CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

21-671

Pharmacology Review(s)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-671**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**July 26, 2003**
DRUG NAME:	**DEPODUR™ (Morphine Sulfate Sustained-Release Liposome Injection)**
INDICATION:	**For administration by the epidural route, at the lumbar or lower thoracic levels, for the treatment of post-operative pain.**
SPONSOR:	**SkyePharma Inc.**
DOCUMENTS REVIEWED:	**Original NDA submitted to the EDR**
REVIEW DIVISION:	**Division of Anesthetic, Critical Care and Addiction Drug Products (HFD-170)**
PHARM/TOX REVIEWER:	**Mamata De, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Robert Rappaport, M.D.**
PROJECT MANAGER:	**Sara Stradley**

Date of review submission to Division File System (DFS): Amended Review Dated May 18, 2004. This review supercedes the previous review, dated May 14, 2004.

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical pharmacology and toxicology perspective, NDA 21-671 is considered to be approvable.

B. Recommendation for nonclinical studies

There are several outstanding issues which should be addressed by the Sponsor. These issues are not considered to be approval issues, however, they should be addressed as Phase 4 commitments, if the NDA is approved on the first cycle. If the NDA is not approved on the first cycle, the sponsor should resolve the issues as part of the second cycle submission.

1. Tricaprylin, an inactive ingredient, has been adequately qualified for epidural administration in only one species. The current practice in CDER follows the draft guidance on inactive ingredients and recommends that new excipients be qualified in two species, at least one non-rodent. As a Phase 4 Commitment, the Sponsor should complete a 28-day epidural repeat-dose toxicity study in a second species.

2. The Sponsor has recently determined that the morphine sulfate drug substance contains an impurity, [redacted]. The Sponsor should provide qualification of this impurity via a minimal genetic toxicology screen (one *in vitro* mutagenicity assay and one *in vitro* cytogenicity assay). If either genetic toxicology study produces a positive or equivocal result, the Sponsor should either reduce the impurity to acceptable levels or qualify the positive finding via a carcinogenicity assessment in a single species.

C. Recommendations on labeling

The Sponsor proposed the following labeling pertaining to the non-clinical pharmacology and toxicology sections of the label. This labeling is identical to that in the Avinza® label (NDA 21-260):

Carcinogenicity/Mutagenicity/Impairment of Fertility

Studies in animals to evaluate the carcinogenic potential of morphine sulfate have not been conducted. No formal studies to assess the mutagenic potential of morphine have been conducted. In the published literature, the results of *in vitro* studies showed that morphine is non-mutagenic in the *Drosophila melanogaster* lethal mutation assay and produced no evidence of chromosomal aberrations when incubated with murine splenocytes. Contrary to these results, morphine was found to increase DNA fragmentation when incubated *in vitro* with a human lymphoma cell line. *In vivo*, morphine has been reported to produce

an increase in the frequency of micronuclei in bone marrow cells and immature red blood cells in the mouse micronucleus test and to induce chromosomal aberrations in murine lymphocytes and spermatids. Some of the *in vivo* clastogenic effects reported with morphine in mice may be directly related to increases in glucocorticoid levels produced by morphine in this species.

Pregnancy

Teratogenic Effects (Pregnancy Category C)

No formal studies to assess the teratogenic effects of morphine in animals have been performed. Several literature reports indicate that morphine administered subcutaneously during the early gestational period in mice and hamsters produced neurological, soft tissue and skeletal abnormalities. With one exception, the effects that have been reported were following doses that were maternally toxic and the abnormalities noted were characteristic of those observed when maternal toxicity is present. In one study, following subcutaneous infusion of doses greater than or equal to 0.15 mg/kg in mice, exencephaly, hydronephrosis, intestinal hemorrhage, split supraoccipital, malformed sternebrae, and malformed xiphoid were noted in the absence of maternal toxicity. In the hamster, morphine sulfate given subcutaneously on gestation day 8 produced exencephaly and cranioschisis. Morphine was not a significant teratogen in the rat at exposure levels significantly beyond that normally encountered in clinical practice. In one study, however, decreased litter size and viability were observed in the offspring of male rats administered morphine at doses approximately 3-fold the maximum recommended human daily dose (MRHDD) for 10 days prior to mating. In two studies performed in the rabbit, no evidence of teratogenicity was reported at subcutaneous doses up to 100 mg/kg.

In humans, the frequency of congenital anomalies has been reported to be no greater than expected among the children of 70 women who were treated with morphine during the first four months of pregnancy or in 448 women treated with this drug anytime during pregnancy. Furthermore, no malformations were observed in the infant of a woman who attempted suicide by taking an overdose of morphine and other medication during the first trimester of pregnancy.

Nonteratogenic Effects

Published literature indicates that exposure to morphine during pregnancy is associated with reduction in growth and a host of behavioral abnormalities in the offspring of animals. Morphine treatment during gestational periods of organogenesis in rats, hamsters, guinea pigs and rabbits resulted in the following treatment-related embryotoxicity and neonatal toxicity in one or more studies: decreased litter size, embryo-fetal viability, fetal and neonatal body weights, absolute brain and cerebellar weights, lengths or widths at birth and during the neonatal period, delayed motor and sexual maturation, and increased neonatal mortality, cyanosis and hypothermia. Decreased fertility in female offspring, and decreased plasma and testicular levels of luteinizing hormone and testosterone, decreased testes weights, seminiferous tubule shrinkage, germinal cell aplasia, and decreased spermatogenesis in male offspring were also observed. Behavioral abnormalities resulting from chronic morphine exposure of fetal animals included altered reflex and motor skill development, mild withdrawal, and altered responsiveness to morphine persisting into adulthood.

Morphine sulfate should be used by a pregnant woman only if the need for opiate analgesia clearly outweighs the potential risks to the fetus.

II. Summary of nonclinical findings:

DEPODUR™ (also referred to as SKY0401, C0401, DepoFoam™ encapsulated morphine sulfate, sustained-release encapsulated morphine, DepoMorphine™, morphine liposome injection and morphine sulfate sustained-release liposome injection) is a sterile,

non-pyrogenic, white to off-white aqueous suspension of lipid-based particles (DepoFoam™ drug delivery system) containing morphine sulfate. The proposed indication is for use as a single epidural injection at the initiation of surgery for management of post-operative regional pain for up to 48 hours following surgery. The proposed clinical dosage is 10, 15 and 20 mg depending on the type of surgery. The particles are suspended in a 0.9% sodium chloride solution. Each vial contains morphine sulfate (expressed as the pentahydrate) at a concentration of 10 mg/ml. The median diameter of the liposome particles is in the range of 17 to 23 microns. The inactive ingredients and their approximate concentrations are listed in the table below:

Inactive Ingredient	Abbreviation	Concentration
1,2-dioleoyl-*sn*-glycero-3-phosphocholine	DOPC	4.2 mg/ml
cholesterol		3.3 mg/ml
1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)	DPPG	0.9 mg/ml
tricaprylin		0.3 mg/ml
triolein		0.1 mg/ml

The nonclinical development program outlined in this NDA submission relies in part on the FDA's previous findings of safety and efficacy of epidural morphine sulfate administration (Duramorph®; NDA 18-565). The nonclinical evaluation of the DepoFoam™ component of the SKY0401 is based upon previously published literature and toxicology studies of DepoFoam™ as tested alone, as a control for SKY0401 studies and as a component of DepoCyt® (NDA 21-041). The DepoCyt NDA was approved by the Division of Oncology Drug Products under 21 CFR Subpart H for use as an intrathecal treatment of lymphomatous meningitis.

As morphine has been approved by FDA for epidural and intrathecal drug delivery, the Sponsor's non-clinical development program was designed to demonstrate the controlled release of morphine following epidural administration in animals. As requested by the Division, the Sponsor also conducted studies to characterize the potential toxicity of inadvertent intravenous or intrathecal administration of the drug product. In addition, nonclinical studies examining the effects of coadministration of DEPODUR with lidocaine or bupivacaine.

Primary Pharmacodynamics: The primary pharmacodynamics of DepoFoam™ encapsulated morphine sulfate was studied to determine the behavioral, physiological and antinociceptive activities of the encapsulated morphine sulfate and results were compared to those of unencapsulated morphine sulfate. Each animal (Beagle dogs) received three test article (bolus epidural) injections in 3-ml dose volumes, with sufficient time between injections to allow for washout of morphine from the CSF: 5 mg morphine sulfate, 10 mg SKY0401 and 30 mg SKY0401. All morphine-containing test articles blocked the skin twitch response to thermal stimulus. The antinociceptive effect occurred earlier after morphine sulfate (5 mg) administration, with 5 of 5 dogs exhibiting maximum antinociception at 2 hours. By contrast, maximum antinociception did not occur until 4 hours after SKY0401 administration (10 or 30 mg). The antinociceptive effect lasted longer after 30 mg SKY0401, with 4 of 5 animals still exhibiting antinociception at 24 hours. Nociceptive responses returned to baseline by 48 to 72 hours in animals

administered 30 mg of SKY0401. In another study in Beagle dogs, the onset of effect defined by the complete block of skin twitch response occurred at 3 to 6 hours and the duration was extended to 24 to 48 hours after intrathecal or epidural administration of SKY0401. SKY0401 administered intravenously resulted in a complete block of skin twitch response similar to morphine sulfate administration with onset of effect at 5 to 60 minutes and duration of 6 to 10 hours.

Secondary Pharmacodynamics: Secondary pharmacodynamics of SKY0401 was assessed in the same studies mentioned above. All three morphine-containing doses resulted in mild to moderate decreases in arousal state, muscle tone and coordination. However, these behavior effects peaked earlier in most animals for 5 mg morphine sulfate administration (30 to 60 minutes) compared with 10 and 30 mg SKY0401 administration (5 and 10 hours, respectively). Moreover, the time to recovery differed for the various treatments. The animals recovered more quickly after 5 mg morphine sulfate administration; scores were back to near baseline by 10 hours. Behavioral scores returned to baseline by 24 and 48 hours after administration of 10 and 30 mg of SKY0401, respectively. All three morphine-containing administrations resulted in mild to moderate depression of heart and respiratory rates and a moderate depression of blood pressure. The peak effect on blood pressure occurred earlier, and animals recovered more quickly after 5-mg morphine sulfate administration than after 10 and 30 mg SKY0401 administration. There was a trend toward a more pronounced and a slightly longer lasting effect on physiological measurements after administration of 30 mg of SKY0401 compared with 10 mg of SKY0401. However, all animals recovered by 24 to 48 hours. SKY0401 administration (10 and 30 mg) also resulted in a dose-dependent decrease in body temperature that was lowest at 24 hours and returned to baseline by 48 hours. A decrease in body temperature was not observed at 24 hours after 5-mg morphine sulfate administration. Bradycardia was observed after morphine sulfate and SKY0401 treatments with a peak at 6 hours and recovery to baseline by 24 hours after intravenous administration and 48 to 72 hours after epidural or intrathecal administration. Respiratory rate was moderately reduced after all morphine treatments with recovery to normal by 24 hours. No safety pharmacology studies have been cited with the present submission. However, studies evaluating the antinociceptive and toxic effect also evaluated behavioral, neurological and physiological effects as mentioned I with the secondary pharmacodynamics.

Pharmacokinetics: The pharmacokinetics studies were carried out (1) to evaluate a single, epidural injection of SKY0401in the beagle dog; (2) to compare the pharmacokinetics of single intravenous, intrathecal, or epidural injections of SKY0401 in the beagle dog; (3) to compare the pharmacokinetics of SKY0401 manufactured at pilot — versus commercial — scales; and (4) to evaluate the effects of co-administration of lidocaine/epinephrine on the pharmacokinetics of SKY0401. After epidural and intrathecal injection of SKY0401, serum morphine levels reached a peak at approximately 4 and 6 hours, respectively, and fell below the limit of detection (LOD = — ng/ml) by 48 hours. Peak serum morphine levels were approximately 2-fold lower after intrathecal administration of SKY0401 compared with epidural administration of SKY0401 at the same dose. Serum morphine concentrations declined thereafter with

roughly first-order kinetics (t½, of approximately 10 hours) for both intrathecal and epidural dose administrations. Morphine concentrations in lumbar CSF peaked within 5 minutes after single epidural administration of a 5-mg bolus of unencapsulated morphine and declined thereafter to below the detection limit (— ng/ml) by 24 to 48 hours post-dose. By contrast, after administration of 10 or 30 mg SKY0401, morphine concentrations in lumbar CSF peaked at approximately 3 to 11 hours post-dose and declined to below the detection limit at 96 and 144 hours post-dose, respectively. Peak morphine concentrations in CSF were approximately 3-fold lower after SKY0401 (30 mg) administration compared with morphine sulfate administration at one-sixth the dose. Morphine pharmacokinetics in serum after SKY0401 or morphine sulfate administration were similar to those observed in lumbar CSF, except that serum concentrations were approximately 150- to 300-fold lower at the peak. Examination of the serum concentration profiles after a single epidural injection of two SKY0401 formulations manufactured at pilot (— versus commercial —) scales showed that both SKY0401 formulations delivered the drug over 24 hours post-dose in beagle dogs. Peak serum concentrations of morphine were reached at 3 hours when either treatment was administered. In a repeat dose study in Beagle dog (once a week for 4 weeks) morphine concentrations in serum and cerebral spinal fluid (CSF) of all SKY0401-treated animals at 24 hours after dosing ranged from 3.7 to 10.1 ng/ml in, males and from 9.3 to 43.5 ng/ml in females. Morphine concentrations in cisternal CSF of SKY0401-treated animals at 24 hours after epidural administration, ranged from 165.6 to 583.6 ng/ml morphine was not detected in the CSF of any animals euthanized 7 to 9 days after administration of the final (fourth) SKY0401 dose (recovery animals). Distribution, metabolism and elimination studies were not done systematically with the current formulation. The lipids that comprise DepoFoam™ are the same as those that make up the cell membranes, mitochondrial membranes, and microsomal membranes of red blood cells and platelets. Thus it is expected that the lipid molecules in the liposomes will be cleared in the epidural space as that of the components of blood. Also, 2 ml (17 mg approximately) DepoFoam™ will pose less concern regarding the clearance of lipid material considering the magnitude lipid material CSF is known to clear (epidural hematoma or blood patch). Local clearance of morphine and DepoFoam™ lipid constituents after subcutaneous administration in rats demonstrated clearance within 10 days after injection. In this study, 90% of the lipid components (DOPC, DPPG, cholesterol, and tricaprylin) measured by HPLC on excised injection site tissue, disappeared from the injection site within 10 days after administration, and none of the injected lipid could be detected at 3 weeks after administration (Triolein, the fifth and lowest-concentration lipid component of SKY0401, could not be distinguished from endogenous triglycerides). Tricaprylin disappeared most rapidly, followed by DOPC, DPPG, and morphine. Cholesterol was the slowest to disappear. The mechanisms of lipid clearance from the subcutaneous space are believed to be very similar to those from other extravascular spaces, including the epidural space, namely restructuring of the particles, phagocytosis by local macrophages, incorporation into local tissues, and clearance via the lymphatic system. In summary, the lipids that comprise the DepoFoam™ liposomes are either naturally occurring or very close analogues of endogenous lipids that should be remodeled, incorporated, metabolized, and/or cleared like any endogenous lipid.

General Toxicology: SkyePharma characterized the general toxicity of DEPODUR in three main studies:

1. SkyePharma Report 033-00025: An acute epidural injection toxicity study of SKY0401 in the Beagle Dog (GLP).

2. SkyePharma Report 033-00028: A single intravenous, intrathecal and epidural injection drug interaction study in male Beagle Dog (GLP). This study characterized the potential toxicity that would be associated with the accidental injection of the drug product either intravascularly or intrathecally.

3. SkyePharma Report 033-00009: DepoFoam encapsulated morphine sulfate (C0401): A bolus epidural multiple-dosing toxicity study in the Beagle Dog (GLP with some exceptions). This study was conducted by Tony Yaksh, University of California, San Diego.

Due to the unique caveats with epidural/intrathecal drug delivery, all these studies were done in the dog model which has a larger spinal column. As the drug product is designed to provide prolonged release of morphine, daily dosing is not possible. The 28-day repeat-dose toxicology study therefore employed once a week dosing, rather than once a day. A full histological assessment was not completed in these studies.

SkyePharma's NDA for SKY0401 also relies, part, on the finding of safety and efficacy of the reference listed drug, DuraMorph®. In addition, SkyePharma's previous work during the development of DepoCyt [redacted] has contributed to our understanding of the potential toxicity associated with DEPODUR®. Thus, the effect of morphine and DepoFoam were determined independently as well as combined into the current formulation for SKY0401.

The toxicity of epidural and intrathecal morphine has been well characterized. In addition, DepoFoam™ as it exists in DepoCyt® has been approved by the FDA. DepoFoam™ formulation alone showed no major toxicity when studied as placebo with cytarabine toxicity studies after IT administration in Rhesus monkeys. Toxicity of DepoFoam™ placebo was limited to histiocytic infiltration only in the injection sites after intrathecal administration. The formulation of DepoFoam™ used in cytarabine study does not contain tricaprylin. The TDI dosage of tricaprylin will be 6 μg with the current formulation. Considering IV non toxic dose for tricaprylin is 1gm/kg in rat (no intrathecal toxicity study in any species other than dogs), it is assumed that since more than 1000-fold safety margin exist, the formulation will be well tolerated by the human population. However, in context of future safety concern and according to draft excipient guidelines it is recommended that toxicity of the tricaprylin be characterized in a second species with similar route of administration.

None of the studies mentioned above with the encapsulated morphine DepoFoam™ showed any major toxicity. One dog was euthanized after IT administration of 30 mg. The fact that naloxone could not reverse the effect shows that morphine overdose might

not be the causative factor. Congestion of lung observed in this dog at autopsy. Although the cause of death was not determined the circumstantial evidence points to a procedural effect. Histopathological assessment in the morphine DepoFoam™ toxicity studies were limited to brain and spinal cord (lumber, thoracic) and the injection sites. Other toxicity findings are limited to slight to minimal nerve root degeneration with similar incidence in controls which most probably attributed to as procedural effect. Minimal invasions of the inflammatory cells were noted after the intrathecal administration as well as epidural administration.

Overall, DepoFoam™ encapsulated morphine sulfate appears to be relatively safe for the use in analgesia. However, the toxicity of tricaprylin was not assessed in two species.

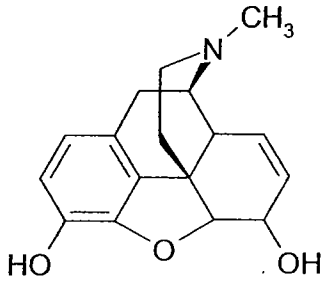
Genetic Toxicology: The Sponsor did **not** conduct formal genetic toxicology studies in support of this NDA. At the pre-NDA meeting with the Division on February 25, 2003, the Sponsor proposed to rely solely on literature to support the NDA. Dr. McGovern (Supervisory Pharmacologist) indicated that, for a 505(b)(2) application, it would be acceptable to **reference published literature** for information on genotoxicity for SKY0401. However, Dr. McGovern indicated that the adequacy of the data will be determined during review of the NDA. Morphine is reported in the published literature to have genotoxic effects in both the *in vivo* chromosomal aberration assay and the in vivo micronucleus assay. All available data for the genotoxicity for the DepoFoam™ components was assessed and submitted with the NDA. DOPC and triolein were found to be non-mutagenic. Standard battery test for clastogenicity was not done for these two above mentioned components. Tricaprylin was found to be mutagenic in one strain of bacteria, negative in other strains. Results from the chromosomal aberration and in vivo micronucleus assays with the tricaprylin are inconclusive. DPPG genotoxicity data was not provided by the Sponsor and is not available in the public domain. Considering all the components of DepoFoam™ are present in other FDA approved products, necessity for required genotoxicity assessment may be limited. However, the approved liposome containing FDA approve drug products are for the indication in oncology.

Carcinogenicity: The Sponsor did **not** conduct a carcinogenicity assessment for SKY0401. However, carcinogenicity assessment is **not required** for this drug product due to the acute indication, as described in the ICH M3 Guidance.

Reproduction and Developmental Toxicology: The Sponsor did **not** conduct reproduction or developmental toxicology studies in support of this NDA. During the pre-NDA Meeting, the Sponsor proposed to **reference published literature** for information on reproductive toxicology for SKY0401. Dr. McGovern (Supervisory Pharmacologist) indicated that the proposal was acceptable; however, the adequacy of the data will be determined during review of the NDA. Previous evaluations of the published data on the potential reproductive toxicity with morphine defined it to be Pregnancy Category C drug. DOPC reproductive toxicity is well characterized. However, DPPG and triolein and tricaprylin reproductive toxicity studies are not well characterized according to today's standard. DPPG and triolein are present in other FDA approved drug products.

Impurity Qualification: Impurity qualification of the residual solvents and drug products are evaluated and was found to be well characterized and qualified except for [ ] which is believed to be an impurity in the morphine drug substance that contains a structural alert for mutagenicity. This issue has been discussed with the DMF holder, [ ] Based upon information in a meeting package received during the review cycle for this NDA, [ ]

[ ] NOTE: This is Proprietary Information from [ ] and can not be relayed to the Sponsor or released to the public). [ ] [ ] that is a structural alert for mutagenicity.



Morphine

Due to the structural alert, the Sponsor should either reduce the levels of [ ] to NMT — in the drug substance or provide adequate qualification of the isolated impurity in a minimal genetic toxicology screen (one *in vitro* mutagenicity study and one *in vitro* chromosome aberration assay) tested up to the limit dose for each assay. If the results of either study are positive, the Sponsor should either reduce specifications to NMT — or conduct carcinogenicity assessment in one species to provide adequate qualification.

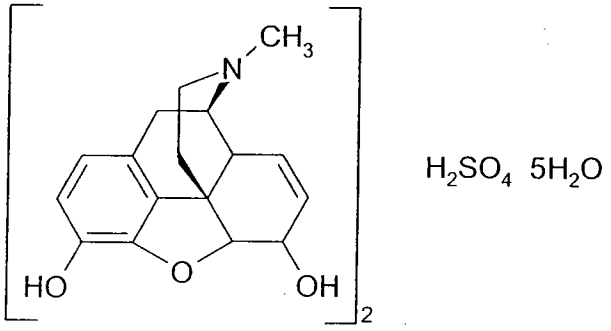
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number:	21-671
Review number:	1
Sequence number/date/type of submission:	000 / July 26, 2004 / Original NDA
Information to Sponsor:	Yes (X) No ( )
Sponsor and/or agent:	SkyePharma Inc. San Diego, CA
Manufacturer for drug substance:	[redacted]
Reviewer name:	Mamata De, Ph.D.
Division name:	Division of Anesthetic, Critical Care and Addiction Drug Products
HFD #:	170
Review completion date:	May 14, 2004

Drug:

Trade name:	DEPODUR™
Generic name:	Morphine Sulfate Sustained-Release Liposome Injection
Code names:	SKY0401, C0401, DepoFoam™ encapsulated morphine sulfate, sustained-release encapsulated morphine, DepoMorphine™, Morphine liposome injection
Chemical name:	7, 8-Didehydro-4,5-epoxy-17-methyl-(5α, 6α)-morphinan-3,6-diol sulfate (2:1) (salt), pentahydrate
CAS registry number:	64-31-3 (morphine sulfate)
Molecular formula/molecular weight:	$C_{17}H_{19}NO_3 \cdot \frac{1}{2}H_2SO_4$ / 758.83
Structure:	



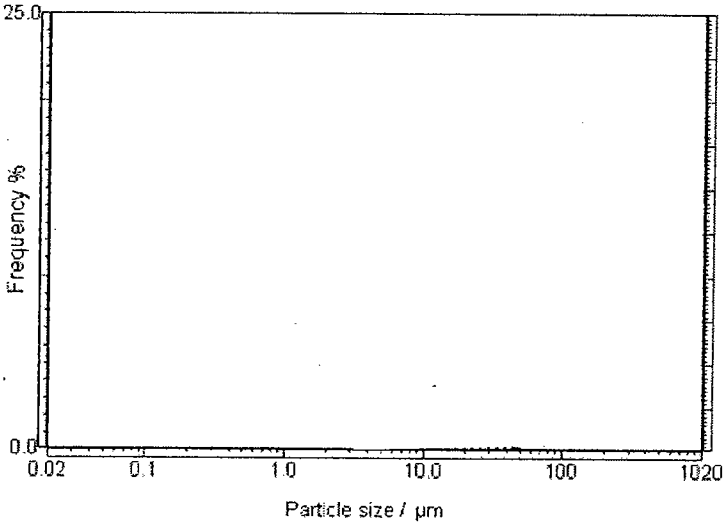
Relevant INDs/NDAs/DMFs:

Type	Number	Component	Company	LOA
IND	29,839	Cytarabine Liposomes	SkyePharma	N/A
IND	48,675	[redacted] (Withdrawn	DepoTech Corporation (name changed to SkyePharma in 1999)	N/A
IND	52,113	Morphine Encapsulated S-R (C0401)	SkyePharma	N/A
NDA	18-565	Duramorph® (Morphine sulfate PF)	Baxter Healthcare	No
NDA	21-041	DepoCyt® (Cytarabin Liposome Inj.)	SkyePharma	N/A
DMF	[redacted]	Morphine Sulfate, USP	[redacted]	Yes
DMF	[redacted]	DOPC	[redacted]	Yes
DMF	[redacted]	DPPG	[redacted]	Yes
DMF	[redacted]	DPPG	[redacted]	Yes
DMF	[redacted]	DPPG	[redacted]	Yes
DMF	[redacted]	Triolein	[redacted]	Yes
DMF	[redacted]	Tricaprylin (Trioctanoin)	[redacted]	Yes

Drug class: Analgesic, Opioid receptor agonist.

Indication: For use as a single epidural injection at the initiation of surgery for management of post-operative regional pain for up to 48 hours following surgery.

Clinical formulation: "SKY0401 (morphine liposome injection) is a sterile, non-pyrogenic, white to off-white aqueous suspension of multivesicular lipid-based particles (DepoFoam™ drug delivery system) containing morphine sulfate, intended for local sustained release following epidural administration." "The multivesicular liposome (MVL) particles form honeycomb-like structures of numerous non-concentric aqueous chambers containing dissolved drug, each chamber being surrounded by a lipid membrane barrier. Morphine sulfate is completely solubilized in the internal aqueous chambers. The MVL particles are generally in the 10 to 30 μm diameter size range. The particle size distribution (Lot 02-4004, initial) is reproduced below:



In the final product, less than [ ] of the total morphine is in the external phase (outside of the chambers. The Sponsor's scanning electron micrograph of an MVL particle and cartoon of rupturing chambers is reproduced below:

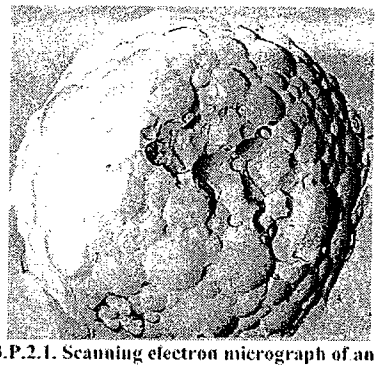
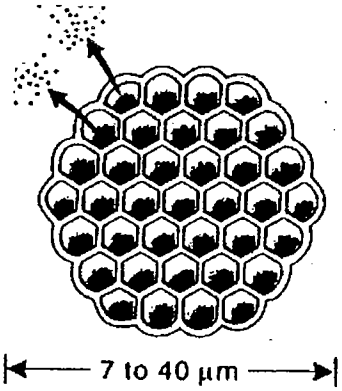


Figure 2.3.P.2.1. Scanning electron micrograph of an MVL particle.



The DepoFoam™ drug delivery system is a sterile, non-pyrogenic, white to off-white, preservative-free homogenous suspension of microscopic, spherical particles composed of non-concentric aqueous chambers that are separated by a bilayer lipid membrane of synthetic analogues of naturally occurring lipids. DepoFoam™ particles are suspended in NaCl 0.9% w/v in water for injection (WFI). The inactive ingredients in SKY0401 include dioleylphosphatidylcholine (DOPC; 1,2-dioleoyl-*sn*-glycero-3-phosphocholine), dipalmitoylphosphatidylglycerol (DPPG; 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol), sodium salt), cholesterol, triolein (glyceryl trioleate), tricaprylin (glyceryl tricaprylate), sodium chloride, water for injection, [ ] The final formulation is based upon the same technology that has already been approved in oncology (DepoCyt®). The reduction of the triolein and substitution of tricaprylin [ ] was completed to produce 48 hours of drug delivery. The table below directly compares the inactive ingredients between DEPODUR® and DEPOCYT® (FDA approved). As highlighted in the table below, the only component in DEPODUR® that

was not evaluated in the studies conducted for the DEPOCYT® NDA is tricaprylin.

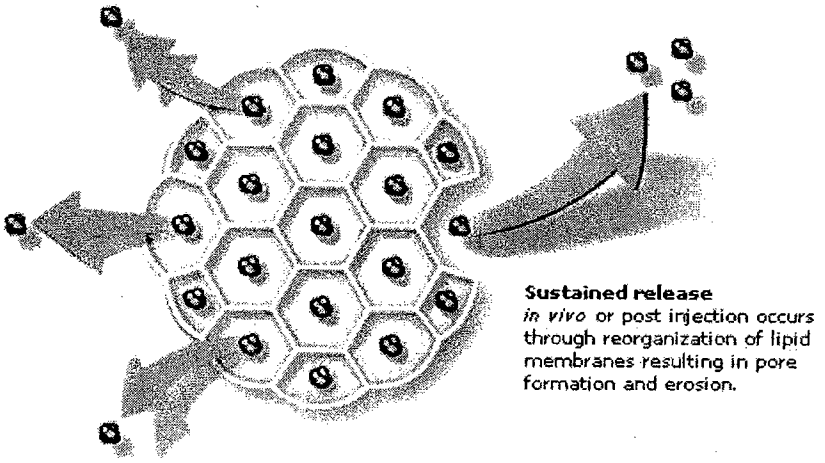
DepoFoam™ Components / Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DEPOCYT® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	5.7
Cholesterol	3.3	4.1
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	1.0
Tricaprylin	0.3	--
Triolein	0.1	1.2

DEPODUR® is supplied in[ ] amber glass vials with [ ] rubber stoppers and [ ] flip-off type caps. [ ]

[ ] The product will be provided in the following fill configurations:

20 mg / 2 ml
15 mg / 1.5 ml
10 mg / 1 ml

The rate of drug release from the liposomal matrix is dependent upon the lipid components, aqueous excipients and manufacturing parameters used in the production of the formulation. The cartoon below was borrowed from the SkyePharma web site:



1 Page(s) Withheld

✓ § 552(b)(4) Trade Secret / Confidential

_____ § 552(b)(5) Deliberative Process

_____ § 552(b)(5) Draft Labeling

NOTE: During the IND review of this NDA, it appeared as though the formulation of the drug product was being adjusted. The composition of these formulations (A-E) are presented in the table below and was described by Dr. Kathleen Haberny during the IND review:

	A	B	C	D	E
	C0401 Formulation in Introductory Statement[A]	C0401 Formulation in Investigators Brochure[B]	D0401 Formulation in Pre-IND Meeting Briefing Book[C]	SKY0401	SKY0401.1
Volume				Not provided	
Particle Size				Not provided	
pH				Not provided	
Morphine Sulfate, USP				10 mg/ml	
Cholesterol				3.3 mg/ml	
Triolein				0.1 mg/dl	
Tricaprylin				0.3 mg/dl	
1,2-dioleoyl-*sn*-glycero-3-phosphocholine (DOPC)				mg/dl	
1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol) (DPPG)				mg/dl	

A: Proposed clinical formulation.
B: Proposed formulation in the IND 52,113, Vol 1; Appendix 4-1.
C: Proposed formulation in pre IND briefing book 195.
D: Original formulation mentioned in IND 52,113.
E: Experimental formulation to adjust release rate.

The final clinical formulation for the drug product described in this NDA has the specifications listed for D.

Formulation SKY0401.1 contained [redacted] of triolein to tricaprylin. This formulation was used in two non-GLP preclinical dog studies and briefly in a human clinical study. This formulation had a slower release rate than SKY0401 *in vitro*. *In vivo*, this formulation had longer morphine release rates after epidural administration in dogs.

Toxicity studies with the DepoFoam™ morphine were done with the clinical formulation; however, the special toxicity studies were done with the formulation A-C, where B and C do not contain tricaprylin.

The impurities in the drug substance were reported by the drug manufacturer and are reproduced in the table below. In addition, SkyePharma analyzed the impurity profile for the same lots of the drug substance independently (also below):

SkyePharma Analytical Results[a] for Impurity Profiles of Lots of Morphine Sulfate, USP, Used in SKY0401 Development

[

]

Impurity Profiles of Lots of Morphine Sulfate, USP,[a] Used in SKY0401 Development

[

]

As presented in the tables above, there are ' impurities in the drug substance

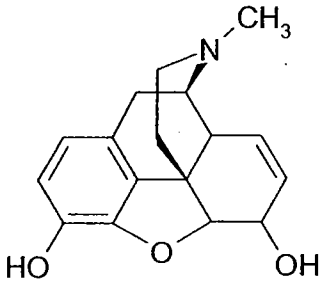
The structures of these impurities are reproduced below:

[redacted]

According to the Sponsor, [redacted] None of the impurities either listed by [redacted] Certificate of Analysis (CoA) or detected by SkyePharma present a safety concern. The specifications for morphine sulfate related substances include unknown related substances (each) at [redacted] area max and total related substances at [redacted] area max.

During communications with [redacted] in January of 2004, the Division became aware that morphine may contain an impurity that [redacted] structure that is a structural alert for mutagenicity. This impurity has been identified by [redacted] examined by [redacted] (Confidential Information from [redacted] This impurity is not listed in the DMF, and therefore, the Sponsor may not have been aware of this issue until early May of 2004. At that time, the Division encouraged the Sponsor to contact their drug substance supplier [redacted] Should this impurity be detected,

further discussions would be required to establish how to limit the levels of the impurity in the pending clinical study. The structures of morphine [ ] are below:



Morphine

Due to the structural alert, the Sponsor should either reduce the specifications for [ ] NMT — in the drug substance or provide adequate qualification of the isolated impurity in a minimal genetic toxicology screen (one *in vitro* mutagenicity study and one *in vitro* chromosome aberration assay) tested up to the limit dose for each assay. If [ ] tests positive in either assay, the sponsor should reduce specifications to NMT — Alternately, the sponsor may conduct carcinogenicity assessment in one species to provide adequate qualification.

The Division has been in discussions with [ ] regarding potential mutagenic impurities [ ] In preparation for a meeting between the Division and [ ] held in January of 2004, the Division received information [ ] rior to that meeting, the Division did not expect either morphine [ ] to have such an impurity [ ] SkyePharma does not appear to be aware of these data. We have informed other sponsors that we will consider a tentative specification in conjunction with a timeline for reaching the required specification. Prior to May 6, 2004, this issue was not previously discussed with the Sponsor.

[ ] contacted Lisa Basham-Cruz, a project manager in the Division in late April 2004 to clarifying a point in the official meeting minutes from the January 2004 meeting. The minutes originally indicated that [ ] However, [ ] Specifically, [ ] tested negative in both the *in vitro* bacterial reverse mutation assay and negative in the *in vitro* chromosome aberration assay. Lisa called the representative from [ ] to determine when they will be submitting the reports to the Division. [ ] indicated that they would send it in 2-3 weeks. Therefore, the data was not available for review prior to the 5/18/2004 action date, thereby leading to a deficiency in the DMF for morphine sulfate.

Route of administration: Injection or instillation into the epidural space only.

Proposed use: SKY0401 is a sustained-release liposome injection of morphine sulfate for administration by the epidural route, at the lumbar or lower thoracic levels, for the treatment of post-operative pain. SKY0401 was designed to provide pain relief for 48 hours without attendant loss of motor, sensory or sympathetic function and with the need for less supplemental analgesia compared to a standard dose of unencapsulated morphine given by epidural injection.

The safety and efficacy of SKY0401 for post-operative pain management have been tested after various surgical procedures, including hip and knee arthroplasty, lower abdominal surgery and cesarean section. These studies are listed in the Sponsor's Table 2.6.1.1 below:

Table 2.6.1.1 Clinical Studies of SKY0401

Clinical Study Number	Pain Model	SKY0401 Doses Studied
C0401-009	Hip arthroplasty	10, 20 and 30 mg
SKY0401-011	Hip arthroplasty	15, 20 and 25 mg
SKY0401-012	Lower abdominal	5, 10, 15, 20 and 25 mg
SKY0401-015	Cesarean section	5, 10 and 15 mg
SKY0401-017	Knee arthroplasty	20, 30 mg

The following table presents the recommended doses of the drug product for each surgical population examined, broken down by surgery type and patient age. No dose adjustment is necessary based on safety and efficacy analyses of subpopulations.

Surgical Populations	Age Categories	
	< 65 Years	≥ 65 Years
Hip and knee orthopedic	20 mg	15 mg
Lower abdominal	20 mg	15 mg
Cesarean section	10 mg	N/A

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

Studies reviewed within this submission:

Study Title	Document #	Module / CTD Description
Pharmacology and Pharmacokinetics		
DepoFoam™ Encapsulated Morphine Sulfate (CO401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog	SkyePharma Report 033-00001.001	4.2.1.1.1 4.2.1.2.1 4.2.2.2.1
Effects of Intravenous Intrathecal and Epidural Administration of Sustained- Release Encapsulated Morphine (CO401) in the Beagle Dog	SkyePharma Report 033-00018.001	4.2.1.1.2 4.2.1.2.2 4.2.2.2.2
An Epidural Injection Bioequivalence Study of — and — Manufacturing Scale SKY0401 in the Beagle Dog	SkyePharma Report 033-00027.001	4.2.2.2.3
Pharmacokinetic Effects of Lidocaine on	SkyePharma Report	

SKY0401 Encapsulated Morphine Formulation Delivered Epidurally in Beagle Dogs	033-00026.001	4.2.2.6.1
Toxicity		
An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog	SkyePharma Report 033-00025	4.2.3.1.1
A Single-Dose Intravenous, Intrathecal and Epidural Injection Drug Interaction Study in Male Beagle Dogs (GLP)	SkyePharma Report No. 033-00028.001	4.2.3.1.2
DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Multiple- Dosing Toxicity Study in the Beagle Dog	SkyePharma Report 033-00009	4.2.3.2.1
Other Toxicity		
Dermal Sensitization Potential of DepoFoam™ - Encapsulated Amikacin (C0201) and Blank DepoFoam™ in Guinea Pigs	SkyePharma Report 032-00006	4.2.3.7.3
29-Day Toxicity Study of DepoFoam™ - Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Mouse	SkyePharma Report 032-00005	4.2.3.7.1
29-Day Toxicity Study of DepoFoam™- Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Dog	SkyePharma Report 032-00004	4.2.3.7.2
21-Day Biocompatibility Study of DepoFoam™ Placebo Administered Subcutaneously to Rats	SkyePharma Report 0333-00020.001	4.2.3.7.6
Ocular Tolerance Study of Various Carrier Formulations After a Single Intravitreal Injection in Rabbits	SkyePharma Report 033-00021.001	4.2.3.7.5
DTC 101 (DepoFoam™ Encapsulated Cytarabine) 4 Cycle Intrathecal Subchronic Toxicity Study in the Rhesus Monkey with a Subsequent Treatment-Free Period	SkyePharma Report 032-00007.003	4.2.3.7.4

Studies not reviewed within this submission: Many of the studies for this NDA were previously reviewed by Dr. Kathleen Habemy during the IND review process; the findings were incorporated into this NDA review and noted in the text. This was done to update the review to the current Good Review Practices (GRPs) within CDER.

Drug Product History:

In 1995, DepoTech Corporation, the manufacturer of the drug product, requested a Pre-IND meeting with the Division of Anesthetic, Critical Care & Addiction Drug Products. This was granted and took place on November 13, 1995.

SkyePharma, the sponsor of the IND, submitted the IND (52,113) on December 6, 1996. The IND was for a new formulation of morphine sulfate with the code name of C0401.

The Sponsor submitted an End of Phase 2 meeting package dated December 10, 1999. Following review of that package, a teleconference was held on January 11, 2000 to notify the Sponsor of the Division's concerns regarding preclinical findings to date. The EOP2 meeting took place on January 13, 2000. According to the meeting minutes of the

EOP2 meeting, the Drs. Rappaport and McCormick clearly indicated the Division's concern that in addition to histological signs of inflammation near the catheter site, there were signs of inflammation distal to the injection site in the 28-day studies (animals were dosed once a week for a total of 4 injections). Dr. McCormick noted that the distal inflammation could have been caused by either the drug product or the DepoFoam™ vehicle. She recommended that the Sponsor complete the single dose toxicology studies with recovery groups to characterize the potential toxicity and reversibility of any histological change following a single-dose treatment without the indwelling catheter.

