

**CENTER FOR DRUG EVALUATION AND  
RESEARCH**

*APPLICATION NUMBER:*

**022496Orig1s000**

**PHARMACOLOGY REVIEW(S)**



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

## Supervisory Pharmacologist Memorandum

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NDA NUMBER: 22-496  
SERIAL NUMBER: 000  
DATE RECEIVED BY CENTER: 9/28/2010  
PRODUCT:  
    **(Proposed) Trade Name:** Exparel  
    **Established Name:** Bupivacaine Extended Release Liposome Injection

INDICATION: Post-Surgical Analgesia  
INTENDED CLINICAL POPULATION: Adult patients undergoing surgery  
SPONSOR: Pacira Pharmaceuticals, Inc.  
DOCUMENTS REVIEWED: Primary review of Dr. Gary Bond;  
eCTD Submission

REVIEW DIVISION: Division of Anesthesia, Analgesia, and  
Addiction Products (HFD-170)

PHARM/TOX REVIEWER: Gary P. Bond, Ph.D.  
PHARM/TOX SUPERVISOR: Adam Wasserman, Ph.D.  
DIVISION DIRECTOR: Bob Rappaport, M.D.  
PROJECT MANAGER: Sharon Turner-Rinehardt

## EXECUTIVE SUMMARY

### I. BACKGROUND

Exparel™, referred to developmentally as SKY0402, is an extended release (ER) injectable depot formulation of bupivacaine hydrochloride (HCl) intended for single-dose use in post-surgical analgesia (b) (4)

Exparel is designed to be infiltrated into the wound during surgical closure and the formulation releases the amide anesthetic slowly (b) (4). The current proposed uses of Exparel allows for bupivacaine dosages (b) (4) of 120 mg and 300 mg are allowed for wound infiltration (b) (4)

For support of a 505(b)(2) approval pathway, the Applicant has identified Marcaine (NDA 16-964) as the listed drug for reliance on the Agency's prior finding of safety and efficacy of the active pharmaceutical ingredient (API) bupivacaine HCl. The liposomal formulation is the Applicant's proprietary DepoFoam® drug delivery system, and is highly similar to two other DepoFoam-containing products currently marketed and for which they were developmentally responsible: DepoDur (NDA 21-671) a marketed epidural extended release morphine product approved in 2004, and DepoCyt (NDA 21-041) an extended release cytarabine product for intrathecal administration in lymphomatous meningitis approved in 1999. The only difference is the substitution of a novel phosphatidylcholine (dierucoylphosphatidylcholine, DEPC). Bupivacaine HCl, a long-acting amide local anesthetic, has been available for infiltrative use since the approval of Marcaine in 1972 and is formulated in 0.25% – 0.75% injection solution strengths with or without epinephrine. Approved indications encompass the production of local or regional anesthesia or analgesia for various surgical procedures including local infiltration, peripheral nerve blocks, and epidural blocks. Bupivacaine HCl is not recommended for intravenous regional anesthesia or obstetrical anesthesia due to observed cases of mortality which are believed due to inadvertent intravascular administration resulting in fatal central nervous system (CNS) and cardiac toxicity. The proposed use of Exparel falls within that of Marcaine.

### II. SUMMARY OF NONCLINICAL DATA SUPPORT

Please see the primary nonclinical review by Dr. Gary Bond for details. Briefly, the Applicant has provided nonclinical studies to address general local safety of the drug product through single- and repeat-dose subcutaneous toxicity evaluations in two species, acute surgical models of wound infiltration in two species, and a series of evaluation with various routes of administration. Acute surgical models and most especially the repeat-dose toxicity studies also contain data to address the systemic safety of a more prolonged bupivacaine exposure.

DEPC safety was characterized both by inclusion as part of the formulation tested in these studies as well as an independent testing strategy comprising general toxicity, genetic toxicity, and reproductive toxicity evaluation of a SKY0402 placebo formulation (comparable to SKY0402 without bupivacaine). The potential for liposome integrity failure and unintentional or uncontrolled release of bupivacaine was explored *in vitro* in interaction studies provided in the Chemistry and Manufacturing section of the application while *in vivo* nonclinical toxicokinetic studies allow indirect examination of the drug product in use performance.

#### *ADME*

Absorption, distribution, metabolism and elimination were acceptably addressed by the Applicant based on Dr. Bond's evaluation. Notably, retention of the liposome at the injection site was performed in guinea pigs and rats using subcutaneous administration of SKY0402 or the SKY0402 placebo formulation. The liposome (as measured by radiolabeled DEPC) appeared to be present for 2-3 weeks, slightly longer than bupivacaine (1-2 weeks) as might be expected with a controlled erosion-associated release. Clearance of the liposome is through normal metabolic channels for the cholesterol (b) (4) components.

#### *Uncontrolled Release or Intravascular Migration of Drug Product*

The drug product is a multivesicular liposomal ER formulation, therefore the issue of potential uncontrolled release or "dose dumping" of bupivacaine through rapid erosion needed to be addressed as defeat of the ER properties of the drug product could produce significant clinical toxicity. Indeed, several instances of significantly elevated plasma concentrations of bupivacaine were noted in the nonclinical toxicology studies. This was most apparent in the acute surgical models, in particular the perineural studies involving brachial plexus administration and to a lesser degree in the wound infiltration study in the rabbit. As described in the primary review, levels spiked in several cases 6-10X the prevailing levels observed in other animals within the group and this was occasionally observed well after dose administration. A less pronounced apparent spike was also observed in the dog model. Upon further evaluation, this appears to be an artifact of the plasma sampling methodology as the validated method used does not discriminate between "free" bupivacaine released from the liposome and encapsulated bupivacaine. Therefore these spikes may simply represent entry of liposomes into the vascular compartment and subsequent release of stored bupivacaine on sample analysis. This is supported by the observation that animals demonstrating these spikes did not exhibit observable bupivacaine toxicity and further suggests the liposome maintains its integrity in blood and a controlled release is still to be expected in the event of intravascular introduction. The entry of these liposomes into the vascular compartment, however, introduces an additional concern of embolism. Notably the liposome size is generally (b) (4) in diameter with an upper range of (b) (4) which exceeds capillary diameter (~5-10 µM). The Applicant evaluated

this worst-case scenario by performing direct intravenous administration of SKY0402 (7.5 mg/kg) in rats which demonstrated a better safety profile than a direct injection of unencapsulated bupivacaine (Sensorcaine, 2.5. mg/kg), the latter resulting in rapid, severe systemic bupivacaine toxicity including mortality. Intravenous administration of the liposomal placebo formulation did not produce evidence of toxicity on clinical observation or post-mortem evaluation using relative administration volumes (b) (4) which are equivalent to the maximal proposed human dosing volume assuming 100% inadvertent intravascular administration. At higher administration levels of SKY0402 placebo (>1 mL/kg) treatment-related mortality was observed.

### *Systemic Safety*

Comparative bioavailability data suggests that while SKY0402 doses  $\leq$  300 mg would likely produce bupivacaine exposures within the use of the listed drug Marcaine, and therefore are supported by prior approval, the (b) (4) dose of SKY0402 would exceed this based on pharmacokinetic modeling by the Clinical Pharmacology reviewer staff (see discussion in Dr. Bond's primary review). However, the systemic safety of SKY0402 at all proposed dose levels are adequately characterized from the nonclinical perspective by the conduct of acute and sub-acute toxicity studies in animal models with SKY0402. (b) (4)

(b) (4)

A similar 4-week intermittent dosing study of SKY0402 conducted in the rabbit was not adequately supportive of systemic safety. Bupivacaine-induced convulsions and mortality was observed at various doses and due to intolerability in this model the high doses utilized did not produce exposure levels which support the upper range of human exposure. Therefore, the rabbit model appears more sensitive than human and not an appropriate species on which to base safety margins. As this product is a reformulation of an approved product, and the clinical toxicity associated with transiently high plasma levels of local anesthetics are well understood, only a single species is necessary for nonclinical support. Notably, the bupivacaine exposure associated with use of 300 mg Exparel is within that of both dog and rabbit NOAELs in 4-week toxicokinetic studies.

### *Local Safety*

The Applicant has evaluated local safety in animal models using acute subcutaneous administration, acute wound infiltration in a hernia repair models, and repeated (bi-weekly) subcutaneous administration of the drug product for 28-days. Additionally, the Applicant has conducted nonclinical studies to evaluate the toxicity produced with intravascular administration to address risks

associated with this inadvertent route. Infiltrative and subcutaneous studies conducted in nonclinical models at clinically equivalent (15 mg/mL) or higher (25 mg/mL) concentrations of SKY0402 were not associated with significant drug product-associated local toxicity. Subcutaneous single-dose administration in rats identified minimal to mild subacute and chronic inflammation at the injection site with the highest concentration of SKY0402. The local effects resolved within a 15-day recovery period and it was noted that SKY0402 appeared to produce similar findings as the immediate release bupivacaine HCl solution (Sensorcaine 7.5 mg/mL) employed as an active control group. Subcutaneous single-dose administration of SKY0402 (15 mg/mL), SKY0402 placebo, bupivacaine solution (5 mg/mL), or saline in dogs revealed a transient thickening and leathery appearance of the skin of a single SKY0402-treated animal without evidence of microscopic changes. No findings were observed on examination after a 14-day recovery period. Most pertinent to the proposed clinical use were models of surgical hernia repair (including prolene mesh insertion) conducted in dog and rabbit in which the effects of wound infiltration of SKY0402 (25 or 15 mg/mL) on wound healing and local toxicity was evaluated in comparison with bupivacaine solution (7.5 mg/mL) and saline. Slightly more erythema and edema were observed upon hernia repair evaluation in the dog with bupivacaine administration in either SKY0402 or solution form than with saline. No effects on the wound bed were noted. The surgical site histology findings were generally similar between groups with the exception of a slightly increased incidence of granulomatous inflammation of mild severity noted with some SKY0402 animals on Study Day 3 (SD3). These were not associated with sutures as was observed in all animals on SD15 and may represent a specific test article-related finding. SD15 examination also appeared to reveal slightly greater fibrosis severity (moderate) in the SKY0402-treated dogs when compared to other groups (mild fibrosis). An analogous model of surgical hernia repair in the rabbit (including prolene mesh insertion) was evaluated with identical single-dose administered concentrations of SKY0402 (15 mg/mL and placebo) in comparison with bupivacaine solution (7.5 mg/mL) or saline. As was noted in the dog, slightly higher hernia repair scores were noted in the rabbit with SKY0402 treatment, driven by a stronger erythematous response which resolved by SD3. No wound bed abnormalities were noted in any animal of any group. Surgical site findings were histologically similar across groups on SD3 evaluation. Granulomatous inflammation was noted by SD15 in SKY0402 group. Local safety was further evaluated in multiple-dose nonclinical studies. Repeated subcutaneous administration of SKY0402 (15 mg/mL or 25 mg/mL) was compared to bupivacaine solution (sensorcaine, 7.5 mg/mL) and saline in 28-day studies conducted in rabbits and dogs to provide an evaluation with a dosing regimen, twice weekly for four weeks, far in excess of that intended for clinical use. Treatment-related findings indicative of local effects were highly similar in rabbits and dogs and were limited to microscopic observation of inflammation, hemorrhage, and neovascularization. These findings correlated with observed discoloration and swelling as well as evidence of vacuolated macrophages at the 25 mg/mL concentration of SKY0402 with an incidence that was greater than

observed in the lower concentration (15 mg/mL) groups. None of these observations were present in either sensorcaine- or saline-treated groups. All treatment-related findings were considered by the study pathologist to be minimal to moderate in severity and were notably reduced in both incidence and severity when examined in animals allowed a 28-day recovery period.

#### *Formulation support*

Support for the (b) (4) formulation DepoFoam used in Exparel derives from a dedicated full nonclinical program designed to qualify the safety of the novel excipient DEPC through testing of a SKY0402 placebo as well as the toxicologic studies conducted with SKY0402. There is also indirect support from prior usage of similar DepoFoam technology in DepoDur (epidural morphine) and to a lesser extent from DepoCyt (intrathecal cytarabine) in which greater toxicity is allowed due to the seriousness of the approved indication, lymphomatous meningitis. 28-Day daily subcutaneous toxicology studies of SKY0402 placebo at the maximum feasible volume of administration in rats and dogs revealed only local effects characterized by granulomatous inflammation and chronic panniculitis in rats with additional findings of vacuolated macrophages, mineralization, edema, and hemorrhage in dogs. Observed histologic changes appeared to be slowly reversible in animals. Reproductive toxicity evaluation demonstrated no apparent DepoFoam/DEPC toxicity with administration of SKY0402 placebo when considering fertility, embryofetal development, or peri/postnatal developmental milestones. The DepoFoam in SKY0402 placebo was not genotoxic or clastogenic *in vitro* in a bacterial reverse mutation (Ames) assay or a chromosomal aberration assay conducted using human peripheral blood lymphocytes, respectively. SKY0402 placebo also lacked evidence of genotoxicity *in vivo* in a Mouse Micronucleus Test.

Impurities appear to be controlled appropriately. Early concerns with potential levels of (b) (4), a known genotoxic impurity, were resolved by the Applicant. More sensitive detection methods were enacted to establish a limit of (b) (4) which ensures human exposure with the (b) (4) SKY0402 dose would be less than 1.5 µg/day, an acceptable totally daily intake level for a genotoxic compound. The impurity (b) (4), previously demonstrated to be carcinogenic in rodents, has been set to a level of (b) (4) based on the limit of quantitation for the Applicant's assay. Based on a recent revision to another bupivacaine-containing product it appears this specification may not meet the criteria of ALARP (as low as reasonably possible) based on existing technical capabilities; however, the Agency has previously considered this level to be acceptable in local anesthetic products and is considered so in the present application. Finally, there are no leachables observed with storage of this drug product for up to 30 months.

### III. PRIMARY REVIEW RECOMMENDATIONS

The primary nonclinical review was completed by Dr. Gary Bond. Dr. Bond recommends the Application may be approved without additional nonclinical studies; however, he has made recommendations involving adjustment of the Pregnancy section to address the inability to reliably adapt the label of the listed drug Marcaine to the currently proposed label.

### IV. SUPERVISORY RECOMMENDATION

The Applicant is utilizing a 505(b)(2) submission pathway referencing Marcaine®, to allow the Agency's prior determination of safety and efficacy for bupivacaine HCl to be used for support of the active pharmaceutical ingredient in Exparel. In addition, the Applicant has provided nonclinical studies to address general local safety of the drug product through repeat-dose subcutaneous toxicity evaluations in two species, acute surgical models of wound infiltration in two species, and a series of evaluation with various routes of administration. Acute surgical models and most especially the 28-day repeat-dose subcutaneous toxicity studies also contain data to address the systemic safety of a more prolonged bupivacaine exposure. DEPC safety was characterized both by inclusion as part of the drug product tested in the previously mentioned studies as well as an independent testing strategy comprising general toxicity, genetic toxicity, and reproductive toxicity evaluation of a SKY0402 placebo formulation. Toxicity studies indicated a relatively benign systemic and local profile with the principle drug product-related toxicities being slightly greater erythema and edema and the non-adverse microscopic observation of granulomatous inflammation. The latter finding was representative of clearance of the liposome and its remnants and was a finding which was also observed at the site of administration in studies of the liposomal placebo. No significant toxicities were apparent with the liposomal placebo containing the novel excipient DEPC. Intravenous administration of SKY0402 at levels which would be considered equivalent to human dosing of Exparel was not associated with significant toxicity or animal morbidity suggesting that inadvertent administration of Exparel in the clinical setting may not be gravely concerning. There was no evidence of uncontrolled release of encapsulated bupivacaine with infiltration or any other investigated route. This includes intravenous administration which allays a significant concern for bupivacaine systemic toxicity with use of the maximal proposed clinical dose of (b) (4). From data in the Chemistry and Manufacturing Modules, it appears there is some risk of rapid erosion of the liposome with co-administration of lidocaine and this will be addressed as part of the label. Lastly, the formulation appears to be of an acceptable quality and lacks significant impurities, degradants, or leachables which would elicit concern.

I concur with Dr. Bond's recommendation that the Application may be approved from the nonclinical perspective without further nonclinical data and agree with his preliminary labeling recommendations.

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/s/  
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ADAM M WASSERMAN  
09/27/2011

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

**PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION**

Application number: 22-496  
Supporting document/s: eCTD submission in DARRTS (SDN 1)  
Applicant's letter date: September 28, 2010  
CDER stamp date: September 28, 2010  
Product: EXPAREL™  
(SKY0402; Bupivacaine Extended Release  
Liposome Injection)  
Indication: Post surgical analgesia  
Applicant: Pacira Pharmaceuticals, Inc.  
Review Division: Division of Anesthesia, Analgesia, and Addiction  
Products  
Reviewer: Gary P. Bond, Ph.D.  
Supervisor/Team Leader: Adam M. Wasserman, Ph.D.  
Division Director: Bob Rappaport, M.D.  
Project Manager: Sharon Turner-Rinehardt

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Any tabular and graphical information are constructed by the applicant or reviewer of NDA 22-496 or the reviewer of IND 69,198 (Adam M. Wasserman) unless cited otherwise. Text may be taken directly and/or modified from the sponsor's submission and reviews of IND 69,198 if considered accurate and acceptable after evaluation by the reviewer of this NDA.

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# 1 Executive Summary

## 1.1 Background and Regulatory Issues

EXPAREL™ (tested as SKY0402) is a bupivacaine-containing extended-release liposome (DepoFoam® drug delivery system). The active pharmacological ingredient, bupivacaine hydrochloride (HCl), is an amide-type local anesthetic proposed for single-injection local administration into a surgical wound to produce postsurgical analgesia. The proposed single doses of SKY0402 are (b) (4)

The human safety of EXPAREL is supported using a 505(b)(2) submission and submitted nonclinical studies. This support includes the Agency's prior findings of bupivacaine safety and efficacy and the acute infiltrative surgical studies in rabbits and dogs and repeat-dose subcutaneous toxicity studies in rats and dogs using SKY0402 compared to the pivotal single dose clinical trials. The submitted nonclinical testing satisfies testing needs as listed in the FDA Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route (March 2008). Bupivacaine HCl has been marketed in the US for over 30 years as Marcaine® (NDA 16-964 - 1972), the listed drug (LD) in the FDA Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations. The highest approved daily dose of bupivacaine is 400 mg.

The human safety of DepoFoam (Inactive Ingredient) is generally supported by the use of this excipient formulation in marketed products DepoCyt® (NDA 21-041, 1999) and DepoDur® (NDA 21-671, 2004). However, the exact composition of DepoFoam is (b) (4) different in each of the three drug products (DepoCyt, DepoDur, and SKY0402). The difference in the DepoFoam in SKY0402 is the novel excipient dierucoylphosphatidylcholine (DEPC). A comprehensive nonclinical testing program was submitted to support the human safety for the DEPC-based DepoFoam in SKY0402. This program included repeated dose toxicity, genotoxicity, and reproductive toxicity testing and is consistent with the FDA Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients (May 2005).

## 1.2 Brief Discussion of Nonclinical Findings

The nonclinical program was designed to support the administration of SKY0402 by local infiltration. Inclusion of bupivacaine in the liposome achieved the intended effect of producing a sustained local release and analgesic effect of bupivacaine compared to bupivacaine HCl alone by extending the systemic absorption time of bupivacaine from the site of injection. Studies in rats and guinea pigs showed that both bupivacaine and DEPC remained at the injection site for several days with bupivacaine leaving more rapidly than DEPC. It could take up to approximately 2 weeks for bupivacaine not to be detected and between 2 and 3 weeks for DEPC not to be detected at the injection site.

The DepoFoam membrane remnants (b) (4) are biocompatible and are cleared through the lymphatic system and metabolized as nutrients.

The safety profile of bupivacaine administered in SKY0402 was anticipated to be the same as bupivacaine HCl. A main focus of the nonclinical testing program was primarily on the evaluation of the local tolerability and potential systemic toxicity of the DepoFoam with DEPC as a novel excipient (SKY0402 Placebo). The potential systemic exposure and toxicity of SKY0402 itself was, however, evaluated in two nonclinical repeat dose toxicity studies. The nonclinical testing of SKY0402 turned out to be necessary to support the human safety of bupivacaine because systemic exposure levels of bupivacaine after dosing with SKY0402 in clinical trials at the highest proposed single dose of (b) (4) could not be determined to be comparable to those supported by the referenced NDA after Clinical Pharmacology evaluation. The systemic levels of bupivacaine after human dosing with SKY0402 at (b) (4) could be potentially greater than those achieved after dosing with the approved bupivacaine HCl at the maximum recommended human dose (MRHD) of 400 mg for the 505(b)(2) referenced drug. Human exposure levels at the MRHD were not tested in any of the clinical trials. This non-comparability was largely due to the lack of human pharmacokinetic data at the MRHD. The proposed 300 mg dose was supported by the referenced NDA.

### **Safety of Exparel based on nonclinical testing of SKY0402**

Human safety is supported for all proposed doses. Only anticipated pharmacological effects of bupivacaine (systemic toxicity) and reversible local injection site effects (local toxicity) were observed after single- and repeated-dose nonclinical studies. Single- and repeated-dose study assessments are combined as the results are very similar.

Local toxicity – SKY0402-specific toxicity was not observed as only local effects were observed and not considered SKY0402-specific as observations were likely the result of dosing method and surgical procedure and were also observed with immediate release bupivacaine HCl treatment included as an active control.

Systemic toxicity – The nonclinical data support all proposed clinical doses based on nonclinical toxicokinetic (TK) and clinical pharmacokinetic (PK) values and nonclinical:clinical (TK:PK) exposure ratios (safety margins - SM) for bupivacaine exposure. SMs of  $\geq 1$  are considered supportive of human safety. Not all SMs are  $\geq 1$  for the proposed 300 and (b) (4) human doses after single- and repeat-dose administration in the rabbit and dog nonclinical studies. However, as all nonclinical doses are the highest doses tested for a given study and species and only resulted in, at most, anticipated pharmacological effects, the nonclinical doses most relevant for comparison to human doses are the ones resulting in the most systemic exposure. The highest TK values observed after repeated dosing in the dog support human safety at doses of 120, 300, & (b) (4) SKY0402. In addition, a long history of human bupivacaine use considered in conjunction with the observed TK and PK values also supports the proposed clinical use.

### **Safety of Exparel based on DEPC exposure in SKY0402 and SKY0402 Placebo nonclinical studies**

Human safety is supported for all proposed doses. No significant DEPC-related toxicity was observed. Anticipated local effects were observed that were considered more dosing method related than a direct result of DEPC treatment. These local effects could also be observed after dosing with SKY0402, SKY0402 Placebo, Bupivacaine HCl, and even saline.

SKY0402 containing the novel excipient DEPC is considered safe for the proposed human use up to a dose of (b) (4) for both local and systemic toxicity. (b) (4)

For potential human local and systemic toxicity, nonclinical:clinical exposure ratios (safety margins) are all indicative of human safety. SMs of  $\geq 1$  are considered supportive of human safety. As all SMs are approximately equal to or greater than 1, human safety of DEPC is supported at the proposed human doses of 120, 300, (b) (4) mg SKY0402.

### **Other safety assessments**

Genotoxicity of SKY0402 Placebo - SKY0402 placebo was not genotoxic in a valid standard battery of three genetic toxicology studies consistent with the ICH S2B guidance (A Standard Battery for Genotoxicity Testing of Pharmaceuticals; Jul 1997).

Inadvertent intravenous dosing – Proposed infiltrative use of SKY0402 containing bupivacaine HCl and the unique DepoFoam excipient DEPC is considered safe in the event of inadvertent intravenous injection at the proposed human doses. A worst case scenario was assumed to be an intravascular injection of 10% of the total dose volume according to the medical reviewer.

Intravenous administration of SKY0402 to rats at bupivacaine doses 3 times larger than the administered bupivacaine HCl injection formulation caused no adverse effects while the immediate-release bupivacaine HCl formulation caused severe toxicity including tremors and death. Based on this information, for the proposed dose route of subcutaneous infiltration only, the applicant was informed that no further nonclinical intravenous toxicity testing was required (IND 69,198 – September 27, 2006). This issue will need to be revisited for any other proposed dose routes.

Intravenous administration of SKY0402 placebo to rats at dose volumes comparable to proposed human doses did not identify any toxicity. An even larger human safety margin exists when assuming a maximal inadvertent intravenous dose volume of 10%.

Local tolerance - Local injection site studies performed in various species (rat, guinea pig, rabbit, and dog) indicate that SKY0402, bupivacaine HCl solution, SKY0402 placebo, or saline produced comparable macroscopic or microscopic changes in

subcutaneous tissue following injection. Transient reactions included reddening of incision lines, edema, or thickened or leathery skin with histopathologic findings such as mild subacute or chronic inflammatory changes confined to the injection site and adjacent muscle. These effects were completely reversed or significantly reversed by 14 days after dosing.

Drug interactions - Subcutaneous co-administration of SKY0402 with lidocaine HCl solution resulted in a more rapid release of bupivacaine from SKY0402 than after SKY0402 alone. This interaction can be reduced by allowing 20 minutes to elapse between a lidocaine dose and administration of SKY0402. In addition, in vitro determinations made as part of CMC-related studies and other nonclinical studies provide support for this concern. In contrast, the composite plasma profiles appeared to be cumulative after subcutaneous co-administration of SKY0402 with bupivacaine HCl.

Safety Pharmacology – While no separate studies were conducted with SKY0402 or placebo, safety pharmacology indices were included as part of the repeat dose studies with no indication of any previously unknown safety concern.

Impurities and degradants in Drug Substance (DS) and Drug Product (DP) – There are no impurity and degradant issues based on submitted DS and DP specifications. ICH Guidances Q3A (Impurities in New Drug Substance; Revision 2, (June 2008) and Q3B(R) (Impurities in New Drug Products; Aug 2006) and FDA guidance (Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches; Dec 2008) specifications were met except for [REDACTED] (b) (4) [REDACTED] which was considered qualified by the nonclinical data provided by the Applicant.

Extractable and Leachables from container closure system - Based on the worst case extractable studies performed, no leachables were detected in any of the analyzed lots of SKY0402 that were stored in the proposed commercial container/closure system for up to 30 months.

Nonclinical Summary – The nonclinical data package supports the proposed human use of SKY0402 with the novel excipient DEPC for the proposed indications and doses.

## **1.3 Recommendations**

### **1.3.1 Approvability**

NDA approval is recommended based on nonclinical data provided.

### **1.3.2 Additional Non Clinical Recommendations - none**

### **1.3.3 Labeling**

Pharm/Tox-related labeling for the reference NDA 16-964 (Marcaine), initially approved in 1972, has essentially not changed in over 30 years and has also been inaccurate for most of that time period for section 8.1 (Pregnancy). Currently, we are working with the

reference NDA owner to update the label to be consistent with current labeling practices and to correct the inaccuracies in animal and human dose comparison information. Once updated, all 505(b)(2)-related bupivacaine NDAs will need to update labels to conform with the reference NDA label. Changes and updating for section 13.1 (Carcinogenesis, mutagenesis, impairment of fertility) are also in progress with the reference NDA owner. However, this section will remain the same as the reference NDA at this time.

At present, we have amended section 8.1 of the proposed label to remove inaccurate information while attempting to provide the most information that we could for labeling purposes within the regulations. In addition, consistency with the Pregnancy and Lactation Labeling Outline structure was attempted. The proposed label language is subject to change upon negotiation with the Applicant.

**[Label starts on next page]**

18 PAGES OF DRAFT LABELING HAVE BEEN WITHHELD IN FULL AS  
b4 (CCI/TS) IMMEDIATELY FOLLOWING THIS PAGE

## 2 Drug Information

### 2.1 Drug

CAS Registry Number - 2180-92-9

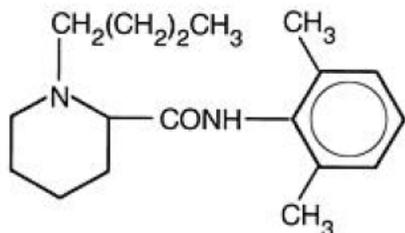
Generic Name - Bupivacaine

Code Name – none reported

Chemical Name - (*RS*)-1-butyl-*N*-(2,6-dimethylphenyl)piperidine-2-carboxamide;  
1-butyl-*N*-(2,6-dimethylphenyl)-2-piperidinecarboxamide

Molecular Formula/Molecular Weight - C<sub>18</sub>H<sub>28</sub>N<sub>2</sub>O/288.43

Structure or Biochemical Description



Pharmacologic Class – amide local anesthetic (FDA established pharmacologic class)

### 2.2 Relevant INDs, NDAs, BLAs and DMFs

1) bupivacaine

- 505(b)(2) submission using NDA 16-964 (Marcaine)

[REDACTED] (b) (4)

- IND 69-198 (original IND for this NDA)

2) DepoFoam

- DepoFoam is a liposomal extended-release formulation contained in the marketed products DepoCyt® (NDA 21-041, 1999) and DepoDur® (NDA 21-671, 2004).

- 1,2-Dierucoylphosphatidylcholine (DEPC)

[REDACTED] (b) (4)

## 2.3 Drug Formulation

### Drug Substance

Bupivacaine clinical doses are proposed (b) (4) as a single administration to a surgical site. At a maximum daily dose of (b) (4), the qualification safety threshold (ICH Guidance Q3A) for impurities is 0.15% or 1.0 mg/day intake (whichever is lower). (b) (4) The proposed specifications for bupivacaine are consistent with ICH standards as listed in the table (column 2). Data for analyzed batches of bupivacaine (other columns) are within specifications. As a result, unless otherwise indicated by ONDQA in their review, the bupivacaine specifications are considered acceptable based on information submitted in the original NDA.

The potential genotoxic/carcinogenic impurities (b) (4) are impurities of concern and will be discussed in section 2.5. Impurities (b) (4) were determined not to be structural alerts or genotoxic for the Ames bacterial mutation assay by CompTox analysis (FDA CDER Informational and Computational Safety Analysis Staff - ICSAS) and are therefore not impurities of concern as they are also within ICH specifications.

**Bupivacaine Batch Analysis Data**

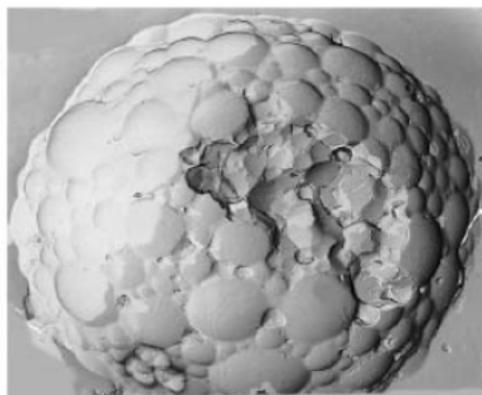
Test	Specification	Batch No: 691665	Batch No: 691745	Batch No: 691776	Batch No: 1069812	Batch No: 1069815
Description	White crystalline powder	complies	complies	complies	complies	complies
Identification	Conforms to reference standard	complies	complies	complies	complies	complies

(b) (4)

### Drug Product

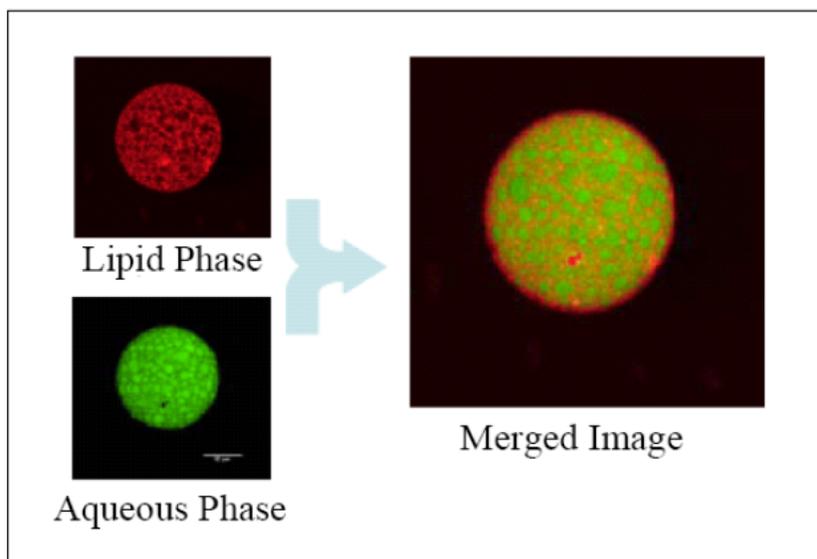
SKY0402 is a sterile, non-pyrogenic, white to off-white, preservative-free, aqueous suspension of multivesicular lipid-based particles (DepoFoam® drug delivery system)

containing bupivacaine, intended for local prolonged release following injection in the management of postsurgical pain. Bupivacaine HCl, the drug substance, is present in SKY0402 at a concentration of (b) (4). Inactive ingredients include dierucoylphosphatidyl-choline (DEPC; 1,2-dierucoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)), dipalmitoylphosphatidyl-glycerol (DPPG; 1,2-dipalmitoyl-*sn*-glycero-3-phospho-*rac*-(1-glycerol)), (b) (4) cholesterol, tricaprylin (b) (4), sodium chloride, and (b) (4).



Scanning electron micrograph of a typical DepoFoil particle

Simply stated, the bupivacaine aqueous phase is merged with the lipid phase to form the DepoFoil particle. See the ONDQA review for a more detailed description.



Confocal microscopy image of a fluorescently labeled MVL particle. The merged image shows the aqueous phase of the vesicles surrounded by the lipid membranes.

All primary stability studies were ongoing and continued to do so during the NDAS review period as production was transitioned from (b) (4) during the course of the NDA review. To this end, as there are no impurity/degradant issues, Pharm/Tox refers to the ONDQA review for current information on stability.

The composition of the drug product (DP) is as listed in the table. For purposes of determining human safety for NDA approval, the drug product was tested and qualified in 28-day repeat dose subcutaneous and acute tissue infiltration surgical studies in rabbits and dogs. In addition, the novel excipient DEPC in SKY0402 was tested in separate nonclinical studies using SKY0402 Placebo (DEPC-based SKY0402 without bupivacaine). Human safety based on nonclinical data is discussed in the Executive Summary in section 1.2 and Integrated Summary and Safety Evaluation in section 11.

**Composition of SKY0402**

Component	Nominal Content per mL	Nominal Content per Vial (10-mL)	Nominal Content per Vial (20-mL)	Molar Ratio (Lipid:Drug Substance)	Weight Ratio (Lipid:Drug Substance)
Bupivacaine	15.0 mg*	150.0 mg*	300.0 mg*	(b) (4)	(b) (4)
Dierucoylphosphatidylcholine (DEPC)	8.2 mg	82 mg	164 mg		
Dipalmitoylphosphatidylglycerol (DPPG)	0.9 mg	9 mg	18 mg		
Cholesterol	4.7 mg	47 mg	94 mg		
Tricaprylin	2.0 mg	20 mg	40 mg		
Sodium Chloride	(b) (4)				

\*Expressed as anhydrous bupivacaine hydrochloride equivalent.

Potential impurities include (b) (4)  
 (b) (4)  
 (b) (4)  
 Except for (b) (4)  
 none of these impurities have been detected in SKY0402 at the time of manufacture or in stability studies to date (i.e., through 36 months at 5°C and 6 months at 25°C) except the impurity (b) (4) in three separate lots (these lots not listed in section 2.3 specification and analysis table. The origin of this impurity in the three lots is reported to be from the bupivacaine base which contained (b) (4) per the certificate of analysis from the vendor. This situation has been corrected so that unless notified of a relevant issue(s) by ONDQA, there are no impurity issues dealing with the drug substance other than (b) (4) (see ONDQA review).

Potential Impurities and Degradation Products of Drug Substance		
Name	Structural Formula	Source
(b) (4)		

Extractable-Leachables from container closure system - Based on the worst case extractable studies performed on the proposed commercial container closure system, SKY0402 lots stored beyond expiration dating were analyzed for potential leachable content. No leachables were detected in any of the analyzed lots of SKY0402 that were stored in the proposed commercial container/closure system for up to 30 months. Pacira has confirmed and FDA ONDQA has concurred that the proposed container closure system is appropriate for SKY0402 and that no additional nonclinical studies to support its use are necessary (See ONDQA review for more detail).

Lots of SKY0402 Used in Nonclinical Toxicology Studies

The following table lists all SKY0402 lots used in nonclinical testing as well lots used in clinical trials. Notable is lot 04-2502 which was used in the Inguinal Hernia clinical trial (study SKY0402-C-201) and which was also used in the acute surgical models in rabbit and dog. Nonclinical study numbers are listed in the studies reviewed section 3.1 and with the individual study review.

## Lots of SKY0402 Used in Nonclinical Toxicology Studies

SKY0402 Batch No. (T)	Study Number	Type of Studies	Species	Route
02BUP04-034	(b) (4) 947-004	Acute local toxicity (pilot)	Rabbit	i.a. Perineural SC (incision site)
03-2001	(b) (4) 947-013	Acute local toxicity (pilot)	Dog	i.a. Perineural SC (incision site)
PP04-048	(b) (4) 947-017	Acute toxicity (pilot)	Dog	SC
PP04-045	947-018	Expanded Acute toxicity	Dog	SC
PP04-048	947-020	Expanded Acute toxicity	Dog	i.t., e.d.
04-2502 (C)	947-031	Expanded Acute toxicity	Rat	e.d.
04-2502 (C) 04-2501	(b) (4) 947-030	Expanded Acute toxicity	Rabbit	SC (incision site)
04-2502 (C) 04-2501	947-029	Expanded Acute toxicity	Dog	SC (incision site)
04-2502 04-2501	(b) (4) 947-035	Expanded Acute toxicity	Rabbit	i.a. (incision site)
04-2502 (C) 05-2501 (C)	947-034	Expanded Acute toxicity	Dog	i.a. (incision site)
01-2009 01-2011	20995	Acute toxicity	Rat	s.s.
02-BUP02-027 (phosphate) (P) 02-BUP02-028 (glucuronate) (P)	RES-0702-D0402-044	Acute local toxicity (pilot)	Guinea pig	SC
02-BUP02-027 (phosphate) (P) 02-BUP02-028 (glucuronate) (P)	RES-0702-D0402-047	Acute local toxicity (pilot)	Guinea pig	i.a.
KP2078 (P)	RES-050524-D0402-611	Acute toxicity (pilot)	Rat	i.v
072501 07PD003	(b) (4) 947-036	28-day toxicology	Rabbit	SC
072501 07PD003	947-037	28-day toxicology	Dog	SC
02BUP04-034	(b) (4) 947-004	Acute local toxicity (pilot)	Rabbit	i.a. Perineural SC (incision site)
03-2001 (P)	(b) (4) 947-013	Acute local toxicity (pilot)	Dog	i.a. Perineural SC (incision site)
PP04-048	(b) (4) 947-017	Acute toxicity (pilot)	Dog	SC
PP04-045	947-018	Expanded Acute toxicity	Dog	SC
PP04-048	947-020	Expanded Acute toxicity	Dog	i.t., e.d.
04-2502 (C)	947-031	Expanded Acute toxicity	Rat	e.d.
04-2502 (C) 04-2501	(b) (4) 947-030	Expanded Acute toxicity	Rabbit	SC (incision site)
04-2502 (C) 04-2501	947-029	Expanded Acute toxicity	Dog	SC (incision site)
04-2502 04-2501	947-035	Expanded Acute toxicity	Rabbit	i.a. (incision site)

C – used in clinical studies

P – used in non-GLP toxicology (and toxicokinetic) studies

T – used in GLP toxicology studies (unless noted)

**Lots of SKY0402 Used in Nonclinical Toxicology Studies**

SKY0402 Batch No. (T)	Study Number	Type of Studies	Species	Route
04-2502 (C) 05-2501 (C)	(b) (4) 947-034	Expanded Acute toxicity	Dog	i.a. (incision site)
01-2009 01-2011	20995	Acute toxicity	Rat	SC
02-BUP02-027 (phosphate) (P) 02-BUP02-028 (glucuronate) (P)	RES-0702-D0402-044	Acute injection site irritation (pilot)	Guinea pig	SC
02-BUP02-027 (phosphate) (P) 02-BUP02-028 (glucuronate) (P)	RES-0702-D0402-047	Acute injection site irritation (pilot)	Guinea pig	i.a.
KP2078 (P)	RES-050524-D0402-611	Acute toxicity (pilot)	Rat	i.v.
082505 (P)	(b) (4) 947-042	Acute toxicity (pilot, juvenile)	Rat	SC
072501 07PD003	947-036	28-day toxicology	Rabbit	SC
072501 07PD003	947-037	28-day toxicology	Dog	SC
02BUP04-034	947-004	Acute local toxicity (pilot)	Rabbit	i.a. Perineural SC (incision site)
03-2001	947-013	Acute local toxicity (pilot)	Dog	i.a. Perineural SC (incision site)
PP04-048	947-017	Acute toxicity (pilot)	Dog	SC
PP04-045	947-018	Expanded acute toxicity	Dog	SC
PP04-048	947-020	Expanded acute toxicity	Dog	i.t., e.d.
04-2502	947-031	Expanded acute toxicity	Rat	e.d.
04-2502 04-2501	947-030	Expanded acute toxicity	Rabbit	SC (incision site)
04-2502 04-2501	947-029	Expanded acute toxicity	Dog	SC (incision site)

C – used in clinical studies

P – used in non-GLP toxicology (and toxicokinetic) studies

T – used in GLP toxicology studies (unless noted)

**2.4 Comments on Novel Excipients**

DEPC is a novel excipient in the DepoFoam system that will be used as the liposome carrier of bupivacaine in SKY0402. A comprehensive nonclinical testing program was conducted for DepoFoam containing DEPC (SKY0402 Placebo). Extensive nonclinical testing for local and systemic toxicity was conducted. The DepoFoam in Sky0402 Placebo was nearly identical to the liposomal formulation but was made with sodium chloride in lieu of bupivacaine. In addition, reference was made to the other approved DepoFoam products Depodur (NDA 21-671) and DepoCyt (NDA 21-041) as supporting the overall safety of the DepoFoam drug delivery system.

(b) (4)

batch analyses. At 15 mg/ml bupivacaine HCl in the DP, the percentages of

(u) (4)

(b) (4) respectively, all in excess of ICH qualification threshold level for DP of (b) (4) bupivacaine/day. The mean batch analysis value is (b) (4) with the 3 standard deviation of the mean value of (b) (4) values in batches of SKY0402 used in 3 of the pivotal clinical trials at doses of 120 mg in 8 mL (bunionectomy), 300 mg in 20 mL (hemorrhoidectomy), (b) (4) (b) (4) and in the 28-dy repeat dose nonclinical studies in rabbits and dogs are (b) (4) and are considered comparable on a µg/mL basis. To this end, the adequate nonclinical-based human safety for SKY0402 and the DEPC excipient alone exist at the proposed human doses of SKY0402. ONDQA will request the DP specification to be set at (b) (4) based on batch analyses and variability.

SKY0402 placebo was not genotoxic in a valid standard battery of three genetic toxicology studies consistent with ICH S2B guidance. Nonclinical animal and human local and systemic doses were compared. Systemic dose comparisons were based on exposure to DEPC on a mg/kg basis with nonclinical animal doses adjusted to a human equivalent dose (HED) resulting in mg dose/m<sup>2</sup> body surface area-based comparisons to the proposed clinical dose of DEPC in the drug product. Local dose comparisons were based on the volume of DEPC-containing dose. In the nonclinical studies conducted (subcutaneous dose - 28 day studies in rats and dogs, embryo-fetal studies in rats and rabbits, and a combined fertility/perinatal-postnatal study in rats), all doses (only one dose level/volume per study) were considered the maximum practicable doses based on the dose volume administered for the respective nonclinical species. All nonclinical doses were NOAEL doses as only local, reversible injection site effects were observed for all of the varied biological indices evaluated in these comprehensive GLP studies. DEPC exposure at the highest proposed dose of (b) (4) is generally comparable to the DEPC level at the NOAEL from the nonclinical studies ( $SM \geq 1$ ), indicating nonclinical support for the proposed human dose of SKY0402 containing the novel DEPC excipient. A more detailed safety assessment is included in Section 11.

Based on these nonclinical results, potential human local and systemic toxicity has been addressed and SKY0402 containing the novel excipient DEPC is considered safe for the proposed human use.

## 2.5 Comments on Impurities/Degradants of Concern

Early in the NDA review process, (b) (4) which is positive in the Ames mutagenicity assay, was identified at levels in one of the supplied bupivacaine drug substances (b) (4) at levels that would exceed the recommended 1.5 µg/day total daily intake as listed in the draft CDER Guidance for Industry: *Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (Dec 2008)*. At a maximum dose of (b) (4) SKY0402/day, the specification can be (b) (4). The limit of detection for (b) (4) is now (b) (4) without any (b) (4) being detected, so this is not a human safety issue. Refer to the ONDQA review for more detail on this matter.

The (b) (4) is genotoxic and has been shown to produce tumors in rodents. Consistent with previous NDA evaluations, the carcinogenicity finding was not relevant to humans (b) (4)

The current generally acceptable level of (b) (4) is to reduce levels to as low as reasonably possible (ALARP) as determined by ONDQA. In the past this has been in the (b) (4) range, of which (b) (4) is the proposed specification for this NDA and is also the limit of quantitation (LOQ). The (b) (4) specification is also considered to be as low as reasonably possible (ALARP) by ONDQA, meaning also as low as technically feasible under current conditions of production and analysis.

(b) (4) which were initially considered as potentially genotoxic/structural alerts, are not structural alerts and would not be genotoxic in the Ames bacterial mutation assay according to CompTox analysis (FDA CDER Informational and Computational Safety Analysis Staff (ICSAS)). As both are currently within ICH specifications, no further action is indicated in regard to human safety.

## 2.6 Proposed Clinical Population and Dosing Regimen

EXPAREL™ is indicated for single-dose local administration into the surgical wound to produce postsurgical analgesia. The recommended dose is based on the (b) (4) type of surgery. EXPAREL has not been studied for use in patients younger than 18 years of age. The dose of EXPAREL in mg is the amount of bupivacaine HCl in the drug product. The actual amount of free bupivacaine has been determined to be 106, 266, (b) (4) of bupivacaine, respectively. The doses will still be referred to as 120, 300, (b) (4) for the purposes of this review.



Pharmacokinetic values from the four pivotal clinical trials are listed in the following table. Blood sampling was up to 96 hours, making the AUC from 0-96h.

**Summary of Pharmacokinetic Parameters  
for Bupivacaine after Administration of  
Single Doses of EXPAREL™ and Bupivacaine HCl**

	EXPAREL™			Bupi- vacaine HCl
	120 mg <3 cm Incision	300 mg ≥3 cm Incision	(b) (4)	100 mg ≥3 cm Incision
	Study 1 <sup>a</sup> (N=26)	Study 2 <sup>b</sup> (N=25)		Study 3 <sup>c</sup> (N=12)
C <sub>max</sub> (ng/mL)	166 (92.7)	867 (353)	(b) (4)	336 (156)
T <sub>max</sub> (h)	2	0.5	(b) (4)	0.6
AUC <sub>(0-96)</sub> (h×ng/mL)	5864 (2038)	16,867 (7868)	(b) (4)	4360 (1559)
AUC <sub>(inf)</sub> (h×ng/mL)	7105 (2283)	18,289 (7569)	(b) (4)	4372 (1560)
t <sub>1/2</sub> (h)	34.1 (17.0)	23.8 (39.4)	(b) (4)	8.47 (2.89)

Note: Arithmetic mean (standard deviation) except T<sub>max</sub> (median).

a – bunionectomy clinical trial (SKY0402-C-203)

b – hemorrhoidectomy clinical trial (SKY0402-C-316)

c – inguinal hernia clinical trial (SKY0402-C-201)

(b) (4)

## 2.7 Regulatory Background

IND 69,168 (original IND submitted May 2, 2007)

NDA submitted September 28, 2010

Marcaine® (NDA 16- 964 – October 3,, 1972) 505(b)(2) reference

### 3 Studies Submitted

#### 3.1 Studies Reviewed

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
Primary Pharmacodynamics	DepoBupivacaine [SKY0402] Efficacy Study in a Dermal Wheal/Pin Prick Model in Guinea Pigs	Guinea pig	Intradermal	SKY0402	RES-0801-D402-059
Secondary Pharmacodynamics	In Vitro Effect of SKY0402 on Whole Blood Coagulation Using Activated Clotting Time	Human whole blood	In vitro	SKY0402	CeeTox 9032-081
Analytical Method and Validation Report	Method Qualification of an LC-MS/MS Assay Method for the Quantitation of Lidocaine and Bupivacaine in Swine Plasma – non-GLP	n/a	n/a	n/a	ABC 62796
Analytical Method and Validation Report	Full Validation of an LC-MS/MS Method for the Determination of Bupivacaine and Lidocaine in Swine Plasma	n/a	n/a	n/a	ABC 62988
Analytical Method and Validation Report	Validation of an LC-MS/MS Assay For Bupivacaine in Dog EDTA Plasma	n/a	n/a	n/a	(b) (4) 947-019
Analytical Method and Validation Report	Validation Transfer of an LC-MS/MS Assay for Bupivacaine in Rat Plasma	n/a	n/a	n/a	(b) (4) 947-032
Analytical Method and Validation Report	Validation Transfer of an LC-MS/MS Assay for Bupivacaine in Rabbit Plasma	n/a	n/a	n/a	(b) (4) 947-033
Analytical Method and Validation Report	Partial Validation of an LC-MS/MS Assay for Bupivacaine in Rabbit Plasma with K <sub>3</sub> EDTA	n/a	n/a	n/a	(b) (4) 999-618

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
Analytical Method and Validation Report	Full Validation of an LC-MS/MS Assay for Bupivacaine in Dog Plasma with K <sub>3</sub> EDTA	n/a	n/a	n/a	(b) (4) 999-619
Analytical Method and Validation Report	Partial Validation of an LC-MS/MS Assay for Bupivacaine in Rat Plasma with K <sub>3</sub> EDTA	n/a	n/a	n/a	(b) (4) 999-646
Analytical Method	Method for Analysis of Bupivacaine in Rat Plasma by Capillary Gas Chromatography	n/a	n/a	n/a	RES-85912
Analytical Method	Comparison of Method for Analysis of Bupivacaine in Rat Plasma by Gas Chromatography and Liquid Chromatography/Mass Spectrometry	n/a	n/a	n/a	RES-91719
Absorption	Pharmacokinetic Profile of Bupivacaine Following Subcutaneous Injection of Bupivacaine HCl Solution in Rats	Rat	SC	Bupivacaine HCl	RES-0703-D402-027 (reviewed in IND 69,198)
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-0703-D0402-026 (reviewed in IND 69,198)
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-041018-D0402-534
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-050502-D0402-608
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-050506-D0402-612

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-050801-D0402-619
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-051010-D0402-627
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-0902-D402-059
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-1102-D0402-072
Absorption	Pharmacokinetic Screening of Newly Manufactured D0402 [SKY0402] Lots in Rats	Rat	SC	SKY0402	RES-0103-D0402-111
Absorption, Toxicokinetics	SKY0402: A Subcutaneous Toxicity Study in Twice-Weekly Dosing for Four Weeks in Rabbits	Rabbit	SC	SKY0402	(b) (4) 947-036  (TK reported with study toxicity review)
Absorption	Non-Decanted and Decanted SKY0402: A Subcutaneous Bioequivalence Study in Male Dogs	Dog	SC	SKY0402	(b) (4) 947-041
Absorption	Decanted SKY0402: A Pharmacokinetic Profile After Hernia Surgery Repair in Male Dogs	Dog	SC	SKY0402	(b) (4) 947-040
Absorption, Toxicokinetics	An Expanded Acute Subcutaneous Toxicity Study of SKY0402 in Dogs	Dog	SC	SKY0402	(b) (4) 947-018  (TK reported with study toxicity review)

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
Absorption, Toxicokinetics	SKY0402: A Subcutaneous Toxicity Study with Twice Weekly Dosing For Four Weeks in Dogs	Dog	SC	SKY0402	(b) (4) 947-037  (TK reported with study toxicity review)
Absorption, Toxicokinetics	Acute Toxicity/Wound Healing Study of SKY0402 in Surgical Model in Rabbits	Rabbit	SC (wound infiltration); Perineural	SKY0402	(b) (4) 947-030  (TK reported with study toxicity review)
Absorption, Toxicokinetics	Acute Toxicity/Wound Healing Study of SKY0402 in a Surgical Model in Dogs	Dog	SC (wound infiltration); Perineural	SKY0402	(b) (4) 947-029  (TK reported with study toxicity review)
Absorption, Toxicokinetics	Expanded Acute Epidural Toxicity Study of SKY0402 in Rats	Rat	e.d.	SKY0402	(b) (4) 947-031  (TK reported with study toxicity review)
Absorption, Toxicokinetics	An Intrathecal and Epidural Acute Toxicity Study of SKY0402 in Male Beagle Dogs	Dog	e.d. i.t.	SKY0402	(b) (4) 947-020  (TK reported with study toxicity review)
(b) (4)					

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
(b) (4)					
Absorption	Retention of Bupivacaine and DEPC Following Intradermal or Subcutaneous Injection of SKY0402 or SKY0402 Placebo in Rats and Guinea Pigs	Rat; Guinea pig	Intradermal; SC	SKY0402; SKY0402-placebo	RES-73965
Distribution	Quantitative Whole-Body Autoradiography Following Single Subcutaneous Injection of [1- <sup>14</sup> C]-2-erucoyl) DEPC DepoFoam Formulation in Rats	Rat	SC	DEPC	QPS 137N-0401
Pharmacokinetic Drug Interaction	Pharmacokinetic Drug Interaction of DepoBupivacaine and Bupivacaine HCl Solution in Rats	Rat	SC	SKY0402	RES-060130-D0402-701
Pharmacokinetic Drug Interaction	A Single Dose Pharmacokinetic Interaction Study of Lidocaine and SKY0402 in Naïve, Sexually Mature, Yucatan Mini-Pigs (non-GLP)	Mini-pig	SC	SKY0402	S07580 (pilot)
Pharmacokinetic Drug Interaction	A Single Dose Pharmacokinetic Interaction Study of Lidocaine and SKY0402 in Naïve, Sexually Mature, Yucatan Mini-Pigs (GLP)	Mini-pig	SC	SKY0402	S07607

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
Pharmacokinetic Drug Interaction	A Single Dose Pharmacokinetic Interaction Study of Bupivacaine and SKY0402 in Naïve, Sexually Mature, Yucatan Mini-Pigs (GLP)	Mini-pig	SC	SKY0402	S08668
Single-dose; Range-finding toxicity	A Dose Range Finding Tolerance Study of SKY0402 Administered by Intravenous Bolus Injection to Rats in Comparison with Bupivacaine HCl Solution	Rat	i.v.	SKY0402	RES-050524 D0402- 611
Single-dose; Local toxicity	Single-Dose Subcutaneous Toxicity Study in Sprague Dawley Rats	Rat	SC	SKY0402	20995 (reviewed in IND 69,198)
Single-dose; Range-finding toxicity	A Dose Range-Finding Subcutaneous Toxicity Study of SKY0402 in Dogs	Dog	SC	SKY0402	(b) (4) 947-017
Single-dose toxicity (+ TK)	An Expanded Acute Subcutaneous Toxicity Study of SKY0402 in Dogs	Dog	SC	SKY0402	(b) (4) 947-018
Single-dose toxicity (+ TK)	Expanded Acute Epidural Toxicity Study of SKY0402 in Rats	Rat	e.d.	SKY0402	(b) (4) 947-031
Single-dose toxicity (+ TK)	An Intrathecal and Epidural Acute Toxicity Study of SKY0402 in Male Beagle Dogs	Dog	i.t., e.d.	SKY0402	(b) (4) 947-020 (reviewed in IND 69,198)
Single-dose toxicity (+ TK)	Acute Toxicity/Wound Healing Study of SKY0402 in a Surgical Model in Rabbits	Rabbit	SC. wound, nerve	SKY0402	(b) (4) 947-030 (reviewed in IND 69,198)

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
Single-dose toxicity (+ TK)	Acute Toxicity/Wound Healing Study of SKY0402 in a Surgical Model in Dogs	Dog	SC. wound, nerve	SKY0402	(b) (4) 947-029
(b) (4)					
Single-dose toxicity	An Expanded Acute Toxicity Study of SKY0402 Placebo in Rats	Rat	i.v.	SKY0402-placebo*	(b) (4) 947-014
Single-dose toxicity	A Dose Range-Finding Intravenous Toxicity Study of SKY0402 Placebo in Rats	Rat	i.v.	SKY0402-placebo*	(b) (4) 947-015
Single-dose ; Range-finding toxicity	An Expanded Acute Toxicity Study of SKY0402 Placebo in Rats	Rat	i.v.	SKY0402-placebo*	(b) (4) 947-016
(b) (4)					
Repeat-dose toxicity (+ TK)	SKY0402: A Subcutaneous Toxicity Study with Twice Weekly Dosing For Four Weeks in Rabbits	Rabbit	SC	SKY0402	(b) (4) 947-036
Repeat-dose toxicity (+ TK)	SKY0402: A Subcutaneous Toxicity Study with Twice Weekly Dosing For Four Weeks in Dogs	Dog	SC	SKY0402	(b) (4) 947-037

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
28-day repeat dose toxicity	A 28-Day Subcutaneous Injection Toxicity Study in Sprague Dawley Rats with a 14-Day Recovery Period with SKY0402 Placebo	Rat	SC	SKY0402-placebo*	LAB00001
28-day repeat dose toxicity	A 4-Week Subcutaneous Toxicity Study of SKY0402 Placebo in Dogs	Dog	SC	SKY0402-placebo	(b) (4) 947-024
Local toxicity (+ PK)	Acute Subcutaneous Toxicity of DepoBupivacaine (made with glucuronic acid or phosphoric acid) in Guinea Pigs (Pilot Study)	Guinea pig	SC	SKY0402	RES-0702-D0402-044
(b) (4)					
Genotoxicity, Bacterial mutation test	SKY0402 Placebo: Bacterial Mutation Test	In vitro	--	SKY0402-placebo*	CTBR 960255
Genotoxicity, Chromosome aberration test	SKY0402 Placebo: Chromosome Aberration Test	In vitro	--	SKY0402-placebo*	CTBR 960331
Genotoxicity, Mouse micronucleus assay	SKY0402 Placebo: Mouse Micronucleus Assay	Mouse	SC	SKY0402-placebo*	CTBR 960257
DART, Segment II (teratology)	Study for Effects on Embryo-Fetal Development in Rats with SKY0402 Placebo Administered Subcutaneously	Rat	SC	SKY0402-placebo	(b) (4) 947-022

Type of Study	Study Title	Test System	Method of Administration	Testing Article	Study No.
DART, Segment II (teratology)	Study for Effects on Embryo-Fetal Development in Rabbits with SKY0402 Placebo Administered Subcutaneously	Rabbit	SC	SKY0402-placebo	(b) (4) 947-023
DART, Segment I/III	Study of Fertility, Reproductive Performance, Maternal Function, and F <sub>1</sub> Prenatal and Postnatal Development in Rats with SKY0402 Placebo	Rat	SC	SKY0402-placebo	(b) (4) 947-026
Repeat-dose toxicity (+ TK)	SKY0402: A 1-Month Subcutaneous Dose Range-Finding Study in Juvenile Rats	Rat (juvenile)	SC	SKY0402	(b) (4) 947-042
Local toxicity	Comparative Evaluation of Local Irritation of SKY0402 and Bupivacaine HCl in Rabbits	Rabbit	SC (wound), nerve, i.a.	SKY0402	(b) (4) 947-004
(b) (4)					

TK: Toxicokinetics; SC: Subcutaneous; i.t.: Intrathecal; i.v.: Intravenous.; (b) (4) e.d: Epidural.  
 \* Included higher concentration products (bupivacaine 25 mg/ml and/or 1.7X higher lipid concentrations).

