream applied to a 12 x 12 cm patch of skin on their lower back. Following drug application the area was covered with an occlusive dressing (Actiderm®) for 24 hours. After 24 hours the covering was removed and the area was cleaned with lukewarm water to remove any cream remnants.

For Phases 2 and 3 the subjects were given either 5 or 25ml of a 20% dispersion of docosanol in [redacted] water. Each dose was followed with 200ml of water to ensure full dosing.

During each treatment blood samples (10ml) were collected at 1, 2, 4, 6, 8, 12, 14, 24, 36, and 48 hours post-dosing. In addition urine samples were collected over the 0-12 and 12-24 hour post-dosing interval, however, in light of the low urinary recovery found in the radiolabel study (LID-01, page 5) these samples were not analyzed.

Results

Analysis of the plasma samples from Phase 1 (topical application) yielded no plasma levels above the limit of detection (10ng/ml). During Phases 2 and 3 low plasma levels were detected and are summarized in the following table:

Oral Absorption Data-Mean (%CV)

	AUC_{0-48} (ng*h/ml)	Cmax (ng/ml)	Tmax (hr)	$Cl_{po/F}$ (l/min)
Phase 2; 1g dose	172 (122%)	19 (95%)	8	201 (90%)
Phase 3; 5g dose	713 (116%)	124 (96%)	6	410 (149%)

The results from this study are highly variable with coefficients of variation >90% for all calculated parameters. This variability is due in some part to the small number of subjects present in this study (5 subjects in Phase 2, and 6 in Phase 3). Even so it is unlikely that the data would be improved if more subjects were used. This is due in no small part to the poor bioavailability of docosanol following oral administration. What is surprising is that even with the large CV's, the data is roughly dose proportional for both AUC and Cmax. As for the observed difference in clearance values, this can be explained by the fact that Cl here is apparent oral clearance (Cl/F) and with drugs with low bioavailability, any variance in F will tend to exaggerate apparent Cl differences. Given that F is probably very low this would account for the observed difference in clearance.

Study Conclusion

The results of this study confirm the previous radiolabel study in that the observed plasma levels are low and erratic. While there does appear to be a "rough" degree of dose proportionality, the

small number of subjects and the high intersubject variability (%CV) makes this conclusion provisional-at best.

Study #95-LID-02

"Pharmacokinetic Study to Assess Plasma Levels of n-Docosanol after Single-Dose and Repeated Topical Dosing with n-Docosanol 10% Cream in Healthy Volunteers with Herpes Labialis Recurrence"

Objective: The objective of this study was to determine the systemic absorption of n-docosanol as a 10% cream in patients with oral-facial Herpes Labials.

Investigator: L. Habbema, M.D.

Study Site: [redacted]

Treatments: 10% n-docosanol cream[1] (Lot# GL019L-95D)

Methods

As noted above this study was designed to assess the systemic absorption of n-docosanol in patients with Herpes Labials. A total of 18 healthy subjects with herpes were screened in the trial and a total of 10 subjects (all female) were enrolled in the trial.

Demographics of Enrolled Subjects

N=10	Mean (%CV)	Range
Age (yrs)	34.1 (38%)	59-22
Weight (kg)	68.9 (13%)	80-55
Height (cm)	169 (4.2%)	182-161

Subject selection for inclusion included the normal requirements (otherwise healthy subjects with herpes labials) and that they have an active recurrence of disease. All screened subjects were instructed to come to the study center upon recognizing the first symptoms of recurrence (burning, tingling, pain, redness, etc.). After re-confirming subject suitability the subjects were released from the study unit with instructions to return when the vesicles appeared.

Once the vesicles appeared the subjects returned to the unit (day 1 of the study) and the vesicles were ruptured using a 21 gauge needle. Following vesicle rupture 10% docosanol cream was applied to the affected area by the investigator. Blood samples (5ml) were collected over the next 24 hours at 0.5, 1, 2, 3, 4, 6, 8, 12 and 24 hours for docosanol levels. Following the 24 hour blood sample the subjects were released from the study unit and instructed to apply the test cream to the affected area 5 times a day (at 4 hour intervals) for the next two days. On day 4 of

[1] To-be-marketed formulation

the study the subjects returned to the study unit and following another application blood samples were again collected at the same timepoints as used on day 1.

Results

A total of 209 blood samples were collected and analyzed for the presence of docosanol. Of these only 1 sample was positive for docosanol at the limit of detection of 10ng/ml. All of the other samples were negative for docosanol.