Dr. McCormick asked the Sponsor how long the DepoFoam™ delivery system remains at the injection site following administration. The Sponsor stated that the DepoFoam™ does break down over time. Specifically, following a subcutaneous administration, some DepoFoam™ was detectable at 10-14 days; however, by 21 days, there was no detectable DepoFoam™ present. Dr. Hertz asked the Sponsor about the histological findings in the monkey study following DepoCyt injection. She was specifically concerned about the evidence for astrocyte activation and mild inflammation in the brain. The Sponsor responded that in that study, the drug was administered via intrathecal lumbar injection. The DepoFoam™ then distributes throughout the CSF via this route. The Sponsor also noted that there were no signs of astrocyte activation or inflammation in the treatment-free period. The meeting minutes go on to reflect the Division's concern and note that the Sponsor must explain to both the Agency as well as the patients who may get this drug why they do not think that the inflammation is due to the drug itself. The Division requested that the Sponsor provide more information about the effects noted and to submit the actual histology slides so the Agency could have a pathology expert examine them. At this point, the Sponsor noted that they would not treat any additional patients until the company has resolved this issue. In the Meeting Minutes, Dr. McCormick "suggested that the sponsor provide a waiver for the acute and chronic toxicology studies. In addition, Dr. McCormick stated that the carcinogenicity was not an issue in this one-time only use product and would not be required." The Sponsor asked if they would have to complete reproductive toxicology studies on the actual drug product (this was not addressed at the time of the meeting). Dr. McCormick indicated that the Division would get back to the Sponsor regarding that issue. She also noted that the Sponsor should provide documentation that the DepoFoam™ particles are not systemically absorbed after epidural administration.

Dr. Kathleen Haberny, the primary pharmacology and toxicology reviewer for the IND responded to the question regarding the adequacy of the nonclinical studies to date to support an NDA. She noted that the IT/IV studies completed to date may not be adequate. Dr. Haberny requested the final study reports for the studies conducted with DepoFoam™ matrix a soon as possible. "Dr. McCormick commented that if the single-dose toxicology study with follow-up is clean and no inflammation is observed in the recovery animals, no additional multiple dose studies would be required."

On April 12, 2000, The Sponsor submitted a proposed preclinical protocol titled "An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog" (N035). This protocol was for a single injection study with both a 72 hour and 21 day recovery period.

The protocol included treatment groups of vehicle control, morphine sulfate, SKY0401 and DepoFoam™ control. Observations included gross pathology and full histopathology according to Dr. Haberny's review.

In her review of IND submission N053 (September 25, 2001, Meeting Request with Questions for the FDA), Dr. Haberny responded to the questions posed by the Sponsor regarding the nonclinical development program the NDA for SKY0401. In that review, Dr. Haberny indicated that the toxicology study on single-dose epidural injection in the dog model adequately addressed the Divisions concerns regarding the histopathological changes in the CNS distal to the injection site. Following review of the single dose studies, Dr. Haberny indicated that the tissue slides from the first study no longer need to be submitted. Further, Dr. Haberny indicated that the proposed study protocol for the "accidental" intrathecal and intravenous administration of SKY0401 will be adequate for evaluation of the potential adverse histopathological changes following these routes of administration.

The pre-NDA meeting was held on March 26, 2003. As discussed during the pre-NDA meeting with the Division, the Sponsor provided evidence demonstrating the release of morphine from their drug product and the safety of the new formulation in the event of accidental administration into the intravenous and epidural route of administration. The Division indicated that the fate of the excipients in the formulation should be characterized for the NDA submission.

On April 24, 2003, a teleconference was held to discuss the Division's request for an evaluation of the fate of the lipid excipients of SKY0401 following injection into the epidural space. The Sponsor maintained that conduction of another study was not necessary given the previous experience with intravenous, intramuscular and intrathecal administration of similar compounds. Further, the Sponsor indicated that the ability to track the lipids in the epidural space was limited. Dr. McGovern indicated that if an animal study was not conducted, the Sponsor "1) prepare a justification, based on literature, about the presence of mechanisms to handle lipids in the epidural space, and 2) demonstrate the movement of lipid out of the epidural space into tissues for metabolism and clearance." The Sponsor submitted their response to this issue on May 15, 2003.

A second teleconference was held between the Sponsor and the Division on June 10, 2003 to discuss the response prepared by the Sponsor. Dr. McGovern inquired whether or not the lipids were endogenous or synthetic. The Sponsor responded that some of the lipids are endogenous and some are synthetic. According to the official memorandum of the telecon, "Dr. McGovern noted that the Sponsor had adequately addressed concerns about the fate of the lipids in the epidural space from a preclinical perspective based upon review of the Sponsor's animal studies, referenced literature and submitted justification."

The NDA was submitted on July 18, 2003.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

Morphine sulfate has been used therapeutically by intravenous, subcutaneous, intramuscular and epidural routes of administration, for many years in the management of pain. It is the most commonly used opioid worldwide and was first approved by the United States (US) Food and Drug Administration (FDA) for intrathecal and epidural administration in 1981 (Duramorph® Preservative-free Injection, Baxter Healthcare, NDA 18-565). Epidural administration of morphine results in analgesia without attendant loss of motor, sensory, or sympathetic function. Compared to the systemic administration of morphine at comparable doses, administration of morphine via the epidural route produces improved analgesia with a longer duration of action.

SKY0401 is manufactured by microencapsulating an aqueous solution of morphine sulfate in multivesicular lipid-based particles (the DepoFoam™ drug delivery system) containing synthetic analogs of common, naturally occurring lipids.

The DepoFoam™ matrix is a proprietary drug delivery system consisting of synthetic analogs of common, naturally occurring lipids that provides sustained release of therapeutic compounds. DepoFoam™ consists of microscopic, spherical particles composed of a honeycomb of numerous nonconcentric internal chambers containing the encapsulated drug in aqueous solution. Each chamber is separated from adjacent chambers by lipid membranes. The liposome particles have a median particle diameter ranging from 17.0 to 23.0 microns with 80% of the particles between 12.0 and 30.0 microns. The particles are suspended in 0.9% sodium chloride solution. Each vial contains morphine sulfate (expressed as the pentahydrate) at a nominal concentration of 10 mg/ml. Inactive ingredients include 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, 1,2- dipalmitoyl-sn-glycero-3-phospho-rac-(1 -glycerol) (DPPG), tricaprylin, and triolein.

The system is designed to provide sustained-release of morphine sulfate to provide post-operative pain control for up to 48 hours after single epidural administration prior to or during surgery. The drug is released from DepoFoam™ particles *in vivo* by a complex mechanism involving reorganization of the barrier lipid membranes allowing release of the drug from the particles. The DepoFoam™ membrane remnants (triglycerides, phospholipids, and cholesterol) are biocompatible and are believed to be cleared through the lymphatic system and metabolized as nutrients.

One of the limitations of epidural analgesia has been the inability to deliver enough opioid to the epidural space to achieve prolonged pain relief without producing excessive side effects. Prolonged pain relief with opioids has been achieved with continuous infusions via epidural catheters or with repeated boluses. Boluses of epidural opioids, however, result in troughs and peaks in intrathecal and plasma concentrations of the opioids, with lower consequent efficacy and a higher incidence of side effects when compared to continuous infusions. In addition, continuous epidural infusions require

specialized equipment, highly trained pain teams, and are associated with complications such as infections and spinal hematomas. A number of the epidural hematomas reported in the literature were associated with the removal of the epidural catheters and caution is especially advised when patients are being concurrently treated with anticoagulant medication such as low molecular weight heparin. Current recommendations are for the removal of the epidural catheter two hours prior to the initiation of low molecular weight heparin, such as enoxaparin. This severely limits the utility of continuous epidural analgesia using a catheter technique in hip replacement surgery, as many surgeons initiate low molecular heparin or other anticoagulants for thromboembolic prophylaxis very early in the postoperative course. SKY0401, when administered as a single epidural dose, does not require the use of epidural catheters or infusion pumps. This simplifies pain management and reduces the risk of infection and spinal hematoma while producing effective analgesia for 48 hours. The simplified technical aspects of this therapy, when compared to continuous infusion should also be associated with reduced cost. Ng et al. demonstrated that at least one or two interventions by the acute pain service were required in 60% of cases to achieve adequate post-operative pain control with epidural catheter infusions. Despite these interventions, one third of patients had their epidural terminated due to inadequate pain control.

Therefore, a formulation of morphine that could provide adequate and continuous pain relief post-operatively for up to 48 hours after a single epidural dose would simplify post-operative pain management, without the need for maintaining a patent epidural catheter, and reduce the need for continuous infusion, epidural PCA and/or repeated parenteral injections as well as minimize episodes of break-through pain. Furthermore, the associated risks and adverse events associated with an indwelling catheter for epidural PCA or bolus epidural injections are non-existent. Thus, the value of prolonged epidural delivery of morphine for post-operative pain is the basis for development of SKY0401.

2.6.2.2 Primary pharmacodynamics

Mechanism of action:

The mechanism of action of morphine pharmacological and toxicological effects is primarily due to high-affinity binding to the mu (μ) opioid receptor, predominantly in the periaqueductal and periventricular gray matter, ventromedial medulla and spinal cord. Binding to the μ-opioid receptor at the supraspinal level is responsible for analgesia, euphoria, respiratory depression and the development of physical dependence. Analgesia at the spinal level, miosis and sedation are mediated in part by action of morphine at the kappa (κ) opioid receptor. Direct μ-opioid receptor mediated effects in the medulla are thought to mediate morphine-induced depression of the cough reflex. Endocrine effects of morphine include increased secretion of anti-diuretic hormone, thyroid stimulating hormone and prolactin, and alterations of glucocorticoid levels, follicle stimulating hormone and luteinizing hormone by effects on corticotrophin releasing factor and gonadotropin releasing hormone (Goodman and Gillman, Pharmacological Basis of Therapeutics, 10th Edition). Chronic treatment with the opioid drugs is known to induce

a state of tolerance to the effects, and therefore abrupt cessation of treatment can lead to withdrawal signs in both animals and humans. Opioid-withdrawal signs after chronic exposure in animals include autonomic (increased pulse and blood pressure, diarrhea, respiratory rate, pupil diameter and body temperature), somatomotor (nociception, neuromuscular reflexes, Straub tail, convulsions) and behavioral symptoms (irritability, eating and drinking sleep, decreased alertness).

Drug activity related to proposed indication:

Morphine produces a wide spectrum of pharmacological effects including analgesia, dysphoria, euphoria, somnolence, respiratory depression, diminished gastrointestinal motility and physical dependence. Opiate analgesia involves at least three anatomical areas of the central nervous system: the periaqueductal gray, periventricular gray matter, the ventromedial medulla and the spinal cord. A systemically administered opiate may produce analgesia by acting at any, all or some combination of these distinct regions. Morphine interacts predominantly with the μ-receptor. The binding sites of opioids are very discretely distributed in the human brain, with high densities of sites found in the posterior amygdala, hypothalamus, thalamus, caudate nucleus, putamen and certain cortical areas. Mu opioid receptors are also found on the terminal axons of primary afferents within lamina 1 and 11 (substantia gelatinosa) of the spinal cord and in the spinal nucleus of the trigeminal nerve.

The Sponsor completed several pharmacology studies to characterize the ability of SKY0401 to produce sustained analgesia:

Study Title: DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog

SkyePharma Report 033-00001
Outside Investigator: T.L. Yaksh, UCSD[1]
Non-GLP Study
C0401 Lot # 96-0030

One of the objectives of this study was to determine the behavioral, physiological and antinociceptive activities of the encapsulated morphine and to compare these effects with that of unencapsulated morphine sulfate. Each dog received the following three test article injections in a 3-ml dose volume (with sufficient time between injections to allow for washout of morphine from the CSF): 5 mg morphine sulfate, 10 mg SKY0401 and 30 mg SKY0401. In addition, two animals also received a 3-ml epidural injection of blank DepoFoam™. Serum and lumbar CSF were collected post-injection until washout. At various times pre- and post-dosing, indices of behavior (arousal, muscle tone and coordination), physiological parameters (body temperature, heart rate, respiratory rate

[1] Yaksh, T.L., Provencher, J.C., Rathburn, M.L., Myers, R.R., Powell, H., Richter, P. and Kohn, F.R. 2000. Safety assessment of encapsulated morphine delivered epidurally in a sustained-release multivesicular liposome preparation in dogs. ***Drug Delivery*** 7:27-36.

and blood pressure) and antinociceptive response were also evaluated. Antinociception was assessed by the application of a heated (62.5°C) probe to shaved lumbar or thoracic areas (1 cm^2 surface area) of the animal's back. Under control conditions this results in a brisk contraction of the underlying musculature in 1 to 3 seconds. The time between application of the probe and muscle contraction was recorded as the latency period. If no contraction had occurred within 6 seconds, the probe was removed and the latency was recorded as 6 seconds. The schedule of test article administration is shown in the following table:

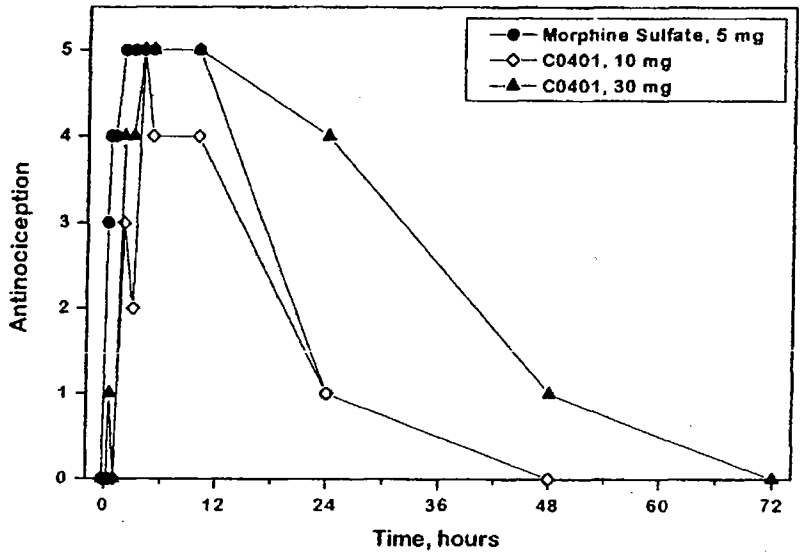
Animal I.D. #	Test Article	Dose*	Study Day
EAT5	1. Morphine sulfate	5 mg	0
	2. C0401	30 mg	2
	3. Blank DepoFoam	3 mL	11
	4. C0401	10 mg	19
JXT5	1. Morphine sulfate	5 mg	0
	2. C0401	30 mg	2
	3. Blank DepoFoam	3 mL	11
	4. C0401	10 mg	19
H6A514	1. Morphine sulfate	5 mg	0
	2. C0401	10 mg	2
	3. C0401	30 mg	12
H6A434	1. Morphine sulfate	5 mg	0
	2. C0401	10 mg	2
	3. C0401	30 mg	12
H6A430	1. Morphine sulfate	5 mg	0
	2. C0401	30 mg	2
	3. C0401	10 mg	12
H6A452	1. Morphine sulfate	5 mg	0
	2. C0401	10 mg	2
	3. C0401	30 mg	12

* All doses were delivered in 3 mL, followed by 0.8 mL 0.9% (w/v) Sodium Chloride for Injection, USP, to flush the port and catheter. The 10 mg C0401 dose was delivered after 1:2 dilution of original vialed suspension with 0.9% (w/v) Sodium Chloride for Injection, USP.

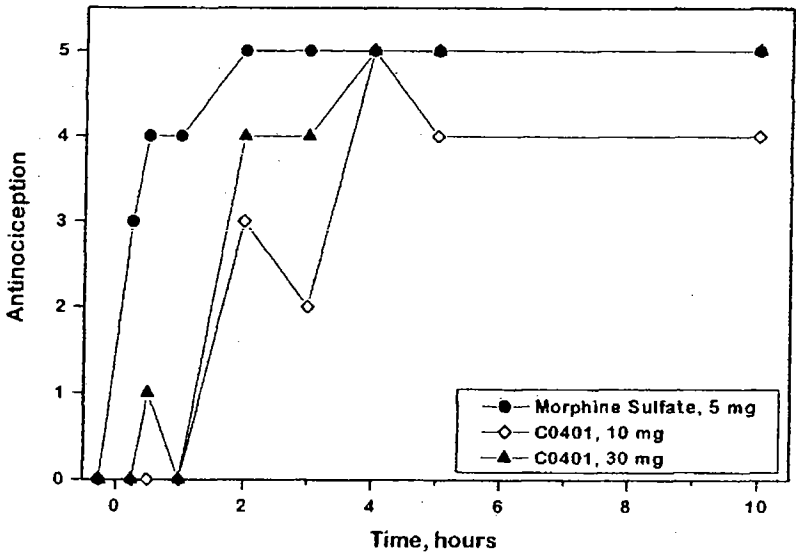
All doses were delivered in 3-ml dose volumes, followed by 0.8 ml saline to flush the port and catheter. The 10 mg SKY0401 dose was delivered after 1:2 dilution (saline) of original SKY0401 suspension.

The results of the study demonstrated that both morphine-containing test articles blocked the skin twitch response to thermal stimulus. The maximum antinociceptive effect (T_{max}) occurred earlier after morphine sulfate (5 mg) administration, with 5 of 5 dogs exhibiting maximum antinociception at 2 hours (note that 1 of the 6 animals used in the experiment was excluded from the analysis because of very high baseline responses throughout most of the study). In contrast, maximum antinociception did not occur until 4 hours after SKY0401 administration (10 or 30 mg). However, antinociception lasted longer after 30 mg SKY0401, with 4 of 5 animals still exhibiting antinociception at 24 hours. In contrast, only 1 of 5 dogs administered morphine sulfate or 10 mg of SKY0401 exhibited antinociception at 24 hours post-dose. Nociceptive responses returned to baseline by 48 to 72 hours in animals administered 30 mg of SKY0401. The antinociceptive effects are depicted in the figure below (extracted from study report):

A.



B.



ure 10. Antinociception: Skin Twitch Assay. Data are number of animals (out of 5 evaluable) exhibiting "criterion antinociception" (%MPE ≥50%). **A.** Full time course **B.** First 10 hours

Study Title: Effects of Intravenous Intrathecal and Epidural Administration of Sustained-Release Encapsulated Morphine (C0401) in the Beagle Dog (SkyePharma Report 033-00018).

The objective of this study was to define the effects of SKY0401 (30 mg/3 ml), administered intravenously (IV), intrathecally (IT), and epidurally (EPI) to adult beagle dogs, on clinical, behavioral, physiological and antinociceptive parameters.

Each of four dogs received an IV bolus injection of SKY0401 (30 mg/ 3 ml) or DepoFoam™ vehicle control article (3 ml) with sufficient time (7 days) between doses to allow for washout. After completion of the IV dosing phase, animals underwent EPI and IT catheter implantation to allow for the procedures described below.

All animals received a bolus injection of 30 mg intrathecal and epidural SKY0401 with at least 7-day washout following each dose (please see the following table). At the completion of the IT/EPI dosing phase, animals received a final IV bolus dose of 30 mg morphine sulfate in saline. The dose volume was 3 ml for each injection.

Animal Number	Treatment Group[a]	Route	Study Day
1	SKY0401	IV	0
	DepoFoam	IV	7
	SKY0401	EPI	21
	SKY0401	IT	29
	Morphine Sulfate	IV	43
2	DepoFoam	IV	0
	SKY0401	IV	7
	SKY0401	EPI	21
	SKY0401	IT	29
	Morphine Sulfate	IV	43
3	SKY0401	IV	0
	DepoFoam	IV	7
	SKY0401	IT	21
	SKY0401	EPI	29
	Morphine Sulfate	IV	43
4	DepoFoam	IV	0
	SKY0401	IV	7
	SKY0401	IT	21

[a] SKY0401 and morphine sulfate doses contained 30 mg morphine sulfate pentahydrate delivered in a 3-mL volume. EPI and IT test article doses were followed by 0.8 mL saline to flush the port and catheter. DepoFoam vehicle was also delivered in a volume of 3-mL.

Throughout all dosing intervals, behavior, motor function and rectal temperatures were monitored daily. Heart and respiratory rates, blood pressures (tail cuff manometer) and

antinociception (thermal-evoked skin twitch) were measured at selected time points. Antinociception was assessed by the application of a heated (62.5°C) probe to shaved lumbar or thoracic areas (1 cm^2 surface area) of the animal's back. Under control conditions this results in a brisk contraction of the underlying musculature in 1 to 3 seconds. The time between application of the probe and muscle contraction was recorded as the latency period. If no contraction had occurred within 6 seconds, the probe was removed and the latency was recorded as 6 seconds.

Blood samples were collected at selected times and prepared as serum for the IT and EPI dosing intervals for determination of free morphine and as plasma for the IV dosing intervals to allow separation of free from encapsulated morphine.

At the end of the study, animals were necropsied to visualize the position of the epidural and intrathecal catheters. An examination of the gross pathology of the spinal cord and dura was performed.

All four animals completed the intravenous SKY0401 and vehicle segments. One of the animals given an intravenous dose of SKY0401 did not receive the full dose and was excluded from this portion of the analysis. One animal developed increasing neurological deficits at 24 hours after intrathecal SKY0401 administration and subsequently died, the reason for the death was determined to be not test article related and will be discussed in the toxicity study review in detail. The neurological findings and death were deemed to be the consequence of misplacement of the IT catheter and not related directly to test article administration. Thus, three animals completed the IT and EPI bolus dosing intervals for SKY0401, and the IV dosing interval for morphine sulfate.

2.6.2.3 Secondary pharmacodynamics

The secondary pharmacodynamics of morphine action in terms of receptor pharmacology has been the subject of several monographs, reviews and texts. The purpose of the following studies was to establish and characterize unique pharmacological syndrome (if any) with morphine delivered in the liposomal drug delivery system. Following is a brief description and conclusion of the studies undertaken by the Sponsor.

Study Title: DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog (SkyePharma Report 033-00001).

One of the objectives of this study was to determine the behavioral, physiological and antinociceptive activities of the compound and results were compared to those of unencapsulated morphine sulfate. Antinociceptive activities were discussed in the primary pharmacodynamics section, behavioral and physiological effects are discussed in this section (study design is same as described above).

All three morphine-containing doses resulted in mild to moderate decreases in arousal state, muscle tone and coordination. However, these behavior effects peaked earlier in most animals for 5 mg morphine sulfate administration (30 to 60 minutes) compared with 10 and 30 mg SKY0401 administration (5 and 10 hours, respectively). Moreover, the time to recovery differed for the various treatments. The animals recovered more quickly after 5 mg morphine sulfate administration; scores were back to near baseline by 10 hours. Behavioral scores returned to baseline by 24 and 48 hours after administration of 10 and 30 mg of SKY0401, respectively.

All three morphine-containing administrations resulted in mild to moderate depression of heart and respiratory rates and a moderate depression of blood pressure. The peak effect on blood pressure occurred earlier, and animals recovered more quickly after 5 mg morphine sulfate administration than after 10 and 30 mg SKY0401 administration. There was a trend toward a more pronounced and a slightly longer lasting effect on physiological measurements after administration of 30 mg of SKY0401 compared with 10 mg of SKY0401. However, all animals recovered by 24 to 48 hours. SKY0401 administration (10 and 30 mg) also resulted in a dose-dependent decrease in body temperature that was lowest at 24 hours and returned to baseline by 48 hours. A decrease in body temperature was not observed at 24 hours after 5-mg morphine sulfate administration.

Parameter	Test Article	Peak Effect	Time to Peak Effect (h)	Time to Recovery (h)
Arousal	Morphine Sulfate - 5 mg	↓↓	0.5	10
	SKY0401 - 10 mg	↓½	5	24
	SKY0401 - 30 mg	↓↓	10	48
Muscle Tone	Morphine Sulfate - 5 mg	↓↓	1	24
	SKY0401 - 10 mg	↓↓	5	24
	SKY0401 - 30 mg	↓↓	5	48
Coordination	Morphine Sulfate - 5 mg	↓½	1	10
	SKY0401 - 10 mg	↓	5	24
	SKY0401 - 30 mg	↓↓	5	48
Heart Rate	Morphine Sulfate - 5 mg	↓	4	24
	SKY0401 - 10 mg	↓	5	24
	SKY0401 - 30 mg	↓	4	48
Resp. Rate	Morphine Sulfate - 5 mg	↓	10	24
	SKY0401 - 10 mg	↓	10	24
	SKY0401 - 30 mg	↓½	10	24
Blood Pressure	Morphine Sulfate - 5 mg	↓↓	2	10
	SKY0401 - 10 mg	↓↓	5	24
	SKY0401 - 30 mg	↓↓	10	24
Body Temp.	Morphine Sulfate - 5 mg	See note	N/A	N/A
	SKY0401 - 10 mg	↓	24	48
	SKY0401 - 30 mg	↓↓	24	48

Note: Body temperature was not measured until 24 hours after dose administration. Therefore, an earlier effect of morphine sulfate may have been missed. N/A = Not Applicable. ↓ = mild. ↓↓ = moderate.

Study Title: Effects of Intravenous Intrathecal and Epidural Administration of Sustained-Release Encapsulated Morphine (C0401) in the Beagle Dog (SkyePharma Report 033-00018).

One of the objectives of this study was to determine the effect of 30 mg morphine sulfate on clinical, behavioral (state of arousal, muscle tone and motor coordination), physiological (heart and respiratory rates, blood pressure and body temperature) and antinociceptive parameters. Antinociceptive activities were discussed in the primary pharmacodynamics section clinical, behavioral (state of arousal, muscle tone and motor coordination), physiological (heart and respiratory rates, blood pressure and body temperature) is described in this section (study design is same as described above).

Intravenous morphine sulfate evoked signs of significant agitation or discomfort, which were not observed after intravenous SKY0401 or vehicle administration. Intravenous injection of SKY0401 and morphine sulfate resulted in quicker onset of the behavioral

effects (5 to 60 minutes) compared with intrathecal or epidural SKY0401 (1 to 3 hours). Behavioral indices returned to baseline values by 3 to 10 hours in animals receiving intravenous morphine sulfate or SKY0401 and by 24 to 48 hours in animals receiving epidural or intrathecal SKY0401. Bradycardia was observed after morphine sulfate and SKY0401 treatments with a peak at 6 hours and recovery to baseline by 24 hours after intravenous administration and 48 to 72 hours after epidural or intrathecal administration. Respiratory rate was moderately reduced after all morphine treatments with recovery to normal by 24 hours. Blood pressure declined moderately after intravenous SKY0401 and morphine sulfate with recovery by 24 hours. Body temperature was unaffected.

Study Title: An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog (SkyePharma Report 033-00025).

The potential acute toxicity following a single lumbar epidural injection of 30 mg SKY0401 in the beagle dog was compared to unencapsulated morphine sulfate (two injections of 15 mg separated by 24 hours). The study was conducted according to current Good Laboratory Practice (GLP) guidelines as described in 21 CFR Part 58. Control animals received either saline or DepoFoam™ placebo. Each animal (6/sex/group) underwent surgery to expose the ligamentum flavum. In the lumbar region at approximately the L6-L7 vertebrae, a needle was inserted to the epidural space and the test or control article was injected.

Day 3 neurological evaluations revealed reduced or absent responses including proprioceptive positioning, hopping/standing on one hind limb, pelvic wheel borrowing, visual/tactile placing reactions (hind limb), righting reaction from lateral recumbency and flexor reflex in several animals treated with unencapsulated morphine sulfate and SKY0401. Disposition/behavior and gait were also abnormal for several of these animals and included decreased activity, difficulty in walking, lateral recumbency, tremors when walking, general weakness and uncoordinated or immobile hind limbs. These observations were considered to be related to the pharmacological properties of morphine. By Day 7, the neurological changes had resolved.

Study Title: DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Multiple-Dosing Toxicity Study in the Beagle Dog (SkyePharma Report No. 033-00009).

The potential toxicity associated with repeated weekly lumbar epidural injections of 30 mg SKY0401 was assessed in beagle dogs. Dogs were surgically implanted with lumbar epidural catheters. Animals (3/sex/group) received SKY0401, DepoFoam™ placebo or saline control on study days 0, 8, 16, and 24 at a constant dose volume of 3-ml. Immediately following administration of the test or control articles, an epidural bolus of saline (0.8 ml) was administered to each dog to flush the port-catheter system. The study was conducted according to current Good Laboratory Practice (GLP) guidelines as described in 21 CFR Part 58.

Clinical observations were recorded twice daily. Food consumption and rectal temperature were monitored daily. Body weights were determined at 4-day intervals throughout the treatment period. Blood samples were obtained from each dog at these same intervals for determination of serum morphine content. CSF samples for morphine determination were collected from each dog on the day of surgery and again at necropsy CSF, urine, and blood samples for clinical chemistry evaluation were obtained from each dog on the day of surgery and on the day of necropsy. Numerical scores for arousal, muscle tone, and coordination were recorded at least twice daily (a.m. and p.m.) for each animal throughout the treatment period. Body temperature, respiratory rate, and blood pressure determinations were conducted immediately prior to each epidural injection, approximately 24 hours after each injection, and on the day of necropsy. Epidural administration of SKY0401 resulted in mild to moderate depression of arousal, muscle tone, coordination and body temperature within 4 to 6 hours of each injection that lasted up to 72 hours. Repeated injections of SKY0401 appeared to result in a decrease in the magnitude of these effects.

Epidural delivery of SKY0401 test article resulted in mild to moderate decrease in mean arousal response scores (postural/behavioral indices of alertness) as compared to scores for animals administered either the DepoFoam™ vehicle or saline control articles. Arousal response was diminished in SKY0401-treated animals within 24 to 48 hours post-injection. This reduced state of alertness returned to normal by the 72-hour post-dose measurement was unrelated to gender, and occurred in most animals with each SKY0401 dosing cycle. Arousal scores for SKY0401- treated recovery animals returned to baseline by 48 hours after administration of the last dose. The DepoFoam™ vehicle and saline control articles each had no effect on arousal throughout the treatment period.

Muscle tone (state of muscle vigor or tension) was decreased within 4 to 6 hours following epidural delivery of SKY0401 test article. Loss of muscle tone associated with SKY0401 administration returned to normal by the 72-hour post-dose measurement and occurred in all animals after each epidural injection. No gender-related effect was evident. The magnitude of effect on muscle tone was diminished after the fourth SKY0401 dose when compared with the first dose. Muscle tone for SKY0401-treated recovery animals returned to baseline by 48 hours after administration of the last dose. Muscle tone remained unchanged throughout the treatment period in animals administered the DepoFoam™ vehicle or saline control articles

A mild to moderate loss of coordination was also observed within 4 to 6 hours after epidural administration of SKY0401 test article. Loss of coordination associated with SKY0401 administration returned to normal by the 72-hours post-dose measurements and was associated in most animals with each epidural injection. The magnitude of this effect diminished with each subsequent SKY0401 dose; such that no evidence of loss of coordination was observed in any SKY0401-treated animal on the day following administration of the last epidural dose (Day 25). Coordination in SKY0401-treated recovery animals returned to normal by 24 hours after administration of the last dose.

Coordination remained unaffected throughout the treatment period in animals administered either the DepoFoam™ vehicle or saline control articles.

Behavioral scores on the day following epidural Delivery of the SKY0401 Test Article:

Time of Evaluation	Mean Behavioral Score [b]					
	Arousal Response		Coordination		Muscle Tone	
	a.m.	p.m.	a.m.	p.m.	a.m.	p.m.
Day -1	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Day 1	-0.2 ± 0.2	-0.2 ± 0.2	2.0 ± 0.3 [c]	1.7 ± 0.2	-2.0 ± 0.3 [c]	-1.8 ± 0.3 [c]
Day 9	-0.8 ± 0.2 [c,d]	-0.2 ± 0.2	0.8 ± 0.5	0.5 ± 0.2	-1.5 ± 0.4 [c]	-0.8 ± 0.2 [d]
Day 17	-0.8 ± 0.2 [c,d]	-0.2 ± 0.2	0.7 ± 0.4 [d]	0.3 ± 0.2	-1.2 ± 0.2 [c]	-0.7 ± 0.2 [d]
Day 25	-1.0 ± 0.0 [c,d]	0.0 ± 0.0	0.0 ± 0.0 [d]	0.0 ± 0.0	-1.0 ± 0.0 [c,d]	-0.5 ± 0.7 [d]

[a] Dogs were administered 3 mL of SKY0401 test article (10 mg morphine/mL) via epidural catheter on study Days 0, 8, 16, and 24.

[b] Mean behavioral score ± Standard Error of the Mean (SEM) for n = 6 animals per evaluation (except for study Day 25, p.m., where n = 2).

[c] Significantly different from respective Day -1 value ($p < 0.05$). All Day -1 mean behavioral scores for the DepoFoam and saline experimental groups were 0.0 ± 0.0.

[d] Significantly different from respective Day 1 value ($p < 0.05$). All Day 1 mean behavioral scores for the DepoFoam and saline experimental groups were 0.0 ± 0.0.

Change in Blood Pressure 24 hrs after Epidural Delivery of Test and Control Articles:

Dose No.	Test/Control Article [a]	Change in Blood Pressure (mm Hg) [b]		
		Systolic	Diastolic	Mean Arterial
1	SKY0401	-36 ± 31	-25 ± 16	-30 ± 20
	DepoFoam	-23 ± 13	-12 ± 8	-21 ± 9
	Saline	-11 ± 25	-6 ± 21	-7 ± 22
2	SKY0401	-44 ± 25	-25 ± 13	-28 ± 17
	DepoFoam	-4 ± 7	-10 ± 10	-6 ± 11
	Saline	10 ± 9	-10 ± 7	-10 ± 7
3	SKY0401	-50 ±18	-26 ± 9 [c,d]	-35 ± 12 [c,d]
	DepoFoam	-3 ± 8	4 ± 5	4 ± 5
	Saline	-10 ± 17	2 ± 7	-1 ± 9
4	SKY0401	-21 ±15 [c]	-7 ± 10	-11 ± 10
	DepoFoam	16 ± 5	15 ± 7	16 ± 6
	Saline	16 ± 9	5 ± 10	6 ± 11

[a] Dogs were administered 3 mL of SKY0401 test article (10 mg morphine/mL), DepoFoam vehicle control article (0 mg morphine/mL), or 0.9% Sodium Chloride for Injection, USP via epidural catheter on study Days 0 (Dose #1). 8 (Dose #2), 16 (Dose #3), and 24 (Dose #4).

[b] Data calculated as pressure prior to dosing minus pressure on the day following dosing. Values represent mean ± SEM for n = 6 animals per evaluation.

[c] Significantly different from respective DepoFoam vehicle control value ($p < 0.05$).

[d] Significantly different from respective saline control value ($p < 0.05$).

Blood pressure data (systolic, diastolic, and arterial pressures) are presented in the above table. Significantly reduced systolic, diastolic, and mean arterial blood pressures were also noted for SKY0401-treated dogs on the day following administration of each dose (study Days 1, 9, 17, and 25). The hypotension associated with epidural administration of SKY0401 appeared unrelated to gender, was transient in nature, and resolved spontaneously prior to administration of each subsequent dose. Heart rate was unaffected by SKY0401 administration.

Change in Body Temperature and Respiratory rate 24 hrs after Epidural Delivery of Test and Control Articles

Dose No.	Test/Control Article [a]	Change in Body Temperature (° Fahrenheit) [b]	Change in Respiratory Rate (breaths/minute) [b]
1	SKY0401	-4.9 ± 0.8 [c,d]	-10 ± 5 [c]
	DepoFoam	0.0 ± 0.2	9 ± 2 [d]
	Saline	-0.1 ± 0.5	-5 ± 2
2	SKY0401	-3.3 ± 0.7 [c,d]	-11 ± 3 [c,d]
	DepoFoam	-0.3 ± 0.2	-4 ± 5
	Saline	-0.2 ± 0.4	-1 ± 7
3	SKY0401	-4.4 ± 0.9 [c,d]	-10 ± 2
	DepoFoam	-0.2 ± 0.3	1 ± 3
	Saline	-0.1 ± 0.5	2 ± 2
4	SKY0401	-3.8 ± 0.4 [c,d]	-5 ± 3
	DepoFoam	0.6 ± 0.2	2 ± 2
	Saline	0.3 ± 0.3	5 ± 5

[a] Dogs were administered 3 mL of SKY0401 test article (10 mg morphine/mL), DepoFoam vehicle control article (0 mg morphine/mL), or 0.9% Sodium Chloride for Injection, USP via epidural catheter on study Days 0 (Dose #1), 8 (Dose #2), 16 (Dose #3), and 24 (Dose #4).

[b] Data calculated as value prior to dosing minus value on the day following dosing. Values represent mean ± SEM for n = 6 animals per evaluation.

[c] Significantly different from respective DepoFoam vehicle control value ($p < 0.05$).

[d] Significantly different from respective saline control value ($p < 0.05$).

Body temperature and respiratory rate data are presented in the above table. Significantly reduced body temperatures and respiratory rates were noted for SKY0401-treated animals on the day following epidural delivery of each dose (study days 1, 9, 17, and 25). These effects appeared unrelated to gender, were transient in nature, and resolved spontaneously prior to administration of each subsequent dose.

2.6.2.4 Safety pharmacology

Formal safety pharmacology studies were not completed by the Sponsor. However, studies evaluating the antinociceptive and toxic effect also evaluated behavioral, neurological and physiological effects. As mentioned in the pharmacodynamic section. Following is a summary of the safety pharmacology studies from the literature. Neurological effects observed after excessively high doses of morphine are lethargy, coma and seizures. Morphine also induces peripheral arteriolar and venous dilatation by stimulating histamine release and blunting reflex vasoconstriction, resulting in hypotension. Morphine-induced reduction in the responsiveness of brain stem respiratory

centers to increased CO_2 tension and depression of pontine and medullary centers mediated by a subpopulation of mu receptors ($\mu 2$) are responsible for respiratory depression, pulmonary edema and respiratory arrest at high morphine doses. Morphine treatment can result in urinary urgency and urinary retention as a result of increased tone of the detrusor muscle and vesical sphincter, and increased tone and amplitude of contractions of the ureter. Dermatological effects, such as pruritus, sweating and cutaneous vasodilation are often reported during treatment with morphine.

Opioid drugs including morphine can induce constipation and delayed gastric emptying. Cowan (1977) demonstrated a dose-related decrease in the rate of passage of a charcoal meal in the intestines of rodents. The gastrointestinal slowing is due to effects on cholinergic, tryptaminergic and encephalinergic receptors in the myenteric plexus of the intestine. Other gastrointestinal effects of morphine are nausea and vomiting, believed to be a result of direct stimulation of the chemoreceptor trigger zone for emesis in the area postrema of the medulla, and spasm of the sphincter of Oddi with increased pressure in the biliary tract.

2.6.2.5 Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies of SKY0401 were not conducted.

Discussion and Conclusion:

Morphine is a commonly used opioid and was approved by the FDA for intrathecal and epidural administration in 1981. The need for prolonged epidural delivery of morphine for post-operative pain was the basis for development of SKY0401.

SKY0401 (also referred to as C0401, DepoFoam™ encapsulated morphine sulfate, sustained-release encapsulated morphine, DepoMorphine™, CTM, morphine liposome injection and morphine sulfate sustained-release liposome injection) is a sterile, non-pyrogenic, white to off-white aqueous suspension of multivesicular lipid-based particles (DepoFoam™ drug delivery system) containing morphine sulfate, USP. It is intended to provide sustained release of the active agent, morphine, following epidural administration. The liposome particles have a median particle diameter ranging from 17.0 to 23.0 microns with 80% of the particles between 12.0 and 30.0 microns. The particles are suspended in 0.9% sodium chloride solution. Each vial contains morphine sulfate (expressed as the pentahydrate) at a nominal concentration of 10 mg/ml. Inactive ingredients include 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC), cholesterol, 1,2-dipalmitoyl-sn-glycero-3-phospho-rac-(1 -glycerol) (DPPG), tricaprylin, and triolein.

The pharmacology and toxicology of morphine sulfate is well known and widely reported in the published literature (Gutstein and Akil 2001). As mentioned above morphine sulfate is approved and marketed in intravenous, epidural and intrathecal formulations (e.g., Astramorph/PF, Duramorph) as well as in oral tablet and solution (e.g., Roxanol)

and suppository (e.g., RMS Suppositories CII) forms. SKY0401 is a sustained-release epidural morphine formulation using DepoFoam™ technology in the management of post-operative pain. The DepoFoam™ drug delivery system is also approved and marketed in the intrathecal cytarabine liposome (DepoCyt®) treatment of lymphomatous meningitis. The DepoFoam™ components include phospholipids (DOPC and DPPG) and neutral lipids (cholesterol, triolein and tricaprylin) that are endogenous compounds. Drug distribution studies in rats found that these compounds when given intrathecally were metabolized and excreted in a manner indistinguishable from the endogenous lipids.

The antinociceptive effects of SKY0401 (DepoFoam™ formulation of Morphine Sulfate) administered via epidural, intravenous, and intrathecal routes were studied in the beagle dog. For these studies, adult beagle dogs were prepared with chronic epidural and intrathecal catheters for dose administration and CSF collection, respectively. The dosing epidural catheters were inserted into the epidural space at the L7-S1 level and passed rostrally to lie at the L1-L2 level in the epidural space. The sampling intrathecal catheters were inserted into the cisterna magna and passed caudally to lie at the L3-L4 level of the spinal cord. This model has been used extensively for nonclinical evaluation of spinally active analgesics (Sabbe et al., 1993; Yaksh et al., 1994). The antinociceptive effects of intravenous unencapsulated morphine sulfate (30 mg) were compared with those of SKY0401 (30 mg) administered by the intravenous, intrathecal and epidural routes in beagle dogs. The maximum effect and duration of antinociception were similar when the intravenous route (onset 5 to 60 minutes; duration gave the compounds 6 to 10 hours). By contrast, SKY0401 administered by either the epidural or intrathecal routes produced antinociception, which peaked later at 3 to 6 hours and lasted 24 to 48 hours.