**3.2 Studies Not Reviewed - none**

**3.3 Previous Reviews Referenced**

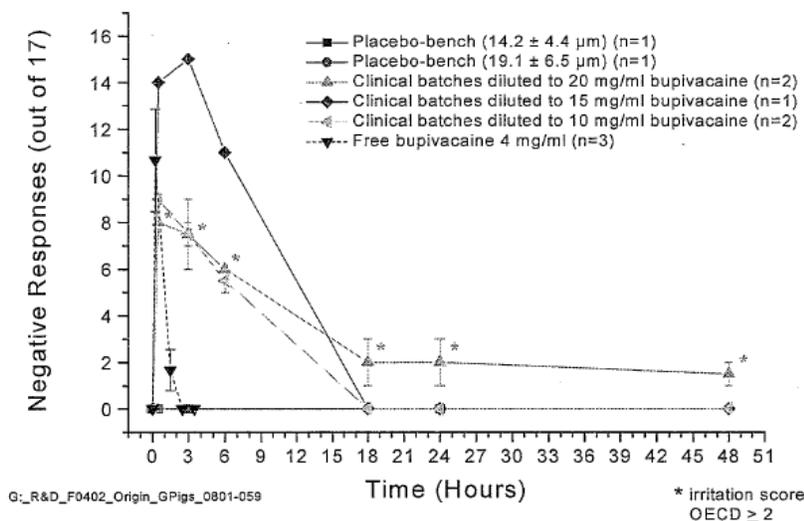
- IND 69,198 (SKY0402) reviewed by Adam M. Wasserman, Ph.D.

## 4 Pharmacology

### 4.1 Primary Pharmacology

**non-GLP study RES-0801-SKY0402-059** – The efficacy of an intradermal DepoBupivacaine (early name for SKY0402 - pooled clinical batches 01-2008 to 1-2011), Bupivacaine Hydrochloride (HCl), or placebo injection for the duration of anesthetic effects and injection site irritation was evaluated in a dermal wheal/pin prick model in male guinea pigs at a dose of 1 mL (~1.4 mL/kg). Slight irritation was observed in placebo animals at 3 hours but not 24 hours post-dosing (see table). Mild to moderate irritation was observed through the last observation time point, 48 hours, in DepoBupivacaine treated animals. Pain efficacy (antinocceptive for pin prick) was observed for at least 6 hours after DepoBupivacaine which was not observed for any time period for placebo animals. Almost complete to complete reversal of pain efficacy was observed by 18 hours depending on the dose of bupivacaine. In a separate study (not submitted other than this notation of data comparison), bupivacaine’s antinocceptive efficacy was not evident at 3 hours post-dosing, indicating the sustained anesthetic effects of DepoBupivacaine.

**RES-0801-SKY0402-059 and 0301-018**  
Bupivacaine Efficacy Study in Guinea Pigs



**Negative Responses to the Prick Recorded in an Area Larger than the Wheal**

**Anesthetic Efficacy and Injection Site Irritation Following Intradermal Injection of DepoBupivacaine (RES-0801- D0402-059)**

Group Number (Number of Animals)	Bupivacaine Concentration (mg/mL)	Lot Number and Formulation Variable	Time Point (h)	Injection Site Irritation <sup>a</sup>	Number of Non-responses per Number of Pin Pricks <sup>b</sup>		
					5	9	17
1 (N=1)	0	AHJ-105A/B Bench placebo, 14.2 µm	0.5	mild	0	0	0
			3	mild	0	0	0
			24	mild	0	0	0
2 (N=1)	0	AHJ-105C Bench placebo, 19.1 µm	0.5	mild	0	0	0
			3	mild	0	0	0
			24	mild	0	0	0
3 (N=2)	20	Pooled DepoBupivacaine clinical batches, Potency adjusted to 2.0%	0.5	mild, mild	5,5	8,8	8,8
			3	mild, mild	5,5	6,9	6,9
			6	mild, mild	5,5	6,6	6,6
			18	mild, moderate	1,3	1,3	1,3
			24	mild, moderate	1,3	1,3	1,3
			48	mild, moderate	1,2	1,2	1,2
4 (N=1)	15	Pooled DepoBupivacaine clinical batches, Potency adjusted to 1.5%	0.5	mild	5	9	14
			3	mild	5	9	15
			6	mild	5	7	11
			18	mild	0	0	0
			24	mild	0	0	0
			48	mild	0	0	0
5 (N=2)	10	Clinical DepoBupivacaine batches, pooled, potency adjusted to 1.0%	0.5	mild, mild	5,5	9,9	9,9
			3	mild, mild	5,5	7,8	7,8
			6	mild, mild	5,5	6,5	6,5
			18	mild, mild	0,0	0,0	0,0
			24	mild, mild	0,0	0,0	0,0
			48	mild, mild	0,0	0,0	0,0

<sup>a</sup>Note: Irritation may enhance or diminish efficacy of anesthesia. <sup>b</sup>Anesthetic efficacy is expressed as the number of non-responses per number of pin pricks (5, 9 or 17).

## 4.2 Secondary Pharmacology

**Non-GLP study 9032-081** – The effect of sustained release bupivacaine from SKY0402 on human whole blood coagulation as measured by activated clotting time (ACT) was evaluated in vitro. Bupivacaine HCl (10 µM Sensorcaine), SKY0402 (5 or 10 µM) and saline were tested at 1 hour with SKY0402 and saline also tested at 3 hours. Both bupivacaine HCl and SKY0402 at high concentrations (10 µM) slightly prolonged ACT in human blood at the 1-hour exposure. No effects were seen with SKY0402 (5 or 10 µM) at 3 hours. The concentrations of SKY0402 and bupivacaine HCl solution used in this experiment are considered clinically relevant. Within the bounds of this in vitro study, prolonged exposure to bupivacaine is not anticipated to significantly prolong clotting time.

## 4.3 Safety Pharmacology – No dedicated studies conducted

- some safety pharmacology indices evaluated in nonclinical studies (e.g., ECGs in dogs)

## 5 Pharmacokinetics/ADME/Toxicokinetics

### 5.1 PK/ADME

Distribution, metabolism and excretion of bupivacaine HCl in SKY0402 is expected to be the same as that of free bupivacaine once the active drug is released from liposome microparticles therefore no studies were done. Phospholipids contained in SKY0402 as well as the neutral lipids are naturally occurring or are synthetic homologues to those found in cells and are expected to undergo a similar metabolic route and fate as their naturally occurring counterparts such as phagocytosis by local macrophages, incorporation into local tissue and clearance via the lymphatic system according to the Applicant (adapted from IND 69,198).

Methods of Analysis (summary review only) - Twelve nonclinical bioanalytical methods were progressively developed and qualified/validated to measure bupivacaine and other analyte levels in plasma and tissue (e.g., DEPC liposome carrier) as the nonclinical research progressed from initial non-GLP testing to full GLP studies. These methods evolved during the research process and included gas chromatography (GC), high performance liquid chromatography (HPLC), liquid chromatography-mass spectrometry (LC-MS), and ultimately liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS). The concentration ranges and limits of quantitation and detection (LOQ and LOD) that could be detected for bupivacaine, the unique DEPC liposome carrier, and lidocaine (bupivacaine interaction studies) were appropriate for the nonclinical studies conducted. Concentration ranges were as low as the detection limit of 1 ng/mL up to the high end of the established range of 10,000 ng/mL for bupivacaine levels, from 0.25 to 100 ng/mL for bupivacaine:lidocaine interaction studies, and a LOD/LOQ for DEPC of 0.38/0.875 mg in tissue.

### Absorption

#### Pharmacokinetic (PK) assessment of SKY0402 using different manufacturing processes

These studies were conducted as the drug was developed to determine how differing the production process and handling could alter the in vivo release profile of bupivacaine. The goal was to develop uniform drug product for ultimate nonclinical and clinical testing and for the final product. These studies are briefly summarized here for information purposes only to illustrate some of the variables involved as they are essentially an ONDQA, ClinPharm, and/or BioPharm issue to be critically addressed in their NDA review.

#### **Non-GLP studies RES-041018-D0402-534, , RES-050606-D0402-612, RES- 051010-D0402-627, and portion of RES-050502-D0402-608 and RES-060130-D0402-701 -**

These five studies evaluated the plasma bupivacaine pharmacokinetics of different lots of SKY0402 (04-2501, 04-2502, 05-2503, and 25BUP02-018) administered by subcutaneous hind limb injection in male rats. Groups of male rats (five per group) were given 0.5 mL of SKY0402 (bupivacaine HCl 15 mg/mL or a dose of approximately 20–25 mg/kg body weight). Blood samples were collected at 0.1, 1, 2, 6, 24, 48, 72, and 96

hours after administration. What was identified is that under the same conditions, the in vivo release profile was similar. However, storage conditions (e.g., 3 months at 25 °C versus 2-8 °C) could lead to an increased release profile.

**Non-GLP study RES-050801-D0402-619** - This study evaluated the plasma bupivacaine pharmacokinetics of different lots of SKY0402 (04-2501, 04-2502, 05-2503, and 25BUP02-018) administered by subcutaneous hind limb injection in male rats. Groups of male rats (five per group) were given 0.5 mL of SKY0402 (bupivacaine HCl 15 mg/mL or a dose of approximately 20–25 mg/kg body weight). Blood samples were collected at 0.1, 1, 2, 6, 24, 48, 72, and 96 hours after administration. What was identified that under the same conditions, is that control SKY0402 (batch 04-2502) made with DPPG (b) (4) DPPG was ultimately used in the final formulation of SKY0402.

**Non-GLP studies RES-0902-D402-059 and RES-1102-D0402-072** - These two studies evaluated the plasma bupivacaine pharmacokinetics of different lots of SKY0402 (b) (4) administered by subcutaneous hind limb injection in male rats. Groups of male rats (six per group) were given 0.5 mL of SKY0402 (bupivacaine HCl 25 mg/mL or a dose of approximately 30 to 40 mg/kg body weight). Blood samples were collected from the saphenous vein at 0.5, 1, 2, 6, 24, 48, 72, and 96 hours after administration. Control batch (02BUP07-027, 25BUP-061,) PK values were less than clinical batches 25-BUP02-017 (study 059) and more than clinical batch 25-BUP02-018 (072).

**Non-GLP study RES-0103-D0402-111** - This study evaluated the plasma bupivacaine pharmacokinetics of two different lots of SKY0402 made by (b) (4) (b) (4) SKY0402 was administered by subcutaneous hind limb injection in male rats. Groups of male rats (six per group) were given 0.5 mL of SKY0402 (bupivacaine HCL 25 mg/mL or a dose of approximately 30 to 40 mg/kg body weight). Blood samples were collected from the saphenous vein at 0.5, 1, 2, 6, 24, 48, 72, and 96 hours after administration. While PK values were comparable, PK did vary, most notably Cmax being lower after using the separate stock solutions in manufacture.

**Non-GLP study RES-050502-608** - This study evaluated the plasma bupivacaine PK of SKY0402 administered by subcutaneous injection in rats after SKY0402 was stored under differing duration and temperature storage conditions (04-2501 at 2-8 °C for 6 months) and 04-2502, at 25 °C for 3 months or 2–8 °C for 6 months). Five males received a 0.5-mL subcutaneous injection of SKY0402 (bupivacaine HCl 15 mg/mL, approximately 20 mg/kg in hindlimbs). The control batch was 25BUP02-018. Blood samples were collected by venipuncture from the saphenous vein at 0.5, 1, 2, 6, 24, 48, 72, and 96 hours after dosing. Four additional animals received a 0.5 mL subcutaneous injection of bupivacaine HCl solution (5 mg/mL or approximately 7.1 mg/kg) in the hind

limb. Similarly, blood samples were collected by venipuncture from the saphenous vein at 0.25, 0.67, 1.1, 2, 6, 9, and 24 hours after dosing. A higher PK release profile was observed when storage was at 25 °C for 3 months compared to 2–8 °C for 6 months, but not compared to the control batch as their PK values were similar. Therefore, storage conditions may impact on the bupivacaine in vivo release profile.

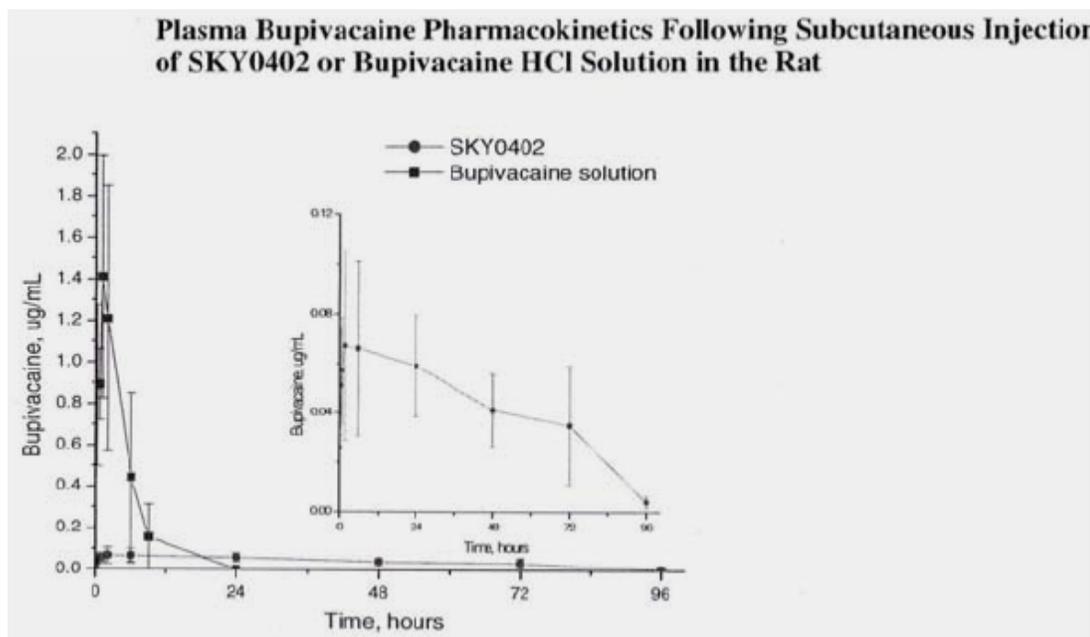
**GLP studies** <sup>(b) (4)</sup> **947-040 and -041** – Study -041 evaluated the potential impact of a final concentration finishing step utilized in making SKY0402 (either decanting/ concentrating or non-decanting/diluting) on the PK profile of SKY0402 after local infiltration in dogs that underwent sham surgery to create a virtual incision line for SKY0402 dosing (simulated hernia repair). Study -040 only tested decanted SKY0402 using an actual surgical incision. The test article was administered once, at a total dose level of 9 mg/kg and a total dose volume of 0.6 mL/kg. SKY0402 was injected at eight points into the subcutaneous tissue around the virtual and actual incision sites to simulate actual use after surgery. Blood samples were collected on Day 0 at predose, 0.5, 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hours after dosing, plus once on days 6 and 9 for determination of bupivacaine. While the two simulated surgery groups (using decanted and non-decanted formulations) did not meet PK bioequivalent limits set for oral doses (90% confidence interval for the ratio falls within the criterion limits of 80–125%) due to data variability, the observed differences in PK were not considered to be clinically relevant. When comparing the nonsurgical (simulated) and surgical models, the decanted SKY0402 profiles were not bioequivalent at the level of 80 to 125% except for C<sub>max</sub>. The higher sustained levels of bupivacaine after wound infiltration of SKY0402 (i.e., AUC) may be explained at least in part, by the surgical procedure that created an extensive raw absorptive surface within the operative field compared to “normal tissues” in dogs.

	<u>Surgical</u>	<u>Non-surgical</u>
T <sub>max</sub> (hr)	6.5	0.5
C <sub>max</sub> (ng/mL)	205	185
AUC <sub>0-inf</sub> (hr*ng/mL)	12,168	9,335
Terminal T <sub>1/2</sub> (hr)	35.1	51.7

#### Sustained release of bupivacaine after dosing with SKY0402 compared to bupivacaine hydrochloride (HCl)

**Non-GLP studies RES-0703-D0402-026 & -027** (adapted from review of IND 69,198) - Six rats received a subcutaneous (SC) injection of SKY0402 in a bupivacaine HCl dose equivalent to 40 mg/kg. Blood samples were collected up to 96 hr after dosing. Four animals received a subcutaneous injection of bupivacaine HCl in a dose of 5 mg/mL (7 mg/kg) with blood sampling out to 24 hr. As can be seen in the figure below, the bupivacaine HCl treated animals had a sooner T<sub>max</sub> and higher C<sub>max</sub> relative to the SKY0402-treated rats which had a lower though prolonged elevation of plasma bupivacaine. SKY0402-treated rats demonstrated relatively stable concentrations of plasma bupivacaine out to 72 hr after administration in contrast to bupivacaine HCl

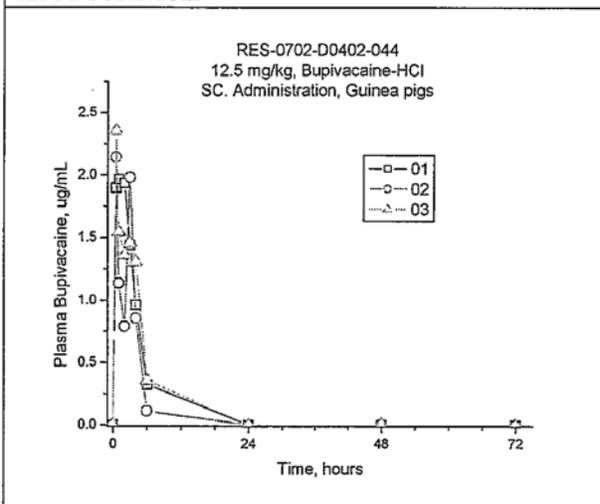
injected animals in which plasma bupivacaine concentration was very low at 12 hr post-administration and was undetectable in plasma by 24 hr.



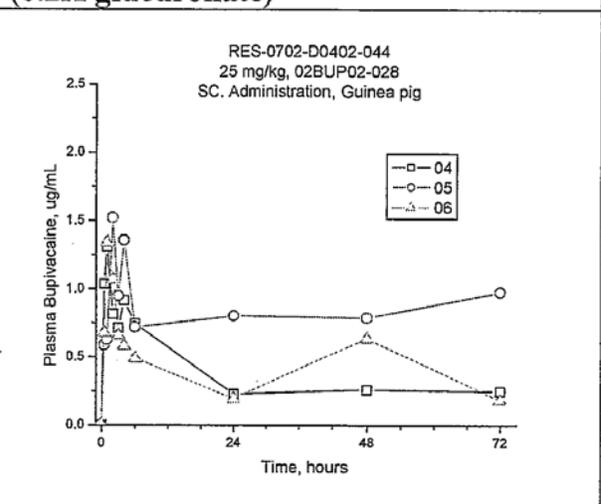
**Non-GLP study RES-0702-D0402-044** - Groups of 3 male guinea pigs per group were injected subcutaneously (SC) with 1 mL/kg of the following on their left and right sides (see table), observed for 13 days, and the skin was evaluated histologically (reported in 10.1 Local Tolerance) and for pharmacokinetics (PK). DepoBupivacaine (phosphate) is the proposed drug product. The pharmacokinetic data indicated that the SC DepoBupivacaine exhibited sustained release compared to SC Bupivacaine HCl (see figures). Local toxicity is reported in section 10.1.

Group Number	Test or Control Article (mg/kg)	
	Left side	Right Side
1	Bupivacaine HCL (12.5 mg/kg)	Saline
2	DepoBupivacaine (glucuronate) (25 mg/kg)	Saline
3	DepoBupivacaine (phosphate)(25 mg/kg)	Placebo

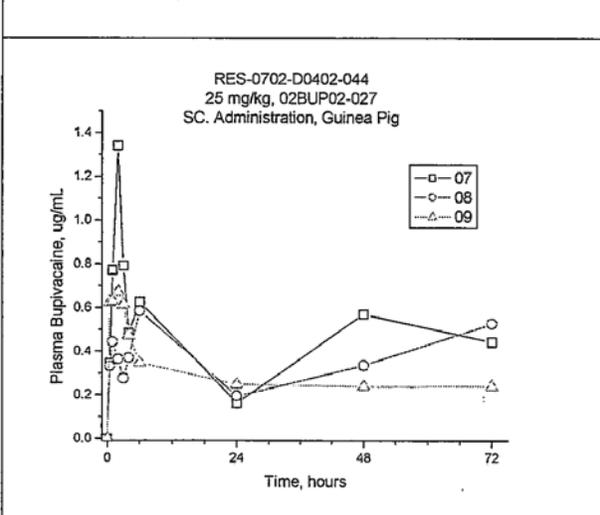
**Figure 1. Individual Pharmacokinetic Profile of Bupivacaine Following Subcutaneous Injection of Bupivacaine-HCl solution**



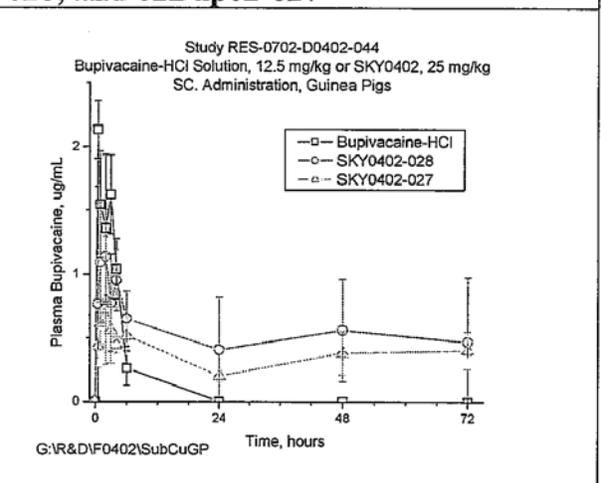
**Figure 2. Individual Pharmacokinetic Profile of Bupivacaine Following Subcutaneous Injection of 02Bup02-028 (0.2X glucuronate)**



**Figure 3. Individual Pharmacokinetic Profile of Bupivacaine Following Subcutaneous Injection of 02Bup02-027 (0.2X phosphate)**



**Figure 4. Comparative Mean Pharmacokinetic Profile of Bupivacaine Following Subcutaneous Injection of Bupivacaine HCL Solution, 02Bup02-028, and 02Bup02-027**



**Non-GLP study RES-0802-D0402-047** - Sustained release of bupivacaine was also observed after intra-articular dosing of guinea pigs with DepoBupivacaine (SKY0402). Comparable PK profiles and values were observed whether the formulations were phosphate- or glucuronate-based compared to bupivacaine HCl. Injection site toxicity is described in section 10.1.

(b) (4)

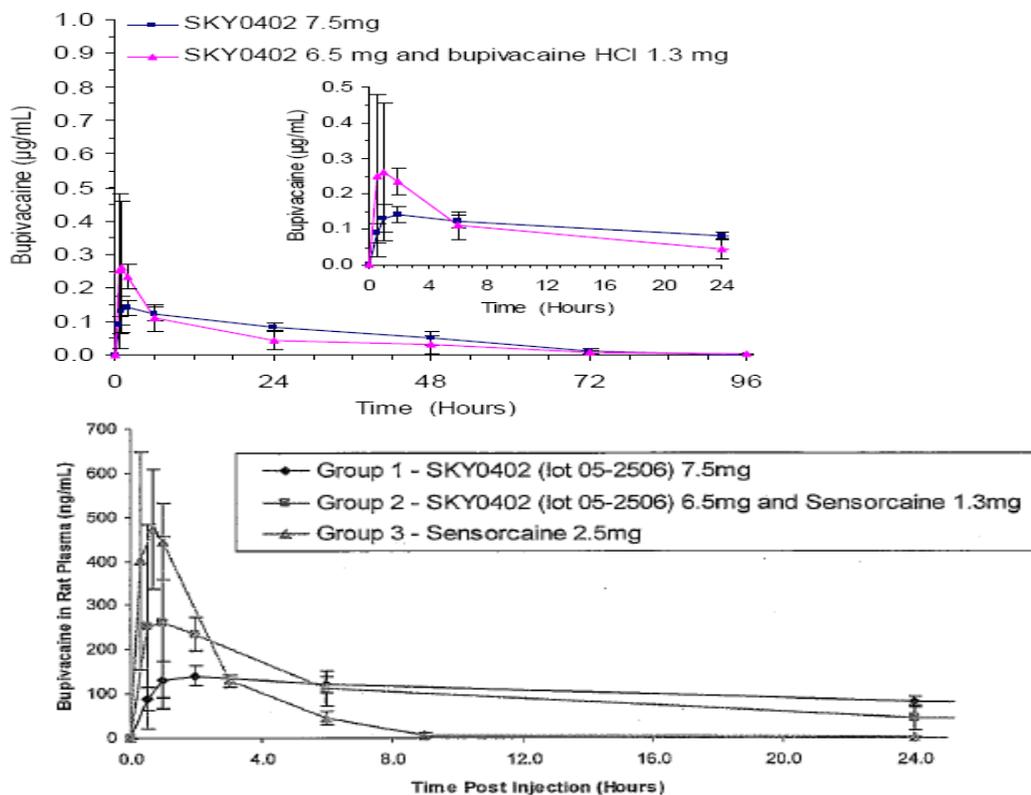
While (b) (4) doses were smaller compared to SC doses (6.25 versus 25 mg/kg), dose volumes were proportionately smaller at 0.25 mL/kg versus 1 mL/kg. The AUC/dose PK values were similar, but  $t_{1/2}$  was smaller after (b) (4) dosing suggesting a shorter time period for bupivacaine in the synovial fluid (data not shown here).

PK profile of bupivacaine after concomitant use of SKY0402 and bupivacaine hydrochloride (HCl) or SKY0402 and lidocaine hydrochloride (HCl)

**Non-GLP study RES-060130-D0402-701** – The effects of combining SKY0402 (lot 05-2506) and bupivacaine HCl (Sensorcaine) dosing was evaluated in rats in order to assess the feasibility of concomitant use of SKY0402 with bupivacaine HCl in the clinic. The PK profile of an admixture of SKY0402 and bupivacaine HCl was compared to that of SKY0402 alone. Co-administration of 6.5 mg SKY0402 and 1.3 mg of bupivacaine HCl solution (admixture) was by a single subcutaneous injection into five male Sprague Dawley rats. The PK profile was compared to that in four male rats that received 7.5 mg SKY0402 alone and in five male rats that received 2.5 mg bupivacaine HCl alone.

The sustained-release profile of SKY0402 was not compromised as the PK profile for the admixture group appeared to be a cumulative profile of SKY0402 and bupivacaine HCl solution (see figure). The bupivacaine alone profile, not shown in the first figure for viewing simplicity, is contained in the second figure that only includes the first 24 hours after dosing as bupivacaine alone was not found in plasma after approximately nine hours.

**Plasma Bupivacaine Pharmacokinetic Following Subcutaneous Injection of SKY0402 or an Admixture of SKY0402 and Bupivacaine HCl Solution in the male Rat. The error bars represent standard deviation of 4-5 animals per group**



**GLP study S08668** – SKY0402 (lot 07PD-002) and bupivacaine HCl were administered to male Yucatan mini-pigs (3/group) either alone or in combination and with differing doses and intervals of no interval up to 15 minutes between co-administration of the two. This dosing schedule was done in order to evaluate the possible PK interactions when used together or separately with a short delay between injections (see table). SKY0402 and bupivacaine HCl were injected subcutaneously as six serial bolus injections along a drawn virtual incision line. All doses were well tolerated.

Treatment Group	No of Animals & Gender	Dose Ratio (SKY0402: Bupivacaine HCL)	SKY0402 14.3 mg/mL (mg/kg)	Bupivacaine HCl Solution 7.5 mg/mL (Sensorcaine®) (mg/kg)	Minutes Post-Bupivacaine HCL Dose	Matrix & Plasma Sample Collection
1	3 males	–	2 <sup>(a)</sup>	–	–	K <sub>3</sub> EDTA – Pre-dose, 5, 10, 15, 30 min, and 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hr
2	3 males	–	4	–	–	
3	3 males	–	–	1 <sup>(a)</sup>	–	
4	3 males	–	–	2	–	
5	3 males	1:1	2 <sup>(a)</sup>	2	1	
6	3 males	4:1	4	1 <sup>(a)</sup>	1	
7	3 males	1:1	2 <sup>(a)</sup>	2	5	
8	3 males	4:1	4	1 <sup>(a)</sup>	5	
9	3 males	1:1	2 <sup>(a)</sup>	2	15	
10	3 males	4:1	4	1 <sup>(a)</sup>	15	
11 <sup>(b)</sup>	3 males	1:1	2 <sup>(a)</sup>	2	NA (pre-mixed)	
12 <sup>(b)</sup>	3 males	4:1	4	1 <sup>(a)</sup>	NA (pre-mixed)	
Volume <sup>(c)</sup> (mL/kg)			0.27mL/kg (Groups 1, 2 and 7-12)	0.27mL/kg (Groups 3-12)		

Note: NA= not applicable; (a) SKY0402 14.3 mg/mL or Sensorcaine® 0.75% (bupivacaine HCL solution) were diluted by half with saline; (b) Groups 3-4 served as a control for potential maximum release of free bupivacaine; (c) Total injected volume was 0.27 mL/kg (Groups 1-4) and 0.54 mL/kg (Groups 5-12).

Co-administration of bupivacaine HCl solution and SKY0402 as a premixture or separated by up to 15 minutes results in higher systemic exposure to bupivacaine than either formulation given alone. However, the systemic exposure was approximately the sum of the systemic exposures of either given alone. The terminal plasma  $t_{1/2}$  was unaffected by co-administration of bupivacaine solution and SKY0402, and  $T_{max}$  was similarly unaffected by co-administration.

The effects on SKY0402 PK were as follows (see table):

- Systemic exposure to bupivacaine HCl ( $C_{max}$ , AUC) was approximately proportional to dose between 2 and 4 mg/kg when bupivacaine HCl was given as SKY0402 (compare Groups 1 and 2). The same was true for bupivacaine HCl given as a solution (1 and 2 mg/kg; compare Groups 3 and 4).
- Consistent with what would be expected with a sustained-release formulation, the mean  $C_{max}$  following SKY0402 (2 mg/kg) was 20% of the  $C_{max}$  following bupivacaine HCl solution (compare Groups 1 and 4).
- The average systemic bioavailability was approximately 87% (based on total AUC) for the 2 mg/kg SKY0402 dose relative to bupivacaine HCl solution administered at the same dose, indicating almost complete absorption.

- Administration of bupivacaine solution concomitant with or prior to SKY0402 produced systemic exposures which were similar to the sum of exposure when each was given separately. This was true for both the 1:1 (Groups 5, 7, and 9) and the 4:1 (Groups 6, 8 and 10) ratios of SKY0402 to bupivacaine solution.
- Apparent half-life and t<sub>max</sub> for bupivacaine were not altered by administration of bupivacaine solution concomitant with or prior to SKY0402.

**Pharmacokinetic Parameters for Bupivacaine in Minipigs by Group**

Group	Bupivacaine dose (mg/kg)		Time <sup>a</sup> (min)	AUC <sub>0-24hr</sub> (hr•ng/mL)	AUC <sub>0-96hr</sub> (hr•ng/mL)	AUC <sub>0-∞</sub> (hr•ng/mL)	C <sub>max</sub> (ng/mL)	t <sub>1/2</sub> (hr)	T <sub>max</sub> (hr)
	as SKY0402	as solution							
1	2	0	NA	1790±595	2810±628	2820±629	181±10.2	10.6±2.15	0.197±0.0462
2	4	0	NA	2020±463	5850±652	6430±1200	234±139	19.6±8.91	16.1±27.6
3	0	1	NA	1050±252	1460±265	1480±263	428±119	14.5±1.91	0.197±0.0462
4	0	2	NA	2570±460	3210±332	3230±355	911±292	10.3±5.77	0.197±0.0462
5	2	2	1	2900±779	5310±1070	5490±1170	592±205	18.1±7.58	0.168±0.0835
6	4	1	1	2920±335	8720±1490	9720±2350	573±120	22.2±11.3	0.223±0.462
7	2	2	5	4490±892	7310±1080	7480±1100	1260±347	15.9±6.05	0.141±0.0502
8	4	1	5	3120±397	8170±716	8380±577	642±183	13.0±5.64	0.141±0.0502
9	2	2	15	3360±676	7480±2110	8890±1240	871±370	36.2±17.5	0.112±0.0502
10	4	1	15	3490±800	7850±562	8160±730	677±258	16.9±2.77	0.168±0.0835
11	2	2	premixed	3320±508	5330±1210	5380±1220	667±224	11.9±1.18	0.197±0.0462
12	4	1	premixed	4260±1710	12800±3200	14300±4460	886±310	22.4±8.28	0.197±0.0462

<sup>a</sup>Time between administration of bupivacaine solution and SKY0402.

Note: All values above were rounded to three significant figures.

NA = not applicable.

In summary, consistent with its slow-release properties, SKY0402 has a relatively modest effect on plasma concentrations when used in combination with bupivacaine HCl solution (pre-mixed) or after sequential administration of bupivacaine HCl solution followed by SKY0402 at doses up to 4 times that of bupivacaine solution and a wait time of up to 15 minutes.

#### PK profile of bupivacaine after concomitant use of SKY0402 and lidocaine hydrochloride

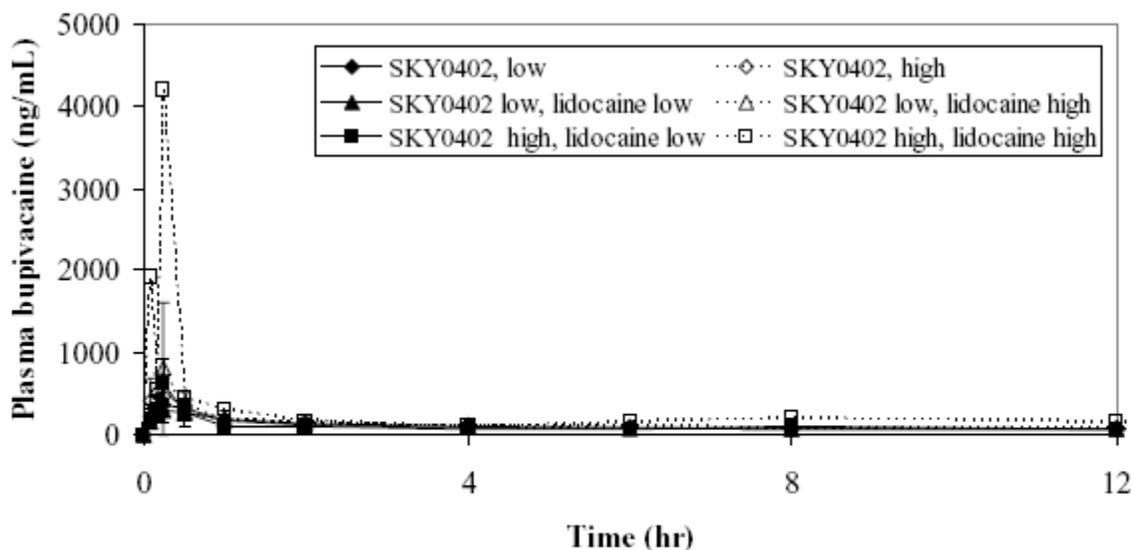
**Non-GLP study S07580 (pilot study) and GLP study S07607** – These PK drug interaction studies were designed to assess the feasibility of concomitant use of SKY0402 with lidocaine HCl with epinephrine (Lido/Epi) in the clinic. Lido/Epi was employed since it is commonly used for local anesthesia/analgesia at the beginning of surgery. In addition, the epinephrine (as a vasoconstrictor) is expected to maximize any potential local interaction by reducing the rate of absorption. The objectives were 1) to quantitate the degree of drug-drug interaction by measuring systemic exposure of lidocaine and bupivacaine in plasma samples, 2) to obtain an estimate of dose response by studying different doses and dose ratios of lidocaine HCl and SKY0402, and 3) to assess the influence of time intervals between the administration of lidocaine HCl and SKY0402 in mitigating a potential drug-drug interaction.

SKY0402 (lot 07PD-002) and Lido/Epi were injected subcutaneously as equally distributed 6 serial bolus injections along a 5 cm long virtual incision line into Yucatan

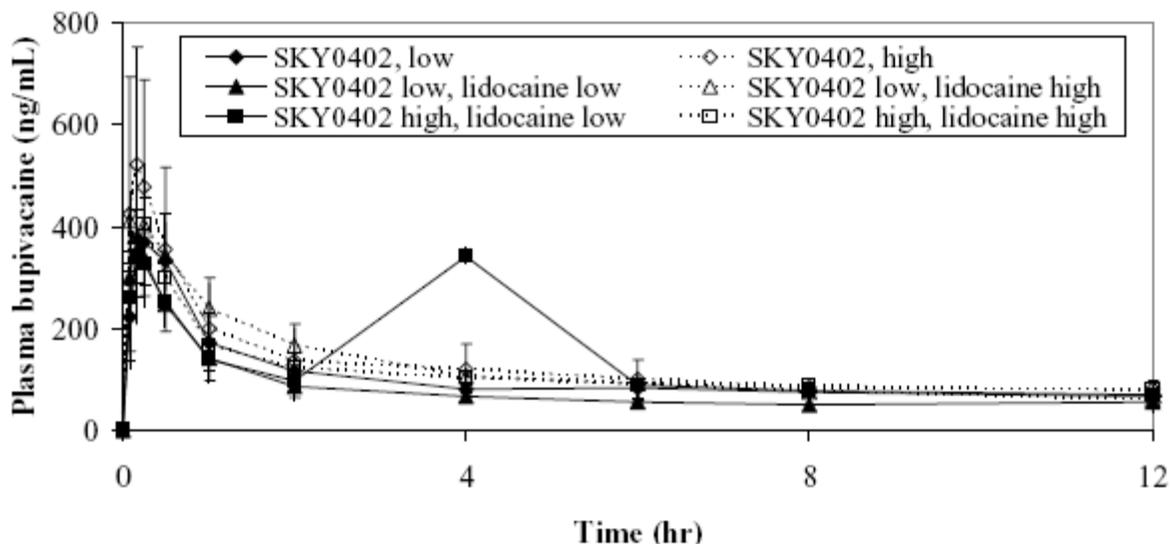
mini-pigs (1 male and 1 female/group in pilot; 3 males per group in the definitive study). The pharmacokinetic profile was compared to the plasma concentration data observed in the control groups that received either SKY0402 or lidocaine HCl alone. All doses of SKY0402 with or without lidocaine/epinephrine were well tolerated in both studies even when two formulations were infiltrated in close physical and temporal proximity at the highest doses of SKY0402 and lidocaine of 4 mg/kg. At high doses of 4 mg/kg the dose would be ~150 mg at the median minipig body weight of 37.5 kg. The highest approved daily human doses are 400 mg (~7 mg/kg) for bupivacaine (Marcaine) and ~250 mg (4 mg/kg) for lidocaine for a 60 kg human.

In the pilot study, the analytical results suggest that the susceptibility of SKY0402 to interact with lidocaine could be reduced with incorporation of a 1-minute time interval or longer (up to 10 minutes tested) after administration of Lido/Epi before SKY0402 injection (no data reported in this summary). In addition, the sustained-release profile of bupivacaine was altered after co-administration of 2 mg/kg SKY0402 and 2 mg/kg Lido/Epi (only admixture group of either study). For systemic exposure to bupivacaine, the C<sub>max</sub> was ~300% higher with the 24 hour, 96 hour, and infinity AUC values also increased by ~200%, 90%, and 70%, respectively. Bupivacaine was also eliminated more rapidly with a ~3-fold decrease in terminal t<sub>1/2</sub>.

The definitive study was conducted to expand on these preliminary findings for up to a 40 minute dosing interval time period. Plasma bupivacaine concentrations produced by SKY0402 did not appear to be affected by pretreatment with lidocaine for up to 40 minutes except when the high dose of lidocaine was given 5 or 10 minutes before the high dose of SKY0402 (see figures for 5 minutes and 20 minutes).

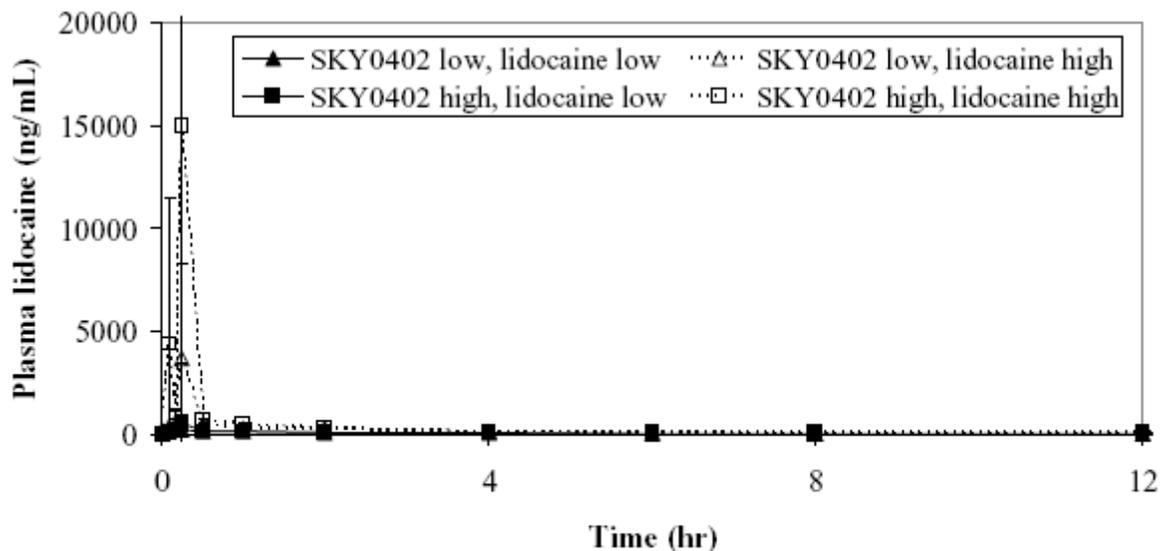


**Mean Plasma Concentrations of Bupivacaine after a 2 mg/kg (Low) or 4 mg/kg (High) Subcutaneous Dose of SKY0402 5 minutes following a 2 or 4 mg/kg Dose of Lidocaine**

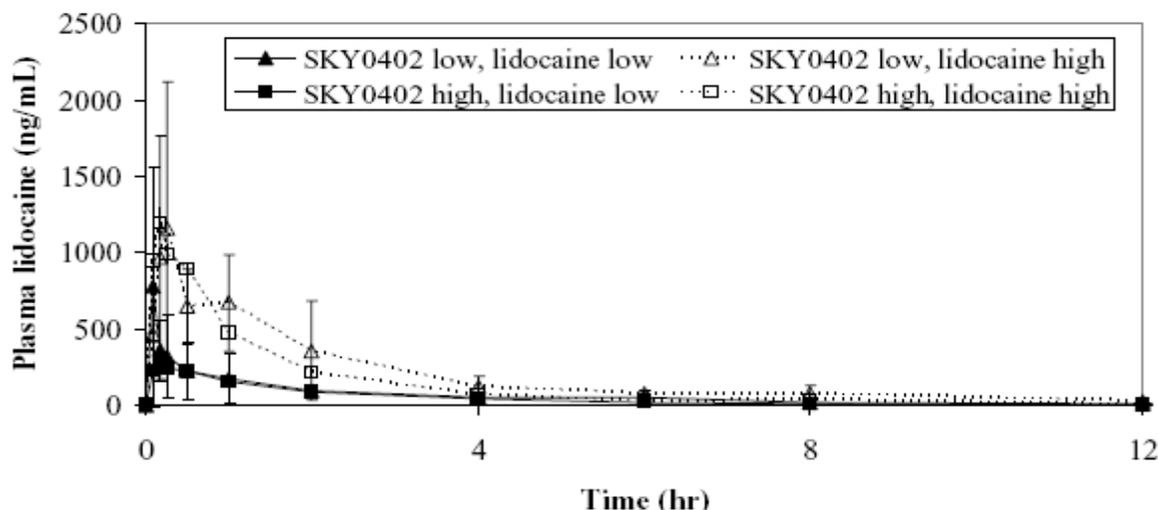


**Mean Plasma Concentrations of Bupivacaine after a 2 mg/kg (Low) or 4 mg/kg (High) Subcutaneous Dose of SKY0402 20 minutes following a 2 or 4 mg/kg Dose of Lidocaine**

Plasma concentrations of lidocaine were not affected with the subsequent administration of SKY0402 except when the high dose of SKY0402 followed the high dose of lidocaine by 5 minutes (see figures for 5 minutes and 10 minutes).



**Mean Plasma Concentrations of Lidocaine after a 2 mg/kg (Low) or 4 mg/kg (High) Subcutaneous Dose of SKY0402 5 minutes following a 2 or 4 mg/kg Dose of Lidocaine**



**Mean Plasma Concentrations of Lidocaine after a 2 mg/kg (Low) or 4 mg/kg (High) Subcutaneous Dose of SKY0402 10 minutes following a 2 or 4 mg/kg Dose of Lidocaine**

The analytical results suggest that the susceptibility of SKY0402 to interact with lidocaine could be reduced with incorporation of a 20- or 40-minute time interval after administration of the Lido/Epi mixture, prior to SKY0402 injection. In both studies, the PK profile of bupivacaine appeared to be altered (i.e., higher C<sub>max</sub> and AUC in combination) when both compounds are injected within short time intervals. A separation of at least 20 minutes between administration of Lido/Epi and SKY0402 appears to substantially reduce the risk of drug interaction (see tables from definitive study below for PK values).

**Pharmacokinetic Parameters for Bupivacaine after Subcutaneous Administration of SKY0402 with or without lidocaine/epinephrine (Mean ±STD; N = 3)**

Group	Lidocaine (1% or 2% with 1:200,000 epinephrine) (mg/kg)	SKY0402 (mg/kg)	Time between Lidocaine and SKY0402 Formulations (min)	AUC <sub>0-24h</sub> (hr•ng/mL)	AUC <sub>0-∞</sub> (hr•ng/mL)	AUC <sub>0-∞</sub> /Dose (hr•ng/mL)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose (ng/mL)	t <sub>1/2</sub> (hr)	t <sub>max</sub> (hr)
1	0	2	NA	1890±171	3970±311	1980±155	372±89.1	186±44.5	12.3±1.86	0.333±0.144
2	0	4	NA	2240±721	6630±1080	1660±269	519±230	130±57.6	16.3±6.16	0.170±0
5	2	2	5	1950±583	4230±1000	2120±502	350±135	175±67.6	17.8±9.06	0.170±0
6	4	2	5	1870±115	4840±429	2420±215	807±808	404±404	39.2±7.54	0.250±0
7	2	4	5	2100±445	7840±1050	1960±263	658±259	165±64.7	31.9±22.5	0.223±0.0462
8	4	4	5	4370 <sup>a</sup>	9580 <sup>a</sup>	2400 <sup>a</sup>	5730 <sup>a</sup>	1430 <sup>a</sup>	21.4 <sup>a</sup>	0.167 <sup>a</sup>
9	2	2	10	1470±236	3560±906	1780±453	404±81.4	202±40.7	27.5±17.6	0.168±0.0835
10	4	2	10	1710±206	3300±881	1650±441	388±76.5	194±38.3	22.0±9.95	0.473±0.458
11	2	4	10	2080±278	6190±393	1550±98.3	359±88.8	89.6±22.2	12.4±1.84	0.197±0.0462
12	4	4	10	3370±1980	9580±3300	2400±825	865±488	216±122	9.48±2.65	0.170±0
13	2	2	20	1490±397	3150±643	1580±321	388±64.5	194±32.2	11.6±3.06	0.141±0.0502
14	4	2	20	1990±261	3850±1340	1920±670	421±44.8	211±22.4	14.4±9.19	0.168±0.0835
15	2	4	20	2380±317	6330±374	1580±93.6	363±28.6	90.8±7.15	14.2±6.82	1.44±2.21
16	4	4	20	2100±180	5890±921	1470±230	412±113	103±28.3	18.0±5.39	0.307±0.172
17	2	2	40	1640±324	3090±380	1550±190	458±137	229±68.7	18.1±12.6	0.197±0.0462
18	4	2	40	1650±312	3430±393	1710±197	303±85.7	152±42.9	13.8±10.2	0.280±0.191
19	2	4	40	2070±513	6390±252	1600±63.1	358±87.2	89.6±21.8	17.4±8.28	0.168±0.0835
20	4	4	40	2150±410	6120±1020	1530±255	422±119	105±29.9	13.7±6.32	0.170±0

<sup>a</sup> N = 2. Animal # 8690 received 55% of the intended dose of bupivacaine. Data were not used in calculation of mean and SD.

**Pharmacokinetic Parameters for Lidocaine after Subcutaneous Administration SKY0402 with or without lidocaine/epinephrine (Mean ±STD; N = 3)**

Lidocaine (1% or 2% with 1:200,000 epinephrine) (mg/kg)	SKY0402	Minutes post Lidocaine dose	AUC <sub>0-24</sub> (hr•ng/mL)	AUC <sub>0-∞</sub> (hr•ng/mL)	AUC <sub>0-∞/Dose</sub> (hr•ng/mL)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose (ng/mL)	t <sub>1/2</sub> (hr)	t <sub>max</sub> (hr)
2 low (1%)	-	NA	932±306	923±294	461±147	315±82.3	158±41.1	2.34±0.882	0.307±0.172
4 high (2%)	-	NA	3490±1160	3540±1200	884±300	1070±82.9	268±20.7	4.20±1.70	0.557±0.418
2 low (1%)	low <sup>(a)</sup>	5	768±184	985±234	492±117	231±72.7	116±36.4	27.9±18.2	0.417±0.144
4 high (2%)	low <sup>(a)</sup>	5	2120±827	2270±871	567±218	3740±4500	935±1130	19.8±10.6	0.223±0.0462
2 low (1%)	high	5	912±369	1360±683	682±342	584±404	292±202	17.9±7.15	0.250±0
4 high (2%)	high	5	5170±1860	5610±2080	1400±519	18600±5472	4640±1370	11.9±3.17	0.194±0.0964
2 low (1%)	low <sup>(a)</sup>	10	792±104	859±127	430±63.3	920±649	460±324	13.1±7.95	0.168±0.0835
4 high (2%)	low <sup>(a)</sup>	10	2460±870	2630±910	657±227	1260±850	316±213	13.0±4.74	0.500±0.433
2 low (1%)	high	10	595±73.6	694±63.0	347±31.5	313±106	156±53.1	14.3±4.17	0.170±0
4 high (2%)	high	10	1720±202	1760±197	440±49.2	1200±104	299±26.0	5.35±1.26	0.170±0
2 low (1%)	low <sup>(a)</sup>	20	979±357	1050±402	523±201	475±92.8	237±46.4	8.57±5.79	0.141±0.0502
4 high (2%)	low <sup>(a)</sup>	20	1870±588	1930±595	483±149	1000±85.3	251±21.3	5.86±1.50	0.083±0
2 low (1%)	high	20	1110±260	1210±274	607±137	354±38.4	177±19.2	16.2±4.14	1.42±2.24
4 high (2%)	high	20	2290±269	2530±327	631±81.6	723±206	181±51.5	11.6±5.45	0.223±0.0462
2 low (1%)	low <sup>(a)</sup>	40	635±249	674±294	337±147	296±14.3	148±7.17	11.9±12.1	0.197±0.0462
4 high (2%)	low <sup>(a)</sup>	40	1640±1310	1720±1400	431±350	491±118	123±29.4	11.6±6.44	0.168±0.0835
2 low (1%)	high	40	658±118	730±133	365±66.4	272±90.9	136±45.4	11.3±3.51	0.141±0.0502
4 high (2%)	high	40	1980±827	2080±880	521±220	774±127	193±31.9	19.3±14.2	0.139±0.0964

NA = Not applicable; low = low dose (2 mg/kg); high = high dose (4 mg/kg)

Note: Total injected volume is 0.27 mL/kg (Groups 1, 2); 0.2 mL/kg (Groups 3, 4); 0.47 mL/kg (0.2 mL lidocaine 1% or 2% and 0.27 mL SKY0402)

<sup>a</sup> SKY0402 (15mg/mL) was diluted by half with saline

### Retention of bupivacaine and liposome carrier after SC dosing

**Non-GLP study RES-73965** – The retention of bupivacaine and the dierucoylphosphatidylcholine (DEPC) component the DepoFoam liposome carrier at the injection site were observed following subcutaneous (SC) injections of DepoBupivacaine (SKY0402 – batch 02Bup01-115) or DepoFoam placebo (containing DEPC) in eight male rats and five male guinea pigs at a dose volume of 0.5 mL (~2 mL/kg for 300 mg rats) or 1 mL (~2 mL/kg for 600 mg guinea pigs). Five additional male guinea pigs also received an intradermal (ID) injection. After injection, animals were sacrificed immediately after dosing with further individual interval animal sacrifices up to 14 days in rats and 7 days in guinea pigs (SC) and 21 days in guinea pigs (ID). The maximum proposed human dose volume is (b) (4) at the highest proposed dose. Injection sites and surrounding tissue were assayed for bupivacaine and DEPC content.

