

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-060

PHARMACOLOGY REVIEW

19 Page(s) Withheld

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

§ 552(b)(5) Deliberative Process

§ 552(b)(5) Draft Labeling



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 21-060
SERIAL NUMBER: N000 AZ
DATE RECEIVED BY CENTER: 06/25/2004
DRUG NAME: ziconotide (Prialt®)
INDICATION: treatment of severe, chronic pain in patients who
require intrathecal (IT) analgesia
SPONSOR: Elan Pharmaceuticals
DOCUMENTS REVIEWED: 10 of 11 volumes
REVIEW DIVISION: Division of Anesthetic, Critical Care, and
Addiction Drug Products (HFD-170)
PHARM/TOX REVIEWER: Suzanne R. Thornton-Jones, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob Rappaport, M.D.
PROJECT MANAGER: Sara Stradley

Date of review submission to Division File System (DFS): 02 December 2004

EXECUTIVE SUMMARY

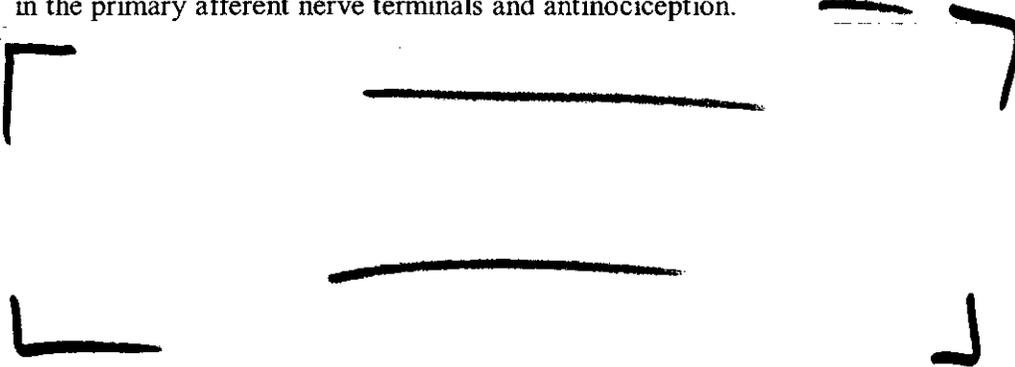
I. Recommendations

- A. Recommendation on acceptability.
The NDA can be approved from a pharmacology and toxicology perspective with appropriate label revision as outlined below.
- B. Recommendation for nonclinical studies.
None.
- C. Recommendations on labeling.

CLINICAL PHARMACOLOGY

Mechanism of Action

Ziconotide binds to N-type calcium channels located on the primary nociceptive (A-δ and C) afferent nerves in the superficial layers (Rexed laminae I and II) of the dorsal horn in the spinal cord. Although the mechanism of action of ziconotide, [REDACTED] results in animals suggest that its binding blocks N-type calcium channels which leads to a blockade of excitatory neurotransmitter release in the primary afferent nerve terminals and antinociception.



Interaction with opioids

Ziconotide does not bind to opioid receptors and its pharmacological effects are not blocked by opioid antagonists. In animal models ziconotide IT potentiated opioid-induced reduction in gastro-intestinal (GI) motility, but did not potentiate morphine-induced respiratory depression. In rats receiving IT ziconotide, additive analgesic effects were observed with concurrent administration of [REDACTED] morphine, baclofen, or clonidine. Concurrent administration of IT ziconotide and morphine did not prevent the development of morphine tolerance in rats.

PHARMACOKINETIC AND BIOLOGICAL DISPOSITION**Metabolism**

Ziconotide is cleaved by endopeptidases and exopeptidases at multiple sites on the peptide. Following passage from the CSF into the systemic circulation during continuous IT administration, ziconotide is expected to be susceptible to proteolytic cleavage by various ubiquitous peptidases/proteases present in most organs (e.g., kidney, liver, lung muscle, etc.), and thus readily degraded to peptide fragments and their individual constituent free amino acids. Human and animal CSF and blood exhibit minimal hydrolytic activity toward ziconotide *in vitro*. The biological activity of the various expected proteolytic degradation products has not been assessed.

CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

No carcinogenicity studies have been conducted in animals.

Ziconotide was negative in the *in vitro* bacterial reverse mutation assay, *in vitro* mouse lymphoma assay, the *in vivo* mouse micronucleus assay, and in the *in vitro* Syrian hamster embryo (SHE) cell transformation assay.

Ziconotide did not affect male fertility in rats given as a continuous intravenous (IV) doses up to 10 mg/kg/day when administered for approximately 8 weeks, including a 28-day pre-mating period, or female fertility at a dose of 3 mg/kg/day when administered for approximately 6 weeks, including a 14-day pre-mating period. Estimated exposure for the male and female rats were approximately 6500-fold and 1700-fold higher, respectively, than the maximum recommended human daily intrathecal (IT) dose based on plasma exposure. Female fertility in rats was significantly affected following continuous IV infusion at a dose of 10 mg/kg/day. Significant reductions in corpora lutea, implantation sites, and number of live fetuses were observed.

PREGNANCY

Pregnancy Category C: Ziconotide was embryolethal in rats when given as a continuous IV infusion during the major period of organogenesis as evidenced by significant increases in post-implantation loss because of an absence or a reduced number of live fetuses.

Ziconotide was not teratogenic in female rats when given as a continuous IV infusion doses up to 30 mg/kg/day or female rabbits up to 5 mg/kg/day during the major period of organ development. Estimated exposures in the female rat and rabbit were approximately 26,000-fold and 940-fold higher than the maximum recommended human daily intrathecal (IT) dose of mcg/hr mcg/day based on plasma exposure. Maternal toxicity in the rat and rabbit, as evidenced

by decreased body weight gain and food consumption, was present at all dose levels. Maternal toxicity lead to reduced fetal weights and transient, delayed ossification of the pubic bones at doses ≥ 15 mg/kg/day which is approximately 8900-fold higher than the maximum recommended human daily IT dose of [redacted] mcg/hr ([redacted] mcg/day) based on plasma exposure. The no observable adverse effect level (NOAEL) for embryo-fetal development in rats was 0.5 mg/kg/day and in rabbits was 5 mg/kg/day. Estimated exposures in the rat and rabbit were approximately 400-fold and 940-fold higher than the maximum recommended human daily IT dose of [redacted] mcg/hr ([redacted] mcg/day) based on plasma exposure.

In a rat pre- and post-natal study in rats, ziconotide given as a continuous IV infusion did not effect pup development or reproductive performance up to a dose of 10 mg/kg/day with is approximately 3800-fold higher, respectively, [redacted] maximum recommended human daily intrathecal (IT) dose of [redacted] mcg/hr ([redacted] mcg/day) based on plasma exposure. Maternal toxicity as evidenced by clinical observations, and decreases in body weight and food consumption were observed at all doses.

No adequate and well-controlled studies have been conducted in pregnant women. Because animal studies are not always predictive of human response, PRIALT should be used during pregnancy only if the potential benefit justifies risk to the fetus.

LABOR AND DELIVERY

The effect of PRIALT on labor and delivery in humans is not known.

NURSING MOTHERS

It is not known whether PRIALT is excreted in human breast milk. Because many drugs are excreted in human milk, [redacted]

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings in 25 June 2004 submission.

Ziconotide up to a concentration of 15 ng/mL did not block hERG [rapidly repolarizing potassium current (I_{KR}), slowly repolarizing cardiac potassium current (I_{KS}), and transient outward potassium current (I_{TO})] channels.

No new toxicities were associated with administration of impurities [redacted] when compared to a high dose of ziconotide (3 μ g/hr) when given as a continuous i.t. infusion in Beagle dogs. Toxicities which associated with both ziconotide and impurity administration included an increase in creatinine, a decrease in triglycerides, decrease in pituitary and ovary weights, increases in liver, thyroid and spleen weights. The common neurological signs observed with ziconotide were not observed with the impurities. The impurities are considered adequately qualified for the Sponsor's specifications.

Three embryo-fetal development studies were conducted where pregnant rats were administered ziconotide as continuous i.v. infusion. Embryo lethality and fetal lethality were observed, however, at doses that were not necessarily dose-related. Common findings included clinical signs (ptosis, stained and thin fur around mouth and eyes and/or labored breathing, dehydration, tremors, hypersensitivity to touch or with handling, convulsions), decreases in body weights, body weight gains, and food consumption, decreases in gravid uterine weight, no/few evaluable fetuses or pups due to no live fetuses or pups due to complete resorptions of the litters in a narrow range of doses (1.5, 4.5, 15 mg/kg/day), increase in plasma concentrations with increase in dose. Only one study revealed fetal variations, incompletely ossified pubic bones, at a dose of 15 mg/kg/day, which was transitory as there were no affects in offspring during the lactation period. The other studies failed to reveal any fetal or pup malformations or variations, even at doses greater than 15 mg/kg/day. A NOAEL of 0.5 mg/kg/day was established.

B. Pharmacologic activity.

Ziconotide is a new molecular entity consisting of a synthetic linear 25-amino acid peptide with three intramolecular disulfide bonds, based upon the structure of a naturally-occurring peptide toxin called ω -conotoxin MVIIA that is produced by a fish-eating sea snail, *Conus magus*. Its pharmacological activity is based upon its ability to block N-type (neuron-specific) voltage-sensitive calcium channels.

C. Nonclinical safety issues relevant to clinical use.

None.

Reviewer Signature Suzanne R. Thornton-Jones, Ph.D.

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

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BACKGROUND: Ziconotide is a new molecular entity investigated as an analgesic to be administered via continuous i.t. infusion to alleviate chronic pain that is resistant to opioid therapy. The original NDA was reviewed and the product received an approvable letter in June 2000. The NDA received another approvable letter in July 2001. The second approvable letter included a non-clinical requirement to conduct another embryo-fetal development study in rats with a study design to include the utilization the double-staining skeletal detection technique to determine if the previously identified absence of pubic bones was due to delayed bone maturation (ossification) or due to absence of bone and cartilage. After the approvable letter was sent to the Sponsor it was determined that there were a significant number of impurities () that were present in the drug substance, as well as in the drug product (alone or while in the Medtronic pump). The impurities of interest were (See table below for further details). Impurities ' ' increase with storage, while the remainder of the impurities do not. To this end the Sponsor was required to decrease the impurity levels to or provide qualification data for their proposed impurity specifications. Per meeting minutes from 12 September 2003 impurity needed to be qualified at the specified level unless it was present at NMT . The Sponsor has provided data that impurity was never detected at levels and therefore did not need to qualify it in the 28-day IT dog study.

The current submission contains three embryo-fetal toxicity studies that address the pubic bone ossification concern and a 28-day continuous intrathecal dog study designed to qualify the impurities. In addition, the Sponsor, at their own discretion, also conducted an *in vitro* cardiovascular safety assessment of ziconotide on hERG channels [repolarizing potassium current (I_{KR}), slowly repolarizing cardiac potassium current (I_{KS}), and transient outward potassium current (I_{TO})].

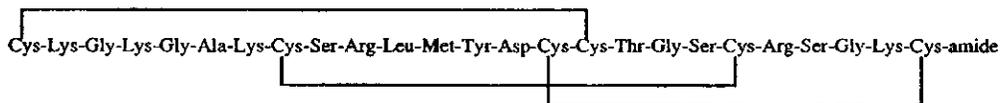
Impurity Code	Impurity Name	Where found	Proposed Specification	Asked to Qualify
┌	_____			┐

└	_____			┘

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

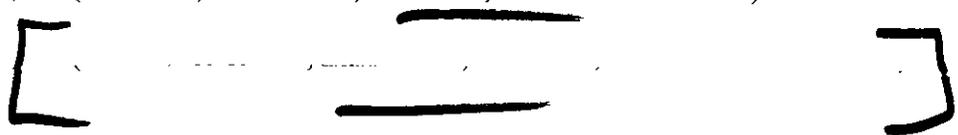
NDA NUMBER: 21-060
REVIEW NUMBER: 4
SEQUENCE NUMBER/DATE/TYPE OF SUBMISSION: N000/25 June 2004/AZ
INFORMATION TO SPONSOR: Yes () No (X)
SPONSOR: Elan Pharmaceuticals
 800 Gateways Blvd
 South San Francisco, CA 94080
 Mallinckrodt (St. Louis, MO)
MANUFACTURER FOR DRUG SUBSTANCE:
MANUFACTURER FOR DRUG PRODUCT:
REVIEWER NAME: Suzanne R. Thornton-Jones, Ph.D.
DIVISION NAME: DACCADP
HFD #: 170
REVIEW COMPLETION DATE: 12 November 2004
DRUG:
TRADE NAME: Prialt®
GENERIC NAME (LIST ALPHABETICALLY): ziconotide
CODE NAME: CI-1009, SNX-111 acetate
CHEMICAL NAME: ω-conotoxin MVIIA (reduced), cyclic (1→6), (8→20), (15→25) tris (disulfide)
CAS REGISTRY NUMBER: 107452-89-1
MOLE FILE NUMBER: not specified
MOLECULAR FORMULA/MOLECULAR WEIGHT: 25 amino acid peptide/2639.18
STRUCTURE:



RELEVANT INDs/NDAs/DMFs:

IND 45,718 (SNX-111, Elan Pharm, HFD-170, active as of 7/5/1994)

IND
 IND
 IND
 DMF



DRUG CLASS: N-type calcium channel antagonist
INTENDED CLINICAL POPULATION: analgesia

CLINICAL FORMULATION: The Ziconotide Drug Product consists of a 0.1 mg/ml solution of ziconotide free base (formulated as the acetate) in isotonic saline containing L-methionine, pH 4.3 ± 0.2. The product is packaged in 5 ml vials and is stored at 5°C.

ROUTE OF ADMINISTRATION: intrathecal (i.t.)

PROPOSED DOSE: 2.4 µg/day (0.1 µg/hr)

Impurity Code	Impurity Name	Where found	Proposed Specification	Asked to Qualify

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

Studies reviewed within this submission:

Study Title	Document/ Study no.	Volume
Effect of ziconotide on cloned hERG hKvLQT1/hminK, and rKv4.3 channels expressed in mammalian cells	410-018-000 (000707.FMB)	1
A 5-day intravenous infusion toxicokinetics study of ziconotide in female Sprague-Dawley rat	410-008-02 (98272)	1
A continuous intravenous infusion teratology study of ziconotide in the rat	410-023-02 (98140)	1
A continuous intravenous infusion range-finding teratology/toxicokinetics study of ziconotide in the rat	410-010-03 (900104)	5
A continuous intravenous infusion teratology study of ziconotide in the rat	410-019-03 (900032)	7
28-day continuous intrathecal infusion toxicity study of ziconotide and ziconotide impurities in beagle dogs	410-021-03 (038-001)	10

Studies not reviewed within this submission: Not applicable.

2.6.2 PHARMACOLOGY:**2.6.2.2 Primary pharmacodynamics:** No new studies were submitted.**2.6.2.3 Secondary pharmacodynamics:** No new studies were submitted.**2.6.2.4 Safety pharmacology:**Neurological effects: No new studies were submitted.

Cardiovascular effects: The potential of ziconotide at concentrations of 0.15, 3, and 15 ng/mL to block rapidly repolarizing potassium current (I_{KR}), slowly repolarizing cardiac potassium current (I_{KS}), and transient outward potassium current (I_{TO}) in HEK, CHO, and L cells, respectively, as well as the potential for frequency-dependent blockade was examined. Ziconotide did not block I_{KR} , I_{KS} , or I_{TO} currents. No frequency-dependent blockage was observed with I_{KR} or I_{TO} at frequencies of 0.3 and 3 Hz, but there was a slight (not statistically significant) block of I_{KS} at 3 Hz and a ziconotide concentration of 15 ng/mL.

Pulmonary effects: No new studies were submitted.Renal effects: No new studies were submitted.Gastrointestinal effects: No new studies were submitted.Abuse liability: No new studies were submitted.Other: NA**2.6.3 PHARMACOLOGY TABULATED SUMMARY:** Not applicable.**2.6.4 PHARMACOKINETICS/TOXICOKINETICS:**

Continuous i.v. infusion of ziconotide at doses of 1.5, 4.5, 10, and 15 mg/kg/day for 5 days to female Sprague-Dawley rats lead to steady state plasma levels by 2 hrs after initiation of dosing, increased with dose, and were minimal by 6 hrs after i.v. dosing cessation.

Text Table 5: Mean (3sds) Plasma Drug Concentrations (ng/mL) of Ziconotide in Female Sprague-Dawley Rats with a 5-Day Continuous Intravenous Infusion

Dose (mg/kg/day)		Day 1 of Treatment (hours post-infusion initiation)			Day 6 of Treatment (hours post-infusion termination)			
		0	2	6	0.5	2	4	6
		1.5	Mean	ND	108.7	106.5	39.3	6.0
	SD		2.5	21.2	9.3	0.8	0.4	0.4
4.5	Mean	ND	291.4	297.0	94.6	13.4	6.2	3.7
	SD		82.2	77.3	12.1	4.9	1.5	0.8
10.0	Mean	ND	413.8	522.3	158.0	19.9	10.5	6.0
	SD		85.3	22.5	27.2	3.0	1.3	1.2
15.0	Mean	ND	733.3	723.7	320.7	44.8	16.9	5.0
	SD		131.0	39.4	156.2	24.2	2.5	0.4

ND = None Detected

* n = 2

2.6.6 TOXICOLOGY**2.6.6.1 Overall toxicology summary:**

General toxicology: No new toxicities were associated with administration of impurities when compared to a high dose of ziconotide (3 µg/hr) when given as a continuous i.t. infusion in Beagle dogs. Toxicities which associated with both ziconotide and impurity administration included an increase in creatinine, a decrease in triglycerides, decrease in pituitary and ovary weights,

increases in liver, thyroid and spleen weights. The common neurological signs observed with ziconotide were not observed with the impurities.

Genetic toxicology: No new information.

Carcinogenicity: No new information.

Reproductive toxicology: Three embryo-fetal development studies were conducted where pregnant rats were administered ziconotide as continuous i.v. infusion. Embryo lethality and fetal lethality were observed, however, at doses that were not necessarily dose-related. Common findings included clinical signs (ptosis, stained and thin fur around mouth and eyes and/or labored breathing, dehydration, tremors, hypersensitivity to touch or with handling, convulsions), decreases in body weights, body weight gains, and food consumption, decreases in gravid uterine weight, no/few evaluable fetuses or pups due to no live fetuses or pups due to complete resorptions of the litters in a narrow range of doses (1.5, 4.5, 15 mg/kg/day), increase in plasma concentrations with increase in dose. Only one study revealed fetal variations, incompletely ossified pubic bones, at a dose of 15 mg/kg/day, which was transitory as there were no affects in offspring during the lactation period. The other studies failed to reveal any fetal or pup malformations or variations, even at doses greater than 15 mg/kg/day. A NOAEL of 0.5 mg/kg/day was established.

Special toxicology: No new information.

2.6.6.2 Single-dose toxicity: No new studies were submitted.

2.6.6.3 Repeat-dose toxicity: No new studies were submitted.

Study title: 28-day continuous intrathecal infusion toxicity study of ziconotide and ziconotide impurities in beagle dogs

Key study findings:

- Clinical signs: hypermetria, dilated pupils, decreased feces, CNS alterations in the ziconotide treated groups
- Hematology: increase in platelets and WBC
- Clinical chemistry: increase in BUN, creatinine, creatine kinase, alkaline phosphatase
- No new toxicities associated with administration of impurities

Study no.: 410-021-03 (038-001)

Volume #, and page #: 10, pp. 1

Conducting laboratory and location:

Date of study initiation: 23 October 2003

GLP compliance/QA report: yes (X) no ()

Drug, lot #, and % purity: ziconotide/920603A — peptide content/

a

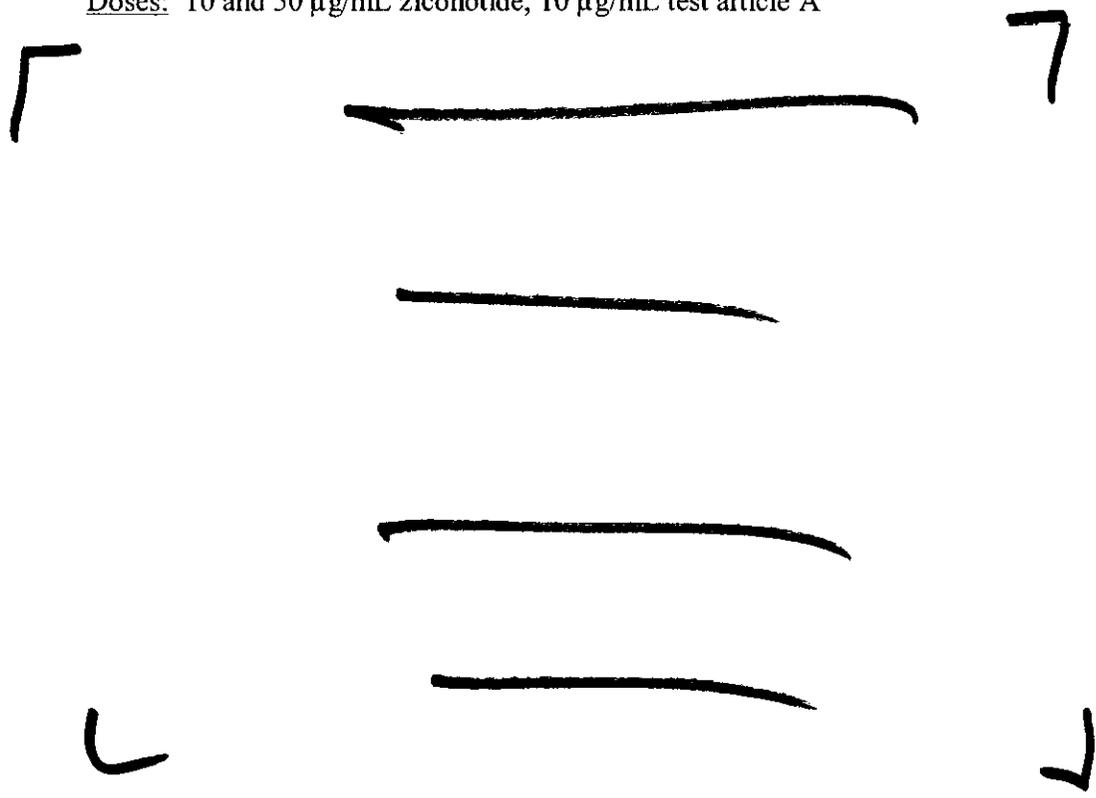
Results
[Redacted]
[Redacted]

Results
[Redacted]
[Redacted]

Appears This Way
On Original

Methods:

Doses: 10 and 50 µg/mL ziconotide; 10 µg/mL test article A



Species/strain: Beagle dogs, [Redacted]

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

Route, formulation, volume, and infusion rate: i.t. in 0.9% sodium chloride injection,
USP @ 0.06 mL/hr for 28 days

Satellite groups used for toxicokinetics or recovery: NA

Age: 14-25 months

Weight: [males] 11.8-14.7 kg; [females] 7.4-13.1 kg

Unique study design or methodology: CADD Micro® Medication Reservoirs were attached to the CADD Micro® Pumps and placed in the jackets for continuous i.t. delivery. I.t. catheter surgery occurred approximately one week before treatment initiation.

Observation times and results:

<u>Observations</u>	<u>Times</u>	<u>Results</u>
Mortality	daily	All survived to scheduled euthanasia.

Clinical signs ¹	daily	Hyperemia, dilated pupils, and decreased feces were observed in male and female dogs during the entire study but only in the ziconotide treated groups.
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Female dogs at a dose of 3 and 0.6 µg/hr Z exhibited constriction, dilatation, and cross reaction papillary reflexes during SW1. Male dogs at a dose of 3 µg/hr Z also exhibited no flexor withdrawal response during SW1. Male and female dogs at a dose of 3 µg/hr Z exhibited no cutaneous reflex during SW1. No knee jerk in male and female dogs was also observed at doses of 3 and 0.6 µg/hr Z during SW1 and/or SW4

Unremarkable for respirations.

Body temperatures were increased in female dogs in the Z treated groups (1-3%) during SW 1 and 4 probably as a result of the tremors and hypermetria.

Body weights	pre-dose, SD 1, then weekly	Unremarkable.
Food consumption	daily	Unremarkable.
Ophthalmoscopy	pre-dose, SW4	Unremarkable.
EKG	pre-dose, SW4	Heart rate was decreased in male (15-27%) and female (41-44%) dogs

3 Page(s) Withheld

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 § 552(b)(5) Deliberative Process

 § 552(b)(5) Draft Labeling

2.6.6.6 Reproductive and developmental toxicology:**A. Study title: A continuous intravenous infusion teratology study of ziconotide in the rat.**Key study findings:

- Clinical signs: ptosis, stained and thin fur around mouth and eyes
- Body weight/food consumption: decrease GD 9-20 with corresponding decreases in body weight gains and food consumption
- Uterine data: decrease in gravid uterine weight, no live fetuses due all early resorptions at doses of 1.5 and 4.5 mg/kg/day
- Fetal data: decrease in body weight and an increased incidence of incomplete ossification of the pubic bones at a dose of 15 mg/kg/day
- The incomplete ossification of the pubic bones was transitory as there were no effects in offspring during the lactation period
- TK: Increase plasma levels with increase in dose
- No NOAEL was established due to complete resorptions or total litter loss, most notably at doses of 1.5 and 4.5 mg/kg/day

Study no: 410-023-02 (98140)Volume #, and page #: 1, pp. 1Conducting laboratory and location: _____Date of study initiation: 27 September 2002GLP compliance/QA report: Yes (X) No ()Drug, lot #, radiolabel, and % purity: ziconotide API/B03020/ _____ peptide content and _____ peptide purityFormulation/vehicle: 0.9% sodium chloride injection, USPMethods:Species/strain: Sprague-Dawley rat/ _____ CD®(SD) IGS BR, _____Doses employed: 1.5, 4.5, 15 mg/kg/day @ 2 mL/kg/hr for 24 hrs/day

In a previous study ('An intravenous infusion male fertility study of ziconotide in the rat) a decrease in the ziconotide concentration was observed in the 1.5 and 4.5 mg/kg/day groups due to adsorption to the _____ ' bad and syringes used in the continuous i.v. delivery system. To compensate for the loss of ziconotide because the same _____ bad and syringes were used in the current study the 1.5 and 4.5 mg/kg/day dosing concentrations were increased upward by 30.77 % (31.25 to 40.87 µg/mL) and 13.93% (93.75 to 106.81 µg/mL), respectively. No changes were made to the 15 mg/kg/day concentration.

Route of administration: continuous i.v. infusionStudy design: daily dosing from GD 6-15Number/sex/group: 24 pregnant dams/group for teratology phase

25-26 pregnant dams/group for littering phase

Parameters and endpoints evaluated: Rats were ordered time-mated and received on GD 0 for both the teratology and littering phases. On GD 0, all pregnant animals underwent i.v. cannula placement under general anesthesia. For both phases of the study, dams were dosed from GD 6-15 via continuous infusion, body weights and food consumption were recorded. TK evaluations were performed on GD 6 at approximately 2 hrs after infusion initiation, on

GD 10 at approximately the same time, and on GD 16 at 0, 0.5, 1, 2, and 4 hrs after the infusion was stopped. The teratology group was euthanized on GD 20 with corpora lutea, no live/dead fetuses, and no implantation sites were recorded; macroscopic examination of dam and fetal body weight and external findings, including gender determination, visceral, and skeletal (Alizarin Red S and Alcian Blue staining) findings were recorded. The littering phase dam and pup body weights were recorded on lactation days (LD) 0 and 4. On LD 0 all pups were externally examined for malformations, the gender determined, and the number of live and dead pups recorded. All dams and pups were euthanized on LD 4. All dams had their cervix, infusion site, mammary glands (thoracic and inguinal), ovaries, uterus, uterine horns, and vagina preserved for possible future evaluation. All pups were externally examined at the time of euthanasia and approximately one-half were processed for skeletal evaluations (Alizarin Red S and Alcian Blue staining) while the other one-half of the pups were not stained but were preserved in alcohol for future evaluation but were later discarded after finalization of the study report. All dams that failed to deliver pups by GD 26 were euthanized and uterine data recorded.

Observation times and results:

TERATOLOGY PHASE

Observations

Times

Results

Mortality

twice daily

One female in the 4.5 mg/kg/day group was euthanized on GD 23 due to self-mutilation of the right hindlimb. Uterine examination revealed 16 implantation sites and embryos. All other animals survived to scheduled euthanasia.

Clinical signs

daily

Eyes partly closed (ptosis), red fur staining of the lower jaw and muzzle, right and left periorbital sites, and fur thin around the right and left periorbital sites were observed at in all treated groups during gestation.

Body weights¹

GD 0, 3, 6, 9, 12, 15, 18, 20

Body weights were statistically significantly decreased in all treated groups from GD 9-20. Body weight gains were also decreased in all treated groups for GD 6-9, GD 12-15, and during treatment, GD 6-15.

Corrected body weight gains for GD 6-20 were decreased in all treated groups (22-24%, statistically significant at doses of 1.5 and 15 mg/kg/day).

Food consumption

GD 0, 3, 6, 9, 12, 15, 18, 20

Statistically significantly decreased during GD 6-9 in all treated groups (41-44%). Sporadic, statistically significant decreases were observed

Food consumption
(cont'd)

during GD 9-12 at a dose of 4.5 mg/kg/day (10%), GD 12-15 at a dose of 1.5 mg/kg/day (13%), and GD 15-19 at doses of 4.5 mg/kg/day (9%).

Terminal and necroscopic evaluations
Dams¹ GD 20

Gravid uterine weights were statistically significantly decreased in all treated groups (41%, 78%, 21% at doses of 1.5, 4.5, and 15 mg/kg/day, respectively).

Dams (cont'd)

Unremarkable gross pathology.