The clinical, behavioral, physiological and antinociceptive effects of SKY0401 were more or less similar in both encapsulated and unencapsulated morphine sulfate. However, with the encapsulated morphine sulfate, SKY0401, the time of onset of action and the duration of action were longer than the unencapsulated injection. Mild to moderate lowering of the mean arousal time, muscle tone and coordination were seen with all different dosage of the morphine sulfate administered. Behavioral score were returned to base line by 48 hrs. Statistically significant decrease in body temperature, heart and respiratory rate and the blood pressure were noted for SKY0401-treated animals on the day following epidural delivery of each dose (Study Days 1, 9, 17, and 25 all animals recovered by 24-48 hrs. The effect were not gender specific were transient in nature.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Type of Study Study Title	Test System	Method of Administration	Testing Facility	Study Number	Location*
Safety Pharmacology					
Not Applicable; see Secondary Pharmacodynamics					
Pharmacodynamic Drug Interactions					
Not Applicable					

* Not applicable for an electronic submission.

Table 2.6.3.2.1 Primary Pharmacodynamics

Test Article: morphine liposome injection (SKY0401)

Study Title: DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog

Species	Method of Administration (vehicle/formulation)	Doses	Gender and No. per Group	Noteworthy Findings	Study Number[a]
Dog Beagle	Epidural Injection DepoFoam Lot No. 96-0029	3 mL	2 males[b]	• None	033-00001
	Epidural Injection Morphine Sulfate Lot No. VS-184	5 mg in 3 mL	6 males[b]	• Blocked thermal-evoked skin twitch response, maximum response within 2 hr, lasting 24 hr in 1 of 5 dogs evaluated	
	Epidural Injection SKY0401[c] Lot No. 96-0030	10 mg in 3 mL[d]	6 males[b]	• Blocked thermal-evoked skin twitch response, maximum response within 4 hr and lasting 24 hr in 1 of 5 dogs evaluated	
	Epidural Injection SKY0401[c] Lot No. 96-0030	30 mg in 3 mL	6 males[b]	• Blocked thermal-evoked skin twitch response, maximum response within 4 hr and lasting 24 hr in 4 of 5 dogs evaluated. Recovery in 48 to 72 hr.	

[a] Study was conducted at University of California, Department of Anesthesiology Research, San Diego, CA. (Study No. TY-96-024, finalized November 22, 1996)

[b] Dogs were prepared with chronic epidural and intrathecal catheters for dose administration and CSF collection. Each animal received the three test article injections with sufficient time between injections to allow for washout of morphine from the CSF.

[c] DepoFoam Encapsulated Morphine Sulfate, 10 mg/mL morphine sulfate.

[d] The 10 mg SKY0401 dose was delivered after 1:2 dilution of original SKY0401 suspension with saline.

Table 2.6.3.2.2 Primary Pharmacodynamics

Test Article: morphine liposome injection (SKY0401)

Study Title: Effects of Intravenous, Intrathecal and Epidural Administration of Sustained–Release Encapsulated Morphine (C0401) in the Beagle Dog

Species	Method of Administration (vehicle or formulation/dose/lot no.)	Gender and No. per Group	Noteworthy Findings	Study Number
Dog Beagle	Intravenous (IV), Intrathecal (IT), Epidural (EPI) Injection DepoFoam (3 mL) Lot No. 96-0029 SKY0401 30 mg in 3 mL Lot No. 97-0004 Morphine Sulfate (MS) 30 mg in 3 mL Lot No. VS-184	Male 4 per group	• Antinociceptive effect (block of skin twitch response): IV-MS<IV-SKY0401<IT-SKY0401<EPI-SKY0401 • Duration of antinociceptive effect: IV-SKY0401<IV-MS<IT-SKY0401<EPI-SKY0401	033-00018

Study was conducted at University of California, Department of Anesthesiology Research, San Diego, CA. (Study No. TY-97-001, finalized July 28, 1998)

Table 2.6.3.3.1 Secondary Pharmacodynamics

Test Article: morphine liposome injection (SKY0401)

Study Title: DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog

Species	Method of Administration (vehicle or formulation)	Doses	Gender and No. per Group	Noteworthy Findings	Study Number [a]
Dog Beagle	Epidural Injection DepoFoam Lot No. 96-0029	3 mL	2 males[b]	• No behavioral or physiological effects after injection	033-00001
	Epidural Injection Morphine Sulfate Lot No. VS-184	5 mg in 3 mL	6 males[b]	• Moderate decrease in arousal, muscle tone, and motor coordination peaked at 30 to 60 minutes with recovery by 10 hours post-dose • Mild to moderate depression of heart and respiratory rates and moderate depression of blood pressure with recovery by 24 to 48 hours post-dose	
	Epidural Injection SKY0401[c] Lot No. 96-0030	10 mg in 3 mL[d]	6 males[b]	• Moderate decrease in arousal, muscle tone, and motor coordination peaked at 5 to 10 hours with recovery by 24 hours post-dose • Mild to moderate depression of heart and respiratory rates, moderate depression of blood pressure and decrease in body temperature with recovery by 24 to 48 hours post-dose	
	Epidural Injection SKY0401[c] Lot No. 96-0030	30 mg in 3 mL	6 males[b]	• Moderate decrease in arousal, muscle tone, and motor coordination peaked at 5 to 10 hours with recovery by 48 hours post-dose • Mild to moderate depression of heart and respiratory rates, moderate depression of blood pressure and decrease in body temperature with recovery by 24 to 48 hours post-dose	

[a] Study was conducted at University of California, Department of Anesthesiology Research, San Diego, CA. (Study No. TY-96-024, finalized November 22, 1996)
[b] Dogs were prepared with chronic epidural and intrathecal catheters for dose administration and CSF collection. Each animal received the three test article injections with sufficient time between injections to allow for washout of morphine from the CSF.
[c] DepoFoam Encapsulated Morphine Sulfate, 10 mg/mL morphine sulfate.
[d] The 10 mg SKY0401 dose was delivered after 1:2 dilution of original SKY0401 suspension with saline.

Table 2.6.3.3.2 Secondary Pharmacodynamics

Test Article: morphine liposome injection (SKY0401)

Study Title: Effects of Intravenous, Intrathecal and Epidural Administration of Sustained-Release Encapsulated Morphine (C0401) in the Beagle Dog

Species	Method of Administration (vehicle or formulation/dose/lot no.)	Gender and No. per Group	Noteworthy Findings	Study Number
Dog Beagle	Intravenous (IV), Intrathecal (IT), Epidural (EPI) Injection DepoFoam (3 mL) Lot No. 96-0029 SKY0401 30 mg in 3 mL Lot No. 97-0004 Intravenous (IV) Injection Morphine Sulfate (MS) 30 mg in 3 mL Lot No. VS-184	Male 4 per group	• All morphine treatments resulted in mild behavioral depression, decrease in arousal, muscle tone and coordination and bradycardia. • The duration of behavioral and physiological effect was consistent with plasma exposure and spinal opioid administration. • Behavior depression: IV-MS>IT-SKY0401>IV-SKY0401>EPI-SKY0401 • Time to onset of behavior response: IV-MS<IV-SKY0401<IT-SKY0401<EPI-SKY0401 • Duration of behavior depression: IV<10 hr.; EPI and IT was 48 to 72 hrs • Antinociceptive effect (block of skin twitch response): IV-MS<IV-SKY0401<IT-SKY0401<EPI-SKY0401 • Duration of antinociceptive effect: IV-SKY0401<IV-MS<IT-SKY0401<EPI-SKY0401 • Bradycardia (duration): IV-MS<IV-SKY0401≈EPI-SKY0401<IT-SKY0401 • Time of peak decrease in heart rate was 360 minutes. Recovery to baseline occurred 24 hours after IV and 48-72 hrs after EPI and IT. • Decreases in blood pressure were observed after IV-MS and IV-SKY0401. • Respiratory rates, 180 to 600 minutes after dose administration, were moderately decreased after all morphine treatments. The magnitude was greatest for IT-SKY0401. • Decrease in body temperature was measured after IT-SKY0401. Body temperature returned to baseline by 24 hrs. • IV-vehicle had no effect on general behavior, heart rate, respiratory rate, blood pressure, body temperature or skin twitch response.	033-00018

Study was conducted at University of California, Department of Anesthesiology Research, San Diego, CA. (Study No. TY-97-001, finalized July 28, 1998)

Table 2.6.3.3.3 Secondary Pharmacodynamics

Test Article: morphine liposome injection (SKY0401)

Study Title: An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog (GLP)

Species	Method of Administration (vehicle or formulation/dose/lot no.)	Gender and No. per Group[4]	Noteworthy Findings	Study Number
Dog Beagle	Epidural Injection (3 mL) Saline: Lot Nos. WOC02B3 WOC13B1 Morphine Sulfate: 2 x 15 mg Lot No. MS003 SKY0401: 30 mg/3 mL Lot No. 99-0007 Vehicle Control: DepoFoam Lot No. 00-4006	3 Males 3 Females Recovery Animals 3 Males 3 Females	• Reduced neurological and decreased behavioral observations were consistent with the pharmacological properties of morphine. • All neurological and behavioral deficits resolved in recovery animals.	033-00025

Study was conducted at [redacted]

[4] Three animals/group were euthanized on Day 4 and the remaining animals were euthanized on Day 22.

Table 2.6.3.3.4 Secondary Pharmacodynamics

Test Article: morphine liposome injection (SKY0401)

Study Title: DepoFoam Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Multiple-Dosing Toxicity Study in the Beagle Dog (GLP)

Species	Method of Administration (vehicle/formulation)	Gender and No. per Group	Noteworthy Findings	Study Number
Dog Beagle	Epidural (EPI) 3 mL Injection SKY0401: 30 mg / 3 mL Lot No. 96-0030 Vehicle Control: DepoFoam Lot No. 96-0029 Saline: Lot No. 16-416-DK (Abbott Labs)	3 Males 3 Females Recovery Animals 1 Male 1 Female	• No effects were seen in the saline or DepoFoam treated control animals. • Reduced neurological and decreased behavioral observations were consistent with the pharmacological properties of morphine. -proprioceptive positioning -hemi-hopping/standing -pelvic wheel barrowing -visual/tactile placing reactions -righting reaction from lateral recumbency -flexor reflex -decreased activity and general weakness -difficulty in walking, including tremors, uncoordinated or immobile hindlimbs -lateral recumbency • All neurological and behavioral deficits resolved in recovery animals.	033-00009

Study was conducted at the University of California, Dept. of Anesthesiology Research, San Diego, CA. (Study Report No. TY-96-023, finalized March 21, 1997).

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The objectives of the pharmacokinetics studies were to evaluate a single, epidural injection of SKY0401in the beagle dog; (2) to compare the pharmacokinetics of single intravenous, intrathecal, or epidural injections of SKY0401 in the beagle dog; (3) to compare the pharmacokinetics of SKY0401 manufactured at pilot ( ) versus commercial ( ) scales; and (4) to evaluate the effects of co-administration of lidocaine/epinephrine on the pharmacokinetics of SKY0401.

For these studies adult beagle dogs were prepared with chronic epidural and/or intrathecal catheters for dose administration and CSF collection, respectively. The sampling intrathecal catheters were inserted into the cisterna magna and passed caudally to lie at the L3-L4 level of the spinal cord. The dosing epidural catheters were inserted into the epidural space at the L7-S1 level and passed rostrally to lie at the L1-L2 level in the epidural space. This model has been used extensively for non-clinical evaluation of spinally active analgesics.

2.6.4.2 Methods of Analysis

Concentrations of morphine in serum and CSF samples were quantified using a commercially available radioimmunoassay (RIA) kit used for screening of human serum (Coat-A-Count IRI Serum Morphine, Diagnostics Products Corporation, Los Angeles, CA). The RIA method was not validated. The assay method used a dog serum standard curve typically consisting of 250, 75, 25, 10, 5, 2.5 and 0 ng/ml. A 5 ng/ml standard (n=5) was assayed at the end of each individual animal's analysis. The criteria for the 5 ng/ml check standard to pass was that the recovery was between [ ] with RSD [ ]%. The limit of quantitation (LOQ) in dog serum and CSF was estimated to be approximately 2.5 ng/ml based on the lowest concentration of the standard curve.

Samples (1 ml serum) for the [ ] method of morphine analysis were spiked with 200 ng of D^3-morphine, and acetonitrile was added to precipitate protein. The samples were centrifuged and 0.5 ml was applied to [ ] cartridges. The cartridges were washed twice with 1 ml 5 mM K_2CO_3, pH 9.3 and then with 1 ml water. Morphine was then eluted with 0.5 ml of 5% acetonitrile in 30 mM K_2HPO_4, pH 2.0. Aliquots of the eluate were [ ] Morphine was detected as an ion at m/z 286.2 and D^3-morphine, the internal standard, is detected as the ion at m/z 289.3. The method accurately and precisely measures morphine. The [ ] method was not validated. The method used [ ] deuterated morphine as an internal standard. The LOQ was —ng/ml in dog serum.

2.6.4.3 Absorption

Study Title: DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog (Study #033-00001).

This study evaluated the pharmacokinetics of morphine in lumbar cerebrospinal fluid (CSF) and serum of beagle dogs receiving a single, epidural injection of SKY0401 (10 or 30 mg SKY0401 manufactured at pilot scale) or unencapsulated morphine (5 mg unencapsulated morphine sulfate).

Pharmacokinetic parameters are summarized below for CSF and serum. Morphine concentrations in lumbar CSF peaked within 5 minutes after epidural administration of a 5 mg bolus of unencapsulated morphine and declined thereafter to below the detection limit (2.5 ng/ml) by 24 to 48 hours post-dose. By contrast, after administration of 10 or 30 mg SKY0401, morphine concentrations in lumbar CSF peaked at approximately 3 to 11 hours post-dose and declined to below the detection limit at 96 and 144 hours post-dose, respectively. Peak morphine concentrations in CSF were approximately 3-fold lower after SKY0401 (30 mg) administration compared with morphine sulfate administration at one-sixth the dose. Morphine pharmacokinetics in serum after SKY0401 or morphine sulfate administration were similar to those observed in lumbar CSF, except that serum concentrations were approximately 150- to 300-fold lower at the peak.

Pharmacokinetics of Morphine in Lumber CSF after Epidural Administration of SKY 0401 or Morphine Sulfate to the Beagle Dog

Parameter [1,2]	Morphine sulfate (5 mg)	SKY0401 (10 mg)	SKY0401 (30 mg)
t_{max} (h)	0.08	2.67	10.67
C_{max} (ng/mL)	34,942 ± 5,578	10,730 ± 2,888	14,483 ± 3,438
$t_{1/2}$ (h)	3.51 ± 0.19	7.45 ± 0.37	9.98 ± 0.60
$AUC_{0-\infty}$ (ng • h/mL)	17,682 ± 1,440	163,181 ± 65,336	236,998 ± 39,750
$AUMC_{0-\infty}$ (ng • h^2/mL)	13,307 ± 1,547	1,552,695 ± 696,600	3,074,651 ± 609,508
MRT (h)	0.77 ± 0.10	8.92 ± 0.76	12.81 ± 1.02

[1] t_{max} was determined from the mean curve, which was a plot of the mean values at each time point.
[2] All other parameters shown above represent the Mean ± SEM of parameter estimates from individual animals.

Pharmacokinetics of Morphine in the Serum after Epidural Administration of SKY0401 or Morphine Sulfate to the Beagle Dog

Parameter [1,2]	Morphine sulfate (5 mg)	SKY0401 (10 mg)	SKY0401 (30 mg)
t_{max} (h)	0.03	2.67	10.67
C_{max} (ng/mL)	230 ± 30.2	34.7 ± 6.5	59.1 ± 4.8
$t_{1/2}$ (h)	0.83 ± 0.15	4.14 ± 0.57	10.72 ± 2.18
$AUC_{0-\infty}$ (ng • h/mL)	129 ± 16.4	312 ± 31.1	1,100 ± 89.9
$AUMC_{0-\infty}$ (ng • h^2/ mL)	134 ± 38.2	2,416 ± 426	17,779 ± 3,473
MRT (h)	0.94 ± 0.16	7.52 ± 0.73	16.18 ± 3.04

[1] t_{max} was determined from the mean curve, which was a plot of the mean values at each time point.
[2] All other parameters shown above represent the Mean ± SEM of parameter estimates from individual animals.

The plasma pharmacokinetics of morphine after injection of SKY0401 or morphine sulfate via intravenous, intrathecal, and epidural routes are summarized in the table below. Plasma morphine concentrations peaked within 5 min after intravenous dosing of either SKY0401 or morphine sulfates and became undetectable by 24 hours. The elimination half-life of morphine in plasma after intravenous administration of morphine sulfate was approximately 2 hours.

After epidural and intrathecal injection of SKY0401, serum morphine levels reached a peak at approximately 4 and 6 hours, respectively, and fell below the limit of detection (LOD = [redacted] ng/ml) by 48 hours. Peak serum morphine levels were approximately 2-fold lower after intrathecal administration of SKY0401 compared with epidural administration of SKY0401 at the same dose. Serum morphine concentrations declined thereafter with roughly first-order kinetics ($t_{1/2}$, of approximately 10 hours) for both intrathecal and epidural dose administrations.

Mean SKY0401 and Morphine Sulfate Pharmacokinetics Parameters After Intravenous, Intrathecal and Epidural Administration to Male Beagle Dogs

	Treatment			
	Intravenous morphine sulfate	**Intravenous SKY0401**	**Intrathecal SKY0401**	**Epidural SKY0401**
Dose (mg)	30	30	30	30
C_{max} (ng/mL)	788 ± 89	377 ± 39	39.3 ± 17.8	78.2 ± 6.1
t_{max}(h)	0.08 ± 0.00	0.08 ± 0.00	6.3 ± 2.0	4.0 ± 1.0
$t_{1/2}$(h)	2.3 ± 0.7	ND	9.7 ± 3.2	10.3 ± 3.3
$AUC_{(0-\infty)}$ (ng • h/mL)	1098 ± 120	ND	727 ± 84	1002 ± 150
$AUMC_{0-\infty}$ (ng • h^2/mL)	2,312 ± 985	ND	12,195 ± 2,563	14,235 ± 4,637
MRT (h)	2.0 ± 0.6	ND	17.7 ± 4.9	13.3 ± 3.0

ND = Not determined; no data or insufficient data to perform PK analysis.

Effects of Intravenous, Intrathecal and Epidural Administration of Sustained-Release Encapsulated Morphine (SKY0401) in the Beagle Dog (Study # 033-00018)

Study Title: An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog (SkyePharma, Report 033-00025).

Animals (6 dogs/sex/treatment) received epidural injections of saline (3 ml), SKY0401 (30 mg) or DepoFoam™ (3 ml) on Day 1 or morphine sulfate (15 mg) on Days 1 and 2.

The dogs underwent surgery to expose the ligamentum flavum and permit visual confirmation of injection into the epidural space. In the lumbar region at approximately the L6-L7 vertebrae, a needle was inserted into the epidural space and the test article or control article was injected over an approximately 15 to 30 second period. The needle was flushed with 0.35 ml of 0.9% saline. The needle was removed and the incision site was closed. Blood samples were taken pre-dose and at 24 hours after the dose (and only after the first dose of morphine sulfate). Serum was prepared and analyzed for morphine by RIA (LOQ — ng/ml).

Morphine was detected in the plasma of 30 mg SKY0401-treated animals 24 hours following administration (10 of 12 dogs, 9.97 ± 5.64 ng/ml). Two dogs treated with SKY0401 were below the LOQ of the assay at 24 hours post-dose. Morphine was not detected in the serum of morphine sulfate-treated animals 24 hours following administration.

Study Title: An Epidural Injection Bioequivalence Study of [ ] Manufacturing Scale SKY0401 in the Beagle Dog

This was a randomized, 2-way cross-over study in 6 male beagle dogs to assess bioequivalence after a single epidural injection of two SKY0401 formulations manufactured at pilot [ ] versus commercial [ ] scales. SKY0401 manufactured at [ ] scale was used in the phase 3 clinical trials, while material manufactured at the [ ] scale was used in the initial Phase 1 and 2 studies.

Examination of the serum concentration profiles showed that both SKY0401 formulations delivered the drug over 24 hours post-dose in beagle dogs. Peak serum concentrations of morphine were reached at 3 hours when either treatment was administered. The mean C_{max} and AUC_{0-4} values are presented in the table below.

Pharmacokinetics of [ ] in the male Beagle Dog

	SKY0401 Lots Tested	
	— SKY0401 (Lot 99-0007)	— SKY0401 (Lot 00-4104)
Parameter		
C_{max} (ng/mL)	166 [557 - 2043][a]	103 [500 - 3188]
AUC_{0-t} (ng • hr/mL)	883 [56 - 468]	1057 [53 - 294]

[a] Values shown in brackets are minimum and maximum values.

The differences in the mean C_{max}, and AUC_{0-t} between the two treatments may have been related to high inter-animal variability and small sample size. The bioequivalence of the two manufacturing scale SKY0401 formulations could not be determined conclusively in this experiment.

Study Title: A Bolus Epidural Multiple-Dosing Toxicity Study in the Beagle Dog (SkyePharma Report 033-00009).

Morphine concentrations in serum after repeated weekly lumbar epidural injections of 30 mg SKY0401 were assessed in beagle dogs. Dogs were surgically implanted with lumbar epidural catheters as described above. Animals (3/sex/group) received SKY0401, DepoFoam™ placebo or saline control on study days 0, 8, 16, and 24 at a constant dose volume of 3-ml. Immediately following administration of the test or control articles, an epidural bolus of saline (0.8 ml) was administered to each dog to flush the port-catheter system.

Morphine concentrations in serum and cerebral spinal fluid (CSF) of all SKY0401-treated animals at 24 hours after dosing ranged from 3.7 to 10.1 ng/ml in, males and from 9.3 to 43.5 ng/ml in females. Morphine concentrations in cisternal CSF of SKY0401-treated animals at 24 hours after epidural administration, ranged from 165.6 to 583.6 ng/ml morphine was not detected in the CSF of any animals euthanized 7 to 9 days after administration of the final (fourth) SKY0401 dose (recovery animals).

2.6.4.4 Distribution

Formal distribution studies following DEPODUR™ administration were not completed.

2.6.4.5 Metabolism

Formal metabolism studies following DEPODUR™ administration were not completed.

2.6.4.6 Excretion

Formal excretion studies following DEPODUR™ administration were not completed.

2.6.4.7 Pharmacokinetic drug interactions

Study Title: Pharmacokinetic Effects of Lidocaine on SKY0401 Encapsulated Morphine Formulation Delivered Epidurally in Beagle Dogs (033-00026).

The objective of this study was to assess the in vivo interaction of lidocaine/epinephrine on the release of morphine sulfate from epidural administration of SKY0401 by evaluating serum pharmacokinetics of morphine. The procedures were designed to mimic the study drug administration procedures as may occur in clinical practice.

The systemic exposure of morphine sulfate was measured in three groups of morphine naive male dogs after epidural administration of 3 ml SKY0401 (equivalent to 30 mg of morphine sulfate). The administration modes studied and results obtained are summarized below. After epidural injection of SKY0401 following Procedure 1, serum morphine levels reached a peak at approximately 2 hours (T_{max}) with a t½ of 25 hours, whereas in Procedures 2 and 3, the T_{max}, AUC and t½ were shortened (see table below). In all treatment procedures, serum morphine concentrations declined with first-order kinetics. Area under the curve was equivalent no matter which treatment procedure was used. However, of the three regimens that mimic clinical practice, only Procedure 2 demonstrated a potential for interaction between lidocaine/epinephrine and SKY0401. The administration of lidocaine/epinephrine immediately prior to dosing with SKY0401 (Procedure 2) resulted in a 2.5-fold to 3.0-fold increase in C_{max}, of morphine occurring 5 minutes post-SKY0401 injection, compared to Procedure I (no lidocaine/epinephrine injection) and Procedure 3 (flushing the catheter with saline after the lidocaine/ epinephrine test dose and waiting 15 minutes), respectively.

Flushing the epidural catheter with saline to remove residual lidocaine/epinephrine, then incorporating a 15-minute wait period prior to SKY0401 administration or not using lidocaine/epinephrine as a test dose prevents the early release of morphine sulfate from SKY0401 in native dogs.

Mean Pharmacokinetic Parameters of Naïve Dogs after Epidural Dosing of 30 mg SKY0401

Parameter (units)	Epidural Dosing Procedures		
	Procedure 1 n=2 Mean (range)	**Procedure 2** n=4 Mean ± SD	**Procedure 3** n=5 Mean ± SD
	• 1.8 mL saline • 3 mL SKY0401 (30 mg) • flush 0.75 mL saline	• 1.8 mL lidocaine[a] • wait 3 min • 3 mL SKY0401 (30 mg) • flush 0.75 mL saline	• 1.8 mL lidocaine[a] • flush with 1 mL saline • wait 15 min • 3 mL SKY0401 (30 mg) • flush 0.75 mL saline
C_{max} (ng/mL)	71.7 (46.1 – 97.3)	180 ± 29	59.1 ± 25.0
t_{max} (h)	2.0 (2.0 – 2.0)	0.08 ± 0.00	0.62 ± 0.39
$t_{1/2}$ (h)	25.2 (17.9 – 32.6)	12.0 ± 3.2	11.2 ± 6.9
$AUC_{0-\infty}$ (ng • h/mL)	607 (536 –677)	791 ± 362	578 ± 254

[a] Xylocaine®-MPF – 1.5% lidocaine with 1:200000 epinephrine for injection (AstraZeneca)

2.6.4.8 Other Pharmacokinetic Studies

N/A

2.6.4.9 Discussion and Conclusions

Morphine, injected into the epidural space, is rapidly absorbed into the general circulation. Absorption is so rapid that the plasma concentration-time profiles closely resemble those obtained after intravenous or intramuscular administration. Peak plasma concentrations averaging 33-40 ng/ml (range 5-62 ng/ml) are achieved within 10 to 15 minutes after administration of 3 mg of morphine. Plasma concentrations decline in a multiexponential fashion. The terminal half-life is reported to range from 39 to 249 minutes (mean of 90 ± 34.3 min) and, though somewhat shorter, is similar in magnitude as values reported after intravenous and intramuscular administration (1.5-4.5 h). CSF concentrations of morphine, after epidural doses of 2 to 6 mg in postoperative patients, have been reported to be 50 to 250 times higher than corresponding plasma concentrations. The CSF levels of morphine exceed those in plasma after only 15 minutes and are detectable for as long as 20 hours after. Following the epidural injection of 2 mg of morphine, approximately 4% of the dose reached the CSF. This corresponds to the relative minimum effective epidural and intrathecal doses of 5 mg and 0.25 mg, respectively. The disposition of morphine in the CSF follows a biphasic pattern, with an early half-life of 1.5 h and a late phase half-life of about 6-h. Morphine crosses the dura slowly, with an absorption half-life across the dura averaging 22 minutes. Maximum CSF concentrations are seen 60-90 minutes after injection. Minimum effective CSF concentrations for postoperative analgesia average 150 ng/ml (range < 1-380 ng/ml).

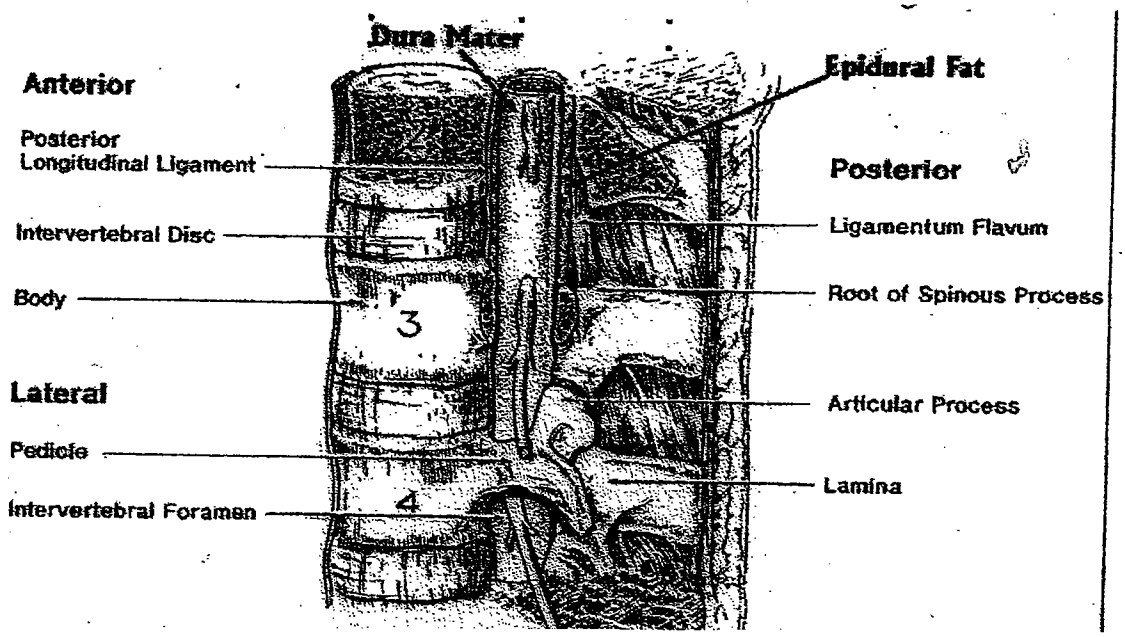
The intrathecal route of administration circumvents meningeal diffusion barriers and, therefore, lower doses of morphine produce comparable analgesia to that induced by the epidural route. After intrathecal bolus injection of morphine, there is a rapid initial distribution phase lasting 15-30 minutes and a half-life in the CSF of 42-136 min (mean 90 ± 16 min). Derived from limited data, it appears that the disposition of morphine in the CSF, from 15 minutes post-intrathecal administration to the end of a six-hour observation period, represents a combination of the distribution and elimination phases. Morphine concentrations in the CSF averaged 332 ± 137 ng/ml at 6 hours, following a bolus dose of 0.3 mg of morphine. The apparent volume of distribution of morphine in the intrathecal space is about 22 ± 8 ml.

Time-to-peak plasma concentrations, however, are similar (5-10 min) after either epidural or intrathecal bolus administration of morphine. Maximum plasma morphine concentrations after 0.3 mg intrathecal morphine have been reported from <1 to 7.8 ng/ml. The minimum analgesic morphine plasma concentration during Patient-Controlled Analgesia (PCA) has been reported as 20-40 ng/ml, suggesting that any analgesic contribution from systemic redistribution would be minimal after the first 30-60 minutes with epidural administration and virtually absent with intrathecal administration of morphine.

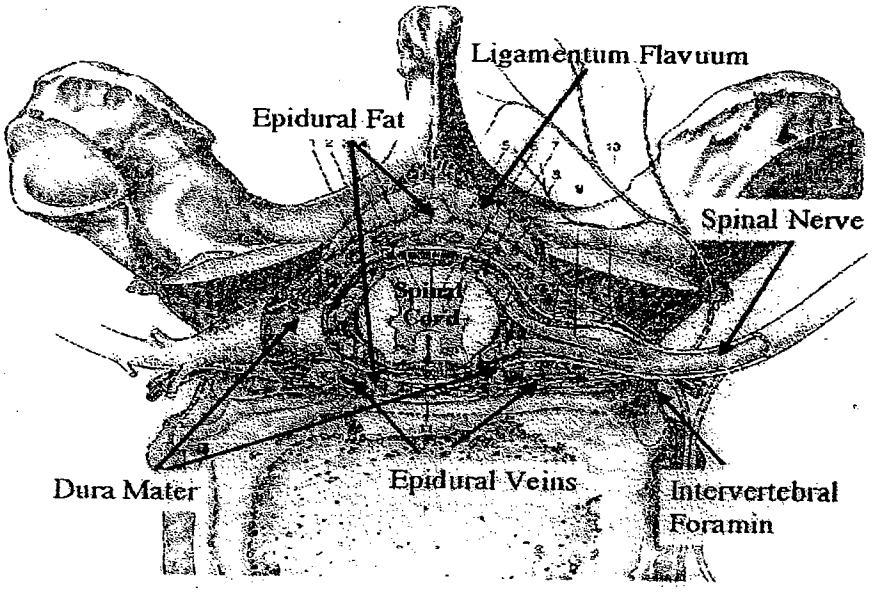
This led to anticipate a need for a sustained release formulation for morphine, which will serve the need for controlling post surgical pain. Liposomal formulation for morphine sulfate seems to serve the purpose of sustained release formulation however; the fate of SKY0401 in the epidural space is subject to immense discussion. The epidural space is a potential space into which anesthesia have been administering a variety of material for more than 100 years. This potential space exit within the vertebral column and bounded medially by the dura mater which in conjunction with the arachnoid mater serves to contain cerebral spinal fluid (CSF). Laterally the epidural space is bounded by the bony vertebral canal (vertebral body, pedicles, and lamina) and the ligaments that hold the vertebra together (primarily the posterior longitudinal ligament and the ligamentum flavum) see following longitudinal and cross-section figure of spinal column. The epidural space is bounded superiorly by the foramen magnum where the dura mater fuses with the periosteum of the skull. This is an important anatomic feature because it prevents material in the epidural space from reaching the intracranial cavity. Inferiorly the epidural space terminates in the sacrum.

Importantly, the epidural space is not an entirely closed space in that it communicates with the paraspinous musculature via the intervertebral foramina through which the spinal nerves exit the vertebral canal en route to innervate peripheral tissues.
Within the boundaries of the vertebral canal there are several tissues that define the potential epidural space. These tissues serve several important physiological functions. The primary constituents of the vertebral canal are the epidural fat, which lies between the dura mater and the anterolateral boundaries of the vertebral canal.

Longitudinal section through spinal column depicting anatomy of the epidural space:



Cross sectional anatomy through spinal column depicting contents of the epidural space:



The fat serves to cushion the spinal cord and is not involved in "fuel storage" as is the fat in the remainder of the body. Consequently, the amount of fat within the epidural space does not vary with patient "size." Pharmacologically, the epidural fat is a site into which drugs placed in the epidural space can and do partition. The extent to which any exogenously administered molecule partitions into the epidural fat depends on the molecules lipid solubility with more lipid soluble molecules partitioning to a greater extent into the epidural fat (Umenhoefer et al, 2000). The dura mater, which forms the medial boundary of the potential epidural space, is composed almost entirely of collagen and elastin fibers. Except for some fibroblasts and macrophages there are few cellular elements within the dura mater. However, the dura mater has a comparatively large vascular supply running almost entirely along its medial border with the arachnoid mater. The vertebral column also contains a very rich venous plexus (Batson's plexus that lies in the anterior aspects of the canal adjacent to the posterior longitudinal ligament this runs the entire length of the vertebral canal and serves to connect the pelvic veins with the basi-vertebral venous system and drains the brain. Physiologically, Batson's plexus functions as a "back-up" to the inferior vena cava. It constitutes the major route for venous blood to return from the lower extremities and pelvis to the heart during pregnancy because the pregnant woman is recumbent.

The epidural space also contains a rich lymphatic network that exits the epidural space through the intervertebral foramina and connects the epidural space with the lymphatic network running along the anterior surface of the vertebral bodies. Like all lymphatics, those in the epidural space function to remove materials that escape the capillaries and venules (e.g., plasma ultra-filtrate) or that are too large to be cleared at the capillary/venule level (e.g., cellular debris).

Under normal physiologic conditions the principal endogenous material that must be cleared from the spinal canal is (1) plasma that has been ultra-filtered in the capillaries of the dura mater and fat, and (2) CSF that has diffused across the spinal meninges to reach the epidural space. The absence of any material in the potential space known as the "epidural space" under normal conditions attests to the efficiency of this system or removing "extraneous" material.

However, there are multiple situations in which the amount of material presented to the epidural space for clearance is much larger than that which enters under normal conditions. For example, CSF can also reach the epidural space in much larger volumes under several iatrogenic pathologic conditions. The rapid clearance of aqueous-based solutions is also seen when local anesthetics are injected epidurally for surgical anesthesia.

It is perhaps not surprising that aqueous solutions are cleared rapidly from the epidural space. The mechanisms responsible for this rapid clearance include bulk flow of fluid out of the epidural space and into the paraspinous space via the intervertebral foramina and clearance via the lymphatic system (Bernards, 2000). Arguably the most dramatic evidence of the ability of the epidural space to clear foreign material is the rapid clearance of clotted blood. The rapid removal of clotted blood from the epidural space has been demonstrated after both spontaneous/traumatic spinal epidural hematoma and after intentional injection of blood into the epidural space. Whether blood enters the spinal epidural space iatrogenically or spontaneously it represents a very different material than other exogenous solutions placed in the epidural space (e.g. local anesthetics, glucocorticoid depot etc). Specifically blood represents a very large mass of RBCs. The soluble blood proteins (i.e., those that do not participate in clot formation; e.g., albumin, alpha, acid glycoprotein) can be readily cleared by the epidural lymphatics. The proteins and platelets that form fibrin clots, as well as the red blood cells that are entrapped therein cannot be cleared by these mechanisms. Instead, the organized clot is cleared primarily by the actions of macrophages and their associated proteases and lipases. The source of the macrophages, at least initially, is the adjacent dura mater, which hosts a readily available population of macrophages. Teleologically, the dural macrophages probably "exist' for just such an eventuality. Just as they do everywhere else in the body when blood clots form, the macrophages "digest" the clot and in so doing release peptides, lipids and other molecules into the epidural space. The lymphatics and capillaries ultimately remove the bulk of these molecules.

However, some components of the clot may initially be "re-cycled" locally. In particular, the phospholipids and cholesterol that make up the cell membranes of both red blood

cells and platelets will preferentially partition into the epidural fat. This is not surprising because the laws of thermodynamics dictate that all molecules will preferentially enter into environments in which they are thermodynamically most stable. For hydrophobic (i.e., lipid soluble) molecules the most stable environment (from a thermodynamic standpoint) is the epidural fat, which constitutes the largest hydrophobic domain in the epidural space. Importantly, the lipids that comprise DepoFoam™ are the same as those that make up the cell membranes, mitochondrial membranes, and microsomal membranes of red blood cells and platelets. Thus it is expected that the lipid molecules in the liposomes will be cleared in the epidural space as that of the components of blood (Bernards 2004). Also, 2 ml (approximately 17 mg) DepoFoam™ will pose less concern regarding the clearance of lipid material considering the magnitude lipid material CSF is known to clear (epidural hematoma or blood patch).

Therefore, it is expected that DepoFoam™ could be cleared by the following mechanisms:

1. Exit via intervertebral foramina: Many studies have demonstrated that material injected into the epidural space can leave the epidural space by way of the intervertebral foramina through which the spinal nerves exit the vertebral canals. Most of the material that exits via this route does so during the injection process as the solution flows through the epidural space. The amount of material that exits in this way increases (as a percentage of the total volume injected) as the volume of the injectate increases. Given the relatively small volume in which the DepoFoam™ is suspended (2 ml) one would expect a very small percentage of the material to exit the epidural space in this way. The material that does exit via the intervertebral foramina will be cleared by macrophages in the paraspinous muscle space.

2. As it is injected, the DepoFoam™ solution will spread out into a very thin film that will disperse throughout the adjacent epidural space. Thus, most (in fact nearly all) of the liposomes injected as part of the DepoFoam™ solution will abut the epidural fat. Consequently, some portion of the lipids that comprise the liposomal membrane will simply diffuse into the much larger lipid volume of the epidural fat. Once in the fat the cholesterol and phospholipids derived from the DepoFoam™ will be indistinguishable from the endogenous phospholipids and cholesterol that make up the fat and they will be stored and/or metabolized exactly as the endogenous lipids are.

3. Some portion of the liposomes will be phagocytosed by local macrophages to generate "foamy macrophages" just as has been shown to occur when DepoFoam™ is injected subcutaneously. The liposomal lipids will be metabolized by the macrophage to produce energy, CO_2 and H_2O.

4. Some portion of the liposomes will spontaneously (or with help from macrophages) release their lipids into the surrounding milieu where local tissues (e.g., arachnoid mater, fibroblasts, fat cells, and nerves) will incorporate them into

their cell membranes or metabolize them for energy. Some portion of the "free' liposomal lipids will be cleared via the capillaries/lymphatics and transported to distant tissues for membrane incorporation/metabolism.

5. Some smaller liposomes or liposomal fragments will be cleared directly via the epidural lymphatics.

Further supporting evidence is provided by results of animal studies conducted by SkyePharma on DepoFoam™ systems. Rats were injected intrathecally with a DepoFoam™ formulation of cytarabine in which DOPC (dieoleylphosphatidylcholine), the primary lipid constituent of DepoFoam™, was ^{14}C and the cytarabine labeled with [^{3}H]Cytarabine resembles morphine in lipid solubility and molecular weight). Injection volume was 20 L, equivalent to a cytarabine dose of 0.7 mg/kg. Over 90% of the ^{14}C label were expired as $^{14}CO_2$, indicating that the injected lipid underwent virtually complete catabolism.

No evidence of a cellular mass or a fibrotic foreign body response was observed in the epidural space following epidural injection of placebo DepoFoam™ in safety studies in dogs, even after four injections. This emphasizes that the DepoFoam™ constituents indeed undergo ready clearance from the epidural space after local delivery.
Local clearance of morphine and DepoFoam™ lipid constituents after subcutaneous administration in rats demonstrated clearance within 10 days after injection. In this study, 90% of the lipid components (DOPC, DPPG, cholesterol, and tricaprylin) measured by HPLC on excised injection site tissue, disappeared from the injection site within 10 days after administration, and none of the injected lipid could be detected at 3 weeks after administration (Triolein, the fifth and lowest-concentration lipid component of SKY0401, could not be distinguished from endogenous triglycerides). Tricaprylin disappeared most rapidly, followed by DOPC, DPPG, and morphine. Cholesterol was the slowest to disappear. The mechanisms of lipid clearance from the subcutaneous space are believed to be very similar to those from other extravascular spaces, including the epidural space, namely restructuring of the particles, phagocytosis by local macrophages, incorporation into local tissues, and clearance via the lymphatics.