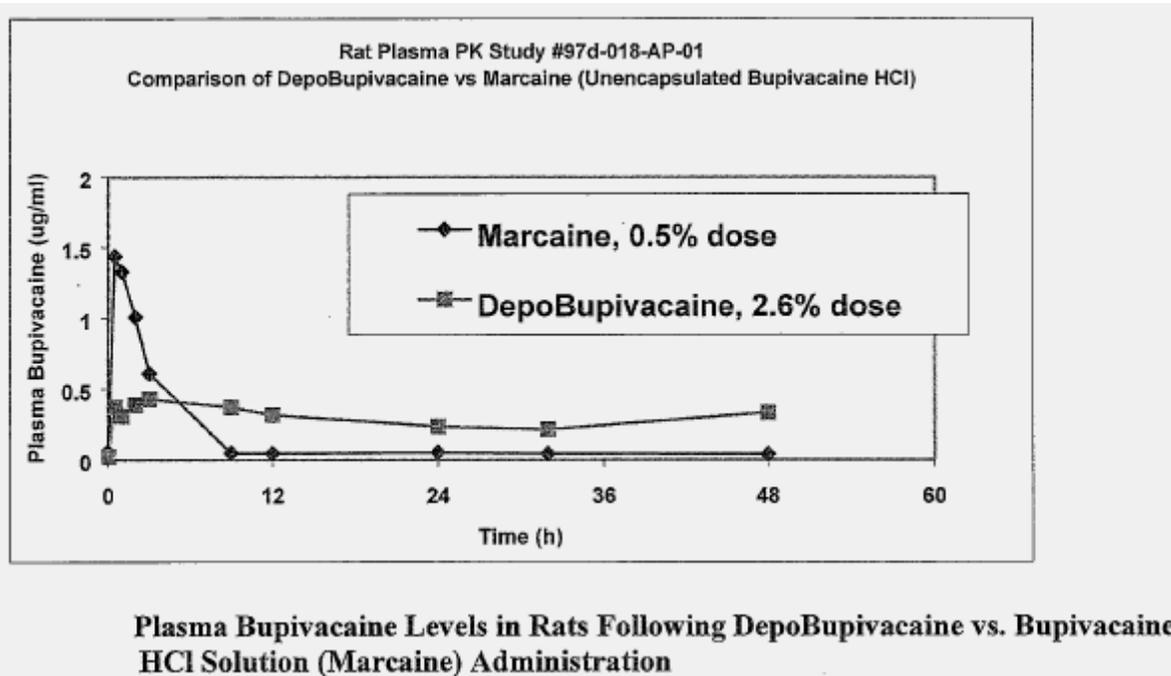
In rats following SC injections, bupivacaine levels were 16% of the original injection site dose after 3 days with no detection by 7 days. DEPC levels were 58% after 3 days and 10% by 14 days after dosing (see table). In guinea pigs following SC injections, bupivacaine levels were 50-60% at 3 days after injection and 23% at 7 days. DEPC levels were ~90% through 7 days after injection. This data indicates the delayed release of bupivacaine and the continued presence of the DepoFoam at the injection site. AS listed in the table, this report contains the results from three separate studies which were not individually submitted by the applicant.

**Remaining Dose of Bupivacaine and Dierucoylphosphatidylcholine at the Injection Site Treated with DepoBupivacaine or Placebo**

Study Number	Species	Route	Bupivacaine Dose mL (mg)	DEPC Dose mL (mg)	Days Post-Dose <sup>b</sup>	Remaining Dose of Bupivacaine, mg (% of injected dose)	Remaining Dose of DEPC, mg (% of injected dose)
RES-0801-D0402-063	Rat	s.c.	0.5 (11.75)	0.5 (3.1)	0 <sup>a</sup>	11.6 (99)	3.7 (119)
					1	5.9 (50)	2.0 (65)
					3	1.9 (16)	3.2 (105)
					7	None detected	1.8 (58)
					14	None detected	0.3 (9.8)
					0	None detected	3.0 (97)
RES-0801-D0402-058	Guinea Pig	i.d.	1.0 (23.5)	1.0 (6.1)	3	1.3 (5)	6.4 (105)
					5	0.4 (1.7)	5.9 (97)
					7	1.0 (4.3)	5.7 (93)
RES-0901-D0402-064	Guinea Pig	s.c.	1.0 (23.5)	1.0 (6.1)	14	0.2 (1)	0.5 (8)
					21	None detected	None detected
					0 <sup>a</sup>	20.8 (89)	6.6 (108)
					1	15.4 (66)	5.2 (85)
					2	14.8 (63)	5.6 (92)
					4	11.2 (48)	5.3 (87)
7	5.3 (23)	5.7 (93)					

i.d.: Intradermal/intracutaneous; s.c.: Subcutaneous; <sup>a</sup>Day 0: Immediately following injection; <sup>b</sup>Relative to Day 0

Gradual disappearance of bupivacaine after DepoBupivacaine exposure was supported by the comparative plasma profiles in rats with prolonged presence for bupivacaine after subcutaneous dosing compared to Marcaine dosing as reported in Abbott Laboratories study 97d-018-AP01 which also was not submitted separately in this NDA (see figure).



The results of these two studies taken together suggest bupivacaine release from the liposome carrier in a zero order fashion over an extended time period compared to initial peak plasma levels with a rapid decline after Marcaine dosing. Based on these results, it could take up to approximately 2 weeks for bupivacaine not to be detected and

greater than 2 weeks for DEPC (i.e., DepoFoam) not to be detected. DEPC was not detected at 21 days post dosing.

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## Distribution

**Non-GLP study QPS 137N-0401** (SkyePharma D0402-94712) - Quantitative whole-body autoradiography (QWBA tissue distribution) was conducted under “GLP-like” conditions (e.g., quality oversight) in male Long Evans rats following a single subcutaneous (SC) dose of ([1-<sup>14</sup>C]-2Erucoyl)DEPC DepoFoam (aka SKY0402 Placebo). Seventeen rats received 2.5 mg of the radioactive formulation SC with a single rat being sacrificed from 1 hour after dosing up to 28 days after dosing.

([1-<sup>14</sup>C]-erucoyl)DEPC-derived radioactivity was widely distributed from 1 to 28 days post-dose with the highest concentrations observed in most tissues occurring at 2 to 4 days post-dose. The highest concentrations observed among a comprehensive list of tissue sections measured were in the lymphatic (up to ~16 mcg equivalents/g tissue), excretory (up to ~17 mcg equivalents/g tissue), and adipose tissues (up to ~8 mcg/equivalents/g tissue) and at the injections site (up to ~9500 mcg equivalents/g tissue) for highest day’s reading. Concentrations of radioactivity in the central nervous system were near or below quantifiable limits (0.6 mcg equivalent/g tissue). In general, radioactivity concentrations in tissues declined with time and most were near or below quantifiable limits by 28 days post-dose, except for high concentrations in lymph nodes proximal to the injection site (~2 mcg equivalents/g tissue). [<sup>14</sup>C]DEPC-related radioactivity was not selectively associated with melanin containing tissues. In summary, based on measurement of the radioactive label, the SKY0402 liposome component was widely distributed in the body with less than quantifiable levels identifiable 28 days after dosing except for concentrations in the lymph nodes proximal to the injection site which were still <1% of the original radioactivity.

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**Metabolism** - metabolism of bupivacaine is well characterized; no metabolism studies were conducted with SKY0402.

**Elimination** - excretion of bupivacaine is well characterized; no excretion studies were conducted with SKY0402.

## 5.2 Toxicokinetics

Information is included with studies reviewed as part of the General Toxicology section.

## 6 General Toxicology

### 6.1 Single-Dose Toxicity

#### Subcutaneous dosing

(b) (4) **study #20995 from IND 69,198** - A GLP single-dose subcutaneous toxicity study of SKY0402 was conducted in Sprague-Dawley rats for the purpose of determining systemic toxicity and local irritancy of different concentrations of bupivacaine in SKY0402. Acute subcutaneous administration was saline (negative control), bupivacaine (reference drug) at 7.5 mg/mL, or SKY0402 at 7.5, 15.0, or 25.0 mg/mL (test article). All dose volumes including saline were 0.25 mL. Two lots of SKY0402 were used for the 25.0 mg/mL concentration (lot #01-2009 and #01-2011) as both these lots are to be used for initial clinical studies. Animals were sacrificed after 2 or 14 days observation. Cage-side observations made daily did not detect evidence of clinical signs and no changes in food consumption, body weight or clinical pathology parameters were noted to be treatment-related. Post-mortem assessment consisted of a full necropsy with macroscopic observation of organs and tissues which were considered normal. Organs weighed included the heart, kidneys, liver and spleen only. On Day 3 sacrifice (2 days observation) female rats were observed to have slightly decreased kidney weights in both the bupivacaine and 25 mg/mL SKY0402 groups (~-10%;  $p < 0.05$ ) though female rats administered the second batch of SKY0402 given at 25 mg/mL demonstrated a non-significant reduction in kidney weight (-6%). This finding was not observed in animals sacrificed on Day 15 (14 days observation). Spleen weights were treatment and dose-relatedly decreased in male rats only on Day 15 compared with saline-treated rats, though a non-significant trend was noted by this reviewer (Adam Wasserman) on Day 3:

Spleen weight (Males)						
	Saline	Bupivacaine 7.5 mg/mL	SKY0402			
			7.5 mg/mL	15 mg/mL	25 mg/mL (lot #01-2009)	25 mg/mL (lot #01-2011)
<b>Day 3</b>	-	0	+2%	0	-8%	-10%
<b>Day 15</b>	-	<b>-13%*</b>	-6%	<b>-17%**</b>	<b>-17%**</b>	<b>-22%**</b>

\*  $p < 0.05$ ; \*\* $p < 0.01$ , by ANCOVA (terminal kill body weight covariate)

No significant difference was detected between the bupivacaine and SKY0402-treated rats in regards to organ weight. Histopathologic assessment was conducted only on the injection site which included the skin around the injection site and the underlying muscle. Day 3 observation indicated evidence of minimal to mild subacute and chronic inflammation at the injection site in the high dose SKY0402 group which did not appear qualitatively different from the bupivacaine reference control. Rats administered lower levels of SKY0402 appeared no different from saline-treated rats though some

inflammatory evidence was found in these groups and was attributed by the sponsor to the injection procedure. Rats sacrificed on Day 15 showed general resolution of the inflammation though some evidence was still seen in the subcutis area in both SKY0402 25 mg/mL and bupivacaine-treated rats. It is unclear why there would be chronic inflammation at the injection site by Day 3 and it is possible that some prior process was underway at the site. Histopathologic assessment of a secondary location not directly part of the injection site was not conducted so no conclusion can be made. The sponsor did not identify a NOAEL for this study but noted that the histopathologic findings were mild but more obvious in the 25 mg/mL SKY0402 which they attributed to the test article but noted that this was not severe, was similar in nature to findings in rats given the reference drug bupivacaine and thus should be considered of acceptable tolerability. The reviewer notes the changes observed in spleen weight which appeared to be dose-related and demonstrated a "delayed" effect, showing statistical significance at 15 days post-drug administration in male rats only but trending towards a decrease by Day 3. With the observation that the SKY0402 DepoFoam remains at the injection site for several weeks (data presented by Sponsor in Pharmacology Summary) it is possible this is a real effect of either the prolonged release of bupivacaine or the DepoFoam lipid microparticles. It is unknown whether recovery of organ weight occurs. Although the histopathology does not contribute to the establishment of the NOAEL the reviewer (Adam Wasserman) identifies the NOAEL in males as 7.5 mg/mL SKY0402 while in female rats the NOAEL is > 25 mg/mL due to changes in absolute and relative spleen weight in males.

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**GLP studies (b) (4) 947-017 and (b) (4) 947-018** - The objective of these studies was to evaluate the potential local and systemic toxicity of SKY0402 compared with bupivacaine hydrochloride (HCl) solution when administered by the subcutaneous (SC) route in beagle dogs.

In the preliminary dose-range finding study ((b) (4) 947-017), one male and one female dog were administered single escalating SC doses of SKY0402 (lot PP04-048) on Day 1 (15 mg/kg, 1 mL/kg, 15 mg/mL) and on Day 5 (30 mg/kg, 2 mL/kg, 15 mL/kg). Following a four-day washout period, a single SC dose of bupivacaine HCl (2 mg/kg bupivacaine, 5 mg/mL Sensorcaine, 0.4 mL/kg) was administered. Each dose was administered as a single bolus injection into the scapular region. SKY0402 was tolerated at 30 mg/kg based on no significant treatment related effects on mortality, clinical signs, body weight, and food consumption. Bupivacaine HCl (2 mg/kg) was also tolerated based on the same criteria.

In an expanded acute SC toxicity study ((b) (4) 947-018), five dogs/sex/group were administered saline, SKY0402 Placebo (lot 02BUP04-185), SKY0402 (lot PP04-045), or Sensorcaine (bupivacaine HCl) at dose volumes of 2 mL/kg except for the bupivacaine HCl groups with a dose volume of 0.4 mL/kg. Animals were sacrificed on days 2 or 15 after dosing.

Group Assignments				
Group Number	Bupivacaine Dose Level (mg/kg)	Treatment	Number of Animals	
			Male <sup>a</sup>	Female <sup>a</sup>
1	0	Saline	5	5
2	0	SKY0402 placebo	5	5
3	30	SKY0402	5	5
4	2.0	Sensorcaine <sup>®</sup>	5	5

<sup>a</sup> Three animals were submitted to necropsy on Day 2 and the two remaining animals were submitted to necropsy on Day 15.

All animals were observed twice daily for morbidity, mortality, injury, and the availability of food and water. Body weights for all animals were measured the day of arrival, prior to randomization, prior to dosing, and daily during the study. Food consumption was recorded daily for all animals scheduled for necropsy on day 15. A comprehensive assessment of clinical chemistry, hematology, urinalysis, ophthalmology, macroscopic pathology, organ weights, toxicokinetics (day 1), and histopathology (full tissues set on Day 2; affected organs site on Day 15) was conducted. Day 15 histopathological evaluations included injection site and skin.

The following organs were weighed and evaluated microscopically:

- Adrenal (2)*	- Larynx
- Aorta	- Liver [3 sections collected; 2 examined]*
- Bone with marrow [femur]	- Lung [2 sections examined]*
- Bone with marrow [rib]	- Lymph node, mandibular [2 collected; 1 examined]
- Bone with marrow [sternum]	- Lymph node, mesenteric
- Bone marrow smear [2 collected]*	- Lymph node, tracheobronchial
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*	- Mammary gland [process females only]
- Epididymis (2)*	- Nictitans gland (2)
- Eye including optic nerve (2)	- Pancreas*
- Gallbladder	- Peyer's patches
- Gastrointestinal tract:	- Pituitary*
esophagus	- Prostate*
stomach [cardia, fundus, and pylorus]	- Salivary gland, mandibular [2 collected; 1 examined; only right was weighed]*
duodenum	- Salivary gland, sublingual [2 collected; 1 examined]
jejunum	- Salivary gland, parotid [2 collected; 1 examined]
ileum	- Sciatic nerve
cecum	- Skeletal muscle, biceps femoris
colon	- Skin
rectum	- Spinal cord [cervical, thoracic, and lumbar]
- Gonads:	- Spleen*
ovary (2)*	- Thymus*
testis (2)*	- Thyroid/parathyroid (2)*
- Gross lesions	- Tongue
- Heart*	- Trachea
- Injection site [scapular]	- Urinary bladder
- Joint, tibiofemoral	- Uterus [both horns] with cervix*
- Kidney (2)*	- Vagina

<sup>a</sup> Bone marrow smears were collected at scheduled necropsies and held  
\* Organ weighed  
(2) Paired organ

In summary, no notable bupivacaine treatment related effects were observed after single SC dosing with SKY0402 or bupivacaine HCl. Systemic effects were also not observed after SC treatment with SKY0402 placebo or saline. A possible transient test article-related effect on the skin (thickened and leathery) was seen in 3/10 animals on Day 2 in the high dose (30 mg/kg) SKY0402 group. Pharmacokinetic (PK) values

obtained on day 1 indicated consistent AUC values after bupivacaine HCl at 24 and 96 hours after dosing, but increasing values for SKY0402 treated animals as AUC increased from approximately 10,000 ng•h/mL to 20,000 ng•h/mL from 24 to 96 hours (see table). As expected because of the differing bupivacaine absorption profile, the  $T_{max}$  for SC bupivacaine was less than for SKY00402 (~2 hours versus ~8 hours).

Mean pharmacokinetic parameters ( $\pm$  standard deviation) for plasma bupivacaine after subcutaneous injection of SKY0402 or bupivacaine HCl solution in dogs.

Treatment	Last timepoint (hr)	Gender	$t_{max}$ (hr)	$C_{max}$ (ng/mL)	$C_{max}/Dose$ (ng•kg/mg•mL)	$AUC_{last}$ (ng•hr/mL)	$AUC_{last}/Dose$ (ng•hr•kg/mg•mL)
Bupivacaine HCl solution (2 mg/kg)	24	Male	3.20 $\pm$ 4.92	145 $\pm$ 41.6	72.5 $\pm$ 20.8	1820 $\pm$ 459	912 $\pm$ 230
		Female	1.00 $\pm$ 0.00	293 $\pm$ 282	147 $\pm$ 141	1910 $\pm$ 802	955 $\pm$ 401
		M+F	2.10 $\pm$ 3.48	219 $\pm$ 205	110 $\pm$ 103	1870 $\pm$ 618	933 $\pm$ 309
	96	Male	a	a	a	2800 <sup>b</sup>	1400 <sup>b</sup>
		Female	a	a	a	1360 <sup>b</sup>	678 <sup>b</sup>
		M+F	a	a	a	2080 $\pm$ 867	1040 $\pm$ 434
SKY0402 (30 mg/kg)	24	Male	7.80 $\pm$ 10.2	804 $\pm$ 358	26.8 $\pm$ 11.9 <sup>c</sup>	9750 $\pm$ 3710	325 $\pm$ 124 <sup>c</sup>
		Female	7.80 $\pm$ 10.2	652 $\pm$ 203	21.7 $\pm$ 6.77	9190 $\pm$ 4560	306 $\pm$ 152 <sup>c</sup>
		M+F	7.80 $\pm$ 9.65	728 $\pm$ 286	24.3 $\pm$ 9.63	9470 $\pm$ 3930	316 $\pm$ 131
	96	Male	a	a	a	20500 <sup>b</sup>	683 <sup>b</sup>
		Female	a	a	a	22800 <sup>b</sup>	761 <sup>b</sup>
		M+F	a	a	a	21700 $\pm$ 4720	722 $\pm$ 157

Note: M= Male; F= Female. <sup>a</sup> These values were determined using only the 24 hour data. <sup>b</sup>SD not calculated for N=2. <sup>c</sup>Significantly different from corresponding value for bupivacaine HCl solution, Student's t test, p<0.05.

In conclusion, SKY0402 was tolerated at SC doses up to 30 mg/kg. No effects related to the test article were observed except for a possible transient effect on the skin. This single dose SC NOAEL for SKY0402 had gender combined mean AUC PK values of 9,470 ng•h/mL at 24 hours after dosing and 21,700 ng•h/mL at 96 hours after dosing. The  $C_{max}$  value for 30 mg/kg SKY0402 was 728 ng/mL for the 24 hour sampling period.

(b) (4)

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### Intravascular dosing

**Non-GLP study RES 050524-D0402-611** – This dose range-finding intravenous study was conducted in female rats to assess the intravascular (IV) toxicity of SKY0402 (lot KP2078) and also Bupivacaine HCl (Sensorcaine – lot 04-2502)) in the event that there was accidental IV exposure instead of the intended subcutaneous exposure. No pharmacokinetic analyses were conducted. The dosing regimens are listed in the table:

**Description of Treatment Groups**

Group	Treatment	Route	Bupivacaine (mg/kg) <sup>a,c</sup>	Bupivacaine concentration <sup>a</sup> (mg/mL)	Dose volume (mL)	Number of animals
1	Sensorcaine	Intravenous	1.0	0.50	0.5	3
2	Sensorcaine	Intravenous	2.5	1.25	0.5	3
3	Sensorcaine	Intravenous	1.75 <sup>b</sup>	0.875	0.5	3
4	SKY0402	Intravenous	2.5	1.25	0.5	3
5	SKY0402	Intravenous	5.0	2.5	0.5	3
6	SKY0402	Intravenous	7.5	3.75	0.5	3

<sup>a</sup> Expressed as anhydrous bupivacaine HCl equivalent; <sup>b</sup> The original 3.75 mg/kg dose level was not tested due to adverse events at 2.5 mg/kg; <sup>c</sup> Based on average body weight of 250 grams

Each dose was administered by slow bolus injection into the tail vein over approximately 3 minutes. The injection volume was 0.5 mL/animal for all groups. All animals were observed for morbidity, mortality, injury, and availability of food and water. A detailed clinical examination was conducted hourly for eight hours after dosing and again at 24 hours, just prior to sacrifice. Limited macroscopic examinations were conducted at necropsy.

Results - No treatment related clinical or macroscopic signs were observed after IV dosing with SKY0402 up to 7.5 mg/kg (HED of 1.2 mg/kg). The highest proposed human dose is (b) (4) with the next lower proposed human dose of 300 mg/day (5 mg/kg).

For bupivacaine HCl, no treatment related clinical signs were observed at 1 mg/kg. In contrast, at 2.5 mg/kg, two of the three treated animals died within 4 to 5 minutes after dosing. Clinical signs included labored breathing progressing to tremors and death. At necropsy, there was evidence of heart hypertrophy with engorged vessels. The cause of death was reported as being due to cardiovascular/respiratory insufficiency or related complications. The rapidity of the observed clinical signs symptoms were reported to be indicative of high systemic exposure to bupivacaine. Subsequently, a lower dose of 1.75 mg/kg was evaluated instead of the planned dose of 3.75 mg/kg. At 1.75 mg/kg, there were no treatment related clinical or macroscopic signs of toxicity observed. Observed clinical signs were hyper-reactivity to handling or noise at 2 and 4 hours post-dosing and somnolence at 4 hours post-dosing. The sponsor considered this dose to be an approximate maximum tolerated dose (MTD).

The results of this study indicate that the purported sustained release delivery of SKY0402 is better tolerated than unencapsulated bupivacaine even if inadvertently injected IV up to at least 3-fold (2.5 mg/kg bupivacaine lethal and 7.5 mg/kg SKY0402 NOAEL).

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**GLP studies 947-014, -015, & -016** - The acute toxicity of intravenous (IV) SKY0402 placebo (SkyePharma lot 25BUP02-019 - 76.2% packed particle volume) was evaluated in rats in three progressively detailed GLP studies that were designed based on the results of the previous study: 1) 10 mL/kg dose (**study 947-014**), 2) dose range finding study (**study 947-015**), and 3) expanded acute toxicity study (**study 947-016**). No pharmacokinetic analyses were conducted.

- 1) During a 5 minute IV dosing period with 10 mL/kg of SKY0402 placebo (lot 25BUP02-19 - 76.2% packed particle volume), the first two treated animals died within the first minute of dosing after exhibiting decreased activity. No notable macroscopic necropsy observations were reported. The first two animals dosed with 10 mL/kg of saline were unaffected. No more of the intended 10 animals per group were treated. Death was reported to be treatment related and may

possibly have been dose volume and administration rate related as upper limit IV dose volume are reported as low as 5 mL/kg (Diehl, K-H et al. 2001). The lethal dose was 10 mL/kg/5 minutes or approximately 2 mL/kg/min.

- 2) In order to estimate the maximum tolerated dose (MTD) of SKY0402 placebo, groups of 4 rats/sex/group received a single IV dose in the tail vein at dose volumes of 0.02, 0.05, 0.10, and 1 mL/kg with no negative control group being tested. Observations for mortality, morbidity and the availability of food and water were conducted twice daily for all animals. Observations for clinical findings, body weights, and food consumption were conducted daily during the study. At study termination (day 9), a complete macroscopic necropsy examination was performed.

No unscheduled deaths occurred during the conduct of this study. Decreased activity was observed in two highest dose volume males on day 1 only with 1 of the males also exhibiting rapid and shallow breathing noted on Day 1. These effects were considered treatment related as it was observed in the previous study. These animals recovered by Day 2 post-dose. In summary, under the conditions of this study, the 0.10 mL/kg dose volume was considered to be a No-Observed-Adverse-Effect-Level (NOAEL). Because of the test article-related decreased activity seen at 1 mL/kg, the MTD would be 0.1 mL/kg (between 0.10 and 1 mL/kg).

- 3) In an expanded acute IV toxicity study with SKY0402 placebo, groups of 10/sex/group were treated with 0.1, 0.5, or 1 mL/kg of SKY0402 placebo or 1 mL/kg of saline in the tail vein. Observations for mortality, morbidity and the availability of food and water were conducted twice daily for all animals. Observations for clinical findings were conducted immediately post-dose and one hour post-dose on Day 1 and daily during the duration of the study. Body weights and food consumption were measured and recorded daily. Blood and urine samples for clinical pathology evaluations were collected from five animals/sex/group on Days 2 and 15. At animal sacrifice (5/sex/group on Days 2 and 15), a complete necropsy examination was performed, organ weights were taken, and selected tissues (adequate test battery) were microscopically examined for animals in the control and 1 mL/kg groups.

One female in the 1 mL/kg SKY0402 placebo dose group died after dosing on Day 1. This animal showed decreased activity with slow shallow breathing and loss of the righting reflex immediately post-dose. One 0.5 mL/kg female also exhibited shallow breathing on day 1. At necropsy there were no macroscopic findings nor were there any notable microscopic findings. No clinical pathology evaluated was conducted on this animal. The cause of death could not be determined, however, this death was considered to be test article-related by the sponsor. The only test article-related clinical signs observed during the study were the signs recorded this female that was found dead. There was no treatment related effects on body weight, food consumption, hematology, clinical

chemistry, urinalysis parameters, macroscopic observations, organ weight changes, or microscopic evaluations.

Under the conditions of this study where rats were dosed intravenously with SKY0402 placebo at dose volumes of 0.1, 0.5, and 1 mL/kg, the treatment related death was associated with clinical signs of decreased activity in one of ten females and zero of ten males dosed at 1 mL/kg. The 0.5 mL/kg was the NOAEL.

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### Epidural dosing

**GLP study 947- 031** – The purpose of this study in CD® [CrI:CD®(SD)] rats was to evaluate potential local and systemic toxicity of the test articles after a single epidural administration under surgical conditions. Four treatment groups of 10 male and 10 female rats were administered either SKY0402 Placebo, SKY0402 (lots 04-2502), SKY0402 in combination with Xylocaine (lidocaine), Bupivacaine HCl (Sensorcaine®), or the negative control of 0.9% Sodium Chloride (see table). Five animals/sex/group were necropsied on days 3 and 15 with TK being evaluated on day 1.

**Epidural Treatment Assignments (MPI 947-031)**

Treatment (Day 1)	Route	Dose		Number of Animals		
		mg <sup>a</sup>	mL	Necropsy Day 3	Necropsy Day 15	Toxicokinetics Day 1 <sup>d</sup>
SKY0402 15 mg/mL	Epidural	1.5	0.1	5/sex	5/sex	9/sex
SKY0402 placebo	Epidural	0	0.1	5/sex	5/sex	-
Bupivacaine HCl <sup>b</sup>	Epidural	0.75	0.1	5/sex	5/sex	9/sex
Saline	Epidural	0	0.1	5/sex	5/sex	-
SKY0402 15 mg/mL (with lido/epinephrine) <sup>c</sup>	Epidural	1.5	0.05 /0.05	5/sex	5/sex	9/sex

<sup>a</sup> Bupivacaine HCl or bupivacaine HCl equivalent; <sup>b</sup> Sensorcaine 0.75%; <sup>c</sup> Xylocaine (1.5% lidocaine with 1:200,000 epinephrine); <sup>d</sup> Toxicokinetic samples were collected up to 96 hours post-dose from satellite animals.

A comprehensive battery of observations were conducted for this GLP study that included daily observations for mortality, morbidity, injury, clinical signs, the availability of food and water, body weights, and food consumption. Blood and urine samples for clinical pathology evaluations were collected from all main study animals on Days 3 and 15. After blood collection, the TK animals were euthanized, and the carcasses were discarded without further evaluation. At each termination on Days 3 and 15, necropsy examinations of the main study animals were performed, organ weights were recorded, and selected tissues, including the spinal cord, were microscopically examined.

The following list constitutes the full complement of organs and tissues for this study:

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- 
- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>- Adrenal (2)*</li> <li>- Aorta</li> <li>- Bone with marrow [femur]</li> <li>- Bone with marrow [sternum]</li> <li>- Bone marrow smear [2 collected]<sup>a</sup></li> <li>- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*</li> <li>- Epididymis (2)*</li> <li>- Esophagus</li> <li>- Eye including optic nerve (2)</li> <li>- Gastrointestinal tract: <ul style="list-style-type: none"> <li>stomach [glandular and nonglandular]</li> <li>duodenum</li> <li>jejunum</li> <li>ileum</li> <li>cecum</li> <li>colon</li> <li>rectum</li> </ul> </li> <li>- Gonads: <ul style="list-style-type: none"> <li>ovary (2)*</li> <li>testis (2)*</li> </ul> </li> <li>- Gross lesions</li> <li>- Heart*</li> <li>- Joint, tibiofemoral</li> <li>- Kidney (2)*</li> <li>- Lacrimal gland, exorbital (2)</li> <li>- Larynx</li> </ul> | <ul style="list-style-type: none"> <li>- Liver [3 sections collected; 2 examined]*</li> <li>- Lung [collected whole; 2 sections examined]*</li> <li>- Lymph node, mandibular [2 collected; 1 examined]</li> <li>- Lymph node, mesenteric</li> <li>- Mammary gland [process females only]</li> <li>- Pancreas</li> <li>- Peyer's Patches</li> <li>- Pituitary*</li> <li>- Prostate*</li> <li>- Salivary gland, mandibular [2 collected; 1 examined]*<sup>b</sup></li> <li>- Salivary gland, parotid [2 collected; 1 examined]</li> <li>- Salivary gland, sublingual [2 collected; 1 examined]*<sup>b</sup></li> <li>- Sciatic nerve</li> <li>- Seminal vesicle (2)</li> <li>- Skeletal muscle, biceps femoris</li> <li>- Skin</li> <li>- Spinal cord [cervical, thoracic, and lumbar; serial sections at injection sites]</li> <li>- Spleen*</li> <li>- Thymus*</li> <li>- Thyroid/parathyroid (2)*</li> <li>- Tongue</li> <li>- Trachea</li> <li>- Ureter (2)</li> <li>- Urinary bladder</li> <li>- Uterus [both horns] with cervix*</li> <li>- Vagina</li> </ul> |
|---|---|
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<sup>a</sup> Bone marrow smears were collected at scheduled necropsies and held

<sup>b</sup> A combined weight of the right mandibular/sublingual salivary gland was obtained

\*Organ weighed

(2) Paired organ

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Treatment related deaths were observed after dosing on day 1 with Sensorcaine as 11 of 38 died. These deaths were all considered to be due to drug overdose (not clear to reviewer if dosing error or dose just too high, but replacement animals did not die). The animals that died on day 1 were replaced. No apparent observations/evaluations were conducted for animals that died (not reported here).

There were no apparent SKY0402-related toxic effects from clinical signs up to and including microscopic evaluations. TK evaluations indicated Bupivacaine disappeared from the plasma of rats given Sensorcaine® more rapidly than it did from the plasma of rats given SKY0402 or SKY0402 in combination with Xylocaine (lidocaine), as would be expected due to the liposome carrier. There was wide gender variability for SKY0402 dosing alone groups but not when administered with xylocaine which also exhibited higher systemic exposure for bupivacaine in the presence of xylocaine. For epidural injection of SKY0402 in rats, the NOAEL was 1.5 mg in 0.1 mL (~6 mg/kg).

Toxicokinetic Values for Bupivacaine in Rats Treated with SKY0402 by a Single Epidural Injection (day of dosing)				
Dose	gender	Cmax (ng/mL)	Tmax (hour)	AUC <sub>0-96h</sub> (ng•hr/mL)
SKY0402 (1.5 mg – ~6 mg/kg)	Male	392	1.56	2510
	Female	1620	2.33	8290
	M+F	1000	1.94	5400
SKY0402 (1.5 mg) and Xylocaine (0.75 mg) (~3 mg/kg bupivacaine)	Male	652	1.11	4950
	Female	714	1.67	5180
	M+F	683	1.39	5070
Bupivacaine HCl (0.75 mg – ~2 mg/kg)	Male	285	1.00	521
	Female	418	1.00	1040
	M+F	352	1.00	778

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**GLP study 947-020** - The purpose of this study in male beagle dogs was to evaluate potential local and systemic toxicity of the test articles after a single epidural administration under surgical conditions. Four treatment groups of 6 males each were administered either SKY0402 Placebo (lot 02BUP14-185), SKY0402 (lot PP04-048), SKY0402 in combination with Xylocaine (lidocaine), Bupivacaine HCl (Sensorcaine®), or the negative control of 0.9% Sodium Chloride. Three animals/group were necropsied on days 4 and 22 with TK being evaluated on day 1 from blood taken from the day 22 necropsy animals.

Treatment Groups for a Single Epidural Dose Study of Male Dogs ( <sup>(b) (4)</sup> 947-020)					
Treatment Group	Dose Bupivacaine		Number of Animals		
	mg <sup>a</sup>	mL	Day 1	Necropsy (Day 4)	Necropsy (Day 22)
Saline	0	3	6	3	3
SKY0402 Placebo	0	3	6	3	3
SKY0402	45	3	6	3	3
SKY0402/Xylocaine	45	3/1.8	6	3	3
Sensorcaine	15	3	6	3	3

a – bupivacaine HCl or its equivalent

A comprehensive battery of observations were conducted for this GLP study that included daily observations for mortality, morbidity, injury, clinical signs, the availability of food and water, body weights, and food consumption. Blood and urine samples for clinical pathology evaluations were collected pretest and on Days 4 and 22. At each termination on Days 4 and 22, necropsy examinations of the main study animals were performed, organ weights were recorded, and selected tissues, including the spinal cord, were microscopically examined. Sections of the spinal cords were embedded,

sectioned, and stained using amino cupric silver stain along with a parallel set of H&E stained slides.

The following list constitutes the full complement of organs and tissues for this study:

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- |   |  |
|---|--|
| - Adrenal (2)*  | - Heart  |
| - Aorta   | - Injection site [1 site collected]                    |
| - Bone with marrow [femur]                              | - Kidney (2)*  |
| - Bone with marrow [rib]                                | - Liver [3 sections collected; 2 examined]*            |
| - Bone with marrow [sternum]                            | - Lung [2 sections examined]*                          |
| - Bone marrow smear [2 collected]                       | - Lymph node, mandibular [2 collected; 1 examined]     |
| - Brain [cerebrum, midbrain, cerebellum, medulla/pons]* | - Lymph node, mesenteric                               |
| - Epididymis (2)  | - Nictitans gland (2)                                  |
| - Eye including optic nerve (2)                         | - Pancreas   |
| - Gallbladder   | - Pituitary  |
| - Gastrointestinal tract:                               | - Prostate   |
| esophagus   | - Salivary gland, mandibular [2 collected; 1 examined] |
| stomach [cardia, fundus, and pylorus]                   | - Sciatic nerve  |
| duodenum  | - Skeletal muscle, biceps femoris                      |
| jejunum   | - Skin   |
| ileum   | - Spinal cord [cervical, thoracic, and lumbar]         |
| cecum   | - Spleen   |
| colon   | - Thymus   |
| rectum  | - Thyroid/parathyroid (2)                              |
| - Gonads:   | - Tongue   |
| testis (2)  | - Trachea  |
| - Gross lesions   | - Urinary bladder                                      |
- 

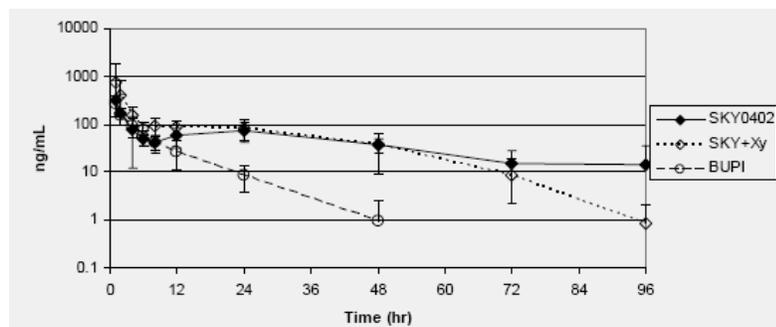
\* Organ weighed  
(2) Paired organ

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No treatment related mortality occurred. As to clinical signs, impaired limb function was observed on the day of dosing in 4 of 6 dogs dosed with Sensorcaine and 5 of 6 dogs dosed with the SKY0402/Xylocaine combination. The impairment was not observed on day 2. There was no associated histopathology with this clinical sign. No clear treatment related effects occurred, but the mean activated partial thromboplastin time (APTT) was prolonged on Day 4 (5%) and Day 22 (27%) for the three SKY0402/Xylocaine dogs but not SKY0402 dogs. Lymphocytes increased 16% relative to pretest on Day 22 in animals receiving Sensorcaine. These increases were reported to be within expected ranges. A slight prolongation of APTT in animals receiving Sensorcaine was considered treatment related by the sponsor. The increased lymphocytes in those receiving Sensorcaine may have been incidental, but a mild antigenic response was not ruled out by the sponsor.

No treatment related effects were observed for food consumption, body weights, other clinical pathology, gross necropsy, organ weights or microscopic evaluation. All spinal cord microscopic observations were within normal limits with no test article-related effects.

In TK evaluations, bupivacaine concentrations were generally measurable out to 96 hours in animals given SKY0402, but generally were below the limit of quantitation after 48 hours in animals given the bupivacaine HCl solution. As expected, exposure to bupivacaine over the first 24 hours was significantly lower after SKY0402 than after bupivacaine HCl solution.



The overall AUC was comparable for the SKY0402 and SKY0402/Xylocaine groups but the  $t_{max}$  was considerable sooner (~4 hr versus ~1 hr) and the  $C_{max}$  approximately double (319 ng/mL versus 759 ng/mL) when the xylocaine was present suggesting an alteration in the release kinetics from the liposome. The SKY0402 dose was a NOAEL.

Summary of Pharmacokinetic Parameters (mean and standard deviation) After an Epidural Injection of SKY0402 (45 mg) or Bupivacaine HCl solution (15 mg) in Male Beagle Dogs							
Route	Treatment	$t_{max}$ (hr)	$t_{1/2}$ (hr)	$C_{max}$ (ng/mL)	$C_{max}/Dose$ (ng•kg/mg•mL)	$AUC_{0-96 hr}$ (ng•hr/mL)	$AUC_{0-96 hr}/Dose$ (ng•hr•kg/mg•mL)
Epidural	Bupivacaine HCl solution (15 mg)	1.00±0.00	6.52±1.95	271±126	178±74.2	1350±767	838±458
	SKY0402 (45 mg)	8.67±11.9	41.6±55.9	319±504	73.4±124	4290±522	885±62.4
	SKY0402 (45 mg) +Xylocaine	1.17±0.41	20.4±22.0	759±1060	155±213	5500±1120	1150±176

### Intrathecal dosing

**GLP study 947-020** - The purpose of this study in male beagle dogs was to evaluate potential local and systemic toxicity of the test articles after a single intrathecal administration under surgical conditions. Four treatment groups of 6 males each were administered either SKY0402 Placebo (02BUP14-185), SKY0402 (lot PP04-048), Bupivacaine HCl (Sensorcaine®), or the negative control of 0.9% Sodium Chloride. Three animals/group were necropsied on days 4 and 22 with TK being evaluated on day 1 from blood taken from the day 22 necropsy animals.

Treatment Groups for a Single Intrathecal Dose Study of Male Dogs (b) (4) 947-020					
Treatment Group	Dose Bupivacaine		Number of Animals		
	mg <sup>a</sup>	mL	Day 1	Necropsy (Day 4)	Necropsy (Day 22)
Saline	0	3	6	3	3
SKY0402 Placebo	0	3	6	3	3
SKY0402	45	3	6	3	3
Sensorcaine	15	3	6	3	3

a – bupivacaine HCl or its equivalent

A comprehensive battery of observations were conducted for this GLP study that included daily observations for mortality, morbidity, injury, clinical signs, the availability of food and water, body weights, and food consumption. Blood and urine samples for clinical pathology evaluations were collected pretest and on Days 4 and 22. At each termination on Days 4 and 22, necropsy examinations of the main study animals were performed, organ weights were recorded, and selected tissues, including the spinal cord, were microscopically examined. Sections of the spinal cords were embedded, sectioned, and stained using amino cupric silver stain along with a parallel set of H&E stained slides.

The following list constitutes the full complement of organs and tissues for this study:

- |   |  |
|---|--|
| - Adrenal (2)*  | - Heart  |
| - Aorta   | - Injection site [1 site collected]                    |
| - Bone with marrow [femur]                              | - Kidney (2)*  |
| - Bone with marrow [rib]                                | - Liver [3 sections collected; 2 examined]*            |
| - Bone with marrow [sternum]                            | - Lung [2 sections examined]*                          |
| - Bone marrow smear [2 collected]                       | - Lymph node, mandibular [2 collected; 1 examined]     |
| - Brain [cerebrum, midbrain, cerebellum, medulla/pons]* | - Lymph node, mesenteric                               |
| - Epididymis (2)  | - Nictitans gland (2)                                  |
| - Eye including optic nerve (2)                         | - Pancreas   |
| - Gallbladder   | - Pituitary  |
| - Gastrointestinal tract:                               | - Prostate   |
| esophagus   | - Salivary gland, mandibular [2 collected; 1 examined] |
| stomach [cardia, fundus, and pylorus]                   | - Sciatic nerve  |
| duodenum  | - Skeletal muscle, biceps femoris                      |
| jejunum   | - Skin   |
| ileum   | - Spinal cord [cervical, thoracic, and lumbar]         |
| cecum   | - Spleen   |
| colon   | - Thymus   |
| rectum  | - Thyroid/parathyroid (2)                              |
| - Gonads:   | - Tongue   |
| testis (2)  | - Trachea  |
| - Gross lesions   | - Urinary bladder                                      |

\* Organ weighed  
(2) Paired organ

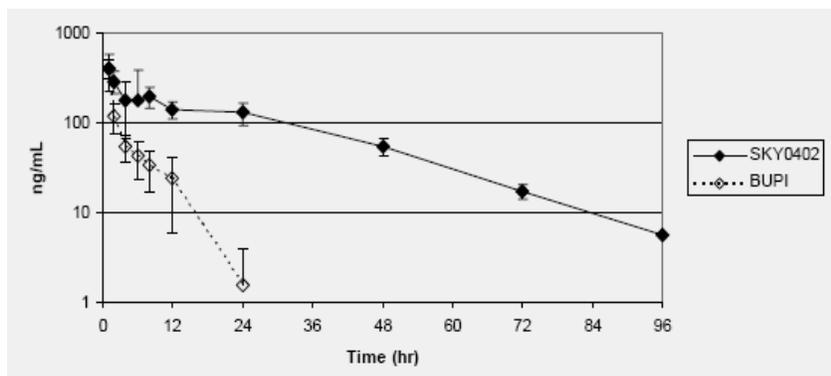
No treatment related mortality occurred. As to clinical signs, impaired limb function was observed on the day of dosing in 4 of 6 dogs dosed with Sensorcaine and 5 of 6 dogs dosed with the SKY0402/Xylocaine combination. The impairment was not observed on day 2. There was no associated histopathology with this clinical sign. No clear treatment related effects occurred, but the mean activated partial thromboplastin time (APTT) was prolonged on Day 4 (5%) and Day 22 (27%) for the three SKY0402/Xylocaine dogs but not SKY0402 dogs. Lymphocytes increased 16% relative to pretest on Day 22 in animals receiving Sensorcaine. These increases were reported to be within expected ranges. APTT in animals receiving Sensorcaine was considered treatment related by the sponsor. The increased lymphocytes in those receiving Sensorcaine may have been incidental, but a mild antigenic response was not ruled out by the sponsor.

Because of the severity of the impaired limb function seen in the one SKY0402 dog, additional exams were conducted on this animal prior to necropsy. The physical examination found this animal number had absent conscious proprioception and hopping reflex in both hind limbs; decreased panniculus reflex (intact to the mid-lumbar region, anterior to the incision, but absent caudally); and decreased superficial pain response (deep pain response was intact.) The complement of the neurologic exam was within normal limits. At the time of the exam, just prior to necropsy, this dog was able to support weight on the hind limbs in a crouched position. This was a significant improvement from the prior day when he was incapable of standing upright or supporting any weight on either hind limb. His condition at this time could be characterized as a bilateral hind limb paresis with upper motor neuron deficits. A myelogram and Faxitron imaging were performed, neither diagnostic demonstrated an etiology. This SKY0402 group dog otherwise appeared to be in good health at that time. Microscopically, hemorrhage and degeneration in sections of the thoracic and lumbar cord cranial to the injection site were seen in this dog. Therefore, the clinical signs and microscopic changes at these sites may also have been a result of vascular injury during the injection as noted previously regarding impairment in control/placebo groups.

The mean activated partial thromboplastin time (APTT) was prolonged on Days 4 (5%) and 22 (26%) in Sensorcaine dogs. Values were reported to be within expected ranges. Lymphocytes were increased 33% relative to pretest on Day 22 in animals receiving Sensorcaine. The lymphocyte counts were reported to be within expected ranges. The prolongation of APTT in animals receiving Sensorcaine was considered treatment related. The increased lymphocytes in those receiving Sensorcaine may have been incidental, but a mild antigenic response was not ruled out by the sponsor.

No treatment related effects were observed for food consumption, body weights, other clinical pathology, gross necropsy, organ weights or microscopic evaluation. All spinal cord microscopic observations were within normal limits with no test article-related effects except for 1 male dosed with SKY0402 and observed on day 4 post dosing. For this male, hemorrhage and degeneration were observed in sections of the thoracic and lumbar cord cranial to the injection site. Changes appeared to be the result of vascular injury during the injection and were not observed in any other males on day 4 or 22.

In TK evaluations, bupivacaine concentrations were generally measurable out to 96 hours in animals given SKY0402, but generally were below the limit of quantitation after 48 hours in animals given the bupivacaine HCl solution. As expected, exposure to bupivacaine over the first 24 hours was significantly lower after SKY0402 than after bupivacaine HCl solution.



$C_{max}/dose$  for bupivacaine was ~3-fold lower for SKY0402 than for bupivacaine HCl (Sensorcaine) while  $AUC_{0-96}$  and  $AUC_{0-96}/dose$  were ~10-fold and ~3-fold higher, respectively, for SKY0402 compared to bupivacaine HCl.

Summary of Pharmacokinetic Parameters (mean and standard deviation) After an Intrathecal Injection of SKY0402 (45 mg) or Bupivacaine HCl solution (15 mg) in Male Beagle Dogs							
Route	Treatment	$t_{max}$ (hr)	$t_{1/2}$ (hr)	$C_{max}$ (ng/mL)	$C_{max}/Dose$ (ng·kg/mg·mL)	$AUC_{0-96}$ (hr·ng/mL)	$AUC_{0-96}/Dose$ (ng·hr·kg/mg·mL)
Intrathecal	Bupivacaine HCl solution (15 mg)	1.00±0.00	3.25±0.96	404±98.3	256±35.6	876±182	510±62.5
	SKY0402 (45 mg)	2.17±2.86	14.2±4.6 <sup>a</sup>	442±251	84.3±41.7 <sup>a</sup>	8410±2920	1680±466 <sup>a</sup>

<sup>a</sup>Significantly different from corresponding value for bupivacaine HCl solution, Student's t test  $p < 0.05$

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**Study title: Acute Toxicity/Wound Healing Study of SKY0402 in a Surgical Model in Dogs**

Reviewer: Dr. Adam Wasserman  
Study no.: 947-029  
Study report location: Pacira Pharmaceuticals, Inc.  
10450 Science Center Drive  
San Diego, CA 92121  
Conducting laboratory and location: (b) (4)  
Date of study initiation: Initiated: June 14, 2005  
Completed April 3, 2006;  
Final Report June 21, 2006  
Amendment (1): May 10, 2006  
Amendment (2): June 21, 2006  
Amendment (3): April 17, 2008  
*(note: Amendments pertained to change in Sponsor as well as the change in high dose level (to 25 mg/kg actually administered from 30 mg/kg intended), and correction of SKY0402 lot used on study.*  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: SKY0402 25 mg/mL, Lot No. 05-2501, manufacture date 5/3/2005. Total bupivacaine 23.9 mg/mL (free 2.2 mg/mL) – 96% nominal. Expiration date 5/2006.  
  
SKY0402 15.1 mg/mL, Lot No. 04-2502 manufacture date 10/8/2004. Total bupivacaine 15.1 mg/mL (free 3.3%) – 101% nominal  
  
Sensorcaine, 7.5 mg/mL (0.75% bupivacaine HCl solution), Lot No. LB2233  
  
0.9% Sodium Chloride Injection, USP

**Key Study Findings**

- Four dogs/sex were received injections of saline, SKY0402 (0, 9, 18 or 25 mg/kg), or reference drug Sensorcaine (9 mg/kg) in two separate phases to the study:  
Phase 1 - subcutaneous (SC) infiltration into incisional site in a hernia repair

model (including prolene mesh)

Phase 2 - perineural injection in a nerve block model

- In both phases two (2) dogs/sex/group were sacrificed on study day 3 (SD3 - acute sacrifice) and SD15 (delayed sacrifice) and subjected to full post-mortem necropsy. SKY0402 bupivacaine concentrations were 15 mg/mL or 25 mg/mL, the latter used to for the highest dose group. The Sensorcaine concentration was 7.5 mg/mL bupivacaine HCl.

*Phase 1 (Wound infiltration) Findings:*

- No apparent treatment-related effects on clinical signs, body weight, food consumption, or clinical pathology.
- Toxicity findings limited to effects noted on a wound healing evaluation on SD2-3 (total Hernia Repair Score) and microscopic findings at the surgical site taken at sacrifice on SD3 and SD15:
  - Total Hernia Repair score generally indicated higher group values (indicating more findings) with SKY0402 in a dose-dependent manner compared to saline control though was generally similar to Sensorcaine control. Observations were almost exclusively erythema and edema, the latter slight in severity and only in SKY0402 25 mg/kg animals. Wound bed appeared normal in all animals and no odor detected.
  - Surgical site findings on SD3 necropsy revealed no clear effects of SKY0402 compared with saline or Sensorcaine controls. All treatments had animals with myofiber degeneration/regeneration and mineralization, hemorrhage, acute inflammation, edema, and abscesses noted around sutures. Minimal to mild granulomatous inflammation was noted in 9 and 18 mg/kg SKY0402 animals but not in 25 mg/kg SKY0402 animals and was not observed with either saline or Sensorcaine control. This was not associated with sutures per report (unlike at SD15 necropsy) so this could possibly represent a specific test article effect though the reviewer noted the lack of dose-dependence for the response.
  - Surgical site findings on SD15 revealed granulomatous inflammation, generally of mild severity around sutures in all animals as would be expected for a foreign body response. Slightly elevated fibrosis severity (mild to moderate) was noted in SKY0402 18 mg/kg and 25 mg/kg dogs compared with dogs in saline and Sensorcaine groups (mild).
- Toxicokinetic evaluation of bupivacaine plasma concentrations with wound infiltration administration of SKY0402 or Sensorcaine revealed  $C_{max}$  was attained at the first blood sampling (1 hr). Group  $C_{max}$  was similar between all SKY0402 dose groups and was slightly more than half that of Sensorcaine at a (generally) lower dose of 9 mg/kg. Dose-adjusted  $C_{max}$  therefore was reduced with increasing doses of SKY0402.  $AUC_{0-96hr}$  was, however, double Sensorcaine at the equivalent 9 mg/kg dose and rose sub-proportionally as SKY0402 dose was increased. Apparent half-life ( $t_{1/2}$ ) was extended in

SKY0402 groups (21-36 hr) compared with Sensorcaine (5 hr). Individual animal data reveals a highly variable exposure to bupivacaine in SKY0402 groups despite the group data, however. Sensorcaine  $C_{max}$  ranged from 562-1450 ng/mL while the equivalent 9 mg/kg dose of SKY0402 produced exposures in dogs that ranged from 185-226 ng/mL except in a single animal in which the very high level of 1230 ng/mL was reached.

*Phase 2 (Perineural injection) Findings:*

- No apparent treatment-related effects on clinical signs, body weight, food consumption, or clinical pathology.
- Toxicity findings related to SKY0402 appeared limited to alterations in tissues around the brachial plexus including subacute minimal to mild granulomatous inflammation of the adipose tissue around nerve roots on SD3 which were still noted in some animals at SD15. All nerve sections were considered normal on histologic evaluation. Hemorrhage was noted in all treatment groups and Sensorcaine animals had an additional finding of skeletal muscle and myofiber degeneration and regeneration which resolved prior to SD15.
- Toxicokinetic evaluation of bupivacaine plasma concentrations with perineural administration in a nerve block model revealed dose-related increases in plasma bupivacaine when comparing SKY0402 groups to Sensorcaine though the highest SKY0402 dose tested, 25 mg/kg, did not produce plasma exposures greater than the 9 mg/kg dose of Sensorcaine.  $AUC_{0-96hr}$  exposure was significantly increased with SKY0402 dose and apparent  $t_{1/2}$  extended compared with the reference Sensorcaine group. Individual animal data revealed highly variable plasma exposures within groups with wide ranges in SKY0402 9 mg/kg (104 – 1170 ng/mL), 18 mg/kg (181 – 1790 ng/mL), and 25 mg/kg (249 – 1630 ng/ml) groups compared with Sensorcaine (1340 – 1650 ng/mL). With the 25 mg/kg SKY0402 group there appeared to be a mildly biphasic release of drug, acutely and around 12 hr.

## Methods

Doses (Phase 1 &amp; 2):

Treatment (Day 1)	No. M/F	Concentration <sup>a</sup> mg/mL	Dose	
			mL/kg	mg/kg <sup>a</sup>
Saline	4/4	0	1.2	0
SKY0402	4/4	15	0.6	9
SKY0402	4/4	15	1.2	18
SKY0402 <sup>b</sup>	4/4	25	1.0 <sup>b</sup>	25 <sup>b</sup>
Sensorcaine <sup>®</sup>	4/4	7.5	1.2	9

a) Bupivacaine

b) Animals in these treatment groups were administered 1.0 mL of SKY0402 rather than the intended 1.2 mL, therefore the 30 mg/kg dose intended was, in fact, 25 mg/kg.

Dose justification: based on published intravenous (IV) lethality of 5-11 mg/kg for bupivacaine and lethality seen in a rabbit study (947-004) the maximum total non-lethal bupivacaine dose was considered by Sponsor to be 9 mg/kg (or 1.2 mL/kg) of Sensorcaine. The highest SKY0402 dose level selected was designed to test the same volume of the highest formulated concentration while the mid-dose was selected to deliver an equal volume at a lower dose and the lowest dose designed to deliver an equivalent bupivacaine dose as reference control.