A significant number of dams at doses of 1.5 and 4.5 mg/kg/day (16/25 and 22/25, respectively) showed no live fetuses, but had all early resorptions. There was a statistically significant decrease in the mean number of live fetuses, a statistically significant increase in the mean number of early resorptions which both lead to an increase in % pre-implantation and post-implantation loss.

A decrease in the mean number of male and female fetuses was observed in all treated groups leading to a slight decrease in the sex ratio at 1.5 and 4.5 mg/kg/day, probably as a result of the decrease in the mean number of live fetuses.

Offspring GD 20

Fetal body weights (male, female, and total fetuses) were statistically significantly decreased at a dose of 15 mg/kg/day (10-11%) which was the only group with evaluable fetuses.

No external or visceral malformations or variations were observed in any treated groups.

There was a significant increase in the number of fetuses with incomplete ossification of the pubic bones at a dose of 15 mg/kg/day. There were no other skeletal findings.

Toxicokinetics ¹	GD 6, GD 10, GD 16	A dose-related increase in plasma levels of ziconotide was observed on GD 6 and 10. On GD 16 there was a significant decrease in plasma concentrations by 4 hrs after dosing stopped, but there were still measurable plasma levels in all treated groups, although at low levels.
Other: Dosing solution analysis	GD 6, 16	All dosing solutions were within $\pm 15\%$ of the nominal concentration.

[Note: GD = gestation day; LD=lactation day; ¹ See Appendix for further details]

LITTERING PHASE:

<u>Observations</u>	<u>Times</u>	<u>Results</u>
Mortality	twice daily	<p>Three dams in the control group, 3 dams at a dose of 1.5 mg/kg/day, and 3 dams at a dose of 4.5 mg/kg/day were euthanized between LD 0 and 3 due to total litter loss. One dam at a dose of 4.5 mg/kg/day was euthanized on GD 23 due to dystocia. The majority of the dams at doses of 1.5 and 4.5 mg/kg/day were euthanized on GD 26 because they had not littered. Uterine examinations of these animals revealed that all of them had been pregnant as evidenced by multiple implantation sites but no live fetuses (e.g., embryoletality) as all fetuses had been completely resorbed.</p> <p>Non-treatment related euthanizes included one dam each at doses of 1.5 and 4.5 mg/kg/day on GD 9 and 8, respectively, due to severe lesions at the i.v. surgical site. One dam at a dose of 15 mg/kg/day that was assigned to the TK group was euthanized on GD 9 due to poor condition after having clinical observations of tremors, labored breathing, decreased activity, and cold to the touch, probably related to TK plasma sampling.</p>
Clinical signs	daily	Eyes partly closed (ptosis), red fur staining of the lower jaw and muzzle, right and left periorbital sites, and fur thin around the right and left periorbital

Clinical signs (cont'd)		sites were observed at in all dose treated groups during gestation. Unremarkable during lactation.
Body weights ¹	GD 0, 3, 6, 9, 12, 15, 18, 20; LD 0, 4	Unremarkable for body weights during gestation and lactation. Body weight gains were decreased during lactation at doses of 1.5 and 4.5 mg/kg/day (54% and 73%, respectively).
Food consumption	GD 0, 3, 6, 9, 12, 15, 18, 20	Statistically significantly decreased during GD 6-9 in all treated groups (41-44%). Sporadic, statistically significant decreases were observed during GD 9-12 at a dose of 4.5 mg/kg/day (10%), GD 12-15 at a dose of 1.5 mg/kg/day (13%), and GD 15-19 at doses of 4.5 mg/kg/day (9%).
Terminal and necroscopic evaluations Dams ¹	LD 4	Unremarkable gross pathology. Due to significant total litter loss at doses of 1.5 and 4.5 mg/kg/day, the gestation index was statistically significantly decreased to 15.4% and 11.5%, respectively, compared to the control which was 100%. The gestation index at a dose of 15 mg/kg/day was decreased (84.6%) but it was not statistically significantly different from control. The viability index on LD 4 was decreased at doses of 1.5 and 4.5 mg/kg/day (50% and 36.4%, respectively) compared to the control (87.7%), as well as a decrease in the mean number of live male and female pups also.
Offspring	LD 4	Pup body weights and clinical observations were unremarkable. There were no external or skeletal malformations or variations.

[Note: GD= gestation day; LD=lactation day; ¹ See Appendix for further details]

B. Study title: A continuous intravenous infusion range-finding teratology/toxicokinetics study of ziconotide in the rat.

Key study findings:

- Clinical signs: ptosis, stained and thin fur around mouth and eyes, labored breathing, dehydration, tremors, hypersensitivity to touch or with handling, convulsions
- Body weight/food consumption: decrease GD 9-20 with corresponding decreases in body weight gains and food consumption in most dose groups
- Uterine data: decrease in gravid uterine weight, no live fetuses due all early resorptions at doses of 1.5 and 4.5 mg/kg/day, and an increase in the number of dams with all early resorptions at a dose of 15 mg/kg/day
- Fetal data: decrease in body weight at doses ≥ 20 mg/kg/day
- TK: Increase plasma levels with increase in dose

Study no: 410-010-03 (900104)

Volume #, and page #: 5, pp. 1

Conducting laboratory and location: _____

Date of study initiation: 07 April 2003

GLP compliance/QA report: Yes (X) No ()

Drug, lot #, radiolabel, and % purity: ziconotide API/B03020/ _____ peptide content and _____ peptide purity

Formulation/vehicle: 0.9% sodium chloride injection, USP

Methods:

Species/strain: Sprague-Dawley rat _____ CD[®](SD) IGS BR, _____

Doses employed: 0.3, 1.5, 4.5, 15, 20, 30 mg/kg/day @ 2 mL/kg/hr for 24 hrs/day

In a previous study ('An intravenous infusion male fertility study of ziconotide in the rat) a decrease in the ziconotide concentration was observed in the 1.5 and 4.5 mg/kg/day (20.83 and 62.50 $\mu\text{g/mL}$, respectively) groups due to adsorption to the _____¹ bad and syringes used in the continuous i.v. delivery system. To compensate for the loss of ziconotide because the same _____ bad and syringes were used in the current study the 0.3, 1.5 and 4.5 mg/kg/day dosing concentrations were increased upward by 112% (6.25 to 13.25 $\mu\text{g/mL}$), 30.77 % (31.25 to 40.87 $\mu\text{g/mL}$) and 13.93% (93.75 to 106.81 $\mu\text{g/mL}$), respectively. No changes were made to the 15, 20, or 30 mg/kg/day dose concentrations.

Route of administration: continuous i.v. infusion

Study design: daily dosing from GD 6-15

Number/sex/group: 5 pregnant dams/group

Parameters and endpoints evaluated: Rats were ordered time-mated and received on GD 0. On GD 0, all pregnant animals underwent i.v. cannula placement under general anesthesia. For both phases of the study, dams were dosed from GD 6-15 via continuous infusion, body weights and food consumption were recorded. TK evaluations were performed on GD 6 at approximately 2 hrs after infusion initiation, on GD 10 at approximately the same time, and on GD 16, 6 hrs after the infusion was stopped. All dams were euthanized on GD 20 with corpora lutea, no. live/dead fetuses, and no. implantation sites were recorded; macroscopic examination of dam and fetal body weight and external findings, including gender determination, were recorded.

Observation times and results:

<u>Observations</u>	<u>Times</u>	<u>Results</u>
Mortality	twice daily	All animals survived to scheduled euthanasia.
Clinical signs ¹	daily	All treated groups exhibited eyes partly closed (ptosis) and a decrease in feces output. Animals at doses ≥ 1.5 mg/kg/day also exhibited labored breathing and decreased activity, at doses ≥ 4.5 mg/kg/day exhibited red fur staining of the muzzle, at doses ≥ 15 mg/kg/day exhibited dehydration and tremors, at doses ≥ 20 mg/kg/day exhibited hypersensitivity to touch or with handling, and at a dose of 30 mg/kg/day exhibited convulsions.
Body weights ¹	GD 0, 3, 6, 9, 12, 15, 18, 20	<p>Decrease in body weights were observed on GD 9 (4.5, 15, and 30 mg/kg/day), GD 15 (1.5 and 4.5 mg/kg/day), and 18-20 (1.5-15 mg/kg/day). Body weight gains were decreased from GD 6-9 and GD 12-20 at doses of 1.5-15 mg/kg/day), and GD 6-15 (during treatment), but increased at doses ≥ 20 mg/kg/day GD 18-20. It is interesting that dams at doses of 1.5 and 4.5 mg/kg/day continued to loss weight after treatment, even though they had no live fetuses (all had been resorbed).</p> <p>Corrected body weight gain for GD 6-20 were decreased in all treated groups with evaluable data (all dams at doses of 1.5 and 4.5 mg/kg/day had all fetuses resorbed) (15-40%).</p>
Food consumption	GD 0, 3, 6, 9, 12, 15, 18, 20	Decreased during GD 6-9 in all treated groups (36-50%). Sporadic, statistically significant decreases were observed during GD 12-15 (20%) and GD 18-20 (16%) at a dose of 1.5 mg/kg/day.
Terminal and necroscopic evaluations Dams ¹	GD 20	Gravid uterine weights were comparable to control for all treated groups.

Dams (cont'd)		Unremarkable gross pathology. All dams at doses of 1.5 and 4.5 mg/kg/day, and dams (3/5) at a dose of 15 mg/kg/day showed no live fetuses, but had all early resorptions. There was an increase in the mean number early resorptions at doses of 15 and 20 mg/kg/day (35X and 7.3X, respectively) which lead to a decrease in the mean number of live fetuses at a dose of 15 mg/kg/day. Pre-implantation (%) was decreased at a dose of 0.3, 4.5, and 15 mg/kg/day, while % post-implantation loss was increased in all treated groups except at a dose of 30 mg/kg/day.
Offspring	GD 20	Fetal body weights (male, female, and total fetuses) were decreased at doses ≥ 20 mg/kg/day (9-11%). No external malformations or variations were observed in any treated groups.
Toxicokinetics ¹	GD 6, GD 10, GD 16	A dose-related increase in plasma levels of ziconotide was observed on GD 6 and 10. On GD 16 there was a significant decrease in plasma concentrations by 6 hrs after dosing stopped, but there were still measurable plasma levels in all treated groups, although at low levels.
Other: Dosing solution analysis	GD 6, 16	All dosing solutions were within $\pm 15\%$ of the nominal concentration.

[Note: GD = gestation day; ¹ See Appendix for further details]

C. Study title: A continuous intravenous infusion teratology study of ziconotide in the rat.

Key study findings:

- Clinical signs: ptosis, stained and thin fur around mouth and eyes, hunched posture, tremors
- Body weight/food consumption: decrease GD 9-20 with corresponding decreases in body weight gains and food consumption
- Uterine data: no or few live fetuses due all early resorptions at doses of ≥ 4.5 mg/kg
- Fetal data: decrease in body weight at a dose of 30 mg/kg/day but no skeletal malformations or variations
- No or few pups delivered during lactation as most were resorbed at doses ≥ 4.5 mg/kg/day

- TK: Increase plasma levels with increase in dose
- NOAEL = 0.5 mg/kg/day

Study no: 410-019-03 (900032)

Volume #, and page #: 7, pp. 1

Conducting laboratory and location: _____

Date of study initiation: 25 July 2003

GLP compliance/QA report: Yes (X) No ()

Drug, lot #, radiolabel, and % purity: ziconotide API/B03020/; ■ peptide content and
 ■ peptide purity

Formulation/vehicle: 0.9% sodium chloride injection, USP

Methods:

Species/strain: Sprague-Dawley rat/ ■CD®(SD) IGS BR, _____

Doses employed: 0.5, 4.5, 30 mg/kg/day @ 2 mL/kg/hr for 24 hrs/day

The compensation for the adsorption of the low dose ziconotide solutions was not performed in this study as was done in the previous studies.

Route of administration: continuous i.v. infusion

Study design: daily dosing from GD 6-15

Number/sex/group: 25-30 pregnant dams/group for teratology phase
 23-25 pregnant dams/group for littering phase

Parameters and endpoints evaluated: Rats were ordered time-mated and received on GD 0 for both the teratology and littering phases. On GD 0, all pregnant animals underwent i.v. cannula placement under general anesthesia. For both phases of the study, dams were dosed from GD 6-15 via continuous infusion, body weights and food consumption were recorded. TK evaluations were performed on GD 6 at approximately 2 hrs after infusion initiation, on GD 10 at approximately the same time, and on GD 16 at 5 mins, 0.5, 1, 2, 3, and 12 hrs after the infusion was stopped. The teratology group was euthanized on GD 20 with corpora lutea, no. live/dead fetuses, and no. implantation sites were recorded; macroscopic examination of dam and fetal body weight and external findings, including gender determination, visceral, and skeletal (Alizarin Red S and Alcian Blue staining) findings were recorded. The littering phase dam and pup body weights were recorded on lactation days (LD) 0 and 4. On LD 0 all pups were externally examined for malformations, the gender determined, and the number of live and dead pups recorded. All dams and pups were euthanized on LD 4. All dams had their cervix, infusion site, mammary glands (thoracic and inguinal), ovaries, uterus, uterine horns, and vagina preserved for possible future evaluation. All pups were externally examined at the time of euthanasia and approximately one-half were processed for skeletal evaluations (Alizarin Red S and Alcian Blue staining) while the other one-half of the pups were not stained but were examined for visceral evaluations using Wilson's technique. All dams that failed to deliver pups by GD 26 were euthanized and uterine data recorded.

Observation times and results:

TERATOLOGY PHASE

<u>Observations</u>	<u>Times</u>	<u>Results</u>
Mortality	twice daily	Two dams in the 30 mg/kg/day group were found dead on GD 6 and 8. Prior to their death they exhibited treatment-related clinical signs of tremors and

Mortality (cont'd)

hunched posture and/or were pale, thin, cold to touch, had decreased activity, diarrhea, ptosis, and clear ocular discharge prior to their death. Uterine examination revealed 16 implantation sites and embryos for each dam.

All other animals survived to scheduled euthanasia.

Clinical signs¹ daily

Decreased activity, cold to touch, decreased feces output, erect fur, red fur staining of the right and left periorbital sites and urogenital area, and fur thin around the right and left periorbital sites, and hunched posture were observed at a dose of 30 mg/kg/day. Eyes partly closed (ptosis) and tremors were also observed at doses ≥ 4.5 mg/kg/day and thin fur on the forelimbs, forepaws and hindpaws in all treated groups.

Body weights¹ GD 0, 3, 6, 9, 12, 15, 18, 20

Decrease in body weights were observed on GD 6-15 in all treated groups, and GD 15-20 in doses ≥ 4.5 mg/kg/day. Body weight gains were decreased from GD 6-9 and GD 6-15 (during treatment) in all treated groups, and GD 15-20 in doses ≥ 4.5 mg/kg/day. It is interesting that dams at doses ≥ 4.5 mg/kg/day continued to loss weight after treatment, even though they had no or few live fetuses (all if not most had been resorbed).

Corrected body weight gains for GD 6-20 were decreased in all treated groups (18-32%, statistically significant at doses of 0.5 and 30 mg/kg/day).

Food consumption GD 0, 3, 6, 9, 12, 15, 18, 20

Statistically significantly decreased during GD 6-9 in all treated groups (39-46%). Sporadic, statistically significant decreases were observed during GD 12-15 (7%) at a dose of 0.5 mg/kg/day, and GD 15-20 (10-27%) at a dose 4.5 mg/kg/day were observed. Also sporadic statistically significant increases were observed on GD 9-18 at a dose of 30 mg/kg/day, and GD 15-20 at a

Food consumption
(cont'd)

dose of 0.5 mg/kg/day.

Terminal and necroscopic evaluations
Dams¹ GD 20

Unremarkable gravid uterine weights
(where evaluable) and gross pathology.

A significant number of dams at doses ≥ 4.5 mg/kg/day showed no live fetuses, but had all early resorptions. There was a statistically significant decrease in the mean number of live fetuses, a statistically significant increase in the mean number of early resorptions which both lead to an increase in % pre-implantation and post-implantation loss at doses ≥ 4.5 mg/kg/day.

Offspring GD 20

Fetal body weights (male, female, and total fetuses) were statistically significantly decreased at a dose of 30 mg/kg/day.

No external, visceral, or skeletal malformations or variations in evaluable fetuses.

Toxicokinetics¹ GD 6, GD 10, GD 16

A dose-related increase in plasma levels of ziconotide was observed on GD 6 and 10. On GD 16 there was a significant decrease in plasma concentrations by 4 hrs after dosing stopped, but there were still measurable plasma levels in all treated groups, although at low levels.

Other: Dosing GD 6, 16
solution analysis

All dosing solutions were within $\pm 15\%$ of the nominal concentration.

[Note: GD = gestation day; LD=lactation day; ¹ See Appendix for further details

LITTERING PHASE:

Observations

Mortality

Times

daily

Results

One dam in the control group, 1 dam at a dose of 0.5 mg/kg/day, and 1 dam at a dose of 30 mg/kg/day were euthanized between LD 0 and 1 due to total litter loss as a result of cannibalism. The majority of the dams at doses of 4.5 and 30 mg/kg/day were euthanized on

Mortality (cont'd)		GD 26 because they had not littered. Uterine examinations of these animals revealed that the majority of them had been pregnant as evidenced by multiple implantation sites but no live fetuses (e.g., embryoletality) as all fetuses had been completely resorbed.
Clinical signs ¹	daily	<p>During gestation, there was decreased activity, cold to touch, decreased feces output, erect fur, red fur staining of the right and left periorbital sites and urogenital area, and fur thin around the right and left periorbital sites, and hunched posture observed at a dose of 30 mg/kg/day. Eyes partly closed (ptosis) and tremors were also observed at doses ≥ 4.5 mg/kg/day and thin fur on the forelimbs, forepaws and hindpaws in all treated groups.</p> <p>During lactation, fur thin around the right and left periorbital sites and ventral cervical area at a dose of 30 mg/kg/day. Also observed at doses ≥ 4.5 mg/kg/day was eyes partly closed (ptosis), red fur staining of the muzzle, and hunched posture.</p>
Body weights ¹	GD 0, 3, 6, 9, 12, 15, 18, 20; LD 0, 4	<p>Body weights were decreased in all treated groups on GD 9, and at doses ≥ 4.5 mg/kg/day on GD 12-20. Body weight gains were decreased in all treated groups on GD 6-9 and GD 6-15 (during treatment), and at doses ≥ 4.5 mg/kg/day on GD 9-20.</p> <p>Unremarkable for body weight and body weight gains during lactation.</p>
Food consumption	GD 0, 3, 6, 9, 12, 15, 18, 20	<p>Statistically significantly decreased during GD 6-9 in all treated groups (32-43%). Statistically significant increases were observed during GD 15-18 at a dose of 0.5 and 30 mg/kg/day (13%).</p>
Terminal and necroscopic evaluations Dams ¹	LD 4	Unremarkable gross pathology.

Dams (cont'd)

The length of gestation was a slightly longer at a dose of 30 mg/kg/day (22.3days vs. 21.4 days for control).

Due to significant total litter loss at doses of 4.5 and 30 mg/kg/day, the gestation index was statistically significantly decreased to 4.2% and 55%, respectively, compared to the control which was 100%. The viability index was unaffected by treatment because there were few, if any, evaluable pups and those pups that were delivered survived to LD 4, as evidenced by the decrease in live birth index.

The litter size was statistically significantly decreased on LD 0 and 4 at a dose of 30 mg/kg/day (12-20%).

Offspring

LD 4

Pup body weights and clinical observations were unremarkable. There were no external or skeletal malformations or variations.

[Note: GD = gestation day; LD=lactation day; ¹ See Appendix for further details]

2.6.6.7 Local tolerance: No new studies were submitted.

2.6.6.8 Special toxicology studies: No new studies were submitted.

2.6.6.9 Discussion and Conclusions:

The 28-day continuous i.t. study was designed to determine if impurities [redacted] that are found in the drug product leads to toxicities which are different from ziconotide. The study showed that the impurities did not produce any untoward toxicities that were not already identified with high doses of ziconotide alone. Based on the results of this study, the impurities are considered adequately qualified at the proposed specification levels.

Impurity Code	Proposed Specs	Impurity level at NOAEL ¹ (µg/mL) (% of NOAEL dose)	Impurity level at NOAEL (ng/kg/hr)	Human exposure to impurity ² (ng/kg/hr)	Multiple of exposure	Qualified
						Yes
						Yes
						Yes
						Yes
						Yes
						Yes
						Yes
						Yes

¹ NOAEL for impurities from 28-day dog study is

Three embryo-fetal development studies were conducted where pregnant rats were administered ziconotide as continuous i.v. infusion. Embryo lethality and fetal lethality were observed, however, at doses that were not necessarily dose-related. Only one study revealed fetal variations, incomplete ossification of the pubic bones, at a dose of 15 mg/kg/day. The incomplete ossification was transitory as there were no effects observed in offspring during the lactation period. The other studies failed to reveal any fetal or pup malformations or variations, even at doses greater than 15 mg/kg/day. The reason for the variability in the study results even at the same doses may be due to differences in exposure, however, the values are not that significantly different. An interesting phenomenon that was observed in the numerous embryo-fetal development studies was embryo lethality at low and intermediate doses (1.5, 4.5, and 15 mg/kg/day), but not at higher doses. Fetal lethality, as evidenced by a decrease in the number of live fetuses due to failure to deliver (all fetuses were completely resorbed), was observed at higher doses only during the littering phases of the studies. It remains unknown why the high doses were comparable to the control values for uterine data parameters when the dams underwent a cesarean section on GD 20, but if the dams were allowed to litter (normally occurring on GD 21/22) there were complete resorptions of the fetuses. An even more quandary is why there were such variations in the uterine data and fetal outcomes clinical signs and changes in body weights and food consumption were consistent between all of the studies. There are numerous unanswered questions about results from the numerous embryo-fetal development toxicity studies, but their answers are not necessary for approval. A NOAEL for embryo-fetal development was finally established at 0.5 mg/kg/day after repeating the study multiple times.

2.6.6.10 Tables and Figures: Not applicable.

2.6.7 TOXICOLOGY TABULATED SUMMARY: Not applicable.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Ziconotide up to a concentration of 15 ng/mL did not block hERG [rapidly repolarizing potassium current (I_{KR}), slowly repolarizing cardiac potassium current (I_{KS}), and transient outward potassium current (I_{TO})] channels. No new toxicities were associated with administration of impurities _____ when compared to a high dose of ziconotide (3 μ g/hr) when given as a continuous i.t. infusion in Beagle dogs. A NOAEL of 0.5 mg/kg/day was for embryo-fetal development was established. The Sponsor was able to definitively show the pubic bones are not absent as previously categorized, but were incompletely ossified.

Unresolved toxicology issues: There are no unresolved toxicology issues.

Recommendations: None at this time.

Suggested labeling: (Note: strike-through indicates corrections to proposed label, double underlines indicate insertions/edits to the proposed label)

CLINICAL PHARMACOLOGY

Mechanism of Action

5 Page(s) Withheld

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

_____ § 552(b)(5) Deliberative Process

_____ § 552(b)(5) Draft Labeling

2 Page(s) Withheld

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

§ 552(b)(5) Deliberative Process

§ 552(b)(5) Draft Labeling

B. A continuous intravenous infusion teratology study of ziconotide in the rat (study no. 410-023-02 [98140])

TERATOLOGY PHASE:

Body weight and body weight gains:

Dose (mg/kg)	1.5		4.5		15	
	GD	BWG	BW	BWG	BW	BWG
9	---	---	---	---	---	---
9-12	---	---	---	---	---	---
12	↓5%*	---	↓6%*	---	↓8%*	---
12-15	---	---	---	---	---	---
15	↓4%*	---	↓5%*	---	↓3%*	---
15-18	---	↓81%*	---	↓58%*	---	↓22%*
18	↓9%*	---	↓8%*	---	↓5%*	---
18-20	---	↓87%*	---	↓108%*	---	↓16%*
20	↓18%*	---	↓20%*	---	↓6%*	---
20	↓22%*	---	↓26%*	---	↓6%*	---
6-15	---	↓66%*	---	↓93%*	---	---
6-15	---	↓61%*	---	↓51%*	---	↓22%*

[* p<0.05; GD=gestation day; BW=body weight; BWG=body weight gain; Values represent % change from control]

Maternal gestational data:

Dose (mg/kg/day)	No. live fetuses [^]	No. early resorptions [^]	Pre-implantation loss (%) ¹	Post-implantation loss (%) ²
1.5	↓79%*	↑10.6X*	17.1*	80.8*
4.5	↓99%*	↑12.9X*	17.3*	98.3*
15	↓12%*	↑2.5X*	18.3*	18.3*

[[^]Values represent % change from control; *p<0.05; ¹control, 14.8% ; ²control, 7.9%]

Toxicokinetics:

Text Table 5: Mean (SD); Plasma Ziconotide Concentrations (ng/mL)

Ziconotide Treatment (mg/kg/day)	GD		GD 16 (hours post-infusion termination)				
	8 [^]	10 [^]	0	0.5	1	2	4
0	0	0	0	0	0.14 [*] (0.04)	0	0.12 (n=1)
1.5	104.9 (21.2)	78.3 (50.3)	111.0 (30.3)	36.1 (4.6)	17.3 (3.8)	5.8 (1.8)	2.4 (0.4)
4.5	176.3 (111.6)	293.3 (30.2)	346.0 (24.6)	100.1 (8.9)	57.0 (16.6)	15.1 (1.4)	7.9 (1.7)
15.0	591.8 (348.7)	895.7 (75.7)	1050.0 [*] (28.3)	368.3 (108.4)	228.0 [*] (5.7)	45.1 (25.4)	14.9 [*] (1.27)

[^] Sample collected 2 hours after initiation of continuous infusion

LITTERING PHASE:

Maternal littering results:

Dose (mg/kg/day)	Gestation Index (%) ¹	No of pups at birth		Sex ratio (%) ²	Live birth index (%) ³
		Live [^]	Dead [^]		
1.5	15.4	↓54%*	---	35.9	39.7
4.5	11.8	↓60%*	↑2.2X	56.6	38
15	84.6q	---	---	47.4	86

[* p<0.05; [^]Values represent % change from control; ¹ control, 100%; ² control, 49.8%; ³ control, 87.7%]

C. A continuous intravenous infusion range-finding teratology/toxicokinetics study of ziconotide in the rat (study no. 410-010-03 [900104])

Clinical signs:

Dose (mg/kg)	0.3	1.5	4.5	15	20	30
Decreased activity		3/5	5/5	5/5	5/5	5/5
Convulsions						
	Non-sustained	---	---	---	1/5	---
Sustained	---	---	---	---	---	1/5
Dehydrated	1/5	3/5	5/5	5/5	5/5	5/5
Eyes partly closed (left and/or right eyes)	1/5	3/5	5/5	5/5	5/5	5/5
Feces output decreased	3/5	2/5	4/5	5/5	5/5	4/5
Fur staining red/muzzle	---	---	1/5	1/5	3/5	2/5
Hypersensitive	---	---	---	---	2/5	4/5
Tremors	---	---	---	1/5	3/5	5/5

Body weight and body weight gains:

Dose (mg/kg/day)	0.3		1.5		4.5		15		20		30		
	GD	BW	BWG	BW	BWG	BW	BWG	BW	BWG	BW	BWG	BW	BWG
			↑17%		↑22%		↑14%		↓152%		↑118%		
9	---	---	---	---	↓11%	---	↓9%	---	---	---	---	↓12%	---
9-12	---	---	↑31%	---	↑23%	---	↑64%	---	---	---	---	---	↑57%
12-15	---	---	---	---	↓99%	---	↓62%	---	↑48%	---	---	---	---
15	---	---	---	↓13%	---	↓10%	---	---	---	---	---	---	---
15-18	---	---	---	---	↓124%	---	↓128%	---	↓98%	---	---	---	---
18	---	---	---	↓24%	---	↓22%	---	↓17%	---	---	---	---	---
18-20	---	---	---	---	↓78%	---	↓90%	---	↓68%	---	↑18%	---	↑12%
20	---	---	---	↓29%	---	↓28%	---	↓21%	---	---	---	---	---
6-15	---	---	↓31%	---	↓67%	---	↓57%	---	↓45%	---	↓24%	---	↓31%

[GD=gestation day; BW=body weight; BWG=body weight gain; Values represent % change from control]

Maternal gestational data:

Dose (mg/kg/day)	No with all early resorptions*	No. live fetuses*	No. early resorptions*	Pre-implantation loss (%) ¹	Post-implantation loss (%) ²
0.3	---	---	---	6.7	4.2
1.5	4/5	None	↑44X	18.4	100
4.5	4/5	None	↑46X	9.5	100
15	3/5	↓69%	↑35X	11.5	75
20	---	---	↑7.3X	25.5	15.5
30	---	---	---	32.7	2.9

[*Values represent % change from control; ¹control, 20.3%; ²control, 1.7%]

Toxicokinetics:

Text Table 5: Mean (SD) Plasma Ziconotide Concentrations (ng/mL)

Group	Dose (mg/kg/day)	GD 6	GD 10	GD 16
		2 hrs post-infusion start		6 hrs post-infusion end
1	0.0	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
2	0.3	22.5 (5.7)	28.2 (4.6)	0.0 (0.0)
3	1.5	76.5 (13.5)	91.4 (24.2)	2.6 (0.7)
4	4.5	287.0 (119.0)	224.3 (29.7)	5.1 (0.8)
5	15.0	603.0 (207.1)	719.0 (145.7)	14.9 (0.8)
6	20.0	596.6 (313.9)	896.2 (431.5)	19.0 (3.5)
7	30.0	813.9 (640.7)	774.0 (264.4)	34.2 (6.5)

D. A continuous intravenous infusion teratology study of ziconotide in the rat (study no. 410-019-03 [900032])

TERATOLOGY PHASE:

Clinical signs:

Dose (mg/kg)	0.5	4.5	30
Decreased activity	---	---	13/25
Cold to touch	---	---	3/25
Eyes partly closed (left and/or right eyes)	---	7/25	14/25
Feces output decreased	---	---	23/25
Fur erect	---	---	6/25
Fur, red stained	---	---	
Periorbital area (left and/or right)			10/25
Urogenital area			4/25
Fur thin			
Forelimb (left and/or right)	2/25	4/25	3/25
Forepaw (left and/or right)	6/25	6/25	4/25
Hindpaw (left and/or right)	6/25	3/25	1/25
Periorbital area (left and/or right)	---	---	6/25
Hunched posture	---	---	25/25
Tremors	---	1/25	25/25

Body weight and body weight gains:

Dose (mg/kg)	0.5		4.5		30	
	BW	BWG	BW	BWG	BW	BWG
9	↓4%*	---	↓8%*	---	↓12%*	---
9-12	---	---	---	---	---	↑26%
12	↓5%*	---	↓7%*	---	↓9%*	---
12-15	---	↑23%	---	↓44%	---	↑53%
15	↓4%*	---	↓8%*	---	↓6%*	---
15-18	---	↑11%	---	↓116%*	---	↓36%*
18	---	---	↓21%*	---	↓10%*	---
18-20	---	↑15%	---	↓86%*	---	↓29%*
20	---	---	↓29%*	---	↓11%*	---
6-18	---	↓33%	---	↓44%	---	↓41%

[* p<0.05; GD=gestation day; BW=body weight; BWG=body weight gain; Values represent % change from control]

Maternal gestational data:

Dose (mg/kg/day)	No with all early resorptions*	No. live fetuses*	No. early resorptions*	Pre-implantation loss (%) ¹	Post-implantation loss (%) ²
0.5	---	---	---	9.6	7.1
4.5	23/25	↓99%*	↑20.7X	13.9	99.3*
30	7/25	↓41%*	↑7.7X	17.2	47.9*

[*Values represent % change from control; ¹control, 11.3%; ²control, 4.5%]

Toxicokinetics:

Text Table 4: Mean (SD) Plasma Ziconotide Concentrations (ng/mL)

Ziconotide Treatment (mg/kg/day)	Gestation Day		Gestation Day 16 (hours post-infusion termination):					
	6	10	0**	0.5	1	2	4	12
0	ND	ND*	ND	ND	ND	ND	ND	ND
0.5	29.2 (8.7)	41.7 (8.7)	33.7 (6.8)	10.6 (3.3)	5.1 (0.3)	1.3 (0.2)	0.8 (0.1)	0.26 (0.04)
4.5	375.3 (55.8)	404.5 (27.8)	374.5 (60.2)	140.5 (24.7)	55.2 (15.4)	19.8 (1.1)	8.0 (0.4)	2.6 (0.3)
30.0	2750.0 (97.0)	2650.0 (279.4)	2432.5 (536.9)	1165.0 (440.2)	468.3 (103.3)	177.0 (51.1)	58.3 (13.3)	20.0 (2.4)

ND = None Detected

ND* = None Detected except for one of the four samples analyzed (see below)

** = Approximately 5 minutes before the end of infusion

LITTERING PHASE:**Clinical observations:**

Dose (mg/kg)	0.5	4.5	30
Eyes partly closed (left and/or right eyes)	---	25/26	21/26
Fur staining red/muzzle	---	5/26	8/26
Fur thin			
Periorbital area (left and/or right)	---	---	12/26
Ventral thoracic area	---	---	4/26
Hunched posture	---	22/26	26/26
Tremors	---	23/26	26/26

Body weight and body weight gains:

Dose (mg/kg)	0.5		4.5		30	
	GD	BW	BWG	BW	BWG	BWG
6-9	---	---	---	---	---	↓195%*
9	---	↓4%*	---	↓8%*	---	↓11%*
9-12	---	---	---	---	---	↑39%*
12	---	---	---	↓6%*	---	↓7%*
12-15	---	---	↓22%*	---	↓53%*	↓24%*
15	---	---	---	↓9%*	---	↓8%*
15-18	---	---	---	---	↓125%*	↓39%*
18	---	---	---	↓23%*	---	↓12%*
18-20	---	---	---	---	↓100%*	↓32%*
20	---	---	---	↓29%*	---	↓13%*
6-15	---	---	↓26%*	---	↓47%*	↓50%*

[* p<0.05; GD=gestation day; BW=body weight; BWG=body weight gain;
Values represent % change from control]

Maternal littering results:

Dose (mg/kg/day)	Gestation Index (%) ¹	No of live pups at birth [^]	Live birth index (%) ²
0.5	96.2	---	89
4.5	4.2	↓96%*	3.6 (↓96%*)
30	55	↓62%*	44.4 (↓52%*)

[* p<0.05; ¹ control, 100%; ² control, 92.1%; [^] Values represent % change from control]

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this page is the manifestation of the electronic signature.**

/s/

Suzanne Thornton-Jones
12/2/04 02:44:38 PM
PHARMACOLOGIST

R. Daniel Mellon
12/2/04 05:51:04 PM
PHARMACOLOGIST
I concur

MEMORANDUM

July 23, 2001

TO: File
FROM: Kenneth L. Hastings, Dr.P.H.
SUBJECT: NDA 21-060

I have reviewed the action package for NDA 21-060 (ziconotide solution; PRIALT[®]) and concur that the application is approvable. Specifically, the sponsor should conduct a nonclinical toxicology study in rats to determine the effect of ziconotide on embryo-fetal development as recommended by the Pharmacology/Toxicology review staff. There are no other recommendations.

/s/

Kenneth L. Hastings, Dr.P.H.
Acting Associate Director for Pharmacology/Toxicology, ODE II

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this page is the manifestation of the electronic signature.**

/s/

Kenneth Hastings
8/2/01 07:49:46 AM
PHARMACOLOGIST

ADRA Review #2 of Action Package for NDA 21-060, Prialt (ziconotide)

Reviewer: Lee Ripper, HFD-102

Date: July 18, 2001

Dates volumes received in HFD-102:

Chem and P/T – July 18, 2001

Admin and Clinical – July 20, 2001

Indication: _____ management of severe, chronic pain in patients
_____ for whom
intrathecal therapy is warranted.

Action type: AE

Drug Classification: 1P

User fee goal date: July 29, 2001

505(b)(1) application

RPM: Laura Governale, 7-7423

Date original NDA received: Dec 28, 1999

ACTION GOAL DATE: July 25, 2001

Patent Info: Yes, acceptable

EER: EER signed AC on 4/2/01. However, _____ testing site needs to be added to EER and inspected. Site information not submitted in time to be inspected during this review cycle.

Clinical Inspection Summary: Two sites inspected. Data from _____ site were excluded from analysis. Addressed in statistics and medical reviews.

OPDRA review of tradename: Yes, Prialt acceptable

DDMAC review of PI: No review by DDMAC in action package, but labeling comments are not being provided to firm at this time due to nature of deficiencies.

Debarment statement: Acceptable

EA: Categorical exclusion

Financial disclosure information/review: See pp. 25-26 of MOR finalized 7/17/01

Safety update: Pp. 7-25 of MOR finalized 7/17/01

Comments:

1. On the eSignature page and pages 1-3, 9, 11, 12, et al. of Chem Rev #2, words are all run together with no spaces in between them. When this happens, need to pull doc up again from DFS and/or run off on another printer to get spacing correct. I reprinted the pages and put them in the jackets.
2. Routing history of draft letter should be included in package.
3. See minor editorial comments on letter.

C:\Data\Wpfiles\N21060AE.doc

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

/s/

Leah Ripper

7/23/01 03:53:33 PM

CSO

MEMORANDUM

June 19, 2000

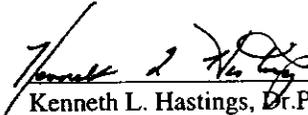
TO: John K. Jenkins, M.D.
Leah Ripper

FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-060 (Ziconotide)

I have reviewed the action package and concur with the conclusions of the Pharmacology/Toxicology Reviewer (Dr. David Brase) and the Pharmacology/Toxicology Team Leader (Dr. Lucy Jean). In particular, I agree with both that the product label should be changed to classify the drug in Pregnancy Category C. There were findings consistent with teratogenic potential in rats at a dose that was not toxic to the dams. In addition, the sponsor should provide appropriate animal to human comparisons (systemic exposure or dose normalized according to relative body surface areas) at which adverse fetal effects were observed in nonclinical reproductive toxicity studies. These appropriate comparisons should also be made in the information on fertility effects in the *Carcinogenesis, Mutagenesis, and Impairment of Fertility* section of the product label. I suggest the following wording to be used in this section concerning carcinogenicity and mutagenicity:

[_____]



Kenneth L. Hastings, Dr.P.H.
Acting Associate Director for Pharmacology/Toxicology

ADDENDUM TO REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA**DIVISION OF ANESTHETIC, CRITICAL CARE, AND ADDICTION DRUG PRODUCTS**

Suzanne R. Thornton, Ph.D.
18 July 2001

NDA NO. 21-060
SUBMISSION NO. N000BP

This addendum supersedes all previous recommendations for the submission.

RECOMMENDATIONS:**INTERNAL COMMENTS**

While there is a significant body of evidence to indicate that the generalized delay in ossification of the pubic area bones were a result of maternal toxicity, the issue of whether the pubic bones are truly absent or unossified remains unanswered. If the bones are truly "absent" then that would make ziconotide a teratogen because absence of bones is a malformation, however, if the bones are unossified, then the finding would be a delay in ossification and ziconotide would not be considered teratogenic. To address this issue, the Sponsor should conduct another rat teratology study so that a definitive answer to the question of the pubic bones being absent or unossified can be determined and to assess the reproducibility of the original findings. If possible, the Sponsor should: 1) utilize the same experimental design as that employed in the original rat teratology study, same supplier, strain, age, and weight of rat; 2) have a vehicle and a 15 mg/kg/day ziconotide treatment group; 3) utilize double-staining techniques (Alizarin Red S/Alcian Blue) for bone and cartilage visualization; and 4) assess exposure levels by performing TK plasma analysis. If the same supplier, strain, age, and weight of rat can not be employed, the Sponsor should: 1) use doses of ziconotide that approach the maximum tolerated dose; 2) utilize double-staining techniques (Alizarin Red S/Alcian Blue) for bone and cartilage visualization; and 3) assess exposure levels by performing TK plasma analysis.

EXTERNAL COMMENTS

The review of the pharmacology and toxicology section of your submission is complete, and we have identified the following deficiency.

Provide a definitive answer regarding the presence/unossified or absence of the pubic and ischial bones and assess the reproducibility of the original findings by conducting another rat embryo-fetal development study. The rat embryo-fetal development study should:

1. utilize the same experimental design as in the previously conducted rat embryo-fetal development study, including using the same supplier, strain, age, and weight of rat;
2. treatment groups – vehicle and 15 mg/kg/day ziconotide;
3. utilize double-staining techniques (Alizarin Red S/Alcian Blue) for bone and cartilage visualization; and
4. assess exposure levels by performing TK plasma analysis.

If the same supplier, strain, age, and weight of rat can not be employed, the Sponsor should:

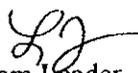
1. use doses of ziconotide that approach the maximum tolerated dose;
2. utilize double-staining techniques (Alizarin Red S/Alcian Blue) for bone and cartilage visualization; and
3. assess exposure levels by performing TK plasma analysis.

Author: Suzanne R. Thornton, Ph.D., Pharmacologist
Supervisory Pharmacologist: Thomas Papoian, Ph.D.

**Appears This Way
On Original**

MEMORANDUM

TO: Cynthia McCormick, M.D., Division Director
Robert Rappaport, M.D., Deputy Division Director

FROM: Lucy Jean, Ph.D. 
Pharmacologist Team Leader

DATE: June 2, 2000

SUBJECT: NDA 21-060 Ziconotide Acetate
Summary of pharmacology and toxicology data and issues

Introduction: Ziconotide, also known as SNX-111 acetate, is a synthetic peptide consisting of 25 amino acids with three disulfide bonds. It is a new molecular (chemical) entity and is a synthetic version of ω -conotoxin MVIIA, a naturally-occurring peptide found in a fish-eating marine snail (*Conus magus*). Ziconotide potently blocks N-type voltage-dependent calcium ion channels in neurons. It is thought to produce analgesia by inhibiting the release of substance P and calcitonin gene-related peptide from primary afferent terminals involved in neuronal pain-signaling pathways in the spinal cord. The proposed indication for ziconotide is for the management of severe chronic pain in whom intraspinal therapy is needed. The drug product, to be administered intrathecally via either an external or an implantable pump, is a  solution containing 0.1 mg/mL of ziconotide and  of L-methionine in 0.9% saline. The recommended initial dose is no more than 0.1 μ g/hr. The dose should not be increased more often than once in a 24 hr period and by not more than 0.1 μ g/hr per increment. Few patients respond to ziconotide doses above 2.4 μ g/hr which is considered as the maximum recommended human dose (MRHD) in the review. It should be noted that during the clinical studies lasting up to 24 months patients who received initial dose of 0.1 μ g/hr or less had a mean dose of 0.42 μ g/hr and a median (range) dose of 0.24 μ g/hr . The corresponding values for initial dose greater than 0.1 μ g/hr were 1.78 μ g/hr, and 0.74 μ g/hg (0.12-10.00), respectively.

Efficacy: Antinociceptive activity of ziconotide was shown in various animal models, mostly in rats. In both acute and chronic inflammatory pain models where both NSAIDs and/or morphine are effective, ziconotide was shown to possess analgesic activity. It is to be noted that in hot-plate and tail-withdrawal tests, which differentiate weak (NSAID) from strong analgesics (morphine), robust antinociceptive activity of intrathecal ziconotide (0.3 μ g/rat by bolus injection or 0.1 μ g/hr/rat by continuous infusion) was usually accompanied by shaking behavior in rats. These doses are equivalent to 1.3 μ g/kg (0.3 μ g/rat) or 0.4 μ g/kg/hr, assuming a body weight of 250 gm. Thus from the rat data, it appears that the separation of efficacy against severe pain and CNS adverse effects is poor for intrathecal (IT) ziconotide.

Safety pharmacology: Dose-dependent CNS adverse effects were observed in mice, rats and dogs following IT administration. In mice, a low dose (0.03 µg, or approx. 1.2 µg/kg) evoked serpentine tail movements and intermittent shaking, mainly involving the hind legs and tail, and higher doses produced whole body shaking. In rats, similar effects were observed at 0.1-10 µg/rat (~ 0.4-40 µg/kg). In dogs, whole body trembling, panting, decreased arousal and activity were observed at 10 µg (~ 1 µg/kg) and infusion of 100-1200 ng/kg/hr (approx. 1-16X the MRHD) caused ataxia, tremors, and hyperactivity. Females appeared to be more sensitive. Some dogs showed decreased or no pupillary light reflex or dilated pupils. In rats IT injection of 10 µg/rat (~ 40 µg/kg) had no significant effect on systemic blood pressure whereas in dogs, IT injection of 10 µg/dog (~ 1 µg/kg) decreased blood pressure and heart rate. In dogs, EKG showed second degree A-V block accompanied by respiratory sinus arrhythmia from 600 ng/kg/hr; the no-effect dose was 300 ng/kg/hr. A respiratory depressant effect was shown in dogs (10 µg/dog, IT) only. In rats ziconotide (0.1 µg/rat, IT) had no respiratory depressant effect, and did not exacerbate the respiratory depressant effect of morphine. In morphine-tolerant rats, no cross tolerance to ziconotide was shown. In mice (ED₅₀ of 0.19 µg/mouse, IT) and rats (1 µg/rat, IT), ziconotide inhibited gastrointestinal transit. The NOEL in rats (0.3 µg/rat) was shown to potentiate morphine-induced decrease in GI motility.

Special Toxicology Studies (abuse liability and hyper-sensitization): Based on the nonclinical data, ziconotide does not have physical dependence potential or abuse liability of the opioid type. This was evaluated in a standard battery of *in vivo* studies (mouse p-phenylquinone abdominal constriction and tail-flick tests, suppression of morphine withdrawal signs in monkeys) and *in vitro* studies (receptor binding assays, electrically stimulated mouse vas deferens). Ziconotide was shown to be a weak sensitizer in a systemic anaphylaxis test in guinea pigs. A test in mice did not show antibody formation. The potential for antibody formation should now be obtained from clinical studies. ✓

ADME/Pharmacokinetics: Pharmacokinetics profile in plasma and cerebrospinal fluid after IT and IV administration was studied in rats, dogs and monkeys. Systemic exposure was low following IT administration in rats and dogs. In dogs following an IT bolus of 10 µg, the AUC values for lumbar CSF and plasma were 3445 and 1.53 ng hr/mL, respectively. IT 48-hr-infusion at 0.069 and 0.35 µg/kg/hr had plasma C_{max} of [redacted] and [redacted] and T_{max} of approx. 35 and 26 hrs, respectively. For comparison, IV injection of 100 µg/kg had plasma C_{max} of [redacted] and CSF C_{max} of [redacted]. Ziconotide is probably cleaved at multiple sites by various peptidases. It is hypothesized that metabolism involves cleavage of hydrophobic and basic residues by endoproteases followed by digestion of the amino and carboxyl terminals by exopeptidases. Clearance from the CSF was fast with a terminal half-life of 1.8 hrs after IT bolus and 1.6 hrs after IV bolus injections. In dogs, the elimination of ziconotide after IT administration was an initial rapid distribution (T_{1/2} 0.4 hr) into the systemic circulation, followed by movement away from the injection site by bulk CSF flow (terminal T_{1/2} 1.8 hrs). The extent of plasma protein binding was high in humans (approx. 90%) and moderate in rats (approx. 56%), dogs (approx. 62%) and monkeys (approx. 73%). Distribution into

sites and live embryos, was observed at 10 mg/kg (1700X the MRHD on a mg/m² basis). The NOAEL was 3 mg/kg (500X the MRHD). Male and female fertility was not affected at doses up to 10 mg/kg (3000X the MRHD based on the comparison with the plasma concentration). Prenatal and postnatal effects included slightly reduced pup body weights at birth, during lactation and post weaning at 10 mg/kg. There were no effects on physical and functional development or reproductive function of the F₁ generation. The NOAEL was 3 mg/kg (500X the MRHD). Therefore, based on the data showing great separations between the doses that either caused or did not cause the adverse reproductive effects and the MRHD, the human risk for adverse reproductive effects following labeled use is considered minimal. The package insert has been amended by the primary reviewer to include the pertinent data.

Genotoxic Effects: Ziconotide was not mutagenic in the *in vitro* Ames mutation assay with *S. typhimurium* or *E. coli*, and mouse lymphoma assay. Ziconotide was not clastogenic in the *in vivo* mouse micronucleus assay.

Carcinogenicity: Long-term animal studies to evaluate the carcinogenic potential of ziconotide have not been conducted; this is due to technical difficulty of intrathecal administration in rodents for 2 years. The PTCC (5/20/99) recommended a SHE cell transformation assay, which was shown to be negative for ziconotide.

CONCLUSIONS and RECOMMENDATIONS: Ziconotide has been extensively studied in nonclinical *in vivo* and *in vitro* studies. The pharmacological and toxicological profiles generated following IT and IV administrations have shown efficacy and reasonable safety for the proposed intrathecal use. Ziconotide, therefore, can be labeled for human use. Before the application can be approved, however, the package insert should be amended to reflect the teratogenic effects in rats and to calculate human multiples based on a mg/m² basis. These deficiencies have been communicated with the applicant.

Nonclinical issues:

The deficiencies under the Pregnancy section of the proposed package insert should be amended and have been communicated with the applicant in a discipline review letter. The amendment should include the teratogenic findings, a change from Pregnancy Category • to Pregnancy Category C and the calculation of human multiples based on PK data or body surface area. Refer to the pharmacology review for details.



FDA CENTER FOR DRUG EVALUATION AND RESEARCH
DIVISION OF ANESTHETIC, CRITICAL CARE, AND ADDICTION DRUG PRODUCTS
HFD-170, ROOM 9B-45, 5600 FISHERS LANE, ROCKVILLE MD 20857

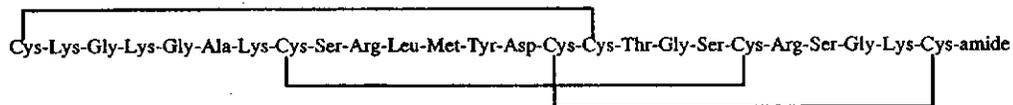
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Suzanne R. Thornton, Ph.D.
21 June 2001

NDA NO. 21-060
SUBMISSION NO. N000BP
SUBMISSION DATE: 24 May 2001
CENTER RECEIPT DATE: 25 May 2001
REVIEWER RECEIPT DATE: 29 May 2001
SPONSOR: Elan Pharmaceuticals
800 Gateway Blvd
South San Francisco, CA 94080

DRUG:

CODE NAME: SNX-111 acetate
GENERIC NAME: Ziconotide
TRADE NAME: Ptialt
CHEMICAL NAME: ω -conotoxin MVIIA (reduced), cyclic (1 \rightarrow 6), (8 \rightarrow 20), (15 \rightarrow 25) tris (disulfide)
CAS REGISTRY NUMBER: 107452-89-1
MOLECULAR WEIGHT: 2639.18 daltons
STRUCTURE:



RELATED INDS/NDAS/DMFS: IND 45,718.
PHARMACOLOGIC CLASS: N-type Calcium channel blocker
PROPOSED CLINICAL INDICATION: Analgesia
FORMULATION: The product is a sterile, preservative-free, isotonic solution containing 100 mcg of ziconotide base with L-methionine and sodium chloride as excipients, pH 4-5.
ROUTE OF ADMINISTRATION: Intrathecal (i.t.)
BACKGROUND: Ziconotide is a new molecular entity investigated as an analgesic to be administered via continuous i.t. infusion to alleviate chronic pain that is resistant to opioid therapy. The original NDA was reviewed and the product received an approvable letter June 2000. This resubmission addresses additions to product label, and responses to questions raised in a Pharmacology/Toxicology Advice letter (25 May 2000) and in an advice letter (09 November 2000) Product label additions proposed by the Sponsor deal primarily with the mechanism of action, pharmacology, and reproductive toxicology.

REPRODUCTIVE TOXICOLOGY

The Sponsor concurs with the Division's interpretation to label ziconotide as a Pregnancy Category C due to the fetal toxicity observed in the rat and rabbit teratology studies. However, the Sponsor does not agree with the Division's past interpretation that ziconotide is teratogenic in rats and present arguments to support their interpretation and to have the word "teratogenic" removed from the label.

Discussion Points presented by the Sponsor:

1. CORRELATION OF REDUCED MATERNAL FEED CONSUMPTION AND RETARDED SKELETAL OSSIFICATION.

The rat teratology study showed a 5% decrease in maternal body weight and body weight gains, and a 25-29% decrease in food consumption on Gestation Day (GD) 9 at all doses (1.5, 4.5, 15 mg/kg/day). Cesarean section data revealed a slight increase in mean number of early resorptions at 15 mg/kg/day. There was no effect of ziconotide on fetal body weight but there were increased incidence of reduced ossification of the pubic bones at all doses. In fetuses treated with 15mg/kg/day ziconotide there was an increased incidence of absent, reduced ossification, irregular ossification, pubic, ischial, and/or ilial bones, absent thoracic vertebral arches, reduced ossification of the ribs, reduced number of caudal vertebrae, and a decreased incidence of irregular ossification of the interparietal skull bones. The historical control data (HCD) supplied by the CRO conducting the study show that the incidence of these findings are outside of the HCD range.

The original pharm/tox reviewer (D. Brase) interpreted the data to show that the delayed development may be a result of maternal toxicity, such as decreased food consumption (review, June 2000). The interpretation of the teratology data is difficult because of the categorization utilized for the fetal findings. In non-USA contract and pharmaceutical laboratories, fetal findings are categorized as major malformations, minor anomalies, and common variants, while in the USA, they are generally categorized as malformations and variations. The definitions between these categories vary between the laboratory, but it is generally recognized that major malformations (non-USA) and malformations (USA) are considered detrimental to survival, while minor anomalies, common variants, and variations are not considered detrimental to survival. One other difference between non-USA and USA companies is in the terminology for differing degrees of ossification, for example categories used include absent, unossified, reduced ossification, incomplete ossification, where "absent" is used as a measure of ossification, rather than a true description of a malformation.

In the rat teratology study submitted for review, this difference becomes important in the interpretation of the data. The primary findings were an increased incidence of absent, reduced ossification, irregular ossification pubic, ischial, and/or ilial bones, absent thoracic vertebral arches, reduced ossification of the ribs, reduced number of caudal vertebrae, and a decreased incidence of irregular ossification of the interparietal skull bones. The difficulty in the interpretation is the following, are the bones truly "absent" or are they just unossified? If the bones are truly "absent" then that would make ziconotide a teratogen because absence of bones is a malformation. However, if the bones are unossified, then

the finding would be a delay in ossification and ziconotide would not be considered teratogenic. A teleconference was held between the Sponsor, the CRO who conducted the rat teratology study and the Division (10 April 2000) to discuss the findings of the pubic/ilial/ischium bones. [See the teleconference meeting minutes for further details]. During the discussion with the Sponsor and CRO, the Division was not able to ascertain if the pubic bones were truly absent or unossified. The Division advised the Sponsor and CRO of the following: 1) since the CRO laboratory had not conducted double staining of the skeletons of the rats (Alizarin Red S/Alcian Blue) which is not required per ICH guidelines, they may be able to salvage the experiment by re-examining the fetuses; 2) the Sponsor could conduct a focused study examining the skeletons using the same experimental design, but only comparing the vehicle and the high dose group, 15 mg/kg/day, where the alterations in the pubic bones occurred being sure to perform double-staining of the skeletons which will allow for the visualization of the bone and cartilage; or 3) submit supporting evidence for their interpretation that the bones in question were not absent, but unossified. The Sponsor chose the latter option and submitted supporting evidence for what they consider delayed ossification as a result of maternal toxicity.

In an attempt to ascertain if the pubic bones were truly absent or just unossified, a re-review of the rat teratology study indicated that pubic bones are "targets" for ziconotide, the incidence of pubic bone findings exceeded the Historical Control Data (HCD) values supplied by the CRO who conducted the study (Table 1). If one examines the pubic bone findings, it becomes apparent that there is an increased incidence above the control group of multiple delayed ossifications of the pubic area (Table 2). In some of the litters, the pubic bones were absent on one side (unilaterally) and reduced ossification on the other side (unilaterally) or there were reduced or irregular ossification of the ischial and/or ilial bones, with absent pubic bones. These observations indicate that there was a generalized delay in the skeletal ossification of the pubic area in fetuses treated with ziconotide.

Table 1. Litter incidence for pubic area HCD and findings in the rat teratology study.

Fetal Observation	% Litters Affected (Incidence of finding/Litter Size)				
	HCD ¹	Control	Ziconotide (mg/kg/day)		
Pubic Bone			1.5	4.5	15
Reduced ossification	7% (40/605)	14% (3/22)	30% (7/23)	20% (5/25)	56% (14/25)
Irregular ossification	0.2% (1/605)	14% (3/22)	13% (3/23)	12% (3/25)	8% (2/25)
Absent	0.5% (3/605)	0% (0/22)	0% (0/23)	4% (1/25)	12% (3/25)
Ischial Bone					
Reduced ossification	3% (20/605)	5% (1/22)	9% (2/23)	8% (2/25)	12% (3/25)
Irregular ossification	0.3% (2/605)	0% (0/22)	4% (1/23)	4% (1/25)	12% (3/25)
Absent	0.2% (1/605)	0% (0/22)	0% (0/23)	0% (0/25)	4% (1/25)
Ilial Bone					
Irregular ossification	0.2% (1/605)	0% (0/22)	4% (1/23)	0% (0/25)	0% (0/25)

¹ - Data represents 28 studies conducted between 1991-1999.

Table 2. Fetal/Litter Incidence of multiple pubic bone findings.

[Note: There were no multiple pubic bone findings in the control group]

Group	Animal no., fetus no.	Fetal Findings
Ziconotide - 1.5	2501, 11	Reduced, bilateral pubic bones

		Reduced, bilateral ischial bones
	2502; 12	Irregular ossification, unilateral pubic bones Irregular ossification, unilateral ilial bones
	2512,16	Reduced, bilateral pubic bones Reduced, unilateral ischial bones
	2515,5	Reduced, unilateral pubic bones Irregular, unilateral pubic bones Irregular, bilateral ischial bones
	Incidence: 17% of litters affected (4/23) [no litters affected/litter size]	
Ziconotide – 4.5	3510,3	Reduced, bilateral pubic bones Reduced, bilateral ischial bones
	3510,4	Reduced, unilateral pubic bones Irregular, unilateral pubic bones
	3512,4	Reduced, unilateral pubic bones Reduced unilateral ischial bones
	3519,17	Absent, unilateral pubic bones Reduced, unilateral pubic bones
	3524,11	Irregular, bilateral pubic bones Irregular, bilateral ischial bones
	Incidence: 16% (4/25)	
Ziconotide – 15	4501,8	Reduced, bilateral pubic bones Reduced, bilateral ischial bones
	4516,2	Reduced, bilateral pubic bones Irregular unilateral ischial bones
	4516,19	Reduced, bilateral pubic bones Irregular unilateral ischial bones
	4520,10	Absent, bilateral pubic bones Reduced, bilateral ischial bones
	4520,12	Reduced, unilateral pubic bone Absent, unilateral pubic bone
	4521,2	Absent, bilateral pubic bones Irregular, bilateral ischial bones
	4521,4	Absent, bilateral pubic bones Irregular, bilateral ischial bones
	4521,6	Absent, bilateral pubic bones Irregular, bilateral ischial bones
	4521,10	Absent, bilateral pubic bones Irregular, bilateral ischial bones

Table 2 (continued).