In summary, the lipids that comprise the DepoFoam™ liposomes are either naturally occurring or very close analogues of endogenous lipids that should be remodeled, incorporated, metabolized, and/or cleared like any endogenous lipid.

The SKY0401 formulation provides a drug delivery system that yields a sustained and controlled-release of morphine after epidural administration. This is demonstrated by the epidural delivery of SKY0401 formulation that provided a sustained release of morphine in CSF and serum with lower peak levels of morphine than those observed after delivery of 5 mg of morphine sulfate in saline. Mean residence time for morphine in the lumbar CSF was increased approximately 17-fold after 30 mg SKY0401 administration as compared with 5-mg morphine sulfate. Administration of SKY0401 intrathecally does not result in higher or more persistent serum concentrations of morphine compared to epidural administration. Pharmacokinetics after the epidural and intrathecal routes are

very similar rapidly. There were no apparent differences in morphine pharmacokinetics in male and female animals following SKY0401. Administration of a lidocaine test dose should have no effects on morphine pharmacokinetics after an epidural dose of SKY0401. The needle however, should be flushed and 15 minutes should be allowed to elapse before SKY0401 administration.

2.6.4.10 Tables and figures to include comparative TK summary

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pharmacokinetics Overview:

Type of Study Study Title	Test System	Method of Administration	Testing Facility	Study Number
Absorption				
DepoFoam Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Pharmacokinetic and Efficacy Study in the Beagle Dog	Beagle Dog	Epidural	University of California, San Diego Dept. of Anesthesiology	033-00001
Effects of Intravenous, Intrathecal and Epidural Administration of Sustained-Release Encapsulated Morphine (C0401) in the Beagle Dog	Beagle Dog	Epidural Intravenous Intrathecal	University of California, San Diego Dept. of Anesthesiology	033-00018
An Epidural Injection Bioequivalence Study of — and — Manufacturing Scale SKY0401 in the Beagle Dog	Beagle Dog	Epidural	[ ]	033-00027
An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog	Beagle Dog	Epidural	[ ]	033-00025
DepoFoam™ Encapsulated Morphine Sulfate (C0401): A Bolus Epidural Multiple-Dosing Toxicity Study in the Beagle Dog	Beagle Dog	Epidural	University of California, San Diego Dept. of Anesthesiology	033-00009
Distribution				
Not applicable				
Metabolism				
Not applicable				
Excretion				
Not applicable				

Type of Study Study Title	Test System	Method of Administration	Testing Facility	Study Number
Pharmacokinetic Drug Interactions				
Pharmacokinetic Effects of Lidocaine on SKY0401 Encapsulated Morphine Formulation Delivered Epidurally in Beagle Dogs	Beagle Dog	Epidural	University of California, San Diego Dept. of Anesthesiology	033-00026

*Not applicable for an electronic submission.

A Bolus Epidural Pharmacokinetic And Efficacy Study In The Beagle Dog

Mean (± SD) Pharmacokinetic Parameters[a]						
	Lumbar CSF			Serum		
Test article[b]	Morphine Sulfate 5 mg in 3 mL Lot No VS-184	SKY0401 10 mg in 3 mL Lot No 96-0030	SKY0401 30 mg in 3 mL Lot No 96-0030	Morphine Sulfate 5 mg in 3 mL Lot No VS-184	SKY0401 10 mg in 3 mL Lot No 96-0030	SKY0401 30 mg in 3 mL Lot No 96-0030
C_{max} (ng/mL)	34,942 ± 5,578	10,730 ± 2,888	14,483 ± 3,438	230 ± 30.2	34.7 ± 6.5	59.1 ± 4.8
$t_{1/2}$(hr)	3.51 ± 0.19	7.45 ± 0.37	9.98 ± 0.60	0.83 ± 0.15	4.14 ± 0.57	10.72 ± 2.18
t_{max} (hr)	0.08	2.67	10.67	0.03	2.67	10.67
$AUMC_{0-\infty}$ (ng·hr²/mL)	13,307 ± 1,547	1,552,695 ± 696,600	3,074,651 ± 609,508	134 ± 38.2	2416 ± 426	17,779 ± 3473
$AUC_{0-\infty}$ (ng·h/mL)	17,682 ± 1,440	163,181 ± 65,336	236,998 ± 39,750	129 ± 16.4	312 ± 31.1	1,100 ± 89.9
MRT (hr)	0.77 ± 0.10	8.92 ± 0.76	12.81 ± 1.02	0.94 ± 0.16	7.52 ± 0.73	16.18 ± 3.04

[a] Study was conducted at University of California, Department of Anesthesiology Research, San Diego, CA. (Study No. TY-96-024, finalized November 22, 1996)

[b] Six dogs were prepared with chronic epidural and intrathecal catheters for dose administration and CSF collection. Each animal received the three test article injections in 3 mL dose volumes, with sufficient time in between injections to allow for washout of morphine from the CSF.

Intravenous, Intrathecal And Epidural Administration Of Sustained-Release Encapsulated Morphine In The Beagle Dog

Mean (± SD) Pharmacokinetic Parameters					
	Intravenous	Intravenous	Intrathecal	Epidural	Intravenous
Morphine State	Free[a]	Encapsulated[b]	Free[c]	Free[c]	Free[c]
Test article[b]	SKY0401 Lot No 97-0004 30 mg in 3 mL	SKY0401 Lot No 97-0004 30 mg in 3 mL	SKY0401 Lot No 97-0004 30 mg in 3 mL	SKY0401 Lot No 97-0004 30 mg in 3 mL	Morphine Sulfate: Lot No VS-184 30 mg in 3 mL
C_{max} (ng/mL)	377 ± 39	125 ± 24	39.3 ± 17.8	78.2 ± 6.1	788 ± 89
$t_{1/2}$(hr)	ND	ND	9.7 ± 3.2	10.3 ± 3.3	2.3 ± 0.7
T_{max} (hr)	0.08 ± 0.0	0.31 ± 0.46	6.3 ± 2.0	4.0 ± 1.0	0.08 ± 0.0
$AUMC_{0-\infty}$ (ng·hr²/mL)	ND	ND	12195 ± 2,563	14235 ± 4637	2312 ± 985
$AUC_{0-\infty}$ (ng·h/mL)	ND	ND	727 ± 84	1002 ± 150	1098 ± 120
MRT (hr)	ND	ND	17.7 ± 4.9	13.3 ± 3.0	2.0 ± 0.6

Study was conducted at University of California, Department of Anesthesiology Research, San Diego, CA. (Study No. TY-97-001, finalized July 27, 1998)

[a] Free morphine plasma concentrations after SKY0401 intravenous injection

[b] Morphine concentrations in pellet (remaining after blood centrifugation) after SKY0401 intravenous injection

[c] Free morphine serum levels after injection

ND = Not Determined; no data or insufficient data to perform PK analysis.

An Epidural Injection Bioequivalence Study of [ ] Manufacturing Scale SKY0401 in the Beagle Dog

Summary of Pharmacokinetic Parameters						
Parameter (unit)	SKY0401	Mean	Geometric Mean	Coefficient of Variation, %	Minimum	Maximum
AUC_{0-t} (ng · hr/mL)	manuf. scale	883	779	65.5	[	
	manuf. scale	1057	815	99.4		
C_{max} (ng/mL)	manuf. scale	166	119	97.9		
	manuf. scale	103	83.1	90.9		
t_{max} (hr)	manuf. scale	3.00	2.81	40.0		
	manuf. scale	3.00	4.76	75.4		]

'Mean' refers to arithmetic mean for AUC_{0-t} and C_{max} and median for t_{max}.
Sample size, n= 6

Least Square Means and 90% Confidence Intervals of Pharmacokinetic Parameters				
Parameter	SKY0401 manuf. scale	SKY0401 manuf. scale	Ratio	90% Confidence Interval
AUC_{0-t} (ng · hr/mL)				
Geometric Mean	779	815	1.05	
ln AUC_{0-t}	6.66	6.70	1.05	(55.2-198)
C_{max} (ng/mL)				
Geometric Mean	119	83.1	0.698	
ln C_{max}	4.78	4.42	0.698	(46.8-104)

A Bolus Epidural Multiple Dosing Toxicity Study in the Beagle Dog

Morphine Concentrations 24 Hours after Epidural Dosing with 30 mg SKY0401 (mean ± std. deviation)					
	Serum				Cerebral Spinal Fluid
Sex (n=3)	Day 1	Day 9	Day 17	Day 25	Day 25 (n=2)
Male	7.1 ± 1.4	6.1 ± 2.2	8.7 ± 1.8	6.9 ± 2.8	165.6 - 398.7
Female	13.4 ± 3.6	21.7 ± 18.9	15.2 ± 0.6	11.1 ± 2.0	216.5 - 583.6

Pharmacokinetic Effect of Lidocaine on SKY0401 Encapsulated Morphine Formulation Delivered Epidurally in the Beagle Dog

Epidural Dosing Procedures			
	Procedure 1 n=2 Mean (range)	Procedure 2 n=4 Mean ± SD	Procedure 3 n=5 Mean ± SD
Parameter (units)	• *1.8 mL saline* • 3 mL SKY0401 (30 mg) • flush 0.75 mL saline	• *1.8 mL lidocaine (1.5%)* • wait 3 min • 3 mL SKY0401 (30 mg) • flush 0.75 mL saline	• *1.8 mL lidocaine (1.5%)* • flush with 1 mL saline • wait 15 min • 3 mL SKY0401 (30 mg) • flush 0.75 mL saline
C_{max} (ng/mL)	71.7 (46.1 – 97.3)	180 ± 29	59.1 ± 25.0
t_{max} (hr)	2.0 (2.0 – 2.0)	0.08 ± 0.00	0.62 ± 0.39
$t_{1/2}$ (hr)	25.2 (17.9 – 32.6)	12.0 ± 3.2	11.2 ± 6.9
AUC_{0-inf} (ng·h/mL)	607 (536 –677)	791 ± 362	578 ± 254

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: SkyePharma characterized the general toxicity of DEPODUR® in three main studies:

4. SkyePharma Report 033-00025: An acute epidural injection toxicity study of SKY0401 in the Beagle Dog (GLP).

5. SkyePharma Report 033-00028: A single intravenous, intrathecal and epidural injection drug interaction study in male Beagle Dog (GLP). This study characterized the potential toxicity that would be associated with the accidental injection of the drug product either intravascularly or intrathecally.

6. SkyePharma Report 033-00009: DepoFoam encapsulated morphine sulfate (C0401): A bolus epidural multiple-dosing toxicity study in the Beagle Dog (GLP with some exceptions). This study was conducted by Tony Yaksh, University of California, San Diego.

Due to the unique caveats with epidural/intrathecal drug delivery, all these studies were done in the dog model which has a larger spinal column. As the drug product is designed to provide prolonged release of morphine, daily dosing is not possible. The 28-day repeat-dose toxicology study therefore employed once a week dosing, rather than once a day. A full histological assessment was not completed in these studies.

SkyePharma's NDA for SKY0401 also relies, part, on the finding of safety and efficacy of the reference listed drug, DuraMorph®. In addition, SkyePharma's previous work during the development of DepoCyt [redacted] has contributed to our understanding of the potential toxicity associated with DEPODUR®. Thus, the effect of morphine and DepoFoam were determined independently as well as combined into the current formulation for SKY0401.

The toxicity of epidural and intrathecal morphine has been well characterized. In addition, DepoFoam™ as it exists in DepoCyt has been approved by the FDA. DepoFoam™ formulation alone showed no major toxicity when studied as placebo with cytarabine toxicity studies after IT administration in Rhesus monkeys. Toxicity of DepoFoam™ placebo was limited to histiocytic infiltration only in the injection sites after intrathecal administration. The formulation of DepoFoam™ used in cytarabine study does not contain tricaprylin. The TDI dosage of tricaprylin will be 6 μg with the current formulation. Considering IV non toxic dose for tricaprylin is 1gm/kg in rat (no intrathecal toxicity study in any species other than dogs), it is assumed that since more than 1000 fold safety margin exist, the formulation will be well tolerated by the human population. However, in context of future safety concern and according to draft excipient guidelines it is recommended that toxicity of the tricaprylin be characterized in a second species with similar route of administration.

None of the studies mentioned above with the encapsulated morphine DepoFoam™ showed any major toxicity. One dog was euthanized after IT administration of 30 mg. The fact that naloxone could not reverse the effect shows that morphine overdose might not be the causative factor. Congestion of lung observed in this dog at autopsy. Although the cause of death was not determined the circumstantial evidence points to a procedural effect. Histopathological assessment in the morphine DepoFoam™ toxicity studies were limited to brain and spinal cord (lumber, thoracic) and the injection sites. Other toxicity findings are limited to slight to minimal nerve root degeneration with similar incidence in controls which most probably attributed to as procedural effect. Minimal invasions of the inflammatory cells were noted after the intrathecal administration as well as epidural administration.

Overall, DepoFoam™ encapsulated morphine sulfate appears to be relatively safe for the use in analgesia. However, the toxicity of tricaprylin was not assessed in two species.

Genetic toxicology: The Sponsor did **not** conduct formal genetic toxicology studies in support of this NDA. At the pre-NDA meeting with the Division on February 25, 2003, the Sponsor proposed to rely solely on literature to support the NDA. Dr. McGovern (Supervisory Pharmacologist) indicated that, for a 505(b)(2) application, it would be acceptable to **reference published literature** for information on genotoxicity for SKY0401. However, Dr. McGovern indicated that the adequacy of the data will be determined during review of the NDA. Morphine is reported in the published literature to have genotoxic effects in both the *in vivo* chromosomal aberration assay and the in vivo micronucleus assay. All available data for the genotoxicity for the DepoFoam™ components was assessed and submitted with the NDA. DOPC and triolein were found to be non-mutagenic. Standard battery test for clastogenicity was not done for these two above mentioned components. Tricaprylin was found to be mutagenic in one strain of bacteria, negative in other strains. Results from the chromosomal aberration and in vivo micronucleus assays with the tricaprylin are inconclusive. DPPG genotoxicity data was not provided by the Sponsor and is not available in the public domain. Considering all the components of DepoFoam™ are present in other FDA approved products, necessity for required genotoxicity assessment may be limited. However, the approved liposome containing FDA approve drug products are for the indication in oncology.

Carcinogenicity: The Sponsor did **not** conduct a carcinogenicity assessment for SKY0401. However, carcinogenicity assessment is **not required** for this drug product due to the acute indication, as described in the ICH M3 Guidance.

Reproductive toxicology: The Sponsor did **not** conduct reproduction or developmental toxicology studies in support of this NDA. During the pre-NDA Meeting, the Sponsor proposed to **reference published literature** for information on reproductive toxicology for SKY0401. Dr. McGovern (Supervisory Pharmacologist) indicated that the proposal was acceptable; however, the adequacy of the data will be determined during review of the NDA. Previous evaluations of the published data on the potential reproductive toxicity with morphine defined it to be Pregnancy Category C drug. DOPC reproductive

toxicity is well characterized. However, DPPG and triolein and tricaprylin reproductive toxicity studies are not well characterized according to today's standard. DPPG and triolein are present in other FDA approved drug products.

Special toxicology: Several special toxicology studies were included in the NDA package:

1. SkyePharma Report 032-00006: Dermal Sensitization Potential of DepoFoam™ - Encapsulated Amikacin (C0201) and Blank DepoFoam™ in Guinea Pigs.

2. SkyePharma Report 032-00005: 29-Day Toxicity Study of DepoFoam™ - Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Mouse.

3. SkyePharma Report 032-00004: 29-Day Toxicity Study of DepoFoam™- Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Dog.

4. SkyePharma Report 0333-00020.001: 21-Day Biocompatibility Study of DepoFoam™ Placebo Administered Subcutaneously to Rats.

5. SkyePharma Report 033-00021.001: Ocular Tolerance Study of Various Carrier Formulations After a Single Intravitreal Injection in Rabbits.

6. SkyePharma Report 032-00007.003: DTC 101 (DepoFoam™ Encapsulated Cytarabine) 4 Cycle Intrathecal Subchronic Toxicity Study in the Rhesus Monkey with a Subsequent.

2.6.6.2 Single-dose toxicity

Study title: An Acute Epidural Injection Toxicity Study of SKY0401 in the Beagle Dog.

NOTE: This study was previously reviewed by Dr. Kathleen Haberny for IND 52,113 (Serial number 040). Portions of the following review were extracted from Dr. Haberny's review.

Key study findings: Single epidural injections of either saline, SKY0401, morphine or the DepoFoam vehicle, suggested the following key findings:

1. One animal in the morphine sulfate control group had to be sacrificed on Day 4 due to moribund condition. The Sponsor indicates that the response of the animal appears to be related to the drug. However, even with necropsy and histological assessment of the animal, the cause of death could not determined.

2. On Day 2, both the morphine sulfate-treated and SKY0401-treated animals demonstrated limited usage of the hind limbs and reduced activity. The severity appeared greater in the SKY0401 group. By Day 4, the animals recovered, indicating the effect was pharmacological rather than pathophysiological in nature.
3. Day 3 neurological examination of the animals detected reduced or absent responses in several animals treated with morphine sulfate in saline and SKY0401 animals. By Day 7, animals responded normally.

Study no.: SkyePharma Report No. 033-00025
Volume #, and page #: EDR
Conducting laboratory and location: [ ]

Date of study initiation: June 8, 2000
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: **SKY0401,** Lot 99-0007, purity assumed to be [ ] for calculations.
Morphine sulfate, Lot MS003, purity assumed to be [ ] for calculations
DepoFoam, Vehicle, Lot 00-4006

Methods: Epidural injection was completed under surgical anesthesia. A needle was inserted into the epidural space in the lumbar region at approximately L6-L7. Test article was injected over a period of approximately 15 to 30 minutes. The test article was injected over a 15-30 minute period, flushed with 0.35 ml of saline and the incision closed.

The initial experimental design was as shown in the sponsor's table below:

DOSING

Group No. Identification	Number of Animals[a] Males	Females	Dose Level[b] (mg)	Dose Volume (mL)
1. Saline Control	6	6	0	3
2. Morphine Sulfate Control	6	6	15 x 2	3 x 2
3. SKY0401	6	6	30	3
4. DepoFoam Vehicle Control	6	6	0	3

[a] Three animals/group were euthanized on Day 4 and the remaining animals were euthanized on Day 22.
[b] Animals in Groups 1, 3 and 4 received a single bolus epidural injection of test or control article (3 mL) on Day 1. Animals in Group 2 received a bolus epidural injection of the morphine sulfate control (15 mg/3 mL) on each of Days 1 and 2.

Animals in Groups 1, 3 and 4 received a single slow bolus injection on day 1 while those in Group 2 (morphine treatment) received injections on days 1 and 2. Half of the animals

in each group were euthanised on day 4 of the study (3 days after treatment) and the remaining animals on day 22 (21 days after treatment).

Observation times and results

The parameters that were evaluated included clinical signs, body weights, food consumption, neurological evaluations and terminal hematology and biochemistry. In addition, blood was collected prior to dosing, approximately 24 hours following dosing (first dose for morphine), and again prior to necropsy.

The following organs and tissues were examined in the necropsy: abnormalities, adrenals, aorta (thoracic), bone and marrow (sternum), brain (including meninges), cecum, colon, duodenum, epididymides, esophagus, eyes, gallbladder, heart (including aorta), ileum, injection site (region L6/7 including meninges), jejunum, kidneys, liver, lungs, lymph nodes (mandibular and mesenteric), mammary gland (inguinal), optic nerves, ovaries, pancreas, pituitary, prostate, rectum, salivary glands, sciatic nerves, skeletal muscle, skin (inguinal), spinal cord (cervical, thoracic, lumbar and cauda equina, including meninges), spleen, stomach, testes, thymus, thyroid lobes (and parathyroids), tongue, trachea, urinary bladder, uterus (with cervix), and vagina. Meninges included examination of the dura mater, arachnoid and pia mater.

Tissues of the central nervous system and injection sites were examined histopathologically. Specifically, the cerebral cortex, piriform cortex, thalamus and hypothalamus, hippocampus, midbrain, cerebellum and medulla oblongata were examined.

Results:

Mortality: One male animal from the morphine sulfate group was euthanised pre-terminally on day 3 due to an apparent adverse reaction to treatment. Clinical signs considered by the Sponsor as being related to the treatment in this animal included: abnormal gait, decreased activity, sustained convulsions, limited usage of hind limb, lying on side, dilated pupil, increased respiration, uncoordination and vomiting. A cause of death was not established for this animal following gross necropsy and histopathological examination.

Clinical signs: Clinical signs in surviving animals administered morphine sulfate and SKY0401 included limited use of hindlimbs, decreased activity and lateral recumbancy on study day 2. These effects generally resolved by day 4. The clinical signs in the surviving dogs administered morphine sulfate (15 mg/d for 2 days) and SKY0401 (30 mg) were: limited use of hindlimbs, decreased activity, and lateral recumbency on Day 2 that resolved by Day 4. There were no differences in the clinical signs in male and female dogs.

Body weights: Body weights were decreased in all groups including the controls for 1 week after dosing, and recovered by Day 21.

Food Consumption: Food consumption was decreased in all groups including the controls on Day 1, and in the morphine-treated dogs (morphine sulfate control 15 mg/d for 2 days and SKY0401 30 mg) on Days 2 and 3.

Hematology and Clinical Chemistry: There were no treatment-related effects of SKY0401 on hematology and clinical biochemistry parameters. In the morphine sulfate control group, there were significantly increased MCH (24.3 pg compared to 23.4 pg in the DepoFoam™ controls, Day 4, M), decreased lymphocytes (Day 4, Fs, 12.0% compared to 32.3% in DepoFoam™ controls), increased neutrophils (Day 4, Fs, 10608 compared to 3371 in saline controls), decreased cholesterol 9140.7 compared to 199.0 mg/dl in saline controls), decreased total protein (5.8 g/dl compared to 6.3 in saline controls in Day 4 M, 6.0 g/dl compared to 6.5 g/dl in the saline controls and 6.7 g/dl in the DepoFoam™ controls in the Day 22 F), decreased creatinine (0.7 mg/dl compared to 0.9 mg/dl in DepoFoam™ controls, Day 4, F), and decreased calcium (9.2 mg/dl compared to 9.9 mg/dl in the saline controls, Day 4, F).

Macroscopic examination revealed red skin, lesions, scabs and/or swelling at the surgical site in all groups following the dosing. On Day 2, animals in the SKY0401 and unencapsulated morphine sulfate dose groups exhibited limited usage of the hind limbs and reduced activity, with an increase in severity (moderate to severe) in the SKY0401-treated animals. By Day 4, the animals recovered.

Neurological exams. On day 3 neurological evaluations revealed reduced or absent responses in several animals treated with unencapsulated morphine sulfate and SKY0401. Disposition/behavior and gait were also abnormal for several of these animals. These observations were considered to be related to the pharmacological properties of morphine. By Day 7, the neurological changes resolved.

Toxicokinetics. Morphine was detected in the plasma of most SKY0401-treated animals at 24 hours following administration, at levels slightly above the lower limit of quantitation —ng/ml). The results of the analysis also indicated detectable morphine levels in the pretreatment samples from 6 animals and Day 22 samples from 2 animals. These findings were felt to be incorrect and anomalous in nature. Due to the timing of the studies and the levels of morphine in question, these observations should not impact the interpretation of the study.

Histopathology: Gross pathological examination was performed on all animals as specified in the protocol. Tissues of the central nervous system and injection sites were examined histopathologically for all animals on this study. The pathology report concludes that there were no test-article related gross lesions observed in these animals other than relatively minor postoperative swelling and dark discoloration at the surgical site on day 4 that had resolved in most animals by the 22nd day. The pathology report indicates that there were minor changes noted that were attributed to the procedure itself. The following descriptions were extracted from the pathology report:

Day 4:

Spinal cord – injection site: An extremely localised, focal hemorrhage on the outer surface of the arachnoid was present at the injection site of one group 2 (Morphine sulfate control) male animal and a focus of perivascular subacute inflammatory cell infiltration on the outer surface of the dura mater was present in the region of the injection site of one group 1 (Saline control) group female animal. The mild severity and highly focal nature of these isolated lesions leads to the conclusion that they were most probably associated with minor local trauma (for example pressure, either by the injection needle or transient increase in hydrostatic pressure) that may have been incurred during the process of injection.

Spinal cord – distal to injection site: A focus of hemorrhage was seen within the lumbar spinal cord of one saline control dog and the thoracic spinal cord of one dog receiving morphine sulfate.

Brain: Highly localised, meningeal foci of subacute inflammatory cells, minimal or slight in degree, were occasionally observed overlying the brains of one group 1 (Saline control) animal and three group 4 (DepoFoam vehicle control) animals. These cellular infiltrations were, in all cases, restricted to the meningeal tissue and there was no appearance of abnormality or inflammatory infiltration of subjacent neural tissues. The slight preponderance of focal meningeal infiltration in animals receiving DepoFoam vehicle control was considered to be probably coincidental, a conclusion that was borne out by the observations made in animals killed on day 22 of the study (*q.v.*). The spatial separation of these lesions from the injection site, the absence of multiplicity of infiltrations and the absence of similar changes at or near the injection site where the concentration of the test article was greatest leads to the conclusion that these changes were probably spontaneous in origin.

Spinal cord – distal to injection site: No abnormalities were seen other than a single focal perivascular inflammatory infiltrate in the spinal cord dura of animal 3032 male (SKY0401). This was considered to be coincidental and unassociated with treatment.

Brain: Occasional, isolated, focal, minimal, meningeal inflammatory changes overlying the brain were similar in nature to that seen on day 4 although they were not seen in animals of either sex receiving SKY0401 or in female saline control animals. There was no evident increase in severity or frequency between day 4 and day 22 and they were therefore similarly considered not to be associated with any of the test articles.

The distribution of these changes is shown in the following tables: (*Note:* severity grades were assigned subjectively on a five point scale (minimal, slight, moderate, marked, severe) with reference to the normally expected range of severity for a given lesion in animals of this age etc.).

Day 22:

Spinal cord – injection site: Animals examined on day 22 exhibited a similar pattern of changes to those seen after only four days with the exception that focal degeneration and gliosis of individual nerve roots at or near the spinal cord injection sites were evident in a proportion of animals from both control and treated groups. In a single female animal (number 3522 receiving SKY0401), some nerve fibre degeneration of a similar nature was evident within the spinal cord at the injection site as well as an the adjacent nerve root. It was not possible to ascertain a connection between this and the damage seen in the adjacent affected nerve root but from the nature of the change it was possible to infer a similar etiology, this being a probability of local trauma having caused their occurrence. At each of these sites only a single nerve root was affected whilst all other nerve roots in the plane of section were apparently normal and unaffected. There was a preponderance of these changes in female animals compared to males but no clear reason for this was apparent. It was considered that these changes probably resulted from minor local trauma incurred at dosing and were unlikely to be associated with any particular control or treatment article.

The distribution of these changes is shown in the following tables: [Note: severity grades were assigned subjectively on a five point scale (minimal, slight, moderate, marked, severe) with reference to the normally expected range of severity for a given lesion in animals of this age etc.]

Day 4	Males				Females			
Group#	1	2	3	4	1	2	3	4
N	3	3	3	3	3	3	3	3
Injection Site								
Hemorrhage (minimal)	0	0	1	0	0	0	0	0
Inflammation (minimal)	0	0	0	0	1	0	0	0
Mineralization	0	0	0	0	0	0	1	0
Meninges								
Inflammation (slight - minimal)	1	0	0	1	0	0	0	2
Spinal Cord Lumbar								
Hemorrhage (minimal)	1	0	0	0	0	0	0	0
Inflammation	1	0	0	0	0	0	0	0
Spinal Cord Thoracic								
Hemorrhage	0	1	0	0	0	0	0	0

Day 22	Males				Females			
Group#	1	2	3	4	1	2	3	4
N	3	3	3	3	3	3	3	3
Brain								
Dilation: ventricle	0	0	0	0	0	1	0	0
Injection Site								
Degeneration: nerve root (minimal)	1	2	3	2	0	0	3	2
Damage to the spinal cord (slight)	0	0	1	0	1	0	1	0
Meninges								
Inflammation (minimal)	1	1	0	1	0	1	0	1
Spinal Cord								
Inflammation	0	0	1	0	0	0	0	0
Spinal Cord Thoracic								
Hemorrhage	0	1	0	0	0	0	0	0

1 = Saline Control 2 = Morphine Control 3= SKY0401

4 = DepoFoam™ Control

In conclusion no treatment related changes were seen that could be ascribed to the administration of any of the control or test articles in this study. Occasional minor, focal inflammatory (day 4) and degenerative (day 22) changes at or near the spinal nerve roots were seen in some animals and were considered to be probably procedure associated changes and the consequence of minor local trauma incurred during the process of the epidural injection process.

Study title: An Epidural Injection Bioequivalence Study of [ ] Manufacturing Scale SKY0401 in the Beagle Dog

Key study findings:

- There were no deaths in the study. There was no indication of a treatment-related difference between the — scale and — scale lots.
- Clinical signs consisted of decreased activity, reduced appetite, mucoid discharge from the prepuce, dilated pupils, wet fur, red fur staining around the anus, limited use of the hind limbs, lying on side, salivation, abnormal gait and vomiting.
- There was no clear difference in severity of the signs between treatment groups.

Study no.:	SkyePharma Report No. 033-00027
Volume #, and page #:	EDR
Conducting laboratory and location:	[ ]
Date of study initiation:	August 29, 2000
GLP compliance:	Yes, except test article analysis which were conducted according to GMPs.
QA report: yes ( ) no ( )	
Drug, lot #, and % purity: [ ]	SKY0401 Lot 99-007 [ ] Purity assumed
	Lot 00-4104 [ ] purity assumed [ ]

Methods

Doses:	30 mg/ml
Species/strain:	Beagle dog, males
Number/sex/group or time point (main study):	3 males per group
Route, formulation, volume, and infusion rate:	Epidural, 3 ml volume (maximum tolerated)
Satellite groups used for toxicokinetics or recovery:	None
Age:	6-7 months
Weight (nonrodents only):	11-12.2 kg

Unique study design or methodology (if any):

All dogs were prepared with a chronic epidural-dosing catheter. Catheter placement was verified radiographically. Each single 3-ml SKY0401 dose (equivalent to 30-mg morphine sulfate) was injected epidurally through the catheter. Test article was administered in a slow bolus injection over a 15-30 second period. At least 7 days was placed between treatments.

The parameters evaluated during the study were clinical condition, body weights and food consumption. Gross necropsy was performed on all animals on Day 15 to confirm catheter placement.

Group No.	Dose 1 (Day 1)	Dose 2 (Day 8)	Number of Males
1	[redacted]		3
2		[redacted]	3

Observation times and results

Mortality: The animals were observed daily for 14 days, no mortality observed.

Body weights: Body weights were measured weekly. Minor body weight loss was observed in some animals following dosing, which correlated with reduced food intake. There was no indication of a treatment-related difference between the [redacted] scale and [redacted] scale lots.

Food consumption: This parameter was checked weekly. No test article related changes were noted.

Clinical signs: Clinical signs consisted of decreased activity, reduced appetite, mucoid discharge from the prepuce, dilated pupils, wet fur, red fur staining around the anus, limited use of the hind limbs, lying on side, salivation, abnormal gait, and vomiting. There was no clear difference in severity of the signs between the [redacted] and [redacted] manufactured lots of SKY0401.

Ophthalmoscopy: Not done
EKG: Not done
Hematology: Not Done
Clinical chemistry: Not Done
Urinalysis: Not Done
Gross pathology: No major findings.
Organ weights (specify organs weighed if not in histopathology table): Not Done
Histopathology: Adequate Battery: Not done.
Peer review: yes ( ), no (x)

Toxicokinetics: Blood samples for serum were taken for pharmacokinetic analyses. Examination of the serum concentration profiles showed that both SKY0401 lots

delivered the drug over 24 hours post-dose in beagle dogs. Peak serum concentrations of morphine were reached at 3 hours (median) when either treatment was administered between the — and — manufactured lots of SKY0401.

Study title: A Single-Dose Intravenous, Intrathecal and Epidural Injection Drug Interaction Study in Male Beagle Dogs (GLP).

Key study findings:

- One dog died was euthanised at 30 mg IT Skye0401 administration, the cause of death could not be determined, however, since naloxone could not reverse debilitating effect observed after the procedure, possible cause of morphine overdose was overruled.
- Under the conditions of this study, epidurally administered SKY0401 was accidentally administered by intravenous or intrathecal administration with regional or local anesthesia. These procedures mimic the mis-administration of drug ("accidental" administration), which might happen in the clinic.
- Test article-related effects included decreased activity and ataxia on the day of dosing.
- When given intrathecally, the decreased activity appeared to last longer than when given intravenously. No test article effects were seen in any other parameter measured including body weight, food consumption, clinical pathology, macroscopic, or microscopic pathology.
- No test article effects were seen in any other parameter measured including body weight, food consumption, clinical pathology, macroscopic, or microscopic pathology.

Study no.: SkyePharma Report No. 033-00028
Volume #, and page #: EDR
Conducting laboratory and location: [redacted]
Date of study initiation: May 8th, 2002
GLP compliance: Yes
QA report: yes ( ) no ( )
Methods

Doses: SKY0401 (30 mg), Bupivacaine (7.5 mg), Lidocaine (22.5 mg) alone and in combination

Species/strain: Beagle Dog

Number/sex/group or time point (main study): 6/male animals/group, 3/6 / group were sacrificed at D4 and the rest were sacrificed at D22

Route, formulation, volume, and infusion rate: IV, IT and Epidural, formulation has similar composition to SKY401 (see page 5 of this review) (1.5 ml- 3 ml), bolus, vehicle used was saline.

Satellite groups used for toxicokinetics or recovery: Toxicokinetics analyses were done from the same animals at D4 and D22
Age: 6-7 months
Weight (nonrodents only): 9.15-12.53 kg

Unique study design or methodology (if any): The animals were treated with IV and IT route of administration with morphine sulfate (SKY0401 formulation) Bupivacaine and Lidocaine. The animals were also, treated with Morphine sulfate together with either Bupivacaine or Lidocaine with 3 different routes of administration (IV, IT and Epidural). The test and interactive articles were administered once by intravenous (IV), intrathecal (IT), or epidural (EPI) administration. For epidural administration, a spinal needle was introduced into the epidural space. For intrathecal administration, a spinal needle was introduced into the intrathecal space. Placement of the needle was verified by fluoroscopy and photographed. The IV dose was by bolus injection administered via the cephalic vein.

DOSING

Group	N	Test Article	Route	SKY0401 (mg)	Other anesthesia (mg)	Dose Volume	Necropsy Day[1]
1	6	Saline	IV, IT, EPI	0	0	3 mL each site	4, 22
2	6	SKY0401	IT	30	0	3 mL	4, 22
3	6	SKY0401	IV	30	0	3 mL	4, 22
4	6	Bupivacaine Lidocaine Lidocaine	IT IV EPI	0 0 0	7.5 22.5 22.5	1.5 mL 1.8 mL 1.8 mL	4, 22
5	6	Bupivacaine/SKY0401	IT	30	7.5	1.5 mL/3 mL	4, 22
6	6	Lidocaine/SKY0401	IV	30	22.5	1.8 mL/3 mL	4, 22
7	6	Lidocaine/SKY0401[2]	EPI	30	22.5	1.8 mL/3 mL	4, 22

[1] Three dogs/group were euthanized and necropsy performed on Study Day 4. The remaining three dogs/group were held for a 21-day recovery period after the end of dosing, and euthanized/necropsied on Study Day 22.
[2] Animals in Group 5 and 7 received an intrathecal or epidural test dose of lidocaine with epinephrine (1.8 mL of 1.5% lidocaine containing 1:200000 epinephrine) followed by a 0.8 mL saline flush. After a 15 min wait, the animals received a 3-mL injection of SKY0401 followed by a 0.8-mL saline flush. The procedure is designed to mimic study drug administration as may occur in clinical practice.

Observation times and results

Mortality: Dogs from all groups were observed twice daily for morbidity and mortality. One dog in the SKY-IT group was euthanized in axis on Day 1. The animal's vocalization during recovery was very pronounced; hind limb function impaired

convulsions and bloody stool were noted. The animal lacked response to naloxone. The dog was ultimately euthanized.

The fact that naloxone could not reverse the condition of a possible morphine overdose in this dog could indicate that the debilitating condition was a result of some procedural complication. The onset of debilitation was evident as the dog was waking from the general anesthesia, and lasted until the decision to euthanize. No direct cause of the debilitating condition could be identified. Severe signs were not seen in 23 of 24 dogs that went through the IT procedure. The fact that IT bupivicaine/SKY0401 did not show any adverse signs in six dogs (Group 5) supports the idea that morphine was not directly involved in inducing the debilitating condition.

Clinical signs: Dogs from all groups were observed twice daily for clinical signs. On the day of dosing, similar clinical observations were recorded for at least half of the animals dosed with SKY0401 either by the IT or IV route. These observations, decreased activity and ataxia, were present on Day 1 but they had essentially disappeared by Day 2 except for the IT treated dogs where decreased activity persisted for several days. SKY0401 in conjunction with bupivacaine seemed to have a reduced incidence of ataxia. Therefore this decrease in clinical findings is considered of no clinical significance. Chronic inflammation of the subcutis of skin injection sites was probably due to trauma of the injection procedure. The Group 2 dog (SKY-IT) that was euthanized in extremis had congestion in the lung, liver, and kidney.

Body weights: Body weights were measured daily. No test article related changes in body weight were observed.

Food Consumption: Food consumption was measured at D4 and weekly thereafter. No test article related changes in food consumption were observed.

Ophthalmoscopy: Not Done
EKG: Not Done

Hematology: Hematological parameters were studied from D4 and D22. No test article related changes were observed.

Clinical chemistry: Clinical chemistry parameters were studied from D4 and D22. No test article related changes were observed.

Urinalysis: Urinalysis was done from D4 and D22. No test article related changes were observed.

Gross pathology: Gross pathology was examined at D4 and D22. No test article related changes were observed. Discoloration (redness) of the injection site in one Group 3 (SKY-IV), one Group 4 (BU-IT/LI-IV/LI-EPI) and one Group 6 (LI-IV/SKY) dog at Day 22 necropsy was probably due to the injection procedure.

One Group 2 dog (SKY-IT) that died on study showed gross lesions of discoloration (redness) in the heart, lung, and thymus. The changes in the lung corresponded microscopically to congestion of vessels.

Organ weights (specify organs weighed if not in histopathology table): Brain, adrenal, liver, lung, kidney were weighed. Statistically significant changes in the organ wt were found at the Day 22 necropsy and included increased liver/body weight in male dogs of Groups 2 (SKY-IT) and 7 (LI- EPI/SKY). These changes were considered not significant and unrelated to the compound.

Histopathology: Adequate Battery: yes ( ), no (x)—explain
Peer review: yes (x), no ( )

Brain, adrenal, liver, lung, kidney, spinal cord (thoracic and lumber) and the injection sites were analyzed microscopically. There were no test article-related microscopic findings. The focal axonal degeneration of the lumbar spinal cord in one dog of Group 2 (SKY-IT) at the Day 22 necropsy and hemorrhage and edema in the epidural injection site of one Group 1 (Saline) dog at the Day 22 necropsy were probably due to trauma from injection.

Toxicokinetics: Not Done
Other: Not Done

2.6.6.3 Repeat-dose toxicity

Study title: DepoFoam™ Encapsulated Morphine Sulfate (CO40l) - A Bolus Epidural Multiple-Dosing Toxicity Study in the Beagle Dog

The study objective was to assess the potential toxicity of DepoFoam™ Encapsulated Morphine Sulfate (SKY0401) and the DepoFoam™ vehicle delivered as a lumbar epidural bolus a total of 4 times at 8-day intervals at the maximum repeatable dose (MRD).

Key study findings:

- Clinical observations noted across experimental groups included diarrhea and/or emesis, slight skin discoloration and/or bruising at the injection port site, slight swelling around the eyes, and excessive salivation. These observations were unrelated to dose group and are of minor toxicological relevance.
- The result of this study indicated that repeat epidural dose of 30 mg SKY 0401 resulted in moderate transient behavioral and physiological effects after in each injection, consistent with morphine administration.
- No changes in the CSF level of morphine were found.

- There were mild to moderate effects noted in spinal cord histopathology, however, there were no changes observed in cerebrospinal fluid nor evidence of altered neurological function.
- These results suggest that 30 mg C0401 and its vehicle DepoFoam™ at the doses and volumes employed, when given in 4 sequential epidural injections in dogs over approximately 28 days, is without significant pathological effect on spinal tissue or function.
- Therefore, the NOAEL is 30 mg of C0401.