Frequency of dosing: Once

Route of administration: SC infiltrative (Phase 1)  
Perineural (Phase 2)

Dose volume: See above table

Formulation/Vehicle: Saline (control) is in sterilized water for injection (SFWI), test article is formulated in SFWI

Species/Strain: Beagle dog (b) (4)

Number/Sex/Group: 4/sex/group

Age: 5-6 months

Weight: Males 6.8-9.7 kg; Females 6.2-7.9 kg at randomization

Satellite groups: None, two sets of necropsies with TK obtained

Unique study  
design:

Group	Treatment (Day 1)	Number of animals (M/F)	Concentration <sup>a</sup> (mg/mL)	Dose		Number of Males/Females	
				mL/kg	mg/kg	Day 3	Day 15
1	Saline Infiltration	4/4	0	1.2	0	2/2	2/2
2	SKY0402 Infiltration	4/4	15	0.6	9	2/2	2/2
3	SKY0402 Infiltration	4/4	15	1.2	18	2/2	2/2
4	SKY0402 Infiltration	4/4	25	1.0 <sup>b</sup>	25 <sup>b</sup>	2/2	2/2
5	Sensorcaine <sup>®</sup> Infiltration	4/4	7.5	1.2	9	2/2	2/2
6	Saline Nerve Block	4/4	0	1.2	0	2/2	2/2
7	SKY0402 Nerve Block	4/4	15	0.6	9	2/2	2/2
8	SKY0402 Nerve Block	4/4	15	1.2	18	2/2	2/2
9	SKY0402 Nerve Block	4/4	25	1.0 <sup>b</sup>	25 <sup>b</sup>	2/2	2/2
10	Sensorcaine <sup>®</sup> Nerve Block	4/4	7.5	1.2	9	2/2	2/2

Note: <sup>a</sup> Bupivacaine concentration; <sup>b</sup> The actual dose is 25 mg/kg (1.0 mL/kg) due to a technical oversight

Two phases to the study each with different sets of animals:

#### Infiltration phase

Anesthesia induced and surgery performed with aseptic technique with hair clipped and site sterilized. An indwelling catheter was inserted into the vein.

Scheduled Medications and Doses		
Drug	Interval, Dose, and Route	
	Surgery (Day 1)	Postsurgery <sup>a</sup>
Atropine sulfate	0.05 mg/kg SC	-
Propofol	6 mg/kg IV to effect	-
Isoflurane	To effect, inhalation intubated	-
Buprenorphine	0.01 mg/kg SC BID	0.01 mg/kg SC BID x 3 days
Cefazolin	20 mg/kg IV prior to surgery	-
Cefazolin	20 mg/kg IM after surgery	-
Cephalexin	-	250 mg PO BID x 3 days
Lactated Ringers Solution	100 mL/hr IV during surgery	-

<sup>a</sup>Postoperative medications were administered for the duration indicated, or until necropsy.  
SC = subcutaneous injection IV = intravenous injection IM = intramuscular injection  
BID = twice daily PO = orally

A blunt dissection into the inguinal canal all the way into the peritoneal cavity was performed. The hernia was then closed by layered closure of the fascia of the muscles plus the inguinal ligament using 3-O PDS II sutures. A Prolene™ mesh (2 x 3 cm) was incorporated into the closure and sutured in place using 4-O prolene between muscle layers to strengthen the incision site. Test article, Sensorcaine or saline was injected in the muscle fascia around the mesh in 4 locations and the remaining fascia and

subcutaneous tissues closed with 3-0 vicryl. Test article, Sensorcaine, or saline was given as 4 additional injections around the mesh and the skin was closed with staples. Animals were followed post-surgery and Elizabethan collars utilized to prevent disruption of the surgical incision.

#### *Peripheral Nerve Block*

Anesthesia (Isoflurane/NO<sub>2</sub> followed by Isoflurane) was administered and a brachial plexus block made in left thoracic limb using a 22 gauge 3.5 inch needle. SKY0402, Sensorcaine or saline was administered slowly as the needle was withdrawn from the site. Following the procedure animals were monitored for excessive bleeding from the injection site.

Deviation from study protocol: Inaccurate administration of the highest intended dose level (equivalent to 1.2 mL/kg or 30 mg/kg) resulted in a smaller volume and dose in the high group (1.0 mL/kg and 25 mg/kg). The reviewer concurs with the Study Director that this does not compromise the integrity of the study or impact conclusions.

## **Observations and Results**

### **Mortality**

Observed twice daily throughout study duration.

There was no treatment-related mortality observed on study in either wound infiltration or peripheral nerve block phase.

### **Clinical Signs**

Detailed clinical examination conducted prior to randomization and then daily thereafter.

No apparent treatment-related clinical signs were noted during the Wound Infiltration phase. General findings in all groups including saline and reference control Sensorcaine included (over various days of the study): red discoloration and swelling at the inguinal region or incision site, injected sclera and lacrimation, soft/mucoid feces. One 9 mg/kg female (animal #116) was noted to have an umbilical hernia. The relationship to treatment is not clear as no incision was made at this site.

No apparent treatment-related clinical signs were noted during the Peripheral Nerve Block phase per study report. Most animals were considered to have no abnormalities throughout the study. It is noted, however, that impaired limb function was observed in 4/8 18 mg/kg SKY0402 dogs (left forelimb), 3/8 25 mg/kg SKY0402 dogs, and 8/8 9 mg/kg Sensorcaine animals. All animals with impaired left forelimbs in the Sensorcaine group were noted with this behavior only on SD1 while animals in the SKY0402 group had evidence of limb impairment through SD3 in several cases. Note that some animals

were sacrificed per protocol on SD3, however animals with limb impairment remained on study past SD3.

### **Body Weights**

Recorded prior to randomization and daily during the study.

No treatment-related effect on body was observed in either wound infiltration or peripheral nerve block phase.

### **Feed Consumption**

Recorded daily during study.

No treatment-related effects were observed in either wound infiltration or peripheral nerve block phase.

### **Ophthalmoscopy**

Not conducted

### **ECG**

Not conducted

### **Clinical Pathology**

All blood samples collected after an overnight fast from the jugular vein and placed into EDTA- or citrate-containing tubes for evaluation of general and coagulation parameters, respectively.

### **Hematology**

Leukocyte count, erythrocyte count, hemoglobin, hematocrit, MCV, MCH, MCHC, Platelets, Differential Leukocyte Count, Absolute Reticulocytes, Percent Reticulocytes, APTT, and PT were determined.

### **Clinical Chemistry**

Sodium, Potassium, Chloride, Calcium, Phosphorus, Alkaline Phosphatase, Total Bilirubin, Gamma Glutamyltransferase (GGT), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Sorbitol Dehydrogenase (SDH), Urea Nitrogen, Creatinine, Total Protein, Albumin, Globulin, A/G Ratio, Cholesterol, Glucose

There were no apparent treatment-related effects on any hematologic or clinical chemistry parameters.

### Urinalysis

Not conducted

### Post-Mortem Findings

Animals were euthanized by an overdose of sodium pentobarbital followed by exsanguination and full and standard necropsy examination with examination of all cavities and organs and tissues designated for histologic examination placed in fixative (neutral buffered formalin for all tissues but eye/optic nerve and testes which were immersed in modified Davidson's fixative).

### Organ Weights

Paired organs weighed together except (right) mandibular salivary gland and thyroid and parathyroid glands were weighed together.

There were no apparent treatment-related effects on organ weight

### Macroscopic Findings

See Histopathology table

### Histopathology

Adequate Battery: Yes

Peer Review: No

Histological Findings: Note, no apparent treatment-related observations except at surgical site.

### *Wound Infiltration Results (reviewer tables)*

#### **Surgical Site Findings, group incidence (males & females combined) – Study Day 3**

Microscopic Finding	Severity	Saline	SKY0402 (mg/kg)			Sensor-caine (mg/kg)	Comments
			9	18	25	9	
Degeneration/Regeneration, Myofiber	Min	1	1	0	1	1	
	Mild	0	1	2	0	0	
	Mod	0	0	1	0	0	
Hemorrhage	Min	0	2	1	0	0	
	Mild	4	2	3	4	4	
Inflammation, acute	Min	1	0	0	0	1	

	Mild	2	0	1	3	0	
Edema	Min	0	1	0	0	0	
	Mild	1	0	0	0	1	
Abscesses, multiple	Min	1	1	1	0	3	Noted around sutures
	Mild	0	0	0	1	0	
Mineralization, myofiber	Min	1	0	2	0	0	
Inflammation, granulomatous	Min	0	12	1	0	0	
	Mild	0	1	1	0	0	

**Surgical Site Findings, group incidence (males & females combined) – Study Day 15**

Microscopic Finding	Severity	Saline	SKY0402 (mg/kg)			Sensor-caine (mg/kg)	Comments
			9	18	25	9	
Degeneration/Regeneration, Myofiber	Min	1	3	2	2	2	
	Mild	0	0	1	1	2	
	Mod	1	0	0	0	0	
Hemorrhage	Min	0	0	1	2	0	
	Mild	0	0	0	0	0	
Inflammation, acute	Min	0	0	0	0	0	
	Mild	0	0	0	0	0	
Inflammation, granulomatous	Min	0	0	0	0	0	Present around sutures
	Mild	4	4	4	3	4	
	Mod	0	0	0	1	0	
Abscesses, multiple	Min	0	0	0	0	0	
	Mild	0	0	0	1	0	
Mineralization, myofiber	Min	1	0	0	1	2	
Fibrosis	Min	0	1	1	0	0	
	Mild	3	2	2	3	4	
	Mod	0	0	1	1	0	

**Individual Animal Findings**

Group	Animal #	Macroscopic Observations	Microscopic Observations
<b>SD3 Evaluation</b>			
0 mg/kg (saline)	101	WNL	- Edema, mild - Hemorrhage, mild - Inflammation, acute, mild
	102	Red fluid, mild (approximately 3 mL)	- Hemorrhage, mild - Inflammation, acute, minimal - Mineralization, myofiber, minimal
	105	Incision site discoloration, red, mild Surgical site,	- Degeneration/regeneration, myofiber, minimal - Hemorrhage, mild (corresponds to macroscopic observation) - Inflammation, acute, mild

			<ul style="list-style-type: none"> <li>- hemorrhage, minimal</li> <li>- inflammation, granulomatous, mild (suture material present)</li> </ul>
9 mg/kg Sensorcaine	135	WNL	<ul style="list-style-type: none"> <li>- degeneration/regeneration, myofiber, mild</li> <li>- fibrosis, mild</li> <li>- inflammation, granulomatous, mild (suture material present)</li> <li>- mineralization, myofiber, minimal</li> </ul>
	136	WNL	<ul style="list-style-type: none"> <li>- degeneration/regeneration, myofiber, minimal</li> <li>- fibrosis, mild</li> <li>- inflammation, granulomatous, mild (suture material present)</li> </ul>
	139	WNL	<ul style="list-style-type: none"> <li>- degeneration/regeneration, myofiber, mild</li> <li>- fibrosis, mild</li> <li>- inflammation, granulomatous, mild (suture material present)</li> <li>- mineralization, myofiber, minimal</li> </ul>
	140	WNL	<ul style="list-style-type: none"> <li>- degeneration/regeneration, myofiber, minimal</li> <li>- fibrosis, mild</li> <li>- inflammation, granulomatous, mild (suture material present)</li> </ul>

**Peripheral Nerve Block Results**

<b>Microscopic Observations</b>
<b>SD3 &amp; SD15 Evaluation Summary</b>
<p>Saline demonstrated no treatment-related microscopic abnormalities at the brachial plexus injection site on SD3 examination though by SD15 examination minimal hemorrhage was occasionally noted. SKY0402 groups were observed to have generally minimal hemorrhage and subacute minimal to mild granulomatous inflammation of the adipose tissue around nerve roots on SD3 which in some cases was still observed on SD15 evaluation. Sensorcaine-treated animals had occasional evidence of minimal hemorrhage of the skeletal muscle and myofiber degeneration/regeneration on SD3 but by SD15 evaluation occasional findings of minimal hemorrhage was observed. All nerve sections were within normal limits.</p>

**Special Evaluation**

**Wound Healing (Phase 1 only)**

Wound healing was determined beginning on Study Day 2 (SD2) with macroscopic examination of the hernia repair site using the following scoring system:

<b>Wound Healing Scoring System for Hernia Repair</b>			
<b>Inflammation</b>	<b>Edema:</b>	<b>Erythema:</b>	<b>Induration:</b>
0 = none	0 = none	0 = none	0 = no
1 = slight	1 = slight	1 = slight	1 = yes
2 = moderate	2 = moderate	2 = moderate	
3 = severe	3 = severe	3 = severe	
<b>Exudate:</b>	<b>Wound Bed:</b>	<b>Odor:</b>	
0 = none	0 = dry	0 = no	
1 = slight	1 = dry/moist	1 = yes	
2 = moderate	2 = moist/dry		
3 = severe	3 = moist		

Scores for inflammation, erythema, induration and exudate were added together for a total possible individual score of 10. The total hernia group score calculated by this reviewer was therefore maximally 40. It was not stated why other scoring categories (such as edema) were not included.

No test article-related findings were noted on wound healing according to the scoring method used per study report though there appears to be an effect of treatment though it does not appreciably differ from the reference drug Sensorcaine. The highest value obtained was 4 in one 25 mg/kg SKY0402 group animal. Saline-treated animals only showed the lowest level of erythema (slight) at the site. For SKY0402, the 9 mg/kg group demonstrated erythema and, in one animal, induration. Induration was not reported in 18 mg/kg animals but slightly greater erythema (moderate) was noted in two animals while in the 25 mg/kg group animals in addition to slight erythema all had evidence of slight edema. Sensorcaine administration produced similar signs as SKY0402 except erythema was more intense in at least one animal, reaching severe on SD2 and becoming moderate on SD3). Generally signs remitted by SD5 with the exception of an 18 mg/kg female (#123, resolving SD11) and the 25 mg/kg animal (male #127) reporting the highest score (4) which peaked on SD5 and maintained a score dwindling to 1 (slight erythema) by SD14, the last day of evaluation. No wound bed findings or reports of odor were noted in any group. See reviewer's table below:

**Total Group Hernia Repair Score\***

Group	Males		Females	
	SD2	SD3	SD2	SD3
Saline	2	2	2	2
SKY0402				
9 mg/kg	2	3	2	3
18 mg/kg	6	5	3	3
25 mg/kg	5	5	7	7
Sensorcaine	5	4	4	5

\*Scored for inflammation, erythema, induration and exudate.  
Note max group score is 40

## Toxicokinetics

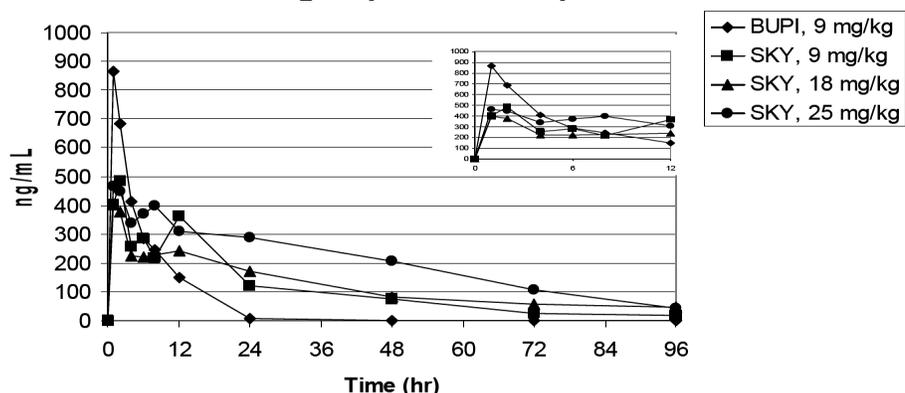
Blood samples (0.5-1.0 mL) obtained from animals destined for SD15 necropsy for evaluation of plasma bupivacaine concentrations. Animals were not fasted except when this was required for collection of clinical pathology samples or for surgery. Two collection locations used: indwelling catheter at pre-dose and 1 hr time-points, from the jugular vein at 2, 4, 6, 8, 12, 24, 48, 72, and 96 hrs post injection. Plasma was stored at -70°C until analyzed. PK modeling and interpretation of results was conducted by (b) (4) PK parameters were calculated by non-compartmental module of WinNonlin™ and was derived from mean concentration-time plasma data.

From the Toxicokinetic Report Summary:

- Following a single administration of Sensorcaine, the maximum plasma concentrations of bupivacaine were generally attained at the first sampling time, i.e., 1 hour post-dose. In contrast, the individual t<sub>max</sub> estimates for SKY0402 were variable, ranging from 1 hour to 48 hours after administration by any given route.
- The half-life appeared to be longer after SKY0402 versus Sensorcaine, but the difference was statistically significant only when the dose was given by SC infiltration.
- At the same given dose, the C<sub>max</sub> values for SKY0402 were on average, more than two-fold lower than Sensorcaine (536 vs. 931 ng/mL and 654 vs. 1490 ng/mL) after SC infiltration or nerve block, respectively.
- The plasma AUC<sub>0-96hrs</sub> were similar, i.e., 6820 vs. 5360hr\*ng/mL, and 7460 vs. 6100hr\*ng/mL after SC infiltration or nerve block, respectively, in comparison with equal doses of SKY0402 or Sensorcaine®.
- The C<sub>max</sub> was, on average, more than two-fold lower (504 vs. 931 ng/mL, and 715 vs. 1500 ng/mL) when SKY0402 was given at the limits of solubility (30 mg, 25 mg/mL) by SC infiltration or nerve block, respectively. Similar findings were reported in the rabbit (307 vs. 620 ng/mL, and 205 vs. 433 ng/mL, respectively).
- Overall, the extent of systemic exposure (C<sub>max</sub> and/or AUC<sub>0-96 hrs</sub>) was near dose proportional. There were no marked differences between male and female dogs in plasma concentrations of bupivacaine.
- The C<sub>max</sub>/Dose was similar across dose levels and between sexes, indicating dose proportionality with no apparent saturating levels, and less variability in females (as noted previously in rabbits). Also, AUC<sub>0-96hrs</sub>/Dose did not vary substantially across dose levels, and between sexes.

Group data indicates 9 mg/kg Sensorcaine administration produces plasma levels well in excess of 9, 18, and 25 mg/kg SKY0402 levels while the duration of exposure to bupivacaine is extended in SKY0402 treatment groups in a somewhat dose-dependent manner.

**Wound infiltration/SC group Plasma Bupivacaine Concentrations**



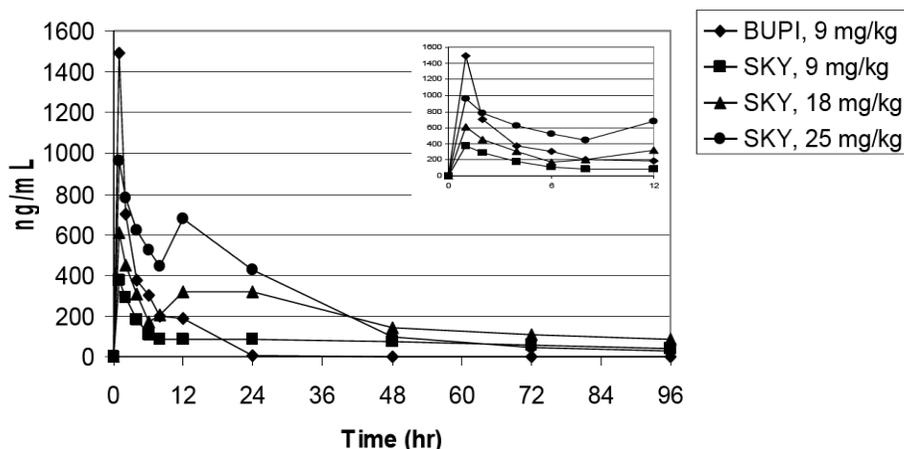
Note however, that individual animal data reveals highly variable exposure with SKY0402 treatment. The male dog #111 at 9 mg/kg demonstrated a  $C_{max}$  (1230 ng/mL) which was far greater than other animals at this dose level (185-504 ng/mL) or higher SKY0402 dose groups and appeared similar to Sensorcaine 9 mg/kg  $C_{max}$  levels. Generally exposure to bupivacaine in SKY0402 groups was not strongly dose-dependent.

Treatment	Animal #	Sex	Dose (mg/kg)	$t_{max}$ (hr)	$t_{1/2}$ (hr)	$C_{max}$ (ng/mL)	$C_{max}/Dose$ (ng/mL·mg/kg)	$AUC_{0-96hrs}$ (hr·ng/mL)	$AUC_{0-96hrs}/Dose$ (hr·ng/mL·mg/kg)
Bupivacaine HCl Infiltration	135	M	9	1.00	3.80	1450	161	7860	873
	136	M	9	2.00	4.98	691	76.8	6540	727
	139	F	9	1.00	7.18	1020	113	4210	468
	140	F	9	1.00	4.65	562	62.4	2850	317
	Mean±STD			1.25±0.500	5.15±1.44	931±396	103±44.0	5360±2260	596±251
SKY0402 Infiltration	111	M	9	2.00	19.4	1230	137	21800	2430
	112	M	9	2.00	23.1	226	25.1	6540	727
	115	F	9	1.00	26.9	504	56.0	9220	1020
	116	F	9	2.00	19.3	185	20.6	4700	522
	Mean±STD			1.75±0.500	22.2±3.61 <sup>b</sup>	536±484	59.6±53.7 <sup>a</sup>	10600±7730	1180±859
	119	M	18	2.00	32.1	701	38.9	11200	620
	120	M	18	1.00	35.8	438	24.3	14800	824
	123	F	18	1.00	31.6	309	17.2	8710	484
	124	F	18	1.00	45.2	292	16.2	10900	605
	Mean±STD			1.25±0.500	36.2±6.33	435±189	24.2±10.5 <sup>a</sup>	11400±2530	633±141
	127	M	25	2.00	18.0	765	30.6	28400	1140
	128	M	25	1.00	25.2	483	19.3	24300	972
	131	F	25	1.00	19.2	476	19.0	14900	596
132	F	25	8.00	21.3	292	11.7	10500	420	
Mean±STD			3.00±3.37	20.9±3.16	504±195	20.2±7.81 <sup>a</sup>	19500±8280	780±331	

<sup>a</sup> p<0.05 vs. bupivacaine HCl solution by same route; <sup>b</sup> p<0.05 vs. bupivacaine HCl solution by same dose

In the peripheral nerve block condition, group plasma bupivacaine exposures appear to be dose-dependent with significant plasma levels out to 24 -48 hours (see Sponsor's graph, below).

### Peripheral Nerve Block Plasma Bupivacaine Concentrations



However, individual animal plasma levels varied considerably – even the 9 mg/kg SKY0402 group contained an animal (#151) with levels (1170 ng/mL) nearly as high as 9 mg/kg Sensorcaine. 18 mg/kg and 25 mg/kg groups contained even more individual animals with levels that approximated the Sensorcaine plasma levels (1340-1650 ng/mL). In fact, the 25 mg/kg group is artificially low as a group mean due to a single animal (#172) having a very low C<sub>max</sub> (249 ng/mL) while other animals were high (1290-1630 ng/mL). See Sponsor’s table, below.

Treatment	Animal #	Sex	Dose (mg/kg)	t <sub>max</sub> (hr)	t <sub>1/2</sub> (hr)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose (ng/mL•mg/kg)	AUC <sub>0-96hrs</sub> (hr•ng/mL)	AUC <sub>0-96</sub> /Dose (hr•ng/mL•mg/kg)
Bupivacaine HCl Nerve Block	175	M	9	1.00	3.41	1650	183	5520	613
	176	M	9	1.00	4.63	1530	171	8130	903
	179	F	9	1.00	6.49	1340	150	6250	694
	180	F	9	1.00	9.17	1450	161	4510	502
	Mean±STD			1.00±0	5.92±2.51	1490±131	166±14.5	6100±1520	678±169
SKY0402 Nerve Block	151	M	9	1.00	25.8	1170	130	6390	710
	152	M	9	24.0	27.1	138	15.3	8430	936
	155	F	9	1.00	323	104	11.6	6190	687
	156	F	9	2.00	94.0	197	21.9	8860	984
	Mean±STD			7.00±11.3	117±141	402±513	44.7±57.0 <sup>a</sup>	7460±1370	829±153
	159	M	18	24.0	61.5	239	13.3	13600	754
	160	M	18	1.00	25.6	1790	99.4	18200	1010
	163	F	18	2.00	75.5	181	10.1	10800	602
	164	F	18	24.0	29.9	648	36.0	30400	1690
	Mean±STD			12.8±13.0	48.1±24.3	715±747	39.7±41.5 <sup>a</sup>	18200±8640	1010±480
167	M	25	1.00	7.08	1630	65.2	19000	760	
168	M	25	12.0	34.7	1340	53.6	42800	1710	
171	F	25	1.00	25.3	1290	51.6	15800	632	
172	F	25	12.0	21.6	249	9.96	12700	508	
Mean±STD			6.50±6.35	22.2±11.5	1130±604	45.1±24.2 <sup>a</sup>	22600±13700	902±549	

<sup>a</sup> p<0.05 vs. bupivacaine HCl solution by same route

Reviewer's graph of individual animals below:

**Dog Wound Healing/Hernia Repair Model**

(b) (4)



**Note:** each line represents a single animal

**Dosing Solution Analysis - Not conducted**

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**Study title: Acute Toxicity/Wound Healing Study of SKY0402 in a Surgical Model in Rabbits**

Reviewer: Dr Adam M. Wasserman  
Study no.: 947-030  
Study report location: SkyePharma Inc  
San Diego, CA 92121  
Conducting laboratory and location: (b) (4)  
Date of study initiation: Initiated May 24, 2005  
Completion June 15, 2006  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity: SKY0402 25 mg/mL, Lot No. 05-2501,  
manufacture date 5/3/2005. Total  
bupivacaine 23.9 mg/mL (free 2.2  
mg/mL) – 96% nominal. Expiration date  
5/2006.  
  
SKY0402 15.1 mg/mL, Lot No. 04-2502  
manufacture date 10/8/2004. Total  
bupivacaine 15.1 mg/mL (free 3.3%) –  
101% nominal  
  
Sensorcaine, 7.5 mg/mL (0.75%  
bupivacaine HCl solution), Lot No.  
LB2233  
  
0.9% Sodium Chloride Injection, USP

**Key Study Findings**

- Four rabbits/sex were received injections of saline, SKY0402 (9, 18 or 30 mg/kg), or reference drug Sensorcaine (9 mg/kg) in two separate phases to the study:
  - Phase 1* - infiltration into incisional site in a hernia repair model (including prolene mesh)
  - Phase 2* - perineural injection in a nerve block model
- In both phases two (2) rabbits/sex/group were sacrificed on study day 3 (SD3 - acute sacrifice) and SD15 (delayed sacrifice) and subjected to full post-mortem necropsy. SKY0402 bupivacaine concentrations were 15 mg/mL or 25 mg/mL, the latter used to produce the highest dose group. The Sensorcaine concentration was 7.5 mg/mL bupivacaine.

*Phase 1 (Wound infiltration) Findings:*

- No apparent treatment-related effects were observed on clinical signs, body weight, food consumption, or clinical pathology.
- Toxicity findings limited to effects noted on a wound healing evaluation on SD2-3 (total Hernia Repair Score) and microscopic findings at the surgical site taken at sacrifice on SD3 and SD15:
  - Total Hernia Repair score generally revealed a slight increase in score with SKY0402 usage compared to saline and Sensorcaine groups principally driven by a stronger erythema on SD2 which was largely resolved by SD3. No wound bed abnormalities or odor was noted in any animal of any group.
  - Surgical site findings on SD3 necropsy revealed generally similar findings between groups with evidence of degeneration and necrosis of myofibers and hemorrhage, both mild in severity. Fibrosis, mineralization, and chronic inflammation of minimal severity were apparent by SD15 without evidence of treatment group effect. SKY0402 animals appeared to develop granulomatous inflammation of minimal to mild severity by SD15 which was not observed in saline or Sensorcaine-injected rabbits which represents the only apparent effect attributable to SKY0402.
- Toxicokinetic evaluation of bupivacaine plasma concentrations with wound infiltration administration of SKY0402 or Sensorcaine revealed bupivacaine  $C_{max}$  does not generally increase with dose of SKY0402, remaining roughly one-third to half that of the 9 mg/kg Sensorcaine dose.  $AUC_{0-96hr}$  exposure is, however, dose-dependently increased with increasing SKY0402 dose along with apparent  $t_{1/2}$ . Of interest, the 9 mg/kg dose of SKY0402 was noted to have nearly double the AUC exposure of the 9 mg/kg dose of Sensorcaine for reasons which are not clear. Also notable is the smooth release profile through 12 hours at a low level with an apparent “dose-dump” release of bupivacaine in 3 animals in the SKY0402 groups with peaks occurring at the 24 or 48 hr time-point (this is even more apparent in the nerve block model). For these 3 animals, only one of the peaks (in a 9 mg/kg SKY0402 animal) approximated the  $C_{max}$  of the Sensorcaine group but release at this later time-point appeared to generate 2-fold to 4-fold the prior prevailing plasma bupivacaine concentration.

*Phase 2 (Perineural injection) Findings:*

- No apparent treatment-related effects on clinical signs, body weight, food consumption, or clinical pathology.
- Toxicity findings related to SKY0402 appeared limited to alterations in tissues around the brachial plexus including a non-dose-related incidence of granulomatous inflammation, generally minimal to mild in severity and hemorrhage on SD3 which by SD15 included some additional mineralization of the local tissues. This was not seen in saline-treated rabbits while in Sensorcaine-injected rabbits, minimal hemorrhage and acute inflammation was noted on SD3 with inflammation not being observed by SD15.

- Toxicokinetic evaluation of bupivacaine plasma concentrations with perineural administration in a nerve block model revealed dose-related increases in plasma bupivacaine when comparing SKY0402 groups to Sensorcaine though the highest SKY0402 dose tested, 25 mg/kg, did not produce plasma exposures greater than the 9 mg/kg dose of Sensorcaine.  $AUC_{0-96hr}$  exposure was significantly increased with SKY0402 dose and apparent  $t_{1/2}$  extended compared with the reference Sensorcaine group. A comparable dose of bupivacaine administered at 9 mg/kg produced AUC levels 1.6-fold greater in the SKY0402 group compared with the Sensorcaine group. Individual animal data revealed highly variable plasma exposures within the 18 mg/kg SKY0402 group with two of four animals demonstrating a significant spike in  $C_{max}$  suggestive of dose-dumping, one animal at 4 hr with a 10-fold higher plasma concentration than generally observed in non-dumping animals and another animal with a delayed release at 48 hours representing a 6-fold plasma increase over the previous observations to that time-point.

## Methods

## Doses:

Group Number	Group Assignments		
	Bupivacaine Dose Level	Number of Animals <sup>a</sup>	
		Male	Female
<u>Infiltration Phase</u>			
1	0 mg/kg (Saline)	4	4
2	9 mg/kg (SKY0402) <sup>b</sup>	4	4
3	18 mg/kg (SKY0402) <sup>b</sup>	4	4
4	30 mg/kg (SKY0402) <sup>c</sup>	4	4
5	9 mg/kg (Sensorcaine <sup>®</sup> ) <sup>d</sup>	4	4
<u>Nerve Block Phase</u>			
6	0 mg/kg (Saline)	4	4
7	9 mg/kg (SKY0402) <sup>b</sup>	4	4
8	18 mg/kg (SKY0402) <sup>b</sup>	4	4
9	30 mg/kg (SKY0402) <sup>c</sup>	4	4
10	9 mg/kg (Sensorcaine <sup>®</sup> ) <sup>d</sup>	4	4

<sup>a</sup>Two animals/sex/group were euthanized on Days 3 and 15, respectively.  
<sup>b</sup>For 9 and 18 mg/kg SKY0402, the bupivacaine concentration was 15 mg/mL.  
<sup>c</sup>For 30 mg/kg, the bupivacaine concentration was 25 mg/mL.  
<sup>d</sup>For 9 mg/kg (Sensorcaine<sup>®</sup>), the bupivacaine concentration was 7.5 mg/mL.

## Per Sponsor (verbatim):

The dose levels and volumes were selected in consultation with the Sponsor on the basis of available data from previous studies and discussed here. Based upon the published intravenous lethality of 5 to 11 mg/kg for bupivacaine and the lethality seen in rabbits in (b) (4) Study Number 947-004, the maximum total non-lethal bupivacaine dose is approximately 9 mg/kg or 1.2 mL/kg of Sensorcaine<sup>®</sup> (7.5 mg/mL). Both the highest volume delivered (1.2 mL/kg) and the highest dose administered (9 mg/kg) of Sensorcaine<sup>®</sup> were evaluated. For the equal volume groups, the volume of SKY0402 or saline to be injected was the same volume as the Sensorcaine<sup>®</sup> volume, 1.2 mL/kg. The mid dose level (18 mg/kg) was selected between the 9 and 30 mg/kg.

Frequency of dosing: Once

Route of administration: Infiltrative or Perineural

Dose volume: 1.2 mL/kg maximum

Formulation/Vehicle: Saline (control) is in sterilized water for injection (SWFI), test article is formulated in SWFI

Species/Strain: New Zealand White Hra (NZW) SPF Albino rabbits

(b) (4)

Number/Sex/Group: 4/sex/group

Age: 5.5 months

Weight: Males 2.6-3.0 kg; Females 2.8-3.7 kg

Satellite groups: None, two sets of necropsies with TK obtained

Unique study design: Two phases to the study each with different sets of animals:

*Infiltration phase*

Anesthesia induced and surgery performed with aseptic technique with hair clipped and site sterilized. An indwelling catheter was inserted into an appropriate vein.

Scheduled Medications and Doses		
Drug	Interval, Dose, and Route	
	Surgery (Day 1)	Postsurgery <sup>a</sup>
Atropine sulfate	0.2 mg/kg SC	-
Ketamine	30 mg/kg SC	-
Isoflurane	To effect, inhalation by mask	-
Buprenorphine	0.01 mg/kg SC BID	0.01 mg/kg SC BID x 3 days
Baytril	5 mg/kg SC SID	5 mg/kg SC SID x 3 days
Lactated Ringers Solution	50 mL/hr IV intravenous during surgery	-

<sup>a</sup>Postoperative medications were administered for the duration indicated, or until necropsy.  
IV = Intravenous SC = subcutaneous injection BID = twice daily SID = once daily

A blunt dissection into the inguinal canal all the way into the peritoneal cavity was performed. The hernia was then closed by layered closure of the fascia of the muscles plus the inguinal ligament using 3-0 PDS II sutures. A Prolene™ mesh (1 x 1 cm) was incorporated into the closure and sutured in place using 4-0 prolene between muscle layers to strengthen the incision site. Test article, Sensorcaine or saline was injected in the muscle fascia around the mesh in 4 locations and the remaining fascia and subcutaneous tissues closed with 3-0 vicryl. Test article, Sensorcaine, or saline was given as 4 additional injections around the mesh and the skin was closed with staples. Animals were followed post-surgery and Elizabethan collars utilized to prevent disruption of the surgical incision.

*Peripheral Nerve Block*

Anesthesia (Isoflurane) was administered and a brachial plexus block made in left thoracic limb using a 22 gauge 3.5 inch needle. SKY0402, Sensorcaine or saline was administered slowly as the needle was withdrawn from the site. Following the procedure animals were monitored for excessive bleeding from the injection site.

Deviation from study protocol: During the closure of the hernia in the Infiltration Phase, the apposition of the internal oblique was performed with 3-O Vicryl.

On several occasions during the study, toxicokinetic blood samples were collected several minutes outside of the allowable window for several animals.

On Day 2, two doses of Baytril 5 mg/kg were administered to one female during the Infiltration Phase at 9 mg/kg Sensorcaine (animal number 158).

On Day 3, the hematology samples were placed on ice after collection when it was not required to do so.

On Day 3, a short serum sample was collected for one female during the Nerve Block Phase at 30 mg/kg SKY0402 (animal number 173).

On Day 3, blood samples could not be collected for two females during the Infiltration Phase at 9 mg/kg Sensorcaine® (animal number 157) and 18 mg/kg SKY0402 (animal number 150), and one female during the Nerve Block Phase at 30 mg/kg SKY0402 (animal number 173) due to the condition of the neck.

On Days 3 and 15, coagulation or hematology parameters were not collected from one female in the Infiltration Phase at 18 mg/kg SKY0402 (animal number 150), one male and one female during the Infiltration Phase at 30 mg/kg SKY0402 (animal numbers 113 and 153, respectively), one male during the Nerve Block Phase at 18 mg/kg SKY0402 (animal number 132), and one male during the Nerve Block Phase at 30 mg/kg SKY0402 (animal number 135).

Plasma samples were analyzed from control animals in the Infiltration and Nerve Block Phases.

At the terminal necropsy, the pituitary gland from one female during the Nerve Block phase at 18 mg/kg SKY0402 (animal number 170) was lost.

None of these deviations were considered by the Study Director to have impacted the quality or result described in the study report to which this reviewer agrees.

## Observations and Results

### Mortality

All animals evaluated for morbidity, mortality twice daily throughout duration of the study.

Two animals died during the study at unscheduled times. One animal was in the Sensorcaine 9 mg/kg group, which occurred shortly after dosing and was attributed to the bupivacaine control article. This dose is considered within that of the minimum lethal dosing range based on published work and a prior range-finding study conducted by the Sponsor ( (b) (4) Study 947-004). The other animal (animal (#157) was from the post-dosing phase on SD14 after collection of blood. Post-mortem evaluation revealed a hemorrhage in the neck region at the site of blood collection and therefore was not attributed to test article. This appears to be reasonable. All other animals survived until scheduled necropsy.

### Clinical Signs

Evaluation of each animal performed daily including signs of disease, toxicity, injury or masses.

There were no clearly test-article-related clinical signs observed in either wound infiltration or peripheral nerve block phase. Findings noted are considered post-surgical in nature and not specific to study drug(s).

### Body Weights

Body weight was recorded at receipt, prior to randomization, and daily during the study.

There was no clearly test-article related effect on body weight in either wound infiltration or peripheral nerve block phase.

### Feed Consumption

Food consumption was recorded daily during the study.

There was no clearly test-article-related effect on food consumption other than a slight reduction in the immediate post-surgical period as would be expected in both wound infiltration and nerve block phases.

### Clinical Pathology

All blood samples collected after an overnight fast from the jugular vein and placed into EDTA- or citrate-containing tubes for evaluation of general and coagulation parameters, respectively.

**Hematology**

Leukocyte count, erythrocyte count, hemoglobin, hematocrit, MCV, MCH, MCHC, Platelets, Differential Leukocyte Count, Absolute Reticulocytes, Percent Reticulocytes, APTT, PT were determined.

**Clinical Chemistry**

Sodium, Potassium, Chloride, Calcium, Phosphorus, Alkaline Phosphatase, Total Bilirubin, Gamma Glutamyltransferase (GGT), Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Sorbitol Dehydrogenase (SDH), Urea Nitrogen, Creatinine, Total Protein, Albumin, Globulin, A/G Ratio, Cholesterol, Glucose

There were no apparent treatment-related effects on any hematologic or clinical chemistry parameter in either wound infiltration or nerve block phases.

**Urinalysis**

Not conducted

**Post-Mortem Findings**

Animals were euthanized by an overdose of sodium pentobarbital followed by exsanguination and full and standard necropsy examination with examination of all cavities and organs and tissues designated for histologic examination placed in fixative (neutral buffered formalin for all tissues but eye/optic nerve and testes which were immersed in modified Davidson's fixative).

**Organ Weights**

Body weight and organ weights recorded at necropsy exam. Organ weights and ratios to body weight and brain weight calculated. Paired organs were weighed together.

There were no apparent treatment-related effects on organ weight

**Macroscopic Findings**

There were no specific treatment-related findings on either SD3 or SD15 evaluation when comparing SKY0402 groups with either Sensorcaine or saline control.

**Histopathology**

Adequate Battery: Yes

Peer Review: No

Tissue embedded in paraffin was sectioned and stained with hematoxylin and eosin to reveal structure. Slides were examined by a veterinary pathologist.

Histological Findings: Note, no apparent treatment-related observations except at surgical site.

**Wound infiltration:**

At the surgical site it was not uncommon even in saline-treated animals to find evidence of degeneration/necrosis of myofiber generally mild in severity as well as mild hemorrhage and minimal to mild inflammation. In SKY0402-treated groups this was occasionally moderate in severity. In some cases (#114 30 mg/kg male on SD3) this was considered severe (hemorrhage). By SD15 saline-treated animals demonstrated mild fibrosis and minimal hemorrhage with some chronic inflammation of minimal severity, and some minimal mineralization. Sensorcaine-treated controls appeared similar. SKY0402-treated animals were similar in presentation with the additional finding of granulomatous inflammation by SD15 being occasionally present but minimal to mild in severity. The following table from the Applicant was verified by this reviewer and is included below:

<b>Test Article-Related Microscopic Findings</b>					
<b>Day 3 Infiltration Phase</b>					
<b>Male</b>					
<b>Dose Level: mg/kg</b>	<b>0</b>	<b>9</b>	<b>18</b>	<b>30</b>	<b>9</b>
	<b>Saline</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>Sensorcaine®</b>
Number Examined	2	2	2	2	2
<b>Surgical Site</b>					
Degeneration/necrosis, myofiber,	2	2	2	2	2
-minimal	0	0	1	0	0
-mild	2	2	0	1	1
-moderate	0	0	1	1	1
Hemorrhage,	2	2	2	2	2
-minimal	0	0	1	0	0
-mild	2	2	0	1	2
-moderate	0	0	1	0	0
-severe	0	0	0	1	0
Inflammation, acute,	2	2	1	2	2
-minimal	0	2	0	1	1
-mild	2	0	0	1	1
-moderate	0	0	1	0	0

Test Article-Related Microscopic Findings					
Day 3 Infiltration Phase					
Female					
Dose Level: mg/kg	0	9	18	30	9
	Saline	SKY0402	SKY0402	SKY0402	Sensorcaine®
Number Examined	2	2	2	2	2
<b>Surgical Site</b>					
Degeneration/necrosis, myofiber,	2	2	2	2	2
-mild	2	2	1	2	2
-moderate	0	0	1	0	0
Hemorrhage,	2	2	2	2	2
-minimal	0	2	1	1	1
-mild	2	0	1	0	1
-moderate	0	0	0	1	0
Inflammation, acute,	2	2	2	2	2
-minimal	1	1	2	1	1
-mild	1	1	0	1	1

(b) (4)

Test Article-Related Microscopic Findings					
Day 15 Infiltration Phase					
Male					
Dose Level: mg/kg	0	9	18	30	9
	Saline	SKY0402	SKY0402	SKY0402	Sensorcaine®
Number Examined	2	2	2	2	2
<b>Surgical Site</b>					
Degeneration/necrosis, myofiber,	1	2	2	2	2
-minimal	0	0	0	1	2
-mild	1	2	2	1	0
Fibrosis,	2	0	2	1	2
-minimal	0	0	0	0	2
-mild	2	0	1	1	0
-moderate	0	0	1	0	0
Hemorrhage, minimal	2	2	1	0	1
Inflammation, acute, mild	0	1	0	0	0
Inflammation, chronic,	1	1	1	0	2
-minimal	1	1	1	0	1
-mild	0	0	0	0	1
Inflammation, chronic active, mild	1	0	0	0	0
Inflammation, granulomatous,	0	0	1	2	0
-minimal	0	0	0	1	0
-mild	0	0	1	1	0
Mineralization, minimal	1	0	0	0	1

<b>Test Article-Related Microscopic Findings</b>					
<b>Day 15 Infiltration Phase</b>					
<b>Female</b>					
<b>Dose Level: mg/kg</b>	<b>0</b>	<b>9</b>	<b>18</b>	<b>30</b>	<b>9</b>
	<b>Saline</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>Sensorcaine®</b>
Number Examined	2	2	2	2	2
<b>Surgical Site</b>					
Degeneration/necrosis, myofiber,	2	2	2	2	2
-minimal	2	0	0	2	1
-mild	0	2	2	0	1
Fibrosis,	2	2	1	2	2
-minimal	1	0	0	1	1
-mild	1	0	0	1	0
-moderate	0	2	1	0	1
Hemorrhage, mild	0	1	1	0	1
Inflammation, chronic,	2	0	0	1	1
-minimal	2	0	0	0	0
-mild	0	0	0	1	1
Inflammation, chronic active, minimal	0	0	0	0	1
Inflammation, granulomatous,	0	2	2	1	0
-minimal	0	0	1	0	0
-mild	0	2	1	1	0

### ***Peripheral Nerve Block***

Saline- and Sensorcaine-injected rabbits had generally normal brachial plexus tissue though in several cases tissue was “not evaluated” for reasons which are unclear or occasionally subacute inflammation, minimal in severity, was reported. SKY0402-treated animals appeared to have a non-dose-related incidence of granulomatous inflammation, generally minimal to mild and occasional hemorrhage by SD3. By SD15, saline- and Sensorcaine-treated animals were generally within normal limits in regard to brachial plexus tissue while SKY0402 animals retained a finding of hemorrhage, granulomatous inflammation and demonstrated some mineralization of tissue within the section.

Test Article-Related Microscopic Findings					
Day 3 Nerve Block Phase					
Male					
Dose Level: mg/kg	0	9	18	30	9
	Saline	SKY0402	SKY0402	SKY0402	Sensorcaine®
Number Examined	2	2	2	2	2
<b>Brachial Plexus, Distal</b>					
Hemorrhage, moderate	0	0	1	0	0
Inflammation, granulomatous, minimal	0	2	1	1	0
<b>Brachial Plexus, Mid</b>					
Hemorrhage, minimal	0	1	0	1	0
Inflammation, granulomatous, minimal	0	0	0	1	0
<b>Brachial Plexus, Proximal</b>					
Inflammation, granulomatous, minimal	0	0	1	1	0

Test Article-Related Microscopic Findings					
Day 3 Nerve Block Phase					
Female					
Dose Level: mg/kg	0	9	18	30	9
	Saline	SKY0402	SKY0402	SKY0402	Sensorcaine®
Number Examined	2	2	2	2	2
<b>Brachial Plexus, Distal</b>					
Hemorrhage,	0	1	1	0	1
-minimal	0	0	1	0	1
-moderate	0	1	0	0	0
Inflammation, granulomatous, minimal	0	1	0	0	0
Inflammation, subacute, minimal	0	0	0	0	1
<b>Brachial Plexus, Mid</b>					
Hemorrhage,	0	2	0	0	0
-minimal	0	1	0	0	0
-moderate	0	1	0	0	0
Inflammation, granulomatous, minimal	0	1	1	2	0
Inflammation, subacute, minimal	0	0	1	0	0
<b>Brachial Plexus, Proximal</b>					
Hemorrhage,	0	2	1	1	0
-minimal	0	0	1	1	0
-mild	0	1	0	0	0
-moderate	0	1	0	0	0
Inflammation, granulomatous, minimal	0	0	1	1	0
Inflammation, subacute, minimal	0	1	1	0	0

<b>Test Article-Related Microscopic Findings</b>					
<b>Day 15 Nerve Block Phase</b>					
<b>Male</b>					
<b>Dose Level: mg/kg</b>	<b>0</b>	<b>9</b>	<b>18</b>	<b>30</b>	<b>9</b>
	<b>Saline</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>Sensorcaine®</b>
Number Examined	2	2	2	2	2
<b>Brachial Plexus, Distal</b>					
Hemorrhage,	0	1	2	1	0
-minimal	0	1	1	0	0
-mild	0	0	1	1	0
Inflammation, subacute, minimal	0	1	0	0	0
<b>Brachial Plexus, Mid</b>					
Hemorrhage,	0	1	1	0	1
-minimal	0	1	1	0	0
-mild	0	0	0	0	1
Inflammation, granulomatous, mild	0	0	1	0	0
Inflammation, subacute, minimal	0	1	0	0	0
Mineralization, minimal	0	0	1	0	0
<b>Brachial Plexus, Proximal</b>					
Hemorrhage	0	2	0	0	0
-minimal	0	1	0	0	0
-mild	0	1	0	0	0

<b>Test Article-Related Microscopic Findings</b>					
<b>Day 15 Nerve Block Phase</b>					
<b>Female</b>					
<b>Dose Level: mg/kg</b>	<b>0</b>	<b>9</b>	<b>18</b>	<b>30</b>	<b>9</b>
	<b>Saline</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>SKY0402</b>	<b>Sensorcaine®</b>
Number Examined	2	2	2	2	2
<b>Brachial Plexus, Distal</b>					
Hemorrhage,	0	0	0	1	1
-mild	0	0	0	0	1
-moderate	0	0	0	1	0
<b>Brachial Plexus, Mid</b>					
Fibrosis, minimal	0	0	0	0	1
Hemorrhage,	0	0	0	1	2
-minimal	0	0	0	0	2
-moderate	0	0	0	1	0
<b>Brachial Plexus, Proximal</b>					
Hemorrhage,	0	1	1	1	1
-minimal	0	1	0	0	0
-mild	0	0	1	1	0
-moderate	0	0	0	0	1

## Special Evaluation

### Wound Healing

The following macroscopic exam scoring system was used starting on SD2.

Wound Healing Scoring System for Hernia Repair			
<b>Inflammation</b>	<b>Edema:</b>	<b>Erythema:</b>	<b>Induration:</b>
:			
0 = none	0 = none	0 = none	0 = no
1 = slight	1 = slight	1 = slight	1 = yes
2 = moderate	2 = moderate	2 = moderate	
3 = severe	3 = severe	3 = severe	
<b>Exudate:</b>	<b>Wound Bed:</b>	<b>Odor:</b>	
0 = none	0 = dry	0 = no	
1 = slight	1 = dry/moist	1 = yes	
2 = moderate	2 = moist/dry		
3 = severe	3 = moist		

Inflammation, erythema, induration, and exudate scores were added (maximum score was 10) to determine a hernia repair score.

### Total Hernia Repair Score

Group	Males		Females	
	SD2	SD3	SD2	SD3
Saline	3	1	2	1
SKY0402				
9 mg/kg	4	1	6	2
18 mg/kg	2	2	1	1
30 mg/kg	2	1	2	0
Sensorcaine	1	1	1	0
*Scored for inflammation, erythema, induration and exudate. Note max group score is 40				

No dose-dependent effects on wound healing were observed. On the first day post-surgery (SD2) hernia repair scores were higher in the 9 mg/kg SKY0402 group, driven primarily by findings of slight-moderate erythema, than saline or Sensorcaine groups though by SD3 this appeared to be generally resolved by SD3. No wound bed findings or reports of odor were noted in any group.

### Toxicokinetics

Blood samples (~1.0 mL) obtained from the jugular vein of animals destined for SD15 necropsy for evaluation of plasma bupivacaine concentrations. Animals were not fasted except when this was required for collection of clinical pathology samples or for surgery. Blood samples were obtained at 1, 2, 4, 6, 8, 12, 24, 48, 72, and 96 hrs post injection.

Plasma was stored at -70°C until analyzed. PK modeling and interpretation of results was conducted by (b) (4). PK parameters were calculated by non-compartmental module of WinNonlin™ and were derived from mean concentration-time plasma data.

**Toxicokinetic Data from Acute Toxicology Study in Rabbit  
(Wound Infiltration & Nerve Block)**

Group	Dose	C <sub>max</sub> (ng/mL)	AUC <sub>0-96</sub> (ng•h/mL)	t <sub>1/2</sub>	T <sub>max</sub>
<i>Infiltration</i>					
Sensorcaine	9 mg/kg	620 ± 90	3550 ± 988	6 hr	1.0
SKY0402	9 mg/kg	234 ± 282	6670 ± 5570	31	17 ± 8
	18 mg/kg	222 ± 28	9050 ± 1400	67	7 ± 11
	30 mg/kg	307 ± 148	18400 ± 7630	17	30 ± 23
<i>Nerve Block</i>					
Sensorcaine	9 mg/kg	433 ± 26	1670 ± 249	3 hr	2.25
SKY0402	9 mg/kg	106 ± 68	2700 ± 781	511	10 ± 10
	18 mg/kg	491 ± 455	8870 ± 6600	341	26 ± 25
	30 mg/kg	205 ± 60	9370 ± 1170	49	36 ± 23

Group data indicate a reduced dose-adjusted C<sub>max</sub> when comparing Sensorcaine with SKY0402 groups in the Wound Infiltration model while AUC<sub>0-96 hr</sub> and t<sub>1/2</sub> was increased and prolonged, respectively and T<sub>max</sub> delayed. Examination of individual animal plasma levels reveals, however, a more complicated picture in which delayed dumping of drug can be seen in a non-dose-dependent fashion (see SKY0402 9 mg/kg animal below and to a lesser extent two animals in the 30 mg/kg dose group).

Reviewer constructed graphs follow (next page):

**Rabbit Wound Healing/Hernia Repair Model**

(b) (4)



**Note:** each line represents a single animal

A similar situation appears in the group data for nerve block but the SKY0402 18 mg/kg data reveals a variability ( $491 \pm 455$  ng/mL; and  $8870 \pm 6600$ ) which is striking and is the result of an apparent major dose dump in two animals, one at 1-6 hrs and the other dumping between 24 and 72 hrs. This dumping appears to be the explanation for an obviously artifact in the  $t_{1/2}$  calculation in the 9 mg/kg and 18 mg/kg groups. The Toxicokinetic Modeling report conducted considers this “biphasic” response and apparent drug dumping a “rebound” in plasma bupivacaine levels. The study report generally ignores these TK issues, as it did with the companion dog study (947-029).

### Brachial Plexus Block (Perineural Route) in Rabbit

(b) (4)



**Note:** each line represents a single animal

**Dosing Solution Analysis - Not conducted**

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## 6.2 Repeat-Dose Toxicity

### Subcutaneous Dosing

**Study title:** SKY0402: A Subcutaneous Toxicity Study with Twice Weekly Dosing For Four Weeks in Rabbits

Study no.: (b) (4) 947-036  
 Study report location: eCTD DARRTS SDN-1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 31, 2007  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: - SKY0402, lot 07-2501 (15 mg/mL dosing solution) and lot 07PD-003 (25 mg/mL dosing solution) in 0.9% NaCl  
 - Sensorcaine, lot ML 2273, 0.75% in 0.9% NaCl  
 - 0.9% NaCl, lots 49-017-JT and 52-124-JT

### Key Study Findings

- Groups of three male and female New Zealand white rabbits were subcutaneously dosed twice a week for 4 weeks with SKY0402 at concentrations of 15 or 25 mg/mL resulting in bupivacaine doses of 0 (1.2 mL/kg), 9 (0.6 mL/kg, 15 mg/mL), 18 (1.2 mL/kg, 15 mg/mL), or 30 (1.2 mL/kg, 25 mg/mL) mg/kg. Sensorcaine (Bupivacaine HCl) was also administered at a dose of 9 mg/kg (1.2 mL/kg, 7.5 mg/mL). Groups of 3/sex were also included for a 4 week recovery period. The largest proposed human dose (b) (4)
- Convulsions were seen on the day of dosing with SKY0402 on a few occasions during the study at 9 and 18 mg/kg, similar to Sensorcaine® at 9 mg/kg but not with SKY0402 at 30 mg/kg. One high dose SKY0402 female died in the 3<sup>rd</sup> week of the study the day after dosing without observed clinical symptoms and without histological observations as to the cause of death other than that of physiologically induced (stress) lymphoid depletion.
- While a true no-observed-adverse-effect-level (NOAEL) was not achieved, the only other treatment related effects were the microscopic changes seen at the injection sites that are expected for subcutaneous administration of a liposomal formulation. Observations included minimal to mild hemorrhage, minimal to moderate numbers of vacuolated macrophages, minimal to moderate neovascularization, and minimal to mild inflammation (chronic-active or subacute). The incidence and severity of these effects were reduced after the recovery period suggesting reversibility.
- While the convulsions appeared to be associated with the bupivacaine and not the liposome formulation, it is unclear why no convulsions were seen at the highest bupivacaine level of 30 mg/kg. The nonclinical-based human safety assessment will

be based on toxicokinetic (TK) levels compared to those in humans, most notably C<sub>max</sub> for convulsions.