Group	Animal no., fetus no.	Fetal Findings
	4521,13	Reduced, unilateral pubic bone Irregular, unilateral pubic bone Irregular, unilateral ischial bone
	4521,16	Absent, bilateral pubic bones Irregular, unilateral ischial bone
	4531,10	Reduced, bilateral pubic bones Irregular, unilateral ischial bone
	Incidence: 20% (5/25)	

Also, a re-review of the pre- and postnatal development reproductive toxicity studies showed that there was the same maternal toxicity, decrease in body weight and food consumption, on GD9 as that observed in the rat teratology study. Pup observations during lactation did not indicate any changes in gait or other behaviors which could indicate a problem with the pubic bones. However, there were significant decreases in the pup body weights at birth and throughout the study. Caveats to the data in the pre- and postnatal data are: 1) the high dose administered in the pre- and postnatal study was 10 mg/kg/day which was below the high dose where the pubic bone findings were observed in the rat teratology study, but the decreases in food consumption on GD 9 were observed at both the 10 and 15 mg/kg/day; and 2) it is difficult to determine affects on gait since pups below the Lactation Day (LD)/Postnatal Day (PD) 14 are not ambulatory. Another caveat to the pubic bones findings in the rat teratology study is the lack of similar findings in the rabbit teratology study (0.2, 1.0, 5.0 mg/kg/day).

Articles available in the literature and articles supplied by the Sponsor, indicate that hypoglycemia due to fasting or decreased caloric intake on GD 9-10 can lead to delays in ossification, especially in the axial skeleton (thoracic vertebral centra, ribs, cervical vertebrae, sternebral bones) (Collins et al., 1987; Hannah and Moore, 1971; Ikemi et al., 1993). After re-examination of the rat and rabbit teratology studies, pre- and postnatal development study, and review of articles available in the literature, it seems reasonable to conclude that the maternal toxicity, decreased body weight and food consumption on GD 9, could be responsible for the generalized delay in skeletal ossification observed in the rat teratology study.

At the time of the original NDA review, the PK/TK exposure level data were unavailable for the rat and rabbit teratology studies. In the teleconference held between the Division and the Sponsor, the status of the TK sample analysis was requested. The Sponsor indicated that the CRO laboratory where the plasma samples had been sent for analysis was no longer in existence and they were attempting to determine if there was any data available. In the most recent submission, the Sponsor has included some PK/TK exposure level data from the rat and rabbit studies. The Sponsor indicates that the data are non-GLP and the accuracy of the results can not be determined. The PK/TK data submitted show that the rats and rabbits were exposed to ziconotide during the studies, and ziconotide exhibited similar $t_{1/2}$, a linear increase in C_{ss} and AUC values. Since the validity of the PK/TK results are questionable,

they will not be used to calculate human safety factors, therefore, the label will reflect human safety factors based on body surface area (mg/m^2).

Based on the weight of evidence in the literature regarding the phenomenon of maternal caloric deficiencies on GD 9, the lack of similar findings in the rabbit teratology study, and re-examination of the rat teratology and pre- and postnatal development studies, this reviewer believes that the generalized delay in the pubic bones is probably a result of maternal toxicity, decreased body weight and food consumption on GD 9. While there is evidence for maternal toxicity causing the delayed ossification, a definitive answer on whether the pubic bones are truly absent or unossified has not been provided by the Sponsor.

2. ELAN'S RESPONSE TO THE DISCIPLINE LETTER (25 MAY 2000)

Discipline letter comments:

1. Low dose of ziconotide (1.5 mg/kg/day) – reduced pubic bones (bilateral) in 2.5% of fetuses, multiple spinal malformations, including the absence of lumbar vertebrae 5 and 6 in one fetus.
2. Mid dose of ziconotide (4.5 mg/kg/day) – multiple fusions and/or anomalies in the thoracic vertebrae, including absence of 5th, 6th, and 12th vertebral arches in one fetus.
3. High dose of ziconotide (15 mg/kg/day)- a statistically significant increase in skeletal abnormalities 40% above controls, reduced pubic bones in 6.2% of the fetuses, reduced number of caudal vertebrae in 1.6% of fetuses, reduced ossification of pubic bones in 9.1% of fetuses, irregular ossification in ischial bones in 1.6% of fetuses, major malformations in 3 fetuses in 2 litters, compared with 1 malformation each in the lower dose and control groups.

Response to comments:

General comment - This reviewer agrees with the Sponsor that fetal findings should be presented as the litter incidence, not the fetal incidence as the dam is the experimental unit in reproductive toxicity studies.

1. Low dose – There was higher litter incidence of reduced pubic bones that exceeded the HCD values and can be attributed to ziconotide treatment, therefore, this reviewer disagrees that the affect in not ziconotide treatment-related. This reviewer agrees with the Sponsor's argument that when the litter incidence is examined for the multiple spinal malformations, including the absence of lumbar vertebrae 5 and 6, that there is no difference between the control and low dose ziconotide treated groups.
2. Mid dose – This reviewer agrees with Sponsor's argument that when the litter incidence is examined for the multiple fusions and/or anomalies in the thoracic vertebrae, that there is no significant difference between the control and mid dose.
3. High dose – This reviewer agrees with Sponsor's argument that when the litter incidence is examined for skeletal abnormalities and major malformations in 3 fetuses in 2 litters, compared with 1 malformation each in the lower dose and control groups, that there is no significant difference between the control and high dose. Also, the fetal findings of reduced pubic bones, absent pubic bones, reduced number of caudal vertebrae, reduced ossification

of pubic bones, and irregular ossification of ischial bones when compared using litter incidence are significantly different than the control group, exceed the HCD values, and can be attributed to ziconotide treatment.

3. RECOMMENDATIONS:

3.1. INTERNAL COMMENTS

While there is a significant body of evidence to indicate that the generalized delay in ossification of the pubic area bones were a result of maternal toxicity, the issue of whether the pubic bones are truly absent or unossified remains unanswered. If the bones are truly 'absent' then that would make ziconotide a teratogen because absence of bones is a malformation, however, if the bones are unossified, then the finding would be a delay in ossification and ziconotide would not be considered teratogenic. A focused rat teratology study needs to be conducted by the Sponsor so that a definitive answer to the question of the pubic bones being absent or unossified can be determined and to assess the reproducibility of the original findings. The focused study should: 1) utilize the same experimental design as that employed in the original rat teratology study, same supplier, strain, age, and weight of rat; 2) have a vehicle and a 15 mg/kg/day ziconotide treatment group; 3) utilize double-staining techniques (Alizarin Red S/Alcian Blue) for bone and cartilage visualization; and 4) assess exposure levels by performing TK plasma analysis.

3.2. EXTERNAL COMMENTS

The review of the pharmacology and toxicology section of your submission is complete, and we have identified the following deficiency.

Provide a definitive answer regarding the presence/unossified or absence of the pubic and ischial bones by conducting a focused rat embryo-fetal development study. The rat embryo-fetal development study should:

1. utilize the same experimental design as in the previously conducted rat embryo-fetal development study, including using the same supplier, strain, age, and weight of rat;
2. treatment groups – vehicle and 15 mg/kg/day ziconotide;
3. utilize double-staining techniques (Alizarin Red S/Alcian Blue) for bone and cartilage visualization; and
4. assess exposure levels by performing TK plasma analysis.

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Hannah, R.S. and K.L. Moore. 1971. Effects of fasting and insulin on skeletal development in rats. *Teratology* 4: 135-140.

Ikemi, N., J. Imada, T. Goto, H. Shimazu, and M. Yasuda. 1993. Effects of food restriction on the fetal development during major organogenesis in rats. *Congenital Anomalies* 33: 363-377.

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Supervisory Pharmacologist: Thomas Papoian, Ph.D.

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/s/

Suzanne Thornton
6/29/01 03:34:24 PM
PHARMACOLOGIST

Thomas Papoian
7/3/01 12:56:24 PM
PHARMACOLOGIST

stored at 5°C. A diluent with formulation identical to the drug product, but without ziconotide is also provided. The following table shows the quantitative composition of the drug product.

Quantitative Composition of Ziconotide Drug Product

Components	Amount/ml	Amount per 5-ml Vial	
Ziconotide	0.1 mg	0.5 mg	
L-methionine USP			
Sodium Chloride USP			
Water for Injection USP qs ad			

Route of administration: Intrathecal (IT)

Disclaimer: Some of the sponsor's submitted material may be used in this review.

Introduction/drug history: Ziconotide is a new molecular entity consisting of a synthetic linear 25-amino acid peptide with three intramolecular disulfide bonds, based upon the structure of a naturally-occurring peptide toxin called ω -conotoxin MVIIA that is produced by a fish-eating sea snail, *Conus magus*. Its pharmacological activity is based upon its ability to block N-type (neuron-specific) voltage-sensitive calcium channels. It was first under development (currently inactive) as a

For this NDA, it is being promoted as an analgesic to be administered by continuous IT infusion for the alleviation of chronic pain that is resistant to opioid therapy.

Studies reviewed within this submission:

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(*Reviewed by Kathleen Haberny)

Effects of SNX-111 Administered Intrathecally and Intracerebroventricularly on the Formalin Test in BALB/c Mice (Study No. 0084, Volume 2.005, p. 212, non-GLP) 16

Antinociceptive Effects of Intrathecally Administered Omega-Conopeptides on the Formalin Test in the Rat (Study No. 4-5-93, Vol. 2.006, p. 002, non-GLP) 16

Analgesic Effects of Spinally-administered (Intrathecal) SNX-111 on Formalin-evoked Cutaneous Pain in the Rat (Study No. 93-6008, Vol. 2.005, p. 238, non-GLP) 18

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Effects of Intrathecal Co-administration of Methionine and SNX-111 on Acute and Persistent Pain Behavior Evoked by Subcutaneous Injection of Dilute Formalin into the Hind Paw of the Rat (Study No. 97-5011, Volume 2.006, p. 112, non-GLP).	18
Effects of Continuous Constant-rate Spinal (Intrathecal) Infusion of SNX-111 on Formalin-evoked Cutaneous Pain in the Rat: Dose-Response Relationship (Study No. 93-5043, Volume 2.006, p. 125, non-GLP).	18
Examination of the Effects of Chronic Spinal Delivery of ω -Conopeptides on Behavior and Antinociception on the Formalin and Hot Plate Test in Rats (Study No. 8-30-94, Volume 2.006, p. 152, non-GLP).	19
In Vitro Release of Histamine by SNX-111 (Study No. 93-6002, Volume 2.005, p. 210, non-GLP).	20
Tremorgenic Potencies of ω -Conopeptides in the Mouse (Study No. 98-5043, Volume 2.005, p. 007, non-GLP).	21
Effect of Diazepam on SNX-111 Induced Shaking Behavior in the Mouse (Study No. 98-5045, Volume 2.005, p. 020, non-GLP).	22
Effects of Spinally-administered SNX-111 and SNX-239 on Systemic Blood Pressure and Motor Behavior in the Rat (Study No. 93-6007, Vol. 2.005, p. 097, non-GLP).	22
Cardiovascular Effects of Ziconotide in Rats when Administered by a Variety of Routes, (Study No. 98-5038, Volume 2.005, p. 106, non-GLP).	22
Effects of Intrathecal SNX-111 on Spinal Clonidine-induced Depressor and Bradycardia Responses in Conscious Rats (Study No. 98-5013, Volume 2.010, p. 221, non-GLP).	23
Effects of SNX-111 on Morphine-induced Respiratory Depression: Acute Morphine Administration (Study No. 94-5019, Volume 2.010, p. 88; non-GLP)	23
Effects of SNX-111 on Morphine-induced Respiratory Depression in Morphine-tolerant Rats (Study No. 95-5009, Volume 2.010, p. 109; non-GLP).	24
Effects of SNX-111 Alone and in Combination with Morphine on Gastrointestinal Transit in the Mouse (Study No. 94-5001, Vol. 2.010, p. 130).	25
Ziconotide (SNX-111): Determination of Physical Dependence Potential and Abuse Liability (Study No. 9120, Volume 2.010, p. 254).	25

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*A Pharmacokinetic Study with Test Article SNX-111 Administered by 24-Hour Intravenous Infusion to Rats (Study No. 309-92, Volume 2.009, p. 002).	26
*Determination of the Kinetics of SNX-111 Infused into Rats: Use of a Radioimmunoassay to Determine the Concentration of SNX-111 in Rat Plasma Samples (Study No. 92-5009, Volume 2.009, p. 051).	27
*Analysis of Dose Preparation Samples from a Pharmacokinetic Study with Test Article SNX-111 Administration by 24-Hour Intravenous Infusion to Rats (Study No. 309-92) (Study No. 98-5068, Volume 2.009, p. 91).	29
*A Pharmacokinetic Study with Test Article SNX-111 Administered by 24-Hour Intravenous Infusion to Cynomolgus Monkeys (Study No. 310-92, Volume 2.009, p. 96).	29
*Analysis of Dose Preparation Samples from a Pharmacokinetic Study with Test Article SNX-111 Administered by 24-Hour Intravenous Infusion to Cynomolgus Monkeys (Study No. 310-92, Volume 2.009, p. 152).	29
*Determination of the Kinetics of SNX-111 Infused into Monkeys: Use of a Radioimmunoassay to Determine the Concentration of SNX-111 in Monkey Plasma (Study No. 92-5010, Volume 2.009, p. 127).	30
*Intrathecal SNX-111 Given as a Bolus or Continuous Infusion in Beagle Dogs: Part I Pharmacokinetics (Study No. 96-019, Volume 2.009, p. 157, non-GLP).	33
*Intrathecal SNX-111 Given as a Bolus or Continuous Infusion in Beagle Dogs: Part II Behavioural and Clinical Observations (Study No. 96-019, Volume 2.009, p. 229, non-GLP).	35
*SNX-111 Flux Through the Spinal Meninges of the Monkey (Study No. 98-5028, Volume 2.009, p. 314, non-GLP).	37
*Blood-Brain Barrier Permeability of [¹²⁵ I]-Ziconotide (SNX-111) (Study No. 0106, Volume 2.009, p. 324, non-GLP).	37
*Analysis of <i>In Vitro</i> Metabolism and Brain Penetration of Ziconotide (SNX-111) in Rats (Study No. 98-5037, Volume 2.009, p. 343, non-GLP).	38
*Possible Modes of Ziconotide Degradation <i>In Vivo</i> (Report No. 0201, Volume 2.010, p. 364).	39
* ¹²⁵ I-SNX-111: An <i>In Vitro</i> Assessment of Plasma Protein Binding and Partitioning in Whole Blood from Four Different Species (Study No. 43767, Vol. 2.010, p. 002).	40

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*Determination of the Protein Binding of CI-1009 in Heparinized Rat Plasma and EDTA Human Plasma by Radioimmunoassay (Study No. 764-03035, Volume 10, p. 288, non-GLP).	41
*Determination of Ziconotide Binding to Proteins in Dog Plasma by Radioimmunoassay (Study No. █ 48031, Volume 2.010, p. 301).	42
A 28-Day Intrathecal Infusion Toxicity Study of SNX-111 in the Beagle Dog Followed by a Recovery Period of Up To 2 Weeks (Study No.: █ 54156 with Addendum No. █ 0190, Volume 2.024, p. 002- and Vol. 2.025, pp. 001-[Addendum, Vol. 2.025, p. 398]).	46
A Study to Evaluate the Effects of SNX-111 Introduced into the Intrathecal Space of Beagle Dogs by 42-Day Continuous Infusion (with Addendum) (Study No.: 2-P46 with Addendum No. █ 0191, Volume 2.026, p. 002 to Vol. 2.027, p. 184 and Addendum, Volume 2.027, p. 185).	57
Clonal Transformation Assay Using Syrian Golden Hamster Embryo (SHE) Cells (Study No. AA18WM.308. █ Volume 2.047, p. 224).	62
Immunogenicity of Ziconotide (SNX-111) in Swiss Webster Mice (Study No. █ 291, Volume 2.048, p. 002).	64
Active Immunization of Rats with SNX-111 (Study No. 95-5020, Volume 2.048, p. 176).	65
Antigenicity Study in Guinea Pigs: Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis Reactions (Study No. █ 742A-NEU-001-94, Vol. 2.048, p. 120).	66
Antigenicity Study in Guinea Pigs: Systemic Anaphylaxis (Study No. █ 742B-NEU-001-94, Volume 2.048, p. 147).	68
Passive Cutaneous Anaphylaxis Reaction in Guinea Pigs (Study No. █ 745GP-NEU-001-95, Volume 2.048, p. 195).	69
An Intravenous Infusion Male Fertility Study of Ziconotide in the Rat (Study No. █ 96428, Volume 2.034, p. 002).	70
A Continuous Intravenous Infusion Female Fertility Study of Ziconotide in the Rat (Study No. █ 96429, Volume 2.036, p. 002).	74
A Continuous Infusion Teratology Study of SNX-111 in the Rat (Study No. █ 95625, Volume 2.037, p. 002).	78

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A Continuous Infusion Teratology Study of SNX-111 in the Rabbit (Study No. █ 95627, Volume 2.039, p. 104).	85
A Continuous Intravenous Infusion Pre- and Postnatal Study of Ziconotide in the Rat (Study No. █ 96589, Volume 2.040, p. 002).	91
Mutagenicity test on SNX-111 in the Salmonella/mammalian-microsome █ Mutation Assay (Ames Test) Preincubation Method (Study No. █ 15766-0-420, Volume 2.047, p. 004).	98
█ Mutation Assay (Study No. G97BK84.502, Volume 2.047, p. 048).	100
Mutagenicity Test of SNX-111 in the L5178yTK+/- Mouse Lymphoma Forward Mutation Assay with an Independent Repeat (Study No. 15766-0431R, Volume 2.047, p. 103).	102
In Vitro Mammalian Cell Gene Mutation Test (Study No. G97BK84.702, Volume 2.047, p. 145).	104
Mutagenicity Test on SNX-111 In Vivo Mouse Micronucleus Assay (Study No. 15766-0455, Volume 2.047, p. 191).	106
Histopathology of the ω-Conopeptide Ziconotide (SNX-111) after Repeated Intrathecal Injections in the Rat (Study No. 7-14-95, Volume 2.048, p. 223).	108
A Study of the Combined Effects of Histamine H ₁ and H ₂ Receptor Blockers on the Toxicity of SNX-111 in Rats (Study No. █ 360-92, Volume 2.048, p. 73).	110

Studies not reviewed within this submission:

A. The following studies were reviewed by Lois M. Freed, Ph.D. (4/13/93) when they were submitted under IND █ and will only be briefly summarized in the "OVERALL SUMMARY AND EVALUATION" section of this document, if relevant to the indication for the intrathecal treatment of pain (Volume numbers refer to current NDA submission):

Effects of intravenous SNX-111 administration on cortical electroencephalographic activity in the rat: a pilot study (Study No. 92-5013, Volume 2.003, p. 027)

Relationship between SNX-111 blood levels and sympatholysis in the pithed rat (Study No. 92-5027, Volume 2.005, p. 062)

NDA 21-060

Pilot study of the effects of antihistamine pretreatment on SNX-111-induced hypotension in the conscious rat (Study No. 92-5014, Volume 2.005, p. 113)

Blood pressure recordings using SNX-111 in infusion studies in the rat 4-VO model of forebrain ischemia (Study No. 92-5023, Volume 2.005, p.128)

Pharmacologic reversibility of hypotension induced by SNX-111 in the rat 4-vessel occlusion model (Study No. 93-6003, Volume 2.005, p. 138)

Acute hemodynamic effects of intravenous infusions of SNX-111 in the open-chest anesthetized dog (Study No. 247-NEU-001-91, Volume 2.005, p. 150)

Effects of SNX-111 in the guinea pig ileum (Study No. 93-6001, Volume 2.005, p. 177)

Neuroprotective effects of slow intravenous infusion of SNX-111 in the rat four-vessel occlusion model of reversible forebrain ischemia (Report No. 92-5012, Volume 2.008, p. 014).

A pharmacokinetic study with test article SNX-111 administered by 24-hour intravenous infusion to rats (Study No. 309-92, Volume 2.011, p. 002).

A pharmacokinetic study with test article SNX-111 administered by 24-hour intravenous infusion to cynomolgus monkeys (Study No. 310-92, Volume 2.011, p. 096)

A pilot single intravenous dose study with SNX-111 in the Sprague-Dawley rat (nonGLP Study No. 53328, Volume 2.013, p. 002)

A single-dose intravenous infusion toxicity study of SNX-111 in the albino rat with a 14-day observation period (Study No. 53224, Volume 2.015, p. 209)

A single-dose intravenous infusion toxicity study of SNX-111 in the cynomolgus monkey with a 14-day observation period (Study No. 53225, Volume 2.016, p. 002)

A 14-day intravenous toxicity study of SNX-111 in the albino rat (Study No. 53226, Volume 2.018, p. 002).

A 3-day preliminary evaluation by continuous intravenous infusion of SNX 111 in the cynomolgus monkey (Study No. 53269, Volume 2.019, p. 252)

A 3-day continuous infusion toxicity study of SNX 111 in the cynomolgus monkey (Study No. 53329, Volume 2.019, p. 268)

A 14-day intravenous toxicity study of SNX-111 in the cynomolgus monkey (Study No. 53227, Volume 2.020, p. 002).

Cardiovascular effects of ω -conopeptides in conscious rats: mechanism of action (*J. Cardiovasc. Pharmacol.* **20**: 756-764, 1992, Volume 2.XXX)

B. The following studies were reviewed by David Brase, Ph.D., when they were submitted under IND 45,718 and will only be briefly summarized in the "OVERALL SUMMARY AND EVALUATION" section of this document, if relevant to the indication for the intrathecal treatment of pain (Volume numbers refer to current NDA submission):

Inhibition of N-type Ca^{2+} Channel Splice Variants by Ziconotide (SNX-111) (Study No. 98-5083, Volume 2.004, p. 002) (Reviewed 10/26/99).

Effects of Intrathecal Ziconotide on Morphine-induced Inhibition of Gastrointestinal Motility in Rats (Study No. 94-5001, Volume 2.010, p. 130) (Reviewed 10/26/99).

Heart Catecholamine Levels in Rats after Acute Intravenous Administration of a High Dose of Ziconotide (SNX-111) (Study No. 98-5036, Volume 2.049, p. 091) (Reviewed 10/26/99).

A Review of Plasma Glucose Levels in Animals after Ziconotide Treatment in Single Dose and Repeated Dose Toxicity Studies and Clinical Studies in Man (Study No. CL0045, Volume 2.049, p. 96) (Reviewed 8/13/99)

C. The following studies involving preliminary dose range-finding studies, method validations, and dosing sample analyses, etc. were not reviewed:

Analysis of Dose Preparation Samples from a Pilot Single Intravenous Dose Study with SNX-111 in the Sprague-Dawley Rat from Study No. 53328 (Study No. 98-5064)

Analysis of Dose Preparation Samples from a Single-Dose Intravenous Infusion Toxicity Study of SNX-111 in the Albino Rat with a 14-Day Observation Period from Study No. 53224 (Study No. 98-5066)

A Single-Dose Intravenous Infusion Toxicity Study of SNX-111 in the Cynomolgus Monkey with a 14-Day Observation Period (Study No. 53225)

Quantification of SNX-111 in Cerebrospinal Fluid Samples from a Single-Dose Intravenous Infusion Toxicity Study of SNX-111 in the Cynomolgus Monkey with a 14-Day Observation Period from Study No. 53225 (Study No. 98-5048)

Analysis of Dose Preparation Samples from a Single-Dose Intravenous Infusion Toxicity Study of SNX-111 in the Cynomolgus Monkey with a 14-Day Observation Period from Study No. 53225 (Study No. 98-5065)

Acute Dose-Ranging Study of SNX-111 Administered via Bolus and Continuous Intrathecal Infusion to Beagle Dogs (Study No. 2-P55)

Quantification of SNX-111 in Plasma Samples from a 14-Day Intravenous Toxicity Study of SNX-111 in the Albino Rat from Study No. 53226 (Study No. 98-5049)
Analysis of Dose Preparation Samples from a 14-Day Intravenous Toxicity Study of SNX-111 in the Albino Rat from Study No. 53226 (Study No. 98-5054)

Analysis of Dose Preparation Samples from a 3-Day Preliminary Evaluation by Continuous Intravenous Infusion of SNX-111 in the Cynomolgus Monkey from Study No. 53269 (Study No. 98-5058)

Quantification of SNX-111 in Cerebrospinal Fluid Samples from a 3-Day Continuous Intravenous Infusion Study of SNX-111 in the Cynomolgus Monkey from Study No. BR 53329 (Study No. 98-5051)

Analysis of Dose Preparation Samples from a 3-Day Continuous Intravenous Infusion Study of SNX-111 in the Cynomolgus Monkey from Study No. 53329 (Study No. 98-5063)

Quantification of SNX-111 in Plasma and Cerebrospinal Fluid Samples from a 14-Day Intravenous Toxicity Study of SNX-111 in the Cynomolgus Monkey from Study No. 53227 (Study No. 98-5050)

Analysis of Dose Preparation Samples from a 14-Day Intravenous Toxicity Study of SNX-111 in the Cynomolgus Monkey from Study No. 53227 (Study No. 98-5060)

Quantification of SNX-111 in Plasma and Cerebrospinal Fluid Samples from a 14-Day Intravenous Toxicity Study of SNX-111 in the Cynomolgus Monkey from Study No. BR 53505 (Study No. 98-5052)

Analysis of Dose Preparation Samples from a 14-Day Intravenous Toxicity Study of SNX-111 in the Cynomolgus Monkey from Study No. 53505 (Study No. 98-5062)

Quantification of SNX-111 in Cerebrospinal Fluid and Serum Samples from a 28-Day Intrathecal Neurotoxicity Study in Male and Female Sprague-Dawley Rats from Study No. 5509-M009-94 (Study No. 98-5053)

A Range-Finding Continuous Intrathecal Infusion Toxicity Study of SNX-111 for up to 14 Days in the Beagle Dog (Study No. 54715)

Addendum to a Range-Finding Continuous Intrathecal Infusion Toxicity Study of SNX-111 for up to 14 Days in the Beagle Dog (Study No. 547189)

Quantification of SNX-111 in Plasma and CSF from a Range-Finding Continuous Intrathecal Infusion Toxicity Study of SNX-111 for up to 14 Days in the Beagle Dog from Study No. 54715 (Study No. 97-5050)

Analysis of Dose Preparation Samples from a Range-Finding Continuous Intrathecal Infusion Toxicity Study of SNX-111 for up to 14 Days in the Beagle Dog from Study No. [REDACTED] 54715 (Study No. 98-5057)

Quantification of SNX-111 in Plasma Samples from a 28-Day Continuous Intrathecal Infusion Toxicity Study of SNX-111 in Beagle Dogs Followed by a Two-Week Recovery Period from Study No. [REDACTED] 54156 (Study No. 97-5051)

Analysis of Dose Preparation Samples from a 28-Day Intrathecal Infusion Toxicity Study of SNX-111 in the Beagle Dog Followed by a Recovery Period of up to Two Weeks from Study No. [REDACTED] 54156 (Study No. 98-5055)

Quantification of SNX-111 in Plasma and Cerebrospinal Fluid Samples from a Study to Evaluate the Effects of SNX-111 Introduced into the Intrathecal Space of Beagle Dogs by 42-Day Continuous Infusion from Study No. 2-P46 (Study No. 98-5047)

Analysis of Dose Preparation Samples from a Study to Evaluate the Effects of SNX-111 Introduced into the Intrathecal Space of Beagle Dogs by 42-Day Continuous Infusion from Study No. 2-P46 (Study No. 98-5067)

Quantification of SNX-111 in Plasma from a 14-Day Epidural Infusion Safety Study of SNX-111 in the Beagle Dog Followed by a Recovery Period of up to Two Weeks from Study No. [REDACTED] 55068 (Study No. 98-5030)

Analysis of Dose Preparation Samples from a 14-Day Epidural Infusion Safety Study of SNX-111 in the Beagle Dog Followed by a Recovery Period of up to Two Weeks from Study No. [REDACTED] 55068 (Study No. 98-5056)

Analysis of Dose Preparation Samples from a Continuous Infusion Teratology Study of SNX-111 in the Rat from Study No. [REDACTED] 95625 (Study No. 98-5061)

Purity of SNX-111 Drug Substance and Drug Product by [REDACTED] Phase High Performance Liquid Chromatography (HPLC) Assay Utilizing [REDACTED] (Testing Procedure No. 3005-00)

Identity, Potency, and Purity of SNX-111 Drug Substance and Drug Product by [REDACTED] -Phase High Performance Liquid Chromatography (HPLC) Assay (Testing Procedure No. 3005-02)

Radioimmunoassay for Determination of SNX-111 Concentrations in Biological Samples (Operating Procedure No. [REDACTED])

NDA 21-060

Validation of a [REDACTED]-Phase HPLC Assay for the Determination of SNX-111 Purity and Potency (Study No. 92-5020)

Validation of a [REDACTED]-Phase HPLC Assay for the Determination of SNX-111 Purity and Potency: Extension of Protocol No. 92-5020 (Study No. 94-5010)

Analytical Method Validation Report for SNX-111 (Study No. C96BM09)
Validation of a Radioimmunoassay for Determination of SNX-111 Concentrations in Plasma Samples (Study No. 92-5021)

Validation of a Radioimmunoassay for Determination of SNX-111 Concentrations in Cerebrospinal Fluid (CSF) (Study No. 93-5027)

Validation of a Radioimmunoassay for Determination of SNX-111 Concentrations in Dog Plasma (Study No. 93-5035)

Validation of a Radioimmunoassay (RIA) for Determination of SNX-111 Concentrations in Dog Plasma and CSF in the Presence of Methionine and Inulin (Study No. 96-5007)

Validation Report Method ICD 85.1: RIA Analysis of Ziconotide in Rat EDTA Plasma (Study No. [REDACTED])