Study no.:	Skye Pharma Report No 033-00009
Volume #, and page #:	EDR
Conducting laboratory and location:	Yaksh T, Dept of Anesthesiology, UCSD
Date of study initiation:	July 8th, 1996
GLP compliance:	Yes
QA report:	yes (x) no ( )
Drug, lot #, and % purity:	C0401 Lot # 96-0030, Purity not specified
Formulation/vehicle:	DepoFoam™ and saline

Methods

Doses:

Species/strain: Beagle Dog

Number/sex/group or time point (main study): 4/sex/group

Route, formulation, volume, and infusion rate: Depo Foam encapsulated (see formulation SKY0401 and C0401 see page 5 of the review), 3 ml, bolus, vehicle used was saline DepoFoam™.

Satellite groups used for toxicokinetics or recovery: 1/4 males and females from each group was retained for recovery for 7-9 days after the last dose administration and was autopsied at day 31-33.

Age: 12-22 months

Weight (nonrodents only): 9-12 kg females
12-18 kg males

Unique study design or methodology (if any):

Male and female adult beagle dogs (N = 9 males / 9 females) were prepared with chronic catheters passed to lie at approximately the L 1-2 level in the epidural space and connected to subcutaneous injection ports following rigid aseptic precautions under halothane anesthesia. Dogs were surgically implanted with lumbar epidural catheters approximately 72 hours prior to initial injection of test or control articles. Catheters remained in place for the duration of the study.

After catheter placement (Day-3), all dogs received 3.8 ml epidural injections of 0.9% (w/v) sodium chloride for injection, USP through the injection port (Day-1). The animals then received (Day 0) epidural bolus injections of 3 ml C0401 test article (10 mg/ml) (N = 3 males / 3 females), DepoFoam™ vehicle control article (N = 3 males / 3 females) or 0.9% (w/v) sodium chloride for injection, USP control (N = 3 males / 3 females). Test

article, vehicle control or control article delivery was followed by a 0.8 ml injection of 0.9% (w/v) sodium chloride for injection, USP to flush the port and catheter. This dosing regimen was repeated 3 more times at 8 day intervals (Days 8, 16, 24). Approximately 24 hours (Day 25) after the last injection all dogs were anesthetized with sodium pentobarbital and euthanized by exsanguination (with the exception of 1 male and 1 female from each group).

Observation times and results

Mortality: The animals were observed daily for mortality and morbidity. There were no deaths during the treatment period. All animals survived to their scheduled date of necropsy.

Clinical signs: Animal responses to the bolus injection of C0401 test article, DepoFoam™ vehicle and saline control articles were noted on Days 1, 0, 8, 16, and 24. Mild to moderate response (vocalization) occurred primarily during the first 2 epidural injections (Day -1 and 0) and appeared unrelated to dosing group. Diarrhea or emesis, where observed, did not appear dosing group related. A slight skins discoloration or braises appeared on several of the animals at the injection port site starting around Day 16. This finding appeared unrelated to group and had no effect on the animal behavior. One animal in the C0401 test article group was observed to have a slight swelling around the eyes after the initial dosing (Day 2). Excessive salivation was observed in the same animal on that day and on the third and fourth dosing, two days prior to dosing (Day 16 and 24). The catheter of one animal in the DepoFoam™ vehicle control group became occluded and required manipulation to administer the third dose (Day 16) and a cut-down for repair to administer the fourth dose (Day 24).

Epidural delivery of C0401 test article resulted in a mild to moderate decrease in mean arousal response scores (postural/behavioral indices of alertness), muscle tone (state of muscle vigor or tension) and loss of coordination was also observed 4 to 6 hours after administration after epidural administration of C0401 test article. Arousal, muscle tone and loss of coordination associated with the first dose of C0401 administration persisted for up to 72 hours post-dose. The magnitude of these effects diminished with each subsequent C0401 dose; no evidence of incoordination was observed in any C0401-treated animal on the day following administration of the last epidural dose (Day 25). Arousal, muscle tone and coordination remained unaffected throughout the treatment period in animals administered either the DepoFoam™ vehicle or saline control articles.

Body weights: Body weights were noted every 4 days. There were no statistically significant test material-related effects on body weight.

Food consumption: Food consumption was assessed daily. Epidural delivery of C0401 test article resulted in a decrease in food consumption for up to 72 hours after administration on. This lasted up to 72 hours, occurred with each injection and appeared unrelated to that animal's sex. In contrast, animals in the DepoFoam™ vehicle and saline control groups showed no change in food consumption throughout the study.

Ophthalmoscopy: Not Done
EKG: Not Done

Hematology: Blood was aspirated at the day of necropsy. No test article related changes were noted.

Clinical chemistry: Blood was aspirated at the day of necropsy. No test article related changes were noted.

Urinalysis: Day 3 and the day of the necropsy urine was collected and urinalysis were done. No test article related changes were noted.

Gross pathology: A necropsy was performed and selected tissues (spinal cord and brain) submitted for histopathological analysis. Six dogs were allowed a 7 to 9 day recovery period before they were anesthetized with sodium pentobarbital, euthanized by exsanguination and a necropsy performed. Gross necropsy revealed all dogs to be normal.

Organ weights (specify organs weighed if not in histopathology table): Organ weights were not measured

Histopathology: Adequate Battery: yes ( ), no (x)—All tissue from histopathology battery were preserved , however, only spinal cord and brain histopathology were done. Peer review: yes (x), no ( )

A comprehensive examination of selected tissues performed at the time of necropsy revealed all animals to be essentially normal. The incidence lesions observed were determined to have been received on the day of necropsy.

Neurohistopathology: All animals presented with evidence of mild to moderate inflammation in the epidural space, which only occasionally affected the integrity of adjacent nerve roots, or dura. Minimal changes were observed in the intrathecal space. Occasional reactive changes were noted in the arachnoid tissue of the brain. The inflammation and reactive changes that was observed in these animals were relatively modest and not unexpected given the presence of a foreign body chronically implanted in the epidural space. All pathology scores were in the lower 1/2 range of the possible values, and most were within the range of 0.5 to 1, which represents minimal pathological findings. However, a vehicle or test article related effect cannot be ruled out as the following trend in overall pathology scores was noted: saline control < DepoFoam™ vehicle < C0401 test article.

Spinal Cord, Nerve Roots and Dura: In all but one case there was minimal evidence of inflammation in the intrathecal space. In one animal there was invasion of inflammatory cells in intrathecal nerve roots and a dense accumulation of inflammatory cells in the perivascular space at a dorsal root entry zone. In other animals with pathology scores of

1.5 or 1, inflammatory cells were penetrating the dura or could be seen in the subdural space. In most instances however, the dura blocked infiltration of epidural inflammatory cells. This was observed in some animals with dense epidural inflammation. Brain: The brains were generally insignificantly affected by the procedures. There was no inflammation, no microglial nodules, and no pathogenic changes in brain. There was occasional diffuse thickening of the arachnoid membrane with increased cellularity consistent with a reaction to injury. The most severe of these changes was seen in one animal (DepoFoam™ vehicle control group), but the changes were minor.

Toxicokinetics: Blood was sampled 24 hrs after each test or control article administration (Days 0, 1, 8, 9, 16, 17, 24 and 25) and on the days of the necropsy for the recovery animal (days 31-33). Morphine levels in sera and CSF were quantitated by radioimmunoassay.

Morphine was not detected at any time in the serum or cisternal CSF of any animal that received DepoFoam™ vehicle control article or saline control article. Morphine was detected in sera of all animals administered C0401 test article at 24 hours after each dose administration. Twenty-four hour levels in sera ranged from 3.7 to 10.1 ng/ ml in male animals and 9.3 to 43.5 ng/ml in female animals. The generally higher levels of morphine in sera of female animals at 24 hours were likely due to their smaller size and therefore smaller total blood volume. Serum morphine levels had returned to baseline immediately prior to each subsequent dose administration. Morphine was also detected in cisternal CSF of animals administered C0401 test article at 24 hours after the final dose administration, i.e., at necropsy. Twenty-four hour levels in CSF ranged from 165.6 to 583.6 ng/ml in these animals. Morphine was not detected in CSF of recovery animals at the time of necropsy, i.e., 7 to 9 days after -the final dose administration. The finding of detectable levels of morphine in sera and relatively high levels of morphine in the cisternal CSF of study animals at 24 hours after C0401 test article administration confirms the sustained release characteristic of the test article.

Other:

Behavior, motor function, food consumption and rectal temperatures were monitored daily. At 4-day intervals animal weights were measured. Blood serum samples, heart rate, blood pressure (using a tail cuff manometer) and respiratory rate measurements were collected immediately prior to each injection and approximately 24 hours after each injection.

Quantitative assessments (see attached figures) of arousal, muscle tone and motor coordination, i.e. lethargy, slackness and unsteadiness, were also assigned numerical scores and presented below:

Arousal: The epidural delivery of C0401 test article resulted in a clear depression of arousal response (postural/behavioral indication of alertness within 4 to 6 hours of injection as indicated by a loss in the animal's attentiveness to its surroundings. This lasted up to 72 hours, occurred with each injection and appeared unrelated to the animal's

sex. Animals in the DepoFoam™ vehicle and saline control groups showed no change in arousal response-throughout the study. There were statistically significant differences between the C0401 test article group and each control article group for observations made on the day after each injection.

Muscle Tone: The epidural delivery of C0401 test article resulted in a clear decrease in muscle tone (the state of muscle vigor or tension) within 4 to 6 hours of injection as indicated by a difficulty to ambulate caused by buckling of the back legs or inability of the animal to lift itself to a standing position. This lasted up to 72 hours, occurred with each injection and appeared unrelated to the animal's sex. Animals in the DepoFoam™ vehicle and saline control groups showed no change in muscle tone throughout the study. There were statistically significant differences between the C0401 test article group and each control article group for observations made on the day after each injection.

Coordination: The epidural delivery of C0401 test article resulted in a clear decrease in coordination (complimentary/balanced muscle activity) within, 4 to 6 hours of injection as indicated by difficulty in ambulation caused by poor paw placement or inability to maintain vertical posture. This lasted up to 72 hours, occurred with each injection and appeared unrelated to the animal's sex. Animals in the DepoFoam™ vehicle and saline control groups showed no change in coordination throughout the study. There were statistically significant differences between the C0401 test article group and each control article group for observations made on the day after each injection.

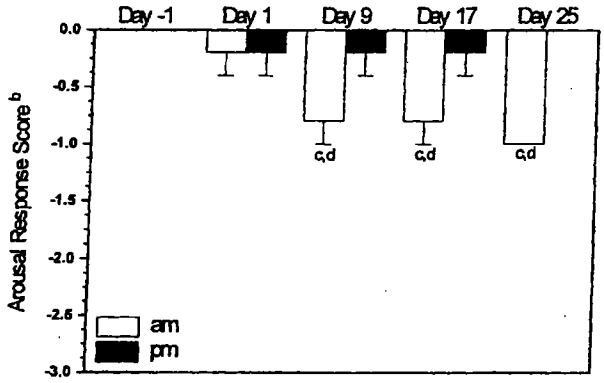
Body Temperature: The epidural delivery of C0401 test article resulted in a clear decrease in body temperature (see figure below). This lasted up to 72 hours, occurred with each injection and appeared unrelated to the animal's sex. Body temperature decreased after each weekly C0401 administration (decrease of 1.5 to 7.7 degrees Fahrenheit) compared to controls and persisted for 24 to 48 hours after dosing. Animals in the DepoFoam™ vehicle and saline control groups showed no change in body temperature throughout the study. There were statistically significant differences between the C0401 test article group and each control article group for observations made on the day after each injection. There were no statistically significant differences between the C0401 test article group and each control article group for observations made on the day after each injection.

Respiratory Rate: Respiratory Rate was measured at days –7, -5, -3, 0, 1, 8, 9, 16, 17, 24, 25 and the day of necropsy. A consistent reduction in mean respiration rate was noted for animals administered C0401 on the day following epidural delivery of each dose on study days 1, 9, 17 and 25 (mean decrease of 5 to 10 breaths per minute). Epidural administration of DepoFoam™ vehicle control article had minor variable effects on respiratory rate throughout the treatment period. Normal respiratory rates were recorded for all recovery animals on the days of sacrifice.

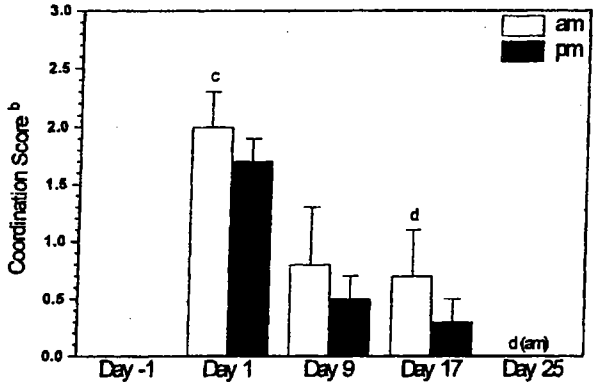
Heart Rate: Heart Rates were measured at days –7, -5, -3, 0, 1, 8, 9, 16, 17, 24, 25 and the day of necropsy.

Blood Pressure: Epidural delivery of C0401 consistently lowered mean systolic, diastolic and arterial pressures by an average of 38, 21, and 26 mm Hg, respectively, following administration of each dose. Epidural administration of DepoFoam™ vehicle control article had variable minor effects on blood pressure throughout the treatment period. No unusual findings with respect to blood pressure were recorded for C0401-treated or DepoFoam™-treated recovery animals.

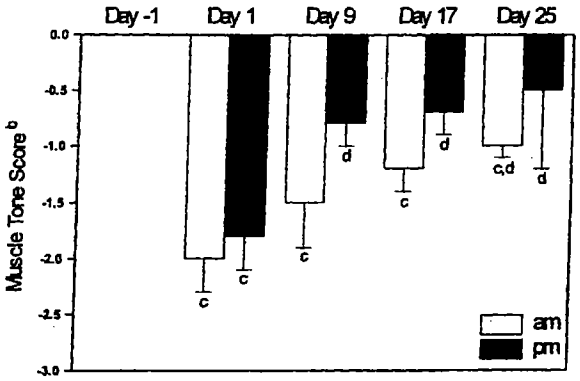
Arousal Response in Dogs



Muscle Tone Scores



Coordination Scores in Dogs



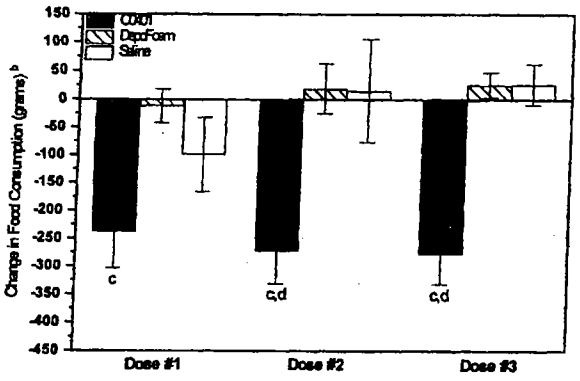
[a] Dogs were administered 3 mL of C0401 test article (10 mg morphine/mL) via epidural catheter on study Days 0, 8, 16, and 24.

[b] Mean muscle tone score ± SEM for n=6 animals per evaluation (except for study Day 25, p.m., where n=2).

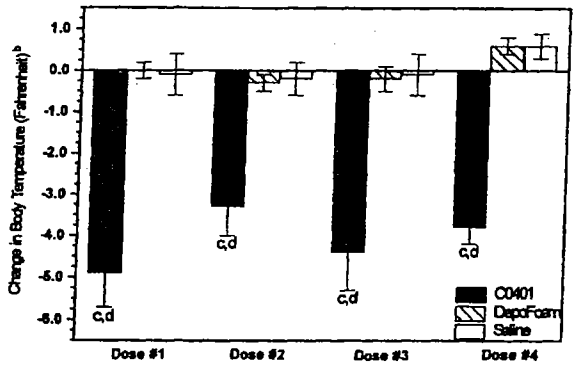
[c] Significantly different from respective Day -1 value ($p < 0.05$)

[d] Significantly different from respective Day 1 value ($p < 0.05$)

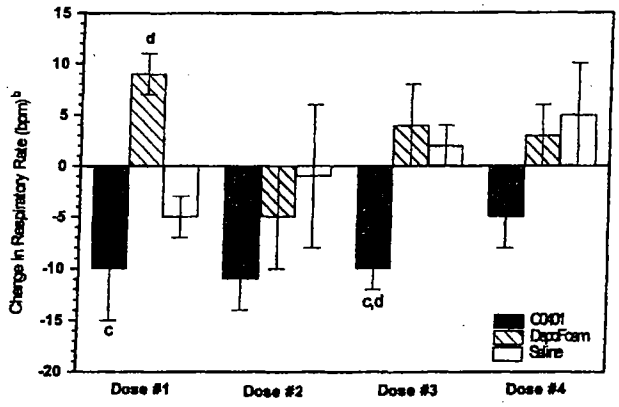
Change in Food Consumption



Change in Body Temperature



Change in Respiratory Rate



[a] Dogs were administered 3 mL of C0401 test article (10 mg morphine/mL), DepoFoam vehicle control article (0 mg morphine/mL), or 0.9% Sodium Chloride for Injection, USP via epidural catheter on study Days 0 (Dose #1), 8 (Dose #2), 16 (Dose #3), and 24 (Dose #4).

[b] Data calculated as # of breaths per minute prior to dosing minus # of breaths per minute on the day following dosing. Values represent mean ± SEM for n=6 animals per evaluation.

[c] Significantly different from respective DepoFoam vehicle control value ($p < 0.05$)

[d] Significantly different from respective saline control value ($p < 0.05$)

2.6.6.4 Genetic toxicology

No genetic toxicology study report has been submitted with the current NDA.

The results of the genotoxicity studies on morphine sulfate, reported in the literature are presented in the following table.

Citation	Assay /Test System	#Animals/Dose Group	Dose Regimen/Formulation/Route	Significant Findings
Knapp and Cramer 1976	Drosophila/ Mutagenicity	Not applicable	In vivo	No evidence of induction of the sex linked recessive or dominant lethal mutation or translocation
Madden et al 1979	Human PBL	Not applicable	IN vitro	Morphine negatively effect DNA damage caused by UV radiation in addicts
Swain et al 1980	In vivo Cytogenetic mice	5/sex/group	Single dose: 0, 3.2, 8, 6, 32, 64 mg/kg IP 17 consecutive days: 3.2 mg/kg/day IP	Increase in chromosomal aberration in bone marrow cells at 3.2 mg/kg No morphine related effect may be due to tolerance
Das and /Swain 1982	Micronucleus mice	5/sex/group	2doses separated by 24 hrs: 0, 3.2, 8, 6, 32, 64 mg/kg IP	Dose related increase in incidence of micronuclei in polychromatic erythrocytes at doses> 3.2 mg/kg
Badr and Rabouh, 1983	Dominant lethal and spermatocyte test in male Mouse	12 male mated with 2 female /dose group	3 consecutive daily doses of 0, 10, 20, 40, 60 mg/kg IP	Increase in # of dominant lethal, particularly early spermatids and types and frequencies of chromosomal aberration in dividing spermatocytes at all dose levels
Fuchs and Pruett, 1993	In vivo and In vitro DNA fragmentation in thymocytes in mice	Females, 4	75 mg SC time release pellet morphine released for 12-48 hrs	DNA fragmentation noted following implantation of morphine In vitro, no DNA fragmentation noted following morphine exposure In vivo DNA fragmentation blocked by opiate and glucocorticoid antagonist suggesting effect mediated atleast partially by hypothalamus pituitary axis
Shafer et al 1994	Mutagenicity/ human HUT–78 cells and HRPT mutant cells	Not applicable	In vitro	Morphine increased DNA fragmentation at 10^9 M concentration. Morphine increased mutation frequency of the mutagen ethyl methane sulfonate over that of the mutagen alone.
Sawant and Couch 1995	In vivo and in vitro micronuclei assay in mice	Female C57 black and DBA strain of mice, # unknown	Single IP dosage of 0-100 mg/kg Single IV dose 20 mg/kg	Dose and time related increase in micronucleated splenocytes & lymphocytes, blocked by adrenalectomy

				increase in micronuclei in peripheral blood cell Morphine added to lymphocytes of cyclophosphamide treated animals at $>10^7$ M increased the # of micronuclei following in vitro stimulation with mitogen
Falek et al 1991	Chromosomal aberration in human PBL	Not applicable	In vitro lymphocyte culture	2 fold increase in chromatid damage among the opiate addicts Increase SCE in opiate addicts

Although only limited information is available, there is evidence that in vivo administration of morphine to mice can increase the frequency of chromosome aberrations in bone marrow cells (Swain et al., 1980) and induce micronuclei in bone marrow cells and lymphocytes (Das and Swain, 1982; Sawant and Couch, 1995). In contrast, *in vitro* morphine treatment has failed to induce chromosome aberrations in cultured human lymphocytes (Falek el al., 1972) or micronuclei in mitogen stimulated murine splenocytes (Sawant and Couch, 1995). It is therefore reasonable to assume that metabolic activation is involved in the induction of chromosome aberrations or micronuclei formation. Furthermore, morphine-induced micronuclei formation in mice can be reduced by naloxone, an opioid antagonist, indicating that this genetic damage is at least in part opioid receptor mediated. Although the principal metabolite of morphine in *in vivo*, morpline-3-glucuronide (Glare and Walsh, 1991), does not participate in receptor-mediated responses, the possibility of the involvement of other metabolites cannot be ruled out (Sawant and Couch, 1995).

Mutagenic effects of morphine were not observed in Drosophilla, Salamonella and yeast test systems (reviewed in Madden et al., 1979). However, one recent study has indicated that the mutation frequency and the frequency of Comet tails of fragmented DNA were dose-dependently increased when human HUT-78 cells were treated with morphine alone for 4 days or morphine in combination with a brief ethyl methane sulfonate (EMS) exposure (Shafer et al, 1994). Since DNA damage from brief EMS exposure were repairable, the result of morphine induced hprt gene mutagenesis suggested that direct or indirect mutagenesis could be initiated if the exposure to morphine persisted through one or or more cell cycle. Thus morphine could act through long term inhibition of replication or repair process, converting transient alteration to permanent mutations. Morphine can also affect the repair of DNA damage caused by UV light (Madden and Falek 1991). Morphine induced DNA fragmentation has been associated with apoptosis in murine thymocytes in vivo (Fuchs and Pruett, 1993). Both opiate and glucocorticoids are involved in morphine induced apoptosis. Recently, it has been reported that administration of morphine to rats increased the ethylation of oesophageal DNA by N-nitrosodiethyl amine and may reduce the first pass clearance of N nitrosodiethyl amine by the liver, although only at high dose of morphine (Ribero and Swain, 1997). Morphine could therefore be classified as a co mutagen.

Chronic opioid abusers (e.g. heroin abusers) and their offspring display higher rates of chromosomal damage. However, the rates of chromosomal abnormalities were similar in unexposed individuals and in heroin users enrolled in long-term opioid maintenance programs.

2.6.6.5 Carcinogenicity

No carcinogenicity study report has been submitted with the current NDA. Consistent with the recommendations described in ICHM3, carcinogenicity assessment is not required for DEPODUR due to the acute use of this drug product.

Long-term studies in animals to evaluate carcinogenic potential of morphine have not been conducted. In the published literature, evidence for indirect involvement of morphine in tumorigenesis was reported. Increased ethylation of oesophageal DNA was observed in rat given doses of 5mg/kg Sc and higher (Ribero-Pinto and Swain, 1997). Immunosuppression by morphine has been observed in several species (which theoretically can contribute to an increase risk in carcinogenesis. No neoplastic lesion or preneoplastic changes were, however observed in rats given upto 25 mg/kg/d and morphine in diet for 124 days (Finnegan et al 1948, Fennesay and Fearn, 1969) or in dog, given upto 5 mg/kg/d, by daily subcutaneous administration for 100 days (Finnegan 1948). Also, inhibition of human cancer cell growth in vitro by morphine (Sueoka et al 1996) and inhibition neuroblastoma and PC 9 (peripheral neuronal cell line) cell growth by morphine-6-glucuronide (Sueoka et al 1996) has been reported.

2.6.6.6 Reproductive and developmental toxicology

No formal reproductive toxicity studies were submitted with the current NDA. The sponsor is relying upon the published literature.

The Sponsor provided several literatures reports on studies to evaluate morphine reproductive toxicology in mice, rats, hamsters, guinea pigs, and rabbits. Exencephaly and axial skeletal fusions were observed in morphine treated mice at doses of 200-400 mg/kg IP on gestation days 8 or 9 (Iuliucci and Gautieri, 1971). Subcutaneous morphine injections produced exencephaly at doses of 300 mg/kg and greater given on gestation day 8, and axial skeletal fusions at doses of 100 mg/kg and greater on gestation day 9 in mice (Harpel and Gautieri, 1968). In another study in mice, continuous subcutaneous infusion of morphine sulfate at 0.15 – 15 mg/kg/d on gestation days 7 through 10 resulted in fetal exencephaly, hydronephrosis, intestinal hemorrhage, split supraoccipital, malformed sternebrae, and malformed xiphoid (Ciociola and Gautieri, 1982). Additional embryotoxic effects of morphine observed in mice in one or more of these studies include decreased crown-rump length, decreased fetal body weights, and partial fetal resorptions. The relative contribution of maternal toxicity including decreased maternal food consumption and body weights, and hypoxia, to the observed toxicity in the fetal mice is unknown.

Reproductive toxicology studies in rats administered morphine in drinking water, and by IP and SC injection were reported in the literature. Oral morphine sulfate decreased fetal viability, body weights and postnatal viability, and increased the sensitivity to morphine-induced analgesia in rats at doses of 12.5 mg/kg/d and greater given on gestation day 5 through postpartum day 2 in rats (Eriksson and Ronnback, 1989). In that study, there were no surviving offspring at doses greater than 25 mg/kg/d PO.

Daily prenatal exposure to morphine in the maternal drinking water at 0.4 g/L on gestation days 0 through 21 resulted in faster acquisition of morphine self administration behavior in the offspring of the morphine treated rats (Glick et al., 1977). Intraperitoneal administration of morphine sulfate in rats at doses up to 40 mg/kg/d beginning several weeks before mating through mating, gestation and 10 days into the postpartum period altered several reproductive parameters in both the dams and offspring (Siddiqui et al., 1997). Abnormal estrus cycles, increased gestational length and increased number of stillbirths were observed in the maternal rats. In the offspring, decreased body weights at birth and body weigh gain during development, delayed sexual maturation, altered mating behavior at adulthood, and decreased plasma estradiol, ovarian estradiol, progesterone, and hypothalamic norepinephrine were observed. Zagon and McLaughlin (1977a and 1977b) observed decreased litter size, embryo-fetal viability, fetal and neonatal body weights, absolute brain and cerebellar weights at birth and during the neonatal period, brain lengths, and cerebral and cerebellar widths, increased neonatal mortality, and cyanotic and hypothermic infants after morphine sulfate administration at 80 mg/kg/d IP from 5 days before mating through gestation and lactation. Similar effects were observed in a study by Siddiqui et al. 1995 in rats given 40 mg/kg/d IP morphine sulfate beginning several weeks before mating through the 10th day postpartum. Also reported in that study were decreased plasma and testicular levels of luteinizing hormone and testosterone, decreased testes weights, seminiferous tubule shrinkage, germinal cell aplasia, and decreased spermatogenesis in the male offspring.

The literature reports of sixteen studies on reproductive toxicology of subcutaneous morphine in rats were summarized (see Johannesson and Becker, 1972; Sovrian, 1977; Kirby, 1982; Vathy et al., 1983; Fujinaga and Mazze, 1988; Vathy and Katay, 1992; Vathy et al., 1994; Vathy et al., 1995; Gagin and Shavit, 1996; Hol et al., 1996; Niesink et *al.*, 1996; Gagin et al., 1997 and 1997b; Shavit et al., 1998). Doses of 10-70 mg/kg by SC injection or perfusion pumps were studied in paradigms ranging from dosing during various gestation periods of organogenesis (e.g., gestation days 5-12, 11-18, etc.), to dosing from pre-mating through gestation day 15. An early study by Sobrian 1977 showed that subcutaneous morphine administered to pregnant rats at 40 mg/kg/d from 5 days before mating through gestation day 15 resulted in decreased fetal viability and body weights, and increased neonatal mortality and postnatal spontaneous motor activity.

Morphine treatment during gestational periods of organogenesis resulted in the following treatment-related embryotoxicity and neonatal toxicity in one or more studies: increased pre- and post-natal mortality, inter-litter variability for vaginal opening, incidence of

enlarged cerebral ventricles, and increased hypothalamic norepinephrine in males, decreased body weights, growth of spinal cord components, female hypothalamic norepinephrine, and immune function.

There are few controlled human reports on possible morphine teratogenesis; however, based on a relatively small number of studied exposures, this agent does not appear to increase the incidence of birth defects in humans (Mellin, 1964; Heinonen, 1977). Newborns of women addicted to morphine and other opioids may develop a withdrawal syndrome during the first several days of life (Cobrinick 1959). It is not known how much morphine must be used before such a withdrawal syndrome is likely.

Administration to women at the time of third trimester fetal blood sampling was associated with a decrease in fetal breathing movements (Chamberline and Wraight 1990). The authors concluded that there was also evidence of placental vascular constriction with the potential for adverse fetal effects; however, their data did not show a significant change in measures of placental vessel constriction. Morphine administration to women in labor also may be associated with neonatal respiratory depression. Although this effect may be seen after administration of any opioid, morphine is believed, based on reports from many years ago, to be more likely to be associated with respiratory depression in the neonate than is meperidine (Campbell, 1961, Eddy, 1957).
There is a body of reports that claim systemically administered morphine (and other opioids) lack analgesic effectiveness for labor pain, but serve primarily to sedate the parturient, and inadvertently, the neonate (Olofsson et al 1996, Reynold et al., 1997). Prenatal or perinatal exposure to opioids may be associated with behavioral abnormalities in the offspring. While such abnormalities can be demonstrated in rodents, persistent human defects attributable to the drug alone have not been proven, largely because of the number of confounding factors involved in studies of human opioid users. Based on the available animal and human data, however, a strong case can be made for persistent alterations in neurobehavioral function attributable to opioid effects on the developing nervous system (Fujinaga, 1988). The timing and dose of morphine that may be associated with such effects in humans is unknown.

Morphine and other opioids depress sexual activity in male rats by reducing serum luteinizing hormone (LH) and testosterone (Cicero et al 1976, Adams et al 1993). This effect may be mediated by direct effects on the testes as well as the inhibition of LH secretion. The prenatal administration of morphine has also been associated with reduced testicular function and spermatogenesis (Siddiqi et al 1995). In a group of 30 subjects, men and both premenopausal and postmenopausal women who received chronic intrathecal morphine for nonmalignant pain had evidence of hypogonadism with low levels of serum testosterone or estrogen coupled with low levels of pituitary gonadotrophins (Finch et al 2000). These effects appear to be reversible once morphine administration is ended. In one report from Thailand, chronic exposure to morphine was associated with the induction of galactorrhea in male cynomolgus monkeys.

Morphine enters breast milk in very small amounts (Feilberg et al, 1989, Oberlander et al2000). Some data suggest that acute morphine administration can inhibit oxytocin

release during breast- feeding, but the clinical significance of this observation has not been established (Lindow et al 1999). Based on a time course of morphine excretion in the milk of five lactating mothers, a milk: plasma ratio of 2.85 was reported, and the estimated maximum concentration of morphine in breast milk was 500 ng/ml milk (Feilberg 1989). A separate report has described one mother addicted to morphine who had comparable milk levels of morphine, but her neonate was found to have unexpected therapeutic concentrations of the drug suggesting much higher excretion levels of morphine. Until this finding is supported by additional data, we believe the previous study to be a more rigorous analysis of the excretion of morphine in human milk. The amount of drug received by a suckling infant is unlikely to cause respiratory depression or drowsiness (Feilberg et al, 1989), and there appears to be no contraindication to nursing in women receiving morphine (Committee on Drugs, AAP, 2001).

2.6.6.7 Local tolerance

There were no specific local tolerance studies conducted for this NDA. Local tolerance was assessed in the acute and repeat dose toxicology studies.

2.6.6.8 Special toxicology studies

THE DEPOFOAM™ MATRIX

Following degradation of DepoFoam™ particles either at the site of local administration or within the cells of the reticuloendothelial system (RES), the triglyceride components of the matrix are expected to behave as endogenous fatty acids, transported throughout the body via lipoprotein complexes available for routine cellular metabolic processing. Free oleic and/or caprylic fatty acids liberated from lipoprotein-associated DepoFoam™ derived triglycerides via the action of endogenous lipases may either circulate bound to albumin, serving as a major source of energy for various organs, or may be transported to adipose cells and reassembled intracellularly into new triglycerides for storage. Published data concerning the tolerability of either systemically- or locally administered triglyceride- containing liposomal formulations are not available. However, the results of several comprehensive toxicology studies conducted in various species with triglyceride-containing DepoFoam™ formulations support the safety of triglycerides as a component of the DepoFoam™ matrix (see below).

SkyePharma Inc. has conducted a nonclinical safety program with DepoFoam™ and DepoFoam™ containing products that also supports the safety of components in SKY0401. The results of the DepoFoam™ nonclinical safety program are presented in below. In some cases, a DepoFoam™ placebo was utilized in a study that was intended to also investigate the toxicity of a DepoFoam™ encapsulated drug, such as Amikacin or cytarabine. In those circumstances, only the results from the DepoFoam™ placebo portion of the study are presented below.

	Formulation lipid content (mg/ml)			
	Studies A	Study B	Study C	Study D
Dioleolyphosphatidylcholine	2.93	5.0	4.1	0.73
Dipalmitoylphophatidylglycerol	0.55	1.1	0.87	0.15
Cholesterol	1.63	4.6	3.3	0.54
Triolein	0.57	1.2	0.1	0.15
Tricaprylin	N/A	N/A	0.3	N/A

Bench-scale formulation: Approximation of formulation lipid content based upon estimated 70% incorporation efficiency of 1 ml emulsion phase lipid combination (containing 10.38 mg/ml DOPC, 2.08 mg/ml DPPG, 7.69 mg/ml cholesterol, and 2.16 mg/ml triolein) and a final formulation volume of 10 ml.
Study A: represent Report # 033-00007
Study B: represent Report # 033-00005, 033-00004, 033-00006, 33-00020
Study C represent: Report # 033-00001, 033-00018, 033-00025, 033-00027, 033-00028, 033-00009.
Study D: represent Report # 033-000021

Brief Summary of the studies conducted with placebo DepoFoam™ is presented below:

Study title: Dermal Sensitization Study of DepoFoam™ Encapsulated Amikacin (C0201) and Blank DepoFoam™ in Guinea Pigs

Key study findings: In a standard guinea pig dermal sensitization assay, there was no evidence that the neither C0201 (DepoAmikacin) nor Blank DepoFoam control produced any evidence of sensitization. This blank contains most of the same excipients as C0401.

Study no.: SkyePharma Report No. 032-00006
Volume #, and page #: EDR
Conducting laboratory and location: [redacted]
Date of study initiation: May 8, 1995
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: C0201, DepoAmikacin (15 mg Amikacin/ml (DepoTech Lot No. 95-2003)
Formulation/vehicle: Blank DepoFoam (DepoTech Lot No. 95-2002)

Methods
This GLP study consisted of two in-life phases: (1) induction and (2) challenge and was conducted according to the Magnusson and Kligman maximization Protocol. Briefly,

adult female Hartley guinea pigs (300-350 g) were randomly allocated into one of the following experimental groups (10 animals/group):

Group 1	Exposed to C0201 prior to challenge with C0201 21 days later
Group 2	Exposed to placebo DepoFoam prior to challenge with placebo DepoFoam 21 days later
Group 3	Not exposed to C0201 prior to challenge with C0201 21 days later
Group 4	Not exposed to placebo DepoFoam prior to challenge with placebo DepoFoam 21 days later

A combined intradermal injection/dermal patch induction protocol was employed. On study Day 0, each animal was administered a series of six intradermal injections to a shaven area on the anterior upper flank. These adjuvant-based intradermal injections served to initiate the induction phase of the study and to maximize potential detection of a potentially weak response upon subsequent challenge. On study Day 6, a 10% suspension (w/v) of sodium lauryl sulfate (SLS) in liquid paraffin was applied to the intradermal injection site to generate a mild inflammatory response, resulting in permeabilization of the epithelial barrier at the induction site. On study Day 7, the SLS suspension was removed, and a dermal patch saturated either with placebo DepoFoam™ (Group 1) or saline (Groups 2) was applied to the primed induction site. The induction phase of the study concluded with removal of the induction patches after a 48-hour exposure period.

On study Day 21, animals were challenged with placebo DepoFoam™ (Group 1) via dermal patch. The challenge patch, saturated with test article and affixed over a naive shaven site on the flank, was left in place for 24 hours. Visual scoring of all challenge sites occurred 24 and 48 hours after patch removal according to the four-point scale reproduced below:

Score	Dermal Reaction
0	No reaction
1	Scattered and mild erythema (no edema)
2	Moderate and diffuse erythema (no edema)
3	Intense erythema and edema

Sensitization Rate (% positive responses)	Grade	Classification
0 - 8	I	Weak
9 - 28	II	Mild
29 - 64	III	Moderate
65 - 80	IV	Strong
81 - 100	V	Extreme

Doses:

Study design: The study designed in summarized in the Sponsor's table 1 below:

Table 1. Design of dermal sensitization study of DepoFoam™ encapsulated amikacin (C0201) and DepoFoam in guinea pigs

	GROUP 1 (n=10)	GROUP 2 (n=10)	GROUP 3 (n=10)	GROUP 4 (n=10)
Induction/Challenge Scheme	C0201/C0201	DF/ DF	NS/C0201	NS/DF
Day 0 i.d. injections (induction)	area 1: FA area 2: 5% C0201 in NS area 3: 5% C0201 in FA	area 1: FA area 2: 5% DF in NS area 3: 5% DF in FA	area 1: FA area 2: NS area 3: 1:1 FA/NS	area 1: FA area 2: NS area 3: 1:1 FA/NS
Day 6	Application of 10% SLS to intradermal injection site	Application of 10% SLS to intradermal injection site	Application of 10% SLS to intradermal injection site	Application of 10% SLS to intradermal injection site
Day 7 (induction)	Remove SLS and apply C0201 induction patch	Remove SLS and apply DF induction patch	Remove SLS and apply NS induction patch	Remove SLS and apply NS induction patch
Day 9	Remove induction patch	Remove induction patch	Remove induction patch	Remove induction patch
Day 21 (challenge)	Apply C0201 challenge patch	Apply DF challenge patch	Apply C0201 challenge patch	Apply DF challenge patch
Day 22	Remove challenge patch	Remove challenge patch	Remove challenge patch	Remove challenge patch
Day 23	24 hr post-challenge evaluation	24 hr post-challenge evaluation	24 hr post-challenge evaluation	24 hr post-challenge evaluation
Day 24	48 hr post-challenge evaluation	48 hr post-challenge evaluation	48 hr post-challenge evaluation	48 hr post-challenge evaluation
Day 24	Terminate	Terminate	Terminate	Terminate

i.d. = Intradermal
FA = Freund's complete adjuvant solution
NS = normal saline
DF = Blank DepoFoam
SLS = sodium lauryl sulfate

A positive control was also completed concurrently. The positive control was dinitrochlorobenzene (DNBC), a known dermal sensitizing agent (i.e., test article in the study design above).

Results: No dermal reactions were noted at the challenge sites of animals exposed to saline prior to placebo DepoFoam™ challenge. Twenty-four hour and forty-eight hour post-challenge dermal sensitization scores of zero (no reaction) were recorded for each animal in this treatment group. Likewise, no dermal evidence of delayed-type sensitization was detected in any animal exposed to and subsequently challenged with placebo DepoFoam™. Twenty-four hour and forty-eight hour post-challenge dermal sensitization scores of zero (no reaction) were recorded for each animal in this treatment group. Based upon the dermal scores recorded in this study, a sensitization rate of 0% was calculated for placebo DepoFoam™. Consequently, placebo DepoFoam™ was classified as a Grade I (weak) sensitizer when tested in guinea pigs using the Magnusson & Kligman maximization model. This is the lowest classification achievable in the model employed.

Table 2. Mean 24- and 48-hour post-challenge dermal reaction scores for sensitized and non-sensitized C0201- and blank DepoFoam-challenged guinea pigs.

	Post-Challenge Dermal Reaction Score*	
Group Number	24-Hour	48-Hour
1	0 (0)	0 (0)
2	0 (0)	0 (0)
3	0 (0)	0 (0)
4	0 (0)	0 (0)
Positive Control Validation	0.9 (0.3)	1 (0.4)

* Values are mean (SD) for n=10 animals/treatment group

The results of this assay indicate that Placebo DepoFoam™ possesses a low potential for eliciting cell-mediated immune responses in *in vivo* test systems having intact, functional immune mechanisms. It is important to note that the DepoAmikan and the DEPODUR® do not contain identical inactive ingredients, as noted in the table below:

DEPODUR® Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DepoAmikan® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	
Cholesterol	3.3	
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	
Tricaprylin	0.3	
[redacted]	--	
[redacted]	--	
[redacted]	--	
Triolein	0.1	

Study title: A 29-Day Toxicity Study of DepoFoam™-Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Mouse

Key study findings: A mild inflammatory response associated with histiocyte infiltration was noted at the injection site. The histiocytes are thought to be the resident macrophages and function to clear damaged tissues.