- As only a single, acute dose will be employed clinically, single dose rabbit TK for bupivacaine are C<sub>max</sub> of 213, 147, & 94 ng/mL and AUC<sub>0-72h</sub> of 2,520, 3,750, & 4,710 ng•h/mL at SKY00402 doses of 9, 18, & 30 mg/kg, respectively. TK for single dose Sensorcaine (Bupivacaine HCl) was a C<sub>max</sub> of 396 ng/mL and an AUC<sub>0-72h</sub> of 261 ng•h/mL. Repeated dosing in the rabbits resulted in accumulation of bupivacaine as reflected by up to 3-fold larger TK values after 4 weeks of dosing. Rabbit TK values were at least 4-fold lower than in dogs at the same subcutaneous mg/kg doses of SKY0402 and Sensorcaine.

## Methods

### Doses:

Group Assignments						
Group Number	Treatment	Number of Animals <sup>a</sup>		Bupivacaine Concentration (mg/mL)	Dose Volume (mL/kg)	Dose Level (mg/kg/dose)
		Male	Female			
1	Saline	6	6	0	1.2	0
2	SKY0402	6	6	15	0.6	9 <sup>b</sup>
3	SKY0402	6	6	15	1.2	18 <sup>b</sup>
4	SKY0402	6	6	25	1.2	30 <sup>b</sup>
5	Sensorcaine <sup>®</sup>	6	6	7.5	1.2	9

<sup>a</sup>Three animals/sex/group maintained for a 28-day recovery period.  
<sup>b</sup>Expressed as bupivacaine HCl equivalent

Frequency of dosing:	2x/week for 4 weeks
Route of administration:	Subcutaneous bolus injection (once per week to right dorsal thoracic side and once per week to left dorsal thoracic side)
Dose volume:	See table
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	New Zealand White Hra:(NZW)SPF albino rabbits
Number/Sex/Group:	6 (3 for main study and 3 for recovery groups)
Age:	6-6.5 months
Weight:	3.06 to 3.61 kg (males) and 3.41 to 4.11 kg (females) at randomization
Satellite groups:	None – main study animals used for toxicokinetics
Unique study design:	4 week recovery groups
Deviation from study protocol:	Nothing significant

## Observations and Results

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily throughout the duration of the study. A detailed clinical examination of each animal was performed weekly.

**Mortality** – nothing treatment related

- 1 high dose SKY0402 female died the day after the day 18 dosing with no abnormal observations prior to death and no cause of death determined after complete examination, including histopathology

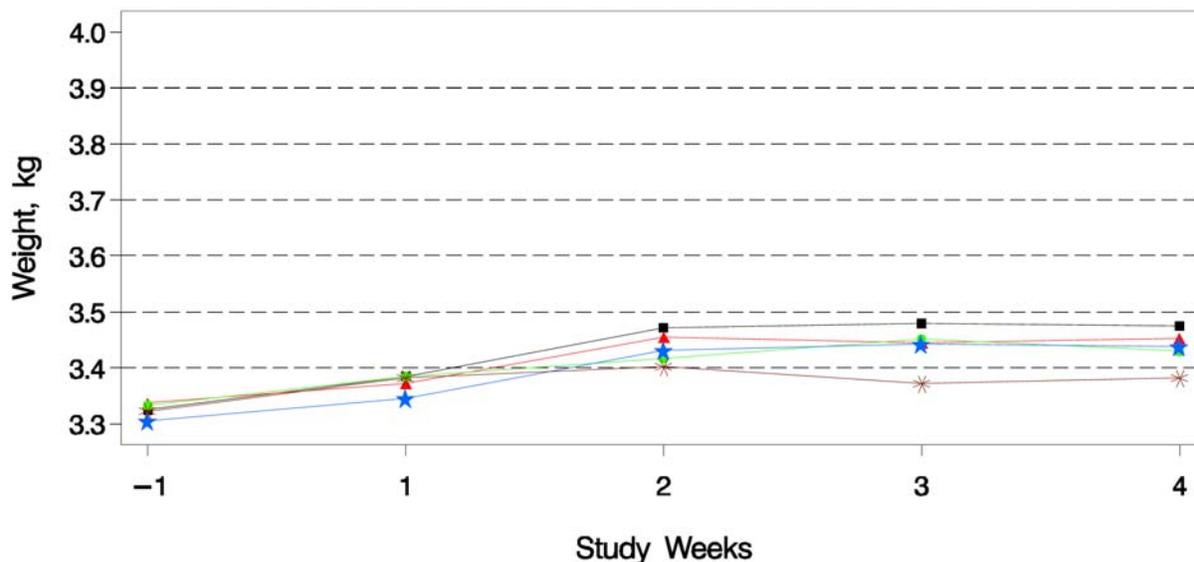
**Clinical Signs** – No adverse clinical observations were noted for the saline control group. Test article-related convulsions were observed on the listed day of dosing in the animals treated with Sensorcaine as well as animals dosed with 9 or 18 mg/kg/dose SKY0402 (see table). While convulsions were not seen at the 30 mg/kg/dose of SKY0402, the convulsions at the 9 and 18 mg/kg/dose of SKY0402 were considered to be bupivacaine related because of the convulsions seen in the Sensorcaine (bupivacaine HCl solution) dose group. Other observations only occurred sporadically with no apparent relationship to treatment. Nothing treatment related was observed during the recovery period for any groups.

		Incidence of Convulsions <sup>a</sup>									
Group	Treatment	Dose (mg/kg)	Week 1		Week 2		Week 3		Week 4		
			Dose 1	Dose 2	Dose 3	Dose 4	Dose 5	Dose 6	Dose 7	Dose 8	
2	SKY0402 15 mg/mL	9 <sup>b</sup>	1(M)	–	–	–	–	–	–	–	–
3	SKY0402 15 mg/mL	18 <sup>b</sup>	–	–	–	1(F)	1(M)	–	–	–	–
4	SKY0402 25 mg/mL	30 <sup>b</sup>	–	–	–	–	–	–	–	–	–
5	Sensorcaine® 0.75%	9	–	–	3(M) 1(F)	–	1(M)	–	–	–	–

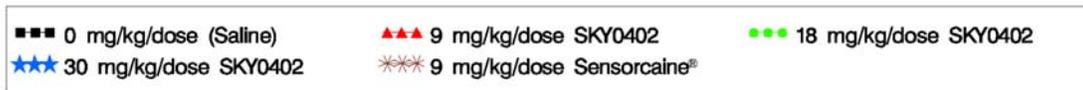
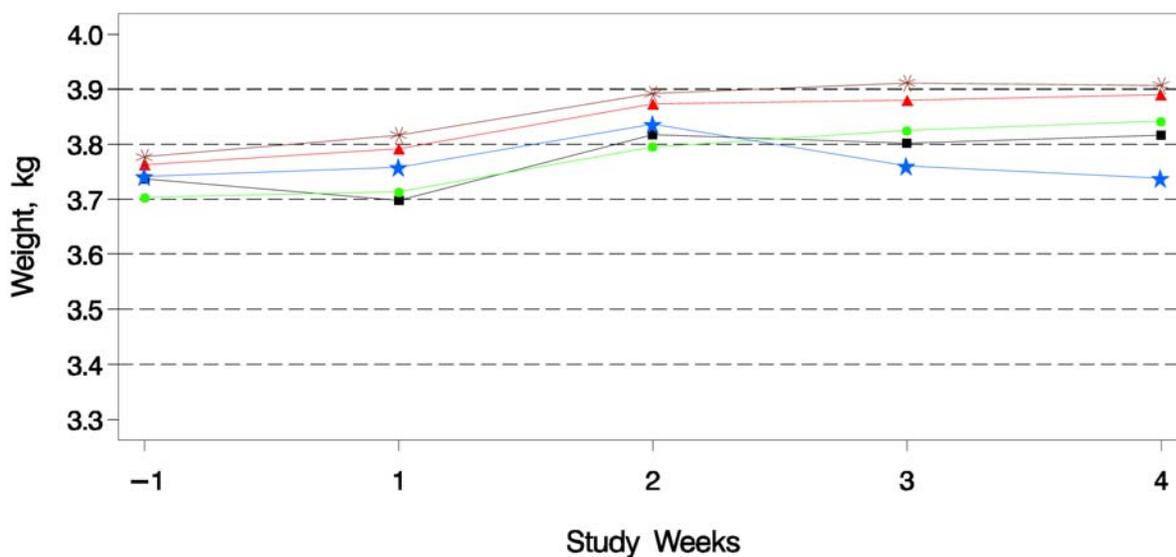
<sup>a</sup>Number of animal affected on each dosing day  
<sup>b</sup>Expressed as bupivacaine HCl equivalent  
M= male F= female  
“–“ No seizures recorded

**Body Weights** – measured weekly with no apparent treatment related effects for main study animals. High dose males exhibited increased body weights and high dose females exhibited decreased body weights during recovery of ~5-10% compared to the other groups.

### Mean Body Weight Values – MALE



### Mean Body Weight Values – FEMALE



**Feed Consumption** – measured daily and variable with results consistent with body weights (no apparent treatment related effects). Food consumption for high dose recovery males and females did not reflect the increased and decreased body weights, respectively, during recovery.

**Ophthalmoscopy** - none

**ECG** - none

**Clinical Pathology Evaluations** - Clinical pathology evaluations were conducted on all animals pretest and prior to terminal and recovery necropsies. The animals had access to drinking water but were fasted overnight prior to sample collection.

**Hematology and Coagulation** – no treatment related effects observed during main study or recovery period. Minimal sporadic alterations were seen in red cell parameters in all groups. These alterations were minimal in magnitude, did not fall outside of clinically expected ranges, and showed no dose dependency.

**Clinical Chemistry** - No definitive test article-related effects were identified, but the control and treated groups exhibited elevations in means for some liver function enzymes [aspartate aminotransferase (AST), alanine aminotransferase (ALT), sorbitol dehydrogenase (SDH), and gamma glutamyltransferase (GGT) at pretest and occasionally at termination] outside of clinically expected control ranges due to one animal of three in each groups. Although a test article related hepatocellular injury cannot wholly be ruled out, the marked increases seen in some controls, as well as the inter-animal variation, and lack of dose dependency makes these changes more consistent with non-test article related changes. No liver correlate was observed during histological observation.

**Urinalysis** - none

**Gross Pathology** - Necropsy examinations were performed under procedures approved by a veterinary pathologist on animals found dead and all surviving animals at the scheduled terminal and recovery necropsies. The following table lists organs collected and weighed.

The following list constitutes the full complement of organs and tissues:

- Adrenal (2)*	- Liver [3 sections collected; 2 examined]*
- Aorta	- Lung [collected whole; 2 sections examined]*
- Bone with marrow [femur]	- Lymph nodes: ileocecolic, mandibular, and tracheobronchial
- Bone with marrow [rib]	- Mammary gland [process females only]
- Bone with marrow [sternum]	- Peyer's patch
- Bone marrow smear [2 collected] <sup>a</sup>	- Pituitary*
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*	- Prostate*
- Epididymis (2)*	- Salivary gland, mandibular [2 collected; 1 examined]*#
- Eye including optic nerve (2)	- Salivary gland, parotid [2 collected; 1 examined]
- Gallbladder	- Salivary gland, sublingual [2 collected; 1 examined]
- Gastrointestinal tract:	- Sciatic nerve
esophagus	- Skeletal muscle, biceps femoris
stomach [cardia, fundus, and pylorus]	- Skin [injection sites]
duodenum	- Skin [untreated]
jejunum	- Spinal cord [cervical, thoracic, and lumbar]
ileum	- Spleen*
cecum	- Thymus*
colon	- Thyroid/parathyroid (2)*
rectum	- Tongue
- Gonads:	- Trachea
ovary (2)*	- Urinary bladder
testis (2)*	- Uterus [both horns]/Cervix*
- Gross lesions	- Vagina
- Heart*	
- Kidney (2)*	
- Larynx	

<sup>a</sup>Bone marrow smears were collected at the scheduled necropsy and held.

\*Weighed organ

#The weight of the right mandibular salivary gland was obtained.

(2) Paired organ

Only injections sites had treatment-related findings. Test article-related macroscopic findings observed in main study animals included an increased incidence of mild to moderate red discoloration and swelling/thickening of both injection sites in both sexes of the 30 mg/kg SKY0402 group. In recovery animals, mild red discoloration was seen at a low incidence in SKY0402-dosed males in all dose groups and in one female at 30 mg/kg of SKY0402. These changes were, for the most part, correlated with the microscopic findings of hemorrhage and neovascularization (see histopathology). Red discoloration and/or swelling/thickening of the injection sites were also seen at a low incidence in lower dose level main study SKY0402 groups, but these incidences were not significantly different from those in controls (both 0 mg/kg of saline and 9 mg/kg of Sensorcaine®).

**Organ Weights** - Body weights and protocol-designated organ weights were recorded for all surviving animals at the scheduled necropsies as listed in the above table and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together.

No apparent treatment related effects were observed. Decreased absolute heart weight at the 18 mg/kg/dose of SKY0402 (↓ 12.4%) and the 30 mg/kg/dose of SKY0402 (↓ 13.5%) were noted compared to control. The heart weight decrease was not accompanied by any microscopic findings and was not seen in the terminal females, suggesting that this finding may be biological variation, rather than a test article effect.

**Histopathology** - Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on listed tissues for all terminal and recovery dose groups.

Adequate Battery - yes

Peer Review - no

**Histological Findings** – No treatment related effects were observed for tissues other than the injections sites which were observed at all dose levels of SKY0402 in the majority of main study animals. The primary microscopic findings (see tables for main study and recovery animals) consisted of minimal to mild hemorrhage, minimal to moderate numbers of vacuolated macrophages, minimal to moderate neovascularization, and minimal to mild inflammation (chronic-active or subacute). There was no consistent dose-dependent response seen, except in increased numbers of vacuolated macrophages at higher dose levels. Incidence and severity of injection site effects were reduced in the recovery groups suggesting reversibility/healing of observed injection site effects.

Test Article-Related Microscopic Findings										
Terminal										
Male and Female										
Dose Level (mg/kg/dose)	0 <sup>a</sup>		9 <sup>b</sup>		18 <sup>b</sup>		30 <sup>b</sup>		9 <sup>c</sup>	
Sex	M	F	M	F	M	F	M	F	M	F
Number Examined	3	3	3	3	3	3	3	4	3	3
<b>Injection Site 1</b>										
Hemorrhage	0	0	2	1	2	2	3	2	0	0
- minimal	0	0	1	1	1	0	2	1	0	0
- mild	0	0	1	0	1	2	1	1	0	0
Inflammation, chronic-active	0	0	0	1	0	1	2	0	0	0
- minimal	0	0	0	1	0	0	1	0	0	0
- mild	0	0	0	0	0	1	1	0	0	0
Macrophages, vacuolated	0	0	2	3	3	3	3	4	0	0
- minimal	0	0	1	2	1	1	0	1	0	0
- mild	0	0	0	1	1	1	3	1	0	0
- moderate	0	0	1	0	1	1	0	2	0	0
Neovascularization	0	0	1	1	1	2	2	2	0	0
- minimal	0	0	0	1	0	1	2	0	0	0
- mild	0	0	1	0	1	0	0	2	0	0
- moderate	0	0	0	0	0	1	0	0	0	0
<b>Injection Site 2</b>										
Hemorrhage	0	0	3	0	3	3	2	3	0	0
- minimal	0	0	2	0	0	2	1	0	0	0
- mild	0	0	1	0	2	1	1	3	0	0
- moderate	0	0	0	0	1	0	0	0	0	0
Inflammation, subacute	0	0	1	0	1	1	2	1	0	0
- minimal	0	0	1	0	0	1	1	1	0	0
- mild	0	0	0	0	1	0	1	0	0	0
Macrophages, vacuolated	0	0	3	0	3	2	2	3	0	0
- minimal	0	0	0	0	0	1	0	0	0	0
- mild	0	0	3	0	2	1	1	1	0	0
- moderate	0	0	0	0	1	0	1	2	0	0
Neovascularization	0	0	2	0	2	0	1	2	0	0
- minimal	0	0	1	0	0	0	0	1	0	0
- mild	0	0	1	0	2	0	1	1	0	0

<sup>a</sup>Saline  
<sup>b</sup>SKY0402  
<sup>c</sup>Sensorcaine®

Test Article-Related Microscopic Findings										
Recovery										
Male and Female										
Dose Level (mg/kg/dose)	0 <sup>a</sup>		9 <sup>b</sup>		18 <sup>b</sup>		30 <sup>b</sup>		9 <sup>c</sup>	
Sex	M	F	M	F	M	F	M	F	M	F
Number Examined	3	3	3	3	3	3	3	3	3	3
<b>Injection Site 1</b>										
Hemorrhage, minimal	0	0	0	0	0	0	0	1	0	0
Macrophages, vacuolated										
- minimal	0	0	0	0	1	0	1	1	0	0
Neovascularization										
- minimal	0	0	0	0	0	0	0	1	0	0
<b>Injection Site 2</b>										
Hemorrhage	0	0	1	0	1	0	1	0	0	0
- minimal	0	0	0	0	0	0	1	0	0	0
- mild	0	0	1	0	1	0	0	0	0	0
Macrophages, vacuolated	0	0	0	0	1	0	1	0	0	0
- minimal	0	0	0	0	0	0	1	0	0	0
- mild	0	0	0	0	1	0	0	0	0	0
Neovascularization, mild	0	0	1	0	0	0	0	0	0	0

<sup>a</sup>Saline  
<sup>b</sup>SKY0402  
<sup>c</sup>Sensorcaine®

One female from the 30 mg/kg SKY0402 high dose group) died on the day after dosing on day 18 of the study without determination of the cause of death. In addition to injection sites effects observed in other SKY0402 treated groups, microscopic findings consisting of splenic lymphoid depletion, lymph node lymphoid depletion, and thymic lymphoid depletion. The sponsor reported this observation to be a physiological stress-associated lymphoid depletion which is a common finding in animals that die on study. Additionally, a mild amount of foreign material consistent with food matter was observed in the lungs, possibly due to perimortem aspiration, as there was no associated lung inflammation.

### Special Evaluation – none

**Toxicokinetics** - Blood samples (approximately 0.5 to 1 mL) were collected from cohorts of three animals/sex/group designated for the 4-week recovery period via the jugular vein for determination of the plasma concentrations of bupivacaine. Samples were collected prior to dosing and at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours post-dose on Days 1 and 25. Only the 1 hour post dose samples from animals at 0 mg/kg were analyzed. The animals were not fasted prior to blood collection.

Based on sampling on day 1 and 25, generally gender nonspecific, dose responsive but not strictly dose-proportional increases in bupivacaine were observed for SKY0402 treated groups (see table). The C<sub>max</sub> for the Sensorcaine group was approximately double that of the comparable SKY0402 dose group (9 mg/kg bupivacaine) on day 1.

Mean ( $\pm$ Standard Deviation) Toxicokinetic Parameters for Bupivacaine after Subcutaneous Administration of Sensorcaine<sup>®</sup> or SKY0402

Dosing Day	Treatment	Bupivacaine (mg/kg/dose)	AUC <sub>0-tlast</sub> (hr•ng/mL)	AUC <sub>0-tlast</sub> /Dose (hr•ng/mL)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose (ng/mL)	t <sub>1/2</sub> (hr)	t <sub>max</sub> (hr)
1	Sensorcaine <sup>®</sup>	9	2,350 $\pm$ 587	261 $\pm$ 65.2	396 $\pm$ 315	44.1 $\pm$ 35.0	9.57 $\pm$ 3.10	2.50 $\pm$ 4.66
		9	2,520 $\pm$ 429	280 $\pm$ 47.6	213 $\pm$ 145	23.7 $\pm$ 16.1	42.0 $\pm$ 26.4	1.42 $\pm$ 1.39
	SKY0402	18	3,750 $\pm$ 503	208 $\pm$ 27.9	147 $\pm$ 59.6	8.18 $\pm$ 3.29	48.2 $\pm$ 32.4	3.33 $\pm$ 3.61
		30	4,710 $\pm$ 1150	157 $\pm$ 38.5	94.4 $\pm$ 45.2	3.15 $\pm$ 1.51	150 $\pm$ 78.8	26.3 $\pm$ 24.2
25	Sensorcaine <sup>®</sup>	9	2,060 $\pm$ 454	228 $\pm$ 50.5	218 $\pm$ 122	24.3 $\pm$ 13.6	11.2 $\pm$ 4.76	2.67 $\pm$ 4.58
		9	2,380 $\pm$ 1170	264 $\pm$ 130	338 $\pm$ 136	37.5 $\pm$ 15.2	14.5 $\pm$ 9.25	0.583 $\pm$ 0.204
	SKY0402	18	5,810 $\pm$ 426	323 $\pm$ 23.7	347 $\pm$ 210	19.3 $\pm$ 11.7	17.2 $\pm$ 7.06	4.67 $\pm$ 9.47
		30	10,100 $\pm$ 4590	336 $\pm$ 153	292 $\pm$ 233	9.73 $\pm$ 7.76	62.9 $\pm$ 42.6	7.50 $\pm$ 6.16

As noted in the table above for actual numbers and the table below for ratios, SKY0402 treated groups exhibited modest accumulation of bupivacaine in plasma, as would be expected from its prolonged absorption from the injection site and twice weekly dosing. The accumulation was more evident at 30 mg/kg/dose and suggests that each dose of SKY0402 was not cleared completely before the next dose was administered.

**Toxicokinetic Parameter Ratios Based on Single- and Multiple-Dose Data (Day 25 versus Day 1)**

Treatment	Bupivacaine (mg/kg/dose)	Ratio (Day 25 vs. Day 1)			
		AUC <sub>0-tlast</sub> (hr•ng/mL)	C <sub>max</sub> (ng/mL)	t <sub>1/2</sub> (hr)	t <sub>max</sub> (hr)
Sensorcaine®	9	0.88	1.02	1.17	1.07
SKY0402	9	0.94	2.30	0.35	0.41
	18	1.55	2.36	0.36	1.40
	30	2.14	3.09	1.42	0.29

**Dosing Solution Analysis**

The Sponsor provided documentation that the test article, SKY0402, was a suspension, homogeneous, and stable for the duration of the study (28 days); therefore, test article analysis was not performed.

=====

**Study title:** SKY0402: A Subcutaneous Toxicity Study with Twice Weekly Dosing For Four Weeks in Dogs

Study no.: (b) (4) 947-037  
 Study report location: eCTD DARRTS SDN-1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: July 31, 2007  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: - SKY0402, lot 07-2501 (15 mg/mL dosing solution) and lot 07PD-003 (25 mg/mL dosing solution) in 0.9% NaCl  
 - Sensorcaine, lot ML 2273, 0.75% in 0.9% NaCl  
 - 0.9% NaCl, lots 49-017-JT and 52-124-JT

**Key Study Findings**

- Groups of three male and female beagle dogs were subcutaneously dosed twice a week for 4 weeks with SKY0402 at concentrations of 15 or 25 mg/mL resulting in bupivacaine doses of 0 (1.2 mL/kg), 9 (0.6 mL/kg, 15 mg/mL), 18 (1.2 mL/kg, 15 mg/mL), or 30 (1.2 mL/kg, 25 mg/mL) mg/kg. Sensorcaine (Bupivacaine HCl) was also administered at a dose of 9 mg/kg (1.2 mL/kg, 7.5 mg/mL). Groups of 3/sex were also included for a 4 week recovery period. The largest proposed human dose volume is (b) (4)

- No treatment-related effects were observed other than injection site effects. Observations included erythema and inflammation. The incidence and severity of these effects were reduced after the recovery period suggesting reversibility. The high dose of 30 mg/kg was considered the systemic NOAEL, excluding local injection site effects.
- As only a single, acute dose will be employed clinically, single dose dog TK for bupivacaine are Cmaxs of 488, 560, & 633 ng/mL and AUCs<sub>0-72h</sub> of 9,100, 12,800, & 25,600 ng•h/mL at SKY00402 doses of 9, 18, & 30 mg/kg, respectively. TK for single dose Sensorcaine (Bupivacaine HCl) was a Cmax of 1,420 ng/mL and an AUCs<sub>0-72h</sub> of 9,720 ng•h/mL. Repeated dosing in the dog resulted in some accumulation of bupivacaine as reflected by up to 2-fold larger TK values after 4 weeks of dosing. Dog TK values were at least 4-fold larger than in rabbits at the same subcutaneous mg/kg doses of SKY0402 and Sensorcaine.

## Methods

### Doses:

Group Assignments						
Group Number	Treatment	Number of Animals <sup>a</sup>		Bupivacaine		
		Male	Female	Concentration (mg/mL)	Dose Volume (mL/kg/dose)	Dose Level (mg/kg/dose)
1	Saline	6	6	0	1.2	0
2	SKY0402	6	6	15	0.6	9 <sup>b</sup>
3	SKY0402	6	6	15	1.2	18 <sup>b</sup>
4	SKY0402	6	6	25	1.2	30 <sup>b</sup>
5	Sensorcaine <sup>®</sup>	6	6	7.5	1.2	9

<sup>a</sup>Three animals/sex/group maintained for a 28 day recovery period.  
<sup>b</sup>Expressed as bupivacaine HCl equivalent

Frequency of dosing:	2x/week for 4 weeks
Route of administration:	Subcutaneous bolus injection to the right of the dorsal midline (Site 1) and to the left of the dorsal midline (Site 2).
Dose volume:	See table
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Beagle dogs
Number/Sex/Group:	6 (3 for main study and 3 for recovery groups)
Age:	5-6 months old
Weight:	male and female animals weighing 8.63 to 11.61 kg and 5.19 to 6.53 kg, respectively, at randomization)
Satellite groups:	None – main study animals used for toxicokinetics
Unique study design:	4 week recovery groups
Deviation from study protocol:	Nothing significant

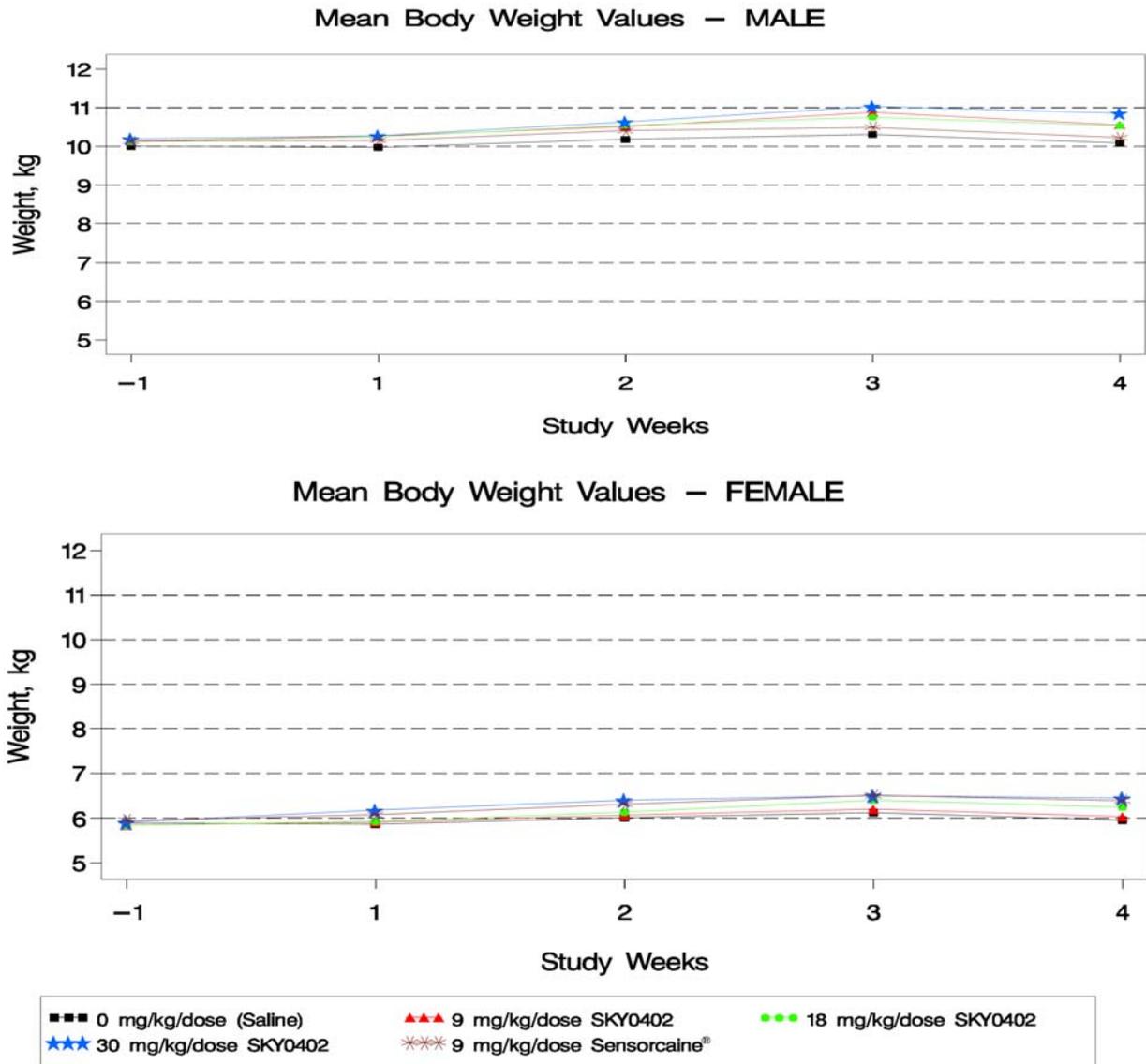
**Observations and Results**

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily throughout the duration of the study. The tray was evaluated for signs of abnormal or reduced defecation. Checks were performed on all cages daily to ensure the cages were securely closed.

**Mortality** – none

**Clinical Signs** - detailed clinical examination of each animal was performed weekly. Nothing treatment related was observed.

**Body Weights** - measured and recorded at receipt, prior to randomization (Week -1), and weekly during the study. Nothing treatment related was observed during the main study (see figures) or during the recovery period.



**Feed Consumption** – measured and recorded daily during the study. Nothing treatment related was observed consistent with the lack of treatment related body weight changes.

### Ophthalmoscopy - none

**ECG** - All animals received an electrocardiographic examination (10 lead) pretest, predose and at 1 hour post-dose ( $\pm 15$  minutes) on Day 22 (Terminal), and on Day 50 (Recovery). Nothing treatment related was observed for SK0402 or Sensorcaine.

**Clinical Pathology** - Clinical pathology evaluations were conducted on all animals pretest and prior to terminal and recovery necropsies. The animals had no access to drinking water and were fasted overnight prior to sample collection.

**Hematology and Coagulation** - Nothing treatment related was observed.

**Clinical Chemistry** - Nothing treatment related was observed.

**Urinalysis** - Urine samples were collected using steel pans placed under the cages for at least 16 hours. Nothing treatment related was observed.

**Gross Pathology** - Necropsy examinations were performed under procedures approved by a veterinary pathologist on all animals at the scheduled terminal and recovery necropsies. The following table lists organs collected and weighed.

The following list constitutes the full complement of organs and tissues:

- Adrenal (2)*	- Lung [collected whole; 2 sections examined]*
- Aorta	- Lymph nodes: mandibular, mesenteric, and tracheobronchial
- Bone with marrow [femur]	- Mammary gland [process females only]
- Bone with marrow [rib]	- Nictitans gland
- Bone with marrow [sternum]	- Pancreas*
- Bone marrow smear [2 collected]*	- Peyer's patch
- Brain [cerebrum, midbrain, cerebellum, medulla/pons]*	- Pituitary*
- Epididymis (2)*	- Prostate*
- Eye including optic nerve (2)	- Salivary gland, mandibular [2 collected; 1 examined]##
- Gallbladder	- Salivary gland, parotid [2 collected; 1 examined]
- Gastrointestinal tract:	- Salivary gland, sublingual [2 collected; 1 examined]
esophagus	- Sciatic nerve
stomach [cardia, fundus, and pylorus]	- Skeletal muscle, biceps femoris
duodenum	- Skin [injection site 1 and 2]
jejunum	- Skin [untreated]
ileum	- Spinal cord [cervical, thoracic, and lumbar]
cecum	- Spleen*
colon	- Thymus*
rectum	- Thyroid/parathyroid (2)*
- Gonads:	- Tongue
ovary (2)*	- Trachea
testis (2)*	- Urinary bladder
- Gross lesions	- Uterus [both horns]/Cervix*
- Heart*	- Vagina
- Joint, tibiofemoral	
- Kidney (2)*	
- Larynx	
- Liver [3 sections collected; 2 examined]*	

\*Bone marrow smears were collected at the scheduled necropsy and held.  
 \*Weighed organ  
 #The weight of the right mandibular salivary gland was obtained.  
 (2) Paired organ

Only injections site effects were treatment related. Swollen or thickened injection sites were noted in one male and one female terminal animal at the 30 mg/kg SKY0402. The swollen or thickened injection sites corresponded with granulomatous inflammation and/or edema and were considered test article-related. Red discoloration at injection sites was noted in a low number of terminal and recovery animals. Often there was no microscopic correlate to the red discoloration at the injection sites. In these cases the toxicological significance of the macroscopic finding was unknown. Rarely the red discoloration corresponded with hemorrhage, edema, and/or subcutaneous subacute inflammation. This macroscopic finding appeared to be a result of physical trauma from the injection process and not a primary test article-related effect.

**Organ Weights** - Body weights and protocol-designated organ weights were recorded for all surviving animals at the scheduled necropsies as listed in the above table and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together. Nothing treatment related was observed and any possible differences did not have microscopic correlates of effect.

**Histopathology** - Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on listed tissues for all terminal and recovery dose groups.

Adequate Battery – yes

Peer Review – no

Histological Findings – No treatment related effects were observed for other than the injections sites which were observed at all dose levels of SKY0402 in the majority of main study animals (see first table below). Observed effects were of decreased incidence and severity after the recovery period, suggesting reversibility of the effects (see second table below).

The primary microscopic findings consisted of minimal to moderate granulomatous inflammation in the subcutaneous tissue of male and female terminal and recovery animals receiving SKY0402. In the terminal animals, granulomatous inflammation was characterized by numerous vacuolated macrophages and fewer lymphocytes, plasma cells, and/or multinucleated giant cells. The inflammation was often associated with edema and/or mineralization. Giant cells were primarily observed when mineralization was present. The mineral deposits may be related to small amounts of foreign matter (i.e. DepoFoam particles) in the loose connective tissues of the subcutaneous space. The mineral deposits were usually surrounded by multinucleated giant cells. In recovery animals, the granulomatous inflammation was observed in a fewer number of animals and was characterized by a greater number of giant cells sometimes associated with mineralization but not edema. In one male recovery animal at the 9 mg/kg/dose of SKY0402 minimal subcutaneous edema not associated with inflammation was noted. This effect was considered an expected response to liposomes. Subcutaneous granulomatous inflammation was not observed in terminal or recovery animals receiving saline or Sensorcaine®.

Test Article-Related Microscopic Findings										
Terminal										
Male and Female										
Dose Level (mg/kg/dose)	0 <sup>a</sup>		9 <sup>b</sup>		18 <sup>b</sup>		30 <sup>b</sup>		9 <sup>c</sup>	
Sex	M	F	M	F	M	F	M	F	M	F
Number Examined	3	3	3	3	3	3	3	3	3	3
<b>Injection Site 1</b>										
Edema										
- minimal	0	0	1	0	1	0	0	0	0	0
- mild	0	0	0	0	1	1	0	0	0	0
Inflammation, granulomatous										
- minimal	0	0	2	0	0	0	2	0	0	0
- mild	0	0	0	0	0	1	0	0	0	0
- moderate	0	0	1	0	3	0	0	1	0	0
Mineralization										
- minimal	0	0	1	0	3	0	0	1	0	0
<b>Injection Site 2</b>										
Edema										
- minimal	0	0	0	0	0	1	0	0	0	0
- mild	0	0	1	0	2	0	1	3	0	0
Inflammation, granulomatous										
- minimal	0	0	0	1	1	1	0	0	0	0
- mild	0	0	1	0	1	1	1	0	0	0
- moderate	0	0	1	0	1	0	1	3	0	0
Mineralization										
- minimal	0	0	2	0	1	1	1	1	0	0
<sup>a</sup> Saline										
<sup>b</sup> SKY0402										
<sup>c</sup> Sensorcaine®										

Test Article-Related Microscopic Findings										
Recovery										
Male and Female										
Dose Level (mg/kg/dose)	0 <sup>a</sup>		9 <sup>b</sup>		18 <sup>b</sup>		30 <sup>b</sup>		9 <sup>c</sup>	
Sex	M	F	M	F	M	F	M	F	M	F
Number Examined	3	3	3	3	3	3	3	3	3	3
<b>Injection Site 1</b>										
Edema										
- mild	0	0	1	0	0	0	0	0	0	0
Inflammation, granulomatous										
- minimal	0	0	0	0	1	0	0	1	0	0
- moderate	0	0	0	0	0	0	1	0	0	0
Mineralization										
- minimal	0	0	0	0	1	0	1	1	0	0
<b>Injection Site 2</b>										
Inflammation, granulomatous										
- minimal	0	0	1	0	0	0	0	0	0	0
- mild	0	0	1	0	0	0	1	0	0	0
Mineralization										
- minimal	0	0	2	0	1	0	0	0	0	0
<sup>a</sup> Saline										
<sup>b</sup> SKY0402										
<sup>c</sup> Sensorcaine®										

**Special Evaluation – none**

**Toxicokinetics** - Blood samples (approximately 0.5-1.0 mL) were collected from three animals/sex/group designated for recovery via the jugular vein for determination of the plasma concentrations of bupivacaine. Samples were collected prior to dosing and at 0.5, 1, 2, 4, 8, 12, 24, 48, and 72 hours post-dose on Days 1 and 25.

Based on sampling on day 1 and 25, generally gender nonspecific, dose responsive but not strictly dose-proportional increases in bupivacaine were observed for SKY0402 treated groups (see table). The C<sub>max</sub> for the Sensorcaine group was approximately three times that of the comparable SKY0402 dose group (9 mg/kg bupivacaine) on day 1.

**Mean (±Standard Deviation) Toxicokinetic Parameters for Bupivacaine after Subcutaneous Administration of Sensorcaine or SKY0402**

Dosing Day	Treatment	Bupivacaine (mg/kg)	AUC <sub>0-tlast</sub> (hr•ng/mL)	AUC <sub>0-tlast</sub> /Dose (hr•ng/mL)	C <sub>max</sub> (ng/mL)	C <sub>max</sub> /Dose (ng/mL)	t <sub>1/2</sub> <sup>a</sup> (hr)	t <sub>max</sub> (hr)
1	Sensorcaine	9	9,720 ± 1,860	1,080 ± 207	1,420 ± 355	158 ± 39.5	16.9 ± 6.05	0.5 ± 0.0
		9	9,100 ± 4,460	1,010 ± 495	488 ± 335	54.2 ± 37.2	59.5 ± 49.1 <sup>a</sup>	0.5 ± 0.0
	SKY0402	18	12,800 ± 2,020	711 ± 112	560 ± 299	31.1 ± 16.6	104 ± 105	0.5 ± 0.0
		30	25,600 ± 8,160	853 ± 272	633 ± 280	21.1 ± 9.34	31.8 <sup>a</sup>	48.1 ± 30.2
25	Sensorcaine	9	9,120 ± 4,090	1,010 ± 455	1,990 ± 304	221 ± 33.8	10.1 ± 8.54	0.5 ± 0.0
		9	17,300 ± 8,710	1,920 ± 968	1,200 ± 301	133 ± 33.4	36.2 ± 12.4	0.5 ± 0.0
	SKY0402	18	24,300 ± 8,960	1,350 ± 498	1,310 ± 521	72.6 ± 28.9	25.7 ± 8.15	0.75 ± 0.61
		30	43,800 ± 23,300	1,460 ± 777	910 ± 433	30.3 ± 14.4	43.9 ± 12.5 <sup>a</sup>	12.8 ± 18.0

NC = not calculated; <sup>a</sup> Parameter not calculated in animals #118 (9 mg/kg, Day 1); #140, 141, 142, and 147 (30 mg/kg, Day 1), and #141 (30 mg/kg, Day 25) due to insufficient number of data points during the terminal phase to estimate a reliable plasma half-life

As noted in the table above for actual numbers and the table below for ratios, SKY0402 treated groups exhibited some non-dose responsive accumulation of bupivacaine in plasma. Comparison of the PK systemic exposure data for Day 1 and Day 25 did not reveal any remarkable or consistent differences in the concentration-time curves or PK parameters between the two sampling periods for Sensorcaine or SKY0402. An accumulation ratio close to 1 was observed for Sensorcaine and close to 2 for SKY0402. This consistency suggests that the animals appear to process bupivacaine similarly after the first dose and after 4 weeks of twice-weekly repeated dosing, especially for the Sensorcaine dosing with the ratio of close to 1.

**Toxicokinetic Parameter Ratios Based on Single and Multiple-Dose Data (Day 25 versus Day 1)**

Treatment	Bupivacaine (mg/kg)	Ratio (Day 25 vs. Day 1)			
		AUC <sub>0-tlast</sub> (hr•ng/mL)	C <sub>max</sub> (ng/mL)	t <sub>1/2</sub> (hr)	t <sub>max</sub> (hr)
Sensorcaine	9	0.94	1.40	0.60	1.0
	9	1.90	2.46	0.61	1.0
SKY0402	18	1.90	2.34	0.25	1.5
	30	1.71	1.44	1.38	0.26

## Dosing Solution Analysis

The Sponsor provided documentation that the test article, SKY0402, was a suspension, homogeneous, and stable for the duration of the study (28 days); therefore, test article analysis was not performed.

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**Study title:** A 28-Day Subcutaneous Injection Toxicity Study in Sprague Dawley Rats with a 14-Day Recovery Period with **SKY0402 Placebo**

Study no.: LAB00001

Study report location: eCTD DARRTS SDN-1

Conducting laboratory and location:

(b) (4)

Date of study initiation: March 25, 2004

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity:

- SKY0402 Placebo, Lot No. 25BUP02-019, 76.2% (packed particle volume)
- 0.9% NaCl, Lot No. J#D629, 0.9%

## Key Study Findings

- Groups of ten male and female rats were subcutaneously dosed daily for 4 weeks with SKY0402 Placebo or 0.9% NaCl at a dose volume of 5 mL/kg. The dose was based on maximum practicable dose possible. Groups of 5/sex were also included for a 14-day recovery period.
- No treatment-related effects were observed for any evaluated parameters other than anticipated injection site effects. The incidence and severity of these effects were reduced after the recovery period suggesting reversibility.
- Test article-related, microscopic changes were observed only at the injection sites. Chronic panniculitis (inflammation) was characterized by granulomatous inflammation and granulomas in animals treated with SKY0402 placebo. In control animals, the inflammation was characterized by a minimal or mild increase in connective tissue. Mean severity scores for chronic panniculitis for test article-treated animals were higher than for control animals. The severity of both treatment groups in both sexes decreased after recovery. The higher mean severity for the test article-treated group compared to the control group persisted after 14 days of recovery, although there was some recovery.
- On this basis, only injection site effects are anticipated in humans at a dose volume of SKY0402 Placebo up to 5 mL/kg based on findings in rats. The largest proposed human dose volume is (b) (4)

## Methods

## Doses:

Group	No. of Animals		Dosage Material	Dosage Level (mL/kg/day)	Dosage Volume (mL/kg)
	Male	Female			
1	10(5)	10(5)	Vehicle Control (Normal Saline)	5.0	5.0
2	10(5)	10(5)	SKY0402 Placebo	5.0	5.0

Note: Animals designated within the parentheses "( )" were utilized for a 14-day drug-free recovery phase.

Frequency of dosing: 1x/day for 28 days

Injections were administered into the scapular region and were rotated daily in a clock-wise manner to a total of four different test areas

Route of administration: Subcutaneous

Dose volume: 5 mL/kg

- The dose was based on maximum recommended dose volume (Diehl et al. J. Appl. Toxicol. 21:15-23, 2001).

Formulation/Vehicle: 0.9% NaCl

Species/Strain: male and female Sprague Dawley  
Crl:CD®(SD)IGS BR rats

Number/Sex/Group: 10/sex/group for main study

Age: ~8 weeks old at study initiation

Weight: 225 to 281 grams for the males and 177 to 214 grams for the females

Satellite groups: 5/sex/group for 14-day recovery groups

Unique study design: Single subcutaneous dose is described as largest practical dose based on previous studies

Deviation from study protocol: Only single dose tested

## Observations and Results

The animals were checked for general health/mortality and moribundity twice daily, in the morning and afternoon. The rats were examined weekly and on the day of scheduled euthanasia for clinical signs of toxicity (detailed clinical observations). In addition, the animals were examined daily for overt toxic effects (cage-side observations) between one-half hour and two hours following dosing. During the recovery phase, detailed clinical observations were performed weekly and cage-side observations were performed daily, except on the days when detailed clinical observations were performed.

### Mortality – none

**Clinical Signs** - The most notable clinical observation was apparent test article retention at the injection site, seen in all test article-treated males and females during

the dosing phase. The incidence of test article retention at the injection site was much lower in the test article-treated males and females during the recovery phase. No other remarkable clinical observations were noted. The subcutaneous injection was well-tolerated in all animals in the control and test article-treated groups.

**Body Weights** - Individual body weights were recorded weekly beginning on Study Day SD) 0. A final fasted body weight was recorded prior to scheduled euthanasia on SD 28 (end of dosing phase) or SD 43 (end of recovery phase).

The only notable observation was a statistically significant decrease in treated male mean body weight gain compared to control during the first week of recovery. This was not observed during the second week of recovery and the toxicological relevance is unknown.

A 28-DAY SUBCUTANEOUS INJECTION TOXICITY STUDY IN RATS WITH A 14-DAY RECOVERY PERIOD					
MALES			SUMMARY OF BODY WEIGHT CHANGES (GRAMS) (RECOVERY PHASE)		
GROUP: LEVEL (ML/KG/DAY) :			1	2	
			5.0	5.0	
DAY	27 TO	34	MEAN	28 t	12*
			S. D.	9.5	7.2
			N	5	5
DAY	34 TO	41	MEAN	22 t	19
			S. D.	5.4	5.5
			N	5	5

STATISTICAL KEY: t=ANOVA/TUKEY-KRAMER \* = P<0.05

**Feed Consumption** - Individual food consumption was measured weekly on the same days as weekly body weights. Nothing treatment related.

**Ophthalmoscopy** - An ocular examination was performed on all animals once prior to in-life initiation (Day -6) and at the end of the dosing phase (SD 28). No test article-related ocular findings were observed in the males or females at the end of the dosing phase (SD 28).

**ECG – none**

**Clinical Pathology Evaluations** - Blood and urine samples were collected from all animals on the day of scheduled euthanasia (SD 28/end of dosing phase or SD 43/end of recovery phase) for evaluation of selected hematology, clinical chemistry and urinalysis parameters. Feed was withheld overnight prior to blood collection; however, water was available. Urine samples were collected overnight prior to the initiation of blood collection.

**Hematology and Coagulation** – parameters evaluated:

Erythrocyte count (RBC)	Hemoglobin concentration (Hgb)
Hematocrit (Hct)	Mean corpuscular hemoglobin (MCH)

Mean corpuscular hemoglobin concentration (MCHC)  
 Mean corpuscular volume (MCV)  
 Platelet count  
 Reticulocyte count

Total and differential leukocyte counts (including RBC morphology)  
 Activated partial thromboplastin time (APTT)  
 Prothrombin time (PT)

Nothing treatment related was observed.

**Clinical Chemistry** – parameters evaluated:

Alanine aminotransferase (ALT)  
 Albumin  
 Albumin/globulin ratio (calculated)  
 Alkaline phosphatase  
 Aspartate aminotransferase (AST)  
 Blood creatinine  
 Blood urea nitrogen (BUN)  
 Calcium  
 Cholesterol

Electrolytes (sodium, potassium and chloride)  
 Gamma glutamyl transferase (GGT)  
 Globulin (calculated)  
 Glucose  
 Phosphorus  
 Total bilirubin  
 Total serum protein

Nothing treatment related observed.

**Urinalysis** – parameters evaluated:

Bilirubin	Ketones	pH
Blood	Leukocytes	Protein
Glucose	Microscopic examination of spun deposit	Specific gravity
Gross appearance	Nitrites	Urobilinogen
		Volume

Nothing treatment related observed.

**Gross Pathology** - All animals were subjected to a complete gross necropsy examination at scheduled euthanasia. Main study animals were euthanized on SD 28 and the recovery animals were euthanized on SD 43. The necropsy examination included evaluation of the external surfaces of the body, all orifices, and the cranial, thoracic, abdominal and pelvic cavities and their contents. The animals were fasted overnight prior to scheduled euthanasia.

With the exception of the bone marrow smear, the following organs/tissues were preserved from all animals in 10% neutral buffered formalin for histopathological examination:

Accessory genital organs (epididymides, seminal vesicles and prostate or uterus and vagina)  
 Brain (including sections of medulla/pons, cerebellar cortex and cerebral cortex)

Adrenals	Mammary gland
All gross lesions	Mediastinal lymph node
Aorta	Mesenteric lymph node
Bone marrow smear (femur)	Pancreas
Cecum	Peripheral nerve (sciatic)
Colon	Pituitary
Duodenum	Rectum
Esophagus	Skeletal muscle (thigh)
Exorbital lachrymal glands	Skin
Eyes	Spinal cord (cervical, midthoracic and lumbar)
Femur (including articular surface) and bone marrow	Spleen
Heart	Stomach (glandular/nonglandular)
Ileum	Submandibular lymph node
Injection site [subcutaneous site (encompassed all injection sites)]	Submaxillary salivary gland
Jejunum	Testes/ovaries
Kidneys	Thymus
Liver (three sections collected)	Thyroids/parathyroids
Lungs (infused with formalin) with bronchi	Tongue
	Trachea
	Urinary bladder

Note: Bone marrow smears were prepared at scheduled necropsies but examination was not deemed necessary by the Veterinary Pathologist.

The only remarkable gross necropsy findings were observed at the injection site for test article-treated males and females at the end of the dosing phase (SD 28) and at the end of the recovery phase (SD 43). Dosing site observations included white material (8 of 10 males and 9 of 10 females from main study; 4 of 5 males and 5 of 5 females from recovery groups) and raised areas (7 of 10 males and 3 of 10 females from main study; 3 of 5 males and 2 of 5 females from recovery groups).

**Organ Weights** - Fresh organ weights were obtained at scheduled euthanasia for the adrenal glands, brain, heart, kidneys, liver, ovaries, pituitary, spleen, testes, thymus and thyroid/parathyroid. Paired organs were weighed together.

No treatment related effects observed.

**Histopathology** - All tissues collected at necropsy from all animals were processed for histopathological examination.

Adequate Battery - yes

Peer Review - no

**Histological Findings** - Test article-related, microscopic changes were observed only at the injection sites. Chronic panniculitis was characterized by granulomatous

inflammation and granulomas at the injection site in animals treated with SKY0402 placebo. In control animals, the inflammation was characterized by a minimal or mild increase in connective tissue. Mean severity scores for chronic panniculitis for test article-treated animals were higher than for control animals. The severity of both treatment groups in both sexes decreased after recovery. The higher mean severity for the test article-treated group compared to the control group persisted after 14 days of recovery, although there was some recovery (group 1 – negative control; group 2 – SKY0402 Placebo).

		Group:		1		2		1		2	
		Necropsied on Day:		28	28	28	28	43	43	43	43
		Sex:		M	F	M	F	M	F	M	F
Diagnosis											
Injection Site		# Ex	10	10	10	10	5	5	5	5	
-Chronic panniculitis, minimal			10	8	-	-	1	2	1	1	
-Chronic panniculitis, mild			-	-	1	7	-	-	3	4	
-Chronic panniculitis, moderate			-	-	9	3	-	-	1	-	

**Special Evaluation - none**

**Toxicokinetics - none**

**Dosing Solution Analysis**

The test article, SKY0402 placebo, was used as received from the Sponsor and no adjustment was made for purity. The test article was administered neat (undiluted). Documentation concerning chemical identity, purity, strength, stability and other required data was the responsibility of the Sponsor. All information on the methods of synthesis and stability and data on the composition and other characteristics that define the test article are on file with the Manufacturer or Sponsor.

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**Study title:** A 4-Week Subcutaneous Toxicity Study of **SKY0402 Placebo** in Dogs

Study no.: (b) (4) 947-024  
Study report location: eCTD DARRTS SDN-1  
Conducting laboratory and location: (b) (4)  
Date of study initiation: January 11, 2005  
GLP compliance: yes  
QA statement: yes  
Drug, lot #, and % purity: - SKY0402 Placebo, Lot Nos. 04PD006 & 04PD007, 40.7% & 46.4% (packed particle volume)  
- 0.9% NaCl, Lot Nos. 21-157-JT, 21-044-JT & 23-209-JT, 0.9%

**Key Study Findings**

- Groups of four male and female dogs were subcutaneously dosed daily for 4 weeks with SKY0402 Placebo or 0.9% NaCl at 2.0 mL/kg to two of six injection sites per day. The dose was based on maximum recommended dose volume (Diehl et al. J. Appl. Toxicol. 21:15-23, 2001). Groups of 5/sex were also included for a 28-day recovery period.
- No other treatment related effects were observed for any other evaluated parameters other than anticipated injection site effects. Only injection site microscopic effects were observed to have occurred. Test article-related microscopic changes in main study animals were identified in both sexes and included accumulations of vacuolated macrophages, mineralization, granulomatous inflammation, edema, and hemorrhage. In recovery animals, the edema and hemorrhage were not observed and the incidence and/or severity of other injection site effects were reduced/reversing suggesting reversibility.
- On this basis, only injection site effects are anticipated in humans at a dose volume of SKY0402 Placebo up to 2 mL/kg based on findings in dogs. The largest proposed human dose volume is (b) (4)

## Methods

Doses:

Group Assignments			
Group Number	Formulation <sup>a</sup>	Number of Animals <sup>b</sup>	
		Male	Female
1	0.9% NaCl	6	6
2	SKY0402 Placebo	6	6

<sup>a</sup>Dose volume was 2 mL/kg/site (2 sites total/day)  
<sup>b</sup>Two animals/sex were maintained for a 28 day recovery period.