Validation of a Radioimmunoassay for the Quantification of Ziconotide in Dog Plasma Containing EDTA (Study No. [REDACTED])

Identification of Ziconotide (SNX-111) Impurities in the [REDACTED] HPLC Chromatographic Profile (Study No. 98-5040)

Stability of Ziconotide at Various Concentrations in the Medtronic SynchroMed Pump Reservoir (Study No. 98-5087)

D. The following studies involving routes of administration other than intrathecal (IT), studies related to other indication(s), or non-GLP studies related to efficacy in redundant rodent tests of antinociception were not reviewed:

Effects of Intracerebroventricular Administration of SNX-111 on Formalin-Evoked Cutaneous Pain in the Rat (Study No. 94-5009)

Antinociceptive Effects of Intrathecally, Intravenously, and Topically-Delivered Omega-Conopeptides on Tactile Allodynia in a Rat Model of Neuropathic Pain (Study No. 10-20-93)

Effects of SNX-111 and SNX-239 on Mechanical Allodynia in Rats with a Painful Peripheral Neuropathy (Study No. 93-5033)

NDA 21-060

Antinociceptive Effects of Intrathecally Administered SNX-273, SNX-279, and SNX-111 in a Rat Model of Painful Peripheral Neuropathy (Study No. 95-5005)

Anti-Nociceptive Effects of Intrathecally Administered SNX-111 in a Rat Model of Painful Peripheral Neuropathy: Methionine/Lactate Buffer Formulation (Study No. 95-5003)

Antinociceptive Properties of SNX-111 Administered by the Spinal Epidural Route in Rats (Study No. 98-5010)

Effects of Subacute Spinal (Intrathecal) SNX-111 Infusion on Mechanical Allodynia in Rats with a Painful Peripheral Neuropathy (Study No. 94-5002)

Effects of Continuous, Constant-Rate Intravenous and Intrathecal SNX-111 Infusions on the Development of Mechanical and Thermal Allodynia in a Rat Model Painful Peripheral Neuropathy (Study No. 96-5005)

Effects of Subacute Intravenous SNX-111 Infusion on Mechanical Allodynia in a Rat Model of Painful Peripheral Neuropathy (Study No. 96-5001)

Synthetic ω -Conopeptides Applied to the Site of Nerve Injury Suppress Neuropathic Pains in Rats (Study No. 96-0122R1)

Analgesic Potencies of Spinally Administered Pharmacological Agents in Rats with an Experimental Peripheral Neuropathy (Study No. 95-5016)

Antinociceptive Effects of SNX-111 in a Rat Chronic Constriction Injury Model of Peripheral Neuropathy (Study No. 94-5013)

SNX-111, a Selective N-Type Calcium Channel Blocker, Suppresses Primary and Secondary Hyperalgesia in Rat Models of Inflammatory Pain (Study No. 97-5044)
The Effect of Calcium Antagonists on Ocular Irritative Responses (Study No. 97-0139)

Effects of Systemic and Intrathecal Ziconotide (SNX-111) Administration on Mechanical Allodynia and Heat Hyperalgesia in a Rat Model of Post-Operative Pain (Study No. 98-5003)

SNX-111, a Selective N-type Neuronal Calcium Channel Blocker, Reduces Inflammatory Edema in Rat Models of Acute Inflammation (Study No. 96-5023)

A Continuous Intravenous Infusion Range-Finding Teratology Study of SNX-111 in the Rabbit (Study No. 95-95626)

NDA 21-060

Effects of NMDA Receptor Antagonists and Calcium Channel Blockers on Acute Glutamate Toxicity (Study No. B-158'386)

Effect of SNX-111 on Glutamate- or Anoxia-Induced Toxicity in Differentiated Cells (Study No. NC0124)

Neuroprotective Effects of Slow Intravenous Infusion of SNX-111 in the Rat Four-Vessel Occlusion Model of Forebrain Ischemia (Study No. 92-5012)

Neuroprotective Activities of Lots No. 1, No. 2, and No. 3 Manufactured by (Study No. 92-5029)

Neuroprotective Properties of Ziconotide (CmTx) in Gerbils and Rats (Study No. 8009, -8219)

Neuroprotective Effects of SNX-111 in the Gerbil 2-Vessel Occlusion Model and the Rat NMDA-Induced Model of Hippocampal Damage (Study No. 0074R1)

The Effect of Treatment with SNX-111 in Transient Focal Ischemia Followed 1 or 7 Days Reperfusion (Study No. 0057)

Optimal SNX-111 Dosing Strategy for Producing Neuroprotection in a Rat Transient Model (Study No. 97-5010)

Administration of an Omega-Conopeptide One Hour Following Traumatic Brain Injury Reduces ⁴⁵Calcium Accumulation (Study No. 0082)

Effects of SNX-111 Infusion on Behavioral Consequences of Traumatic Brain Injury in Rats (Study No. 97-5013)

Effect of Neuron-Specific Calcium Entry Blocker SNX-111 (Neurex) on Neurologic Outcome after Normothermic Ventricular Fibrillation Cardiac Arrest in Dogs (Study No. 0069R1)

General Progress Report on SNX-111 in Global Cerebral Ischemia in Dogs and Renal Ischemia in Rats (Study No. 0141)

The Neuroprotective Effects of Ziconotide (SNX-111) in a Rat Model of Spinal Ischemia (Study No. 98-5027)

Retina/Degenerative Diseases Report (Study No. 0109)

PHARMACOLOGY

Mechanism of action: Ziconotide is a synthetic version of the naturally-occurring peptide, ω -conotoxin MVIIA, which blocks N-type voltage-dependent calcium ion channels (Olivera *et al.*, 1987). Electrophysiological studies on cultured cells expressing the dominant isoforms of the N-type voltage-dependent calcium ion channels in the central or peripheral nervous systems after transfection with the corresponding cDNA's showed similar subnanomolar sensitivities of the isoforms to inhibition by ziconotide *in vitro*, with IC_{50} s around 0.5 nM (Study No. 98-5083, Vol. 2.004). Several findings suggest that N-type calcium channels are important in spinal sensory processing and transmission of the pain signal.

- Binding studies with an ^{125}I -labeled ω -conopeptide to determine spinal localization of high-affinity sites showed the highest density of binding in the superficial laminae of the dorsal horn, where nerve endings of the primary afferent sensory neurons terminate (Gohil *et al.*, 1994).
- Many of the nerve terminals found to be immunoreactive for N-type calcium channels also contained substance P, an important neuropeptide in pain pathways (Westenbroek *et al.*, 1998).
- The release of calcitonin gene-related peptide (CGRP) and substance P from primary afferent terminals is sensitive to inhibition by ω -conopeptide (Maggi *et al.*, 1990; Santicioli *et al.*, 1992).
- Agents that produce potent antinociception when administered spinally, such as opioids and α_2 -adrenergic agonists, decrease neuronal calcium currents (Anwyl, 1991) and inhibit the spinal release of CGRP and substance P (Pohl *et al.*, 1989; Takano *et al.*, 1993).

Drug Activity Related to Proposed Indication:

Study No.	Species, group size	Test System	Doses, $\mu\text{g}/\text{kg}$	Dosing duration	Outcome
NC 0084	Mouse, 8 males/dose	Formalin, paw flinch & vocalization	0, 0.15, 1.5, 15 & 150	Bolus	Phases 1 (acute) and 2 (persistent) were both suppressed.
93-6008	Rat, 8 males/dose	Formalin, paw flinch	0 and 0.087	Bolus*	Only phase 2 was suppressed.
4-5-93	Rat, 1-10 males/dose	Formalin, paw flinch	0, 0.01, 0.033, 0.1, 1, 33, 100 & 330	Bolus	$ED_{50}=0.036 \mu\text{g}/\text{kg}^*$ for both Phases 1 and 2. Only phase 2 was \downarrow w/ 0.1 $\mu\text{g}/\text{kg}$ 5 hr before or 9 min after formalin.
92-5016	Rat, 3-21 males/dose	Formalin, paw flinch	0, 0.03, 0.09, 0.3 & 0.9	Bolus	$ED_{50}=0.033 \mu\text{g}/\text{kg}^*$ for Phase 2. Neither phase was suppressed with 0.3 $\mu\text{g}/\text{kg}$ when given 2 or 4 hr before formalin.

97-5011	Rat, 8-16 males/dose	Formalin, paw flinch	0 or 0.4	Bolus*	Adding methionine at 0.5 or 5X ziconotide did not affect analgesia.
93-5043	Rat, 6-7 males/dose	Formalin, paw flinch	0, 0.003, 0.01, 0.033, 0.1 & 0.33	72-hour infusion	ED ₅₀ =0.014 µg/hr for Phase 1. ED ₅₀ <0.001 µg/hr for Phase 2.
8-30-94	Rat, 5-7 males/dose	Formalin, paw flinch; Hot plate, limb withdrawal	0, 0.028 or 0.28 µg/kg/hr	2- or 7-day infusion	Produced reversible antinociception w/little or no loss of potency over a 7-day treatment period.
10-20-93	Rat, 3-6 males/dose	Tight spinal nerve ligat', mechanical allodynia	0, 0.2, 6.1 and 20	Bolus	High dose blocked mechanical allodynia
93-5033	Rat, 8-24 males/dose	Tight spinal nerve ligat', mechanical allodynia	0, 0.12, 0.4 and 1.2	Bolus	Mid and high dose produced dose-dependent blockade of mechanical allodynia.
95-5005	Rat, 8 males/dose	Tight spinal nerve ligat'n	0 or 0.33	Bolus	Suppressed mechanical allodynia
95-5003	Rat, 7 males/dose	Tight spinal nerve ligat'n	0 or 0.33	Bolus	Adding methionine did not affect analgesia
94-5002	Rat, 7-8 males/dose	Tight spinal nerve ligat'n	0, 0.0036, 0.036 or .36µg/kg/h	7-Day infusion	Reversible dose-related block of mechanical allodynia w/o tolerance.
96-5005	Rat, 7-10 males/dose	Tight spinal nerve ligat'n	0 or 0.4 µg/kg/hr	14-Day infusion	Reversible block of mechanical and cold allodynia throughout 14-day infusion.
PUB Ref. #90	Rat, 6-16 males/dose	Carrageenan+kaolin-induced knee joint inflammat'n.	0.001-0.1 nM (by spinal microdialysis)	Continuous infusion	Blocked pain behavior when administered 1 hr before or 4 hr after the induction of inflammation
96-5023	Rat, 6 males/dose	Carrageenan+kaolin-induced knee joint inflammat'n.	0 or 1.2	Bolus (0.5 hr before C/K injection)	No effect on joint inflammation as measured by joint diameter.
98-5003	Rat, 5-6 males/dose	Incisional pain model, heat hyper-	0, 0.09, 0.3 or 0.9	Bolus (day after surgery)	ED ₅₀ =0.3 µg/hr for blocking heat hyperalgesia. No effect on

		algnesia and mechanical allodynia			withdrawal response to heat stimulation of the contralateral hind paw.
96-019	Dog	Thermally-evoked skin-twitch response	10 µg ----- 1 and 5 µg/hr	Bolus ----- 48-Hour infusion	No effect on skin-twitch response by bolus. Complete blockade at 24-48 hr during infusion.

* Intrathecal bolus administered 10 minutes prior to the formalin injection.

The effects of ziconotide administered either IT (0.003, 0.03, 0.3 or 3.0 µg/mouse) or ICV (0.0001, 0.0003 or 0.001 µg/mouse) were studied in a non-GLP study (Study No. NC0084, Effects of SNX-111 Administered Intrathecally and Intracerebroventricularly on the Formalin Test in BALB/c Mice). Antinociception employing flinching behavior and licking behavior in two phases (phase 1 = 0-10 minutes; phase 2 = 10-60 minutes) immediately following the injection of 20 µl of 5% formalin into the right hind paws of adult male BALB/c mice was studied in comparison with an IT or ICV injection of saline. Ziconotide showed dose-related antinociceptive activity for both parameters in both phases by both routes of administration, but appeared to be around 40-300 times more potent after ICV than IT administration. Although the report did not provide %MPE data from individual animals or treatment groups, nor provide a calculation of the ED₅₀s, visual inspection of the graphs indicates the following estimated antinociceptive potencies (in µg/mouse) of ziconotide for the IT route:

- Phase 1, flinching: ED₅₀ = ~0.03
- Phase 1, licking: 0.003 < ED₅₀ < 0.03
- Phase 2, flinching: 0.03 < ED₅₀ < 0.3
- Phase 2, licking: 0.003 < ED₅₀ < 0.03

Acute IT administration to rats of ω-cono-peptides, produced a potent and dose-dependent antinociception (ED₅₀ = 3 fmol for ziconotide) in the formalin test (Malmberg and Yaksh, 1994). In this non-GLP study, the formalin test (50 µl of 5% formalin injected into the dorsal surface of the right hind paw) was conducted in male Sprague-Dawley rats, ~~weighing 275-325 grams~~ weighing 275-325 grams and surgically prepared 5-7 days before use with spinal indwelling cannulas for making lumbar IT injections (Study No. 4-5-93, Antinociceptive Effects of Intrathecally Administered Omega-Cono-peptides on the Formalin Test in the Rat, Vol. 2.006). Ziconotide doses of 0 (control), 0.003, 0.01 and 0.03 µg/rat, administered intrathecally 10 minutes before the formalin, were tested for effects on number of flinches occurring during Phase 1 (0-9 minutes after arousal from anesthesia with 3% halothane) and during Phase 2 (10-60 minutes). ED₅₀s were calculated on the basis of a 50% reduction of the responses exhibited by the controls, although the study report did not show data for the control responses for this part of the study. The ED₅₀s (with 95% confidence limits) of ziconotide for phases 1 and 2 were reported to be 0.011 (0.005-0.022) and 0.011

(0.007–0.015) µg/rat, respectively. Additional experiments were conducted with the ziconotide dose of 0.03 µg/rat, given at different times relative to the formalin injection: 24 hours prior, 5 hours prior, 10 minutes prior and 9 minutes after the formalin injection.

Time of ziconotide injection, 0.03 µg/rat	Mean (± S.D.) Number of Flinches during Phase		n
	Phase 1 – (% of control)	Phase 2 – (% of control)	
–24 hours	15.0 ± 0.8 (85.2%)	115 ± 22.1 (93.5%)	4
–5 hours	11.0 ± 3.8 (62.5%)	37.5 ± 30.9 (30.5%)	4
–10 minutes	7.5 ± 1.9 (42.6%)	31.3 ± 30.2 (25.4%)	6
9 min. after formalin	15.5 ± 2.1 (88.1%)	85.3 ± 32.0 (69.3%)	6
Control values	17.6 ± 2.5	123 ± 15.5	8

Administration of ziconotide 10 minutes and 5 hours before and 9 minutes after, but not 24 hours before, formalin injection caused significant suppression of phase 2, although a significantly lesser effect was observed for post-formalin than pre-formalin (10 minutes and 5 hours) ziconotide treatment.

In a similar non-GLP study, the formalin test (50 µl of 5% formalin injected into the dorsal surface of the right hind paw) was conducted in male Sprague-Dawley rats weighing 325-375 grams and implanted with IT spinal catheters that were filled with sterile saline 3-9 days prior to testing [Study No. 92-5016, Analgesic Effects of Spinally Administered (Intrathecal) SNX-111 on the Formalin-evoked Cutaneous Pain in the Rat: Dose-response Characteristics and Duration of Action, Vol. 2.006). Ziconotide doses of 0 (control), 0.01, 0.03, 0.1 and 0.3 µg/rat, administered ITly 10 minutes before the formalin, were tested for effects on number of flinches occurring during Phase 1 (0-9 minutes after arousal from anesthesia with 2% halothane) and during Phase 2 (10-90 minutes). Two additional groups were given 0.1 µg/rat, IT, either 2 hours or 4 hours before the formalin test. For the dose-response study, ED₅₀s and 95% confidence limits were determined by fitting the dose-response data to a 4-parameter logistic function using a non-linear, least squares curve fitting algorithm written in BASIC for a Hewlett-Packard 9000, series 300 computer. Phase 1 responses were not consistently affected. The ED₅₀ (with 95% confidence limits) of ziconotide for phase 2 was reported to be 0.11 (0.055–0.19) µg/rat. ANOVA of the group differences in phase 2, with subsequent analysis by Student's t-test indicated significantly lower flinch counts for the rats receiving 0.1 µg (p=0.052) or 0.3 µg (p=0.01).

Time of ziconotide injection, 0.1 µg/rat	Mean (± S.D.) Number of Flinches during Phase		n
	Phase 1 – (% of control)	Phase 2 – (% of control)	
–4 hours	8.3 ± 4.8 (64.8%)	137 ± 45.5 (91.3%)	9
–2 hours	6.4 ± 2.8 (50.0%)	98.0 ± 61.5 (65.3%)	5
–10 minutes	13.0 ± 12.4 (102%)	87.5 ± 63.2 (58.3%)	10
Control values	12.8 ± 10.8	150 ± 84.7	21

Unlike the previous study, in which IT ziconotide (0.03 µg/rat) given 5 hours before formalin reduced the number of flinches to 30% of the controls for phase 2, the present study with a higher dose (0.1 µg/rat) given 4 or 2 hours before formalin failed to cause a significant reduction at –2 hours (p=0.22) or –4 hours (p=0.64), although the data at –2

hours may suffer from the smaller number of rats tested for that time of pre-treatment than for the other time periods.

Another acute non-GLP study employing the formalin test (50 μ l of 5% formalin injected into the dorsal surface of the right hind paw) was conducted in male Sprague-Dawley rats weighing 325-375 grams, except that the single IT dose of ziconotide used was, 0.03 μ g/rat [Study No. 93-6008, Analgesic Effects of Spinally-administered (Intrathecal) SNX-111 on Formalin-evoked Cutaneous Pain in the Rat, Vol. 2.005]. Numbers of flinches (mean \pm S.D.) during Phase 1 (first 10 minutes after arousal from anesthesia with 2% halothane) and during Phase 2 (10-90 minutes) in the ziconotide-treated group (n=15) were compared with those in rats given IT saline (n=11), as shown below:

- Phase 1, saline control: 10 \pm 6 flinches
- Phase 1, ziconotide, 0.03 μ g: 14 \pm 7 flinches
- Phase 2, saline control: 137 \pm 70 flinches
- Phase 2, ziconotide, 0.03 μ g: 63 \pm 43 flinches

Ziconotide did not significantly affect the number of flinches in Phase 1 (p=0.108), and although ziconotide appeared to significantly decrease the number of flinches in Phase 2 (p=0.0028), the t-test used by this reviewer indicated that the test was not valid because of significantly unequal variance (p=0.04) within the two groups compared.

The effect of adding methionine (50 or 500 ng) to ziconotide (100 ng) for IT administration in sterile, preservative-free 0.9% sodium chloride was tested in a non-GLP single-dose study employing the formalin test in male Sprague-Dawley rats weighing 220-280 grams ~~_____~~ seven days after surgical implantation of spinal indwelling cannulas for making lumbar IT injections. The 5% formalin solution (μ l) was injected into right hind paw 10 minutes after the IT bolus injection of 0.9% saline vehicle (10 μ l, n=16), ziconotide (100 ng, n=16), methionine (500 ng, n=8), ziconotide + methionine (100 + 50 ng, n=16) or ziconotide + methionine (100 + 50 ng, n=16). It was reported that neither ziconotide (100 ng), methionine (500 ng), nor the combination of ziconotide (100 ng) and methionine (500 ng) inhibited flinch responses in phase 1 of the formalin test. In phase 2 (10-90 minutes after formalin), however, ziconotide (100 ng) significantly decreased the number of flinch responses by 47% (p<0.05) and methionine (50 or 500 ng) did not alter the ziconotide-induced inhibition when co-administered with ziconotide (Study No. 97-5011, Effects of Intrathecal Co-administration of Methionine and SNX-111 on Acute and Persistent Pain Behavior Evoked by Subcutaneous Injection of Dilute Formalin into the Hind Paw of the Rat, Vol. 2.006).

In a subacute non-GLP study, the formalin test was conducted in male Sprague-Dawley rats ~~_____~~ weighing approximately 300-325 grams (individual animal data not given) and surgically prepared several days before use with spinal indwelling cannulas for making lumbar IT injections (Study No. 93-5043, Effects of Continuous Constant-rate Spinal (Intrathecal) Infusion of SNX-111 on Formalin-evoked Cutaneous Pain in the Rat: Dose-Response Relationship Vol. 2.006). Ziconotide doses of 0 (control), 0.001, 0.003, 0.01, 0.03 and 0.1 μ g/hr were infused for

72 hours before the injection of formalin (50 μ l of 5% formalin injected into the dorsal surface of the right hind paw) were tested for effects on number of flinches occurring during Phase 1 (0-9 minutes) and during Phase 2 (10-90 minutes). ED₅₀s were calculated on the basis of a 50% reduction from the maximal values. Dose-response data for the phase 1 and phase 2 responses to formalin are shown in the table below:

Dose of Ziconotide (μ g/hr, IT, for 72 hr)	N	Mean (\pm S.D.) Number of Flinches during Phase			
		Phase 1	p-Value	Phase 2	p-Value
0 (saline)	7	13.9 \pm 4.2		171.6 \pm 27.2	
0.001	7	9.6 \pm 3.0	0.423	83.1 \pm 23.9	0.0309
0.003	6	11.3 \pm 2.4	0.630	60.3 \pm 24.7	0.0125
0.01	7	6.7 \pm 1.3	0.132	28.7 \pm 21.4	0.001
0.03	7	5.9 \pm 1.9	0.109	23.3 \pm 8.6	0.0002
0.1	6	3.0 \pm 0.8	0.034	15.5 \pm 5.9	0.0003

The ED₅₀s of ziconotide in rats for phases 1 and 2 of the formalin test were reported to be 0.014 and <0.001 μ g/hour, respectively, at 72 hours after the start of continuous infusion.

Chronic IT infusion of rats with ziconotide (0.003 or 0.03 nmol/hr) produced a potent antinociceptive effect in both the hot plate (52.5°C) and formalin tests, with less tolerance development over 7 days than the loss of antinociceptive effect of morphine (20 nmol/hr) over the same period of time (Malmberg and Yaksh, 1995; Study No. 8-30-94, Examination of the Effects of Chronic Spinal Delivery of ω -Conopeptides on Behavior and Antinociception on the Formalin and Hot Plate Test in Rats, Vol. 2.006).

Treatment (μ g/hr, IT)	N	Mean (\pm SEM) Number of Flinches in Formalin Test			
		Phase 1		Phase 2	
		Day 2	Day 7	Day 2	Day 7
Saline (1 μ l/hr)	6	16.0 \pm 1.0	16.7 \pm 0.8	130 \pm 8.1	142 \pm 11.4
Ziconotide (0.01)	6	9.2 \pm 1.5	13.3 \pm 1.0	74.3 \pm 11.3	58.0 \pm 15.5
Ziconotide (0.1)	6	6.2 \pm 0.7	9.5 \pm 1.2	18.2 \pm 2.0	19.7 \pm 7.6
Morphine (5.7 μ g/hr)	6	5.0 \pm 0.9	17.3 \pm 1.1	44.0 \pm 11.9	137 \pm 11.1
		Day 9		Day 9	
48 hours after 7 days of ziconotide (0.1)	6	Data Missing from Final Study Report		Data Missing from Final Study Report	

Treatment (μ g/hr, IT)	Mean (\pm S.D.) Hot Plate Response Latency (sec)		
	Day 2	Day 7	Day 9 (48-hr recovery)
Saline (1 μ l/hr)	13.9 \pm 1.4 (6)	11.6 \pm 1.2 (6)	
Ziconotide (0.01)	42.8 \pm 13.3 (6)	27.7 \pm 9.0* (6)	
Ziconotide (0.1)	51.4 \pm 10.6 (6)	35.3 \pm 9.3* (5)	17.6 \pm 3.8
Morphine (5.7 μ g/hr)	36.6 \pm 7.1 (6)	13.6 \pm 2.2 ^a (6)	

* P<0.05, compared with the Day 2 values (t-test for unpaired data).

^a T-test for unpaired data not valid due to unequal variance.

As expected, the continuous IT infusion of morphine led to marked tolerance development in both the formalin and hot plate tests. The response to ziconotide at both doses also was significantly reduced on Day 7 compared with Day 2, but not reduced to the same extent as the response to morphine. It was not determined whether the diminished response to ziconotide was due to tolerance development or to deterioration of the peptide in the subcutaneous — minipump — reservoir.

Ancillary Pharmacology Studies: The ability of ziconotide to release histamine from mast cells was tested *in vitro* using a broad range of concentrations incubated with rat connective tissue mast cells or human foreskin mast cells for 30 minutes at 37°C (Study No. 93-6002, In Vitro Release of Histamine by SNX-111, Vol. 2.005). The results indicate that ziconotide can release histamine from mast cells in a concentration-dependent manner and is much more potent with human foreskin mast cells ($EC_{50} = 0.14 \mu\text{g/ml}$) than with rat connective tissue mast cells ($EC_{50} = 37 \mu\text{g/ml}$), although the amount of histamine released by ziconotide was less than that released by a positive control agent, as shown in the table below:

Releasing Agent	Concentration, $\mu\text{g/ml}$	Mean (\pm S.D.) % Specific Histamine Release	
		Rat Mast Cell	Human Mast Cell
Control Buffer	—	5.2 \pm 1.7	10.4 \pm 1.1
Ziconotide	0.02	6.9 \pm 7.5	9.3 \pm 1.8
	0.2	5.4 \pm 0.5	21.3 \pm 0.5
	2	10.2 \pm 8.3	23.2 \pm 15.6
	20	24.4 \pm 3.7	25.0 \pm 6.9
	200	45.9 \pm 4.5	33.1 \pm 8.8
—	1	80.6 \pm 1.6	Not tested
—	1	Not tested	51.0 \pm 1.3

Summary of Pharmacology: Ziconotide is a synthetic version of ω -conotoxin MVIIA, a naturally-occurring peptide toxin found in a marine snail (*Conus magus*), which potently blocks N-type voltage-dependent calcium ion channels in neurons at sub-nanomolar concentrations. It is thought to produce analgesia by inhibiting the spinal release of neurotransmitters from primary afferent terminals which are involved in neuronal pain-signaling pathways. Efficacy studies of antinociceptive activity after IT administration were conducted (mostly in rats) using the formalin test; suppression of mechanical allodynia and/or heat hypersensitivity following tight spinal nerve ligation, in an incisional pain model, or UV burn model of inflammatory pain; the hot-plate test; the tail-immersion test; the paw-pressure test; carrageenan plus kaolin-induced knee joint inflammation, and a thermally-evoked skin-twitch test (in dogs). In the formalin test in rats, IT ziconotide did not reliably or reproducibly affect the acute phase (Phase 1) of this test (e.g., compare studies 93-6008 and 4-5-93) but significantly reduced paw flinches in the persistent phase (Phase 2) when administered by bolus within 10 minutes of the formalin or when administered by infusion. Ziconotide had ED_{50} s on the

order of 0.033-0.036 $\mu\text{g}/\text{kg}$ by IT bolus and $<0.003 \mu\text{g}/\text{kg}/\text{hr}$ by continuous IT infusion in suppressing Phase 2 of the formalin test. There is some disagreement regarding whether Phase 2 is suppressed when a bolus is given 2-5 hours before the formalin (compare studies 4-5-93 and 92-5016). It should be noted that several analgesic NSAIDS (e.g., aspirin, indomethacin, flurbiprofen, ibuprofen, acetaminophen) cause significant antinociception in the rat formalin test, after either IP (both phases) or IT (phase 2) bolus administration (Malmberg and Yaksh, 1992). Several studies also used rats with tight ligation of L5 and L6 spinal nerves as a model of peripheral neuropathy (Kim and Chung, 1992). IT ziconotide produced significant suppression of mechanical allodynia in this model at bolus doses of 0.33-0.4 $\mu\text{g}/\text{kg}$ (and higher), and at infusion rates of 0.036 $\mu\text{g}/\text{kg}/\text{hr}$ (and higher), whereas Lashbrook *et al.* (1999) reported that IT morphine, ketorolac or piroxicam were without significant antiallodynic effect in this model when administered as single agents. Intrathecal ziconotide was active in the 52.5°C hot-plate test in rats after both bolus (0.03 μg) and infusion (0.028 and 0.28 $\mu\text{g}/\text{kg}/\text{hr}$), but had both greater efficacy and longer duration with the infusion mode. An infusion dose that was active in the hot-plate test (0.03 $\mu\text{g}/\text{hr}$) was not active in the tail-immersion (50°C hot water) test, and larger bolus IT doses that were active in the tail-immersion test also produced tremor. Shaking behavior also was observed to be coincidental with robust antinociception after IT ziconotide (0.3-1.0 μg) in the rat paw pressure test. Local infusion of ziconotide (1.2-11.4 $\mu\text{g}/\text{hr}$) directly into the spinal cord through microdialysis of the dorsal horn delayed the radiant heat-evoked paw-withdrawal response in rats when the infusion was started 1 hour prior to, or 4 hours following, the induction of acute inflammation of the ipsilateral knee joint by the intra-articular injection of carrageenan plus kaolin (3% each). In general, ziconotide exhibits antinociceptive activity in a variety of pain models in which NSAIDS and/or morphine are also antinociceptive. In pain models that separate weak analgesics (NSAIDS) from strong analgesics (e.g., morphine), there is not a good separation of strong antinociceptive activity and the elicitation of shaking behavior by IT ziconotide, both of which occur at around 0.3 $\mu\text{g}/\text{rat}$ by bolus injection or 0.1 $\mu\text{g}/\text{hr}/\text{rat}$ by continuous infusion.