Study no.:	(SkyePharma Report No. 032-00005)
Volume #, and page #:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	March 28, 1995
GLP compliance:	Yes
QA reports:	yes (X) no ( )
Drug, lot #, and % purity:	C0201 (DepoAmikacin), Lot 95-2003
Formulation/vehicle:	Blank DepoFoam, DepoTech, Lot 95-2002

Methods: Placebo DepoFoam™ was evaluated for potential systemic and local toxicity in adult albino mice following repeated (i.e., 5 times) subcutaneous administration. In this GLP study, non-Swiss albino CFI mice (16 to 26 g at the start of the treatment period, n = 10/sex/group) were administered 0.75 ml subcutaneous injections of either placebo DepoFoam™ or physiologic saline (controls). Test and control articles were administered by subcutaneous injection every seven days over a 29-day study period. Each animal on study received a total of five (5) injections throughout the in-life period. Data collected from this study included weekly body weights, daily clinical observations, daily food/water consumption, baseline and terminal clinical urinalysis, terminal clinical hematology/chemistry, and postmortem gross necropsy and histopathology.

Doses: 0.75 ml via subcutaneous injection.

Study design: The dosing groups are outlined in the table below with regard to the Amikacin:

Table 2. Design of treatment groups for 29 day toxicity study of C0201 (DepoFoam encapsulated amikacin) administered subcutaneously at 7 day intervals to mice for 29 days.

Group Number	Treatment	Dose of amikacin		Number of Animals	
		mg/kg	mg/m^2*	Males	Females
1	Normal Saline Control	0	0	10	10
2	Blank DepoFoam Control	0	0	10	10
3	C0201 Low Dose	20	60	10	10
4	C0201 Mid Dose	100	300	10	10
5	C0201 High Dose	250	750	10	10

* Assuming an average 20 g mouse

Results:

Body weight and Food Consumption: A significant increase in mean body weight (9% of saline control) was noted at the terminal time point (Day 29) for males administered Blank DepoFoam™. The only notable clinical sip observed during the in-life period of

the study was transient mild behavioral depression in one placebo DepoFoam™ mouse on study Day 1 only. These body weight and clinical findings are considered to be of no biological or toxicological relevance. No differences in food and water consumption were noted for treated animals vs. saline controls.

Hematology, Clinical Chemistry Urinalysis: There were no treatment-related alterations in terminal clinical hematology, chemistry, or urinalysis parameters were noted for mice of either sex.

Histopathology: The only test-article related histopathology noted was trace to mild subacute inflammation at the injection site. The incidence of subacute inflammation in both males and females administered placebo DepoFoam™ was 90%. In comparison, 20% of males and 80% of females administered normal saline on the same repeated-injection schedule were noted for subacute inflammation at the injection site.

Table 6. Injection-site histopathology findings in female and male mice administered C0201 (20, 100, or 250 mg amikacin/kg), blank DepoFoam or normal saline subcutaneously every 7 days for 29 days.

	Normal Saline		Blank DepoFoam		C0201 Low Dose		C0201 Mid Dose		C0201 High Dose	
Sex	M	F	M	F	M	F	M	F	M	F
n	10	10	10	10	10	10	10	10	10	10
Hemorrhage	0	1	0	0	1	0	1	2	7	6
Trace	0	1	0	0	1	0	1	2	5	6
Mild	0	0	0	0	0	0	0	0	2	0
Inflammation, subacute	2	8	9	9	10	10	10	9	10	10
Trace	2	8	2	7	3	4	1	1	1	0
Mild	0	0	7	2	7	6	9	8	9	10

No other test article-related findings were observed in any of the tissues examined.

The inflammatory response in DepoFoam™ treated animals was characterized histologically by the presence of polymorphonuclear leukocytes (PMNs), lymphoid cells, and numerous histiocytes. Injection-site tissues from saline-treated mice contained notably fewer histiocytes than those from DepoFoam™ and C0201 treated animals, which contain.

Organ Weights: A significant increase in brain-relative heart weight was noted for males administered placebo DepoFoam™ (vs. saline control). The slight (14%) increase in mean brain-relative heart weight was not considered to be treatment-related since no supporting evidence of cardiac abnormality was demonstrated upon histological examination of the hearts of DepoFoam™ treated males.

Table 7. Heart (relative) and adrenal (absolute and relative) organ weight data for male mice administered C0201 (20, 100, or 250 mg amikacin/kg), blank DepoFoam or normal saline subcutaneously every 7 days for 29 days.

Treatment	Heart (% brain weight)*	Adrenal (g)*	Adrenal (% body weight)*	Adrenal (% brain weight)*
Normal Saline	36.02 (4.87)	0.014 (0.003)	0.045 (0.008)	3.32 (0.68)
Blank DepoFoam	41.06 (4.07)†	0.014 (0.004)	0.041 (0.010)	3.42 (1.11)
C0201 Low Dose	36.95 (5.19)	0.010 (0.003)	0.030 (0.007)†	2.30 (0.55)†
C0201 Mid Dose	35.52 (2.59)	0.011 (0.003)	0.033 (0.008)†	2.42 (0.54)†
C0201 High Dose	38.72 (4.53)	0.015 (0.006)	0.042 (0.017)	3.36 (1.46)

* Data are expressed as mean (SD) for 10 animals per sex per treatment group.
† $p \leq 0.05$ vs. saline control

The results of this study provide evidence that repeated subcutaneous administration of the DepoFoam Formulation used in this study produces a mild inflammatory response. In contrast to saline, the cellular infiltrate following injection of the blank DepoFoam formulation contains numerous histocytes. These cells are resident macrophages which help to clear the tissue of damaged cells and foreign material.

DEPODUR® Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DepoAmikan® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	
Cholesterol	3.3	
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	
Tricaprylin	0.3	
[ ]	--	
[ ]	--	
[ ]	--	
Triolein	0.1	

Study title: 29-Day Toxicity Study of DepoFoam™-Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Dog

Key study findings: Subcutaneous injection of DepoFoam Matrix has the potential to cause a local inflammatory reaction at the injection site. The reaction is mild and resolves.

Study no.: SkyePharma Report No. 032-00004
Volume #, and page #: EDR
Conducting laboratory and location: [ ]
Date of study initiation: April 4, 1995
GLP compliance: GLP
QA reports: yes (X) no ( )
Drug, lot #, and % purity: C0201 (Lot No. 95-2003), Purity unspecified

Formulation/vehicle: Blank DepoFoam (Lot No. 95-2003)
Purity unspecified

Methods: Placebo DepoFoam™ was evaluated for potential systemic and local toxicity in adult beagle dogs following repeated (5X) subcutaneous administration. In this GLP study, adult beagle dogs (6.2 - 10.1 kg at the start of the treatment period, n=3/sex/group) were administered 32.0 ml subcutaneous injections of placebo DepoFoam™ or physiologic saline (controls). Test and control articles were administered by subcutaneous injection every seven days over a 29-day study period. Each animal on study received a total of five (5) injections throughout the in-life period. Data collected from this study included: weekly body weights; daily clinical observations; daily food/water consumption; baseline and terminal clinical urinalysis; baseline and terminal clinical hematology/chemistry; and postmortem gross necropsy and histopathology.

Doses: C0201 was dosed at 0, 3, 15 or 40 mg/kg Amikacin. Group 1 and Group 2 provide a comparison between saline and the DepoFoam control for Amikacin, which similar, although not identical to the DEPODUR vehicle.

Study design:

Table 2. Design of treatment groups for 29 day toxicity study of C0201 (DepoFoam encapsulated amikacin) administered subcutaneously at 7 day intervals to beagle dogs for 29 days.

Group Number	Treatment	Dose of Amikacin		Number of Animals	
		mg/kg	mg/m^2*	Males	Females
1	Normal Saline Control	0	0	3	3
2	Blank DepoFoam Control	0	0	3	3
3	C0201 Low Dose	3	59	3	3
4	C0201 Mid Dose	15	297	3	3
5	C0201 High Dose	40	792	3	3

* Assuming an 8 kg dog

Results: Results for this NDA are primarily comparing the saline and Blank DepoFoam groups as it pertains to the safety of the excipients in the DEPODUR Drug Product.

Body weights: There were no significant differences in mean body weight between treatment groups of either sex throughout the in-life period.

Food and water consumption: No between-group differences in food consumption were noted for either sex throughout the in-life period. A significant increase in water consumption was noted for female dogs in the blank DepoFoam™ treatment group (vs. saline) at the last time point evaluated (Day 27); this finding was not considered to be treatment-related.

Clinical signs: Neither sex demonstrated clinical signs that could be attributed to the test article.

Hematology, clinical chemistry and urinalysis: There were no treatment-related alterations in terminal clinical hematology, chemistry, or urinalysis parameters between treatment groups for either male or female animals.

Histopathology: The only test-article related histopathologic lesion noted was trace to mild subacute inflammation at the injection-site. When saline control responses were evaluated vs. blank DepoFoam™ an increase in the frequency and severity of subacute inflammation was noted in DepoFoam™ treated animals. Whereas 50% of saline-treated animals were noted for subacute inflammation at the injection site, 100% of DepoFoam™ treated animals were noted for this response. Likewise, an increase in response severity was noted in DepoFoam™ treated animals vs. saline controls (6 mild vs. 2 trace/1 mild, respectively). The subacute inflammatory response in tissues of saline-treated animals was characterized by the presence of PMNs and lymphoid cells with very few histiocytes present. Injection-site tissues from DepoFoam™ treated dogs contained numerous histiocytes in addition to PMNs and lymphoid cells. One DepoFoam™treated female was also noted for multifocal trace hemorrhage at the injection site.

Table 7. Injection-site histopathology findings in female and male dogs administered C0201 (3, 15, or 40 mg amikacin/kg), blank DepoFoam or normal saline subcutaneously every 7 days for 29 days.

	Normal Saline		Blank DepoFoam		C0201 Low Dose		C0201 Mid Dose		C0201 High Dose	
Sex	M	F	M	F	M	F	M	F	M	F
n	3	3	3	3	3	3	3	3	3	3
Inflammation, subacute	2	1	3	3	3	3	3	3	3	3
Trace	2	0	0	0	1	0	0	0	0	0
Mild	0	1	3	3	2	3	0	3	1	1
Moderate	0	0	0	0	0	0	3	0	2	2

The above results suggest that the DEPODUR excipients would likely produce an inflammatory response following subcutaneous injection. The formulation for the DepoAmikacin® control is not identical to the formulation proposed for DEPODUR®, however, there are clear similarities, as noted in the summary table below:

DEPODUR® Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DepoAmikan® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	
Cholesterol	3.3	
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	
Tricaprylin	0.3	
[redacted]	--	
[redacted]	--	
[redacted]	--	
Triolein	0.1	

Study title: 21-Day Biocompatibility Study of DepoFoam™ Placebo Administered Subcutaneously to Rats

Key study findings: DepoFoam lipid components were found to be biocompatible.

Study no.: SkyePharma Report No. 0333-00020
Volume #, and page #: EDR
Conducting laboratory and location: [redacted]

Date of study initiation:
GLP compliance: Non-GLP
QA reports: yes ( ) no (X)
Drug, lot #, and % purity: DepoFoam Placebo, Lot 95-2002
Formulation/vehicle:

Methods: The biocompatibility of placebo DepoFoam™ was evaluated in rats over a 21-day period following administration of a single subcutaneous dose. Sprague-Dawley rats (approximately 250 g at start of study, n = 5 injection sites/time point) were "implanted" subcutaneously with sterile placebo DepoFoam™ (2.5 ml/site) on study Day 0. On post-injection days 3, 7, 14, and 21, groups of animals were euthanized and the implantation sites were removed and fixed by immersion in phosphate buffered saline (PBS) containing 10% formaldehyde solution. Following fixation, the specimens were dehydrated with increasing concentrations of ethanol, infiltrated with xylene, embedded in paraffin, and thinly sectioned. Specimens were examined for cellularity and number of cells (hematoxylin and eosin stain) and fibrous capsule formation (Mason's Trichrome stain).

Treatment Groups	Duration (Days)
Group 1	3
Group 2	7
Group 3	14
Group 4	21

Slides were examined via light microscopy and the tissue responses were evaluated by a single investigator [redacted] using the scoring system to the right.

Score	Response
0	No response
+1	Minimal response or presence
+2	Mild response or presence
+3	Moderate response or presence
+4	Extensive response or presence

Results:

Results: No evidence of acute or chronic inflammation at the implant site was observed in this study. The implant sites, when identifiable, were seen as ovoid areas lined by foamy macrophages. No foreign body giant cells were noted in any of the implant sites. Minimal to no fibrous capsule formation was identified. The presence of macrophages was identified as early as post-implantation day 3; foamy macrophages were most predominant at post-implantation day 7. By day 14, the size of the implant sites was reduced, and a collapse of the adjacent tissue with minimal to mild presence of foamy

macrophages was noted. The foamy macrophages were identified as macrophages containing vacuoles whose histological appearance would be consistent with the phagocytosis of DepoFoam™ lipids. Implant sites were unidentifiable in four out of the five day-21 specimens. An alteration in the subcutaneous fibrous connective tissue, which could be construed as an implant site, was identified in one day-21 specimen. Minimal fibrous capsule formation demarcating the subcutaneous implant sites was noted in day-3, -7, and -14 specimens; however, this finding was not interpreted to mean that fibrous capsules were actually forming around the implanted DepoFoam™ material as no increase in collagenous fibrous capsule formation/deposition was noted over time.

DepoTech DepoFoam Placebo Implant Study

HISTOLOGY EVALUATION

Animal Histology I.D.#	Implant Duration, days	Acute Inflammation	Chronic Inflammation	Foamy Macrophages	Foreign Body Giant Cells	Fibrous Capsule Formation
GF-102	3	0	0	+2	0	+1
GF-103	3	0	0	+2	0	+1
GF-104	3	0	0	+2	0	+1
GF-105	3	0	0	+2	0	+1
GF-106	3	0	0	+2	0	+1
GF-107	7	0	0	+3	0	+1
GF-108	7	0	0	+3	0	+1
GF-109	7	0	0	+3	0	+1
GF-110	7	0	0	+3	0	+1
GF-111	7	0	0	+3	0	+1
GF-112	14	[a]	-	-	-	-
GF-113	14	0	0	+1	0	+1
GF-114	14	0	0	+1	0	+1
GF-115	14	0	0	+2	0	+1
GF-116	14	0	0	+1	0	+1
GF-117	21	0	0	0	0	+1
GF-118	21	[a]	-	-	-	-
GF-119	21	[a]	-	-	-	-
GF-120	21	[a]	-	-	-	-
GF-121	21	[a]	-	-	-	-

[a]No Implant/Injection Site Identified

Conclusion: The DepoFoam™ drug delivery matrix is considered to be a histological biocompatible formulation. Based upon the Lot number of the DepoFoam™ tested by [redacted] the material is similar to the DEPODUR in terms of Nominal Content. However, the material did not contain tricaprylin and has an overall lower concentration of most of the components.

DEPODUR® Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DepoAmikan® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	[redacted]
Cholesterol	3.3	[redacted]
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	[redacted]
Tricaprylin	0.3	[redacted]
		[redacted]

Triolein	0.1

Study title: DTC 101 (DepoFoam™ Encapsulated Cytarabine) 4 Cycle Intrathecal Subchronic Toxicity Study in the Rhesus Monkey with a Subsequent Treatment-Free Period

Key study findings: Repeated I.T. administration of DepoFoam did not cause any local or systemic toxicity in monkeys.

Study no.: SkyePharma Report No. 033-00007.003
Volume #, and page #: EDR
Conducting laboratory and location: [redacted]

Date of study initiation: May 16, 1995
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Blank DepoFoam (0101) (DepoTech Lot# 95-0020) DepoCyt + Dexamethasone (see table below)
Formulation/vehicle: Saline + Dexamethasone

DepoFoam™ Components / Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DEPOCYT® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	5.0
Cholesterol	3.3	4.6
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	1.1
Tricaprylin	0.3	--
Triolein	0.1	1.2

Doses and study design:

Appears This Way On Original

Table 1. Experimental design of HE Study No. 1363-002.

Group #	Treatment	# of animals		Dose (mg)	Dose vol. (mL) ‡	Day of Necropsy *			
						Treatment		Recovery	
		M	F			M	F	M	F
1	Saline + Dex †	1	1	0	1.0	57	57		
2	DepoFoam + Dex	3	3	0	1.0	57 (2)	57 (2)	150	259
3	DTC 101 + Dex	2	2	5	0.5	57 (2)	57 (2)		
4	DTC 101 + Dex	3	3	10	1.0	57 (2)	57 (2)	165	259

† Dex = Dexamethasone (2 mg/mL). Dosing regimen: 0.5 mL, single dose on day of dosing; 0.25 mL twice daily days 2 - 7 post-dose.

‡ Dosing volume was independent of body weight. DTC 101 test material was 10 mg/mL.

* The majority of animals on-study ("treatment") were euthanized 14 days after administration of the fourth dose (day 57 post-dose). Selected animals from Groups 2 and 4 ("recovery") were sacrificed after a treatment-free period of 93 - 202 days after completion of the fourth (last) 14-day dosing cycle.

Methods

The potential toxicity of placebo DepoFoam™ multiple intrathecal doses via an implanted port-catheter system (L3-L6) was examined in adult rhesus monkeys. In this GLP study, placebo DepoFoam™ and normal saline served as vehicle and diluent control materials, respectively, for an active DepoFoam™ test formulation containing a cytotoxic chemotherapeutic agent. Eight (8) animals were divided into two (2) control groups as follows:

1. Normal saline + Dexamethasone (1 animal/sex)
2. Placebo DepoFoam™ + Dexamethasone (3 animals/sex)

Each animal on study received a total of four (4) lumbar injections administered at 14-day intervals. Veterinary-grade dexamethasone (2 mg/ml) was co-administered with the active DepoFoam™ test article to counter any neurological effects associated with lumbar administration of the cytotoxic active agent. Dexamethasone was also administered to vehicle (DepoFoam™) and diluent- (saline) treated animals to control for possible confounding effect(s) due to dexamethasone co-therapy. Dexamethasone was administered to each animal as a single 0.5 ml intramuscular injection on each of the four dosing days. Additional 0.5 ml doses of dexamethasone were administered daily to each animal for at least one week after lumbar administration of the test or control articles.

Prior to the commencement of dosing, baseline clinical pathology data (hematology panel, coagulation profile, serum clinical chemistry panel, and urinalysis panel) were obtained, and baseline neurologic, ophthalmologic, and electrocardiographic examinations were performed. Once dosing was initiated, body weight and food consumption data for each animal were monitored on a weekly basis. Throughout the eight-week in-life period, neurologic exams were performed prior to and on the day after administration of each lumbar dose. Electrocardiographic examinations were performed prior to administration of the third lumbar dose and during the final week of the in-life period. Blood and urine samples were collected from each animal during the final week of the in- life period for analysis of terminal clinical pathology. An ophthalmologic

examination was also conducted during the final week of the in-life period. Clinical observations were performed daily throughout the eight-week in-life period.

On the 14th day after administration of the fourth lumbar dose of test or control articles, all animals from the saline control group and two animals per sex from the placebo treatment group were euthanized. The remaining two animals (one per sex) in the placebo DepoFoam™ treatment group were allowed to survive and entered a recovery phase of the study. At necropsy, the cerebral spinal fluid from each animal was drained and collected for analysis. The CSF volume from each animal was replaced by 10% neutral buffered formalin and a whole body formalin perfusion was performed to preserve the brain and spinal cord for neuropathologic examination. Post-mortem organ weight data were collected, and representative samples of various tissues were collected and prepared for complete histopathologic evaluation.

Results: The results of this study demonstrate that repeated intrathecal administration of excipient-only DepoFoam™ to monkeys is well tolerated. No treatment-related alterations in behavior, body weight, food consumption, neurologic status, ophthalmoscopy, cardiovascular condition, hematology, serum and CSF clinical chemistry, or urinalysis were observed in DepoFoam™ treated animals during either the treatment or recovery phase of the study. Likewise, no unusual necropsy findings were reported for DepoFoam™ animals during either study phase.

The most prominent finding of this study was neurohistopathologic alterations observed in all DepoFoam™ treated animals, characterized by meningeal inflammation and/or astrocytic activation in brain and/or spinal cord tissues. The observed meningeal inflammation was minimal to slight in severity and diffusely observed throughout the spinal column. No inflammation was present in the peripheral nervous tissue or eyes of treated animals. Moreover, no evidence of meningeal inflammation was reported for treated animals following a suitable treatment-free period. These findings suggest that the meningeal inflammation noted in animals administered DepoFoam™ is most likely attributable to the combined physical presence of the indwelling catheter system and the DepoFoam™ material.

Glial stimulation demonstrated immunohistochemically in neural tissues from treated animals, was not accompanied by significant findings of demyelination or other notable neuropathology. Moreover, this neuroglial activation resolved spontaneously upon cessation of treatment. Taken together, these findings suggest that the glial activation reported in DepoFoam™ treated animals is most likely a result of nonspecific, reversible irritation due to the presence of the DepoFoam™ matrix in the intrathecal space.

Conclusion: Repeated intrathecal administration of DepoFoam™ to humans is unlikely to result in significant treatment-related local or systemic toxicity.

Study title: Ocular Tolerance Study of Various Carrier Formulations after a Single Intravitreal Injection in Rabbits

Key study findings: DepoFoam matrix is well tolerated following acute intravitreal injection.

Study no.:	SkyePharma Report No. 033-00021.001
Volume #, and page #:	EDR
Conducting laboratory and location:	[redacted]
Date of study initiation:	[redacted]
GLP compliance:	non-GLP
QA reports:	yes ( ) no ( )
Drug, lot #, and % purity:	Unclear from report
Formulation/vehicle:	Saline

Methods: The ocular tolerance of placebo DepoFoam™ was evaluated in rabbits over an 8-day period following administration of a single intraocular dose. In this non-GLP exploratory study, anesthetized adult male [redacted] (DB)SPF rabbits (1.4-1.8 kg, n=3) were injected intraocularly with either placebo DepoFoam™ or normal saline on study Day 1. Intraocular dosing was accomplished using a 30G, 0.5-inch needle; both eyes of each animal were injected with either placebo DepoFoam™ or saline. Parameters evaluated for treatment effect during the 8-day in-life period included clinical observations, body weight data, and ophthalmic examination. On study Day 8, animals were euthanized, macroscopic observations were recorded, and both eyes were removed for microscopic examination. Anterior and posterior ocular segments (a narrow section through the entire globe) were collected, embedded in paraffin, stained with Hematoxylin and Eosin, and examined microscopically. Globes were dissected under an operating microscope by a veterinary ophthalmologist.

Doses and study design: The two groups we are concerned with regarding the DEPODUR are Group 1 Saline control and Group 7 Placebo DepoFoam™.

Group	Dose Material[a]	Needle Size[b]	Number of Animals
1	Saline Control	30G	3
2	Vehicle for Microspheres	30G	3
3	Vehicle for Microspheres	27G	3
4	Microsphere A	27G	3
5	Liposomes A	30G	3
6	Liposomes B	30G	3
7	Depofoam	30G	3

a The dose volume for each material was 0.05 mL/eye.
b A 27-gauge, 0.5-inch or 30-gauge, 0.5-inch needle was used for dose administration.

Results: All animals survived to the scheduled sacrifice (study Day 8). No treatment-related effects on body weight, body weight gain, or qualitative food consumption were noted during the in-life period. Mild cyclitis (inflammation of the ciliary body) was noted in one of six DepoFoam™ treated eyes upon ophthalmic examination on study Day 8. No gross abnormalities were noted in DepoFoam™ treated animals at necropsy. No microscopic lesions or other histopathologic findings were noted in DepoFoam™ treated eyes. The mild cyclitis noted in one of six DepoFoam™ treated eyes was not, associated with adverse histopathologic changes in the ciliary body of the affected eye.

Conclusion: The DepoFoam™ matrix is well tolerated following acute intravitreal injection and is a potentially suitable vehicle for intravitreal delivery of therapeutic agents in humans. Comprehensive multiple-dosing studies and toxicity studies demonstrate that the DepoFoam™ matrix is associated with a very low potential for producing significant local or systemic toxicity when administered by subcutaneous (mouse, dog), epidural (dog), or intrathecal injection (monkey). Furthermore, DepoFoam™ is non-immunogenic (guinea pig), is well tolerated in the vitreal cavity (rabbits), and is biocompatible in subcutaneous tissues (rat).

Toxicology Review: DepoFoam™ Constituent Lipids:

The lipid-based DepoFoam™ drug delivery matrix is composed of microscopic spherical particles (50 - 200 μm), containing multiple nonconcentric aqueous chambers bounded by single-bilayer lipid membranes. An almost limitless variety of water-stable drug molecules may be efficiently entrapped within the multiple internal aqueous chambers of DepoFoam™ particles to produce sustained-release formulations of the active ingredient. The synthetic lipid used to formulate the DepoFoam™ matrix is analogs of common, naturally occurring lipids. Although the body of literature supporting the safety of liposome-based pharmaceuticals is growing, a paucity of toxicological information is available regarding the individual lipids that constitute the various drug delivery matrices.

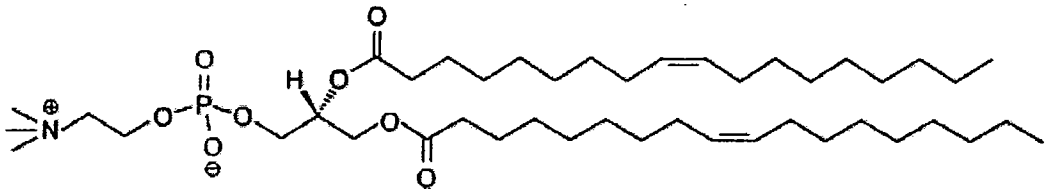
As noted previously the DepoFoam used in the DEPODUR drug product is similar to the DepoFoam used in the DepoCyt Drug product. The following table again compares the two vehicles:

DepoFoam™ Components / Inactive Ingredients	DEPODUR® Nominal Content mg per ml	DEPOCYT® Nominal Content mg per ml
Dioleoyl phosphatidylcholine (DOPC)	4.2	5.0
Cholesterol	3.3	4.6
Dipalmitoyl phosphatidylglycerol (DPPG)	0.9	1.1
Tricaprylin	0.3	--
Triolein	0.1	1.2

The Sponsor has elected to supplement the non-clinical studies with references from the literature to support the safety of the DEPODUR drug product. The following provides a review of the toxicological data available for the DepoFoam™ constituents.

Dioleoyl phosphatidylcholine (DOPC)

DOPC is a phosphatidylcholine (PC) molecule with two oleic acid residues connected to the glycerol backbone, as depicted below:



DOPC (1,2-Dioleoyl-*sn*-glycero-3-phosphocholine)

According to the Sponsor, there are no toxicology studies with the purified version of DOPC. DOPC is a naturally occurring unsaturated PC and there is a large database on information on the mixture of PCs found in the highly purified soya PC. In addition, DOPC is found in the FDA approved drug DepoCyt® at levels higher than found in DEPODUR® on an mg/ml basis. PC is a purified extract from lecithin. The word "lecithin" generally refers to a complex, naturally occurring mixture of phosphatides (phospholipids) derived from a range of animal or vegetable sources. Lecithin was first isolated from egg yolk in 1850. Currently, lecithins are widely used in the food and pharmaceutical industries. Since that time, lecithins have also been derived from vegetable sources, including soybeans, corn, cottonseed, rapeseed, and sunflowers. The primary component of lecithin is phosphatidylcholine, which itself is a mixture of fatty acid diesters of glycerophosphorylcholine. There is an extensive toxicology database available on the mixture of phosphatidylcholines (PC) that is found in highly purified soya phosphatidylcholine. The fatty acid constituents of egg PC, which is used in a different SkyePharma drug product and soya PC are comprised of a similar mixture of

saturated and unsaturated fatty acids. Based on the unsaturated/saturated (U/S) ratios of egg PC and soya PC, these mixtures of phosphatidylcholines are predominately unsaturated phosphatidylcholines. The major unsaturated fatty acid in egg PC is oleic acid, 29-33%, whereas the major unsaturated fatty acid in soya PC is 61% linoleic acid. The total unsaturated fatty acid content in egg PC is 51- 61 %, whereas soya PC contains approximately 77% unsaturated fatty acids. The table below summarizes the component fatty acids which may make up a triglyceride.

Fatty Acid Content of Egg Phosphatidylcholine and Soya Phosaphatidylcholine			
Fatty Acid	Carbon Atoms DoubleBonds	Egg PC %	Soya PC%
Pentadecanoic acid	15:0	--	--
Palmitic acid	16:0	30-33	--
Palmitelaidic acid	16:1	3	--
Stearic acid	18:0	11-15	4.3
Oleic acid	18:1	29-33	11.0
Linoleic acid	18:2	14-16	61.2
Linolenic acid	18:3	--	5.1
Arachidonic acid	20:4	3-4	--
Higher Unsaturated Fatty	C20-C22	2-5	--
Unsaturated/Saturated ratio	NA	1.6:1.1	3.3:1
[a] PC [ ]			
[b] Soya PC [ ]	Note that the % of soy and egg PC are approximates only		

A synopsis of the findings from the extensive safety program conducted with purified soya phosphatidylchholine is presented below.

Metabolism of DOPC: The fate of DOPC, the predominant phospholipid component of DepoCyt® was examined following intrathecal administration of the DepoFoam in a rat model. The results indicated that the DOPC enters standard catabolic pathways following breakdown of the DepoFoam™ particles in the intrathecal space. Only a small fraction of the parent lipid, or hydrolysis products thereof, remained incorporated in the CNS or peripheral tissues at 504 hours following administration of the DepoFoam™ (Kohn et al 1997). The ^{14}C-labeled lipid components originating from DepoFoam™ [1-^{14}C]oleic acid-labeled-DOPC, ie. phospholipid, -diglyceride, -monoglyceride, and -fatty acid were detected early in the plasma and decreased in level throughout the study. Non-lipid ^{14}C-labeled material was found in plasma early (160 minutes), and, at completion of the study, 80% of the ^{14}C-DOPC label could be accounted for. The authors concluded that the major fraction of the (1-^{14}C)-oleic acid label from DepoFoam™ DOPC was liberated as CO_2.

The results obtained for DOPC in DepoCyt® are consistent with the literature. For example, acute and chronic subcutaneous or intravascular administration of Soybean Lecithin (soya phosphatidylcholine) to dogs, rabbits, rats and mice is well tolerated at doses of 0.1 to 1.0 mg/kg (Fiume MZ, 2001). That DOPC enters standard metabolic routes and that the oleic acid enter into beta oxidation to CO_2 (Stumpf, 1969; Bremmer

and Osmundsen, 1984) may be demonstrated by demonstration that isolated rat hepatocytes produce CO_2 from phospholipid within 2 minutes of introduction (Kanoh and Akesson, 1977) and rats injected intravenous with (1-^{14}C) oleic acid enriched lipid emulsions generated ^{14}C-CO_2 in breath after a delay of only 30 minutes and 40-50% of (Redgrave et al 1995) the administered radiolabel was expired by 120 minutes.

Acute Toxicity of Soya Phosphatidylcholine: The acute toxicity of purified soya phospholipids containing 75-98% phosphatidylcholine on single administration has been examined in the mouse, rat, and rabbit by oral, subcutaneous, intravenous, and intraperitoneal administration. No mortality was observed over the 7-day post-dosing period, indicating that under the conditions employed in these studies, soya phosphatidylcholine is essentially non-toxic.

Table 2. Acute Toxicity Testing of Soya Phosphatidylcholine in Multiple Species (Phospholipon® 80/Phospholipon® 90)

Species	Route of Administration	Maximal Non-Toxic* Dose (g/kg)
Mouse	p.o.	20.0
	i.v.	4.0
	i.p.	10.0
	s.c.	10.0
Rat	p.o.	20.0
	i.v.	2.0
	i.p.	4.0
	s.c.	4.0
Rabbit	p.o.	5.0
	i.v.	0.5
	i.p.	1.0
	s.c.	1.0
Dog	p.o.	10.0

*Measured as lethality.

Subacute and Chronic Toxicity of Soya Phosphatidylcholine: Soya phosphatidylcholine has been submitted to extensive subacute and chronic toxicity testing in various species Even at exceptionally high doses, no toxicity is observed with soya phosphatidylcholine. The only compound-related effect observed in these studies was a slight increase in plasma cholesterol observed in rats administered 1 g/kg of the phospholipid intravenously; however, this was an expected effect of compensation given the massive phospholipid load administered to these animals. Values for No Observed Effect Level (NOEL) calculated for soya phosphatidylcholine from these subacute and chronic toxicity tests are presented in below.

Table 3. Subchronic and Chronic Toxicity Testing of Soya Phosphatidylcholine in Rats and Dogs (Phospholipon® 80/Phospholipon® 90)

Species	Route of Administration	Exposure Period (weeks)	NOEL* (mg/kg/day)
Rat	p.o.	4	> 800
	p.o.	6	> 1350
	p.o.	12	> 2800
	p.o.	24	> 2800
	p.o.	48	> 2800
	i.v.	4	316-1000
	i.v.	12	100-1000
Dog	p.o.	6	> 1900
	p.o.	52	> 750
	i.v.	4	> 100

* No Observed Effect Level

Similar subchronic studies conducted by Rosseneu et al. (1979), have reported no overt evidence of toxicity in adult chimpanzees fed a diet rich in polyunsaturated phosphatidylcholine for one month (37.5 g phospholipid/day; 67% linoleic, 11% oleic, 4% stearic, 13% palmitic, and 6% linolenic acid). The available scientific literature (1920-1974) concerning the subacute toxicity of soya phosphatidylcholine has been extensively reviewed in the GRAS report of the FDA (1974). Notable effects described in that report are limited to a vasodepressor effect of aged, oxidized crude soya phospholipids (3% w/v) administered intravenously to cats, and a slight reversible reduction in erythrocyte count over 25 days in dogs orally administered 5 g phosphatidylcholine per day. These notable effects were attributed specifically to the peroxide content of the oxidized crude soya phospholipid test material. The GRAS report also describes the beneficial effects of oral or i.v. administration of soya phosphatidylcholine on plasma cholesterol levels in mice, rabbits, baboon, and humans. In fact, purified soya phosphatidylcholine, the active ingredient of the drug Lipostabil, has been widely used both orally and intravenously over the past 30 years for the clinical lowering of plasma cholesterol in atherosclerosis and other hypercholesterolemic conditions. An extensive literature also exists on the experimental and clinical use of soya phosphatidylcholine as the active ingredient of the drug essentiale for the treatment of hepatic conditions.

Reproductive Toxicity and Teratogenicity of Soya Phosphatidylecholine: There is no indication of any embryotoxicity, teratogenic, peri- or post-natal toxicity, nor adverse effects on fertility in a standard battery of reproductive toxicity/teratogenicity studies conducted with soya phosphatidylcholine at very high doses. Similarly, no effects on nidation or maternal/fetal survival were demonstrated with soya phosphatidylcholine in mice, rats (1800 mg/kg for 10 days), or rabbits (475 mg/kg for 13 days) (FDA 1974). High doses of dilinolcoyl phosphatidylcholine have been shown to sensitize the pregnant rabbit uterus to the parturient action of oxytocin, but this is almost certainly due to

enhancement of the physiological generation of uterine prostaglandins (Lanman et al. 1972). Soya phosphatidylcholine had no *in vitro* effect on human sperm motility (Hong et al 1981). Similar effects have not been reported for DOPC.

Table 4. Reproductive Toxicity and Teratogenicity Testing with Soya Phosphatidylcholine (Phospholipon® 80/Phospholipon® 90)

Species	Route	Parameters Examined	Treatment Period (gestation days)	LOEL* (mg/kg/day)
Rat	p.o.	Maternal survival and teratogenicity	6-15	> 750
	i.v.	Maternal survival and teratogenicity	6-15	> 1000
Rabbit **	p.o.	Maternal survival and teratogenicity	1-6 5-18	> 1000 > 500
Rat	p.o.	Peri- and post-natal toxicity	16-end of 3rd week post partum	> 2800
	i.v.	Peri- and post-natal toxicity	16-end of 3rd week post partum	> 1000
	p.o.	Fertility	16-end of 3rd week post partum	> 2800

* Lowest Observed Effect Level
** Results of two independent studies

Mutagenicity of Soya Phosphatidylcholine: Highly purified soya phosphatidylcholine was not demonstrated to be mutagenic *in vitro* in 5 Salmonella strains (Ames test), 3 yeast strains, and human embryonic epithelial cell; negative results were also demonstrated using the mouse host-mediated and urinary assays in vivo. The addition of soy phosphatidylcholine to incubation of TA98 and 1,8-dinitropyrene reduced mutagenicity (Shaw et al 1991). However the reduction, in mutagenicity was less than that seen with uninduced S9.

Carcinogenicity: There are no carcinogenicity studies reported in the FDA's GRAS report for soya phosphatidylcholine. However, the FDA's GRAS documents did report results of a study where soya phosphatidylcholine inhibited experimental tumor induction in mice.

Dermal Safety of Soya Phosphatidylcholine in Humans: Topically-applied soya phosphatidylcholine (1%) has been used for over 30 years as a component of Essaven Gel for the treatment of peripheral venous insufficiency. No cases of intolerance have been reported.

General Tolerance of Soya Phosphatidylcholine in Humans: As much as 100 g/day of commercial soya phosphatidylcholine has been administered orally to humans without evidence of toxicity. Generally, 25 g/day is tolerated without side effects (FDA 1981). In isolated cases, gastrointestinal complaints such as diarrhea, constipation, soft stool, and loss of appetite have been reported. Vascular irritation associated with rapid intravascular infusion has also been documented. In addition to the well- documented reduction of plasma triglycerides and cholesterol, and the beneficial effects of soya

phosphatidylcholine on diseased liver, some studies indicate clinical benefit of very high oral doses of soya phosphatidylcholine (25-80 g/day) on tardive dyskinesia and other central degenerative disorders (e.g., Alzheimer's disease, Parkinson's disease). This benefit is attributable to the enhanced choline intake associated with high dose phosphatidylcholine.

Further evidence supporting the safety of naturally occurring L-α -phosphatidylcholines (L-α -PCs) such as DOPC is contained in the body of literature available on liposomal formulations of this phospholipid. Traditionally, natural phospholipids, such as L-α-PCs with neutral net charges in physiologic conditions, are used to construct liposomes that closely resemble biologic membranes. Liposomes made of naturally occurring phospholipids are generally considered safe for parenteral use. Little or no toxicity is observed with doses as large as 1 g of intravenously administered liposomes containing phosphatidylcholine or phosphatidylglycerol (Kimelberg and Mayhew, 1978, Olsen et al, 1982). A similar lack of toxicity has been reported for other synthetic L-α -PCs, such as L-α-dipalmitoyl- and L-α-dimyristoyl- phosphatidylcholine. In contrast, investigations into the toxicity of liposomes after administration into the central nervous system have yielded conflicting results. Adams et al (1977) reported that intracerebral administration of 5 to 10 mg of positively charged liposomes containing a mixture of phosphatidylcholine, stearylamine, and cholesterol (5:1:5) produced epileptic seizures and respiratory failure in mice. However, in a study by Kimelberg (1980), similar liposome preparations (up to 24 mg/kg) injected into the lateral cerebral ventricles of monkeys and rats did not produce obvious toxicity for 60 days post-administration. Similarly, Frth et al. (1984) reported that liposomes containing phosphatidylcholine and cholesterol (without stearylamine) did not produce toxicity when administered intracerebrally to rats.

Intracerebral administration of 10 mg L-α-dipalmitoyl-phosphatidylcholine has been reported not to produce any toxicity in mice (Storm et al 1993). However, in the course of studying the spinal delivery of L-α-dipalmitoyl-phosphatidylcholine liposome-encapsulated alfentanil in rats, Wallace et al. (1994) observed unusual behavior in animals receiving non-drug- containing liposomes. The effect elicited by blank liposomes appeared as touch-evoked agitation/pain (allodynia) that began 39-60 minutes after intrathecal injection of up to 1.6 mg of lipid. Further studies were conducted to explore the allodynic response observed with phospholipid preparations in this intrathecal model. Results of studies involving a series of phospholipids with various acyl substitutes, administered intrathecally at doses up to 1000 μg/animal, indicated that the dose-related allodynia observed with phospholipid preparations in this model may be associated with the production of lipid metabolites by the action of phospholipases in the intrathecal space (Yanez et al 1995).

The local and systemic toxicity of freshly-prepared and aged mixed micelles containing phosphatidylcholine and glycocholic acid as the main constituents was examined in rats, rabbits, and dogs following sub-chronic intravenous administration (Teelman et al 1984). Administration of freshly prepared mixed micelles was well tolerated in rats and dogs at doses up to 0.75 ml/kg/day for four weeks. Slight toxicity was noted at the high dose of

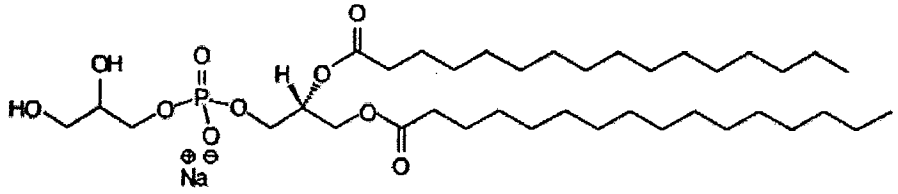
freshly prepared micelles (2.25 ml/kg/day) in both rats and dogs. Evidence of toxicity at the high dose included vomiting (dogs), slight liver enzyme elevation (rats and dogs), and slightly increased absolute liver weights (dogs). The dose at which these signs of toxicity were noted corresponded to some 30 to 50 times the anticipated clinical injection volume for this particular micellular preparation.