Frequency of dosing: 2 of 6 sites/day at same time of day for 28 days  
Route of administration: Subcutaneous to dorsal surface  
Dose volume: 2 mL/kg/site (2 sites/day)  
- The dose was based on maximum recommended dose volume (Diehl et al. J. Appl. Toxicol. 21:15-23, 2001).  
Formulation/Vehicle: NaCl  
Species/Strain: Beagle dogs  
Number/Sex/Group: 4/sex/group  
Age: 5-5.5 months old at receipt  
Weight: 7.81- 9.97 kg (males) & 5.49 - 7.48 kg (females)  
Satellite groups: 2/sex/group for 28 day recovery  
Unique study design: no  
Deviation from study protocol: Nothing significant

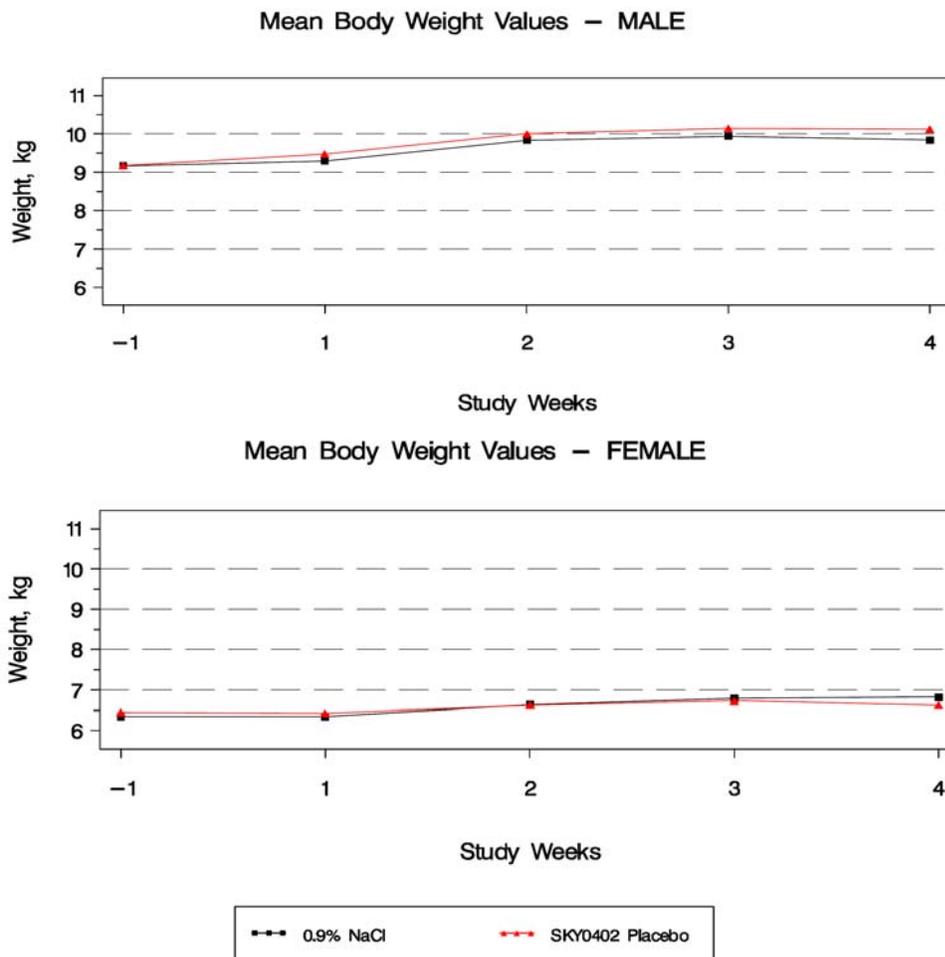
**Observations and Results**

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily throughout the duration of the study. A detailed clinical examination of each animal was performed once during each study week. A complete physical examination was conducted on all animals pretest.

**Mortality** - none

**Clinical Signs** – nothing treatment related.

**Body Weights** - Body weights for all animals were measured on the day of arrival, prior to randomization, and weekly during the study. No treatment related body weight effects occurred.



**Feed Consumption** - Food consumption was measured weekly during the study. No treatment related food consumption effects occurred.

**Ophthalmoscopy** - Ophthalmoscopic examinations were conducted on all animals pretest and prior to each scheduled necropsy. No treatment related effects occurred.

**ECG** - All animals received an electrocardiographic examination (10 lead) pretest, prior to dosing, 1 hour post dose during Week 4 (Day 27), and prior to the recovery necropsy. No treatment related effects occurred.

**Clinical Pathology Evaluations** - Clinical pathology evaluations were conducted on all animals pretest and prior to the terminal and recovery necropsies. The animals had access to drinking water, but were fasted overnight prior to sample collection.

## Hematology

The following parameters were evaluated:

Leukocyte Count	Platelets	Prothrombin Time (PT)
Erythrocyte Count	Differential Leukocyte	Activated Partial
Hemoglobin	Count	Thromboplastin
Hematocrit	Absolute Reticulocytes	Time (APTT)
MCV, MCH, MCHC	Percent Reticulocytes	

No treatment related effects occurred.

## Clinical Chemistry

The following parameters were evaluated:

Sodium	Gamma	Urea Nitrogen
Potassium	Glutamyltransferase	Creatinine
Chloride	(GGT)	Total Protein
Calcium	Aspartate	Albumin
Phosphorus	Aminotransferase (AST)	Globulin
Alkaline Phosphatase	Alanine	Albumin/Globulin (A/G)
Total Bilirubin	Aminotransferase (ALT)	Cholesterol
	Sorbitol Dehydrogenase	Glucose
	(SDH)	

No treatment related effects occurred.

## Urinalysis

The following parameters were evaluated:

Color and Appearance	pH	Bilirubin
Volume	Protein	Occult Blood
Specific Gravity	Glucose	Urobilinogen
Microscopic Elements	Ketones	

No treatment related effects occurred. There was an increased specific gravity (SG) in SKY0402 Placebo males appropriate relative to the urine volume.

**Gross Pathology** - Complete necropsy examinations were performed at the scheduled terminal and recovery necropsies. The animals were examined carefully for external abnormalities, including masses. The skin was reflected from a ventral midline incision, and any abnormalities were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and placed in fixative. All designated tissues were fixed in neutral buffered formalin, except for the eye (including the optic nerve) and testes were

fixed using a modified Davidson's fixative. Formalin was infused into the lung via the trachea. A full complement of tissues and organs was collected from all animals.

The following list constitutes the full complement of organs and tissues for this study:

- |   |   |
|---|---|
| <ul style="list-style-type: none"> <li>- Adrenal (2) #</li> <li>- Aorta</li> <li>- Bone with marrow [femur]</li> <li>- Bone with marrow [rib]</li> <li>- Bone with marrow [sternum]</li> <li>- Bone marrow smear*</li> <li>- Brain [cerebrum, midbrain, cerebellum, medulla/pons] #</li> <li>- Epididymis #</li> <li>- Esophagus</li> <li>- Eye including optic nerve (2)</li> <li>- Gallbladder</li> <li>- Gastrointestinal tract: <ul style="list-style-type: none"> <li>stomach [cardia, fundus, and pylorus]</li> <li>duodenum</li> <li>jejunum</li> <li>ileum</li> <li>cecum</li> <li>colon</li> <li>rectum</li> </ul> </li> <li>- Gonads: <ul style="list-style-type: none"> <li>ovary (2) #</li> <li>testis (2) #</li> </ul> </li> <li>- Gross lesions</li> <li>- Heart #</li> <li>- Injection sites</li> <li>- Joint, tibiofemoral</li> <li>- Kidney (2) #</li> </ul> | <ul style="list-style-type: none"> <li>- Larynx</li> <li>- Liver [3 sections collected; 2 examined] #</li> <li>- Lung [2 sections examined] #</li> <li>- Lymph node, mandibular [2 collected; 1 examined]</li> <li>- Lymph node, mesenteric</li> <li>- Lymph node, tracheobronchial</li> <li>- Mammary gland [process females only]</li> <li>- Nictitans gland</li> <li>- Pancreas #</li> <li>- Peyer's patch</li> <li>- Pituitary #</li> <li>- Prostate #</li> <li>- Salivary gland, mandibular [2 collected; 1 examined] #</li> <li>- Salivary gland, parotid</li> <li>- Salivary gland, sublingual</li> <li>- Sciatic nerve</li> <li>- Skeletal muscle, biceps femoris</li> <li>- Skin</li> <li>- Spinal cord [cervical, thoracic, and lumbar]</li> <li>- Spleen #</li> <li>- Thymus #</li> <li>- Thyroid/parathyroid (2) #</li> <li>- Tongue</li> <li>- Trachea</li> <li>- Urinary bladder</li> <li>- Uterus with cervix #</li> <li>- Vagina</li> </ul> |
|---|---|

\*Collected at scheduled necropsies and held  
# Organ weighed  
(2) Paired organ

Observed macroscopic effects were limited to the injection sites. Test article-related, minimal to moderate thickening of the injection sites was frequently observed in animals of both sexes treated with SKY0402 Placebo. Similar changes were not observed in animals treated with saline. Thickened injection sites typically corresponded microscopically with accumulation of vacuolated macrophages. Mild to moderate red discoloration was occasionally observed in the injection sites of saline control animals, as well as one animal treated with SKY0402 Placebo. Red discoloration correlated microscopically with hemorrhage that was likely due to trauma associated with injection procedures. Effects were reversible as no treatment related effects were observed at recovery necropsy.

**Organ Weights** - Body weights and listed organ weights (see table above) were recorded for all surviving animals at the scheduled necropsy and appropriate organ weight ratios were calculated (relative to body and brain weights). Paired organs were weighed together except that only the right mandibular salivary gland was weighed, and a combined weight of the thyroid/bilateral parathyroid glands was obtained.

No treatment related effects occurred.

**Histopathology** - Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections was performed on sections of tissues from all animals.

Adequate Battery - yes

Peer Review - no

Histological Findings – Only injection site microscopic effects were observed to have occurred. Test article-related microscopic changes in main study animals were identified in both sexes and included accumulations of vacuolated macrophages, mineralization, granulomatous inflammation, edema, and hemorrhage. In recovery animals, the edema and hemorrhage were not observed and the incidence and/or severity of other injection site effects were reduced/reversing. Results for one of the six injection sites in main study (terminal) and recovery group males is included for illustration purposes (see table on next page).

In main study animals, minimal to severe accumulations of vacuolated macrophages were present in all injection sites of all animals treated with SKY0402 Placebo but not saline controls. Abundant, poorly-defined, clear cytoplasmic vacuoles and small numbers of lymphocytes within the subcutis were also observed. Occasionally, test article related minimal to mild foci of mineralization was associated with minimal to mild granulomatous inflammatory cell infiltrate. Granulomatous inflammation was characterized by small numbers of macrophages and multinucleated giant cells with variable quantities of intracellular and extracellular basophilic mineralized material.

In recovery animals, minimal to mild accumulation of vacuolated macrophages was observed in all injection sites of all SKY0402 Placebo animals but not saline controls. Macrophages were similar in appearance to that noted in main study animals, however, they were often slightly smaller and flattened with less prominent cytoplasmic vacuolation. The severity of cellular infiltrates was reduced compared to main study animals indicating reversibility of effects. Minimal mineralization and granulomatous inflammation similar to that seen at the terminal necropsy were occasionally noted only in the injection sites of SKY0402 Placebo treated males at a reduced incidence of main study animals. Hemorrhage and edema were not present in the injection sites of animals at the recovery necropsy.

**Summary of Microscopic Observations - MALE**

		Terminal	
		0.9% NaCl	SKY0402 Placebo
Tissue Observation	Severity		
Number of Animals Examined		4	4
edema	- minimal	0	3
fibrosis	- minimal	1	0
hemorrhage	- minimal	0	1
hyperplasia, epidermal	- minimal	0	1
inflammation, subacute	- minimal	2	1
macrophages, vacuolated		0	4
	- minimal	0	1
	- moderate	0	3
mineralization	- minimal	0	1
within normal limits		2	0

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**Summary of Microscopic Observations - MALE**

		Recovery	
		0.9% NaCl	SKY0402 Placebo
Tissue Observation	Severity		
Number of Animals Examined		2	2
<b>injection site 3</b>		(2)	(2)
exudate, epidermal surface	- minimal	1	0
inflammation, subacute	- minimal	1	0
macrophages, vacuolated	- minimal	0	2

**Special Evaluation – none**

**Toxicokinetics – none**

**Dosing Solution Analysis**

The test article, SKY0402 placebo, was used as received from the Sponsor and no adjustment was made for purity. The test article was administered neat (undiluted). The Sponsor has provided documentation of the strength, stability, purity, and composition for the lots of test article used on study. Documentation of the strength, purity, composition, stability, and method of synthesis, fabrication, and/or derivation for each lot of control article used on study was limited to that information listed on the label of this commercially available product.

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## 7 Genetic Toxicology

### 7.1 *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames)

**Study title:** SKY0402 Placebo: Bacterial Mutation Test

Study no.: (b) (4) 960255

Study report location: eCTD DARRTS SDN-1

Conducting laboratory and location: (b) (4)

Date of study initiation: February 20, 2004

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity:

- SKY0402 Placebo, Lot 25BUP02-019 (PD-019), 76.2% packed particle volume
- 0.9% NaCl, Lot W3F12B2, 0.9%

#### Key Study Findings

- SKY0402 Placebo was not genotoxic in a valid in vitro Ames assay up to the limit dose of 5000 µg/plate using plate incorporation and preincubation methods +/- rat S9 metabolizing enzymes.

## Methods

Strains: *S. typhimurium* TA1535 *hisG46 rfa ΔuvrB*  
*S. typhimurium* TA1537 *hisC3076 rfa ΔuvrB*  
*S. typhimurium* TA98 *hisD3052 rfa ΔuvrB*  
 pKM101  
*S. typhimurium* TA100 *hisG46 rfa ΔuvrB*  
 pKM101  
*E. coli* WP2 *trp uvrA*

Concentrations in definitive study:

Material	Conc.*		No. of replicates		No. of strains
	( $\mu$ L/plate)	( $\mu$ g/plate)	No S9	+S9	
<i>Initial test (plate incorporation method)</i>					
Saline	-	-	3	3	5
SKY0402 Placebo	0.082	1.58	3	3	5
	0.259	5.0	3	3	5
	0.819	15.8	3	3	5
	2.59	50	3	3	5
	8.19	158	3	3	5
	25.9	500	3	3	5
	81.9	1581	3	3	5
	259	5000	3	3	5
Positive control		‡	3	3	5
<i>Confirmatory test (pre-incubation method)</i>					
Saline	-	-	3	3	5
SKY0402 Placebo	0.819	15.8	3	3	5
	2.59	50	3	3	5
	8.19	158	3	3	5
	25.9	500	3	3	5
	81.9	1581	3	3	5
	259	5000 †	3	3	5
	Positive control		‡	3	3

\* In terms of material as supplied ( $\mu$ L) and total lipid content ( $\mu$ g) where 5000  $\mu$ g/plate is the maximum recommended concentration for this test (OECD 1997)

† Estimated lowest toxic or limit dose level based on the results of the initial test

‡ Depends on the test organism and the positive control agent used, refer to results tables

Basis of concentration selection: Limit dose of 5000  $\mu$ g/plate with decreased doses of  $\sim 1/3$  (approximate log dose reductions)

Negative control: NaCl

Positive control: Appropriate for each strain

Formulation/Vehicle: High dose is neat material then diluted with 0.9% NaCl to achieve other doses

Incubation & sampling time: 48-72 hours

Dosing materials: Chemical analysis of dilutions of the test article formulation for concentration,

homogeneity, and stability was not performed by the sponsor as part of this study. However, these parameters were determined by the Sponsor for the undiluted material.

### **Study Validity – the study was considered valid**

The plates from at least five non-toxic dose levels of the test article were assessed in each experiment, *i.e.* the five highest levels below the toxic level.

The mean revertant colony counts of the vehicle controls for each strain were close or within the current historical control range of the laboratory. All positive control articles produced increases in revertant colony numbers to at least twice the concurrent vehicle control levels with the appropriate bacterial strain (1.5x for strain TA100).

**Results** - Based on the following test results, SKY0402 Placebo was not genotoxic +/- S9 metabolizing enzymes (see summary tables for plate incorporation and preincubation tests below).

The test article did not cause any notable increase in the revertant colony counts of any strain in the initial or confirmatory test in either the absence or presence of S9 mix. Except for toxicity (*i.e.*, reduced colony count) observed in the confirmatory test with strain TA1537 at the highest dose level (5000 µg/plate) in the absence of S9 mix no visible thinning of the background lawn of non-revertant bacteria was obtained following exposure to SKY0402 Placebo in the initial or confirmatory test. Precipitation on plates was observed in both the initial and confirmatory tests at the three highest dose levels of the test article. In the confirmatory test, replica plating was used for plates exposed to the highest dose level (5000 µg/plate) to clearly distinguish between revertant colonies and precipitate of the test article.

Initial plate incorporation test results (see tables next page):

**SKY0402 Placebo — Initial (plate incorporation) test in the absence of S9**

Strain	Conc.@ (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	mean	SD	<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	
TA1535	saline	0	25	31	21	<b>26</b>	5				1.0
	50	0	32	22	25	<b>26</b>	5				1.0
	158	0	22	20	20	<b>21</b>	1				0.8
	500	0	20	26	25	<b>24</b>	3	ppt	ppt	ppt	0.9
	1581	0	29	29	27	<b>28</b>	1	ppt	ppt	ppt	1.1
	5000	0	17	19	19	<b>18</b>	1	ppt	ppt	ppt	0.7
TA1537	saline	0	17	13	16	<b>15</b>	2				1.0
	50	0	11	16	21	<b>16</b>	5				1.0
	158	0	15	17	20	<b>17</b>	3				1.1
	500	0	23	21	20	<b>21</b>	2	ppt	ppt	ppt	1.4
	1581	0	25	21	22	<b>23</b>	2	ppt	ppt	ppt	1.5
	5000	0	7	19	19	<b>15</b>	7	ppt	ppt	ppt	1.0
TA98	saline	0	28	22	22	<b>24</b>	3				1.0
	50	0	32	32	26	<b>30</b>	3				1.3
	158	0	29	23	30	<b>27</b>	4				1.1
	500	0	23	47	30	<b>33</b>	12	ppt	ppt	ppt	1.4
	1581	0	29	20	23	<b>24</b>	5	ppt	ppt	ppt	1.0
	5000	0	32	32	30	<b>31</b>	1	ppt	ppt	ppt	1.3
TA100	saline	0	140	125	134	<b>133</b>	8				1.0
	50	0	147	150	127	<b>141</b>	13				1.1
	158	0	126	119	171	<b>139</b>	28				1.0
	500	0	135	119	141	<b>132</b>	11	ppt	ppt	ppt	1.0
	1581	0	135	135	146	<b>139</b>	6	ppt	ppt	ppt	1.0
	5000	0	145	124	152	<b>140</b>	15	ppt	ppt	ppt	1.1
WP2 <i>uvrA</i>	saline	0	30	32	21	<b>28</b>	6				1.0
	50	0	28	22	27	<b>26</b>	3				0.9
	158	0	22	23	33	<b>26</b>	6				0.9
	500	0	29	27	16	<b>24</b>	7	ppt	ppt	ppt	0.9
	1581	0	20	19	32	<b>24</b>	7	ppt	ppt	ppt	0.9
	5000	0	44	42	38	<b>41</b>	3	ppt	ppt	ppt	1.5

@ Expressed in terms of total lipid  
 \* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)  
 † Fold response in mean revertants compared to concurrent vehicle control  
 SD Sample standard deviation

**SKY0402 Placebo — Initial (plate incorporation) test in the presence of S9**

Strain	Conc.@ (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	mean	SD	<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	
TA1535	saline	+	17	11	29	<b>19</b>	9				1.0
	50	+	27	30	28	<b>28</b>	2				1.5
	158	+	25	28	33	<b>29</b>	4				1.5
	500	+	36	26	19	<b>27</b>	9	ppt	ppt	ppt	1.4
	1581	+	36	26	31	<b>31</b>	5	ppt	ppt	ppt	1.6
	5000	+	20	25	27	<b>24</b>	4	ppt	ppt	ppt	1.3
TA1537	saline	+	17	22	17	<b>19</b>	3				1.0
	50	+	19	19	20	<b>19</b>	1				1.0
	158	+	25	21	18	<b>21</b>	4				1.1
	500	+	18	15	13	<b>15</b>	3	ppt	ppt	ppt	0.8
	1581	+	30	33	25	<b>29</b>	4	ppt	ppt	ppt	1.6
	5000	+	8	15	13	<b>12</b>	4	ppt	ppt	ppt	0.6
TA98	saline	+	23	40	44	<b>36</b>	11				1.0
	50	+	49	48	42	<b>46</b>	4				1.3
	158	+	54	42	48	<b>48</b>	6				1.3
	500	+	37	31	51	<b>40</b>	10	ppt	ppt	ppt	1.1
	1581	+	52	44	38	<b>45</b>	7	ppt	ppt	ppt	1.3
	5000	+	37	49	39	<b>42</b>	6	ppt	ppt	ppt	1.2
TA100	saline	+	100	114	131	<b>115</b>	16				1.0
	50	+	142	185	129	<b>152</b>	29				1.3
	158	+	125	124	123	<b>124</b>	1				1.1
	500	+	123	118	154	<b>132</b>	20	ppt	ppt	ppt	1.1
	1581	+	133	148	137	<b>139</b>	8	ppt	ppt	ppt	1.2
	5000	+	145	136	176	<b>152</b>	21	ppt	ppt	ppt	1.3
WP2 <i>uvrA</i>	saline	+	21	29	26	<b>25</b>	4				1.0
	50	+	38	31	28	<b>32</b>	5				1.3
	158	+	37	25	33	<b>32</b>	6				1.3
	500	+	41	31	35	<b>36</b>	5	ppt	ppt	ppt	1.4
	1581	+	16	30	25	<b>24</b>	7	ppt	ppt	ppt	0.9
	5000	+	41	31	27	<b>33</b>	7	ppt	ppt	ppt	1.3

@ Expressed in terms of total lipid  
 \* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)  
 † Fold response in mean revertants compared to concurrent vehicle control  
 SD Sample standard deviation

**Positive controls for the initial (plate incorporation) test**

Strain	Treatment	Conc. (µg/plate)	S9	Number of Revertants					SD	Fold response †
				<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	mean			
TA1535	NaAz	0.5	0	299	294	291	<b>295</b>	4	11	
TA1537	9AC	50	0	716	762	648	<b>709</b>	57	46	
TA98	2NF	1	0	202	238	259	<b>233</b>	29	9.7	
TA100	NaAz	0.5	0	670	573	633	<b>625</b>	49	4.7	
WP2 <i>uvrA</i>	NQO	1.5	0	735	790	806	<b>777</b>	37	28	
TA1535	2AA	5	+	305	328	323	<b>319</b>	12	17	
TA1537	BaP	5	+	119	97	141	<b>119</b>	22	6.4	
TA98	BaP	5	+	481	472	386	<b>446</b>	52	13	
TA100	BaP	5	+	1146	835	918	<b>966</b>	161	8.4	
WP2 <i>uvrA</i>	2AA	15	+	459	509	435	<b>468</b>	38	18	

† Fold response in mean revertants compared to concurrent vehicle control  
SD Sample standard deviation

Confirmatory preincubation test results:

**SKY0402 Placebo — Confirmatory (pre-incubation) test in the absence of S9**

Strain	Conc.@ (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	mean	SD	<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	
TA1535	saline	0	20	21	26	<b>22</b>	3				1.0
	50	0	31	32	19	<b>27</b>	7				1.2
	158	0	19	20	21	<b>20</b>	1				0.9
	500	0	28	32	20	<b>27</b>	6	ppt	ppt	ppt	1.2
	1581	0	26	17	23	<b>22</b>	5	ppt	ppt	ppt	1.0
	5000	0	22	19	20	<b>20</b>	2	ppt	ppt	ppt	0.9 ‡
TA1537	saline	0	19	16	23	<b>19</b>	4				1.0
	15.8	0	10	15	15	<b>13</b>	3				0.7
	50	0	11	16	16	<b>14</b>	3				0.7
	158	0	21	15	11	<b>16</b>	5				0.8
	500	0	21	20	20	<b>20</b>	1	ppt	ppt	ppt	1.1
	1581	0	15	10	17	<b>14</b>	4	ppt	ppt	ppt	0.7
TA98	5000	0	11	11	8	<b>10</b>	2	ppt	ppt	ppt	0.5 T ‡
	saline	0	36	33	21	<b>30</b>	8				1.0
	50	0	32	44	40	<b>39</b>	6				1.3
	158	0	34	34	34	<b>34</b>	0				1.1
	500	0	28	35	24	<b>29</b>	6	ppt	ppt	ppt	1.0
	1581	0	37	41	25	<b>34</b>	8	ppt	ppt	ppt	1.1
TA100	5000	0	19	23	24	<b>22</b>	3	ppt	ppt	ppt	0.7 ‡
	saline	0	127	136	108	<b>124</b>	14				1.0
	50	0	115	128	106	<b>116</b>	11				0.9
	158	0	146	145	119	<b>137</b>	15				1.1
	500	0	133	147	147	<b>142</b>	8	ppt	ppt	ppt	1.2
	1581	0	151	144	154	<b>150</b>	5	ppt	ppt	ppt	1.2
WP2 <i>uvrA</i>	5000	0	74	- <sup>a</sup>	100	<b>87</b>	18	ppt	ppt	ppt	0.7 ‡
	saline	0	20	24	20	<b>21</b>	2				1.0
	50	0	23	23	27	<b>24</b>	2				1.1
	158	0	25	28	20	<b>24</b>	4				1.1
	500	0	24	19	23	<b>22</b>	3	ppt	ppt	ppt	1.0
	1581	0	26	26	18	<b>23</b>	5	ppt	ppt	ppt	1.1
WP2 <i>uvrA</i>	5000	0	21	32	20	<b>24</b>	7	ppt	ppt	ppt	1.1 ‡

@ Expressed in terms of total lipid  
\* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)  
† Fold response in mean revertants compared to concurrent vehicle control  
SD Sample standard deviation (note that SDs based on two values may be unreliable)  
‡ Colony counts as per replica plating to distinguish from precipitate  
<sup>a</sup> Overlapping colonies prevented the count of individual colonies during replica plating

**SKY0402 Placebo — Confirmatory (pre-incubation) test in the presence of S9**

Strain	Conc.@ (µg/plate)	S9	Number of revertants					Plate observations *			Fold response †
			<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	mean	SD	<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	
TA1535	saline	+	23	23	16	<b>21</b>	4				1.0
	50	+	34	21	20	<b>25</b>	8				1.2
	158	+	25	19	23	<b>22</b>	3				1.1
	500	+	14	21	21	<b>19</b>	4	ppt	ppt	ppt	0.9
	1581	+	24	21	14	<b>20</b>	5	ppt	ppt	ppt	1.0
	5000	+	18	30	33	<b>27</b>	8	ppt	ppt	ppt	1.3 ‡
TA1537	saline	+	18	20	15	<b>18</b>	3				1.0
	50	+	19	19	17	<b>18</b>	1				1.0
	158	+	20	15	14	<b>16</b>	3				0.9
	500	+	17	11	17	<b>15</b>	3	ppt	ppt	ppt	0.8
	1581	+	10	16	17	<b>14</b>	4	ppt	ppt	ppt	0.8
	5000	+	18	13	9	<b>13</b>	5	ppt	ppt	ppt	0.8 ‡
TA98	saline	+	43	51	51	<b>48</b>	5				1.0
	50	+	31	47	50	<b>43</b>	10				0.9
	158	+	34	46	45	<b>42</b>	7				0.9
	500	+	49	42	52	<b>48</b>	5	ppt	ppt	ppt	1.0
	1581	+	52	49	44	<b>48</b>	4	ppt	ppt	ppt	1.0
	5000	+	36	41	34	<b>37</b>	4	ppt	ppt	ppt	0.8 ‡
TA100	saline	+	121	136	157	<b>138</b>	18				1.0
	50	+	97	119	118	<b>111</b>	12				0.8
	158	+	129	120	123	<b>124</b>	5				0.9
	500	+	113	123	116	<b>117</b>	5	ppt	ppt	ppt	0.9
	1581	+	140	132	129	<b>134</b>	6	ppt	ppt	ppt	1.0
	5000	+	88	93	76	<b>86</b>	9	ppt	ppt	ppt	0.6 ‡
WP2 <i>uvrA</i>	saline	+	27	29	23	<b>26</b>	3				1.0
	50	+	20	35	29	<b>28</b>	8				1.1
	158	+	25	32	24	<b>27</b>	4				1.0
	500	+	28	26	15	<b>23</b>	7	ppt	ppt	ppt	0.9
	1581	+	31	19	38	<b>29</b>	10	ppt	ppt	ppt	1.1
	5000	+	28	23	28	<b>26</b>	3	ppt	ppt	ppt	1.0 ‡

@ Expressed in terms of total lipid  
 \* Comments on the plate or background lawn if applicable: contamination (C), incomplete lawn (IL), no lawn (NL), not required (NR), poor lawn (PL), precipitate (ppt)  
 † Fold response in mean revertants compared to concurrent vehicle control  
 ‡ Colony counts as per replica plating to distinguish from precipitate  
 SD Sample standard deviation

**Positive controls for the confirmatory (pre-incubation) test**

Strain	Treatment	Conc. (µg/plate)	S9	Number of Revertants					Fold response †
				<i>x</i> <sub>1</sub>	<i>x</i> <sub>2</sub>	<i>x</i> <sub>3</sub>	mean	SD	
TA1535	NaAz	0.5	0	321	331	329	<b>327</b>	5	15
TA1537	9AC	50	0	398	516	307	<b>407</b>	105	21
TA98	2NF	1	0	179	179	153	<b>170</b>	15	5.7
TA100	NaAz	0.5	0	610	566	627	<b>601</b>	31	4.9
WP2 <i>uvrA</i>	NQO	1.5	0	1663	1805	1706	<b>1725</b>	73	81
TA1535	2AA	5	+	220	249	260	<b>243</b>	21	12
TA1537	BaP	5	+	118	59	112	<b>96</b>	32	5.5
TA98	BaP	5	+	315	316	282	<b>304</b>	19	6.3
TA100	BaP	5	+	1125	1270	1081	<b>1159</b>	99	8.4
WP2 <i>uvrA</i>	2AA	15	+	406	462	471	<b>446</b>	35	17

† Fold response in mean revertants compared to concurrent vehicle control  
 SD Sample standard deviation

Historical positive control data was consistent with results of this test (see table):

Historical positive control results							
Strain	Treatment	Conc. (µg/plate)	S9	Mean	SD	Range	
TA1535	NaAz	0.5	0	<b>260</b>	73	102	- 452
TA1537	9AC	50	0	<b>381</b>	270	53	- 2428
TA98	2NF	1	0	<b>189</b>	54	58	- 349
TA100	NaAz	0.5	0	<b>510</b>	120	199	- 725
WP2 <i>uvr:A</i>	NQO	1.5	0	<b>1089</b>	399	100	- 1926
TA1535	2AA	5	+	<b>277</b>	78	26	- 532
TA1537	BaP	5	+	<b>97</b>	20	48	- 164
TA98	BaP	5	+	<b>323</b>	80	148	- 855
TA100	BaP	5	+	<b>914</b>	189	487	- 1555
WP2 <i>uvr:A</i>	2AA	15	+	<b>344</b>	114	86	- 626

Results from non-GLP and QA-audited GLP studies from 27-Nov-2001 to prior to the present study.

## 7.2 *In Vitro* Assays in Mammalian Cells

**Study title: SKY0402 Placebo:** Chromosome Aberration Test

Study no.: (b) (4) 960331

Study report location: eCTD DARRTS SDN-1

Conducting laboratory and location: (b) (4)

Date of study initiation: April 1, 2004

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: - SKY0402 Placebo, lot 25BUP02-019 (PD-019), 76.2 % packed particle volume  
- 0.9% NaCl, lot W3F12B2

### Key Study Findings

- SKY0402 Placebo was not genotoxic in a valid in vitro chromosomal aberration assay using peripheral human lymphocytes up to the highest dose tested +/- rat S9 metabolizing enzymes. The high dose level (100 µL/mL) represents the test article dosed neat as supplied at the maximal practical volume of 500 µL per 5 mL of culture.

## Methods

Cell line: Peripheral human male blood lymphocytes

Concentrations in definitive study:

Group	Material	Final conc. ( $\mu\text{L}/\text{mL}$ ) ‡	Culture numbers		
			4 Hours (0S9)	4 Hours (+S9)	21 Hours (0S9)
1	Saline	-	2	2	2
2	SKY0402 Placebo	0.20	2	2	2
3		0.40	2	2	2
4		0.80	2	2	2
5		1.60	2	2	2
6		3.20	2	2	2
7		6.40	2	2	2
8		12.8	2	2	2
9	Mitomycin C	25.6	2	2	2
10		51.2	2	2	2
11		100	2	2	2
12A		0.05	2		2
12B	0.10	2		2	
12C	0.20	2		2	
12A	Cyclophosphamide	8.0		2	
12B		12		2	
12C		16		2	

‡ Expressed in terms of test article as supplied and 100  $\mu\text{L}/\text{mL}$  corresponds to the maximum practical dose volume. Final concentrations for Mitomycin C and Cyclophosphamide are in terms of  $\mu\text{g}/\text{mL}$ .

Note that the 21 hour incubation ('confirmatory phase') is required by regulatory authorities where the initial phase does not show any indication of genotoxicity for the test article; however, it is routinely performed at the same time as the initial phase to minimize potential delays in performance of the study.

- Basis of concentration selection: High dose is neat test article (100  $\mu\text{L}/\text{mL}$ ) with 50% dilutions of that level down to 0.2  $\mu\text{L}/\text{mL}$
- Negative control: NaCl
- Positive control: Mitomycin C in absence of S9 and cyclophosphamide in presence of S9
- Formulation/Vehicle: 0-.9% NaCl
- Incubation & sampling time: All treatments were performed approximately 48 hours after culture initiation. Cultures were treated as indicated in the study design then returned to the incubator for either 4 or 21 hours.
- Dosing material: Chemical analysis of dilutions of the test article formulation for concentration, homogeneity, and

stability was not performed by the sponsor as part of this study. However, these parameters were determined by the Sponsor for the undiluted material

### **Study Validity – study considered valid**

The vehicle/negative control results were within or close to the historical control range, while the positive control produced a significant increase in the incidence of aberrant cells compared with the concurrent control (see table).

### **Results**

SKY0402 Placebo did not show any evidence of genotoxic activity for the induction of chromosome damage in this in vitro test.

Cell toxicity was not observed at any dose level of test article using the 4 hour treatments +/- S9. Dose-related decreases in the mitotic indices were obtained after 21 hours exposure to the test article. On this basis, the highest dose level of the test article selected for detailed analysis was the highest dose evaluated, 100 µL/mL. The next two lower dose levels were also subjected to examination to yield three analyzable levels for each treatment regime as required by protocol.

SKY0402 Placebo did not cause any statistically significant increases in the proportion of aberrant metaphases at any experimental point (see table). In addition, the proportion of aberrant metaphases for all vehicle and test article groups was within the laboratory historical control range. The positive control agents caused large and highly significant increases in the proportion of aberrant metaphases in each phase of the study confirming the sensitivity of the test system and the effectiveness of the S9 mix (see table next page).

Summary of results and statistical analysis													
Treatment	Conc. ( $\mu\text{L}/\text{mL}$ ) ‡	MI	RMI (%)	No. cells examined	% Aberrant	No. of aberrations					Incidental observations		
						b	e	B	E	other	(g	G	P)
<i>4 hours treatment in the absence of S9 (0S9)</i>													
Saline	-	10.8	100	200	0	0	0	0	0	0	1	0	0
SKY0402 Placebo	25.6	9.4	87	200	0	0	0	0	0	0	0	0	1
	51.2	9.3	86	200	0	0	0	0	0	0	2	0	0
	100	10.5	97	200	0	0	0	0	0	0	2	0	0
Mitomycin C	0.20	10.4	96	200	17 **	21	13	4	0	0	11	2	0
<i>4 hours treatment in the presence of S9 (+S9)</i>													
Saline	-	7.5	100	200	0	0	0	0	0	0	2	0	0
SKY0402 Placebo	25.6	7.8	103	200	0.5	1	0	0	0	0	0	0	0
	51.2	8.9	118	200	0	0	0	0	0	0	0	0	0
	100	8.9	118	200	0	0	0	0	0	0	1	0	0
Cyclophosphamide	8.0	6.2	82	200	18.5 ***	28	6	12	0	1	18	3	0
<i>21 hours treatment in the absence of S9 (0S9)</i>													
Saline	-	11.0	100	200	0.5	0	0	1	0	0	0	0	0
SKY0402 Placebo	25.6	9.3	84	200	0	0	0	0	0	0	1	1	0
	51.2	8.5	77	200	1.5	3	0	0	0	0	0	0	0
	100	8.4	76	200	0	0	0	0	0	0	1	0	1
Mitomycin C	0.10	6.4	58	200	17 **	21	9	11	0	0	7	5	0

‡ Expressed in terms of test article as supplied and 100  $\mu\text{L}/\text{mL}$  corresponds to the maximum practical dose volume. Final concentrations for Mitomycin C and Cyclophosphamide are in terms of  $\mu\text{g}/\text{mL}$ .

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%)  
b, c, g Chromatid break, exchange, gap  
B, E, G Chromosome break, exchange, gap  
other Includes pulverized chromosomes and cells with > 8 aberrations  
P Polyploidy and endoreduplication  
† g, G and P are excluded from the calculation of % aberrant cells

Results of statistical analysis using one-tailed Fisher's exact test  
\*  $p \leq 0.01$  (significant)  
\*\*  $p \leq 0.001$  (highly significant)  
otherwise  $p > 0.01$  (not significant)

### 7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

**Study title: SKY0402 Placebo:** Mouse Micronucleus Test

- bone marrow assay

Study no: (b) (4) 960257

Study report location: eCTD DARRTS SDN-1

Conducting laboratory and location: (b) (4)

Date of study initiation: May 18, 2004

GLP compliance: yes

QA statement: yes

Drug, lot #, and % purity: - SKY0402 Placebo, lot 25BUP02-019  
(PD-019), 76.2 % packed particle  
volume  
- 0.9% NaCl, lot W4D22B2, 0.9%

- Mitomycin C, lot 31K2501, purity not found (2 mg aliquots with ca. 48 mg NaCl)

**Key Study Findings**

- Male and female Swiss mice received a single subcutaneous injection up to the maximum practicable dose of SKY0402 Placebo of 20 mL/kg (0, 25, 50, & 100% SKY0402 Placebo). The positive control, mitomycin C, was administered by oral gavage. All groups were sacrificed at 24 hours post dosing and negative control and high dose SKY0402 Placebo groups were also sacrificed at 48 hours post dosing. Both femurs were evaluated for micronucleated polychromatic erythrocytes
- No adverse clinical signs of treatment were observed at doses up to including the maximum feasible dose
- SKY0402 Placebo did not show any evidence of genotoxic activity in this *in vivo* test for induction of chromosome damage when tested in accordance with regulatory guidelines as dosing with SKY0402 Placebo at a maximum feasible dose did not result in chromosomal damage as indicated by an increase in micronucleated erythrocytes

## Methods

## Doses in definitive study:

Group No	Identification	Dosage (mL/kg)	Dose volume (mL/kg)	Concentration (% v/v)	Sampling time (hours)	Animal numbers	
						Male	Female
1	Vehicle control	-	20	0	24 48	1001-1005 1006-1010	1501-1505 1506-1510
2	SKY0402 Placebo	5	20	25	24	2001-2005	2501-2505
3	SKY0402 Placebo	10	20	50	24	3001-3005	3501-3505
4	SKY0402 Placebo	20*	20	100	24 48	4001-4005 4006-4010	4501-4505 4506-4510
5	Mitomycin C	6 mg/kg	10	0.6 mg/mL	24	5001-5003	5501-5503

\* Maximum practical dosage; material administered undiluted as supplied at the maximal recommended dose volume for this species and route (Hull 1995).

- Frequency of dosing: Single dose with 24 (all groups) or 48 hour (negative control and high dose SKY0402) sacrifice
- Route of administration: Subcutaneous (interscapular region) for negative control and SKY0402 Placebo groups, oral gavage for positive control group
- Dose volume: 20 mL/kg for negative control and SKY0402 groups, 10 mL/kg for positive control group
- Formulation/Vehicle: 0.9% NaCl
- Species/Strain: CD®-1 (Swiss CrI:CD®-1(ICR)BR) albino outbred mice (*Mus musculus*)
- Number/Sex/Group: 5/sex/group; 3/sex/group for positive control group
- Satellite groups: none
- Basis of dose selection: Up to maximum practical subcutaneous dose of 20 ml/kg of 100% material (see table above)
- Negative control: 0.9% NaCl for SKY0402 Placebo, sterile water for mitomycin C
- Positive control: Mitomycin C, Lot 31K2501
- Assessment:
- Clinical signs: All animals were examined twice daily for mortality and signs of ill health or reaction to treatment.
  - Body weights: measured but not reported
  - Bone marrow source: both femurs
  - Evaluation: A total of 2000 immature erythrocytes per animal were examined for the presence of micronuclei. Only one smear was examined per animal, the remaining smears were held temporarily in reserve in case of technical problems with the first smear.

## Study Validity – study considered valid

### Criteria for valid study

- dosing appeared to be adequate based upon the reported practicality of the dose/dose volume. This is consistent with Diehl et al. J. Appl. Toxicol. 21:15-23, 2001.
- preparation and administration of the test substance was acceptable
- the species and number of animals/sex/group were acceptable
- tissue sampling and analysis was acceptable
- positive controls exhibited appropriate responses
- the proportion of immature erythrocytes among total erythrocytes was not less than 20% of the control value.

## Results

### Criteria for positive results:

- statistically significant increase in the incidence of micronucleated immature erythrocytes for the treatment group compared with the concurrent control group ( $p < 0.01$ ).
- individual and/or group mean values should also exceed the laboratory historical control range
- the median number of micronucleated immature erythrocytes per 2000 cells evaluated should not be less than 5 or 3 for 24 hour and 48 hour sampling, respectively.

Test results indicate that dosing with SKY0402 Placebo at a maximum feasible dose did not result in chromosomal damage as indicated by an increase in micronucleated erythrocytes compared to negative controls. The positive control, mitomycin C, resulted in the expected genotoxicity.

Treatment	Dose (mL/kg)	% IE/(IE+ME) †	Incidence mic male	Incidence mic female	Incidence mic (M+F)	Incidence mme (M+F)
<i>24 Hour sampling time</i>						
Vehicle control	-	54	0.2	0.6	0.4	0.0
SKY0402 Placebo	5	57	0.0	1.4	0.7 <sup>ns</sup>	0.0
	10	56	1.0	0.8	0.9 <sup>ns</sup>	0.0
	20	53	1.2	0.6	0.9 <sup>ns</sup>	0.4
Mytomycin C	6 mg/kg	52	14.0	21.0	17.5 <sup>**</sup>	0.0
<i>48 Hour sampling time</i>						
Vehicle control	-	51	0.8	0.2	0.5	0.0
SKY0402 Placebo	20	55	1.0	0.6	0.8	0.0
%IE/(IE+ME)	Proportion of immature erythrocytes					
mic	Number of micronucleated cells observed per 2000 immature erythrocytes examined					
mme	Number of micronucleated mature erythrocytes observed (mean value expressed per 2000 mature erythrocytes examined, see results for individual animals in appendices for actual numbers of cells examined)					
Results of statistical analysis (one-sided probabilities):						
**	P < 0.001 (highly significant)					
*	P ≤ 0.01 (significant)					
ns	P > 0.01 (not significant)					
† Occasional apparent errors of ± 1% may occur due to rounding of values for presentation in the table						

**7.4 Other Genetic Toxicity Studies - none****8 Carcinogenicity - none****9 Reproductive and Developmental Toxicology****9.1 Fertility and Early Embryonic Development and Prenatal and Post Natal Development**

**Study title:** Study of Fertility, Reproductive Performance, Maternal Function, and F<sub>1</sub> Prenatal and Postnatal Development in Rats with **SKY0402 Placebo**

Study no.: (b) (4) 947-026  
 Study report location: eCTD DARRTS SDN-1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: January 24, 2005  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: - SKY0402 Placebo, lot 04PD007 & 008, 44.6-46.4% packed particle volume  
 - 0.9% NaCl, lot – 11 documented lots, 0.9%

**Key Study Findings**

- Male and female rats were treated with SKY0402 Placebo or saline (negative control) subcutaneously at maximum practicable doses of 10 mL/kg (3 injection sites at ~3.3 mL/kg/site) from before mating until lactation day 20 or its equivalent (males) in a combined fertility (P and F<sub>1</sub>), peri-/post-natal (F<sub>1</sub>), and early embryonic development (F<sub>1</sub> parents) study.
- The only effect of treatment with SKY0402 Placebo was observed in the P generation animals and involved thickening of the skin and subcutaneous collections of a white fluid, presumed to be unabsorbed test article, at injection sites.
- These results appear to indicate safety for fertility, early embryonic development, and peri-/post-natal effects as the largest proposed human dose volume is (b) (4) as a single dose. While early embryonic development was not assessed at the usual time after direct dosing of the dams, as it was assessed in F<sub>1</sub> dams (P dams treated during lactation), the lack of any treatment related effects in P females and F<sub>1</sub> deliveries suggests no early embryonic effect of treatment with SKY0402 Placebo.

## Methods

## Doses:

Group Number	Article Administered	Dose Volume (mL/kg)	Group Assignment	
			Number of P Animals	
			Male	Female
1	Saline	10	25	25
2	SKY0402 Placebo (10 mL/kg)	10	25	25

## Frequency of dosing:

- 25 F<sub>1</sub> males and females/group were mated
  - Once daily
  - Dosing of the females began at least 14 days prior to pairing and continued through the ensuing mating period. Mated females were treated daily throughout gestation and females with litters were treated to Lactation Day (LD) 20. Unmated females continued to be treated for at least 20 days following completion of the mating period.
  - Dosing of the P males began at least 28 days prior to pairing and continued through to euthanasia. Males continued on treatment until all deliveries were complete and fertility was assessed.
  - The bolus injection was administered between the skin and underlying layers of tissue in the scapular and lumbar regions of each animal. The scapular dose was administered in three sites (right shoulder, interscapular region, and the left shoulder). The lumbar dose was also administered in three sites (right flank, central lumbar, and left flank). The dose volume was distributed approximately equally between the three sites. The administration alternated daily between the scapular and lumbar regions. Therefore the dose volume for any individual site was ~3.3 mL/kg.

Dose volume: 10 mL/kg (considered the highest volumes which could humanely be administered by the chosen route). This is consistent with Diehl et al. J. Appl. Toxicol. 21:15-23, 2001.

Route of administration: subcutaneous

Formulation/Vehicle: 0.9% NaCl

Species/Strain: Male and female Sprague-Dawley [CrI: CD® (SD)] rats

Number/Sex/Group: 25

Satellite groups: None: no toxicokinetic groups

Study design: This is a combined study for fertility and peri-/post-natal development using P and F<sub>1</sub> animals. Early embryonic development was conducted using 25 paired/mated F<sub>1</sub> females per group.

P animals were mated after the above noted dosing schedule. F<sub>1</sub> litters were culled to 4/sex/litter on LD 4 and litters were weaned on LD 21. On Postnatal Day (PD) 28, 25 male and female F<sub>1</sub> pups were randomly selected from each group to continue on study for evaluation of growth, sexual maturation, and behavioral assessments (motor activity and learning and memory). Reproductive/fertility assessment was conducted on these selected pups when at least 80 days old upon after completion of behavioral and developmental testing by pairing 1 male with 1 female within treatment groups for mating. Mated F<sub>1</sub> females were subjected to a uterine examination on PD 13 in which the total number of corpora lutea, implantations, resorptions, and viable and nonviable embryos was recorded.

Deviation from study protocol: Nothing significant

## **METHODS and OBSERVATIONS**

### **Mortality and Clinical Signs**

All animals (P and F<sub>1</sub> selected animals) were observed for morbidity, mortality, injury, and the availability of food and water twice daily throughout the duration of the study. Daily during treatment, each P animal was removed from the cage and given a detailed clinical examination (30-90 minutes post-dose). The examination included, but was not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration. Selected F<sub>1</sub> animals were given a similar detailed examination weekly. These examinations began after all animals have been selected (Week 6 of F<sub>1</sub> phase).

### **Body Weight**

Individual body weights of P males were measured and recorded at initiation of dose administration and twice weekly at 3- and 4-day intervals for the remainder of the study until terminal euthanasia. P females were weighed at initiation of dose administration, twice weekly at 3 and 4-day intervals prior to and during cohabitation. Mated females were weighed on Gestation Day (GD) 0, 4, 7, 10, 14, 17, and 20 and females with litters were weighed on LD 0, 4, 7, 10, 14, 17, and 21. Pups were individually weighed and examined externally on LD 0, 4, 7, 14, and 21. F<sub>1</sub> animals selected to continue on study

to assess sexual maturation, behavior, and reproductive performance were weighed on PD 28, 35, 42, 49, 56, 63, 70, 77, and 84. F<sub>1</sub> males were weighed again at the start of the mating period and weekly thereafter. A terminal body weight was also recorded. Females were weighed at the start of the mating period and mated females were weighed on GD 0, 4, 7, 10, and 13. Unmated females were weighed weekly during the mating period and over the 2-week postmating period.

### **Feed Consumption**

Food consumption for all P rats was measured and recorded weekly prior but not during pairing. Following the cohabitation period, food consumption was measured and recorded weekly for P males until euthanasia. Food consumption was measured and recorded on corresponding body weight intervals for P females during gestation and lactation periods. Food consumption was not recorded for the F<sub>1</sub> animals.

### **Toxicokinetics - none**

**Dosing Solution Analysis** - no dosing solution analysis as the single test material dose was administered neat. The sponsor provided documentation that the test compound maintained stability over the study period under refrigerated conditions.

**Estrous Cycle and Sperm Analysis** - At initiation of test article administration (P females) and until evidence of copulation was observed or the cohabitation period had ended, the P females were examined daily to establish estrous cycles. Vaginal smears were performed daily (F<sub>1</sub> females) until there was evidence of mating. Sperm analysis was conducted on P males at sacrifice.

### **Necropsy, Organ Weights, and Histology**

Necropsy times - All unmated P females that appeared to be nonpregnant based on body weight and shape were euthanized 32 days after the last scheduled pairing day. After the last P females had delivered, all P males were observed externally, euthanized, and subjected to a necropsy. After weighing on LD 4, each litter was reduced to the eight randomly selected pups from Day 0. The culled pups were euthanized and examined for external abnormalities, and the carcasses were discarded. Only pups with abnormalities were saved in 10% neutral buffered formalin for possible further evaluation. On LD 28, the unselected pups from each litter were euthanized and subjected to a necropsy. On GD 13, each F<sub>1</sub> female was euthanized and the uteri examined. After the last GD 13 uterine examinations were complete, the F<sub>1</sub> males were euthanized.

Necropsy procedures - At necropsy, the dosing sites on the P animals were evaluated. A uterine examination was conducted on all F<sub>1</sub> females (mated and nonmated). Body weights, gravid uterus, and ovary (combined) weights from females euthanized at study termination were recorded. The body weight and testes, epididymides, seminal vesicles, and prostate weights of P males were recorded. The left and right testis was weighed

separately, and a combined weight is presented. The left testis and epididymis were fixed in Bouin's fixative for possible histopathologic examination. Protocol-designated sections of tissues/organs from all P animals were removed, examined and placed in neutral buffered formalin, except where noted otherwise, for possible microscopic examination. Gross lesions from all animals on study were saved in 10% neutral buffered formalin. Sufficient corresponding tissues from controls were collected for comparison purposes as needed. The carcasses were then discarded.

Mated F1 female were euthanized on GD 13. The location of viable embryos, resorptions, and the number of total implantations were recorded. The number of corpora lutea on each ovary was recorded. Uteri from females that appear nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites.

P animal tissue collection:

Organs to be Weighed and Tissues to be Preserved

Tissue	Organ Weight Taken	Collected and Preserved
Epididymis, left (fixed in Bouin's fixative)	X	X
Ovary	X	X
Prostate	X	X
Seminal vesicle with coagulating gland	X	X
Testis, left (fixed in Bouin's fixative)	X	X
Uterus (both horns) with cervix	X	X
Vagina		X
Gross lesions		X
Identified target organs <sup>b</sup>		X

<sup>b</sup> Target organs (and target organ gross lesions) will be designated by the Study Director, Pathologist, and/or Sponsor based on experimental findings

## F1 Behavioral and Developmental Indices

Pups that did not respond/satisfy the respective index on the first day were examined daily until the developmental landmark was achieved unless otherwise noted.

On LD 2, each pup was tested for a complete righting response. On LD 2, each pup was observed for unfolding of the pinna. On LD 11, prior to eye opening, each pup was tested for cliff aversion. On LD 13, each pup was observed for eye opening. On LD 16, each pup was tested for air drop righting reflex. On LD 21, after weaning was complete, each pup was given an Irwin neuropharmacological evaluation. Each pup was evaluated at 22 days of age for auditory (Preyer's) response.

Beginning on Day 28 of age, F1 female pups selected to continue on study for behavioral and reproductive assessment were examined for the presence of vaginal

opening. A body weight was measured and recorded on the day each animal achieved this landmark. Beginning on Day 35 of age, F1 male pups selected to continue on study for behavioral and reproductive assessment were examined for preputial separation. A body weight was measured and recorded on the day each animal achieved this landmark. Motor activity was evaluated on pups selected to continue on study for behavioral and reproductive evaluations. The activity of each pup was assessed at approximately LD 35. Learning and memory were evaluated on pups selected to continue on study using the step-through passive avoidance test. Animals were considered to have learned the appropriate response (i.e., not to leave the light compartment) if they did not pass into the dark compartment for two consecutive 3-minute trials. Animals were tested for a maximum of five trials on the day of testing.

The following indices were evaluated/reported:

<b>Parental In-life Data</b>	<b>Pathology</b>
P and F <sub>1</sub> pre mating body weights - males and females	P male reproduction organ weights
P and F <sub>1</sub> pre mating body weight change between intervals and over the entire pre mating period - males and females	P female reproduction organ weights
P pre mating food consumption	P Spermatogenesis evaluations
Gestation body weights (P, F <sub>1</sub> )	% Abnormal
Gestation body weight changes between all intervals and over the entire gestation period (P - GD 0-20 and F <sub>1</sub> GD 0-13)	% Motility
Gestation food consumption (P)	Concentration
Lactation body weights (P)	F <sub>1</sub> female reproduction organ weights
Lactation body weight change (between all intervals and over the entire LD 0-21 period)	<b>Uterine Exam (F<sub>1</sub> GD 13 evaluations)</b>
Lactation food consumption (P)	Total number of corpora lutea
Postmating body weights and body weight change (week -to-week) – P and F <sub>1</sub> males	Total number implantations/dam
	Viable embryos/dam
	Number resorptions/dam
	Preimplantation Loss
	Postimplantation Loss
<b>Fertility Indices</b>	<b>F<sub>1</sub> Litter</b>
Gestation Index	Litter size
Copulatory Interval	Viable pups
Male fertility index	Pup Sex Ratio (% viable males/litter)
Female fertility index	Stillborn Pups
Female mating index	Stillborn Index
Male mating index	Pup weights
Female fecundity index	Pup survival (LD 0-4 precull and 4 postcull-21)
Male fecundity index	<b>Developmental Indices</b>
P estrous cycle (mean cycle length)	Pinna detachment
P estrous cycle (# cycles/period)	Eye opening
	Preputial separation
	Vaginal opening (body weight)

Behavioral Tests
Static righting reflex
Air Drop Righting Reflex
Auditory Response
Cliff Aversion
Motor activity
Urination Endpoint for Motor Activity
Passive avoidance

## Results

### Mortality and Clinical Signs

P animals – No mortality and no clinical signs were treatment related during pre mating/mating, gestation, and lactation. Treated animals had an increased incidence (up to 4 of 25 animals) and frequency (days observed) of sparse hair on differing parts of the body.

F<sub>1</sub> animals – No mortality occurred in F<sub>1</sub> animals selected to continue on study. No treatment related clinical signs were observed in these animals. Treated animals had an increased incidence (up to 4 of 25 animals) and frequency (days observed) of sparse hair on differing parts of the body.

### Body Weight

P animals – Overall, no treatment related effects for mean body weights and body weight gains were identified. In females, mean body weight change during the pre mating period in the SKY0402 Placebo group was lower than the saline control. These differences were statistically significant for Days 11-15 (-15.9%) and over the entire period (Days 1-15, -12%). The toxicological relevance is unknown as no treatment related effects were observed for gestation and lactation. In males, mean body weights and body weight gains were not affected by treatment during the pre mating, pairing, and post mating periods.

F<sub>1</sub> animals - No treatment related effect of treatment with SKY0402 Placebo was evident from F<sub>1</sub> pup body weights and body weight change data during the pre mating, pairing, and post pairing (males) periods. Likewise, no effect of treatment was evident from F<sub>1</sub> gestation body weights and body weight gain.

### Feed Consumption

P animals - Overall, no treatment related effects food consumption were identified. Consistent with P female body weight changes, food consumption during the 2 week pre mating period was lower than controls with the differences at 4-6%.

F<sub>1</sub> animals – none measured

**Estrous Cycle and Sperm Analysis** - P female estrous cycles were not affected by treatment with SKY0402 Placebo. Percent motile sperm in the treated group at a mean of 86.2% was statistically different ( $p < 0.05$ ) from the 92.4% motility observed in the control (see table). Sperm motility values ranged from 55 to 98% in the treated group and from 84-99% in the control. This lab's historical range for means was 87.8 to 98.0%, so the mean sperm motility of 86.2% in the treated group was just outside the low end of the historical control range. By excluding one treated male with a motility of 55%, the mean sperm motility for the remainder of the SKY0402 Placebo group was 87.8%, the low end of the historical control range. The toxicological significance of this observation is unknown as there were no treatment related effects on the remaining sperm parameters evaluated (i.e., caudal epididymal sperm concentration counts, absolute and per gram tissue, and percent abnormal) and reproductive performance and fertility.