SAFETY PHARMACOLOGY

Neurological effects: In study no. 94-5001 (see Gastrointestinal effects below) mice were given acute IT injections of ziconotide in doses ranging from 0.03-1.0 μg in a 5- μl volume (free-hand) under halothane anesthesia. It was reported that, following recovery from anesthesia, the lowest dose "evoked serpentine tail movements and intermittent shaking, predominantly involving the hind legs and tail. Higher doses produced whole-body shaking, which increased in severity and duration as the dose was increased." In another non-GLP study (Study No. 98-5043, Tremorgenic Potencies of ω -Conopeptides in the Mouse, Vol. 2.005), attempts to determine the ED_{50} for ziconotide-induced shaking behavior in male Swiss Webster mice weighing 11-15 grams after ICV administration were made. This ED_{50} was defined as the dose that

caused 50% of the mice to exhibit any shaking behavior. These experiments resulted in four ED₅₀ estimates ranging from 0.002 to 0.06 µg/mouse (mean = 0.03 µg/mouse). A non-GLP study of the effect of diazepam (0.3, 1.0, 3.2 or 10 mg/kg, IP) administered 15 minutes before a tremorigenic ICV dose (0.1 µg) of ziconotide in 70-90% of control mice indicated some reduction in the incidence of tremor, but the decrease was unrelated to the dose of diazepam, and at least 40% of the mice in each diazepam-pretreated group still showed tremors (Study No. 98-5045, Effect of Diazepam on SNX-111 Induced Shaking Behavior in the Mouse, Vol. 2.005).

Shaking behavior was also studied in rats after the ICV administration of 1 to 3-µg doses of ziconotide (Study No. 98-5044, not reviewed) and was quantified in conscious male Sprague-Dawley rats weighing 250-280 grams previously cannulated for making lumbar IT injections and also acutely cannulated in the femoral artery for monitoring systemic blood pressure (Study No. 93-6007, Effects of Spinally-administered SNX-111 and SNX-239 on Systemic Blood Pressure and Motor Behavior in the Rat, Vol. 2.005). At 90 minutes following the acute IT administration of ziconotide (0.1, 0.3, 3.0 and 10 µg/rat), shaking behavior was scored according to the following criteria:

Score	Criteria
0	No effect.
1	No spontaneous tremor; tail is stiff; slight head-bobbing when handled.
1.5	No spontaneous tremor; slight tremor of head and body when handled.
2	No spontaneous tremor; tremor is clearly discernible upon touch or handling and continues for a few seconds after handling.
2.5	Spontaneous tremor, but only when animal attempts to move.
3	Continuous, uncontrollable shaking in the absence of gross body movements.
3.5	Continuous, uncontrollable shaking; splayed limbs; stereotypical "burrowing".

The results with ziconotide are shown in the following table:

Dose, µg/rat, IT	No. rats/group	Mean (± S.D.) Tremor Score
0.1	5	0.60 ± 0.55
0.3	5	1.40 ± 0.82
1.0	6	2.08 ± 1.16
10	5	3.10 ± 0.22

Thus, the IT administration of ziconotide (0.1-10 µg/rat) caused a dose-related increase in shaking behavior.

Cardiovascular effects: In a non-GLP study, the cardiovascular effects of ziconotide by various routes of administration (IV, IP, SC and oral) were investigated in male Sprague-Dawley rats fitted with a catheter in a femoral artery to measure systemic blood pressure and heart rate. Ziconotide administered by the IV, IP, SC and oral routes had no effect on heart rate. Blood pressure was decreased with similar potency when ziconotide was injected by the IV (ED₅₀ = 0.48 mg/kg), IP (ED₅₀ = 0.36 mg/kg) or SC (ED₅₀ = 0.39 mg/kg) routes. However, the maximum decrease was greater after IV

($E_{max} = -70$ mm Hg) than after IP ($E_{max} = -48$ mm Hg) or SC ($E_{max} = -43$ mm Hg) administration (Study No. 98-5038, Cardiovascular Effects of Ziconotide in Rats when Administered by a Variety of Routes, Vol. 2.005).

In a literature report, the acute IV administration of ziconotide to rats 0.87-2,900 nmol/kg caused a biphasic decrease in mean arterial blood pressure, with ED_{50} values for the two components being 3.5 and 2,800 nmol/kg and the first component accounting for approximately 25% of the reduction. The second component occurring at the higher doses (870 and 2,900 nmol/kg) was attenuated by pretreatment of rats with a combination of cimetidine (5 mg/kg) and chlorpheniramine (2.5 mg/kg), indicating the possible role of histamine release in the second component of the hypotensive effect of ziconotide (Bowersox *et al.*, 1992). Unlike reserpine, which decreased rat heart catecholamines by 96% in 24 hours, ziconotide (15 mg/kg) failed to show an effect on heart catecholamines at 72 hours after its IV administration. Thus, the sympatholytic effect (e.g., hypotension) of intravenously administered ziconotide reported by Bowersox *et al.* (1992) is not due to catecholamine depletion in sympathetic neurons (Study No. 98-5036, Vol. 2.049).

In a non-GLP study in rats that also involved the quantification of shaking behavior (Study No. 93-6007), systemic blood pressure was monitored from a cannulated femoral artery before (baseline) and 30, 60 and 90 minutes after the acute IT administration of ziconotide, as shown in the table below:

Dose, $\mu\text{g}/\text{rat}$, IT	Mean (\pm S.D.) Blood Pressure, mm Hg			
	Baseline	30 minutes	60 minutes	90 minutes
0.1	129 \pm 4	134 \pm 5	136 \pm 5	133 \pm 5
0.3	133 \pm 6	136 \pm 8	137 \pm 8	137 \pm 8
3	126 \pm 12	130 \pm 10	130 \pm 11	128 \pm 11
10	138 \pm 8	124 \pm 6	126 \pm 9	128 \pm 10

No significant effects on systemic blood pressure were observed up to 90 minutes after the IT injection of rats with ziconotide in doses up to 10 $\mu\text{g}/\text{rat}$.

In another non-GLP study (Study No. 98-5013, Effects of Intrathecal SNX-111 on Spinal Clonidine-induced Depressor and Bradycardia Responses in Conscious Rats, Vol. 2.010) to investigate the interactions between IT ziconotide and IT clonidine in rats, a 0.3 μg dose of ziconotide, IT, was followed 10 minutes later by a 3- μg dose of clonidine, IT. Ten minutes after ziconotide, mean (\pm S.E.) arterial blood pressure (baseline, 125 \pm 2 mm Hg) was unaffected (127 \pm 2 mm Hg). The same was found for heart rate (baseline, 399 \pm 11 bpm *versus* 422 \pm 14 at 10 minutes). It was also reported that "ziconotide pretreatment did not significantly alter IT clonidine-induced hypotension and bradycardia," although it is not clear from the figure presented in the study report that the dose of clonidine used (3 μg) caused a significant decrease in these parameters from the baseline values in the saline-pretreated rats. Data from individual animals was not provided in this report.

Pulmonary effects: In a non-GLP study (Study No. 94-5019, Effects of SNX-111 on Morphine-induced Respiratory Depression: Acute Morphine Administration, Vol. 2.010), the effects of IT ziconotide (0.1 µg) and SC morphine (10 and 30 mg/kg), alone and in combination, on the maximum respiratory response to a 10% carbon dioxide in air atmosphere were studied in awake, unrestrained rats before, and 30, 60, 90 and 120 minutes after administration, using a whole-body plethysmographic method to measure respiratory minute volume. Maximum minute volume responses to CO₂ (mean of last 6 minute volume values of CO₂ challenge, except for 3 values for the 30 mg/kg dose of morphine) are summarized in the following table:

Treatment		No. of Rats	% of Baseline max. Min. Vol. Response to CO ₂			
Intrathecal	SC (mg/kg)		30 min	60 min	90 min	120 min
Saline	Saline	4	105.1	96.2	90.8	90.3
SNX-0.1 µg	Saline	4	102.6	101.7	95.8	97.1
Saline	Morphine-10	4	60.8	59.4	67.5	103.9
SNX-0.1 µg	Morphine-10	4	71.8	71.7	93.0	109.8
Saline	Morphine-30	2	63.2	57.7	53.9	68.8
SNX-0.1 µg	Morphine-30	2	69.9	58.9	60.9	59.9

Ziconotide (0.1 µg/rat, IT bolus) had no respiratory depressant effects alone and did not exacerbate the respiratory depressant effects of subcutaneous morphine in rats during exposure to a 10% CO₂ respiratory stimulus. Rather, ziconotide appeared to attenuate the maximum and duration of the respiratory depressant effect of an acute 10 mg/kg SC dose of morphine over a 2-hour period.

A similar non-GLP study was conducted using rats pretreated twice/day for 7 days with saline or morphine (10 mg/kg, SC) and given ziconotide (0.1 µg, IT) 30 minutes before SC morphine on Day 7 (Study No. 95-5009, Effects of SNX-111 on Morphine-induced Respiratory Depression in Morphine-tolerant Rats). Rats receiving twice daily morphine injections exhibited tolerance to the respiratory depressant effect of morphine, as tested during exposure to a 10% CO₂ respiratory stimulus, by treatment Day 5. The IT administration of ziconotide on Day 7 did not depress the respiratory response to a 10% CO₂ respiratory stimulus in either the saline-treated or the morphine-tolerant rats, confirming that ziconotide does not depress respiration itself and indicating that it did not reverse the state of tolerance to morphine-induced respiratory depression.

Renal effects: No specific renal studies were conducted.

Gastrointestinal effects: In a non-GLP study conducted in male Swiss Webster mice fasted overnight and weighing 20-25 grams (n=10/group), the effects of IT ziconotide, without and with systemic morphine sulfate (3 mg/kg, SC), were studied on the transit of a charcoal meal over a period of 30 minutes. Intrathecal injections at the L5-L6 spinal level were done under anesthesia with halothane, and approximately 15 minutes later, the mice received an SC injection of saline or morphine sulfate and the oral gavage of a

charcoal meal (0.2 ml of charcoal, flour and water in a 1:2:6 ratio) and then were euthanized with CO₂ inhalation 30 minutes after the charcoal to determine the distance it traveled in the intestine. In saline-treated control mice, the charcoal traveled a mean of 59% of the length of the intestine. This was shortened by ziconotide (0.03, 0.1, 0.3 and 1.0 µg, IT) in a dose-related manner to 45%, 38%, 29% and 21%, respectively, whereas morphine (3 mg/kg, SC) shortened the distance to 17% in mice given IT saline. In mice given ziconotide (1.0 µg, IT) plus morphine (3 mg/kg, SC), the distance traveled appeared to be only about 13% (estimated from a graph) which was not significantly different from the 17% length in mice given morphine alone. The ED₅₀ of ziconotide for inhibiting gastrointestinal transit after IT administration was estimated to be 0.19 µg/mouse.

In another non-GLP study, the IT administration of ziconotide to rats at doses of 1 and 10 µg/rat inhibited GI transit, whereas doses above that were necessary for inhibition of GI transit by the IV route. The IT administration of a NOEL dose for GI transit inhibition (but active for antinociception), 0.3 µg/rat, caused approximately a 4-fold shift to the left in the log dose-response curve for morphine administered SC. Thus, inhibition of GI transit by IT ziconotide occurs by a non-systemic mechanism and at supra-analgesic doses, but an IT analgesic dose of ziconotide can potentiate the potency of systemic morphine for inhibiting GI transit in the rat (Study No. 94-5001, Effects of SNX-111 Alone and in Combination with Morphine on Gastrointestinal Transit in the Mouse, Vol. 2.010).

Abuse liability: In a series of non-GLP but standardized battery of *in vivo* and *in vitro* studies, ziconotide was evaluated for dependence and abuse liability within the analgesic drug testing program of the Drug Evaluation Committee, which operates under the auspices of the College on Problems of Drug Dependence [Study No. 0120, Ziconotide (SNX-111): Determination of Physical Dependence Potential and Abuse Liability, Volume 2.010]. In binding assays with labeled ligands of opioid *mu* (DAMGO, 1 nM), *delta* (p-CI-DPDPE, 1 nM) and *kappa* (U-69,593, 1.5 nM) receptors in monkey cortical membranes using ziconotide concentrations up to 6 µM, this concentration inhibited ligand binding to *mu* and *kappa* receptors by <2% and the K_i for inhibition of binding to *delta* receptors was quite weak at 1.19 µM. Although ziconotide was a potent inhibitor in the electrically-stimulated mouse vas deferens assay (ED₅₀, 4.78±0.58 nM), its potency was not affected by the presence of 100 nM naltrexone (ED₅₀, 5.02±0.47 nM). In the mouse tail-flick and p-phenylquinone abdominal constriction tests for antinociception, IV ziconotide had ED₅₀s of 17.7 and 3.16 mg/kg, respectively. During the tail-flick tests, ptosis and clonic convulsions were observed with IV doses of 20 and 30 mg/kg. Administered IV in single doses of 0.25, 1 or 2 mg/kg, ziconotide partially attenuated withdrawal signs in morphine-dependent monkeys during abstinence in a somewhat dose-related manner, except that the 2-mg/kg dose was not more effective than a 1 mg/kg dose, and both doses were less effective than morphine. This partial attenuation is likely due to a sympatholytic rather than opioid-like effect of ziconotide, as demonstrated in the mouse vas deferens assay. When administered to 3 morphine-dependent monkeys trained to discriminate saline

from naltrexone, ziconotide in IV doses of 0.0032, 0.01, and 0.032 mg/kg in place of the next-scheduled morphine administration produced relatively little saline-appropriate responding, indicating the lack of activity of ziconotide in attenuating the discrimination of morphine withdrawal. In self-administration studies with 3 morphine-dependent monkeys, ziconotide in IV doses over the range of 1 ng/kg to 0.01 mg/kg did not produce any effects different from saline. Thus, ziconotide does not appear to have physical dependence potential or abuse liability of the opioid type.

Conclusions:

- The spinal action of ziconotide is not specific for nociceptive pathways, as it causes signs of motor dysfunction (shaking behavior) at or slightly above significantly antinociceptive doses in some rodent tests.
- The studies of the interaction of ziconotide with the respiratory depressant effects of morphine in rats do not assess potential respiratory depressant effects of ziconotide (i.e., at higher doses), and therefore, do not comprehensively assess possible interactions with morphine-induced respiratory depression.
- It is not known whether ziconotide has any effects on renal function, but a dose-related increase in BUN was observed in a 28-day toxicology study in dogs.
- Ziconotide inhibits GI transit and markedly potentiates the effect of morphine.
- Ziconotide does not have physical dependence or abuse liability of the opioid type.

Summary: The main neurological effect of acute IT injection of ziconotide is shaking behavior, with a mean quantal ED₅₀ in mice of 0.03 µg/mouse, and a range of signs increasing intensity in rats over the dosage range of 0.1-10 µg/rat – a range including doses (≤0.3 µg/rat) required to produce significant antinociception in some tests. No significant effects on systemic blood pressure were observed up to 90 minutes after the IT injection of rats with ziconotide in doses up to 10 µg/rat. A non-respiratory depressant dose of ziconotide (0.1 µg/rat, IT bolus) did not potentiate a respiratory depressant effect of morphine (10 and 30 mg/kg, SC bolus) in rats and did not have a respiratory depressant effect in rats tolerant to the respiratory depressant effects of morphine, whereas a dose of ziconotide (0.3 µg/rat) not inhibitory for GI transit, caused approximately a 4-fold shift to the left in the log dose-response curve for SC morphine-induced inhibition of GI transit. Although like morphine, ziconotide has inhibitory effects on mouse vas deferens (ED₅₀, ~5 nM), intestinal smooth muscle and GI transit (ED₅₀, 0.19 µg/mouse), ziconotide does not have physical dependence or abuse liability of the opioid type.

PHARMACOKINETICS/TOXICOKINETICS

PK parameters:

Study Title: A Pharmacokinetic Study with Test Article SNX-111 Administered by 24-Hour Intravenous Infusion to Rats

Study No: 309-92

Vol # 009, Page #s 002-046

Conducting laboratory and location: _____

Date of study initiation: October 9, 1992

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Methods:

Dosing:

- **species/strain:** Rat / Sprague-Dawley _____
- **#/sex/group or time point:** 5
- **age:** 7-11 weeks
- **weight:** 223-275 g males and 170-203 g females
- **dosage groups in administered units:** 10 and 40 mg/kg/day
- **route, form, volume, and infusion rate:** Continuous intravenous infusion at 2 ml/kg/hr for 24 hours, at 0.21 and 0.83 mg/ml

Drug, lot# 8068SJJ004, % purity: _____

Formulation/vehicle: in 0.9% Sodium Chloride for Injection U.S.P. _____

Observations and times: Mortalities, clinical signs and body weights daily for 3 days. 0.5 ml blood samples collected via jugular catheter or orbital sinus at following times after start of infusion: Groups 1 and 2 (10 and 40 mg/kg/d): 0, 0.25, 1, 2, 6, 12 and 24 hours; Groups 3 and 4 (10 and 40 mg/kg/d): 0, 5, 15, 30., 60, 120 and 180 minutes; Groups 5 and 6 (10 and 40 mg/kg/d): 0, 3, 6, 8, 10, 12 and 18 hours.

Results:

Mortalities: 12 deaths: 2/15 males and 2/15 females at 10 mg/kg/d (at 18 hours after the end of infusion), 8/15 females at 40 mg/kg/d (1 at 6 hours, 1 at 12 hours and 1 at 24 hours after start of infusion; 5 at up to 18 hours after the end of infusion).

Clinical signs:

Tremors in 5/30 (17%) low dose and nearly all 30 high dose rats (93%) on day 1, in 2/30 low dose and 2/30 high dose rats on day 2 and 1/30 high dose rat on day 3.

Loss of skin elasticity (indicating dehydration) in 1 low dose rat on days 1 and 2.

Loss of coordination in 1 low dose rat on day 2 and 1 high dose rat on days 1 and 2.

Cold skin and palleness in 1 low dose rat on day 1 and 1 high dose rat on day 3.

Comments: Results of pharmacokinetic analysis presented in Study No. 92-5009.

Summary: There was a dose-related increase in mortality and tremors associated with continuous intravenous infusion of SNX-111 at 10 and 40 mg/kg/day for 24 hours in Sprague-Dawley rats. Mortalities occurred up to 18 hours after the end of the infusion. The clinical signs reversed by 2 days after the end of the infusion.

Study Title: Determination of the Kinetics of SNX-111 Infused into Rats: Use of a Radioimmunoassay to Determine the Concentration of SNX-111 in Rat Plasma Samples

Study No: 92-5009

occurred at 2 hours at both doses and were 1013 ng/ml at 10 mg/kg/d and 4401 ng/ml at 40 mg/kg/d. Peak plasma levels in both sexes were observed at 2 hours at the low dose and 24 hours at the high dose, and were slightly higher in female than in male rats. The Cmax and AUC values indicated dose proportionality. The disposition was characterized by 2 exponential components, with a short initial half-life of 0.5-0.9 hours and a longer terminal half-life of 4.9-5.6 hours. Clearance from plasma was 99% complete at 3 hours after end of the infusion.

Study Title: Analysis of Dose Preparation Samples from a Pharmacokinetic Study with Test Article SNX-111 Administration by 24-Hour Intravenous Infusion to Rats (Study 309-92)

Study No: 98-5068

Vol # 009, Page #s 091-095

Conducting laboratory and location: Neurex Corporation, 3760 Haven Avenue, Menlo Park, CA 94025

Date of study initiation: January, 1993

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Methods: For dosing methods, see under Study 309-92 above.

Samples of the SNX-111 solutions administered in the 24-hour intravenous infusion study in rats (Study 309-92) were collected on the day of infusion and analyzed for test article concentration using High Performance Liquid Chromatography (HPLC). Target concentrations were 0.210 and 0.830 mg/ml in the low and high dose solutions respectively. The reference standard was ziconotide Lot No. 8068SJJ004. The limit of detection was [redacted] and limit for quantitation [redacted].

Results: SNX-111 concentrations [redacted] of the target concentrations. Purity [redacted]

Summary: The target concentrations of test article used in the 24-hour intravenous infusion study on SNX-111 pharmacokinetics in rats were confirmed using HPLC at [redacted] and purity was [redacted]

Study Title: *A Pharmacokinetic Study with Test Article SNX-111 Administered by 24-Hour Intravenous Infusion to Cynomolgus Monkeys*

Study No: [redacted] 310-92

Vol # 009, Page #s 096-125

Conducting laboratory and location: [redacted]

Date of study initiation: October 6, 1992

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Methods:

Dosing:

- species/strain: *Macaca fascicularis* (Cynomolgus) Monkeys, [redacted]
- #/sex/group or time point: 2
- age: Young adults

- **weight:** 3.5-4.4 kg males and 3.2-3.5 kg females
- **dosage groups in administered units:** 11.2 and 25 mg/kg/day
- **route, form, volume, and infusion rate:** Continuous intravenous infusion via catheter inserted through the femoral vein in to the vena cava, in 1 ml/kg/h at 0.47 and 1.04 mg/ml

Drug, lot#, % purity: 8068SJJ004, _____

Formulation/vehicle: 0.9% Sodium Chloride for Injection U.S.P. _____

Observations and times: Mortalities and clinical signs daily for 3 days. Body weights (day 1). Systolic blood pressure (baseline, 1, 3, 7 and 22 hours after start of infusion, 25 hours after end of infusion). 1.0 ml blood samples collected via femoral, brachial or saphenous vein at following times after start of infusion: 0, 0.25, 2, 4, 8, 12 and 24 hours; 5, 15, 30. minutes and 1, 2, 3, 6, 8, 10, 12 and 24 hours after the end of infusion.

Results:

Mortalities: No deaths.

Clinical Signs:

Hindlimb tremors in 2/2 low dose females on day 1, all 4 low dose monkeys on day 2 and 4/4 high dose monkeys on day 1.

Whole body tremors in 2/2 high dose females within 30 minutes of start of infusion, and all high dose animals on day 2. Tremors persisted through day 3 in all but one animal.

No feces in all treated monkeys on days 2 and 3.

Lying down in one low dose animal on day 2 and one high dose animal on days 2 & 3.

Vomiting in one high dose male on day 1 and one high dose female on day 2.

Blood Pressure: Decreased in one high dose male monkey 46-60 mm Hg compared to baseline at 1 hour after start of infusion.

Comments: Results of pharmacokinetic analysis presented in Study No. 92-5010.

Summary: SNX-111 infused intravenously in monkeys at 11.2 and 25 mg/kg/day was associated with hindlimb and whole body tremors beginning at 30 minutes after the initiation of the infusion, and persisting up to 3 days. Absent feces was observed for 2 days after the 24-hour infusion ended. A reversible decrease in blood pressure was observed in one of four monkeys given 25 mg/kg/day during the first hour of infusion. Vomiting was also observed in one high dose animal three hours after the start of the infusion.

Study Title: *Determination of the Kinetics of SNX-111 Infused into Monkeys: Use of a Radioimmunoassay to Determine the Concentration of SNX-111 in Monkey Plasma*

Study No: 92-5010

Vol # 009, Page #s 127-151

Conducting laboratory and location: Treatment: _____

Date of study initiation: December 4, 1992

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Summary: The plasma drug concentrations associated with 11.2 and 25 mg/kg/day SNX-111 were dose proportional. There were no differences in the results of the pharmacokinetics analysis on the basis of sex or dose. Plasma levels reached 50%, 90% and 95% steady-state level at 0.75, 2.7 and 3.7 hours respectively. Steady-state plasma concentrations of approximately 1.4 mg/l at the low dose and 3.2 mg/l at the high dose were reached by 4 hours after the initiation of the infusion. The tremors observed at 30 minutes were associated with plasma levels less than 1.6 mg/l. The disposition showed two components, with a fast phase of 0.72 hours composing 97% and slow phase of 6.5 hours composing 3% of the AUC.

In the noncompartmental analysis, maximum plasma levels were reached at 2-24 hours. Steady-state plasma concentrations for males and females combined were 1324 ng/ml at 11.2 mg/kg/day and 2785 ng/ml at 25 mg/kg/day, and were reached at 4 hours. Steady-state plasma levels and the AUC values were dose proportional. The elimination of the drug showed two phases with an initial short component of 0.8-1.1 hours and long terminal component of 5.5-8.9 hours. There were no differences in half-life or clearance on the basis of dose and no differences in any parameter on the basis of sex. SNX-111 was 99% cleared by 6 hours after the end of the infusion.

Study Title: Analysis of Dose Preparation Samples from a Pharmacokinetic Study with Test Article SNX-111 Administered by 24-Hour Intravenous Infusion to Cynomolgus Monkeys

Study No: — 310-92

Vol # 009, Page #s 152

Conducting laboratory and location: Neurex Corporation, 3760 Haven Avenue, Menlo Park, CA 94025

Date of study initiation: January 28, 1992

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Methods: : For dosing methods, see under Study — 310-92 above.

Samples of the SNX-111 solutions administered in the 24-hour intravenous infusion study in cynomolgus monkeys were collected on the day of infusion and analyzed for test article concentration using high performance liquid chromatography (HPLC). Target concentrations were 0.470 and 1.040 mg/ml in the low (11.2 mg/kg/day) and high (25 mg/kg/day) dose solutions respectively. The reference standard was ziconotide Lot No. 8068SJJ004. The limit of detection was — and limit for quantitation —

Results: SNX-111 concentrations of 0.482 and 1.096 mg/ml were 102.5%-105.4% of the target concentrations of 0.470 and 1.040 mg/ml for the 11.2 and 25 mg/kg/day doses respectively. Purity —

Summary: The target concentrations of test article used in the 24-hour intravenous infusion study on SNX-111 pharmacokinetics in rats were confirmed using HPLC at — and purity was —

Study Title: Intrathecal SNX-111 Given as a Bolus or Continuous Infusion in Beagle Dogs: Part I Pharmacokinetics

Study No: UCSD Study No. -96-019

Vol # 009, Page #s 157-217

Conducting laboratory and location: [REDACTED]

Date of study initiation: March 2, 1996

GLP compliance: Yes () No (x)

QA- Report: Yes () No (x)

Methods:

Dosing:

- **species/strain:** Beagle dogs [REDACTED]

- **#/sex/group or time point:** 5 males

- **age:** 9-18 months

- **weight:** 12-17.2 kg

- **dosage groups in administered units:** each dog received SNX-111 by IT bolus injection at 0.01 mg (1 ml over 60-120 seconds), IT continuous infusion for 48 hours at 1 µg/h (0.1 ml/h) and for 48 hours at 5 µg/h (0.1 ml/h) and IV bolus injection at 0.1 mg/kg, with 3-day washout between each treatment as described in the following table:

Dog ID	Dose 1	Dose 2	Dose 3	Dose 4
[REDACTED]	0.01 mg IT bolus	1 µg/d IT infusion	5 µg/h IT infusion	0.1 mg/kg IV bolus
[REDACTED]	1µg/h IT infusion	5 µg/h IT infusion	0.01 mg IT bolus	0.1 mg/kg IV bolus
[REDACTED]	0.01 mg IT bolus	1 µg/h IT infusion	5 µg/h IT infusion	0.1 mg/kg IV bolus
[REDACTED]	0.01 mg IT bolus	1 µg/h IT infusion	5 µg/h IT infusion	0.1 mg/kg IV bolus
[REDACTED]	0.01 mg IT bolus	1 µg/h IT infusion	5 µg/h IT infusion	0.1 mg/kg IV bolus

IT bolus injections included 1 µCi ³H-Inulin with 0.3 ml 0.9% Sodium Chloride for Injection USP flush.

Drug, lot#: SNX-111 for Injection Lot # NUP-001 (Neurex Corporation) , SNX-111 Peptide Lot # SMC 012 (Neurex Corporation)

% purity: SNX-111 for Injection [REDACTED] SNX-111 Peptide [REDACTED]

Formulation/vehicle: 0.1 mg/ml in 0.9% w/v Sodium Chloride for Injection, USP

Observations and times: Plasma (2 ml blood samples), lumbar CSF (0.3 ml) and cisternal CSF (0.3 ml) samples as follows:

IT bolus: baseline, and at 2, 5, 15, 45, 90, 180, 360 and 480 minutes

IT infusion: baseline and at 30, 60, 90, 120, 240 and 480 minutes, 24 and 48 hours and 30 minutes before end of infusion

IV bolus: baseline and at 2, 5, 15, 45, 90, 180, 360 and 480 minutes

Plasma and CSF SNX-111 levels assayed by radioimmunoassay (see Neurex Report No. 96-5007), limit of quantification [REDACTED] Pharmacokinetic parameters analyzed using non-compartmental methods.