Effects such as intravascular hemolysis, hepatic enzyme alterations, and intrahepatic cholestasis (dogs) were demonstrated following repeated administration of artificially aged micelles. In these aged micelles, approximately 25% of the constituent phosphatidylcholine was hydrolyzed to free fatty acid and lysophosphatidylcholine. The toxicity associated with aged micelles was observed only at the highest dose examined (2.25 ml/kg/day), corresponding to approximately 40 to 60 mg lysolipid /kg body weight. The toxicity demonstrated with high-dose aged micelle treatment was found to be reversible after cessation of treatment. Further studies conducted with artificially aged micelles in rats and rabbits demonstrated a lack of embryotoxicity and teratogenicity at doses up to 2 ml/kg/day. Additional studies with the aged material confirmed a lack of mutagenicity in vitro.

DOPC Conclusion: The results of the extensive safety program conducted with highly purified soya phosphatidylcholine coupled with available literature reports concerning the overall lack of toxicity associated with phosphatidylcholines in general support the safety of unsaturated phosphatidylcholines such as DOPC in pharmaceutical preparations. The available data indicate that unsaturated phosphatidylcholines are well tolerated even when administered at doses in the g/kg range. This exposure far exceeds the lipid load associated with administration of DepoFoam™-based products, which is maximally anticipated to be in the mg/kg range.

Dipalmitoyl phosphatidylglycerol (DPPG)

Dipalmitoyl phosphatidylglycerol (DPPG) is a phosphoglyceride containing two saturated C 16 palmitic acid residues esterified to a glycerol backbone.



DPPG (1,2-Dipalmitoyl-*sn*-Glycero-3-[Phospho-rac-(1-glycerol)], sodium salt)

As is the case with DOPC, a paucity of published data exists regarding the toxicology of DPPG. However, the data that are available suggest that liposomes formulated with phosphatidylglycerol (PG), which bears a net negative charge, are associated with a lower potential, for toxicity both *in vivo* than are liposomes that bear a net positive charge. For example, negatively charged liposomes composed of PG:phosphatidylcholine:cholesterol (1:4:5) and positively charged liposomes formulated with cationic stearylamine (SA) (SA:phosphatidlycholine:cholesterol; 1:4:5) were administered to mice by intravenous injection at doses ranging from 0.5 to 10 g/kg; and animals were observed for signs of

toxicity for up to 120 days post-dose. These studies reported an intravenous LD_{50} of 1.1 g/kg for the positively charged SA-containing liposomes compared with an LD_{50} of 7.5 g/kg for negatively charged PG-containing liposomes. In vitro, SA-containing liposomes bearing a net positive charge (10^{-4}M lipid) were demonstrated to be highly toxic to chick myocardial cells, whereas little effect was noted when cells were incubated in the presence of equimolar concentrations of negatively-charged PG-containing liposomes. Limited information regarding the effects of this specific lipid on in vivo test systems is also available from kinetic or therapeutic studies conducted with DPPG-containing liposomal formulations. For example, no apparent evidence of acute toxicity was reported in biodistribution studies conducted with DPPG liposomes in mice (Oku et al 1992). In these intravenous-dosing studies, normal and tumor-bear-ing mice were administered [^{3}H]-inulin-labeled DPPG liposomes (30 μmol lipid) and sacrificed at various intervals up to 12 hours post-dosing. No unusual clinical observations or gross pathology indicative of potential lipid toxicity were reported.

Oku et al. (1994) reported no evidence of toxicity following administration of liposomes containing DPPG and adriamycin to tumor-bearing mice. In these studies, the therapeutic benefit of "conventional" adriamycin-containing liposomes composed of DPPC, cholesterol, and DPPG (40:40:10) was compared with that of long-circulating adriamycin liposomes containing a modified uronic acid derivative, in place of DPPG. Liposomal preparations (5.3 mmol or 3.49 mg in the form of DPPC) were administered intravenously to Meth-A sarcoma bearing mice according to various administration schedules. Therapeutic endpoints (body weight, tumor volume, tumor weight, and survival) were monitored throughout a 25-day post-doing experimental period. Relevant findings (i.e., decreased body weight gain, decreased survival) noted for liposome-treated animals during the experimental period were attributed to the known toxicity of the active chemotherapeutic agent, adriamycin, or to the temporal progression of the experimental sarcoma. No obvious toxicity ascribable to the lipid components of the formulations was reported.

Conclusion of DPPG: The available literature concerning the *in vivo* and *in vitro* effects of PG-containing liposomes suggests that liposomes formulated with negatively charged phospholipids such as DPPG will be well tolerated at concentrations normally used in pharmaceutical preparations. The available data indicate that PG-containing liposomes are well tolerated even when administered at doses in the g/kg range. This exposure far exceeds the lipid load associated with administration of DepoFoam™ based products, which is maximally anticipated to be in the mg/kg range.

Cholesterol

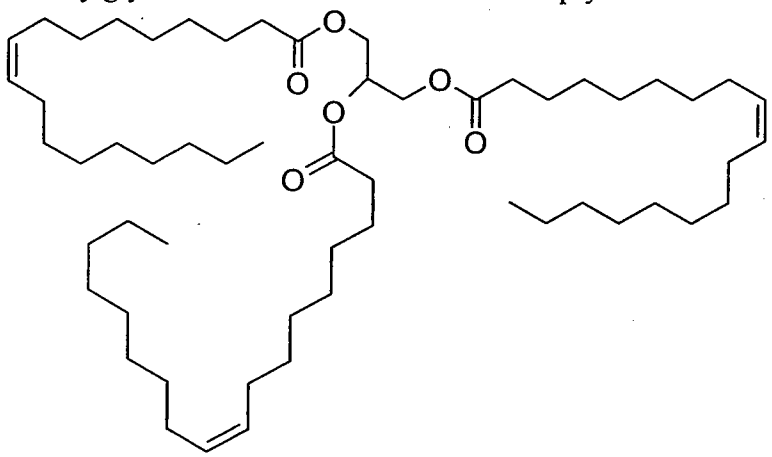
Besides phospholipids, the DepoFoam™ matrix also contains appreciable amounts of triglycerides (neutral fat) and cholesterol. Following degradation of DepoFoam™ particles either at the site of local administration or within the cells of the reticuloendothelial system (RES), the cholesterol component of the matrix will become available for routine cellular metabolic processes. Like dietary and endogenous cholesterol, cholesterol liberated from DepoFoam™ particles will be transported via

lipoprotein complexes to the liver where it may ultimately be excreted into bile converted to bile acids or packaged into lipoproteins and secreted back into blood.

Fuji and Epstein 1979 reviewed limited body of data addressing the gross toxicity of various liposome preparations in various animal models. While many of these studies involve cholesterol-containing liposomal formulations, the vast majority of studies were conducted using systemic routes of administration (e.g., i.v., i.p.) and thus provide little relevant data regarding the tolerability of locally-administered DepoFoam™ formulations. Nevertheless, it is generally accepted that the presence of cholesterol in liposome formulations will not adversely affect their safety.

Triolein

All DepoFoam™ formulations also contain one or more neutral lipid component(s) in the form of simple triacylglycerols such as triolein and/or tricaprylin.



The safety of these triacylglycerols has been established in numerous studies, which were recently reviewed by Johnson, 2001.

Triolein contains three residues of oleic acid (monounsaturated C18 fatty acid), a naturally occurring free fatty acid, esterified to the glycerol backbone. Triolein is a normal constituent of fat cell and chylomicrons (Bryson and Bischoff, 1967).

The effect of triolein on the monocyte-macrophage system was evaluated using four groups of Wistar female rats (average body weight = 150 ± 20 g). Dosing with triolein was administered according to the following schedule: single i.v. injection with 50 mg/100 g (group A); two i.v. doses of 25 mg/100 g, separated by 24 hours (group B); single i.v. dose of 16.7 mg/100 g (group C); and single i.v. dose of 25 mg/100 g (group D). Phagocytic activity indices were determined over a period of 7 days (at intervals of 24 hours after the last i.v. injection) by measuring the rate of clearance of 8 mg of colloidal carbon in 1% calfskin gelatin per 100-g body weight. Compared with untreated controls, the overall phagocytic activity of group A increased 100% within 24 to 48 hours

after dosing. In group B, the overall phagocytic activity increased 500% within 24 hours after the second dose. Results for group C indicated a fourfold increase in carbon clearance within 24 hours. This degree of phagocytic stimulation was twice as great as that induced by a single i.v. dose of triolein (50 mg/100 g) in group A. Results for group D indicated a greater degree of phagocytic stimulation than a dose of 50 mg/100 g (group A); however, in group D, a period of 72 hours was required for reaching peak activity (Altura and Hershey, 1970), Triolein has been described as one of the most potent stimulants of macrophages (Mouton et al., 1975).

Acute Intravenous Toxicity: Triolein was injected intravenously into two mongrel dogs (weights = 10 to 15 kg). Injection was repeated at 30, 60, 120, 180 and 240 minutes and after 24 hours. The animals were killed and lungs were prepared for gross and microscopic examination. No changes in the following parameters were noted: lung compliance, arterial gases, platelet counts, prothrombin times, activated partial thromboplastin times, serum lipase, or plasma lactate. Mild tachypnea was noted after 4 hours but not after 24 hours. Hypotension was not observed in either animal. Focal areas of hemorrhage (not extensive) were observed at gross and microscopic examination. Surfactant activity was reduced in hemorrhagic areas. No alterations in any of the parameters studied were reported for the three saline-treated control dogs (Baker et al., 1969).

Short-Term Oral Toxicity: The short-term oral toxicity of triolein was evaluated along with two other glyceryl esters, trilaurin (C12) and tristearin (C18), using four groups of ten weanling rats, respectively. Each glyceryl ester was administered orally at a concentration of 25% in the diet for a period of 10 weeks. Equal gains in body weight were reported for all groups tested. No lesions were found at necropsy or microscopic examinations that were attributable to administration of test diets.

Short-term Parenteral Toxicity: Over a period of one hour, triolein (dose 30% of the LD_{50}, where LD_{50} equals 1.5x the weight of the animal in kg) was infused into the ascending aorta of each of six spontaneously breathing dogs. Four of the six dogs were made hypoxic with air supply containing 10%:90% oxygen:nitrogen. An additional six dogs were ventilated with a respirator during triolein infusion. Three control dogs received a slow saline infusion into the ascending aorta. The respiratory rate increased in spontaneously breathing dogs; two of the four dogs made hypoxic during the infusion had a respiratory arrest. Atelectasis, hemorrhages and intestinal edema were observed in the lungs in biopsy postmortem specimens of test animals but not in control animals. Severe intravascular accumulation of fat was noted in sections of the brain, heart, and kidneys stained for fat. However, accumulation of fat in the lungs was small (Shaffer et al, 1975).

Genotoxicity: The mutagenicity of triolein was evaluated using *Salmonella typhimurium* strains TA1535, TA100, TA1537, TA1538, and TA98, with and without metabolic activation, according to the procedure of Ames, McCann, and Yamasaki (1975). At a test concentration of 1 mg per plate, triolein was not mutagenic, with or without metabolic activation, in any of the strains tested. The concentration of 1 mg/plate was the greatest dose tested due to limits of solubility, which did not allow testing at concentrations great

enough to cause lethality. Based on the results of this test, triolein was not mutagenic (Ames et al 1975). In an *in vitro* differential DNA repair assay using Escherichia coli, triolein reduced nitrosamine-induced DNA damage in the 1-10 mg/ml dose ranges. In an *in vitro* liquid preincubation assay, triolein also prevented nitrosamine-induced genotoxicity.

Co-carcinogenicity: The co-carcinogenicity of triolein was evaluated using groups of 33 castrated male Marsh mice and groups of 28 intact male BALB/c mice. Groups of Marsh mice were injected subcutaneously with 60-hydroxyperoxy-4-cholesten-3-one (in triolein or sesame oil) and with sesame oil (control). Groups of BALB/c mice were injected with 6-β-hydroxyperoxy-4-cholesten-3-one in triolein, sesame oil, or 2% Balb serum, or with triolein, or sesame oil (controls) alone. Comparisons between groups were made up to age 19 months. In Marsh mice, 6-β-hydroxyperoxy-4-cholesten-3-one (5 mg) in sesame oil and triolein produced 9% and 18% sarcomas respectively. In Balb/c mice, 60-hydroxyperoxy-4-cholesten-3-one (10 mg) alone did not produce local sarcomas but caused 7% local hemorrhagic cysts when tested in sesame oil. Tumors were not observed in any of the groups (both strains) injected with triolein or sesame oil alone. In another comparison, 6-β-hydroxyperoxy-4-cholesten-3-one did not increase the incidence of lung adenomas in Marsh mice over those observed in triolein and sesame oil control groups (Bryson and Bischoff, 1964). However, in Balb/c mice, the incidence of 6-β-hydroxyperoxy-4-cholesten-3-one (in saline)-induced lung adenomas (39%) was significantly greater when compared with controls.
The researchers in the preceding study added that, to date, 6-β-hydroxyperoxy-4-cholesten-3-one in sesame oil, cottonseed oil, and/or triolein has produced sarcomas in Marsh and C57 mice and in Evans rats, but not in Swiss and Balb mice. Additionally, 6-β-hydroxyperoxy-4-cholesten-3-one, administered as an isotonic aqueous suspension, did not produce neoplasms in Marsh, Balb, or Evans strains. The investigators also stated that, when effective, triolein (a major constituent of the oils tested) acts as the local co-carcinogenic factor.

Clinical Assessment of Safety: Eight adult subjects (ages 21 to 51) were fed 10 μCi [^{14}C]-triolein in 5 g olive oil together with a standard breakfast. The collection and assay of expired air began 1 hour after dosing and continued until lunchtime. The maximum rate of excretion of glyceryl- trioleate-l-^{14}C as $^{14}CO_2$ in expired air occurred 5 to 6 hours after the beginning of the experiment (Bloomstrand and Kager, 1973).

The fate of orally administered [1-^{13}C] and [8-^{13}C]-triolein was evaluated using four healthy human subjects (two males, two females). In the first experiment, 100 mg of [1-^{13}C]-triolein was administered orally (postprandial). One week later in a second experiment, the four subjects received 100 mg of [8-^{13}C]-triolein. The fate of both radioactive compounds was traced in serum lipids. A trend of an increase in absolute concentration of triglyceride oleic acid was noted. [^{13}C]-enrichment in palmitic, stearic, linoleic, and oleic acids of these fractions was determined using gas chromatography/isotope ratio mass spectrometry. At 1, 2, 4, 7, and 9 hours after dosing, a range of 2% to 24% of [1-^{13}C]-triolein was recovered in the serum triglyceride fraction, compared to 10% to 60% of the [8-^{13}C]-triolein dose. Thus, after administration of [8-

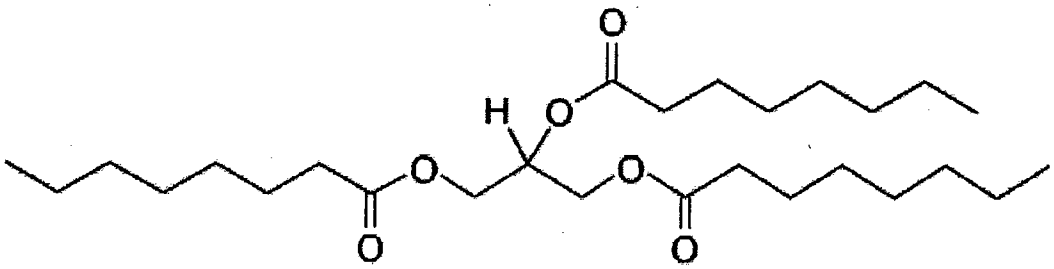
^{13}C]-triolein, oleic acid was significantly more highly enriched in [^{13}C] than after [1-^{13}C]-triolein administration. This difference could have been due to faster elimination of after [1-^{13}C] from serum. [^{13}C] enrichment in other fatty acids of the triglyceride fraction as well as phospholipid and cholesterol ester fractions was in the range of [^{13}C] abundance (Metges at al., 1974).

Eight patients with chronic pancreatitis were fed a breakfast containing [^{14}C]-triolein (10 gCi). These patients, regarded as having normal lipid assimilation (< 7 g of fat per day excreted), excreted in the feces <10.4% of the ingested dose of ^{14}C. Thirteen eczema patients (median age = 68 years) with contact allergy to olive oil were patch-tested with triolein (30% in petrolatum). Patch tests were applied to normal skin of the back using Finn chambers secured with Scanpor tape. The chambers were removed after 48 hours, and reactions were recorded according to International Contact Dermatitis Research Group recommendations. All patch tests were negative (Malmkvist, Pettersson, and Svensson, 1990). In another case report, patch tests with triolein (30% in Vaseline) on 2 patients with dermatitis (one woman, age = 71; one man, age = 60 yrs) also yielded negative results.

Conclusion: Based on the available animal and clinical data, triolein is expected to be safe for pharmaceutical use in DepoFoam™.

Tricaprylin

Tricaprylin contains three residues of caprylic acid, a naturally occurring medium chain (C8) saturated fatty acid, esterified to the glycerol backbone. Tricaprylin is not only a normal constituent of fat cells and chylomicrons (Bryson and Bischoff, 1969), but it has also been used as an energy source for bum patients and for patients having difficulty digesting long-chain fatty acids (NTEI report 1994).



Tricaprylin (1,2,3-Trioctanoylglycerol)

Acute Oral Toxicity: The acute oral LD_{50} values for tricaprylin in male and female mice have been reported to be 34.2 and 29.6 g/kg, respectively. Likewise, the acute oral LD_{50} values for tricaprylin in male and female rats have been reported to be 34.2 and 33.3 g/kg, respectively. Collectively, the acute oral toxicity of tricaprylin is very low.

Acute Intravenous Toxicity: The acute intravenous toxicity of tricaprylin was evaluated using six groups of 10 mice (strain not stated; 13-29 g). Tricaprylin (25% emulsion) was

injected into the tail vein of each animal. Motor uneasiness developed immediately after injection, which was followed by hind leg spasms, respiratory distress, urination, lateral recumbency, as well as froth at the nose. A mean acute intravenous LD_{50} of 3,700 ± 194 mg/kg was reported (Wretlind, 1957). In another study, a minimum lethal intravenous dose of 4 g/kg for a tricaprylin emulsion was reported for two groups of male and female mice, respectively, and two groups of male and female rats, respectively. The researchers concluded that tricaprylin induced very low acute toxicity when administered intravenously to mice and rats.

Acute Intraperitoneal Toxicity: The minimal lethal dose for tricaprylin in two groups of male and female mice, respectively, and in two groups of male and female rats (number of animals not stated), respectively, was >27.8 g/kg. The authors concluded that tricaprylin induced very low toxicity when administered intraperitonealy to mice and rats.

Acute Subcutaneous Toxicity: The minimum lethal dose of tricaprylin was determined to be >27.8 g/kg in two groups of male and female mice, respectively, and in two groups of male and female rats, respectively (number of animals not stated). The researchers concluded that tricaprylin induced very low toxicity when administered subcutaneously to mice and rats.

Short-Term Oral Toxicity: The short-term oral toxicity of tricaprylin was evaluated using groups of male and female Wistar rats (7 to 10 per group). The groups were dosed for 31 days with 2, 5, or 10 ml/kg. Compared with controls dosed with distilled water, statistically significant ($0.01 < p < 0.05$) differences in the following clinical chemistry and hematological parameters were noted: urea nitrogen (mg/dl) significantly lower in groups of female rats dosed with 5 or 10 ml/kg, glutamic oxaloacetic transaminase (GOT) activity and erythrocyte counts (x $10^4/mm^3$) significantly lower in males dosed with 2 ml/kg, glutamic pyruvic transaminase (GPT) activity significantly lower in females dosed with 10 ml/kg, and leukocyte counts (x10^2 /mm^3) significantly higher in females dosed with 10 ml/kg. Glucose concentration (mg/dl) was significantly greater ($p < 0.01$) in males dosed with 2 ml/kg (Ohta et al., 1970). Compared with distilled water controls, there was significant reduction in heart weight ($0.01 < p < 0.05$) in males dosed with 2, 5, or 10 ml/kg; significant reduction in spleen weight ($0.01 < p < 0.05$) in males dosed with 5 ml/kg or 10 ml/kg; significant reduction in right and left kidney weights ($p < 0.01$) in males dosed with 5 ml/kg or 10 ml/kg; significant reduction in left testis weight in males dosed with 2 ml/kg ($0.01 < p < 0.05$), 5 ml/kg ($p < .01$), and 10 ml/kg ($0.01 < p < 0.05$); and significant reduction in right testis weight in 2 males dosed with 2 ml/kg ($0.01 < p < 0.05$) and 10 ml/kg ($p < 0.01$). At microscopic examination, no lesions were found in either test or control groups.

Short-Term Subcutaneous Toxicity: Tricaprylin was used as a vehicle control in a study evaluating the short-term subcutaneous toxicity of monocrotaline pyrrole. Ten male and ten female rats of the SPF CSE strain (average weight = 1200 g) were injected with 0.25 ml tricaprylin twice a we7ek for 5 weeks (total of 10 injections in right flank, same site). The animals were killed in pairs (one male, one female) 24 hours after the first injection and all subsequent injections. Subcutaneous tissue at the injection site was

removed and prepared for histological examination. Initially, subcutaneous injections of tricaprylin produced a granulomatous reaction characterized by numerous oil deposits surrounded by several layers of macrophages, accompanied by mononuclear cells and a few polymorphs. Additionally, from the third injection until the end of the experiment, fibrous tissue formed around the oil globules and fibroblasts (together with other chronic inflammatory cells) were observed between strands of collagen (Hooson and Grasso 1976).

Chronic Oral Toxicity: The chronic oral toxicity of tricaprylin was evaluated using groups of male Wistar rats (8 to 12 per group). The groups were dosed with Tricaprylin for 26 weeks. Compared with rats dosed with distilled water, significant-reductions in GOT activity and hemoglobin concentration were noted in rats dosed with 10 ml/kg. Statistically significant increases in organ weight ($0.01 < p < 0.05$) were reported for the liver (2 ml/kg dose group) and adrenal glands (2 ml/kg and 10 ml/kg dose groups) (Ohta et al. 1970). In another chronic oral toxicity experiment, tricaprylin caused few lesions in the kidneys, myocardium, and the aorta of Wistar rats.

Genotoxicity: The mutagenicity of tricaprylin was evaluated using the Ames preincubation test procedure with and without metabolic activation in the following *Salmonella typhimurium* strains: TA97, TA98, TA100, and TA1535. Tricaprylin was mutagenic only in strain TA1535 (with metabolic activation) at doses > 6666 µg/plate (Zeiger et al., 1996).

The mutagenicity of tricaprylin was evaluated in a dominant lethal study using T-stock and (C3H xC57BL) F-1 female mice. In the first experiment, 44 T-stock females were used. Forty-seven T-stock females and 37 (C3H x C57BL) F-1 females were used in the second experiment. In both experiments, a single intraperitoneal dose (0.2 ml) of tricaprylin was administered to female mice of both strains. Over a period of six days post dosing, the females were mated with (C3H x C57BL) F-1 males. Tricaprylin did not induce dominant lethal mutations in female germ cells (Generoso et al., 1985).

Tricaprylin was used as a vehicle control in a host-mediated mutagenicity assay to evaluate mitotic gene conversion, which is strongly correlated with the induction of mutation. In this study, male BDII rats (weight = 200 g) each received an oral dose of tricaprylin (3 ml), after which 10^9 to 10^{10} yeast cells (*Saccharomyces cerevisiae* strain D4-RDII) were injected into the intraperitoneal cavity. *S. cerevisiae* strain D4-RDII requires adenine and tryptophan for growth, but after gene conversion, growth becomes independent of these two nutrients. The animals were then killed by cervical dislocation, yeast cells were withdrawn, and cultures were incubated for 8 hours. No difference in the spontaneous frequency of convertants was observed between vehicle control cultures and yeast suspensions that were not injected into rats (Seibert et al., 1979).

Tricaprylin served as the solvent control for a study in Chinese hamsters (between 8 and 20 weeks old) evaluating the mutagenicity of polyaromatic hydrocarbons in three assays: chromosome aberration assay, micronucleus test, and sister chromatid exchange assay.

In the chromosome aberration assay, tricaprylin (1 ml) was injected intraperitoneally into Chinese hamsters, and animals were sacrificed 24 hours later. Bone marrow from both femurs was used for chromosome preparations. Chromosome preparations from untreated animals served as controls. The results were pooled because there were no differences between preparations from treated and untreated animals. Of the 3,564 bone marrow cells, the incidence of chromosome aberration was 1.36% (1.3% gaps and 0.06% breaks). The researchers noted that this control value corresponds to the control value achieved in another laboratory (1.33 % gaps and breaks).

In the micronucleus test, tricaprylin (1 ml) was injected intraperitoneally into Chinese hamsters. The animals were sacrificed 30 hours later and bone marrow smears were prepared. The number of micronucleated polychromatic erythrocytes was determined. Because no differences were found between preparations from treated (12 hamsters) and untreated animals (12 hamsters), the results were pooled. In each of the 12 animals, at least 2000 erythrocytes were counted and the polychromatic to normochromatic cell ratio was 1:3.93. The control value for micronucleated polychromatic cells was 5.08%.

In a sister chromatid exchange (SCE) assay, one group of eight Chinese hamsters was pretreated with bromodeoxyuridine (BUDR) and fluorodeoxyuridine (FUDR), and then injected intraperitoneally with 1 ml of tricaprylin. The eight untreated hamsters were pretreated with BUDR and FUDR. Bone marrow was prepared according to the procedure used in the chromosome aberration assay. It was noted that the crucial prerequisite for the *in vivo* SCE test is the inhibition of thymidine kinase and the incorporation the *in vivo* SCE test is the inhibition of thymidine kinase and the incorporation of BUDR into the DNA. Because no differences were found between preparations from treated and untreated animals, the results were pooled. Based on pooled results, a control value of 3.2 ± 0.07 SCEs per cell (500 cells studied) was determined.

Carcinogenicity: Tricaprylin served as the vehicle control in a study evaluating the tumorigenicity of manufactured gas plant residue. The vehicle control group and an untreated control group both consisted A/J mice (6 weeks old). Both groups of mice were fed NIH-07 pellet diet. Each vehicle control mouse was injected intraperitoneally with tricaprylin (0.25 ml; single injection). The mice were killed by cervical dislocation at 260 days post injection. The lungs and stomach were removed from each animal and examined microscopically for tumors. Lung tumors were observed in 37% of vehicle controls and in 23% of untreated control mice. Pulmonary adenomas predominated. When the two groups were compared, values for the mean number of tumors per mouse were not significantly different. Gastric tumors involving the squamous portion were not observed in either group (Weyland et al 1995).

The carcinogenicity of tricaprylin also was evaluated using three groups of 60 male F344/N rats (average weights 145 g). The three groups received 2.5, 5, and 10 ml of tricaprylin/kg body weight by gavage 5 days per week for 2 years. Sixty untreated rats (average weight = 146 g) served as controls. Groups of rats were also dosed with corn oil and sunflower oil according to the same procedure to evaluate the carcinogenicity of

these two oils. Untreated control groups were also used. Groups of 50 rats (instead of 60) were used for the corn oil experiment. After a period of 15 months, 10 rats from each group were selected for interim hematologic evaluations. Rats found in a moribund state, selected for the 15-month interim evaluations, or surviving to the end of the 2-year study were killed by CO_2 asphyxiation. Necropsy and histopathologic evaluation were performed on all animals. The numbers of rats that survived to study termination are listed as follows. 2.5 ml/kg group (30 rats), 5 ml/kg group (31 rats), and 10 ml/kg group (23 rats), and untreated-control group (31 rats). Compared with untreated controls, statistically significant differences were noted in hematocrit (%), hemoglobin (g/dl), and erythrocytes (10^6/L) for the 10-ml/kg dose group (15-month interim evaluation). Results relating to incidences of neoplasia are summarized below:

Compared with untreated controls, a statistically significant dose-related increase in the incidence of pancreatic acinar cell hyperplasia and adenoma was reported for groups dosed with corn oil, safflower oil, and tricaprylin. Tricaprylin did not induce any acinar cell carcinomas, although one acinar carcinoma was found in the mid-dose corn oil group, and one was found in the mid-dose safflower oil group. One rat in the high dose safflower oil group had multiple pancreatic acinar tumors. The incidence of skin neoplasms was greater in untreated controls (skin tumor incidence = 7 of 50 rats) than in saline controls (skin tumor incidence = 1 of 50 rats). Skin neoplasms included papillomas, trichoepitheliomas, keratoacanthomas, squamous cell carcinomas, and basal cell carcinomas. This finding was not considered biologically significant because no statistically significant differences were found between saline controls and corn oil or sunflower oil untreated control groups.

Results for tricaprylin are as follows: Compared with untreated controls, a statistically significant dose-related increase in the incidence of pancreatic acinar cell hyperplasia and adenoma was reported for groups dosed with tricaprylin. Tricaprylin did not induce any acinar cell carcinomas. Additionally, a dose-related decrease (non-significant) in the incidence of pancreatic islet cell hyperplasia and adenoma or carcinoma combined was noted in rats dosed with tricaprylin. The incidence of squamous cell papilloma in the squamous portion of the stomach of rats of the highest dose group (10 ml/kg) was significantly greater when compared with the tumor incidence in untreated controls. Squamous cell papilloma was accompanied by focal to diffuse cell hyperplasia of the nonglandular stomach. The incidence of mononuclear cell leukemia in the 10 ml/kg dose group (9/53, 17% incidence) was much less than that noted for the untreated control group (23/50, 46% incidence). Additionally, compared with untreated controls, both the incidence and severity of nephropathy were diminished in the highest dose group (10 ml/kg). The researchers noted that the results of this study demonstrated that tricaprylin and sunflower oil do not offer significant advantages over corn oil as a gavage vehicle in long- term rodent studies. This is based on results indicating that each of the three caused hyperplasia and adenoma of the exocrine pancreas, decreased incidences of mononuclear cell leukemia, and reduced incidences and severity of nephropathy in male F344/N rats.

Tricaprylin served as the vehicle control in a study evaluating the neoplastic potential of monocrotaline pyrrole. Two control groups of SPF CFE rats (5 males, 5 females/group;

average weight = 100 g) were used. In one of the groups, tricaprylin (0.2 ml) was injected subcutaneously twice weekly for 30 weeks (60 injections). Dosing was followed by a 36-week nontreatment period. In the second group, injections were made twice weekly for a total of 75 weeks (150 injections). Animals with tumors were killed when their health deteriorated or when the neoplasm became ulcerated. Of the 20 rats treated, tumors were observed in 2 animals (at 50 and 61 weeks, respectively). Both tumors were sarcomas arising from the connective tissue at the injection site. According to the Investigators the occurrence of tumors in control rats was unexpected.

Tricaprylin also served as a vehicle control in a study evaluating the carcinogenicity of the pesticide, maleic hydrazide. Tricaprylin was injected subcutaneously into 61 newborn mice (of 16 litters) in volumes of 0.1, 0.1, 0.2, and 0.2 ml on days 1, 7, 14, and 21 after birth, respectively. The results were reported based on the number of survivors at the time that the first tumor was observed (23 males, 22 females injected with tricaprylin). There were 47 male and 47 female survivors in the untreated control group. Sixteen and 14 tumors were reported for the 23 male and 22 female survivors injected with tricaprylin, respectively. In both males and females, most of the tumors were found in the lymphoid tissues. Tumors of the lung and liver were observed in male mice but were not observed in females. Of the tricaprylin-treated mice that survived (23 males, 22 females), the percentages of males and females with tumors were 60.9% and 59.1%, respectively. In untreated controls (47 male and 47 female survivors) the percentages of males and females with tumors were 51.1 %and 42.6%, respectively (Cabral and Ponomarkov et al 1982).

In a study evaluating the carcinogenicity of di- and tri-functional a-chloro ethers and 1,4-Dichlorobutene-2 in ICR/Ha female Swiss mice (4 to 6 weeks old), tricaprylin (vehicle), and untreated controls were used. Tricaprylin was injected either subcutaneously or intraperitoneally weekly for 502 to 569 days (depending on level of survival). With the exception of the cranial region, all mice were evaluated by necropsy either at the end of the experiment or at the time of interim death. Tissues were subjected to histopathologic evaluation. In the subcutaneous injection (left flank; 0.05 ml weekly), the vehicle control group consisted of 50 mice and the untreated control group consisted 85 mice. No tumors were observed in untreated control mice or mice injected subcutaneously with tricaprylin. In the intraperitoneal injection experiment (lower abdomen; 0.05 ml once weekly) the vehicle-control group consisted of 30 female mice and the untreated control group consisted of 85 female mice. No tumors were observed in untreated controls or mice injected intraperitoneally with tricaprylin (Van Duren et al 1975).

In addition to the preceding study, tricaprylin has been used as a negative/solvent control in a number of carcinogenicity/co-carcinogenicity or tumorigenicity studies. An untreated control group was not used in either of these studies (Fuji et al, 1979, Prahlad et al. 1997, Nensnow et al. 1994).

Tumor Inhibition: Inbred Nb rats with implants of Nb2 lymphoma (liver implantation) were treated orally with two 150-mg doses of tricaprylin. Extensive dwnage to tumor cells was evident microscopically 4 to 11 hours after implantation; hepatocytes were

unaffected. On day 17, nuclei were pyknotic and angular, and cells were not in close contact.

Reproductive and Developmental Toxicity: Two groups of female mice received oral doses of 2 ml/kg and 10 ml/kg tricaprylin, respectively, during gestation. Of the 220 live fetuses from the 2-niL/kg-dose group, six fetuses exhibited malformations: cleft palate (1 fetus), club foot (3 fetuses), and assimilation of the ribs (2 fetuses). Of the 219 live fetuses from the 10-ml/kg group, eight exhibited the following malformations: cleft palate (3 fetuses), club foot (4 fetuses), and assimilation of the cervical vertebrae (I fetus). Curled tail (1 fetus), cleft palate (1 fetus), and club foot (1 fetus) were the only malformations reported for 3 of 220 live control fetuses. The investigators concluded that tricaprylin was not teratogenic in mice (Ohta et al. 1970). In another experiment from this study, eight female mice were dosed orally with tricaprylin during gestation. No malformation was reported for any of the 61 live fetuses.

Tricaprylin was effective in producing fusion of the endometrial epithelium (symplasma formation) and decidualization of the stroma in pseudopregnant New Zealand white rabbits (with this in mind, the investigators noted that many of the oils used as vehicles for fat-soluble materials, such as the steroidal sex hormones, have significant-estrogenic activity.) On day 0, tricaprylin (0. 1 ml) was injected into isolated segments of the uterus in which pseudopregnancy had been induced by intravenous injection of human chorionic gonadotropin. The animals were killed as groups of three on days 1, 2, 3, 4, and 6. Saline, simple ligation of the uterus, or uterine trauma served as control treatments in other uterine segments in the same animals. Trauma and ligation with saline, but not ligation alone, induced formation of symplasma. Decidualization was observed after trauma but not after ligation or saline injection alone. Compared with control treatments, tricaprylin was much more effective in inducing symplasma fon-nation. Symplasma, most typically observed in the rabbit, has been specifically described as a fusion of originally columnar cells into large, multinucleated cells with intensely acidophilic cytoplasm. According to the researchers, it is usually found at the implantation site and covering areas of decidua at the margin of the placenta, as well as in areas of decidualization induced by trauma or other artificial means ((Davis and Davenport 1975).

Tricaprylin was used as a vehicle control in a study evaluating the developmental toxicity of dichloroacetonitrile. Tricaprylin (dose not stated) was administered orally to pregnant female Long-Evans hooded rats (65 to 80 days old) on days 6 to 18 of gestation. Another control group was dosed with water according to the same procedure. Pregnant females were killed on day 20 of gestation. No statistically significant differences were found in reproductive parameters between tricaprylin and water control groups (Smith et al 1989). Conclusion: Based on the available data, tricaprylin is expected to be safe when used at levels present in pharmaceutical doses of DepoFoam™.

2.6.6.9 Discussion and Conclusions:

Toxicity of epidural and intrathecal as well as oral administration of morphine has been extensively studied and reviewed by FDA under the Duramorph® and Avinza® submission. The present formulation of morphine in DepoFoam™, the liposomal constituents have been studied in three single dose toxicity studies and one repeat dose toxicity studies (see table attached. In the acute dose toxicity the compound was injected either epidurally or intrathecally or intravenously. The maximum dose tested was 30 mg. The toxicity evaluation consists of histopathological observation as well as clinical observations. Neurological and behavioral changes observed were due to CNS related activity of Morphine. No additional toxicity was noted. Histopathological changes observed at day 4 were reversible by day 22. The repeat dose toxicity study worth discussing for the safety of the drug product. All animals on study survived repeated epidural administration of C0401 test article, DepoFoam™ vehicle control article, or saline control article without significant evidence of local or systemic toxicity. CNS exposure to the test article was confirmed in C0401-treated animals by the presence of relatively high levels of morphine in the CSF 24 hours after epidural administration of test article. Systemic morphine exposure in C0401-treated dogs was confirmed by the presence of measurable levels of drug in the serum 24 hours after epidural administration of each 30 mg C0401 dose. The generally higher levels of morphine detected in sera of female animals are consistent with their smaller size and therefore smaller total blood volume.

Behavioral effects demonstrated in this study with epidural delivery of C0401 included decreased alertness, reduction in muscle tone, and loss of coordination. These observations are consistent with the known pharmacological actions of p-agonists such as morphine at spinal and supraspinal sites. The magnitude of effect with respect to muscle tone and coordination in C0401-treated animals appeared to diminish with each subsequent dose suggesting development of behavioral tolerance in these animals over time. Interestingly, the magnitude of effect on arousal response in C0401-treated animals remained relatively constant over the course of the study.

Transient inhibitions in feeding behavior were also noted in C0401 -treated dogs with each dosing cycle. This finding is likely ascribable to the combined centrally induced somnolence and muscle weakness associated with C0401 administration. It is also possible that the nauseant properties of morphine may have contributed to decreased food consumption in C0401-treated animals since post-dosing emesis was noted in 3 of 6 animals either on the day of or on the day following C0401 administration. Conversely, post-dosing emesis was not noted in any DepoFoam™ or saline-treated animals. While epidural administration of C0401 test article produced a significant reduction in food consumption for up to 48 hours after administration of each dose, no significant effects on body weight were noted in C0401-treated animals over the course of the treatment period. The observed acute changes in feeding behavior associated with spinal delivery of C0401 did not result in a deleterious influence on general metabolic function or viability over the course of the treatment period and are therefore considered to be of minor toxicological relevance.

In addition to the various behavioral alterations associated with epidural delivery of C0401, changes in physiologic parameters were also observed following epidural delivery of the test material. Decreases in respiratory rate, blood pressure, and body temperature were demonstrated following administration of each C0401 dose. The observed respiratory rate decrease and hypotension are consistent with the known pharmacologic properties of morphine. Morphine has also been shown to alter the equilibrium point of hypothalamic heat regulatory mechanisms in dogs resulting in a decrease in body temperature (Martin, 1983; Sabbe et al, 1993). This may explain the transient hypothermia observed in the pre-sent study following administration of each C0401 epidural dose. The effects of C0401 on these physiologic parameters were fully reversible between doses and no apparent ill effects on animal health and/or viability resulted from these physiological alterations.

No evidence of systemic toxicity was demonstrated in this study following repeated epidural administration of either C0401 test article or DepoFoam™ vehicle control article. No significant treatment-related alterations in serum clinical chemistry, hematology, blood coagulation, or urinalysis parameters were noted for any C0401- or DepoFoam™ treated animal at the conclusion of the in-life period. Additionally, measurement of the clinical values of cisternal CSF sampled from all animals at the time of surgery and at necropsy fell within the parameters previously reported for this model (Sabbe et al, 1993; Yaksh et al, 1993, 1994). The findings of mildly increased CSF protein levels in two C0401-treated dogs and one saline-treated animal at necropsy indicate that a modest increase in CSF protein may occur 24 hours after epidural injection in the epidural dog model. The normal CSF protein levels reported for recovery animals indicate that this potential effect on CSF protein content is likely transient. The finding of increased cell numbers in several terminal CSF and urine samples is most likely artifactual and not related to administration of either the test or vehicle control article. Since erythrocytes were the predominant cell type in these samples and occult blood was detected in the urine samples in question, contamination of CSF and urine with blood at the time of sampling was considered a likely factor for this finding. The finding of increased white cell presence in the CSF of a single C0401-treated animal is difficult to interpret.

While increased WBC presence in CSF may suggest infection and/or trauma, the CSF white cell values for this animal were only minimally elevated. The single occurrence of this observation coupled with the slight magnitude of the effect suggests that this finding is of questionable toxicologic relevance. The absence of demonstrable significant effects of C0401 treatment on cisternal CSF composition is consistent with previous work in which daily epidural bolus delivery of morphine in comparable doses for 14 days was observed to have no effect upon CSF composition (Sabbe et al, 1993).

With respect to the potential local effects of epidurally-administered C0401 test article or DepoFoam™ vehicle control article, a mild to moderate local reaction to the implanted epidural catheter was demonstrated histologically in all animals across each of the three experimental groups. An increased density of fibroblasts and the presence of a moderate number of inflammatory cells in the pericatheter environment characterized this local

reaction. Minimal changes were observed in the intrathecal space. Interestingly, cervical block pathology scores in two of six C0401-treated animals were greater than the corresponding lumbar block scores. This finding was unexpected given the placement of the epidural catheter in the lumbar area and the absence in both animals of remarkable gross pathology in the cervical cord area at necropsy. While there was a tendency for the local inflammatory reaction to be somewhat greater in animals treated with C0401 test article, overall neuropathology scores for all animals on study were in the lower half range of the possible values (maximum score of 2 on a 0 - 4 scale), and most were within the range of scores representing minimal pathological findings (0.5 - 1.0).