Study Conclusions

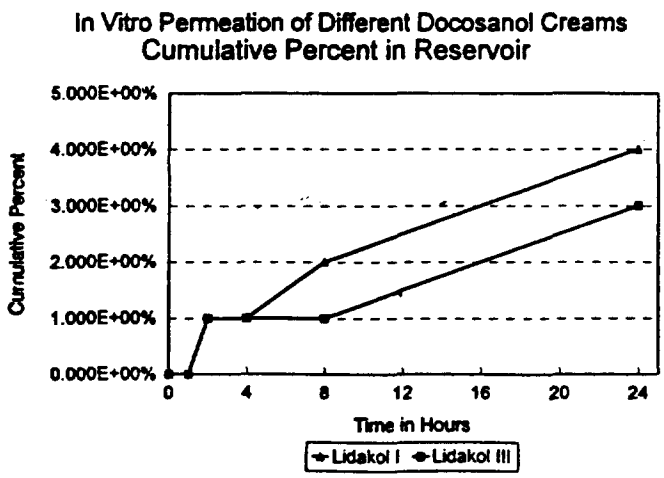
The results of this study suggest that docosanol is not significantly absorbed topically in subjects with active herpes labials. This is not a surprising finding given that docosanol is a long chain unsaturated alcohol with a molecular weight over 300 and is insoluble in water.

VII. Supportive In Vitro Studies

In Vitro Permeation of radiolabeled n-docosanol 10% Cream

As a complement to the in vivo pharmacokinetic studies the sponsor undertook an in vitro assessment of dermal penetration using Franz diffusion cells and cadaver skin. A total of 16 test chambers were prepared using split thickness (200 micron) human cadaver skin. Of these 16 chambers 8 used a developmental formulation of docosanol cream (a/k/a Lidakol I) and the to-be-marketed cream (a/k/a Lidakol III). The reservoir chamber was filled 6-10ml of 4% bovine serum albumen isotonic buffered saline solution. A total of 10mg/cm^2 of ^{14}C-labeled docosanol 10% cream (containing 55µCi) was applied to the skin in each test chamber.

During the study 1ml samples were collected from the reservoir side of each test chamber at 0, 1, 2, 4, 8 and 24 hours with fresh replacement. At the end of the observation period, the skin tissue was cleaned with gauze and warm water to remove any remaining cream. Following this the stratum corneum from each skin sample was removed using skin stripping (~22 strips) and the dermal and epidermal layers were split for individual analysis. The results from this study are summarized below:



Total Percent Recovery

	Reservoir	Dermis	Epi-dermis	Tape	Gauze	Wash	Total
Lidakol I	0.004	0.02	0.4	0.5	1.8	78.3	81
Lidokol III*	0.003	0.04	0.7	1.2	3.9	63.8	69.7

*To-be-marketed cream

The results of this in vitro study confirm the results of the in vivo studies, namely that the dermal penetration of docosanol is minimal and appears to be limited to the dermis and stratum corneum (tape data in table above). A limitation of these types of studies is that the skin tested is normal skin, i.e, the effect of disease state is not assessed. While this is true, it is also true in this case that the in vitro permeation data is consistent with that seen in patients. The net result from this study is that if docosanol cream is inadvertently applied to normal healthy skin, there will be minimal if any absorption beyond the top layers of the skin.

VIII. Labeling

At the present time the application for topical docosanol cream is deficient from the medical standpoint. As the sponsor will be asked to undertake new clinical trials a review of the labeling will be deferred at this time.

IX. Conclusions

From our review of the submitted information the following conclusions can be drawn:

1. Docosanol cream is minimally absorbed following topical administration to either diseased or healthy skin.
2. Low amounts of docosanol are absorbed systematically following oral administration. As herpes labials is a disease found on, in and around the mouth it is likely that small amounts will be inadvertently swallowed. Even so the amounts are highly variable and low even with systemic administration of large doses (1 and 5g).
3. While a formal analysis of gender was not undertaken by the sponsor, the inclusion of male and female subjects in the development of this product (albeit in separate studies) did not reveal a significant gender effect.
4. In vitro studies revealed that, in healthy cadaver skin, docosanol penetration was limited primarily to the stratum corneum and epi-dermis, with very small amounts present in either the receptor fluid or the dermal layers.

X. Comments

1. In future studies with this or other products the sponsor needs to consider using larger number of subjects. In this NDA it was only the fact that there was essentially no systemic absorption that allowed the sponsor to use the number of subjects in their trials that they did. Had there been significant systemic absorption these studies would have been judged to be supportive and not pivotal in nature and design.

/S/ 8/10/98

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