Results:**SNX-111 Pharmacokinetic Parameters after IT Bolus Dose (0.01 mg)
in Beagle Dogs: Mean (SD)**

Parameter	Lumbar CSF	Plasma
Cmax (ng/ml)	7687.00 (2873.42)	—
Tmax (hr)	0.09 (0.09)	—
AUC _{0-∞} (ng·hr/ml)	3445.39 (3641.90)	1.53 (1.58)
Cl (ml/min)	0.08 (0.04)	np
Vd ini (ml)	1.41 (0.38)	np
Vss (ml)	1.29 (0.59)	np
MRT _{0-∞} (hr)	0.28 (0.04)	2.68 (2.49)
t _{1/2} α (hr)	0.40 (0.38)	np
t _{1/2} β (hr)	1.79 (2.67)	1.75 (1.56)

np: not provided

**SNX Pharmacokinetic Parameters after IT Continuous Infusion
in Beagle Dogs: Mean (SD)**

PK Parameter	1 µg/hr		5 µg/hr	
	Lumbar CSF	Plasma	Lumbar CSF	Plasma
Cmax (ng/ml)	473.82 (211.39)	0.07 (0.13)	2072.40 (1284.98)	1.24 (0.21)
Tmax (hr)	17.60 (8.76)	34.67 (23.09)	14.40 (8.76)	25.60 (14.31)
AUC _{0-∞} (ng·hr/ml)	np	np	76505.31 (44435.28)	51.07 (8.07)
Cl (ml/min)	np	np	0.13 (0.15)	np
Cl(lumbar)(ml/min)	0.16 (0.12)	np	0.18 (0.13)	np
Vd ini (ml)	np	np	np	np
t _{1/2} α (hr)	np	np	np	np
t _{1/2} β (hr)	np	np	1.34 (0.52)	1.12 (0.50)

np: not provided

**SNX Pharmacokinetic Parameters after IV Bolus (0.1 mg/kg) in Beagle Dogs:
Mean (SD)**

PK Parameter	Lumbar CSF	Plasma
Cmax (ng/ml)	17.39 (17.84)	639.00 (215.47)
Tmax (hr)	0.75 (0.00)	0.03 (0.00)
AUC _{0-∞} (ng·hr/ml)	37.69 (33.03)	267.14 (106.79)
MRT _{0-∞} (hr)	2.27 (0.50)	0.59 (0.25)
Cl (ml/min)	np	102.07 (40.74)
Cl(lumbar) (ml/min)	np	-
Vd ini (ml)	np	2564.60 (1161.73)
Vss (ml)	np	3604.03 (2526.12)
t _{1/2} α (hr)	np	0.08 (0.08)
t _{1/2} β (hr)	1.64 (0.52)	0.75 (0.91)

np: not provided

Summary: After an IT bolus injection of SNX-111 at 0.01 mg, a peak lumbar concentration of 7687 ng/ml was observed at 5 minutes and peak plasma concentration 0.53 ng/ml at 30 minutes (ratio of CSF to plasma concentrations 20,000:1 at 5 minutes and 30:1 at 8 hours). The AUC values were 3445 and 1.53 ng·h/ml in lumbar CSF and plasma respectively.

The peak lumbar CSF concentration reached 473 ng/ml at 17.6 hours and 2072 ng/ml at 14.4 hours after continuous IT infusion of 1 µg/h and 5 µg/h respectively for 48 hours. Continuous infusion of SNX-111 at these doses resulted in peak plasma levels of 0.07 ng/ml at 34.67 hours and 1.24 ng/ml at 35.6 hours respectively. The CSF (lumbar and cisternal) and plasma concentrations showed approximate dose proportionality. The lumbar CSF to plasma ratio at CSF C_{max} was 2400:1, 1200:1 and 600:1 at 8, 24 and 48 hours respectively after infusion at the rate of 0.005 mg/h. At 48 hours, the ratio of lumbar CSF to cisternal CSF to plasma concentrations was 1:0.001:0.001 after infusion with 0.001 mg/h and 1:0.02 :0.002 after 0.005 mg/h infusion, indicating considerably greater CNS exposure by the IT route.

After IV bolus injection of 0.1 mg/kg, the plasma C_{max} was 642 ng/ml at 0 minutes and the CSF C_{max} was 17.39 ng/ml at 45 minutes, indicating rapid distribution into the CSF. The ratio of plasma to lumbar CSF concentrations was 850:1 at 0 minutes, 3:1 at 45 minutes and 1:1 at 8 hours. The plasma to CSF AUC ratio was 4:1. CSF availability was lower after IV than after IT infusion. The half-life in CSF (1.5 h) was longer than in plasma (0.75 h). The plasma and CSF concentrations and AUC values showed dose proportionality.

Study Title: Intrathecal SNX-111 Given as a Bolus or Continuous Infusion in Beagle Dogs: Part II Behavioural and Clinical Observations

Study No: 96-019

Vol # 009, Page #s 229-313

Conducting laboratory and location: _____

Date of study initiation: March 2, 1996

GLP compliance: Yes () No (x)

QA- Report: Yes () No (x)

Methods: The animals were treated under _____ Study No. 96-019. For detailed methods, see under the study entitled *Intrathecal SNX-111 Given as a Bolus or Continuous Infusion in Beagle Dogs: Part I Pharmacokinetics* above.

Observations were: morbidity, mortality, behavioral indices and clinical signs twice daily; body weights, food consumption, body temperature, heart rate and blood pressures, and presence of stool and urine daily. Behavioral indices were arousal, muscle tone and coordination (twice daily). Nociception was evaluated using the thermally evoked skin twitch response at the following times: IT bolus: baseline, and 20, 30, 60, 120, 180, 240, 300 and 360 minutes; IV bolus: baseline and 2, 5, 15, 45, 90, 180, 360 and

480 minutes; IT infusion: baseline and at 3, 8, 24 and 48 hours after the start of infusion and 180, 360 and 480 minutes after the termination of infusion.

Results: No deaths.

General Behavior: IT bolus (0.01 mg): whole body trembling and panting in 3/5 dogs at 1 hour, decreased arousal and decreased activity (5/5 dogs) for 24 hours after injection; IV bolus (0.1 mg/kg): vomiting (4/5 dogs), scleral vasodilation (2/5 dogs) and facial erythema (2/5 dogs) immediately after injection, whole body trembling, laryngeal spasms, decreased muscle tone, sedation (3/5 dogs) at 5 minutes; 1 µg/h IT infusion: whole body trembling and ataxia (5/5 dogs) at 4-8 hours lasting 48 hours; 5 µg/h IT infusion: whole body trembling and ataxia (5/5 dogs) throughout 48 hour infusion with increasing severity, diarrhea (3/5 dogs) and hematuria (1/5 dogs) 24 hours after end of infusion.

Antinociception: No effects after IV bolus and IT bolus. Complete blockade of skin twitch response at 24-48 hours during infusion of 1 and 5 µg/h.

Body Temperature: No effects after IV bolus and IT bolus. Increased body temperature (1.3°C at 24 h and 1.7°C at 48 hours) after 1 µg/h IT infusion. Additional increase of 0.2°C at 24 h and 0.8°C at 48 h after 5 µg/h infusion, reversible within 24-72 hours after end of infusion.

Blood Pressure: IT bolus: Decrease in mean, diastolic and systolic blood pressure; IV bolus: severe decrease in mean, diastolic and systolic blood pressure (~40 mm Hg) lasting 8 hours; 1 µg/h IT infusion: no effect; 5 µg/h infusion: severe decrease in mean, diastolic and systolic blood pressure lasting for duration of 48 h infusion.

Heart Rate: Mild tachycardia after IT bolus, IV bolus (lasting 8 h) and 5 µg/h IT infusion (lasting duration of infusion).

Respiratory Rate: Panting in 3/5 dogs after IT bolus, lasting 20 minutes. Decreased respiratory rate for 3 hours after IV bolus. No effect after IT infusions.

Summary: Intravenous SNX-111 in a 0.1 mg/kg bolus induced severe behavioural effects including vomiting, scleral vasodilation and facial erythema, and whole body trembling, laryngeal spasms, decreased muscle tone and sedation starting from 0-5 minutes after the injection. While having no effect on antinociception and body temperature, IV SNX-111 injection resulted in profound decreases in mean, diastolic and systolic blood pressure associated with tachycardia that persisted 8 hours after the injection. Respiration was decreased for 3 hours. This dose produced a plasma C_{max} of 639 ng/ml at 0.03 h and AUC of 267 ng·h/ml.

Intrathecal bolus injection of SNX-111 at 0.01 mg resulted in whole body trembling and panting at 1 hour, and decreased arousal and decreased activity for up to 24 hours after injection. The C_{max} at this dose was 0.53 ng/ml at 0.5 hour. No effects on antinociception and body temperature were observed, but mean, diastolic and systolic blood pressures were reduced and heart and respiratory rates were increased.

Continuous IT infusion of SNX-111 completely blocked the skin twitch response, an index of antinociception, from 24-48 hours after the start of infusion at both 1 and 5 µg/h (peak plasma levels 0.07 ng/ml at 34.67 hours for 1 µg/h and 1.24 ng/ml at 35.6 hours for 5 µg/h). The AUC following 5 µg/h was 76505 ng·h/ml. Cardiovascular effects: severe decrease in mean, diastolic and systolic blood pressure lasting for the

duration of the 48-hr infusion and mild tachycardia were observed after infusion of 5 µg/hr. Body temperature increased after both infusions.

Distribution:

Study Title: SNX-111 Flux Through the Spinal Meninges of the Monkey

Study No: 98-5028

Vol # 009, Page #s 314-323

Conducting laboratory and location: Not provided.

Date of study initiation: Not provided.

GLP compliance: Yes () No (x)

QA- Report: Yes () No (x)

Methods: *In vitro* model used to investigate the transmeningeal flux rate of SNX-111

Dosing:

- **species/strain:** *Macaque nemestrina* monkeys

Drug lot#, % purity: Not provided.

Procedure: Dura, arachnoid and pia matter were isolated from the spinal cord at levels T5-L5 from three *M. nemestrina* monkeys. Meninges were mounted in a diffusion cell containing artificial cerebrospinal fluid

glucose in both reservoirs. I-SNX-111 with ¹²⁵I-SNX-111 were added to donor reservoir at 37°C, and 100-500 µl samples were collected from both reservoirs periodically up to 360 minutes. Samples were counted using gamma counter. Flux and permeability were determined by SNX-111 concentrations in recipient reservoir. Dura and pia-arachnoid membranes were analyzed for SNX-111 binding using competition binding with SNX-111 analogue [Nle¹²⁵]SNX-111.

Results: Diffusion across intact meninges non-linear: Flux showed early phase (0-2 h) and late phase (3-5 h). Permeability coefficient for early phase $0.137 \pm 0.045 \times 10^3$ cm/min, late phase $0.962 \pm 0.21 \times 10^3$ cm/min. Permeability linear over time in dura mater alone, but changed in pia-arachnoid mater over time as in intact meninges. Low affinity binding of ¹²⁵I-SNX-111 to [Nle¹²⁵]SNX-111 binding sites in pia-arachnoid and dura. Low non-specific binding. Competition binding curves suggested single binding sites in pia-arachnoid and dura membranes.

Summary: Non-linear ¹²⁵I-SNX-111 flux through the meninges demonstrated an early phase probably due to hydrophilicity of the drug and late phase consistent with the presence of a molecular transport system. The transport system was suggested by low affinity [Nle¹²⁵]SNX-111 binding sites on the dura and pia-arachnoid membranes in the competitive binding assay.

Study Title: Blood-Brain Barrier Permeability of [¹²⁵I]-Ziconotide (SNX-111)

Study No: 0106

Vol # 009, Page #s 324-342

Conducting laboratory and location: Not provided.

Date of study initiation: Not provided.

GLP compliance: Yes () No (x)

QA- Report: Yes () No (x)

Methods: The blood-brain permeability coefficient (PC) of SNX-194 was determined with the assumption that permeability is identical to that of ziconotide (SNX-111). SNX 194 is structurally identical to ziconotide with the exception of an isomeric norleucine moiety instead of methionine in the position-13 amino acid in the 25 amino acid polycationic peptide.

Procedure: _____ model used: test peptides including SNX-194 (Synthesis Group, Neurex Corporation) permeability coefficients determined according to method of Green *et al.* (Enkephalin analog Prodrugs: Assessment of In Vitro Conversion, Enzyme Cleavage Characterization and Blood-Brain Barrier Permeability, *J. Pharmacol. Exp. Ther.* **277**(3): 1366-75, 1996). Data collected at 30 and 120 minutes.

Results: Passage into donor compartment linear from 30-120 minutes. PC for SNX-194 $10.04 \pm 0.47 \times 10^{-4}$ cm/min.

Summary: The permeability coefficient for SNX-194 of $10.04 \pm 0.47 \times 10^{-4}$ cm/min is assumed to be identical to that of SNX-111 due to structural similarity of the two peptides.

Metabolism:

Study Title: Analysis of *In Vitro* Metabolism and Brain Penetration of Ziconotide (SNX-111) in Rats

Study No: 98-5037

Vol # 009, Page #s 343-356

Conducting laboratory and location: Not provided.

Date of study initiation: Not provided.

GLP compliance: Yes () No (x)

QA- Report: Yes () No (x)

Methods:

Dosing:

- **species/strain:** Sprague-Dawley rats.
- **#/sex/group or time point:** Males; numbers not provided.
- **dosage groups in administered units:** IV: 0.4 mg; Intrahippocampal dialysis: 8 and 270 μ M unlabeled ziconotide with [¹²⁵I]-ziconotide.
- **route, form, volume, and infusion rate:** IV (tail vein) at 1 mg/ml over 5-7 seconds; intrahippocampal; perfusion over 2-2.5 hours.
- **procedure** Blood (3 ml) and brain (hindbrain, cerebellum, neocortex, striatum, globus pallidus, caudate putamen, hippocampus, thalamus, hypothalamus) were sampled at various intervals after intravenous injection of ziconotide and after administration of the drug directly into the brain via microdialysis, including radiolabeled ziconotide by both routes. Samples were analyzed by HPLC for parent drug and metabolites. Brain was

sectioned and processed for autoradiography. Radioactivity in serial sections determined using gamma counting.

Results:

Mean percent injected [¹²⁵I]-ziconotide per gram of tissue after IV injection (numbers of samples in parentheses)

Time	Plasma	Neocortex	Subcortex
3 min	1.0	0.0056	0.0051
10 min (n=2)	0.81	0.0032	0.0046
20 min (n=4)	0.31	0.0032	0.0064
40 min (n=3)	0.19	0.0012	0.0018
1 hr (n=2)	0.060	0.0003	0.0007
2 hrs (n=1 or 2)	0.0050	0.00025	0.0006
24 hrs (n=1)	<0.0002	0.00002	<0.00025

The radiolabeled ziconotide was found within 0.5 mm of the dialysis probe in brain at 2.5 hours after perfusion.

Summary: Parent drug content was constant in brain at 0.003-0.006% injected dose, for 3-20 minutes after IV injection, and reduced to 0.0003-0.0006% at 4 hours. Overall, the maximum brain concentration of the parent drug was approximately 0.005% per gram of tissue, or 50 nM. Degradation products in blood and brain were products of [redacted]. Degradation of the parent drug was similar after direct administration into the brain to that after IV administration. Diffusion in brain tissue was less than 1 mm at 2 hours.

Elimination:

Report Title: Possible Modes of Ziconotide Degradation *In Vivo*

Report No: NC0201

Vol # 010, Page #s 364-374

Summary: Based on the known peptide degrading enzymes in mammals and the chemical structure of the 25 amino acid peptide ziconotide, metabolism of the drug is hypothesized to proceed by initial cleavage of internal sites followed by exopeptidase digestion. Evidence is provided by the presence of [redacted] fragments found on HPLC chromatograms after intravenous ziconotide administration. Peptide degradation involves cleavage of [redacted] in both brain and circulatory system. Thereafter, the amino and carboxy terminals are digested by [redacted]. The actual order of cleavages is unknown and difficult to predict. The predicted final products of ziconotide metabolism are free amino acids. Little degradation is expected in the cerebrospinal fluid because the aminopeptidase available has no activity without free amino and carboxy terminals. Ziconotide was found stable in undiluted rat and dog cerebrospinal fluid in a previous study. Metabolism of ziconotide is expected to occur by peptidases found in cellular

membranes. Ziconotide that is transported into the systemic circulation will undergo further proteolysis in the vasculature and liver.

Other studies:

Study Title: ¹²⁵I-SNX-111: An *In Vitro* Assessment of Plasma Protein Binding and Partitioning in Whole Blood From Four Different Species

Study No: 43767

Vol # 010, Page #s 002-287

Conducting laboratory and location: _____

Date of study initiation: January 20, 1993

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Methods:

Dosing:

- **species/strain:** Whole blood collected from healthy male rats (*Rattus norvegicus*), beagle dogs (*Canis familiaris*), cynomolgus monkeys (*Macaca fascicularis*), and human volunteers.

- **#/sex/group or time point:** 10 rats, 16 dogs, 14 monkeys and 4 humans, whole blood and plasma samples pooled for each species (3-3.5 ml/species)

Drug lot# 8068 SJK006, **purity:** Radiolabeled ¹²⁵I-SNX-111 lot number 92NO1FY

- **procedure:** Plasma and whole blood samples from each species incubated with following test drug concentrations:

- 1 pM ¹²⁵I-SNX-111 + 1 nM SNX-111
- 10 pM ¹²⁵I-SNX-111 + 10 nM SNX-111
- 100 pM ¹²⁵I-SNX-111 + 100 nM SNX-111
- 100 pM ¹²⁵I-SNX-111 + 1000 nM SNX-111

- 1 pM ¹²⁵I-SNX-111 + 0.1 nM SNX-111
- 10 pM ¹²⁵I-SNX-111 + 1 nM SNX-111
- 100 pM ¹²⁵I-SNX-111 + 10 nM SNX-111
- 100 pM ¹²⁵I-SNX-111 + 100 nM SNX-111

Free fraction calculated to determine binding. Samples centrifuged and compartments counted in _____ to determine radioactivity in each component. Assays conducted in triplicate.

Results:

Non-specific membrane binding: Percentage, corrected for recovery of ¹²⁵I-SNX-111

Ccn. ¹²⁵ I-SNX-111	100 pM	100 pM	10 pM	1 pM	100 pM	100 pM	10 pM	1 pM
Ccn. SNX-111	100 μM	10 μM	1 μM	0.1 μM	1000 nM	100 nM	10 nM	1 nM
Human	_____							
Dog	_____							
Rat	_____							
Monkey	_____							

Bound SNX-111 in Plasma (M)

[¹²⁵ I-SNX-111]	100 pM	100 pM	10 pM	1 pM	100 pM	100 pM	10 pM	1 pM
Ccn. SNX-111	100 μM	10 μM	1 μM	0.1 μM	1000 nM	100 nM	10 nM	1 nM
Human	_____							
Dog	_____							
Rat	_____							
Monkey	_____							

Partitioning corrected for recovery of ¹²⁵I-SNX-111 between plasma and cellular components (Kd values for pooled samples)

[¹²⁵ I-SNX-111]	100 pM	100 pM	10 pM	1 pM	100 pM	100 pM	10 pM	1 pM
Ccn. SNX-111	100 μM	10 μM	1 μM	0.1 μM	1000 nM	100 nM	10 nM	1 nM
Human	_____							
Dog	_____							
Rat	_____							
Monkey	_____							

Summary: Protein binding was similar across species, but the maximum plasma protein binding was 89.8% in human, 73.4% in monkey, 61.8% in dog, and 55.9% in rat plasma. Binding in all species increased with concentration. Whole blood partitioning was similar across species; the Kd values were 0.3804-0.4254 in dog, 0.3527-0.4010 in human, 0.3400-0.3829 in monkey and 0.2366-0.3106 in rat. Protein and blood cell binding in all species was non-saturable, low-affinity, and non-specific.

Study Title: Determination of the Protein Binding of CI-1009 in Heparinized Rat Plasma and EDTA Human Plasma by Radioimmunoassay

Study No: 764-03035

Vol # 010, Page #s 288-300

Conducting laboratory and location _____

Date of study initiation: Not provided.

GLP compliance: Yes () No (x)

QA- Report: Yes () No (x)

Methods:

Dosing:

- **species/strain:** Rat _____ and human plasma _____
 _____ were pooled within species.

Drug, lot# 92N01FY

Formulation/vehicle: Lyophilized CI-1009 powder reconstituted with distilled water to 1.000 mg/ml, pH 7.4

Procedure: CI-1009 from Neurex Corporation, Menlo Park, CA added to plasma at 1, 10, 100, 1000 and 10,000 ng/ml. Ultrafiltrate quantitated using validated radioimmunoassay and bound and free CI-1009 concentrations calculated. Assays conducted in triplicate.

Results: Nonspecific binding to the [redacted] cartridges was negligible.

CI-1009 Bound to Rat Plasma Proteins

CI-1009 Concentration (ng/ml)			Bound (%)	Free (%)
Nominal	Plasma	Ultrafiltrate		
1	[redacted]	[redacted]	53.6	46.4
10				
100				
1000				
10000				
Overall Mean			53.6	46.4

CI-1009 Bound to Human Plasma Proteins

CI-1009 Concentration (ng/ml)			Bound (%)	Free (%)
Nominal	Plasma	Ultrafiltrate		
1	[redacted]	[redacted]	53.3	46.7
10				
100				
1000				
10000				
Overall Mean			53.3	46.7

Summary: Protein binding of CI-1009 was similar in rat (53.6 %) and in human (53.3%) plasma. Binding was not related to concentration in human plasma, but decreased in rat plasma from 64% to 40% with increased concentration from 1 to 10,000 ng/ml respectively. In comparison, target concentrations for clinical use are 150 ng/ml.

Study Title: Determination of Ziconotide Binding to Proteins in Dog Plasma by Radioimmunoassay

Study No: [redacted] 48031

Vol # 010, Page #s 301-363

Conducting laboratory and location: [redacted]

Date of study initiation: August 27, 1999

GLP compliance: Yes (x) No ()

QA- Report: Yes (x) No ()

Methods:

Dosing:

- species/strain: Beagle dog plasma ([redacted])

Drug, lot# REF007; purity [redacted]

Formulation/vehicle: 100 µg/ml ziconotide [redacted] aqueous solution in isotonic saline containing 50 µg/ml L-methionine. Assay buffer [redacted]

(0.05 M disodium EDTA, 0.05 M sodium phosphate, 0.02% sodium azide and 0.9% sodium chloride), pH 7.4.

Procedure: Plasma samples treated with ziconotide at 0.1, 1, 10, 100 and 1000 ng/ml. After incubation, 1 ml samples were analyzed by radioimmunoassay for ziconotide concentration in buffer and ultrafiltrate for determination of free and bound drug. Assay conducted in triplicate.

Results:

Binding of ziconotide binding in dog plasma:

Ziconotide Concentration (ng/ml)	% Bound to Plasma Proteins	% Non-specific Binding
0.1	Not detected	Not detected
1		
10		
100		
1000		

Summary: Plasma protein binding of ziconotide in dog was slightly decreased from _____ with increasing drug concentration from 1 to 1000 ng/ml. The mean percentage of ziconotide bound over the range of concentrations was 62%. Non-specific binding was negligible.

Comments:

Toxicokinetic measurements were also made in rats during the continuous IV administration of ziconotide in studies of male fertility, female fertility and peri/postnatal development. The results are summarized in the following table:

Sex/Study type	Sampling time after start of IV	Mean±SD plasma ziconotide concentration, ng/ml		
		1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Male fertility	Day 1	93 ± 3	245 ± 34	651 ± 223
	Day 28	105 ± 28	276 ± 54	1374 ± 1111
	Day 49	154 ± 42	375 ± 189	2460 ± 3136
Female fertility	Day 1	85 ± 23	178 ± 21	570 ± 90
	Day 14	81 ± 11	199 ± 44	697 ± 75
	Gestation day 7	87 ± 16	205 ± 46	1232 ± 1248†
Female, peri- and postnatal	Gestation day 6	87 ± 12	200 ± 48	384 ± 252
	Gestation d 17	91 ± 16	191 ± 24	512 ± 106
	Postpartum d 4	67 ± 21	204 ± 49	448 ± 425
	Postpart day 21	82 ± 45	236 ± 25	633 ± 158
Mean (± S.D.) of Female Means:		83 ± 8	202 ± 18	639 ± 107

† One of the high-dose females had the disproportionately high concentration of 3440 ng/ml. The mean of the other four females was 680 ng/ml.

In male rats, ziconotide accumulated over time, especially at the high dose. Female rats maintained steady plasma levels of ziconotide over time. Both sexes showed

proportionality between plasma level and dose administered by the IV route. The plasma concentrations at 10 mg/kg/day for Day 1 (means of 651 ng/ml in males and 570 ng/ml in females) in these studies are somewhat lower than the C_{ss} of 811 ng/ml in males and 1215 ng/ml in females that were reported for the 24-hour IV infusion of 10 mg/kg in Study No. 92-5009.

SUMMARY OF PHARMACOKINETICS AND ADME

The pharmacokinetics profile of intravenous and IT ziconotide in plasma and cerebrospinal fluid was studied in rats, dogs and monkeys. There were no differences in the pharmacokinetic parameters between males and females, after continuous 24-hour intravenous infusion in rats at 10 and 40 mg/kg/day and in monkeys at 11.2 and 25 mg/kg/day. Steady state plasma levels of 1013 ng/ml at 10 mg/kg/day and 4401 ng/ml at 40 mg/kg/day were observed at 2 hours in rats. In the monkeys, steady state concentrations were 1.4 µg/ml and 3.2 µg/ml at the low and high doses respectively at 4 hours. Dose proportionality was observed for both steady-state and AUC values in the monkeys and rats.

In dogs administered ziconotide by IT bolus at 0.69 µg/kg, IT 48-hour infusion at 0.069 and 0.35 µg/kg/hr and intravenous bolus at 0.1 mg/kg, maximum plasma levels were _____ respectively at 0.5, 34.67, 25.60 and 0.03 hours respectively. In comparison, the mean CSF C_{max} levels were 7687, 474.82, 2072.40 and 17.39 ng/ml respectively at 0.09, 17.6, 14.4 and 0.75 hours. These results show a high lumbar CSF to plasma ratio (20,000:1) at peak CSF concentrations after the IT bolus (0.5 hours) and 2400:1 at 8 hours after continuous IT administration of 0.35 µg/kg/h. The plasma to lumbar CSF ratios after intravenous injection were 850:1 at 0 minutes, 3:1 at 45 minutes and 1:1 at 8 hours. Lumbar CSF and plasma AUC values were 3445 and 1.53 ng·h/ml respectively after the IT bolus injection at 0.01 mg. Distribution of ziconotide out of the CSF into the general circulation was rapid with a decline in ratio to 30:1 at 8 hours after the bolus and to 1200:1 at 24 hours after the continuous infusion. Little ziconotide passed into the CSF after intravenous bolus injection, resulting in CSF bioavailability of 0.01%.

Elimination of ziconotide given intravenously was characterized by two components with a short initial half-life of 0.5-0.9 hours in rats and 0.8-1.1 hours in monkeys, and a long terminal half-life of 4.9-5.6 hours in rats and 5.5-8.9 hours in monkeys. Clearance out of the CSF was faster with a terminal half-life of 1.6 hours after intravenous bolus and 1.8 hours after the IT bolus injections. There were no effects of sex or dose on clearance. Ziconotide clearance was 99% at 3 hours in rats and 6 hours in monkeys after the end of the continuous infusion.

Continuous intravenous administration of ziconotide in rats at 10 and 40 mg/kg/day for 24 hours was associated with a dose-related increase in mortality and tremors beginning during the infusion and decreasing in incidence during the subsequent two days after the end of the infusion. Monkeys given intravenous injection of ziconotide at

11.2 and 25 mg/kg/day showed hindlimb and whole body tremors associated with plasma levels < 1600 ng/ml, lasting up to three days. Decreased blood pressure was observed in a high dose monkey during the first hour of infusion and vomiting was seen in one high dose monkey at three hours after the start of the 24-hour infusion.

Intravenous bolus injection of ziconotide at 0.1 mg/kg in dogs induced vomiting, scleral vasodilation, facial erythema, whole body trembling, laryngeal spasms, decreased muscle tone and sedation from 0-5 minutes after the injection and profound decreases in mean, diastolic and systolic blood pressure with tachycardia for 8 hours after the injection. These effects were associated with a C_{max} of 639 ng/ml and AUC of 267 ng·h/ml. After IT bolus injection at 0.01 mg, whole body trembling, panting, decreased arousal and activity and decreased blood pressure, heart rate and respiratory rate were observed in the dogs. Intravenous and IT bolus injections had no effect on antinociception. Antinociception in the thermally-evoked skin-twitch response and elevation of body temperature (1.3 – 2.5°C) were produced during continuous IT infusion of 1 and 5 µg/h, doses that resulted in peak plasma levels of 0.07 ng/ml and 1.24 ng/ml respectively. At 5 µg/h, decreased blood pressure and mild tachycardia were observed. The AUC after continuous IT infusion of 5 µg/h was 76505 ng·h/ml.

Distribution of ziconotide through the spinal meninges was studied *in vitro* using tissues obtained from monkeys. Diffusion across the intact meninges including dura, arachnoid and pia was non-linear, showing two phases. The early phase from 0-2 hours was consistent with hydrophilicity of the drug, and the late phase from 3-5 hours suggested transport system activity. Low affinity binding was observed in isolated pia-arachnoid and dura membranes, also suggesting the presence of a molecular transport system.

Distribution of ziconotide into the brain was studied after intravenous injection in rats. Ziconotide concentration in brain was 0.003-0.006% of the injected dose (0.4 mg) at 3-20 minutes and 0.0003-0.0006% at 4 hours. The overall maximum brain concentration of parent drug was 0.005% per gram of tissue. When infused directly into the hippocampus, ziconotide diffusion in brain tissue was less than 1 mm at 2 hours. The degradation products were products of  and were similar after intravenous administration and direct administration into the brain.

Studies were conducted to evaluate plasma protein binding in rats, dogs, monkeys and humans. Plasma protein binding was  in rats and humans tested at 1-10,000 ng/ml and in dogs at 0.1-1000 ng/ml. Maximum protein binding was  in rat, dog, monkey and human plasma. Binding increased with concentration in all species tested in one study, but decreased slightly with increasing drug concentrations in another study in dogs. Binding was not saturable at clinically relevant concentrations. Partitioning into cellular components was comparable across species and was independent of drug concentration, with ratios of 0.24-0.31, 0.38-0.43, 0.34-0.38 and 0.35-0.40 in rats, dogs, monkeys and humans respectively.

Ziconotide metabolism was studied after intravenous and intrahippocampal administration in rat brain and plasma. Ziconotide, a 25 amino acid peptide, was cleaved at multiple sites via multiple peptidases. Metabolism was similar in brain and plasma after intrahippocampal infusion and intravenous administration. It is hypothesized that the peptide degradation involves cleavage of _____ and basic residues by _____ followed by digestion of the amino and carboxy terminals by _____

The elimination of ziconotide after IT administration in dogs was characterized by an initial rapid distribution into the systemic circulation (initial CSF half-life 0.4 hour) followed by movement away from the injection site by bulk CSF flow (terminal half-life 1.8 hours). The terminal half-life in plasma after IT injection was similar at 1.75 hours and after IV injection was 0.75 hour.