The mild inflammatory reaction observed in this study across all three experimental groups has been previously reported in several studies investigating the histopathological effects of epidurally delivered saline, morphine, sufentanil, alfentanil, (Sabbe et al, 1994) clonidine (Yaksh, et al, 1994), and baclofen (Sabbe, et al, 1993) in chronically-implanted dogs. An inflammatory reaction in the epidural space to the catheter as a foreign body, even in vehicle-treated animals, has also been described in rats (Durant and Yaksh, 1986), pigs and guinea pigs (Edwards et a, 1986), and sheep (Coombs et al, 1993). Moreover, inflammatory responses in the epidural space above the site of the catheter, as observed in two of six C0401-treated animals in the present study, have also been reported following saline administration in a chronic epidural dog model (Sabbe et al, 1994).

The presence of an inflammatory response to the epidural catheter is not limited to animal models. In humans, abnormal contrast dispersion from epidural catheters within 1 week after catheter placement, this was believed to be a consequence of inflammation. Nagaro (1986) demonstrated that following 1 week of anesthetic infusion for continuous epidural block, drug dispersion within the epidural space was limited by an inflammatory envelope of tissue located at the tip of the epidural catheter. Similarly, Samuelsson et al (1987) demonstrated a significant change in redistribution from an implanted catheter over an extended period of time in a human case involving epidural morphine therapy. Fibrotic reactions to chronically implanted catheters in humans have also been described in limited autopsy reports of long-term spinal implantations (Coombs et al, 1985) and epidurograms or computerized tomographic findings after long-term epidural implants (Crul and Delhaas, 1991; Brems-Dalgaard et al, 1991).

With regard to the inflammatory potential of epidural morphine, King et al (1984) showed no histopathologic changes in dogs after a single epidural bolus injection of 0.07 mg morphine/kg in 2 ml. Edwards and coworkers (1986), using a model of long-term epidural implanted guinea pigs, came to the same conclusion that morphine was without toxic effect. In contrast, Larsen et al (1986) concluded out of a limited study with 6 goats that morphine induces an increase in cellular inflammatory response in the epidural space that is occasionally accompanied by acute exudative inflammation and fat cell necrosis. In the present study, a trend toward higher overall neuropathology scores was observed for DepoFoam™-treated and C0401-treated. Morphine and/or DepoFoam™ particles present in the test article may have slightly exacerbated the ongoing foreign body reaction to the catheter. However, the mild histopathological effects observed in the

present study in neural tissues following repeated epidural delivery of either C0401 test article or DepoFoam™ vehicle control article were considered to be of minor toxicologic significance.

In conclusion, the present study demonstrates that repetitive epidural administration of C0401 test article or DepoFoam™ vehicle control article is unlikely to result in significant systemic or local toxicity. Behavioral and systemic physiological effects associated with epidural delivery of C0401 are considered to be the consequence of the pharmacological action of morphine at spinal sites or after redistribution to extraspinal sites following epidural administration of the test article. The DepoFoam™ vehicle control article delivered in equal volumes had no effect upon behavior or physiologic function. Epidural catheter implantation resulted in a local reaction across all three experimental groups. However, CSF clinical chemistry data and independent histopathological evaluation of the epidural and meningeal tissues from C0401- or DepoFoam™ treated animals provide no evidence of specific test article related effects. These observations indicate that C0401 test article and its vehicle, DepoFoam™, are without notable effect upon spinal tissue or function in the canine chronic epidural model when administered by repeated epidural injection at the maximum repeatable dose.

All animals survived to the scheduled sacrifice (study Day 8). No treatment-related effects on body weight or qualitative food consumption were noted during the in-life period. Mild cyclitis (inflammation of the ciliary body) was noted in one of six DepoFoam™ treated eyes upon ophthalmic examination on study Day 8. No gross abnormalities were noted in DepoFoam™ treated animals at necropsy. No microscopic lesions or other histopathological findings were noted in DepoFoam™ -treated eyes. The mild cyclitis noted in one of six DepoFoam™ treated eyes was not associated with adverse histopathological changes in the ciliary body of the affected eye.

DepoFoam™ matrix administered subcutaneously at 7-day intervals over a 29-day period in rat and dog produced trace to mild subacute inflammation at the injection site. No evidence of systemic effect was associated with repeated subcutaneous administration of the DepoFoam™ matrix in either species. A similar lack of systemic effect was demonstrated in Rhesus monkeys administered a total of four intrathecal injections of the DepoFoam™ matrix via implanted catheter at 14 day. The only notable finding in DepoFoam™ treated monkeys were a mild neuro-histopathological response characterized by meningeal inflammation (minimal to slight) and/or astrocytic (glial) stimulation in brain and/or spinal cord tissues. These effects were demonstrated to be reversible upon cessation of treatment and were attributed to either the combined physical presence of the indwelling catheter and the DepoFoam™ material (meningeal inflammation) or were considered to be a result of nonspecific, reversible irritation due to the presence of the DepoFoam™ matrix in the intrathecal space (astrocytic activation). In another study where an indwelling catheter was not used and the intrathecal injection was performed by direct injection, no meningeal inflammation was seen.

In a GLP study conducted in guinea pigs (dermal reaction scores were determined for "sensitized" animals (exposed to the DepoFoam™ matrix prior to challenge with the DepoFoam™ matrix 21 days later) and "non-sensitized" control animal (exposed to saline prior to challenge with the DepoFoam™ matrix 21 days later). Based upon the dermal scores recorded in this study, a sensitization rate of 0% was calculated for the DepoFoam™ matrix. Consequently, the DepoFoam™ matrix was classified as a Grade I (weak) sensitizer when tested in guinea pigs using the maximization model. This is the lowest classification achievable in the model employed.

The biocompatibility of the DepoFoam™ matrix was demonstrated histologically in rats over a 21-day period following administration of a single subcutaneous dose ("implant"). No evidence of acute or chronic inflammation at the implant site was observed at any time over the 21-day period. The implant sites, when identifiable, were seen as ovoid areas lined by foamy macrophages. No foreign body giant cells were noted in any of the implant sites, and minimal to no fibrous capsule formation was identified. The results of this study demonstrated that the DepoFoam™ drug delivery matrix is a histologically biocompatible formulation.

In a later study the ocular tolerance of DepoFoam™ was evaluated in rabbits over an 8-day period following administration of a single intraocular dose. No microscopic lesions or other histopathological ophthalmologic findings were noted in DepoFoam™ treated eyes. Mild inflammation of the ciliary body was noted in one of six DepoFoam™ treated eyes upon ophthalmic examination on study Day 8. This finding was not associated with adverse histopathological changes in the ciliary body of the affected eye. These findings demonstrated that the DepoFoam™ matrix is well tolerated following acute intravitreal injection and is a potentially suitable vehicle for intravitreal delivery of therapeutic agents.

In summary, the safety of the DepoFoam™ drug delivery matrix has been demonstrated in several animal species including rats, mice, guinea pigs, dogs, rabbits, and primates. Comprehensive multiple-dosing toxicity studies demonstrate that the DepoFoam™ matrix is associated with a very low potential for producing significant local or systemic toxicity when administered either subcutaneously (mouse, dog), epidurally (dog), or by intrathecal injection (monkey). Furthermore, the DepoFoam™ matrix is non-immunogenic (guinea pig), is well tolerated in the vitreal cavity (rabbits), and is biocompatible in subcutaneous tissues (rat).

2.6.6.10 Tables and Figures

2.6.7 TOXICOLOGY TABULATED SUMMARY

Acute epidural Injection toxicity study of SKY 0401 in the Beagle Dog:

Species	Method of Administration (vehicle or formulation/dose/lot no)	Gender and No. per Group*	Noteworthy Findings	Study Number
Dog Beagle	Epidural Injection (3 mL) Saline: Lot Nos. WOC02B3 WOC13B1 Morphine Sulfate: 2 x 15 mg Lot No. MS003 SKY0401: 30 mg/3 mL Lot No. 99-0007 Vehicle Control: DepoFoam Lot No. 00-4006	3 Males 3 Females Recovery Animals 3 Males 3 Females	• There were no SKY0401-related effects on body weight, food consumption, hematology or biochemistry. • One male dog that received morphine sulfate was euthanized for humane reasons following the second dose. The adverse reaction was considered related to the morphine. • Morphine was detected in the plasma of the animals 24 hours after treatment with 30 mg SKY0401. The 24 hr morphine levels were consistent with previous studies conducted in beagle dogs. • There were no deaths associated with SKY0401. • Reduced neurological and decreased behavioral observations were consistent with the pharmacological properties of morphine. • There were no gross or histopathological changes associated with SKY0401. Local trauma was observed at the surgical/injection site of all treatment groups by Day 4. • Minor changes considered procedural-related were observed across most groups on Day 4 and included: -focal hemorrhage on the outer surface of the arachnoid -inflammatory cell infiltration (surface of the dura mater) -meningeal foci of inflammatory cells -hemorrhage within the lumbar and thoracic spinal cord -focal degeneration and gliosis of nerve roots -nerve fiber degeneration • There was no increase in severity or frequency in local trauma by Day 22. No lesions were seen in the SKY0401 treated group. • The no observable adverse effect level (NOAEL) is 30 mg SKY0401.	033-00025

Study was conducted at [ ], finalized November 14, 2000).

*Three animals/group were euthanized on Day 4 and the remaining animals were euthanized on Day 22.

An Epidural Injection Bioequivalence Study of [ ] Manufacturing Scale of SKY0401 in the Beagle Dog

Species	Method of Administration (vehicle/formulation)	Doses (mg)	Gender and No.	Noteworthy Findings	Study Number
Dog Beagle	Epidural Injection 3 mL SKY0401 — Lot No. 99-0007 SKY0401 — Lot No. 00-4104	30 mg	6 males	• There were no deaths. • Minor body weight loss was observed following epidural dosing. • Reduced food intake followed each day of dosing. • No difference in severity of clinical signs between dose groups. Clinical signs consisted of: – Decreased activity – Reduced appetite – Mucoid discharge from prepuce – Dilated pupils – Wet fur – Red fur staining around anus – Lying on side – Salivation – Abnormal gait – Vomitus • No treatment related gross pathological changes observed. • Bioequivalence could not be demonstrated.	033-00027

Study was conducted at [ ], finalized February 26, 2003).

A Single Dose Intravenous, Intrathecal and Epidural injection drug interactionstudy in male beagle dogs

Type of Study Study Title	Test System	Method of Administration	GLP Compliance	Testing Facility	Study Number	Location*
Other Toxicity Studies: Nonclinical Safety Studies with DepoFoam						
A 29-Day Toxicity Study of DepoFoam-Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Mouse	Mouse	Subcutaneous	Yes		032-00005	
A 29-Day Toxicity Study of Depofoam-Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Dog	Dog	Subcutaneous	Yes		032-00004	
DTC 101 (DepoFoam Encapsulated Cytabarine) 4 Cycle Intrathecal Subchronic Toxicity Study in the Rhesus Monkey with a Subsequent Treatment-Free Period	Rhesus Monkey	Intrathecal	Yes		033-00007	
Dermal Sensitization Study of DepoFoam-Encapsulated Amikacin (C0201) in Guinea Pigs	Guinea Pig	Topical	Yes		032-00006	
A 21-Day Biocompatibility Study of DepoFoam Placebo Administered Subcutaneously to Rats	Rat	Subcutaneous	No		033-00020	
Ocular Tolerance Study of Various Carrier Formulations After a Single Intravitreal Injection in Rabbits	Rabbit	Intravitreal	No		033-00021	

*Not applicable for an electronic submission.

Non Clinical Safety Studies with DepoFoam™:

Type of Study Study Title	Test System	Method of Administration	GLP Compliance	Testing Facility	Study Number	Location*
Other Toxicity Studies: Nonclinical Safety Studies with DepoFoam						
A 29-Day Toxicity Study of DepoFoam-Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Mouse	Mouse	Subcutaneous	Yes		032-00005	
A 29-Day Toxicity Study of Depofoam-Encapsulated Amikacin (C0201) Administered Subcutaneously at 7-Day Intervals in the Dog	Dog	Subcutaneous	Yes		032-00004	
DTC 101 (DepoFoam Encapsulated Cytabarine) 4 Cycle Intrathecal Subchronic Toxicity Study in the Rhesus Monkey with a Subsequent Treatment-Free Period	Rhesus Monkey	Intrathecal	Yes		033-00007	
Dermal Sensitization Study of DepoFoam-Encapsulated Amikacin (C0201) in Guinea Pigs	Guinea Pig	Topical	Yes		032-00006	
A 21-Day Biocompatibility Study of DepoFoam Placebo Administered Subcutaneously to Rats	Rat	Subcutaneous	No		033-00020	
Ocular Tolerance Study of Various Carrier Formulations After a Single Intravitreal Injection in Rabbits	Rabbit	Intravitreal	No		033-00021	

*Not applicable for an electronic submission.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

This Sponsor has submitted studies that collectively form a bridging study that ties together the existing data on morphine sulfate with the new formation using the DepoMorph™ drug delivery system. There do not appear to be any significant outstanding concerns at this time regarding the morphine sulfate. The excipients are either endogenous substances or are synthetic versions of endogenous substances which produce only minimal damage to the local tissues. The tricaprylin, however, should be qualified in a second species.

Based upon previous communication with [ ] the manufacturer of the drug substance, [ ] lots of morphine sulfate were determined to contain [ ] (Confidential Information from [ ] (structure below) contains a [ ] structure that is a structural alert for mutagenicity. The Sponsor has only recently obtained this information.

Due to the structural alert, the Sponsor should either reduce the specifications for [ ] to NMT [ ] in the drug substance or provide adequate qualification of the isolated impurity in a minimal genetic toxicology screen (one *in vitro* mutagenicity study and one *in vitro* chromosome aberration assay) tested up to the limit dose for each assay. If [ ] tests positive in either assay, the Sponsor should reduce specifications to NMT [ ] Alternately, the Sponsor may conduct carcinogenicity assessment in one species to provide adequate qualification.

Unresolved toxicology issues:

1. Tricaprylin toxicity was studied in one species. As this is a new excipient via the epidural/intrathecal route of administration, additional qualification should be completed. However, the exposure is very low and there does not appear to be any major toxicity associated with the compound. As such, this does not appear to present a significant safety concern. However, this should be further examined by the Sponsor.
2. According to [ ] the drug substance manufacturer, morphine drug substance contains an impurity [ ] that contains [ ] which is a structural alert for mutagenicity. This was not known at the time of NDA submission and the Sponsor was not informed of this impurity until completion of this review. At this time, the Sponsor should set an interim specification for [ ] in the drug substance acceptance criteria and limit [ ] levels to not more than (NMT) [ ] or provide adequate qualification of the [ ] impurity. Qualification would include a minimal genetic toxicology screen (one *in vitro* chromosome aberrations assay and on *in vitro* mutagenicity assay).

Recommendations:

From the non-clinical pharmacology and toxicology perspective, the NDA is approvable.

Suggested labeling:

Carcinogenicity/Mutagenicity/Impairment of Fertility

Studies in animals to evaluate the carcinogenic potential of morphine sulfate have not been conducted. No formal studies to assess the mutagenic potential of morphine have been conducted. In the published literature, the results of *in vitro* studies showed that morphine is non-mutagenic in the *Drosophila melanogaster* lethal mutation assay and produced no evidence of chromosomal aberrations when incubated with murine splenocytes. Contrary to these results, morphine was found to increase DNA fragmentation when incubated *in vitro* with a human lymphoma cell line. *In vivo*, morphine has been reported to produce an increase in the frequency of micronuclei in bone marrow cells and immature red blood cells in the mouse micronucleus test and to induce chromosomal aberrations in murine lymphocytes and spermatids. Some of the *in vivo* clastogenic effects reported with morphine in mice may be directly related to increases in glucocorticoid levels produced by morphine in this species.

Pregnancy

Teratogenic Effects (Pregnancy Category C)

No formal studies to assess the teratogenic effects of morphine in animals have been performed. Several literature reports indicate that morphine administered subcutaneously during the early gestational period in mice and hamsters produced neurological, soft tissue and skeletal abnormalities. With one exception, the effects that have been reported were following doses that were maternally toxic and the abnormalities noted were characteristic of those observed when maternal toxicity is present. In one study, following subcutaneous infusion of doses greater than or equal to 0.15 mg/kg in mice, exencephaly, hydronephrosis, intestinal hemorrhage, split supraoccipital, malformed sternebrae, and malformed xiphoid were noted in the absence of maternal toxicity. In the hamster, morphine sulfate given subcutaneously on gestation day 8 produced exencephaly and cranioschisis. Morphine was not a significant teratogen in the rat at exposure levels significantly beyond that normally encountered in clinical practice. In one study, however, decreased litter size and viability were observed in the offspring of male rats administered morphine at doses approximately 3-fold the maximum recommended human daily dose (MRHDD) for 10 days prior to mating. In two studies performed in the rabbit, no evidence of teratogenicity was reported at subcutaneous doses up to 100 mg/kg.

In humans, the frequency of congenital anomalies has been reported to be no greater than expected among the children of 70 women who were treated with morphine during the first four months of pregnancy or in 448 women treated with this drug anytime during pregnancy. Furthermore, no malformations were observed in the infant of a woman who attempted suicide by taking an overdose of morphine and other medication during the first trimester of pregnancy.

Nonteratogenic Effects

Published literature indicates that exposure to morphine during pregnancy is associated with reduction in growth and a host of behavioral abnormalities in the offspring of animals. Morphine treatment during gestational periods of organogenesis in rats, hamsters, guinea pigs and rabbits resulted in the following treatment-related embryotoxicity and neonatal toxicity in one or more studies: decreased litter size, embryo-fetal viability, fetal and neonatal body weights, absolute brain and cerebellar weights, lengths or widths at birth and during the neonatal period, delayed motor and sexual maturation, and increased neonatal mortality, cyanosis and hypothermia. Decreased fertility in female offspring, and decreased plasma and testicular levels of luteinizing hormone and testosterone, decreased testes weights, seminiferous tubule shrinkage, germinal cell aplasia, and decreased spermatogenesis in male offspring were also observed. Behavioral abnormalities resulting from chronic morphine exposure of fetal animals included altered reflex

and motor skill development, mild withdrawal, and altered responsiveness to morphine persisting into adulthood.

Morphine sulfate should be used by a pregnant woman only if the need for opiate analgesia clearly outweighs the potential risks to the fetus.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature R. Daniel Mellon, Ph.D. Concurrence Yes ___ No ___

REFRENCES:

Adams, M.L., Sewing, B., Forman, J.B., Meyer, E.R. and Cicero, T.J. Opioid-induced suppression of rat testicular function. J Pharmacol Exp Ther 266: 323-8, 1993

Allen, T.M. Toxicity and Systemic Effects of Phospholipids. In: Phospholipids handbook / edited by Gregory Cevc. 801-816. 1993. Publisher: New York: Marcel Dekker

Altura, B.M. and Hershey, S.G. Effects of gyceryl trioleate on the reticulo- endothelial system and survival after experimental shock. Journal of Pharmacology and Experimental Therapeutics 175(3), 555-564, 1970

Ames, B.N., McCann, J. and Yamasaki, E. Methods for detecting carcinogenesis and mutagens with the Salmonella/mammalian microsome mutagenicity test. Mutation Research (31), 347-364, 1975

Badr, F.M. and Rabouh, S. Effects of morphine sulfate on the germ cells of male mice. Terato Carcino Mutagen 3:19-26, 1983

Baker, P.L., Kuenzig, M.C. and Peltier, L.F. Experimental Fat Embolism in Dogs. The Journal of Trauma 9(7), 577-586. 1969

Bremer, J. and Osmundsen, H. In Fatty Acid Metabolism and Its Regulation, Publisher: Amsterdam; New York: Elsevier, Chapter 5, 1998

Brems-Dalgaard, E., Just, S.L. and Pedersen, H. Encapsulated epidural catheter: CT findings. Neuroradiology, 33:81-82, 1991

Blomstrand, R. and Kager, L. The combustion of Triolein-l-CI4 and its inhibition by alcohol in man. Life Sciences 13,113-123, 1973

Bryson, G. and Bischoff, F. Triolein as a Co-Carcinogen. Fed.Proc. 23, 106, 1964.

Bryson, G. and Bischoff, F. Carcinogenesis Viewed as Function Agent - The Limitations of Safety Testing. Session 2, 100-133, 1967

Cabral, J. R. P. and Ponomarkov, V. Carcinogenicity study of the pesticide maleic hydrazine in mice. Toxicology 24, 169-173, 1982

Campbell, C. Analgesia during labor: a comparison of pentobarbital, meperidine, and morphine. Obstet Gynecol 17:714- 8, 1961

Chamberlain, G., Wraight, A., and Steer, P. Pain and its relief in childbirth: report of the 1990 NBT survey. Edinburgh: Churchill Livingstone, 1993

Cicero, T.J., Meyer E.R., Bell R.D. and Koch, G.A. Effects of morphine and methadone on serum testosterone and luteinizing hormone levels on the secondary sex organs of the male rat. Endocrinology 98:367-72, 1976

Ciociola, A.A. and Gautieri, R.F. Evaluation of the teratogenicity of morphine sulfate administered via a miniature implantable pump. J Pharm Sci 72:742-745, 1983

Couch, D.B. and Sawant, S.G. The clastogenicity of morphine sulfate *in vivo*. In: Sharp B et al., Editors. The Brain Immune Axis and Substance Abuse. New York: Plenum Press, 123-9, 1995

Cowan, A., Doxey, J.C. and Harry, E.J. The animal pharmacology of buprenorphine, an oripavine analgesic agent. British J. Pharmacol 60:547-54, 1977

Cobrinik, R.W. The effect of maternal narcotic addiction on the newborn infant. Pediatrics 24:288-304, 1959

Coombs, D., Colburn, R.W., DeLeo, J.A. and Twitchell, B.B. Testing an implantable intraspinal drug delivery device in the ewe. Regional Anesthesia, 18:230-237, 1993

Coombs, D.W., Fratkin, J.D., Meier, F.A., Nierenber, D.W. and Saunders, R.L. Neuropathologic lesions and CSF morphine concentrations during chronic continuous intraspinal morphine infusion: A clinical and postmortem study. Pain, 22:337-351, 1985

Committee on Drugs, American Academy of Pediatrics. The transfer of drugs and other chemicals into human breast milk. Pediatrics 108:776-789, 2001

Crul, B.J. P. and Delhaas, E.M. Technical complications during long-term subarachnoid or epidural administration of morphine in terminally ill cancer patients. A review of 140 cases. Regional Anesthesia, 16:209-213, 1991

Das, R.K. and Swain, N. Mutagenic evaluation of morphine sulfate and pethidine hydrochloride in mice by the micronucleus test. Ind J Med Res 75:112-117, 1982

Davies, J. and Davenport, G.R. Symplasma formation and decidualization in the pseudopregnant rabbit after intraluminal instillation of tricaprylin. Journal Reproduction and Fertility (55), 141-145, 1979

DHHS/NIDA; Research Monographs Series 52; Testing Drugs for physical dependence potential and abuse liability. DHHS Pub no. (ADM) 87-1332:37. 1984

Durant, P.A.C. and Yaksh, T.L. Epidural injections of bupivacaine, morphine, fentanyl, lofentanil, and DADL in chronically implanted rats: A pharmacologic and pathologic study. Anesthesiology, 64-.43-53, 1986

Eddy, N.B. Synthetic substances with morphine-like effect. Clinical experience: potency, side effects, and addiction liability. Bull WHO 17:569-863, 1957

Edwards, W.T., DeGirolami, U., Burney, R.G., Cappadona, D. and Brickley, R. Histo-pathologic changes in the epidural space of the guinea pig during long-term morphine infusion. Regional Anesthesia, 11:14- 19, 1986

Eriksson P.S., and Ronnback, L. Effects of prenatal morphine treatment of rats on mortality, body weight, and analgesic response in the offspring. Drug Alcohol Depend 24:187-194, 1989

Falek, A., Jordan, R.B. and King, B.J. Human chromosomes and opiates. Arch Gen Psychiat; 27:511-515, 1972

FDA. GRAS Report PB-241970, 1974

FDA. GRAS Report PB-234874, 1974

FDA. Effects of consumption of choline and lecithin on neurological and cardiovascular systems. FDA Report PB82-133257, 1981

Feilberg, V.L. Excretion of morphine in human breast milk. Acta Anaesthesiol Scand 33:426-8, 1989

Fennessy, M.R, and Fearn, H.J. Some observations on the toxicology of morphine-N-oxide. J Pharm Pharmacol 21:668-673, 1969

Finnegan, J.K, Haag H.B, Larxon P.S. and Dreyfuss, M.L. Observations on the comparative pharmacological actions of 6-dimethylamino-4, 4-diphenyl-3-heptanone (amidone) and morphine. J Pharmacol Exp Ther 92:269-276, 1948

Finch, P.M., Roberts L.J., Price L., Hadlow, N.C. and Pullan, P.T. Hypogonadism in patients treated with intrathecal morphine. Clin J Pain. 16(3):251-4, 2000

Fiume, M.Z. Final Report on the Safety Assessment of Lecithin and Hydrogenated Lecithin. Reviewed by Cosmetic Ingredient Review Expert Panel. International Journal of Toxicology 20(Suppl. 1), 21-45, 2001

Fuchs, B.A. and Pruett, S.B. Morphine induces apoptosis in murine thymocytes *in vivo* but not *in vitro*: Involvement of both opiates and glucocorticoid receptors. J Pharm Exp Ther 266:417-423, 1993

Fujii, K. and Epstein, S.E. Effects of Piperonyl Butoxide on the toxicity and hepatocarcinogenicity of 2-acetylaminofluorene and 4-acetylarninobiphenyl, and their N-hydroxylated derivatives, following administration to newborn mice, Oncology 36, 105-112, 1979

Fujinaga, M. and Mazze, R.I. Teratogenic and postnatal developmental studies of morphine in Sprague-Dawley rats. Teratology 38:401-410, 1988

Gagin, R., Cohen, E. and Shavit, Y. Prenatal exposure to morphine alters analgesic responses and preference for sweet solutions in adult rats. Pharm Biochem Behav 55:629-634, 1996

Gagin, R., Cohen, E. and Shavit, Y. Prenatal exposure to morphine feminizes male sexual behavior in the adult rat. Pharm Biochem Behav 58:345-348, 1997

Gagin, R., Kook, N. and Cohen E. Prenatal morphine enhances morphine-conditioned place preference in adult rats. Pharm Biochem Behav 58:525-528, 1997

Geber, W.F. and Schramm, L.C. Congenital malformations of the central nervous system produced by narcotic analgesics in the hamster. Am J Obstet Gynecol 123:705-713, 1975

Generoso, W.M., Cain, K.T. and Hughes, P.M. Test for dominant-lethal effects of 1,2-dibromo-3-chloropropane (DBCP) in male and female mice. Mutation Research 156,103-108, 1985

Glick, S.D., Strumpf, A.J. and Zimmerburg, B. Effect of *in utero* administration of morphine on the subsequent development of self-administration behavior. Brain Res 132:194-196, 1977

Gutstain, H.B. and Akil, H., Pharmacologival Basis of Therapeutics, Goodman and Gillman, Xth Edition, Chap 23 pp569-642, 2001

Harpel, H.S. and Gautieri, R.F. Morphine-induced fetal malformations I. J Pharm Sci 57:1590-1597.NDA 21-260 Morphelan™ (morphine sulfate) Extended-Release Capsules 21, 1968

Heinonen, O.P. Birth Defects and Drugs in Pregnancy, Littleton, Publishing Sciences Group, 287-95, 1977

Hol, T., Niesink, M., Van Ree, J.M. Parenatal exposure to morphine affects juvenile play behavior and adult social behavior in rats. Pharm Biochem Behav 55:615-618, 1968

Hong, C.Y., Chaput de Saintonage, D.M. and Turner, P. A simple method to measure drug effects on human sperm motility. Br.J Clin Pharmacol. 11, 385. 1981

Hooson, J. and Grasso, P. Cytotoxic and carcinogenic response to monocrotaline pyrole. Journal of Path. II 8, 121-131, 1976

Hunter, M,A,, Vangelisti ,G.R., and Olsen, G,D. Chronic intermittent *in utero* exposure to morphine; Effects on respiratory control in the neonatal guinea pig. Biol Neonate 72:293-304, 1997

Iuliucci, J.D. and Gautieri, R.F. Morphine-induced fetal malformations II: influence of histamine and diphenhydramine. J Pharm Sci; 60:420-425, 1971

Johnson, W. Final report on the safety assessment of trilaurin, triarachidin, tribehenin, tricaprin, tricaprylin, trierucin, triheptanoin, triheptylundecanoin, triisononanoin, triisopalmitin, triisostearin, trilinolein, trimyristin, trioctanoin, tfiolein, tripalmitin, tripalmitolein, triricinolein, tristearin, triundecanoin, glyceryl triacetyl hydroxystearate, glyceryl triacetyl ricinoleate, and glyceryl stearate diacetate. International Journal of Toxicology 20 (Suppl. 4), 61-94, 2001

Johannesson, T. and Becker, B.A. The effect of maternally administered morphine on rat foetal development and resultant tolerance to the analgesic effect of morphine. Acta Pharmacol et Toxicol 1972; 31:305-313, 1972

Johnston, H.M., Payne, A.P., and Gilmore, D.P. Effect of exposure to morphine throughout gestation on feminine and masculine adult sexual behavior in golden hamsters. J Reproduction and Fertility 100:173-176, 1994

King, F.G., Baxter, A.D., and Mathieson, G. Tissue reaction of morphine applied to the epidural space of dogs. Can. Anaesth. Soc. J., 31:268-271, 1984

Kirby M.L. Recovery of spinal cord volume in postnatal rats following prenatal exposure to morphine. Dev Brain Res 6:211-217, 1983

Kimelberg, H.K., in Liposomes in biological systems, G. Gregoriadis and A.C. Allison, Eds (John Wiley and Sons, Chichester, 1980), Chap.8.

Knaap, A.G. and Kramers, P.N. Absence of mutagenic effects of morphine in drosophila. Mutation Res 40:97-100, 1976

Kohn, F.R., Malkmus, S. A., Randolph, E., Rossi, S. and Yaksh, T.L., Fate of Predominant Phospholipid Component of DepoFoam™ Drug Delivery Matrix After Intrathecal Administration of Sustained-Release Encapsulated Cytarabine in Rats. AAPS Revolutions in Pharmaceutical Sciences, 1997

Kopecky, E.A., Ryan, M.L., Barrett, J.R., Seaward, P.R., Ryan, R., Koren, G., and Amankwah, K. Fetal response to maternally administered morphine. Am J Obstet Gynecol 2000; 1 83:424-30, 2000

Kyfta, J., Rosenberg, P.H., Wahistrom, T.R., Effects of continuous epidural administration of bupivacaine through a catheter in pigs. Eur. J. Anaesthesiol., 3:473-480, 1986

Larson, J.J., Svendsen, O., and Andersen H.B. Microscopic epidural lesions in goats given repeated epidural injections of morphine: Use of a modified autopsy procedure. Acta Pharmacol. Toxicol., 58:5-10, 1986

Li, J.H., Lin L.F., Genetic toxicology of abused drugs: a brief review. Mutagenesis 13:557-565, 1998

Lindow, S.W., Hendricks M.S. Nugent, F.A., Dunne T.T., and van der Spuy Z.M., Morphine suppresses the oxytocin response in breast-feeding women. Gynecol Obstet Invest. 48(l):33-7, 1999

Martin, W.R. Pharmacology of opioids. Pharmacol. Rev., 35:283-323, 1983

Mellin, G.W. Drugs in the first trimester of pregnancy and fetal life of Homo sapiens. Am J Obstet Gynecol 90:1169-80, 1964

Metges, C.C., Kempe, K., and Wolfram, G. Enrichment of selected serum fatty acids after a small ora dosage of (I-CI3)-and 8-CI3)triolein in human volunteers analyzed by gas chromatography/combustion isotope ratio mass spectrometry. Biological mass spectrometry 23, 295-301, 1994

Mouton, D., Bouthillier, Y., Feingold, N., Feingold, J., Decreusefond, C., Stiffel, C., and Biozzi, G. Genetic control of Macrophage Functions. Journal of Experimental Medicine 141, 306-321, 1975

Nagaro, T. The histopathological changes of epidural space and effects of anesthetic after long term continuous epidural block. Japan Journal of Anesthesiology, 35:227-235, 1986

National Toxicology Program (NTP). NTP Technical Report on the comparative toxicology studies of Corn Oil, Safflower Oil, and Tricaprylin in male F344/N rats as vehicles for gavage. NTIS Report No. PB95-103958, 1994

Nestmann E.R., Lee, E. G. H., Matula, T.I., Douglas G.R., and Mueller, J.C. Mutagenicity of consitiuents identified in pulp and paper mill effluents using the salmonella/mammalian-microsome assay. Mutation Research 79, 203-212, 1980

Nesnow, S., Ross, J. A., Nelson, G., Wilson, K., Roop, B. C., Jeffers A.J., Galati, A.J., Stoner, G.D., Sangiah, R., Gold, A., and Mass, M.J. Cyclopenta[cd]pyrene- induced tamorigenicity, Ki-ras codon 12 mutations and DNA adducts in Strain A/J mouse lung. Carcinogenesis 15 (4), 601-606, 1994

Niesink, R.M., VanDerschuren, L.J., and Van Ree, J.M. Social play in juvenile rats after *in utero* exposure to morphine. Neurotoxicology 1996; 17:905-912, 1996

Oberlander, T.F., Robeson P., Ward V., Huckin, R.S., Kamani A., Harpur, A., and McDonald W. Prenatal and breast milk morphine exposure following matemal intrathecal morphine treatment. J Hum Lact. 16(2):137-42, 2000

Olofsson, C., Ekblom A., Ekman-Ordeberg , and Hjelm A., Irestedt Lack of analgesic effect of systemically administered morphine or pethidine on labour pain. Br J Obstet Gynaecol 103: 968-72, 1996

Pederson, N.T. and Marqverson, J. Metabolism of ingested C14-Triolein. Eur J Nucl Med 6, 327-329, 1981

Pedersen, N.T. and Halgreen, H. Simultaneous Assessment of Fat Maldigestion and Fat Malabsorption by a Double-Isotope Method Using Fecal Radioactivity. Gastroenterology 88, 47-54, 1985

Prahalad, A.K., Ross, J.A., Nelson, G.B., Roop, B.C., King, L.C., Nesnow, S., and Mass, M.J. Dibenzo[a,l]pyrene-induced DNA adduction, tumorigenicity, and Ki-ras oncogene mutations in strain A/J mouse lung. Careinogenesis 18(10), 1955-1963, 1997

Ribeiro-Pinto, L.F., and, Swann, P.F. Opium and oesophageal cancer: effect of morphine and opium on the metabolism of Nnitrosodimethylamine and N-nitrosodiethylamine in the rat. Carcinogenesis 18:365-369, 1997

Robieux I., Koren G., Vandenbergh H., and Schneiderman J.: Morphine excretion in breast milk and resultant exposure of a nursing infant. J Toxicol Clin Toxicol 28: 365-70, 1990

Sabbe, M.B., Grafe, M.R., Mjanger, E., Tiseo, P.J., Hill, H.F. and Yaksh, T.L. Spinal delivery of sufentanil, alfentanil, and morphine in dogs. Anesthesiology, 81(4):899-920, 1994

Sabbe, M.B., Grafe, M.R., Pfeifer, B.L., Mirzai, T.H.M. and Yaksh, T.L. Toxicology of baclofen continuously infused into the spinal intrathecal space of the dog. Neurotoxicology, 14:397-410, 1993

Sabbe, M.B., Grafe M.R., Pfeifer BL., Mirazi H.M.T. and Yaksh., T.L., Toxicology of Baclofen continuously Infused into the spinal intrathecal space of the Dog. Neurotoxicology 14(4): 397-420, 1993

Sarmuelsson, H., Nordberg, G., Hedner, T. and Lindquist, J. CSF and plasma morphine concentrations in cancer patients during chronic epidural morphine therapy and its reaction to.pain relief. Pain, 30:303-310, 1987

Sawant, S.G. and Couch, D.B. Induction of micronuclei in murine lymphocytes by morphine. Envi Mol Mutagen 25:279-283, 1995

Scherphof, G.L. Phospholipid Metabolism In: Phospholipids Handbook edited by Gregory Cevc. 777-800. Publisher: New York: Marcel Dekker, Inc., 1993

Siebert, D., Bayer, U., and Marquardt, H. The application of mitotic gene conversion in Saccharomyces cerevisiae in a pattern of four assays, in vitro and in vivo, for mutagenicity testing. Mutation Research 67, 145-156, 1979

Shafer D.A., Xie Y. and Falek A. Detection of opiate-enhanced increases in DNA damage, HPRT mutants, and the mutation frequency in human HUT-78 cells. Envi Mol Mutagen 23:37-44, 1994

Shaffer, J.W., Sealy, W.C., Seaber, A.V., and Goldner, J.L. Etiology of fat embolism syndrome: early morphologic lung changes of respiratory distress syndrome produced by triolein. Orthopaedic Surgery 5:17-521, 1975

Shavit, Y., Cohen, E., and Gagin, R , Effects of prenatal exposure on NK cytotoxicity and responsiveness to LPS in rats. Pharm Biochem Behav 59:835-841, 1998

Siddiqui, A, Haq, S., and Shaharyar, S. Morphine induces reproductive changes in female rats and their offspring. Repro Toxicol 9:143-151, 1995

Siddiqui, A., Haq, S., and Shah, R.H. Perinatal exposure to morphine disrupts brain norepinephrine, ovarian cyclicity, and sexual receptivity in rats. Pharm Biochem Behav 58:243-248, 1997

Sueoka, N., Sueoka, E., Okabe, S, and Fujiki, H. Anti-cancer effects of morphine through inhibition of tumor necrosis factor-αrelease and mRNA expression. Carcinogenesis 17:2337-2341, 1996

Shah, A.B., Combes, R.D., and Rowland, I.R. Interaction with microsomal lipid as a major factor responsible for S9-mediated inhibition of 1,8-dinitropyrene mutagenicity. Mutat Res 249, 93-104, 1991

Sueoka, E., Sueoka, N. and Kai, Y. , anticancer activity of morphine and its synthetic derivative, KT-90, mediated through apoptosis and inhibition of NF-kB activation. Biochem Biophys Res Commun 252:566-570, 1998

Swain, N, Das R.K., and Paul, M. Cytogenetic assay of potential mutagenicity *in vivo* of two narcotic analgesics. Mutat Res 78:97-100, 1980

Thomas, J.A., Korach, K,S., and Mclachlan J.A. Endocrine Toxicology. New York, NY: Raben Press, Ltd., 254, 1985

Van Duuren, B. L., Goldschinidt B.M., and Seidman,I. Carcinogenic Activity of Di- and Trifunctional alpha-chloro Ethers and of 1,4-dichlorobutene-2 in ICR/HA Swiss Mice. Cancer Research 35, 2553-2557, 1975

Vathy, I, Rimanoczy, A., Eaton, R.C., Katay, L. Sex dimorphic alterations in postnatal brain catecholamines aftergestational morphine. Brain Res Bull 36:185-193, 1995

Van Joost, T., Smitt, S., J. H., and Ketel, W .G. Sensitization to olive oil (olea europeae). Contact Dermatitis (7),309-310, 1981

Wretlind, A. The toxicity of Low-Molecular Triglycerides. Acta Physiol.Scand. 40, 338-343, 1957

Weyland, E. H., Chen, Y., Wu, Y., Koganti, A., Dunsford, H. A., and Rodriguez, L. V. Differences in the Tumorigenic Activity of a Pure Hydrocarbon and a Complex Mixture following Ingestion: Benzo[a]pyrene vs Manufactured Gas Plant Residue. Chem.Res.Toxicol. 8, 949-954, 1995

Yaksh, T.L. The spinal actions of opioids. In: Herz, A. (ed.) Handbook of Experimental Pharmacology, Vol 104/11 Springer-Verlag Berlin Heidelberg. pp. 53-90, 1993

Yaksh, T.L., Rathbun, M., Jage, J., Mirzai, T., Grafe, M., and Hiles, R. Pharmacology and toxicology of chronically infused epidural clonidine HCI in dogs. Fundamental and Applied Toxicology, 23:319-335, 1994

Yaksh,T .L., Rathbun, M., Mirazi, T., Grafe, MR ., Hiles RA., Pharmacology and Toxicology of Chronically Infused Epidural Clonidine - HCl in Dogs. Fundam.Appl Toxicol. 23, 319-335, 1994

Zagon I.S., McLaughlin P.J. Morphine and brain growth retardation in the rat. Pharmacology 1977; 15:276-282, 1977

Zeiger, E., Ashby, J., Bakale, G., Enslein, K., Klopman, G., and Resenkranz, H. S. Prediction of Salmonella mutagenicity. Mutagenesis II, 471-484, 1996

APPENDIX/ATTACHMENTS

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

/s/

Mamata De
5/18/04 08:07:44 PM
PHARMACOLOGIST

R. Daniel Mellon
5/18/04 08:08:40 PM
PHARMACOLOGIST