Endpoint	Summary of P Sperm Evaluation					
	Saline			SKY0402 Placebo (10 mL/kg)		
	Mean	SD	N	Mean	SD	N
Sperm Motility Percent Motility	92.4	4.74	22	86.2 <sup>a</sup>	10.60	21
Total Sperm Concentration per Cauda Epididymis x 10 <sup>8</sup>	2.462	0.2894	25	2.501	0.3234	25
Sperm Concentration per gram Cauda Epididymis x 10 <sup>8</sup>	8.271	1.1210	25	8.544	1.0572	25
Percent Abnormal	2.4	2.52	25	1.9	1.37	25

N - Number of measures used to calculate mean  
SD - Standard Deviation  
No. - Number

<sup>a</sup>Significantly different from control; ( $p < 0.05$ )

**P Generation Reproductive Performance** – There was no treatment related effect on reproductive/fertility indices for the P generation animals as mating, fertility, and fecundity were 100% in the treated and control groups. The mean number of days to mating (copulatory interval) was also no different between the two groups.

**P Generation Parturition and F<sub>1</sub> Litter Data** - No treatment related effects were observed in rats treated with SKY0402 Placebo compared to control for parturition and litter size data. Additional indices that were comparable were: difficult or protracted delivery, mean gestation length (21.9 days in control vs. 22.0 days in the treated group), and the mean number of liveborn, stillborn, and total pups per litter. Gestation and stillborn indices, mean litter size at LD 4 (pre and postcull) and for the remainder of lactation to weaning (LD 7, 14, and 21) were also comparable between the two groups.

**F<sub>1</sub> Pups** – No treatment related effects were observed for F<sub>1</sub> pup survival to weaning, viability and lactation indices, F<sub>1</sub> pup sex ratios, clinical observations, and pup body weights at birth or during the 21 day lactation period even though SKY0402 placebo

treated pup body weights were 5.4-7.6% heavier than control. Mean pup weights on PND 28 were comparable between the control and treated group. No treatment related effects were observed for F<sub>1</sub> pup macroscopic examinations.

## P Generation Postmortem Study Evaluations

Macroscopic Observations – The only notable macroscopic observations for the P generation animals included thickening, red/tan discoloration, and collections of white viscous fluid at the injection sites in most of the SKY0402 Placebo animals. The white viscous fluid was considered unabsorbed test article. Similar thickening of the injection sites was not seen in the saline control animals. No histological evaluations were performed.

Summary of P Macroscopic Observations - MALE			
		Terminal	
Tissue		Saline	SKY0402 Placebo (10 mL/kg)
Observation	Severity		
Number of Animals Examined		25	25
<b>all tissues</b>			
within normal limits		24	1
<b>injection site</b>			
discoloration, tan	- moderate	0	1
discoloration, white	- mild	0	24
	- moderate	0	2
	- moderate	0	22
thickened		0	24
	- mild	0	2
	- moderate	0	22
<b>kidneys</b>			
dilatation, pelvic	- mild	0	1
<b>seminal vesicles w/coagulating glands</b>			
small	- mild	1	1

Summary of P Macroscopic Observations - FEMALE			
		Terminal	
Tissue		Saline	SKY0402 Placebo (10 mL/kg)
Observation	Severity		
Number of Animals Examined		25	25
<b>all tissues</b>			
within normal limits		21	2
<b>injection site</b>			
discoloration, red		0	2
	- minimal	0	1
	- mild	0	1
discoloration, tan	- mild	0	3
discoloration, white		0	20
	- minimal	0	1
	- mild	0	12
	- moderate	0	7
thickened		0	22
	- minimal	0	1
	- mild	0	14
	- moderate	0	7
<b>liver</b>			
focus/foci, red	- mild	0	1
<b>mammary gland</b>			
mass	- present	1	0
thickened	- moderate	1	0
<b>uterus with cervix</b>			
cyst	- minimal	1	0
distended with fluid	- minimal	1	0

Organ Weights - No treatment related effects were observed in the reproductive organ weights of SKY0402 Placebo animals. The toxicological relevance is unknown for a statistically significant decrease in prostate weights, absolute (-13.1%) and relative to body weights (-11.1%) for treated males in the absence of effects on the weights (absolute and relative) of the other reproductive organs (i.e., testes, epididymides, and seminal vesicles). Ovary and uterine weights, absolute and relative to body weight, in the SKY0402 Placebo group were comparable to the saline control.

**F<sub>1</sub> Behavioral, Sensory, and Developmental Evaluations** – No treatment related effects were observed for F<sub>1</sub> pups for the testing of reflexes (static and air drop righting), sensory responses (cliff aversion and auditory), development (age of pinna detachment and eye opening), and neuropharmacological observations.

**F<sub>1</sub> Sexual Maturation** – No treatment related effects were observed for sexual maturation (mean age at vaginal opening and preputial separation), and mean body weight of females at vaginal opening. In males, mean body weight at sexual maturation in the SKY0402 Placebo group was about 6% lower than control with the toxicological relevance of this finding being unknown.

**F<sub>1</sub> Motor Activity, Emotionality, and Learning and Memory Assessments** - No treatment related effects were observed with SKY0402 Placebo for motor activity evaluations on PD 35 F<sub>1</sub> pups, emotionality parameters evaluated during the first 5 minutes of the motor activity assessment (i.e., urination counts, defecation, grooming, backing, and rearing), and on learning and memory as evaluated with the passive avoidance test.

**F<sub>1</sub> Parental Reproductive Performance (Reproductive/Fertility Assessment)** – No treatment related effects were observed for F<sub>1</sub> reproductive performance and fertility of the F<sub>1</sub> pups. Reproductive indices (mating, fertility, and fecundity) and the mean number of days-to-mating (copulatory interval) in the treated F<sub>1</sub> animals were comparable to control.

### **F<sub>1</sub> Postmortem Study Evaluations**

Macroscopic Observations - No treatment related effects were observed

Organ Weights – No treatment related effects were observed for ovary and gravid GD 13 uterine weights. Absolute and relative to GD 13 body weights for the treated group were comparable to control.

**F<sub>1</sub> Gestation Day 13 Uterine Examinations (Early Embryonic Development)** - No treatment related effects were observed with SKY0402 Placebo from the GD 13 uterine evaluations. The mean number of corpora lutea, uterine implantation sites, viable embryos, and preimplantation loss in the treated group was comparable to control. The mean number of resorptions per dam (0.8) and mean postimplantation loss (5.10%) in

the treated group was higher than control (0.3 and 1.65%, respectively). While the differences were statistically significant ( $p < 0.05$ ), the values were within the range of recent historical control data for the testing laboratory with a mean resorptions/dam ranged from 0.4 to 1.20 and postimplantation loss ranged from 2.58 to 8.21%.

Summary of F <sub>1</sub> Maternal and Developmental Observations at Uterine Examination			
Endpoint		Saline	SKY0402 Placebo (10 mL/kg)
Corpora Lutea No. per Animal	Mean	17.2	18.5
	SD	2.67	2.50
	N	22	22
Implantation Sites No. per Animal	Mean	15.7	16.3
	SD	1.39	2.01
	N	22	22
Preimplantation Loss % per Animal	Mean	7.40	11.07
	SD	8.460	10.169
	N	22	22
Viable Embryos No. per Animal	Mean	15.5	15.5
	SD	1.26	2.20
	N	22	22
Postimplantation Loss % per Animal	Mean	1.65	5.10 <sup>a</sup>
	SD	2.765	4.979
	N	22	22
Resorptions: Early No. per Animal	Mean	0.3	0.8 <sup>a</sup>
	SD	0.46	0.81
	N	22	22

No. - Number  
SD - Standard Deviation  
N - Number of measures used to calculate mean

<sup>a</sup>Significantly different from control; ( $p < 0.05$ )

## 9.2 Embryonic Fetal Development

**Study title:** Study for Effects on Embryo-Fetal Development in Rats with SKY0402 Placebo Administered Subcutaneously

Study no.: (b) (4) 947-022  
 Study report location: eCTD DARRTS SDN-1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: October 29, 2004  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: - SKY0402 Placebo, lot 04PD006, 40.7% packed particle volume  
 - 0.9% NaCl, lot 20-116-JT, 0.9%

### Key Study Findings

- Time-mated female rats were treated subcutaneously with the maximum recommended volume (10 mL/kg) of saline (negative control) or SKY0402 Placebo from gestation days 6 to 15 with sacrifice on gestation day 20 for the evaluation of potential maternal and developmental toxicity.
- Treatment did not produce any maternal or developmental toxicity.
- These results appear to indicate safety for embryo-fetal effects as the largest proposed human dose volume is (b) (4) as a single dose.

### Methods

Doses:	0 (negative control) and 10 mL/kg SKY0402 Placebo
Frequency of dosing:	Once daily from days 6 to 15 of gestation
Dose volume:	10 mL/kg (considered the highest volumes which could humanely be administered by the chosen route). This is consistent with Diehl et al. J. Appl. Toxicol. 21:15-23, 2001.
Route of administration:	subcutaneous
Formulation/Vehicle:	0.9% NaCl
Species/Strain:	Sprague-Dawley female rats [CrI: CD® (SD)IGS BR]
Number/Sex/Group:	25 females/group
Satellite groups:	None – only potential replacement animals - on Gestation Day (GD) 6, prior to dosing, two control females and one treated female were replaced due to low body weight gain
Study design:	- Time mated female rats were administered the negative control or SKY0402 Placebo from Days 6 to 15 of gestation, at approximately the same time each day in the scapular and lumbar regions of each animal. Three sites in each area were used, alternating daily between scapular and lumbar, to distribute the dose volume. Therefore the dose volume for any individual site was ~3.3 mL/kg.
Deviation from study protocol:	Nothing significant

### Observations and Results

All rats were observed twice daily for morbidity, mortality, signs of injury, and the availability of food and water. Observations of the animals included clinical signs, gestation body weights, and gestation food consumption. On GD 20, each female was euthanized and subjected to a complete necropsy, including a uterine examination in which the total number of implantations, early and late resorptions, and live and dead fetuses, and the position of the cervix were recorded. The total number of corpora lutea

on each ovary was also recorded. Gravid uterine weights were recorded and adjusted body weight changes calculated. All fetuses were weighed, sexed externally, and given a gross external examination. Approximately one-half of the fetuses in each litter were given a visceral examination (Bouin's fixed and razor-blade sectioning technique), and the other half eviscerated and stained with Alizarin Red S for skeletal examination. Malformations and developmental variations were recorded for all animals. Fetal findings were classified as malformations or developmental variations.

The following endpoints were analyzed:

<b>Parental In-life Data</b>	Total Implantations/dam
Gestation Body Weights	Litter Size/dam
Gestation Body Weight Changes	Viable Fetuses/dam
Gestation Food Consumption	Nonviable Fetuses/dam
Adjusted Body Weights	Total Number Resorptions/dam
Adjusted Body Weight Changes (Days 0-20)	Number Early Resorptions/dam
<b>Fertility Indices</b>	Number Late Resorptions/dam
Pregnancy Index	% Preimplantation Loss
<b>Uterine and Ovarian Exam</b>	% Postimplantation Loss
Gravid Uterine Weights	Mean Fetal Body Weights
Corpora Lutea/dam	Malformations by finding and exam type (external, visceral, and skeletal)-litter incidence <sup>a</sup>
Fetal Sex Ratio (% males/litter)	Variations by finding and exam type (external, visceral, and skeletal)-litter incidence <sup>a</sup>
	Total Malformations (external, visceral, and skeletal combined)-litter incidence <sup>a</sup>

a – fetal and litter incidences were reported, but only litter incidences were statistically analyzed

### **Mortality – none**

### **Clinical Signs – nothing treatment related**

### **Body Weight**

Individual body weights were recorded on GD 0, 6, 9, 12, 16, 18, and 20.

Individual body weight change was calculated for the following GD intervals: 0-6, 6-9, 9-12, 12-16, 16-18, 18-20, 6-16, 6-20, and 0-20. Adjusted body weight (Day 20 gestation body weight minus the gravid uterine weight) and adjusted body weight change (GD 0-20) were also calculated.

Body weights and body weight change throughout gestation for the SKY0402 Placebo-treated animals were comparable to the saline controls and unaffected by treatment (see tables).

Summary of Gestation Body Weight Values							
Endpoint	Study Interval (Day)	Saline			SKY0402 Placebo (10 mL/kg)		
		Mean	SD	N	Mean	SD	N
Body Weight Values g							
	0	209.4	12.17	25	211.0	14.17	25
	6	242.9	15.69	25	245.6	15.78	25
	9	259.5	17.74	25	259.4	17.24	25
	12	282.4	20.71	25	281.9	18.66	25
	16	313.5	22.31	24	312.4	21.60	25
	18	338.2	25.41	25	338.0	23.65	25
	20	371.8	26.96	25	369.3	26.44	24

Summary of Gestation Body Weight Change Values							
Endpoint	Study Interval (Day)	Saline			SKY0402 Placebo (10 mL/kg)		
		Mean	SD	N	Mean	SD	N
Body Weight Change Values g							
	0-6	33.6	9.48	25	34.6	8.10	25
	6-9	16.6	13.59	25	13.8	3.92	25
	9-12	22.9	5.28	25	22.5	5.95	25
	12-16	30.3	6.10	24	30.6	7.87	25
	16-18	25.6	9.13	24	25.5	7.09	25
	18-20	33.6	4.75	25	31.4	5.82	24
	6-16	69.8	16.45	24	66.8	10.59	25
	6-20	128.9	22.38	25	124.4	15.54	24
	0-20	162.4	23.24	25	158.6	18.22	24

### Feed Consumption

Food consumption was recorded on the corresponding body weight days and calculated for the following GD intervals: 0-6, 6-9, 9-12, 12-16, 16-18, 18-20, 6-16, 6-20, and 0-20.

Nothing treatment related was observed for food consumption.

### Toxicokinetics - none

**Dosing Solution Analysis** – no dosing solution analysis as the single test material dose was administered neat. The sponsor provided documentation that the test compound maintained stability over the study period under refrigerated conditions.

### Necropsy

A complete necropsy was performed on all dams. Each fetus was individually weighed, sexed, tagged, and examined for external malformations and variations.

**Cesarean Section Data** (Pregnancy Rates, Implantation Sites, Pre- and Post-Implantation Loss, Viable Fetuses, Fetal Sex Ratios, Live and Dead Fetuses, and Resorptions)

No effect of treatment was observed from maternal macroscopic evaluations.

No treatment related effect was observed for pregnancy rates as they were 100% in the saline control and SKY0402 Placebo groups providing 25 litters with viable fetuses in each group for evaluation on GD 20.

No effect of treatment with SKY0402 Placebo was observed for the uterine implantation data (see tables). This included the mean numbers of corpora lutea, uterine implantations, viable fetuses, resorptions sites per dam, mean postimplantation loss indices, and fetal sex ratio, as indicated by the percentage of male fetuses. The only statistically significant change seen in uterine implantation data between the two groups was an increase in preimplantation loss in the SKY0402 Placebo group. This difference was not of toxicologically concern as it was appeared to be attributable to a very low preimplantation loss index in the saline group compared to the historical control index. This index in the saline controls (6.07 %) was outside the historical control data range for the laboratory (6.78% to 18.11%), while this index in the SKY0402 Placebo group (11.91%) was well within this range.

Summary of Maternal and Developmental Observations at Uterine Examination			
Endpoint		Saline	SKY0402 Placebo (10 mL/kg)
Corpora Lutea No. per Animal	Mean	13.5	14.0
	SD	2.65	2.26
	N	25	25
Implantation Sites No. per Animal	Mean	12.6	12.2
	SD	2.04	1.57
	N	25	25
Preimplantation Loss % per Animal	Mean	6.07	11.91 <sup>a</sup>
	SD	7.756	10.752
	N	25	25
Viable Fetuses No. per Animal	Mean	11.9	11.8
	SD	2.13	1.56
	N	25	25
Fetal Sex Ratio % Males per Animal	Mean	52.1	51.2
	SD	14.94	15.26
	N	25	25

Summary of Maternal and Developmental Observations at Uterine Examination					
Endpoint		Saline		SKY0402 Placebo (10 mL/kg)	
		Mean	SD	Mean	SD
Postimplantation Loss % Implants per Animal	Mean	5.39		3.13	
	SD	8.209		5.838	
	N	25		25	
Nonviable Fetuses No. per Animal	Mean	0.0		0.0	
	SD	0.00		0.00	
	N	25		25	
Litter Size No. per Animal	Mean	11.9		11.8	
	SD	2.13		1.56	
	N	25		25	
Resorptions: Early + Late No. per Animal	Mean	0.7		0.4	
	SD	0.99		0.76	
	N	25		25	
Resorptions: Early No. per Animal	Mean	0.7		0.4	
	SD	0.99		0.76	
	N	25		25	
Resorptions: Late No. per Animal	Mean	0.0		0.0	
	SD	0.00		0.00	
	N	25		25	

No. - Number  
SD - Standard Deviation  
N - Number of measures used to calculate mean

There were no treatment related effects for gravid uterine weights, adjusted GD 20 body weights, and adjusted body weight gain (GD 0-20) in the SKY0402 Placebo group compared to the saline controls (see table).

Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values						
Endpoint	Saline			SKY0402 Placebo (10 mL/kg)		
	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, g	76.3	12.48	25	73.1	9.07	25
Final Body Weight, g	371.8	26.96	25	369.3	26.44	24
Adjusted Final Body Weight, g	295.5	22.61	25	296.1	20.50	24
Weight Change from Day 0, g	162.4	23.24	25	158.6	18.22	24
Adjusted Weight Change from Day 0, g	86.2	19.25	25	85.3	13.73	24

### Offspring (Malformations, Variations, etc.)

No treatment related effects were observed for fetal body weights by sex and for both sexes combined (see table).

Summary of Fetal Body Weight Values, g				
			Saline	SKY0402 Placebo (10 mL/kg)
Fetal Weight	Males	Mean	4.28 (4.28)	4.10 (4.10)
		SD	0.599	0.306
		N	25	24
	Females	Mean	4.09 (4.09)	3.97 (3.96)
		SD	0.551	0.250
		N	25	25
	Males + Females	Mean	4.19 (4.19)	4.03 (4.02)
		SD	0.586	0.258
		N	25	25

No treatment related effects were observed for the fetal external examinations (see table). Malformations involving the palate (cleft) and eye (microphthalmia) were seen in a single fetus in the SKY0402 Placebo group. In the absence of similar findings in the remaining 293 fetuses (25 litters) in this group, these findings are considered spontaneous in origin and unrelated to treatment. No developmental variations were seen among the saline control or SKY0402 Placebo fetuses. The observations were within historical control data values as reported in this study report.

Summary of Individual Fetal External Observations				
Observation	Classification	Saline	SKY0402 Placebo (10 mL/kg)	
No. Litters Evaluated		25	25	
No. Fetuses Evaluated		297	294	
<b>Head</b>				
Eye(s), Microphthalmia	M			
No. Litters (%)		0 (0.0)	1 (4.0)	
No. Fetuses (%) <sup>1</sup>		0 (0.0)	1 (0.3)	
Palate, Cleft palate	M			
No. Litters (%)		0 (0.0)	1 (4.0)	
No. Fetuses (%) <sup>1</sup>		0 (0.0)	1 (0.3)	
Palate, Misshapen	M			
No. Litters (%)		0 (0.0)	1 (4.0)	
No. Fetuses (%) <sup>1</sup>		0 (0.0)	1 (0.3)	
<b>Placenta</b>				
Entire, Larger than normal	P			
No. Litters (%)		1 (4.0)	0 (0.0)	
No. Fetuses (%) <sup>1</sup>		3 (1.0)	0 (0.0)	

No.-Number  
M- Malformation  
P- Pathological

<sup>1</sup>Not statistically analyzed

No treatment related malformations or developmental variations were observed for visceral examinations (see table on next page).

Summary of Visceral Malformations and Developmental Variations		
	Saline	SKY0402 Placebo (10 mL/kg)
No. Litters Evaluated	25	25
No. Fetuses Evaluated	149	149
<b>Total Malformations</b>		
No. Litters (%)	0 (0.0)	0 (0.0)
No. Fetuses (%) <sup>1</sup>	0 (0.0)	0 (0.0)
<b>Total Variations</b>		
No. Litters (%)	0 (0.0)	0 (0.0)
No. Fetuses (%) <sup>1</sup>	0 (0.0)	0 (0.0)

No treatment related effects were observed for fetal skeletal examinations that included malformations and ossification variations (see table). The litter incidences for the few skeletal findings were not statistically significant.

Summary of Individual Fetal Skeletal Observations			
Observation	Classification	Saline	SKY0402 Placebo (10 mL/kg)
No. Litters Evaluated		25	25
No. Fetuses Evaluated		149	149
<b>Cervical vertebra(e)</b>			
Neural arch(es), Additional ossification center	V		
No. Litters (%)		2 (8.0)	2 (8.0)
No. Fetuses (%) <sup>1</sup>		2 (1.3)	2 (1.3)
<b>Rib(s)</b>			
Rib(s), Bent	V		
No. Litters (%)		0 (0.0)	1 (4.0)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	2 (1.3)
Rib(s), Rudimentary	V		
No. Litters (%)		14 (56.0)	10 (40.0)
No. Fetuses (%) <sup>1</sup>		30 (20.1)	21 (14.1)
<b>Skull</b>			
Basioccipital, Incompletely ossified	V		
No. Litters (%)		0 (0.0)	1 (4.0)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	1 (0.7)

1 – not statistically analyzed

In summary, the overall incidence of litters containing fetuses with malformations during the external, visceral, and/or skeletal examinations was 4% (1/25 litters) in the SKY0402 Placebo group which did not differ statistically from the 0% litter incidence in the saline controls. On this basis, no treatment related effect was observed for fetal malformations (see table).

Summary of External Malformations and Developmental Variations		
	Saline	SKY0402 Placebo (10 mL/kg)
No. Litters Evaluated	25	25
No. Fetuses Evaluated	297	294
<b>Total Malformations</b>		
No. Litters (%)	0 (0.0)	1 (4.0)
No. Fetuses (%) <sup>1</sup>	0 (0.0)	1 (0.3)
<b>Total Variations</b>		
No. Litters (%)	0 (0.0)	0 (0.0)
No. Fetuses (%) <sup>1</sup>	0 (0.0)	0 (0.0)
Summary of External, Visceral, and Skeletal Malformations		
	Saline	SKY0402 Placebo (10 mL/kg)
No. Litters Evaluated	25	25
No. Fetuses Evaluated	297	294
<b>Total Malformations</b>		
No. Litters (%)	0 (0.0)	1 (4.0)
No. Fetuses (%) <sup>1</sup>	0 (0.0)	1 (0.3)

=====

**Study title:** Study for Effects on Embryo-Fetal Development in Rabbits with SKY0402 Placebo Administered Subcutaneously

Study no.: (b) (4) 947-023  
 Study report location: eCTD DARRTS SDN-1  
 Conducting laboratory and location: (b) (4)  
 Date of study initiation: October 29, 2004  
 GLP compliance: yes  
 QA statement: yes  
 Drug, lot #, and % purity: - SKY0402 Placebo, lot 04PD006, 40.7% packed particle volume  
 - 0.9% NaCl, lot 18-1135-JT, 0.9%

**Key Study Findings**

- Time-mated female rabbits were treated subcutaneously with the maximum recommended volume (2 mL/kg) of saline (negative control) or SKY0402 Placebo from gestation days 6 to 18 with sacrifice on gestation day 29 for the evaluation of potential maternal and developmental toxicity.
- Treatment did not produce any maternal or developmental toxicity.
- These results appear to indicate safety for embryo-fetal effects as the largest proposed human dose volume is (b) (4) as a single dose.

## Methods

## Doses:

Group Assignment			
Group Number	Article Administered	Dose Volume (mL/kg)	Number of Time-mated Female Rabbits
1	Saline	2	23
2	SKY0402 Placebo	2	23

Frequency of dosing: Daily from Days 6 to 18 of gestation  
 Dose volume: 2 mL/kg (considered the highest volumes which could humanely be administered by the chosen route). This is consistent with Diehl et al. J. Appl. Toxicol. 21:15-23, 2001.

Route of administration: subcutaneous  
 Formulation/Vehicle: 0.9% NaCl  
 Species/Strain: New Zealand White [Hra: (NZW) SPF] female rabbits  
 Number/Sex/Group: 23  
 Satellite groups: None – only potential replacement animals - on Gestation Day (GD) 6, prior to dosing, one control female was replaced due to low food consumption

Study design: Time mated female rabbits were administered the negative control or SKY0402 Placebo at approximately the same time each day in the scapular and lumbar regions of each animal. Three sites in each area were used, alternating daily between scapular and lumbar, to distribute the dose volume. Therefore the dose volume for any individual site was ~0.67 mL/kg, which is less than the maximum proposed human dose volume of (b) (4). Sacrifice was on day 29 of gestation.

Deviation from study protocol: Nothing significant

### Observations and Results

All rabbits were observed twice daily for morbidity, mortality, signs of injury, and availability of food and water. Daily from GD 6 through 29 (approximately 1 hour post-dose during the dosing period), each rabbit was removed from the cage and given a detailed clinical examination. The examination included, but was not limited to, evaluation of the skin, fur, eyes, ears, nose, oral cavity, thorax, abdomen, external genitalia, limbs and feet, as well as evaluation of respiration.

On Day 29 of gestation, each female was euthanized which was then followed immediately by a cesarean section. The skin was reflected from a ventral midline

incision to examine mammary tissue and locate any subcutaneous masses. The skin was also reflected dorsally at the injection sites (scapular and lumbar regions) and examined. The abdominal cavity was then opened and the uterus was exposed. Beginning at the distal end of the left uterine horn, the location of viable and nonviable fetuses, early and late resorptions for each uterine horn, position of the cervix, and the number of corpora lutea on each ovary were recorded. Viable fetuses responded to touch while nonviable fetuses did not respond to touch and showed no signs of autolysis. Early resorptions were characterized as implantation sites that had no recognizable fetal characteristics, while late resorptions had recognizable fetal form, but were undergoing autolysis. The uterus was excised, and the gravid uterine weight was recorded. The fetuses were removed by making a dorsal incision longitudinally along both uterine horns. The embryonic membrane of each fetus was gently removed, and each fetus was pulled away from the placenta, fully extending the umbilical cord. The placenta was grossly examined. Uteri from the females that appeared nongravid were opened and placed in 10% ammonium sulfide solution for detection of implantation sites<sup>1</sup>. If no foci were seen the females were considered to be nonpregnant.

### Mortality – none

**Clinical Signs** - No treatment related effect was observed with SKY0402 Placebo. Clinical observation in the treated group were comparable to the saline controls and are considered typical for this strain and age of rabbit in this laboratory.

### Body Weight

Individual body weights were recorded on GD 0, 6, 10, 13, 16, 19, 21, 25, and 29. Individual body weight change was calculated for the following GD intervals: 0-6, 6-10, 10-13, 13-16, 16-19, 19-21, 21-25, 25-29, 6-19, 19-29, and 0-29. Adjusted body weight (GD 29 body weight minus the gravid uterine weight) and adjusted body weight change (GD 0-29) were also calculated.

No treatment related body weights and body weight changes were observed throughout gestation for the SKY0402 Placebo treated animals compared to the saline controls (see tables).

Endpoint	Study Interval (Day)	Summary of Gestation Body Weight Values					
		Saline			SKY0402 Placebo (2 mL/kg)		
Body Weight Values kg		Mean	SD	N	Mean	SD	N
	0	3.622	0.3601	22	3.678	0.2829	22
	6	3.748	0.3205	22	3.792	0.2756	22
	10	3.763	0.3275	22	3.799	0.3004	22
	13	3.839	0.3520	22	3.871	0.3217	22
	16	3.919	0.3635	22	3.987	0.3949	22
	19	3.946	0.3555	22	3.967	0.3463	22
	21	3.920	0.3494	22	3.951	0.3368	22
	25	3.998	0.3496	22	4.025	0.3401	22
	29	4.012	0.3606	22	4.038	0.3304	22

Summary of Gestation Body Weight Change Values							
Endpoint	Study Interval (Day)	Saline			SKY0402 Placebo (2 mL/kg)		
		Mean	SD	N	Mean	SD	N
Body Weight Change Values kg							
	0-6	0.126	0.0739	22	0.114	0.0796	22
	6-10	0.015	0.0589	22	0.006	0.0820	22
	10-13	0.076	0.0837	22	0.072	0.0818	22
	13-16	0.080	0.1775	22	0.116	0.2243	22
	16-19	0.027	0.1656	22	-0.020	0.2449	22
	19-21	-0.026	0.0731	22	-0.016	0.0754	22
	21-25	0.078	0.0791	22	0.074	0.0886	22
	25-29	0.014	0.0980	22	0.013	0.0840	22
	6-19	0.198	0.1062	22	0.175	0.1660	22
	19-29	0.066	0.1688	22	0.071	0.1500	22
	0-29	0.390	0.1560	22	0.360	0.2188	22

### Feed Consumption

Food consumption was recorded daily and reported on the corresponding body weight intervals. Daily food consumption values are presented individually, but not statistically analyzed.

No treatment related effects on food consumption were observed throughout gestation in the SKY0402 Placebo-treated group compared to the saline controls.

### Toxicokinetics - none

**Dosing Solution Analysis** - Test article analyses were not conducted because the test article was administered as received. The sponsor provided documentation that the test compound maintained stability over the study period under refrigerated conditions.

### Necropsy

**Maternal Necropsy** - A complete necropsy was performed on all females. Special emphasis was placed on structural abnormalities or pathologic changes that may have influenced the pregnancy. Gross lesions from the does were saved in 10% neutral buffered formalin for possible histopathologic examination, and carcasses were discarded. Collection of gross lesions and/or target organs from treated animals necessitated collection of sufficient, corresponding tissues from controls (three animals) for comparison purposes if required.

**Fetal examinations** - Before being cut, the umbilical cord of each fetus was momentarily clamped with forceps to prevent bleeding and promote clotting. Each fetus was individually weighed and examined for external malformations and variations. Each fetus was deeply anesthetized, making every attempt to avoid injection of internal organs, and subjected to a fresh fetal soft tissue dissection. Any visceral abnormalities

were recorded. The sex of each fetus was documented. After dissection and examination of internal organs was complete, each fetus was eviscerated, skinned, and fixed in alcohol. All fetuses were macerated in potassium hydroxide, stained with Alizarin Red S, and cleared with glycerin for subsequent skeletal examination. Each fetus was examined for malformations and developmental variations. Fetal findings were classified as malformations or developmental variations.

The following endpoints were analyzed:

Endpoint	
<b>Parental In-life Data</b>	Viable Fetuses/doe
Gestation Body Weights	Nonviable Fetuses/doe
Gestation Body Weight Changes	Total Number Resorptions/doe
Gestation Food Consumption	Number Early Resorptions/doe
Adjusted Body Weights	Number Late Resorptions/doe
Adjusted Body Weight Changes (Days 0-29)	% Preimplantation Loss
<b>Fertility Indices</b>	% Postimplantation Loss
Pregnancy Index	Mean Fetal Body Weights
<b>Uterine and Ovarian Exam</b>	Malformations by finding and exam type (external, visceral, and skeletal)-litter incidence <sup>a</sup>
Gravid Uterine Weights	Variations by finding and exam type (external, visceral, and skeletal)-litter incidence <sup>a</sup>
Corpora Lutea/doe	Total Malformations (external, visceral, and skeletal combined)-litter incidence <sup>a</sup>
Fetal Sex Ratio (% males/litter)	
Total Implantations/doe	
Litter Size/doe	

a - Fetal and litter incidences were reported, but only the litter incidences were statistically analyzed.

### Cesarean Section Data

(Pregnancy Rates, Implantation Sites, Pre- and Post-Implantation Loss, Viable Fetuses, Fetal Sex Ratios, Live and Dead Fetuses, and Resorptions)

No treatment related effects were observed for maternal macroscopic evaluations.

Pregnancy rates were 95.7% in each of the groups providing 22 litters with viable fetuses for evaluation on GD 29 (see table on next page). No animals in either group aborted or delivered early.

Summary of Maternal and Developmental Observations at Uterine Examination		
Endpoint	Saline	SKY0402 Placebo (2 mL/kg)
No. Females on Study	23	23
No. Not Pregnant	1	1
No. Pregnant	22	22
Pregnancy Index Percent	95.7	95.7
No. Died Pregnant	0	0
No. Abortions	0	0
No. Early Deliveries	0	0
No. Females with All Resorptions	0	0
No. Females with Viable Fetuses Day 29 Gestation	22	22

No treatment related effects were observed for uterine implantation data (see table). The mean number of corpora lutea, implantation sites, preimplantation loss, viable fetuses, and fetal sex ratios were comparable to the saline controls.

Summary of Maternal and Developmental Observations at Uterine Examination			
Endpoint		Saline	SKY0402 Placebo (2 mL/kg)
Corpora Lutea No. per Animal	Mean	10.0	10.3
	SD	2.06	1.78
	N	22	22
Implantation Sites No. per Animal	Mean	8.6	9.5
	SD	2.19	1.60
	N	22	22
Preimplantation Loss % per Animal	Mean	12.92	6.70
	SD	17.830	7.105
	N	22	22
Viable Fetuses No. per Animal	Mean	8.2	9.2
	SD	1.94	1.51
	N	22	22
Fetal Sex Ratio % Males per Animal	Mean	42.3	47.1
	SD	19.63	20.66
	N	22	22

No. - Number  
SD - Standard Deviation  
N - Number of measures used to calculate mean

There were no treatment related effects observed for pre and postimplantation loss indices (see table on next page). Litter sizes and resorptions were comparable with the saline controls.

Summary of Maternal and Developmental Observations at Uterine Examination					
Endpoint		Saline		SKY0402 Placebo (2 mL/kg)	
		Mean	SD	Mean	SD
Postimplantation Loss % Implants per Animal	Mean	4.45		3.15	
	SD	7.313		4.777	
	N	22		22	
Nonviable Fetuses No. per Animal	Mean	0.0		0.0	
	SD	0.00		0.00	
	N	22		22	
Litter Size No. per Animal	Mean	8.2		9.2	
	SD	1.94		1.51	
	N	22		22	
Resorptions: Early + Late No. per Animal	Mean	0.5		0.3	
	SD	0.80		0.48	
	N	22		22	
Resorptions: Early No. per Animal	Mean	0.3		0.2	
	SD	0.72		0.39	
	N	22		22	
Resorptions: Late No. per Animal	Mean	0.1		0.1	
	SD	0.35		0.35	
	N	22		22	

No. - Number  
SD - Standard Deviation  
N - Number of measures used to calculate mean

No treatment related effects were observed for gravid uterine weights, adjusted gestation day (GD) 29 body weights and adjusted weight change GD 0-29 in the SKY0402 Placebo-treated group compared to the saline controls (see table).

Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values						
Endpoint	Saline			SKY0402 Placebo (2 mL/kg)		
	Mean	SD	N	Mean	SD	N
Gravid Uterine Weight, kg	0.488	0.1028	22	0.539	0.0975	22
Final Body Weight, kg	4.012	0.3606	22	4.038	0.3304	22
Adjusted Final Body Weight, kg	3.524	0.3212	22	3.498	0.3125	22
Weight Change from Day 0, kg	0.390	0.1560	22	0.360	0.2188	22
Adjusted Weight Change from Day 0, kg	-0.098	0.1689	22	-0.180	0.1931	22

### Offspring (Malformations, Variations, etc.)

No treatment related effects were observed for mean fetal body weights, distinguished by sex and for both sexes combined, for the SKY0402 Placebo group compared to the saline controls (see table on next page).

Summary of Fetal Body Weight Values, g				
			Saline	SKY0402 Placebo (2 mL/kg)
Fetal Weight	Males	Mean	41.79 (41.38)	41.53 (41.94)
		SD	3.967	5.006
		N	21	21
	Females	Mean	41.26 (41.32)	40.03 (39.98)
		SD	3.563	4.634
		N	22	22
	Males + Females	Mean	41.59 (41.22)	40.76 (41.13)
		SD	3.305	4.430
		N	22	22

SD - Standard Deviation; ( ) - Least square mean; N - Number of measures used to calculate mean

No treatment related effects observed for the SKY0402 Placebo for fetal external examinations (see table). No external malformations or developmental variations were seen among the treated fetuses. In the saline controls, cleft palate was seen in a single fetus.

Summary of Individual Fetal External Observations				
Observation	Classification		Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated			22	22
No. Fetuses Evaluated			180	203
<b>Head</b>				
Palate, Cleft palate	M			
No. Litters (%)			1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>			1 (0.6)	0 (0.0)

No.-Number; 1 - Not statistically analyzed; M- Malformation

No treatment related effects were observed for the fetal visceral examinations (see tables on next page).

Summary of Individual Fetal Visceral Observations			
Observation	Classification	Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated		22	22
No. Fetuses Evaluated		180	203
<b>Abdominal cavity</b>			
Adrenal, Larger than normal	V		
No. Litters (%)		0 (0.0)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	2 (1.0)
Gallbladder, Smaller than normal	V		
No. Litters (%)		1 (4.5)	2 (9.1)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	6 (3.0)
Spleen, Discontinuous	M		
No. Litters (%)		0 (0.0)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	1 (0.5)
Ureter, Malpositioned	V		
No. Litters (%)		1 (4.5)	4 (18.2)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	6 (3.0)
<b>Thoracic cavity</b>			
Aortic arch, Dilated	M		
No. Litters (%)		2 (9.1)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		2 (1.1)	1 (0.5)
		<sup>1</sup> Not statistically analyzed	
No.-Number			
M- Malformation			
V- Variation			

Summary of Individual Fetal Visceral Observations			
Observation	Classification	Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated		22	22
No. Fetuses Evaluated		180	203
Common carotid artery, Arising from innominate artery	V		
No. Litters (%)		3 (13.6)	3 (13.6)
No. Fetuses (%) <sup>1</sup>		4 (2.2)	3 (1.5)
Common carotid artery, Extra	V		
No. Litters (%)		1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	0 (0.0)
Heart - entire, Transposition of great vessels	M		
No. Litters (%)		1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	0 (0.0)
Interventricular septum, Discontinuous	M		
No. Litters (%)		1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	0 (0.0)
Right lung, Azygous lobe absent	V		
No. Litters (%)		0 (0.0)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	2 (1.0)
Subclavian artery, Extra	V		
No. Litters (%)		1 (4.5)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	1 (0.5)
		<sup>1</sup> Not statistically analyzed	
No.-Number			
M- Malformation			
V- Variation			

No treatment related effects were observed for fetal skeletal evaluations and ossification variations (see tables). The fused sternbrae at 3% of the fetuses is within the historical control of 7.1% provided by the applicant in the study report.

Summary of Individual Fetal Skeletal Observations			
Observation	Classification	Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated		22	22
No. Fetuses Evaluated		180	203
<b>Cervical vertebra(e)</b>			
Neural arch(es), Additional ossification center	V		
No. Litters (%)		3 (13.6)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		4 (2.2)	0 (0.0)
<b>Hind limb(s)</b>			
Talus, Not ossified	V		
No. Litters (%)		1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	0 (0.0)
<b>Rib(s)</b>			
Rib(s), Rudimentary	V		
No. Litters (%)		19 (86.4)	21 (95.5)
No. Fetuses (%) <sup>1</sup>		53 (29.4)	70 (34.5)
Rib(s), Unilateral full rib	V		
No. Litters (%)		15 (68.2)	18 (81.8)
No. Fetuses (%) <sup>1</sup>		26 (14.4)	33 (16.3)
No.-Number		<sup>1</sup> Not statistically analyzed	
V- Variation			

Summary of Individual Fetal Skeletal Observations			
Observation	Classification	Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated		22	22
No. Fetuses Evaluated		180	203
<b>Skull</b>			
Frontal bone, Additional ossification center	V		
No. Litters (%)		0 (0.0)	2 (9.1)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	2 (1.0)
Hyoid arch, Bent	V		
No. Litters (%)		4 (18.2)	5 (22.7)
No. Fetuses (%) <sup>1</sup>		6 (3.3)	8 (3.9)
Hyoid body, Not ossified	V		
No. Litters (%)		0 (0.0)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		0 (0.0)	1 (0.5)
Nasal bone, Additional ossification center	V		
No. Litters (%)		1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	0 (0.0)
<b>Sternum</b>			
Entire, Fused	M		
No. Litters (%)		1 (4.5)	0 (0.0)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	0 (0.0)
No.-Number		<sup>1</sup> Not statistically analyzed	
M- Malformation			
V- Variation			

Summary of Individual Fetal Skeletal Observations			
Observation	Classification	Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated		22	22
No. Fetuses Evaluated		180	203
Sternebra(e), Additional ossification center	V		
No. Litters (%)		1 (4.5)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		1 (0.6)	1 (0.5)
Sternebra(e), Fused	M		
No. Litters (%)		2 (9.1)	4 (18.2)
No. Fetuses (%) <sup>1</sup>		2 (1.1)	6 (3.0)
Sternebra(e), Misaligned	V		
No. Litters (%)		3 (13.6)	1 (4.5)
No. Fetuses (%) <sup>1</sup>		3 (1.7)	1 (0.5)
Sternebra(e), Not ossified	V		
No. Litters (%)		7 (31.8)	11 (50.0)
No. Fetuses (%) <sup>1</sup>		11 (6.1)	27 (13.3)

No treatment related effects were observed for litters for total fetal malformations as determined during the external, visceral and/or skeletal examinations (see table).

Summary of External, Visceral, and Skeletal Malformations		
	Saline	SKY0402 Placebo (2 mL/kg)
No. Litters Evaluated	22	22
No. Fetuses Evaluated	180	203
<b>Total Malformations</b>		
No. Litters (%)	4 (18.2)	6 (27.3)
No. Fetuses (%) <sup>1</sup>	6 (3.3)	8 (3.9)

### 9.3 Prenatal and Postnatal Development

**Study title:** Study of Fertility, Reproductive Performance, Maternal Function, and F<sub>1</sub> Prenatal and Postnatal Development in Rats with **SKY0402 Placebo**

Study no.: (b) (4) 947-026

Study report location: eCTD DARRTS SDN-1

Conducting laboratory and location: (b) (4)



Combined study reported in section 9.1

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### 9.4 Juvenile Toxicity (pilot)

**Non-GLP study 947-042** - The objective of this study conducted by (b) (4) was to evaluate the toxicity and tolerability of SKY0402 (lot 08-2505) when administered by subcutaneous (SC) injection to juvenile rats once or twice per week for 4 weeks to aid in the selection of doses for a subsequent definitive toxicity study in juvenile rats. The rats

were dosed from postnatal day (PD) 7 to 35. Time mated females were used as the source of the juvenile rats. On the day of parturition (PD 0), the pups from all females delivering were pooled and distributed (cross-fostered) to these same females so that each dam would have a litter that contained eight pups (four/sex), when possible. On PND 5, eight dams that retained all eight normal appearing pups were randomly selected to provide pups for the eight main study groups. The litter from each selected dam was the dose group. In addition, 13 dams with litters of varying size were randomly selected from the remaining dams with litters to provide toxicokinetic (TK) groups. One litter was randomly selected as control (0.9% sodium chloride), and contained the eight control pups, and two dams with litters were selected for each TK group. Three pups of each sex in these litters were dosed, while the remaining pups were untreated. Pups were weaned on PND 21. The number of pups assigned to each dose group is described in the table below.

Group Assignments			
Group Number	Dose Level (mg/kg/dose)	Number of Animals <sup>a</sup>	
		Male	Female
<b>Main Study</b>			
1	Control (Weekly)	4	4
2	9 (Weekly)	4	4
3	18 (Weekly)	4	4
4	30 (Weekly)	4	4
5	Control (Bi-weekly)	4	4
6	9 (Bi-weekly)	4	4
7	18 (Bi-weekly)	4	4
8	30 (Bi-weekly)	4	4
<b>Toxicokinetic</b>			
9	Control (Weekly)	2	2
10	9 (Weekly)	6	6
11	18 (Weekly)	6	6
12	30 (Weekly)	6	6
13	Control (Bi-weekly)	2	2
14	9 (Bi-weekly)	6	6
15	18 (Bi-weekly)	6	6
16	30 (Bi-weekly)	6	6

<sup>a</sup> Pups were evenly distributed across groups to provide 8 pups/litter, 4 pups/sex. This was conducted on PND 0 (day of parturition). Each litter contained cross-fostered pups and were allocated to a dose group. Thus, there were a total of 8 litters that contained main study animals and approximately 13 litters (1 control and 12 treated groups) with TK animals.

Dosing was by subcutaneous bolus injection to the control and treated main study and TK groups at dose levels of 0, 9, 18, or 30 mg/kg/dose (weekly PND 7, 14, 21, 28, and 35), or bi-weekly (PND 7, 10, 14, 17, 21, 24, 28, 31 and 35), at dose volumes of 2.0, 0.6, 1.2, or 2.0 mL/kg, respectively. The control or test article were injected between the skin and underlying layers of tissue in the scapular (thoracic) [injection site 1] or lumbar [injection site 2] regions on the back of each animal.

All animals were observed for morbidity, mortality, injury, and the availability of food and water twice daily throughout the duration of the study. Body weights of main study pups were measured and recorded on PND 7, 10, 14, 17, 21, 24, 28, 31, and 35. Body weight change was calculated between each weighing interval and over the entire PND 7 to 35 period. Following weaning, food consumption for main study animals was measured

and recorded between each weighing interval. Plasma samples for TK analysis were collected from two animals/sex/treated groups at 1, 8, and 24 hours post-dose on PD 35.

Complete necropsy examinations were performed on all main study animals. The animals were examined carefully for external abnormalities including masses. The skin was reflected from a ventral midline incision and any abnormalities were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and, where required, placed in fixative. A full complement of tissues and organs was collected from all animals for weighing and fixed without histological evaluation.

The following list constitutes the full complement of organs and tissues:

- |   |   |
|---|---|
| - Adrenal (2)*  | - Larynx  |
| - Aorta   | - Liver [3 sections collected]*                         |
| - Bone with marrow [femur]                              | - Lung with bronchi [collected whole]*                  |
| - Bone with marrow [sternum]                            | - Lymph nodes: mandibular and mesenteric                |
| - Brain [cerebrum, midbrain, cerebellum, medulla/pons]* | - Mammary gland   |
| - Epididymis (2)  | - Pancreas  |
| - Eye including optic nerve (2)                         | - Peyer's patch   |
| - Gastrointestinal tract:                               | - Pituitary*  |
| esophagus   | - Seminal vesicles with coagulating glands (2)          |
| stomach [glandular and nonglandular]                    | - Salivary gland, mandibular/sublingual [2 collected]** |
| duodenum  | - Salivary gland, parotid [2 collected]                 |
| jejunum   | - Sciatic nerve   |
| ileum   | - Skeletal muscle, biceps femoris                       |
| cecum   | - Skin  |
| colon   | - Spinal cord [cervical, thoracic, and lumbar]          |
| rectum  | - Spleen*   |
| - Gonads:   | - Thymus*   |
| ovary (2)* with oviduct (2)                             | - Thyroid/parathyroid (2)*                              |
| testis (2)*   | - Tongue  |
| - Gross lesions   | - Trachea   |
| - Heart*  | - Ureter (2)  |
| - Joint, tibiofemoral                                   | - Urinary bladder                                       |
| - Kidney (2)*   | - Uterus [both horns]/Cervix                            |
| - Lacrimal gland, exorbital (2)                         | - Vagina  |

\*The combined weight of the mandibular/sublingual salivary gland was obtained.

\*Organ weighed  
(2) Paired organ

No treatment related effects were observed for mortality, clinical signs, body weights or body weight gain, post-weaning food consumption, macroscopic examinations, or organ weight data. In the once weekly dosing regimen, testes to body weight ratios were statistically higher than control in each of the treated groups with a dose-responsiveness of 11, 12, & 13% for the low, mid and high dose groups, respectively. Only the high dose group was increased on an absolute organ weight basis at 10%. The toxicological relevance of this effect cannot be determined as no similar effects were

observed in males dosed biweekly which amounted to twice the weekly dose compared to the rats dosed once weekly. Systemic absorption of bupivacaine was confirmed with bupivacaine present up to the last sampling period of 24-hours post-dosing on PD 35 after weekly and twice weekly dosing.

Group	Dose Level (mg/kg/dose)	Weekly Dosing Regimen								
		Mean Plasma Bupivacaine Concentrations <sup>a</sup> (ng/mL) – Postnatal Day 35 (range of individual values) [Standard Deviation]								
		1-hour post-dose			8-hour post-dose			24-hour post-dose		
		Male	Female	Combined sexes	Male	Female	Combined sexes	Male	Female	Combined sexes
10	9	210 (158-261)	86.3 (54.6-118.0)	147.9 [86.59]	27.0 (14.9-39.1)	18.6 (14.4-22.8)	22.8 [11.53]	10.4 (0 <sup>b</sup> -20.7)	22.0 (10.6-33.4)	16.2 [14.26]
11	18	345 (312-377)	296 (181-410)	320 [101.22]	36.3 (22.0-50.6)	40.0 (31.0-48.9)	38.1 [13.93]	30.1 (20.2-40.0)	24.8 (0 <sup>b</sup> -49.6)	27.5 [22.02]
12	30	777 (692-861)	454 (364-544)	615.3 [211.73]	94.9 (71.8-118.0)	103 (74.1-131.0)	98.7 [30.25]	45.4 (40.2-50.6)	46.8 (36.4-57.1)	46.1 [9.49]

<sup>a</sup> Mean of two values (N=2) for each sex, and mean of four values (N=4, 2/sex) for combined sexes at each time point.  
<sup>b</sup> Values BLQ (Below Limit of Quantification) are represented as 0.

Group	Dose Level (mg/kg/dose)	Twice Weekly Dosing Regimen								
		Mean Plasma Bupivacaine Concentrations <sup>a</sup> (ng/mL) – Postnatal Day 35 (range of individual values) [Standard Deviation]								
		1-hour post-dose			8-hour post-dose			24-hour post-dose		
		Male	Female	Combined sexes	Male	Female	Combined sexes	Male	Female	Combined sexes
14	9	144 (67.6-221.0)	242 (204.0-280.0)	193.2 [89.81]	20.4 (18.2-22.5)	12.7 (12.6-12.8)	16.5 [4.75]	21.9 (17.8-25.9)	22.3 (20.5-24.1)	22.1 [3.63]
15	18	315 (187.0-442.0)	230 (194.0-266.0)	272.3 [118.67]	32.3 (31.2-33.4)	32.1 (21.5-42.6)	32.2 [8.66]	22.1 (19.3-24.8)	30.0 (17.7-42.3)	26.0 [11.27]
16	30	480 (360-599)	362 (289-435)	420.8 [133.0]	66.9 (52.2-81.6)	49.1 (31.0-67.1)	58.0 [21.62]	69.2 (41.8-96.6)	31.3 (19.8-42.7)	50.2 [32.68]

<sup>a</sup> Mean of two values (N=2) for each sex, and mean of four values (N=4, 2/sex) for combined sexes at each time point.

On the basis of these data, the NOAEL was 30 mg/kg/dose administered subcutaneously, twice weekly to juvenile rats for 4 weeks starting on PND 7.

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## 10 Special Toxicology Studies

### 10.1 Local tolerance

**Study title:** Comparative Evaluation of Local Irritation of **SKY0402** and Bupivacaine HCl in Rabbits

Study no.: 947-004  
Study report location: eCTD DARRTS SDN-1  
Conducting laboratory and location: (b) (4)  
Date of study initiation: August 8, 2002  
GLP compliance: Yes  
QA statement: Yes  
Drug, lot #, and % purity:  
- SYK0402, lot 02BUP02-034, NA  
- SKY0402 placebo, lot AKA017, NA  
- Administered neat as supplied by sponsor with no analysis

#### Key Study Findings

- In a test of potential local irritation from dosing with a single dose of SKY0402 or bupivacaine HCl, four male New Zealand White Hra:(NZW)SPF Albino rabbits/group were assigned to one of two treatment groups. Each group received the test or control articles via injection into three sites (the area surrounding the femoral nerve, the area immediately surrounding an approximate one-inch incision site on the dorsal side of the animal, and intra-articularly). The left side of the animal received the appropriate test article while the right side served as the control and received SKY0402 placebo or 0.9% NaCl.
- Animals were observed for 15 days after dosing with microscopic evaluation of dosing site.
- Body weights for both treatment groups decreased by ~7% over the observation period
- The incidence and severity of skin reddening was noted at a higher rate in the SKY0402 skin incisions than SKY0402 placebo sites, but skin reddening was noted equally among SKY0402, Bupivacaine HCl, and saline sites.
- No test article-related microscopic effects were noted. All changes in the skin appeared to be directly associated with the skin incisions as no microscopic effects were observed for the other two treatment sites.
- The actual value of this information relative to human risk for injection of the drug around an incision is unknown as the total dose volumes of SKY0402 was 0.17 mL/kg for the area immediately surrounding an approximate one-inch incision site and the proposed highest human dose is (b) (4) at a dose of 10 mg/kg. However, based on the information, local tolerance at the incision site in suggested.

## Methods

## Doses:

Group Assignments					
Group	N	Test Article	Injection Site	Dose Level at each Injection Site	Dose Volume
1	4	SKY0402	Intra-articular	15 mg/kg	0.6 mL/kg
			Femoral Nerve	10 mg/kg	0.4 mL/kg
			Incision Site	0.05 mL <sup>a</sup>	0.3 mL
2	2 <sup>b</sup>	Bupivacaine HCl	Intra-articular	7.5 mg/kg	1.5 mL/kg
			Femoral Nerve	5.0 mg/kg	1.0 mL/kg
			Incision Site	0.083 mL <sup>a</sup>	0.5 mL
3	4	Bupivacaine HCl	Intra-articular	3.75 mg/kg	0.75 mL/kg
			Femoral Nerve	2.5 mg/kg	0.5 mL/kg
			Incision Site	0.083 mL <sup>a</sup>	0.5 mL

<sup>a</sup>The incision site received six injections (0.05 or 0.083 mL each) of one of the test articles at a fixed dose level.

<sup>b</sup>Because the first two animals at this dose level died, this Group was terminated and a new group (Group 3) started at a lower dose level.

Frequency of dosing: Single dose

Dosing: Following surgery (incised through the skin on the dorsal side of each rabbit), the test and control articles were administered into three separate sites. Each group received one of the two formulations and the corresponding placebo or saline control. The left side of the animal was dosed with the formulation and the right side of the animal received the SKY0402 placebo or 0.9% sodium chloride and served as the corresponding control site.

The sites were identified as follows: intra-articular area (inside the tibiofemoral joint), area immediately surrounding the Femoral nerve, and the area immediately surrounding the incision site. The dose was administered by bolus injection into the intra-articular and femoral sites while the incision area received six injections.

Dose volume: See table

Formulation/Vehicle: SKY0402 placebo or NaCl

Species/Strain: male New Zealand White Hra:(NZW)SPF Albino rabbits

Number/Sex/Group: 4 males

Deviation from study protocol: Nothing significant

## Observations

Animals were observed for moribundity, mortality, and signs of ill health twice daily.

Body weights were measured and recorded on the day of arrival, once prior to randomization, and on Days 1 and 15.

At study termination, complete necropsy examinations were performed on all animals in Groups 1 and 3. Because the death of the first two Group 2 animals was believed to be due to drug overdose, no necropsy was performed on these animals. The animals were examined carefully for external abnormalities including palpable masses. The skin was reflected from a ventral midline incision and any subcutaneous masses were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities, and the organs were removed and examined.

Microscopic examination of fixed hematoxylin and eosin-stained paraffin sections were performed on sections of tissues from all animals in Groups 1 and 3. The tibiofemoral joints, femoral nerves, and injection site (two sections) from each animal were fixed in neutral buffered formalin for histopathologic evaluation.

## Results

**Mortality** – The first two animals that were dosed with Bupivacaine died (Group 2). As these deaths occurred shortly after dosing, they were considered by the sponsor to be due to a drug overdose produced by the total combined Bupivacaine from all dose sites. Therefore, this group was terminated and a new group (Group 3) started using half the dose for the intra-articular and femoral nerve sites. No other unscheduled deaths occurred during the conduct of this study, so the sponsor may have been correct as to cause of death.

**Clinical Signs** - No clinical symptoms reported.

**Body Weights** – Both groups had a slight body weight loss at the termination of the study of approximately 7%

**Gross Pathology** – While the incidence and severity of skin reddening was noted at a higher rate in the SKY0402 skin incisions (three of four sites, trace to mild) compared to the SKY0402 placebo sites (all normal), this skin reddening was noted equally in the Bupivacaine HCl (three of four sites), and saline (three of four sites) treated animals. The reddening in incision lines was reported to be the result of wound healing, specifically fibroplasia, and granulomatous inflammation associated with foreign material (hair).

The right side is the vehicle/placebo injections side.

TISSUE OBSERVATION	Group 1 (SKY0402)	Group 3 (Bupivacaine HCl)
NUMBER OF ANIMALS EXAMINED	4	4
NUMBER WITHIN NORMAL LIMITS	1	0
<hr/>		
<u>All Tissues</u>	(1)	(0)
Within normal limits	1	0
<u>Injection Site, Left</u>	(3)	(3)
Discoloration, red,	3	3
-trace	1	1
-mild	2	2
<u>Injection Site, Right</u>	(0)	(3)
Discoloration, red, mild	0	3
<hr/>		
CODE: ( ) = NUMBER OF ANIMALS WITH MACROSCOPIC OBSERVATIONS		

**Histopathology** – All changes noted in the skin were comparable for SKY0402, Bupivacaine HCl, and placebo/HCl injections. The changes noted as typical of surgical wound healing. Granulomatous inflammation was present in the incision line and was most reported to be likely associated with foreign material (hair segments) in the incision or the surgical staples used to close the skin. No effects were observed at the intra-articular and femoral nerve sites (treatment or vehicle/placebo).

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**Study title:** Local Toxicity after Intra-articular, Nerve Block, and Subcutaneous Administration of **SKY0402** in Male Dogs (unaudited final letter report – May 12, 2004)

Study no.:	947-013
Study report location:	eCTD DARRTS SDN-1
Conducting laboratory and location:	(b) (4)
Date of study initiation:	December 12, 2003
GLP compliance:	No (study based on GLP)
QA statement:	No
Drug, lot #, and % purity:	- SYK0402, lot 03-2001, NA - SKY0402 placebo, lot BUP-019, NA Administered neat as supplied by sponsor with no analysis

## Key Study Findings

- In a test of potential local irritation from dosing with SKY0402 or bupivacaine HCl, four male Beagle dogs/group were assigned to one of three treatment groups. Each group received the test (SKY0402 or Bupivacaine HCL) or control (SKY00402 placebo or saline) articles via injection into five sites (there were 3 dorsal sites and a femoral and intra-articular site with the right side getting injected on SD1 and the left side on SD12). The right sides of the animals were dosed on day 1 and the left side on day 12 with termination on day 15.
- No notable treatment related effects were observed for mortality, clinical signs, body weights and food consumption, and clinical pathology.
- Macroscopically, the major findings in the dorsal subcutis of test dogs consisted of the presence of clear or red fluid and discolored red areas. Comparison of Day 1 and Day 12 injection sites revealed the SKY0402 sites to have similar findings to the Bupivacaine sites. No differences were observed on Day 1 or Day 12 for the femoral and intra-articular sites.
- The principle microscopic findings in the incision injected dogs were cysts and hemorrhage in the dorsal subcutis which correlated with the macroscopic findings of fluid and red discoloration.
- Overall, no differences were observed after intra-articular, nerve block, and subcutaneous administration in male dogs of Bupivacaine (10 mg), SKY0402 (30 mg), placebo or saline at a dose volume of 2 mL/injection (4 injections of 0.5 mL each).
- The actual value of this information relative to human risk for injection of the drug around an incision is unknown as the total dose volumes of SKY0402 was 0.17 mL/kg for the area immediately surrounding an approximate one-inch incision site and the proposed highest human dose is (b) (4) at a dose of 10 mg/kg. However, based on the information, local tolerance at the incision site is suggested.

## Methods

## Dose groups:

Dose Sites and Control or Test Articles Administered per Group					
Group Number	Dose Sites <sup>a</sup>				
	Dorsal Site 1	Dorsal Site 2	Dorsal Site 3	Femoral Nerve	Intra-articular/Stifle joint
1	Saline	Placebo	SKY0402	Bupivacaine	Placebo
2	Saline	Placebo	Placebo	SKY0402	Bupivacaine
3	Saline	Placebo	Bupivacaine	Placebo	SKY0402

<sup>a</sup>Doses were administered on Days 1 (right side) and 12 (left side).

Doses: 30 mg (~3.5 mg/kg) for SKY0402, 10 mg (~1.2 mg/kg for Bupivacaine HCl)

Frequency of dosing: Single doses on day 1 and 12

Dosing: Dosing with SKY0402, Bupivacaine HCL, SKY0402 placebo, or saline occurred following surgery on Days 1 (right side) and 12 (left side) to the surgical site, stifle joint (intra-articular), and inguinal area.

The incision area received four divided injections on each day.

Dose volume: 2 mL per injection site or 0.17 mL/kg with the incision receiving 4 injections of 0.5 mL

Formulation/Vehicle: SKY0402 placebo or NaCl

Species/Strain: male Beagle dogs

Number/Sex/Group: 4

Deviation from study protocol: Nothing significant

**Observations**

All animals were observed at least twice daily throughout the study for morbidity, mortality, injury, and availability of food and water. A detailed clinical examination of each animal was performed daily. All animals were evaluated and graded daily for injection site swelling and hindlimb function. In addition, surgical sites were checked daily for signs of edema, erythema, drainage, etc.

Body weights and food consumption of all animals were measured and recorded the day of arrival, prior to randomization, and daily throughout the study.

Clinical pathology evaluations were conducted on all animals pretest and at study termination. Animals had free access to drinking water but were fasted overnight prior to sample collection.

Complete necropsy examinations were performed on all animals at study termination (Day 15). The animals were examined carefully for external abnormalities including masses. The skin was reflected from a ventral midline incision and any subcutaneous abnormalities were identified and correlated with antemortem findings. The abdominal, thoracic, and cranial cavities were examined for abnormalities and the organs removed, examined, and, where required, placed in neutral buffered formalin.

Tissue	Organ Weight Taken	Collected and Preserved	Microscopic Examination
Adrenal gland	X	X	
Brain (cerebrum, midbrain, cerebellum, medulla/pons)	X	X	
Epididymis	X	X	
Heart	X	X	
Kidney	X	X	
Liver	X	X	
Parathyroid gland <sup>a</sup>	X	X	
Spleen	X	X	
Testis	X	X	
Thyroid	X	X	
Injection sites (Dorsal Sites 1, 2 and 3, femoral area, and intra-articular area (stifle) on each side)		X	X

<sup>a</sup> Parathyroid gland will be collected and weighed with the thyroid gland.

## Results

**Mortality and Clinical Signs** – no deaths or test article related clinical signs. Surgical site swelling was observed in test article, placebo, and saline groups. No test article injection site swelling was observed. There were no test article-related effects for hindlimb function.

**Body Weights and Feed Consumption** – nothing test article related

**Hematology and Clinical Chemistry** – nothing test article related

**Gross Pathology** - The major macroscopic findings in the dorsal subcutis of test dogs consisted of the presence of clear or red fluid (up to 30 mL) and discolored red areas. Comparison of Day 1 and Day 12 injection sites revealed the SKY0402 sites to have similar findings to the Bupivacaine sites. Similarly, no biologically significant differences at Day 1 or Day 12 at the femoral and intra-articular sites were observed.

**Organ Weights** – nothing treatment related

**Histopathology** – No treatment related effects were observed on Day 1 or Day 12 for the femoral and intra-articular injection sites.

The principle microscopic findings in the incision injected dogs were cysts and hemorrhage in the dorsal subcutis which correlated with the macroscopic findings of fluid

and red discoloration, respectively. Comparison of Day 1 and Day 12 injection sites revealed the SKY0402 sites to have similar findings to the Bupivacaine sites.

**Summary of Microscopic Observations - MALE  
Day 1 Injection Sites - Dorsal**

Tissue Observation	Severity	Saline	Placebo	30 mg SKY0402	10 mg Bupivacaine
		Number of Sites Examined	12	16	4
within normal limits		0	0	1	0
cyst		10	16	2	4
-minimal		2	1	0	0
-mild		4	4	0	2
-moderate		4	9	2	2
-severe		0	2	0	0
Fibrosis	-minimal	1	0	0	0
hemorrhage		6	7	2	0
-minimal		5	1	1	0
-mild		1	6	1	0
inflammation, subacute		1	2	0	0
-minimal		0	2	0	0
-mild		1	0	0	0
regeneration, minimal	-minimal	2	0	0	1

**Summary of Microscopic Observations - MALE  
Day 12 Injection Sites - Dorsal**

Tissue Observation	Severity	Saline	Placebo	30 mg SKY0402	10 mg Bupivacaine
		Number of Sites Examined	12	16	4
within normal limits		0	0	0	0
cyst	-minimal	10	12	3	2
hemorrhage		11	16	4	4
-minimal		10	15	4	4
-mild		1	1	0	0
inflammation, acute	-minimal	1	1	0	1
inflammation, granulomatous	-minimal	0	1	0	0
inflammation, subacute	-minimal	2	9	1	0
necrosis	-minimal	1	1	0	1
regeneration, minimal	-minimal	1	2	1	0

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**non-GLP study RES-0702-D0402-044** – In a study conducted by Pacira Pharmaceuticals, groups of 3 male guinea pigs per group were injected subcutaneously (SC) with 1 mL/kg of the following on their left and right sides (see table), observed for 13 days, and the skin was evaluated histologically. DepoBupivacaine (phosphate), aka SKY0402, is the proposed drug product.

Group Number	Test or Control Article (mg/kg)	
	Left side	Right Side
1	Bupivacaine HCL (12.5 mg/kg)	Saline
2	DepoBupivacaine (glucuronate) (25 mg/kg)	Saline
3	DepoBupivacaine (phosphate)(25 mg/kg)	Placebo

No adverse clinical symptoms were reported. Saline treated skin exhibited no treatment related histological changes. Microscopic observations for all other treatment sites were

comparable, whether SKY0402 placebo bupivacaine HCl, or DepoBupivacaine (see table). Observations included mild to moderate, diffuse, subacute dermatitis consisting mainly of macrophages present between the SC tissue and muscle, all consistent with a foreign body reaction. Therefore SC placebo or DepoBupivacaine caused no differing local toxicity than SC Bupivacaine HCl.

Treatment Group	Incidence
Saline	<ul style="list-style-type: none"> <li>• Normal (3/3)</li> </ul>
Placebo	<ul style="list-style-type: none"> <li>• Normal (1/3)</li> <li>• Mild to moderate diffuse subacute dermatitis (2/3)</li> <li>• Presence of foamy macrophages present in deep dermal tissue underlying the layer of muscle (2/3)</li> </ul>
Bupivacaine HCl	<ul style="list-style-type: none"> <li>• Normal (2/3)</li> <li>• Mild to moderate diffuse subacute dermatitis (1/3)</li> <li>• Presence of foamy macrophages present in deep dermal tissue underlying the layer of muscle (1/3)</li> </ul>
02Bup02-028 (glucuronate)	<ul style="list-style-type: none"> <li>• Normal (0/3)</li> <li>• Mild to moderate diffuse subacute dermatitis (3/3)</li> <li>• Presence of foamy macrophages present in deep dermal tissue underlying the layer of muscle (2/3)</li> </ul>
02Bup02-027 (phosphate )	<ul style="list-style-type: none"> <li>• Normal (1/3)</li> <li>• Mild to moderate diffuse subacute dermatitis(1/3)</li> <li>• Presence of foamy macrophages present in deep dermal tissue underlying the layer of muscle (1/3)</li> <li>• Active lesion containing neutrophils (1/3)</li> </ul>

Also noteworthy from this study was pharmacokinetic data that indicated that the SC DepoBupivacaine exhibited sustained release compared to SC Bupivacaine HCl (see section 5.1 PK/ADME).

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**non-GLP study RES-0702-D0402-047** – In a study by Pacira Pharmaceuticals, groups of 3 male guinea pigs per group were dosed by intrasynovial intra-articular (IA) injections with 0.25 mL/kg of the following on their left and right sides (see table), observed for 5 days, and the skin was evaluated histologically. DepoBupivacaine (phosphate), aka SKYU0402, is the proposed drug product.

#### Treatment Assignment by Group

Group Number	Test or Control Article (mg/kg)	
	Left side	Right Side
1	Bupivacaine HCL solution (1.25 mg/kg)	Saline
2	DepoBupivacaine glucuronate (6.25 mg/kg)	Saline
3	DepoBupivacaine phosphate (6.25 mg/kg)	Placebo

All doses were well tolerated with some inflammation of the injection site observed for the glucuronate formulation only, described as focal, mild to moderate, subacute myositis with mostly foamy macrophage exudate in the muscle tissue along the side of the bone.

(b) (4)

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## 11 Integrated Summary and Safety Evaluation

**NOTE:** While nonclinical studies were performed with SKY0402 administered by several routes such as local infiltration, intradermal, subcutaneous, perineural, epidural, intrathecal, and intra-articular routes in rat, guinea pig, rabbit, mini-pig, and/or dog models, only those routes relevant to the proposed indication are discussed in this section (i.e., subcutaneous and local infiltration). However, individual study report reviews of different dose routes are included in the body of this review for future reference.

### Background/Prior Regulatory History:

The original IND application (IND 69,198) for SKY0402 (EXPAREL™) was submitted on December 9, 2004. On January 6, 2005 the IND was inactivated and later re-submitted on March 7, 2006. The FDA accepted the IND on November 13, 2006. The original submission was placed on clinical hold due to lack of sufficient nonclinical information required to assess the risks to subjects of the proposed studies. The submission contained primarily local irritation studies of SKY0402 with subcutaneous, intra-articular, and perineural administration in rats and dogs while the proposed clinical studies involved SKY0402 wound infiltration after hernia repair for control of pain post-operatively as well as a regional ankle-block anesthesia for bunionectomy. With submission of appropriate nonclinical studies and other review discipline information, the IND was reactivated (Nonclinical Review in DARRTS April 16, 2007). Only subcutaneous infiltration of SKY0402 is proposed for this NDA submitted September 28, 2010.

SKY0402 is composed of the active pharmaceutical ingredient (API), bupivacaine, contained in a suspension with the liposomal DepoFoam® drug delivery system. This suspension is intended to lead to a slower release of bupivacaine allowing a longer duration of action and, from a toxicological standpoint, to a slower uptake into the systemic circulation avoiding high plasma concentrations resulting in less potential systemic toxicity (e.g., CNS effects). The DepoFoam in SKY0402 contains a novel excipient, dierucoylphosphatidylcholine (DEPC).

## Bupivacaine

Bupivacaine HCl has been marketed in the US for almost 40 years as Marcaine® (NDA 16-964 - 1972), the reference listed drug (RLD) in the FDA Orange Book: Approved Drug Products with Therapeutic Equivalence Evaluations. The highest approved daily dose is 400 mg (Maximum Recommended Human Dose – MRHD).

In this 505(b)(2) submission, any proposed doses which produce exposures outside of the approved bupivacaine exposure limits require additional nonclinical studies to support the human safety of bupivacaine in SKY0402. (b) (4)

Systemic exposure levels of bupivacaine after dosing with SKY0402 in clinical trials (b) (4) could not be determined to be comparable to those supported by the referenced NDA as human exposure levels at the MRHD could not be found and were not tested in any of the clinical trials. To this end, systemic levels of bupivacaine after human dosing with SKY0402 at (b) (4) are potentially greater than those achieved after dosing with the approved bupivacaine HCl in the 505(b)(2) referenced drug according to ClinPharm evaluation. This noncomparability was largely due to the lack of human pharmacokinetic data for the approved bupivacaine HCl at the highest approved dose of 400 mg/day (MRHD).

The human safety of bupivacaine is supported using a 505(b)(2) submission in combination with acute infiltrative surgical studies in rabbits and dogs and repeat dose subcutaneous studies in rats and dogs compared to the pivotal single dose clinical trials. The sponsor submitted repeat dose toxicity studies (one month) in two species which satisfies the requirement for at least 1 species to be tested as listed in the FDA Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route (March 2008). The nonclinical testing of SKY0402 also turned out to be necessary to support the human safety of bupivacaine (b) (4)

## DEPC-based DepoFoam

The human safety of DepoFoam (Inactive Ingredient) is generally supported by the marketed products DepoCyt® (NDA 21-041, 1999) and DepoDur® (NDA 21-671, 2004) and the submitted DEPC-based DepoFoam toxicity studies. The exact composition of DepoFoam is (b) (4) different in each of the three drug products (DepoCyt, DepoDur, and SKY0402). The difference in the DepoFoam in SKY0402 is the novel excipient dierucoylphosphatidylcholine (DEPC).

A comprehensive nonclinical testing program was submitted to support the human safety for the DEPC-based DepoFoam in SKY0402. This program included repeat dose toxicity studies (one month) in two species (one nonrodent), genotoxicity, and reproductive toxicity, consistent with the FDA Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients (May 2005).

**Proposed Clinical Use:** SKY0402 is indicated for single-dose local administration into the surgical wound to produce post-surgical analgesia. (b) (4)

(b) (4)  
SKY0402 has not been studied for use in patients younger than 18 years of age.

#### **Formulation/Impurities-Degradants Issues/Excipients:**

SKY0402 consists of microscopic spherical, multivesicular liposomes (DepoFoam® drug delivery system), which is composed of a honeycomb-like structure of numerous non-concentric internal aqueous chambers containing bupivacaine. Each chamber is separated from adjacent chambers by lipid membranes. Bupivacaine is released from the DepoFoam particles over an extended period of time. (b) (4)

Drug Substance (DS) and Drug Product (DP) Specifications – Except as noted in the next section, based on submitted DS and DP specifications, all components specified impurities and degradants are within ICH Guidances Q3A and Q3B(R) limits, respectively. The SKY0402-specific DepoFoam containing DEPC has been qualified by nonclinical testing and is also supported by related, approved DepoFoam NDAs for DepoCyt and DepoDur.

Drug Substance (DS) and Drug Product (DP) Impurities and Degradants – Identified chemicals of concern for potential genotoxicity/carcinogenicity that are related to bupivacaine are (b) (4)

(b) (4), a bacterial assay mutagen, was reduced to acceptable levels resulting in potential exposure to less than 1.5 µg/day as recommended in by the draft CDER Guidance for Industry: *Genotoxic and Carcinogenic Impurities in Drug Substances and Products: Recommended Approaches (Dec 2008)*.

(b) (4) has previously been noted to produce tumors in rodents but this has been considered to have little relevance to human (b) (4)

(b) (4) As it is genotoxic, the applicant will be asked by ONDQA to lower (b) (4) levels to as low as reasonably possible (ALARP), meaning also as low as technically feasible. This level has been identified as (b) (4) for this submission. This value is consistent with allowed levels in other previously approved drugs in which (b) (4) is an impurity. Another factor for consideration is that the proposed indication for this NDA application is a single dose, meaning very limited exposure duration.

(b) (4) although initially identified as possessing structural alerts by ONDQA, are not structural alerts and are not predicted to be genotoxic in the Ames bacterial mutation assay according to an FDA CompTox analysis (FDA CDER Informational and Computational Safety Analysis Staff – ICSAS). As both are currently within ICH specifications, no further action is indicated in regard to human safety.

(b) (4)

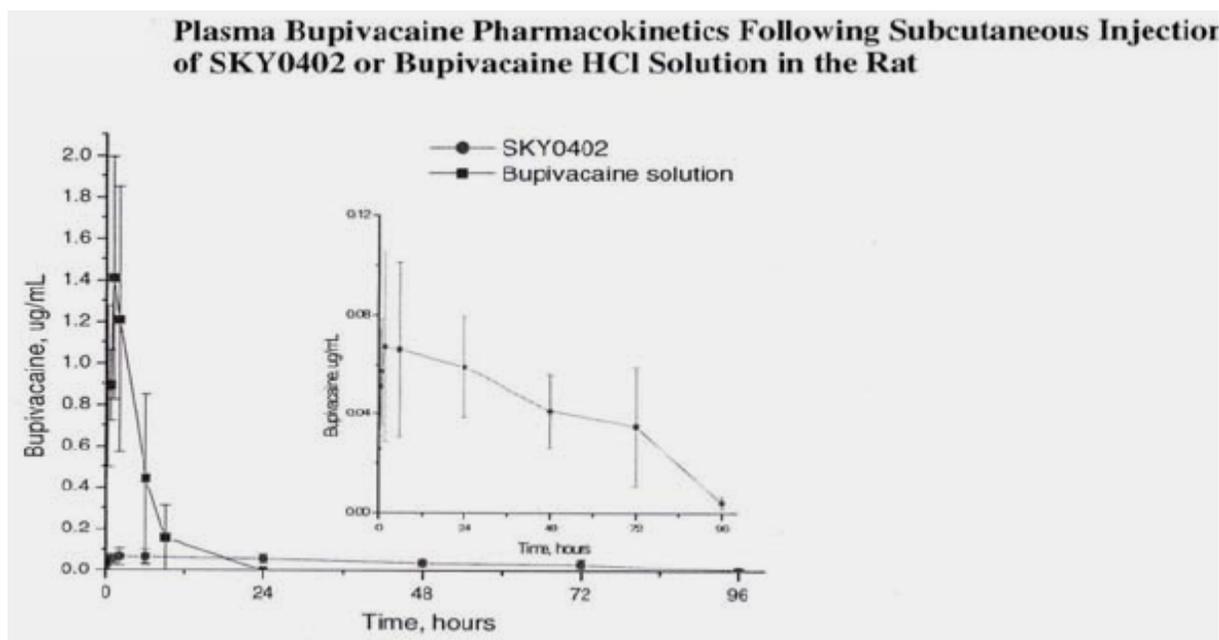
Excipients – The novel excipient DEPC and other excipients in the drug product are qualified by the nonclinical test program for SKY0402 Placebo (DEPC-specific DepoFoam). In addition, the human safety of the excipients, other than DEPC, used in the creation of the DepoFoam liposomal formulation are supported by DepoFoam NDAs 21-041 (DepoCyt®) and 21-671 (DepoDur®).

Extractable-Leachables from container closure system - Based on the worst case extractable studies performed on the proposed commercial container closure system, SKY0402 lots stored beyond expiration dating were analyzed for potential leachable content. No leachables were detected in any of the analyzed lots of SKY0402 that were stored in the proposed commercial container/closure system for up to 30 months. The applicant has confirmed and FDA ONDQA has concurred that the proposed container closure system is appropriate for SKY0402 and that no additional nonclinical studies are necessary to support its use.

**Safety Pharmacology:** While no separate studies were conducted with SKY0402 or SKY0402 Placebo, safety pharmacology indices were included as part of the repeat dose studies with no indication of any previously unknown safety concerns for bupivacaine or other than local, reversible injection site effects for the DEPC-based DepoFoam (SKY0402 Placebo).

**Pharmacology:** In a primary pharmacology study, bupivacaine HCl's pain efficacy (dermal wheel/pin prick test) was not evident at 3 hours post-dosing in guinea pigs. The same dose of SKY0402 indicated the sustained anesthetic effects of SKY0402 as pain efficacy was observed for at least 6 hours after dosing. In a secondary pharmacology study, the effect of sustained release bupivacaine from SKY0402 on human whole blood coagulation was evaluated using activated clotting time (ACT) in vitro. At clinically comparable doses to bupivacaine HCl, there was no indication that clotting time would be prolonged after dosing with SKY0402.

Pharmacokinetics/Toxicokinetics – Doses were selected to relate to the potential human exposure. The nonclinical species used for generating toxicokinetic data were those that were used in the toxicology evaluation of SKY0402 in non-surgical and surgical models. Dedicated PK evaluation was limited to a small group of studies to confirm that SKY0402 produces a sustained bupivacaine profile compared to bupivacaine HCl solution. The following figure for rats illustrates this sustained profile of bupivacaine release from the site application for SKY0402 compared to bupivacaine HCl dosing.



DepoFoam clearance - The DepoFoam membrane remnants (b) (4) are biocompatible and are believed to be cleared through the lymphatic system and metabolized as nutrients.

The retention of bupivacaine and dierucoylphosphatidylcholine (DEPC, one of the lipid components of SKY0402) at the injection site was studied following administration of SKY0402 or SKY0402 placebo in guinea pigs and rats. Both bupivacaine and DEPC remained at the injection site for several days with bupivacaine leaving more rapidly than DEPC. Radio-label studies identified the highest concentrations of radioactivity in lymphatic, excretory, and adipose tissues consistent with known pathways of lipid distribution and metabolism. Based on subcutaneous testing of the injection site in rats

and guinea pigs, bupivacaine radioactivity was not detected at the injection site by approximately 2 weeks and DEPC was not detected at the injection site after between 2 and 3 weeks after injection.

Drug interactions - Subcutaneous co-administration of SKY0402 with lidocaine HCl solution resulted in a more rapid release of bupivacaine from SKY0402 than after SKY0402 alone. This interaction can be reduced by allowing 20 minutes to elapse between a lidocaine dose and administration of SKY0402. In contrast, the composite plasma profiles appeared to be cumulative after subcutaneous co-administration of SKY0402 with bupivacaine HCl.

### **Pivotal Single Dose Toxicology Studies For Protocol Support:**

These studies will be discussed in some detail as they are the only studies that actually involve wound infiltration and healing, which is directly related to the proposed clinical indications. These studies cover both local toxicity (e.g., novel excipient DEPC in DepoFoam) and systemic toxicity (e.g., bupivacaine in SKY0402).

Single dose wound infiltration studies were conducted with SKY0402 in groups of four rabbits and four dogs per group in a nonclinical model of a hernia operation that included using a prolene mesh. A comprehensive nonclinical testing protocol was employed that included microscopic evaluation on days 3 (2 animals/group) and 15 (2 animals per group) after surgery and an assessment of wound healing (total hernia repair score). Doses were 0 (saline), 9, 18, or 25 mg/kg (30 mg/kg in rabbits) with bupivacaine concentrations at 15 mg/mL (same as proposed clinical drug product) or 25 mg/mL (only high dose groups). A Sensorcaine group was included at 9 mg/kg bupivacaine and a 7.5 mg/mL bupivacaine concentration. Dose volumes were 0.6 mL/kg for the low dose and 1.2 mL/kg for all other dose groups (1 mL/kg for the high dose dog group), (b) (4)

No apparent treatment-related effects were observed for clinical signs, body weight, food consumption, or clinical pathology. Toxicity findings were limited to effects noted on a wound healing evaluation on days 2-3 (total Hernia Repair Score) and microscopic findings at the surgical site taken at sacrifice on SD3 and SD15. The Total Hernia Repair score (inflammation, erythema, induration, and exudate) was generally higher for the SKY0402 groups in a dose-dependent manner compared to saline control although generally similar to the Sensorcaine control. Observations were almost exclusively erythema and edema, the latter slight in severity and only in SKY0402 25 mg/kg animals (dogs). Observed effects were reversed within 5 days in all but 1 high dose dog with a decrease to a severity of slight erythema and edema by the end of the study.

The wound bed appeared normal in all animals with no detected odor. Surgical site findings on SD3 necropsy revealed no clear effects of SKY0402 compared with saline or Sensorcaine controls. All groups exhibited myofiber de-/regeneration, and edema, mineralization, hemorrhage, acute inflammation, and abscesses around sutures.

The suture site-related observation of granulomatous inflammation was observed on day 3, but only in dogs. Minimal to mild effects were observed for 3 low dose animals (1 male and 2 females), 2 mid dose animals (females), and no high dose animals. On day SD15, minimal to mild granulomatous inflammation was noted in all rabbit SKY0402 dose groups (only females at low dose group) and in all dog dose groups. This inflammation was not observed with either saline or Sensorcaine control in rabbits but was observed to comparable incidence and severity in dog saline and Sensorcaine control groups. This occurrence of granulomatous inflammation could possibly represent a specific test article effect although the lack of a dose- response in dogs on day SD3 and a reduced incidence in high dose rabbits (incidence of 1 of 4 versus 2 of 4 for other groups) on day SD15 (incidence of 1 of 4 versus 2 of 4 for other groups) contributes raises doubts. Animal numbers are insufficient for any definitive conclusions. Overall, these observations findings were not considered clearly related to SKY0-402 specific treatment related.

In summary, the high doses were considered NOAELs, most notable for microscopic effects at the wound and for wound healing as only local, treatment site effects were observed. As toxicokinetic (TK) values are comparable to the Pharmacokinetic (PK) values from the human clinical trials, these nonclinical studies support the proposed clinical dosing (see the nonclinical-based human safety assessment section below for a more detailed discussion).

Other single dose studies - At two- to three-fold higher doses of bupivacaine in SKY0402 compared to bupivacaine HCl solution, there was no evidence of local or systemic toxicities toxicity when SKY0402 was administered by varied dose routes at doses up to 30 mg/kg bupivacaine. Acute toxicology studies conducted with SKY0402 placebo administered by subcutaneous bolus have indicated that it is without significant local or systemic toxicity when administered as a single dose.

### **Pivotal Repeat-Dose Toxicology Studies for Protocol Support:**

These studies cover both local toxicity (e.g., novel excipient DEPC in DepoFoam) and systemic toxicity (e.g., bupivacaine).

SKY0402 – 4-Week studies in rabbits and dogs at twice weekly doses of 0, 9, 18, & 30 mg/kg with a 4 week recovery period were the pivotal nonclinical studies for SKY0402. In rabbits, convulsions were observed in 1 low dose male on week 1, one mid dose female on week 4, and 1 mid dose male on week 5 after one of the two weekly injections, but not in any high dose animals. These non dose-responsive, anticipated effects are considered clinically monitorable so the high dose is used for animal:human dose ratio calculations. In dogs, the high dose was considered the No Observed Adverse Effect Level (NOAEL). As the Toxicokinetic (TK) values at these nonclinical doses are comparable to the Pharmacokinetic (PK) values from the human clinical trials, these nonclinical studies support the proposed clinical dosing (see the nonclinical-based human safety assessment section below for a more detailed discussion).

SKY0402 Placebo – 28-Day repeat-dose toxicity studies in rats and dogs using daily doses of a SKY0402 placebo formulation at the maximum practicable dose volume per species of 5 mL/kg/day to a single site in rats and 2 mL/kg/site to 2 sites (4 mL/kg/day) in dogs were the pivotal studies submitted to support the human safety of the novel excipient DEPC in SKY0402 Placebo. 14-Day and 28-day recovery periods, respectively, were also included in the study design. The administered doses were considered NOAELs. As the DEPC exposures at these nonclinical doses are comparable to the DEPC exposures in the human clinical trials, these nonclinical studies support the proposed clinical dosing with the novel excipient DEPC in DepoFoam (see the nonclinical-based human safety assessment section below for a more detailed discussion).

### **Genetic Toxicology:**

SKY0402 - No studies were submitted as part of the nonclinical program to assess the genotoxic potential of SKY0402. As listed in the label of the 505(b)(2) reference NDA, no genotoxicity studies have been conducted with bupivacaine. Assessment of potentially genotoxic impurities/degradants is as described in the earlier Formulation section and all are within acceptable levels.

SKY0402 Placebo - The genotoxic potential of DEPC, the novel DepoFoam component of SKY0402 placebo, was tested in a conventional battery of tests that included an in vitro bacterial mutation test (*S. typhimurium* and *E. coli* in the presence or absence of metabolic activation), an in vitro chromosomal aberration test (human peripheral lymphocytes in the presence or absence of metabolic activation), and an in vivo mouse micronucleus test. SKY0402 was not genotoxic in these valid assays.

### **Reproductive Toxicology:**

SKY0402 - No testing was conducted. Information on reproductive toxicity of bupivacaine and safety of use in humans for bupivacaine is based on the label for the 505(b)(2) reference NDA 16-964 (Marcaine).

SKY0402 Placebo – Rat and rabbit embryo-fetal studies and a combined fertility and peri-/post-natal study were conducted with SKY0402 Placebo in order to assess the potential human toxicity of DEPC in SKY0402. As appropriate for a given study, gestational/post-gestational dosing with SKY0402 Placebo was at the maximum practicable dose volume per species for the embryo-fetal studies in rats (3.3 mL/kg/site to 3 sites, 10 mL/kg/day) and rabbits (0.67 mL/kg/site to 3 sites – 2 mL/kg/day) and the combined fertility/perinatal-postnatal study in rats (3.3 mL/kg/sit to 3 sites – 10 mL/kg/day). The administered doses were considered NOAELs and therefore support DEPC use in humans. See section below on safety of proposed SKY0402 Placebo human dosing for additional discussion of these studies. Human safety will be assessed based on the dose of DEPC to each species at the NOAEL as a Human Equivalent Dose (HED) for systemic toxicity and as a dose volume for local toxicity compared to human exposure to DEPC at the proposed human doses.

Effects on Juvenile Animals - A 4-week subcutaneous repeat dose range finding study of SKY0402 (nonGLP) was conducted in juvenile rats as the initial study to support the approvability and labeling of SKY0402 for pediatric use (not part of current application). SKY0402 was tested at previously tested adult rat doses levels of 0, 9, 18, & 30 mg/kg by once or twice weekly dosing starting on postnatal day 7 and continuing for 4 weeks. No treatment related effects were observed for a comprehensive set of biological assessments. Systemic exposure to bupivacaine was confirmed by blood analysis with a generally dose-responsive increase in bupivacaine blood levels. Systemic plasma concentrations of bupivacaine were observed up to 24-hours after the last dose. Blood levels were comparable between the once weekly and twice weekly dose groups for a given dose. The NOAEL was 30 mg/kg as was the case for adult rats.

**Inadvertent intravenous dosing risk:**

Proposed human dosing with SKY0402 containing the unique DepoFoam excipient DEPC is considered safe in the event of inadvertent intravenous injection.

(b) (4)

Single intravenous administration of SKY0402 in rats with SKY0402 at doses 3 times larger than the administered bupivacaine HCl caused no adverse effects while the bupivacaine HCl cause severe toxicity including tremors and death over the 24 hour observation period. Based on this information, for the proposed dose route of subcutaneous infiltration only, the applicant was informed that no further nonclinical intravenous toxicity testing was required (IND 69,198 – September 27, 2006).

The SKY0402 dose volume was 0.5 mL/kg at a dose of 7.5 mg/kg (HED of 1.2 mg/kg). Proposed human dose volumes and doses are 0.13 mL/kg and 2 mg/kg for the proposed human dose of 120 mg dose, 0.3 mL/kg and 5 mg/kg for the proposed human dose of 300 mg dose of SKY0402, and 1 mL/kg and 10 mg/kg (b) (4)

This nonclinical study indicates relative intravenous safety of SKY0402 compared to bupivacaine HCl, but only SKY0402 human dosing safety at the lowest proposed human dose of 120 mg SKY0402 when assuming injection of the total dose intravenously in humans. Safety can be inferred for the other proposed human doses as it is unlikely that more than a minimal amount of the dose would be inadvertently injected intravenously (maximum 10% of dose under worst case conditions per the medical reviewer).

SKY0402 Placebo – Potential inadvertent intravascular injection of SKY0402 was not considered to present a human safety issue under a worst case injection of 10% of the total intended subcutaneous dose volume.

In three GLP studies, single intravenous administration of SKY0402 Placebo was conducted in rats at different dose volumes. Proposed human dose volumes are 0.13, 0.3, & (b) (4) (b) (4)

At 20 mL/kg, the first two rats dosed died within 5 minutes so this study was terminated. The lethal dose was 10 mL/kg/5 minutes or ~2 mL/kg/minute (only dose tested). The highest proposed humans dose is 1/10 the lethal volume in this study (b) (4)

In a second study that lasted 9 days (doses of 0.02, 0.05, 0.1, & 1 mL/kg), reversible decreased activity was observed on the first day at the high dose in 2 of 8 animals (1 mL/kg). (b) (4)

In a third study (doses of 0.1, 0.5, & (b) (4) death occurred in 1 of 20 animals after intravenous administration on the first day of this study. A complete biological assessment was part of this study conducted using a complete protocol that included sacrifices and histology on days 3 and 15 after dosing. No other adverse effects were observed except for the animal that died (decreased activity and shallow breathing) and 1 of 20 animals in the 0.5 mL/kg mid dose group (reversible shallow breathing). No adverse histopathology was observed on any treated animals and the animal that died (e.g., no pulmonary embolism). (b) (4)

## Summary

Intravenous SKY0402 (7.5 mg/kg) was not toxic at lethal doses of bupivacaine HCl (2.5 mg/kg) when injected into rats. However, the dose of SKY0402 tested (0.5 mL/kg and 1.2 mg/kg HED) was not high enough to be able to conduct a nonclinical-based human safety assessment for all the proposed clinical doses assuming inadvertent human intravenous injection is 100% of the total dose. However, this occurrence is not likely. Assuming inadvertent intravenous injection of 10% of the highest proposed human dose (b) (4) all proposed human doses would be supported for inadvertent intravenous dosing. The medical reviewer considered this 10% level a worst case possibility for inadvertent intravenous dosing. In a Division and sponsor communication (IND 69,198 – September 27, 2006), exposure by the intravenous route was considered highly unlikely with the proposed subcutaneous infiltrative dose route. As a result, for the proposed clinical dose route of subcutaneous infiltration only, the applicant was informed that no further nonclinical intravenous toxicity testing was required.

Intravenous administration of SKY0402 Placebo resulted in clinical signs in one study and lethality in another at the dose volume of 1 mL/kg using the same batch of SKY0402 placebo. That would be 1 death in 28 rats and clinical signs in 3 of 28 including the one that died at a dose volume comparable to that of the highest proposed human dose of SKY0402. At a 10% inadvertent intravenous injection in the clinic, the

inadvertent intravenous clinical dose would be equal to the NOAEL in the two nonclinical studies just mentioned (0.1 mL/kg SKY0402 Placebo)

NOTE: Should the Applicant propose dose routes other than subcutaneous tissue infiltration, we will need to revisit this issue. This issue may also need to be revisited based on the data above and if dosing experience indicates that inadvertent intravenous injection is substantially greater than 10% of the administered dose.

## NONCLINICAL-BASED HUMAN SAFETY ASSESSMENTS

### Safety of proposed SKY0402 human dosing based on bupivacaine exposure in SKY0402

The proposed highest dose of SKY0402 in humans is (b) (4) bupivacaine HCl (b) (4). Other proposed doses are (b) (4) hemorrhoidectomy and (b) (4) for bunionectomy. Based on these clinical trials, the human indications are broad and include incisional infiltration (b) (4). Bupivacaine-based exposure comparisons between animals and humans were performed using data from single dose wound healing studies in rabbits and dogs and 4 week repeat dose studies in rabbits and dogs compared to the pivotal single dose clinical studies with SKY04502 as reported for the indications just noted. Exposure was to SKY0402 in these cases.

For the following safety assessments of both single and repeated dose studies, as only anticipated pharmacological effects of bupivacaine and reversible local injection site effects were observed, the listed animal doses are considered the No Observed Adverse Effect Levels (NOAELs) for local and systemic toxicity. Single and repeated study assessments are combined as the results are very similar.

Local toxicity – SKY0402-related toxicity was not observed as only anticipated local effects were observed and not considered specifically SKY0402 related as observations were more dosing method, surgical procedure related (single dose studies only), and also observed with bupivacaine HCl treatment.

Systemic toxicity – The single and repeat dose rabbit and dog data support all proposed clinical doses. The nonclinical doses reported are the high doses of the studies and considered NOAELs. Nonclinical toxicokinetic (TK) and clinical pharmacokinetic (PK) values and nonclinical:clinical exposure ratios (TK:PK) are listed below in separate tables for single and repeat dose studies.

At the animal NOAEL, the nonclinical:human exposure ratios are defined as safety margins (SMs) and should be  $\geq 1$ . While not all SMs are  $\geq 1$  (b) (4) as the nonclinical doses are the highest doses tested with no more than clinically monitorable anticipated pharmacological effects observed (rabbits only), the larger TK values after repeated dosing in dogs are most relevant to support human safety (yellow

highlight). The high Cmaxs in the human bunionectomy (b) (4) clinical trials resulted in SMs < 1, but this is not considered indication of the lack of nonclinical support for the proposed human doses as those human bupivacaine Cmaxs are still less than the bupivacaine lower human toxicity threshold of 1000 ng/mL. In addition, a long history of human bupivacaine use considered in conjunction with the nonclinical-based TK and PK values also supports the proposed clinical use.

Single Dose Studies in Rabbits and Dogs					
Bupivacaine Nonclinical TK:Human PK based Safety Margins (SM) for SKY0402					
	Human PK at proposed dose			Nonclinical Species and NOAEL TK	
Index	120 mg <sup>a</sup>	300 mg <sup>b</sup>	(b) (4)	Rabbit	Dog
Cmax (ng/mL)	166	365 & 867 <sup>d</sup>	(b) (4)	307	504
AUC (ng·h/mL)	7,015	16,758 & 18,289	(b) (4)	18,400	19,500
SAFETY MARGINS					
Index	Rabbit-based SMs			Dog-based SMs	
	120 mg	300 mg	(b) (4)	120 mg	300 mg
Cmax SM	1.8	0.84 & 0.35	(b) (4)	3.0	1.4 & 0.58
AUC SM	2.6	1.1 & 1.0	(b) (4)	2.8	1.2 & 1.1

a – bunionectomy (study SKY0402-C-317)

b - inguinal hernia (study SKY0402-C-201) and hemorrhoidectomy (study SKY0402-C-316), respectively

(b) (4)

d – PK below generally accepted human toxicity lower threshold of ~1,000 ng/mL  
 - rabbit Cmax reverse dose-responsive (e.g., 2x at ~1/3 NOAEL dose at 1 day)

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Repeat Dose Studies in Rabbits and Dogs					
Bupivacaine Nonclinical TK:Human PK based Safety Margins (SM) for SKY0402					
	Human PK at proposed dose			Nonclinical Species and NOAEL TK	
Index	120 mg <sup>a</sup>	300 mg <sup>b</sup>	(b) (4)	Rabbit	Dog
Cmax (ng/mL)	166	365 & 867 <sup>d</sup>	(b) (4)	292	910
AUC (ng·h/mL) <sup>e</sup>	7,015	16,758 & 18,289	(b) (4)	13,467	58,400
SAFETY MARGINS					
Index	Rabbit-based SMs			Dog-based SMs	
	120 mg	300 mg	(b) (4)	120 mg	300 mg
Cmax SM	1.8	0.80 & 0.34	(b) (4)	5.5	2.5 & 1.1
AUC SM	1.9	0.80 & 0.73	(b) (4)	8.3	3.5 & 3.2

a – bunionectomy (study SKY0402-C-317)

b - inguinal hernia (study SKY0402-C-201) and hemorrhoidectomy (study SKY0402-C-316), respectively

(b) (4)

d – PK below generally accepted human toxicity lower threshold of ~1,000 ng/mL  
 - rabbit Cmax reverse dose-responsive (e.g., 2x at ~1/3 NOAEL dose at 1 day)

e - nonclinical AUC values extrapolated to 96 hours from 72 hour measurements

**Safety of proposed SKY0402 human dosing based on DEPC exposure in SKY0402 and SKY0402 Placebo**

SKY0402 and SKY0402 Placebo, which contain the novel excipient DEPC as part of the DepoFoam drug delivery system, received fairly extensive nonclinical testing for local and systemic toxicity. SKY0402 Placebo was made with sodium chloride in lieu of bupivacaine. In addition, reference is made to the other approved DepoFoam products DepoDur and DepoCyt as supporting the safety of DepoFoam, leaving the DEPC alone to be evaluated for safety.

Nonclinical subcutaneous studies conducted were 28 days in rabbits and dogs with SKY0402 and 28 days in rats and dogs, embryo-fetal studies in rats and rabbits, and a combined fertility/perinatal-postnatal study in rats with SKY0402 Placebo. For the SKY0402 Placebo dosing, all doses were considered the maximum practicable dose based on the dose volume administered for the respective nonclinical species (Diehl et al. J. Appl. Toxicol. 21:15-23, 2001).

All nonclinical doses were NOAEL doses as only local, reversible injection site effects were observed for all of the varied biological indices evaluated in these comprehensive GLP studies and also anticipated pharmacological effects of bupivacaine for the SKY0402 studies.

On this basis, DEPC-related toxicity was not observed as only anticipated local effects were observed and not considered specifically DEPC related as observations were more dosing method related and also observed with bupivacaine HCl treatment (SKY0402 only).

SKY0402 containing the novel excipient DEPC is considered safe (b) (4) for both local and systemic toxicity. Comparison of nonclinical animal and human doses for potential local toxicity were based on the exposure to DEPC on a mL/kg basis at comparable levels of DEPC in SKY0402 and SKY0402 Placebo. Comparison of nonclinical animal and human doses for potential systemic toxicity were based on the exposure to DEPC on a mg/kg basis with the nonclinical animal doses adjusted to a human equivalent dose (HED) resulting in mg dose/m<sup>2</sup> body surface area-based comparisons (BSA). The proposed highest human dose is (b) (4) SKY0402 (b) (4)

Potential human local and systemic toxicity Safety Margins (SMs) are listed in separate tables (local toxicity and systemic toxicity tables). SMs of  $\geq 1$  are considered indicative of human safety. As all SMs are approximately equal to or greater than 1, human safety is supported at the proposed human doses of 120, 300, (b) (4) SKY0402 for potential local and systemic toxicity due to the DEPC-based DepoFoam.

<b>Local Toxicity<sup>a</sup>: Injection Site Dose Volume-Based Safety Margins (SMs) - Nonclinical Animal NOAEL Dose<sup>b</sup> to Highest Human Dose<sup>c</sup> Ratios for Local Site Dosing with Novel Excipient DEPC in SKY0402 and SKY0402 Placebo (nonclinical) and SKY0402 (human)</b>					
Nonclinical study	species	Local Dose	(b) (4)		SMs
<b>SKY0402</b>					
Single dose and 28 day	Rabbit	1.2 mL/kg			1.2
	Dog	1.2 mL/kg			1.2
<b>SKY0402 Placebo</b>					
28 day	Rat	5 mL/kg			5
	Dog	2 sites at 2 mL/kg each			2
Embryo-fetal	Rat	3 sites at ~3.3 mL/kg each			3.3
	Rabbit	3 sites at ~0.7 mL/kg each			0.7
Fertility-Perinatal/Postnatal	Rat	3 sites at ~3.3 mL/kg each			3.3

a – assume level of DEPC comparable in SKY0402 and SKY0402 Placebo (SY0402 DEPC ~12% more than in SKY0402 Placebo)  
 - conduct conservative assessment by dividing local animal dose at each local site (e.g., 2 ml/kg SKY0402 Placebo in dog) by total human dose even though human doses also administered in divided doses

b - nonclinical values were No Observed Effect Levels at 1.2 mL/kg for SYO0402 and the maximum practicable subcutaneous dose level in mL/kg/species for SKY0402 Placebo  
 (b) (4)

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<b>Systemic Toxicity: Human Equivalent Dose-Based Safety Margins (SMs) - Nonclinical Animal NOAEL Dose<sup>a</sup> to Highest Human Dose<sup>b</sup> Ratios for Total Dose with Novel Excipient DEPC in SKY0402 and SKY0402 Placebo (nonclinical) and SKY0402 (human)</b>					
Nonclinical study	species	Total Dose <sup>c</sup> (mL/kg)	Dose (mg/kg)	HED <sup>d</sup> (mg/kg)	SMs
<b>SKY0402</b>					
Single dose and 28 day	Rabbit	1.2	9.9	3.2	0.6
	Dog	1.2	9.9	5.3	0.97
<b>SKY0402 Placebo</b>					
28 day	Rat	5	37	5.9	1.1
	Dog	4	29.6	15.9	2.9
Embryo-fetal	Rat	10	74	11.8	2.1
	Rabbit	2	14.8	4.7	0.85
Fertility-Perinatal/Postnatal	Rat	10	74	11.8	2.1

a - nonclinical values were No Observed Effect Levels at highest dose (SKY0402) or maximum practicable subcutaneous dose level in mL/kg/species (SKY0402 Placebo)  
 (b) (4)

- (b) (4)
- total dose of 60 mL SKY0402 at 5.5 mg/mL DEPC = total dose of 5.5 mg/kg for 60 kg human
  - c - nonclinical SKY0402 Placebo DEPC levels of 7.4 mg/mL
  - d - human equivalent dose based on nonclinical animal:human conversion on a mg dose/m<sup>2</sup> body surface area comparison (BSA)

### **Brief Discussion/Summary:** (issues raised by data)

An issue raised by the nonclinical data is that at the highest doses tested, essentially little toxicity was observed other than known pharmacological effects of bupivacaine, local, injection site effects most likely a result of the subcutaneous/infiltrative dosing method, and other general effects. Nonclinical dosing with SKY0402 was near maximum tolerated dose (MTD) levels and nonclinical dosing with SKY0402 Placebo was at maximum practicable levels for each species tested. On this basis and considering the long history of clinical experience with bupivacaine, that all nonclinical animal:human safety margins did not achieve a level of  $\geq 1$  is not a safety concern.

### Bupivacaine

Proposed human doses are 120, 300, (b) (4) SKY0402. Systemic exposure levels of bupivacaine after dosing with SKY0402 in clinical trials at the highest proposed single dose of (b) (4) could not be determined to be comparable to those supported by the referenced NDA as human exposure levels at the maximum recommended human dose could not be found and were not tested in any of the clinical trials. The systemic levels of bupivacaine after human dosing with SKY0402 at (b) (4) were potentially greater than those achieved after dosing with the approved bupivacaine HCl in the 505(b)(2) referenced drug Marcaine after ClinPharm evaluation. This noncomparability was largely due to the lack of human pharmacokinetic data for the approved bupivacaine HCl at the highest approved dose of 400 mg/day.

The human safety of bupivacaine is supported using a 505(b)(2) submission in combination with acute infiltrative surgical studies in rabbits and dogs, and repeat dose subcutaneous studies in rats and dogs compared to the pivotal single dose clinical trials. The sponsor submitted repeat dose toxicity studies (one month) in two species which satisfies the requirement for at least 1 species to be tested as listed in the FDA Guidance for Industry and Review Staff: Nonclinical Safety Evaluation of Reformulated Drug Products and Products Intended for Administration by an Alternate Route (March 2008). As noted, nonclinical testing of SKY0402 turned out to be necessary to support the human safety of bupivacaine at the proposed high dose of (b) (4)

### DEPC-based DepoFoam

The human safety of DepoFoam (Inactive Ingredient) is generally supported by the marketed products DepoCyt® (NDA 21-041, 1999) and DepoDur® (NDA 21-671, 2004) and the DEPC-based DepoFoam toxicity studies submitted. The exact composition of DepoFoam is slightly different in each of the three drug products (DepoCyt, DepoDur,

and SKY0402). The difference in the DepoFoam in SKY0402 is the novel excipient dierucoylphosphatidylcholine (DEPC).

A comprehensive nonclinical testing program was submitted to support the human safety for the DEPC-based DepoFoam in SKY0402. This program included repeat dose toxicity studies (one month) in two species (one nonrodent), genotoxicity, and reproductive toxicity, consistent with the FDA Guidance for Industry: Nonclinical Safety Studies for the Safety Evaluations of Pharmaceutical Excipients (May 2005). These nonclinical studies supported proposed human dosing with SKY0402.

## **12 Appendix/Attachments** - none

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/s/  
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GARY P BOND

09/23/2011

Primary nonclinical review for new NDA

ADAM M WASSERMAN

09/23/2011

I concur the NDA may be approved based on assessment of nonclinical data provided.

## PHARMACOLOGY/TOXICOLOGY NDA FILING CHECKLIST

**NDA Number:** 22,496

**Applicant:** Pacira Pharmaceuticals

**Stamp Date:** 09/28/2010

**Drug Name:** SKY0402

**NDA Type:** Standard

On **initial** overview of the NDA/BLA application for filing:

	Content Parameter	Yes	No	Comment
1	Is the pharmacology/toxicology section organized in accord with current regulations and guidelines for format and content in a manner to allow substantive review to begin?	X		
2	Is the pharmacology/toxicology section indexed and paginated in a manner allowing substantive review to begin?	X		
3	Is the pharmacology/toxicology section legible so that substantive review can begin?	X		
4	Are all required (*) and requested IND studies (in accord with 505 b1 and b2 including referenced literature) completed and submitted (carcinogenicity, mutagenicity, teratogenicity, effects on fertility, juvenile studies, acute and repeat dose adult animal studies, animal ADME studies, safety pharmacology, etc)?	X		
5	If the formulation to be marketed is different from the formulation used in the toxicology studies, have studies by the appropriate route been conducted with appropriate formulations? (For other than the oral route, some studies may be by routes different from the clinical route intentionally and by desire of the FDA).	NA		Applicant must demonstrate comparability of drug product and placebo batches used in nonclinical and clinical testing in order to validate nonclinical data as being able to be predictive of toxicity. Applicant will be asked to do so in 74 day letter.
6	Does the route of administration used in the animal studies appear to be the same as the intended human exposure route? If not, has the applicant <u>submitted</u> a rationale to justify the alternative route?	X		
7	Has the applicant <u>submitted</u> a statement(s) that all of the pivotal pharm/tox studies have been performed in accordance with the GLP regulations (21 CFR 58) <u>or</u> an explanation for any significant deviations?	X		
8	Has the applicant submitted all special studies/data requested by the Division during pre-submission discussions?	X		Previously requested information about potential local injection site effects and compatibility issues appears to have been addressed in the submission so it is now a review issue.

## PHARMACOLOGY/TOXICOLOGY NDA FILING CHECKLIST

	Content Parameter	Yes	No	Comment
9	Are the proposed labeling sections relative to pharmacology/toxicology appropriate (including human dose multiples expressed in either mg/m <sup>2</sup> or comparative serum/plasma levels) and in accordance with 201.57?		X	505(b)(2) submission uses label from reference drug Marcaine (NDA 16-964) where dose ratios are noted but basis for those ratios are not stated (e.g., mg/kg or mg/m <sup>2</sup> ). Label will need to be modified to describe animal to human dose ratios in section 8.1 Pregnancy that are relevant to the proposed drug and its systemic exposure.
10	Have any impurity – etc. issues been addressed? (New toxicity studies may not be needed.)	X		Full nonclinical dataset submitted for novel excipient DEPC.
11	Has the applicant addressed any abuse potential issues in the submission?	NA		Drug substance is not an addictive/abuse potential concern drug.
12	If this NDA/BLA is to support an Rx to OTC switch, have all relevant studies been submitted?	NA		

NA – not applicable

### IS THE PHARMACOLOGY/TOXICOLOGY SECTION OF THE APPLICATION FILEABLE? X Yes \_\_\_ No

If the NDA/BLA is not fileable from the pharmacology/toxicology perspective, state the reasons and provide comments to be sent to the Applicant.

- NA

Please identify and list any potential review issues to be forwarded to the Applicant for the 74-day letter.

1. In the label, applicant must describe animal to human dose ratios in section 8.1 Pregnancy (embryo-fetal studies) that are appropriate for systemic bupivacaine exposure at the Maximum Recommended Human Dose for SKY0402 (b) (4) and for the bupivacaine reference drug Marcaine (400 mg).
2. Applicant must demonstrate comparability of drug product batches and placebo batches used in nonclinical and clinical testing in order to validate nonclinical data as being able to be predictive of potential human toxicity.

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/s/  
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GARY P BOND

11/04/2010

Pharm/Tox NDA filing review - fileable

ADAM M WASSERMAN

11/05/2010