TOXICOLOGY

General Comments: It is difficult to determine the human equivalent doses in dogs for the IT route of administration, as there are no guidelines for this route. The ratio of dog:human body weights (in kg) in this study is approximately 10:60 or 1/6. The ratio of dog:human body surface areas (in m²) is approximately 0.50:1.62 or 1/3. The ratio of dog:human CSF volumes (in ml) is approximately 12.5:150 or 1/12. The highest daily dose per dog attempted in the following study approximated 288 µg, whereas the maximum recommended daily dose in humans is 57.6 µg (24x2.4 µg/hr), i.e., 1/5 the highest daily dose in the dog.

Study Title: A 28-Day Intrathecal Infusion Toxicity Study of SNX-111 in the Beagle Dog Followed by a Recovery Period of Up To 2 Weeks.
(with Addendum)

Study/Project No.: _____ 54156 (Addendum No., _____0190)

Vol. 2.024 (371 pp.) and Vol. 2.025, pp. 1- (Addendum, Vol. 2.025, p. 398)

Conducting laboratory and location: _____

Date of study initiation: Animals received on _____ (n=58) and _____ (n=24), _____
Surgical preparation occurred at least 2 weeks after receipt;
start of treatment was 32-39 days after receipt.

GLP Compliance: Yes, with two exceptions: 1) Analysis of dose preparation solutions; and 2) analysis of plasma samples at Neurex Corporation were conducted in a non-GLP environment.

QA Report: (X) Yes () No

Methods: Dogs were exposed to the following drugs surrounding and/or following the time of surgical placement of a _____ catheter intrathecally. All dogs underwent a microlaminectomy procedure at the L5 vertebra, and after a durotomy, the catheter was inserted such that its tip lay approximately between the L2 and L4 vertebrae.

Drug, dose (mg/kg), route	Administration times	Purpose or function
Tribissen®, 30 mg/kg/d x3, SC	Day before, day of and day after surgery	Systemic antibiotic for all but 6 dogs.
Kefzol, 22.5 mg/kg, IV	Presurgery and b.i.d. for 3 days postsurgery.	Systemic antibiotic for the other 6 dogs.
Banamine®, 1 mg/kg, IM	Presurgery and ~48 hr postsurgery.	NSAID given to 4 group-1 and all group-3 & -4 dogs.
Buprenix, 0.005 mg/kg, IM	B.I.D. on day of surgery.	Analgesic
AC-Promazine, 0.05 mg/kg, IM	Prior to surgery.	Pre-anesthetic medication.
Butorphanol, 0.2 mg/kg, IM		
Glycopyrrolate, 0.01 mg/kg, IM		
Hibitane (4% chlorhexidine)		
70 % isopropanol		
Betadine (10% Povidone I ₂)		
Isoflurane	During surgery	General anesthetic
Marcaine®, 0.25%, 5 ml, SC	Prior to skin incision.	Local anesthetic
Penicillin G sodium	Irrigation of surgical site	Antibiotic
Isovue®, 0.7 ml, IT	Post surgery	Radiographic evaluation of catheter placement.
"Preanesthetic cocktail"	3 days prior to treatment initiation.	
Isovue®, 1.0 ml, IT		
0.9% NaCl for Injection, USP		
Neosporin® topical	daily at catheter exit site	Antibiotic

Dosing:

Species/strain: Dog / beagle \ _____

#/sex/group:

Group #	Treatment Identification	Dose, ng/kg, IT		% of target dose by dose analysis	No. of animals	
		per hr	per day		Male	Female
1	Saline control	0	0		5	9
2	Vehicle "	0	0		5	5
3	Low dose	100	2400	26-34%	4	4
4	Mid dose	300	7200	58-65%	4	4
5	High dose-1	600	14,400	92-94%	5	5
6	High dose-2	1200	28,800	87-93%	1	5

Age: 6-7 months

Weight: Males, 7.8-10.7 kg; females, 6.0-10.4 kg

Satellite groups: 1 dog/sex from groups 1, 2 and 5 were retained for a 2-week recovery period.

Dosage groups: See table immediately above.

Route, form, volume and infusion rate: IT, solution, 0.15 ml/hr.

Drug, lot # and purity: SNX-111 Lot #NUT001-TOX; purity _____

Vehicle control Lot #NUP002 \ _____

Saline control: Nine lot #'s of sodium chloride for injection. USP _____

Formulation/vehicle: SNX-111, 0.1 mg/ml solution in 0.9% saline, USP, with 50 µg/ml L-methionine, USP.

Observations and times:

Clinical signs: Observed twice daily for mortality and ill health, and examined in detail twice during pretreatment period and twice daily during treatment and recovery periods. A neurological exam was given prior to initiation of treatment and on treatment days 2 (~24 hours after start of infusion), 7, 14, 28 and during week 6 (recovery animals only). Shaking behavior, when seen, was scored as follows:

Score	Criteria
0	no effect
1	no spontaneous shaking, tail is stiff, slight head bobbing and/or slight shaking of head and body when animal is touched.
2	sporadic spontaneous shaking at rest; shaking when animal attempts to move.
3	continuous vigorous shaking, both in presence and absence of gross body movements; may be associated with splayed limbs.

Body weights: Measured once weekly, twice during the first treatment week in groups 1, 2, 5 and 6, with additional (unreported) measurements for calculation of pre-anesthetic doses at surgery and radiographic evaluation. A fasted weight was taken before euthanasia.

Food consumption: Measured daily, starting two weeks prior to treatment.

Ophthalmoscopy: Complete fundoscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed during pretreatment period and during weeks 4 and 6.

EKG: Electrocardiograms (from limb leads I, II, III, aVR, aVL and aVF) and blood pressure recordings (indirect systolic vial tail cuff) were taken once during pre-treatment, at 24 and 72 hours after beginning of treatment and weekly thereafter.

Hematology: Blood collected using EDTA or citrate as anticoagulant had the following parameters examined:

- | | |
|---------------------------------------|--|
| activated partial thromboplastin time | prothrombin time |
| blood cell morphology | red cell distribution width |
| hematocrit | red blood cell count |
| hemoglobin | reticulocyte count |
| mean platelet volume | white blood cell count (total, absolute, and percent differential) |
| platelet count | Wintrobe's constants |

Clinical chemistry: The following parameters were examined:

- | | |
|----------------------------|-----------------------|
| A/G ratio (calculated) | creatinine |
| alanine aminotransferase | globulin (calculated) |
| alkaline phosphatase | glucose |
| aspartate aminotransferase | phosphorus |
| blood urea nitrogen | potassium |
| calcium | sodium |
| chloride | total protein |

cholesterol
albumin

total bilirubin
triglycerides

Urinalysis: Urine aliquots were frozen and sent to the sponsor. In addition, the following parameters were examined:

bilirubin	microscopy of centrifuged deposit
blood	nitrite
color and appearance	pH
glucose	protein
ketones	specific gravity
volume	urobilinogen

Organs weighed: Paired organs were weighed together and organ-to-bodyweight ratios were calculated for the following:

adrenals	ovaries	thyroid lobes and parathyroids
brain	pituitary	(lobes weighed together)
heart	spleen	uterus
kidneys	testes	
liver	thymus	

Gross pathology: Complete necropsy was conducted on each dog immediately upon euthanasia by thoracotomy under deep pentobarbital anesthesia after pretreatment with ketamine and xylazine. Animals scheduled for euthanasia were fasted overnight. Dye was injected into the catheter before exposing the spinal cord to establish location of catheter tip without disturbing it and the epidural fat.

Histopathology: Samples from a number of tissues and organs were preserved in neutral buffered 10% formalin (see check list below).

Abnormal tissues	X
Adrenals	X
Aorta (thoracic)	X
Bone and marrow (femur)	X
Brain with meninges* [@]	X
Cecum	X
Colon	X
Duodenum	X
Epididymes	X
Esophagus	X
Eyes	X
Fallopian tube	
Gall bladder	X
Gross lesions	
Harderian gland	
Heart (including section of aorta)	X
Ileum**	X
Infusion site (catheter tip including meninges)* ^b	X
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	

Liver (2 lobes sampled)	X
Lungs (2 lobes sampled)	X
Lymph nodes, cervical	
Lymph nodes, mandibular	X
Lymph nodes, submaxillary	
Lymph nodes, mesenteric	X
Mammary glands (inguinal)*	X
Nasal cavity	
Ovaries	X
Optic nerves*	X
Pancreas	X
Parathyroids*	X
Peripheral nerve (sciatic)	X
Pharynx	
Pituitary	X
Prostate	X
Rectum*	X
Salivary glands	X
Seminal vesicles	
Skeletal muscle	X
Skin (inguinal)	X
Spinal cord (with meninges)**	X
Spleen	X
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	X
Trachea	X
Urinary bladder	X
Uterus , horns and body	X
Vagina	X

*Appears This Way
On Original*

+Examined only when present in routine sections of appropriate tissue/organ.

++Included cervical, thoracic and lumbar sections and L5 at catheter entry. Any remaining spinal cord including the cauda equina was retained for possible future evaluation.

^aRetained but not processed or evaluated.

*Meninges included the dura mater, arachnoid and pia mater.

**Including Peyers patches where found.

@Includes caudate putamen, cerebral cortex, piriform cortex, thalamus and hypothalamus, hippocampus, midbrain, cerebellum and medulla oblongata.

^bThe catheter tip included 2 cm caudal and cranial from the tip. Four sections were processed for histopathological examination: at entry of the catheter, at the catheter tip, 1 cm cranial and 1 cm caudal from the catheter tip.

Toxicokinetics: Pretreatment blood samples (~1 ml in EDTA) from dogs in the second batch (groups 3 & 4 and some group 1) and from all animals during weeks 2 and 4 and from recovery animals during week 6 were collected, centrifuged and the resulting plasma was stored frozen (-80°C) until being shipped to the sponsor in dry ice for analysis.

Results:

Mortality: Four dogs were euthanized during the treatment period due to a poor deteriorating condition and one for losing its catheter by getting out of its jacket:

Animal No./Sex	Dosage, ng/kg/hr	Day of euthanasia	Clinical observations on day of euthanasia
5511/F	600	6	Both eyes hypersensitive to light. Protruding nictitating membrane, both eyes. Increased vascularization in eyes. Moderate head bobbing, tremors and ataxia. Vocalization and hypersensitive during flexor reflex test. Difficulty standing. Severe uncoordination. Moderate whole body tremors while moving. Grade 3 induced shaking behavior.
6511/F	1200	6	Slight head bobbing and tremors. Difficulty standing. Ataxia. Activity decreased. Both eyes partly closed. Increased vascularization in eyes. Protruding nictitating membrane, both eyes. Labored respiration.
6553/F	1200	6	Protruding nictitating membrane, both eyes. Increased vascularization in eyes. Mucoïd discharge and opacity in eyes. Moderate uncoordination. Severe whole body tremors. Ataxia. Activity decreased. Partly digested food in tray.
5145/M	600	12	Both pupils dilated, even in bright light. Slight whole body tremors. Severe uncoordination. Unable to stand properly due to spasticity in all limbs.
1111/M	Vehicle	11	Catheter came out of the intrathecal space.

Clinical signs: Signs observed included those listed above for the euthanized dogs. When examined 24 hours after the beginning of treatment, all but one dog given 300 ng/kg/hr showed ataxia, tremors and/or hyperactivity at the three highest dose levels. At least half of these dogs also exhibited decreased or no pupillary light reflex and some exhibited dilated pupils. These signs were also observed by Day 7 in the low dose group (100 ng/kg/hr), so incidence and/or time of onset tended to be dose-related. The signs tended to be more severe in females, perhaps because dosing was done under the assumption that each dog weighed 10 kg, and the females tended to weigh less than the males. Two dogs per treatment group also displayed a decreased flexor reflex during the first week, whereas one (at 600 ng/kg/hr) showed an increased flexor reflex on days 7 and 14.

Body weights: No treatment-related effects on body weight or body weight gain were reported. After the first couple of days of infusion, however, special efforts were made to increase the appetite of many of the dogs receiving ziconotide by supplementing their pelleted diet with canned food.

Food consumption: Decreased food consumption during the first couple of days of ziconotide infusion prompted the contract laboratory to supplement the pelleted diet

_____ with canned food _____
 _____ for most of the dogs receiving the test article. Food consumption was reported to return to normal following initiation of this supplementation.

Ophthalmoscopy: The board-certified veterinary ophthalmologist reported that, due to marked shaking and hyperactivity, some of the dogs could not be evaluated thoroughly during the week 4 examinations, including 2 dogs in group 3, 5 dogs in group 5 and 1 dog in group 6. With this caveat, no treatment-related ocular changes could be identified by this expert.

EKG: Recordings were examined by _____ who indicated that the only change indicating an effect of treatment was an increase in the incidence of 2° A-V block in groups 5 and 6 accompanied by increases in respiratory sinus arrhythmia in groups 3, 4, 5 and 6, and by ventricular escape beats in one dog. He suggested that this could be due to an increase in vagal tone through a central effect on vagal nuclei, or less likely, due to an increase in the sensitivity of the S-A and A-V nodes to vagal input. He concluded that these changes were within the historical normal range and that there was no EKG evidence of cardiotoxicity *per se* in this study.

The percentages of the mean baseline heart rates of the different male and female group means that were measured at various times after initiation of treatment are summarized in the table below:

Group	Sex	% of Mean Pretreatment Heart Rates in Dogs During Infusion					
		24 hours	72 hours	Day 8	Day 15	Day 22	Day 29
1 (saline control)	Male	100.0	93.3	98.5	105.3	110.7	81.3
	Female	102.2	99.6	101.7	99.5	100.5	88.3
2 vehicle control)	Male	93.1	86.0	85.2	86.8	91.0	88.5
	Female	94.1	94.5	95.2	95.9	91.2	86.7
3 (100 ng/kg/hr)	Male	106.0	86.8	81.5	87.3	63.9*	63.0
	Female	97.2	74.0 ^a	62.7	81.3	82.7	76.1
4 (300 ng/kg/hr)	Male	96.3	72.6	66.6	75.4	74.5*	67.9
	Female	75.1	58.6 ^a	84.0	71.5	60.5	55.3
5 (600 ng/kg/hr)	Male	86.3	88.4	77.3	85.5	101.7	80.1
	Female	87.9	64.7 ^{*a}	96.0	103.5	72.2	76.8
6 (1200 ng/kg/hr)	Male	91.4	69.3	41.4	66.4	51.4	53.6
	Female	74.1	69.0	71.2	92.2	87.6	80.5

*Significantly different from saline control value, $p < 0.05$ (Dunnett's test).

^aSignificantly different from vehicle control value, $p < 0.05$ (Dunnett's test).

Hematology: There were sporadic significant differences from either saline or vehicle controls in some hematological parameters, such as white blood cell count, segmented neutrophils, monocytes, red blood cell count, hemoglobin and hematocrit, most of which appeared to be unrelated to dose and not biologically significant, as they remained within the normal physiological range. There were two changes that appeared to be dose-related during week 4, but since they occurred

only in females, their biological significance is unknown. These are shown in the shaded areas of the table below:

Parameter Measured	Sex	Group No.	Sampling time for parameter measured		
			Pretreatment	2 Weeks	4 Weeks
Mean no. of segmented neutrophils	F	1	6893	5774	7207
		2	9128	5060	4814
		3	5924†	6469	5279
		4	5708†	5169	5576
		5	7570	8094	9308†
		6	8294	8345	11607* ‡
Activated partial thromboplastin time (sec)	F	1	10.3	9.8	9.8
		2	10.1	9.7	9.6
		3	10.9	11.2** †	10.8
		4	10.3	10.5	10.9
		5	10.6	11.3** †	11.5*
		6	10.6	10.8	11.5*

*P<0.05 and **p<0.01 compared with saline (group 1) controls (Dunnett's test).

†P<0.05 and ‡p<0.01, compared with vehicle (group 2) controls (Dunnett's test).

Clinical chemistry: There were sporadic significant differences from either saline or vehicle controls in some chemistry parameters, such as creatinine, ALT, calcium, phosphorus, sodium and chloride, most of which appeared to be unrelated to dose and not biologically significant, as they remained within the normal physiological range. There were some changes that appeared to be dose-related, and three of these (elevated BUN, decreased albumin/globulin ratio and elevated potassium) occurred in both males and females. At most doses (all doses in males), the elevated BUN appeared to be greater at week 4 than at week 2. Dose-related changes are shown in the shaded areas of the table below:

Parameter Measured	Sex	Group No.	Sampling time for parameter measured		
			Pretreatment	Week 2	Week 4
Mean BUN	M/F	1	11.3 / 12.0	10.8 / 12.0	10.7 / 12.3
		2	10.9 / 11.4	10.7 / 11.7	10.9 / 13.6
		3	11.5 / 14.4	14.7 / 17.8*	15.1 / 15.1
		4	13.5 / 12.8	15.2 / 16.2	15.9 / 18.4*
		5	12.1 / 11.4	15.8*†/18.2**	18.9*†/18.4**
		6	9.5 / 11.3	13.6 / 15.8	15.9 / 20.9**
Mean glucose (mg/dl)	M/F	1	93 / 92	105 / 100	102 / 96
		2	98 / 102	103 / 105	102 / 111*
		3	106 / 94	100 / 98	89 / 91†
		4	98 / 104	99 / 94	91 / 85‡
		5	101 / 97	90 / 75**‡	94 / 83‡
		6	106 / 95	94 / 84†	83 / 82‡

Albumin/globulin ratio	M/F	1	1.11 / 1.28	1.18 / 1.30	1.26 / 1.23
		2	1.28 / 1.26	1.26 / 1.31	1.19 / 1.32
		3	0.97‡ / 1.13	1.00‡ / 1.15	1.10 / 1.17
		4	1.03† / 1.14	0.95*‡ / 1.13†	0.95**‡ / 1.10
		5	1.14 / 1.32	1.06† / 1.10*†	0.86**‡ / 1.03
		6	1.15 / 1.23	1.00 / 1.13	0.93 / 0.80**‡
Mean potassium (meq/l)	M/F	1	4.72 / 4.65	4.64 / 4.69	4.61 / 4.69
		2	4.67 / 4.69	4.32 / 4.52	4.62 / 4.66
		3	4.33 / 4.54	4.91 / 5.07	5.03 / 4.97
		4	4.56 / 4.51	5.07 / 5.31	5.26* / 5.16
		5	4.97 / 4.70	5.50*‡ / 5.94**‡	5.71**‡ / 5.53
		6	4.42 / 4.66	5.34 / 5.72*†	4.86 / 5.64*

*P<0.05 and **p<0.01 compared with saline (group 1) controls (Dunnett's test).

†P<0.05 and ‡p<0.01, compared with vehicle (group 2) controls (Dunnett's test).

Urinalysis: Sponsor reported no treatment-related changes.

Organ weights: Male dogs in low-dose and mid-dose groups had significantly higher body weights, absolute heart weights and absolute thymus weights that one or both of the control means. Heart weights normalized when expressed relative to body weight, whereas thymus weights remained elevated and kidney weights were significantly decreased. Females also showed a significant decrease in relative kidney weights, as shown in the following table:

Organ	Sex	Mean organ weight relative to body wt. in following groups					
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Body wt. (kg)	M	8.6±1.0	8.5±0.7	11.2±0.6 ^a	10.4±1.2*	9.4±0.4	8.9 (n=1)
Kidneys, g/kg	M	7.6±0.9	7.3±0.7	5.5±0.5 ^a	5.5±0.3 ^a	7.0±0.9	8.0 (n=1)
Thymus, g/kg	M	0.6±0.4	0.4±0.1	0.8±0.2*	0.8±0.4*	0.5±0.1	0.2 (n=1)
Body wt. (kg)	F	8.1±1.3	7.6±1.9	9.3±0.5	9.1±0.6	6.1±0.8	7.0±0.3
Kidneys, g/kg	F	7.4±1.3	7.1±0.5	5.7±0.3**	6.1±0.2**	7.6±0.4	6.6±0.5

*P<0.05 or **p<0.01, compared with vehicle (group 2) controls (Dunnett's test).

^aP<0.01 compared with both saline (group 1) and vehicle (group 2) controls (Dunnett's test).

Gross pathology: Abnormal observations were either attributed to the experimental procedure, the most common being dilation of the brain ventricles, or to incidental findings commonly seen in laboratory dogs. This reviewer noted, however, that there appeared to be a somewhat dose-related increase in the incidence of reports of a small prostate in the male dogs as shown in the following table:

Prostate	Gross pathology reports of small prostate in male dogs ^a					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Number examined	4	4	4	4	5	1
No. reported small	0	0	1	3	5	1
% Incidence small	0%	0%	25%	75%	100%	100%

^a Does not include recovery males (one each in groups 1, 2 and 5), all of which had prostates of normal size.

Histopathology:

Catheter placement	Sex	Dog Identification Numbers					
		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Inadequate ¹	M	1011 ²	2135				
	F		2511			5522 ²	6553 ³
Subdural	M	1011 1111				5022	
	F	1523					
Unknown	M					5033	

¹ Gross examination after injecting dye through catheter. ² Recovery animal. ³ Euthanized on day 6.

The adverse microscopic findings were considered to be secondary to the mechanical damage and resulting inflammation/infection caused by the catheter in all animals in both control and ziconotide-treated animals. Compression of the spinal cord was often associated with degeneration of nerve fibers characterized by myelin sheath dilation, axonal swelling and/or axonal debris with or without macrophages surrounded by a myelin sheath. Purulent meningitis occurred at and above the infusion site in some dogs, including the brain of one female saline control (group 1) dog. Mixed cell infiltration observed in the brain and various spinal levels was thought to be an extension of the meningeal inflammation and not drug-related.

Toxicokinetics: As indicated in one of the tables above, the HPLC analysis of the dosing solutions found lower concentrations of ziconotide than what were targeted. This deficiency was attributed to a concentration-related loss due to freezing and thawing, as well as long-term frozen storage of samples before their analysis, such that the lower concentrations of dosing solutions showed greater percentage losses than did the higher concentrations (Addendum, Vol. 23, pp. 398-402). It should be noted that similar storage-related deficiencies might occur in samples saved for toxicokinetic studies with plasma samples, which were analyzed by RIA. A summary of the toxicokinetic analyses appears in the following table:

	Plasma ziconotide (ng/ml) in the 28-day dog toxicity study					
	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Number examined	≤14	10	8	8	9	4
Week 2: N >0-level	0	0	6	5	8	4
Mean±SD of N>0			0.11±.08 ^a	0.45±.17	1.26±.47	2.04±1.25
Week 4: N >0-level	0	0	3	7	6*	3**
Mean±SD of N>0			0.05±.07 ^a	0.43±.15	1.29±.53	2.16±1.22
Recovery animals	0	0			0	

^aIncludes 0-level measurements.

*Excludes 2 dogs (one euthanized on day 12 and one dosed only through day 16) with no sample.

**Excludes 1 dog with low value (0.22 ng/ml) where the pump occluded starting the day before sampling.

Key Study Findings:

Dose-related changes occurred in both sexes in severity of clinical/neurological signs (motor dysfunction, pupillary light reflex, nictitating membrane function), incidence of EKG abnormalities (2° A-V block and respiration-related arrhythmias), blood urea nitrogen (increased), glucose (decrease significant only in females), potassium (increased) and the albumin/globulin ratio (decreased). Two dose-related changes occurred only in females (increases in both number of segmented neutrophils and activated partial thromboplastin time), and one was anatomically restricted to males (incidence of small prostate size) that was not observed in any of the male controls.

General Comments: Dose-related changes in hematological parameters seen only in females (increases in both number of segmented neutrophils and activated partial thromboplastin time) are not likely of biological significance.

- A decrease in the albumin/globulin ratio may be caused by the release of interleukin-1 and/or other cytokines (stimulated by bacterial lipopolysaccharide or an infection), so it is not clear why this should be dose-dependent.
- The decrease in blood glucose is difficult to interpret, as it is not known whether the carbohydrate intake was the same in the control and ziconotide-treated dogs, due to the fact that many of the treated dogs were supplemented with canned food, whereas the controls had just pelleted food.
- The dose-related increase in BUN, accompanied by sporadically significant slight increases in creatinine, as well as the dose-related increase in potassium, could be symptomatic of dehydration. Although water was available *ad libitum* to the dogs, water consumption was not measured. Consequently, the motor deficits caused by ziconotide may have caused a decrease in water consumption. However, a comparison of the specific gravities of the pretreatment urines from dogs in the two high-dose groups with those at week 2 and at week 4 did not show any increase in specific gravity with treatment.
- The elevations of BUN and potassium are not likely due to ziconotide-induced kidney disease, as the only kidney disease identified by histopathology was interstitial nephritis in one of the saline control dogs. The increased potassium, however, may have contributed to the 2° A-V block and reduction in heart rate observed with ziconotide treatment.

Summary: Dogs given continuous IT infusions of ziconotide of 100, 300, 600 or 1200 ng/kg/hr displayed ataxia, tremors and/or hyperactivity with onset and severity related to dose and females being more sensitive than males. Four dogs (3 females) at 600 or 1200 ng/kg/hr were euthanized in poor condition. At least half of the ziconotide-treated dogs exhibited decreased or no pupillary light reflex and some exhibited dilated pupils. Two dogs per treatment group also displayed a decreased flexor reflex during the first week, whereas one (at 600 ng/kg/hr) showed an increased flexor reflex on days 7 and 14. Appetite and body weight were maintained by supplementing treated groups with canned food. Dose-related decreases in albumin/globulin ratio, increases in BUN and increases in serum potassium were observed. In females only, dose-related increases in number of segmented neutrophils and activated partial thromboplastin time were

observed. A dose-related increase in the incidence of reports of a small prostate in the male dogs (5/5 at 600 and 1/1 at 1200 ng/kg/hr) was not seen in the one recovery male treated with 600 ng/kg/hr. The contract lab concluded 300 ng/kg/hr to be the NOAEL dose.

The sponsor revised the NOAEL to ≥ 180 ng/kg/hr (from 300) following analysis of the dosing solutions. Considering, however, that only one dog receiving the target rate of 300 ng/kg/hr failed to show signs of ataxia, tremors and/or hyperactivity, and that two dogs receiving the next highest target rate of 600 ng/kg/hr required humane euthanasia before the midpoint of the intended infusion duration because of toxicity, it is this reviewer's opinion that the NOAEL in this study was the target dosing rate of 100 ng/kg/hr, IT (but analyzed at 26-34% of target dose). On a body surface area basis, this is approximately equivalent to $2.0 \mu\text{g}/\text{m}^2/\text{hr}$, which is slightly greater than the maximum recommended human dose of $1.5 \mu\text{g}/\text{m}^2/\text{hr}$ [$(2.4 \mu\text{g}/\text{patient}/\text{hr})/(1.6 \text{m}^2/\text{patient})$].

Comments: The following study was initially reviewed by Dr. [redacted] when submitted to IND 45,718. However, the sponsor's addendum submitted with the NDA led this reviewer to re-examine this study and to conclude that this study was inadequate for assessing the toxicity of ziconotide administered by the IT route.

Study Title: A Study to Evaluate the Effects of SNX-111 Introduced into the Intrathecal Space of Beagle Dogs by 42-Day Continuous Infusion.
(With Addendum)

Study No.: 2-P46 (Addendum, NC0191)

Volume #2.026, page #2 to Vol. 2.027, page #184 (Addendum, Vol. 2.027, page #185)

Conducting laboratory and location: [redacted]

Date of study initiation: July 13, 1992 (randomization)

GLP compliance: Yes, except for analysis of dose preparation solutions, analysis of CSF and plasma samples and inadequate record-keeping of the latter by Neurex Corp.

QA-Report: Yes (X) No ()

Methods:

Five groups of male and female beagle dogs about 9 months of age were given saline, vehicle or one of three concentrations of ziconotide intrathecally at a rate of 0.1 ml/hr through a catheter implanted surgically in the lumbar region (under methohexital, 5.5 mg/kg, plus isoflurane anesthesia) approximately 2 weeks prior to initiating the dosing infusion. Two days before study initiation, all dogs were given dexamethasone (0.55 mg/kg/day, 2 days) to decrease cervical nerve swelling. The continuous infusion was intended to last 6 weeks (42 days).

Dosing:

The following table provides a summary of the intended dosing protocol and deviations therefrom, which includes studies #2-P46 (main study), #98-5067 (analysis of dosing solution batches) and #98-5047 (analysis of CSF and plasma levels):

Grp. No.	N M/F	Treatment (0.1 ml/hr)	Dose, µg/kg/day		42-Day completers	Conc. (ng/ml)*	
			Target	% of target ¹		CSF ^a	Plasma
1	3/3	0.9% Saline	Control	—	3 M, 3 F	—	—
2	3/3	Mannitol ²	~4	—	2 M, 3 F	—	—
3	4/4	Ziconotide	0.01	0%	4 M, 3 F ³	0.48	N.D.
4	4/4	Ziconotide	0.1	22-34%	3 M, 4 F	0.06	N.D.
5	4/4	Ziconotide ²	1	31-49%	4 M, 1 F	1.09	N.D.

* Blood (peripheral vessel) and CSF (cisterna magna) samples were collected at necropsy on Day 43 and analyzed by RIA with a limit of quantification of [REDACTED]

¹ Based upon HPLC analysis of dosing solution batches stored frozen for 10 months and not well sealed; the limit of quantification by HPLC was approximately [REDACTED]

² Mannitol concentration was [REDACTED]

³ One female was found dead on day 38; the other 5 dogs that failed to complete 42 days of treatment had breaks in their infusion lines (between days 10 and 23).

^a Mean of 7-8 dogs, but the majority (4-6/group) had no detectable CSF levels.

N.D. = Not detected in any samples.

- Species/strain: Dog / Beagle
- No./sex/group: 3 in two control groups; 4 in three ziconotide treatment groups.
- Age: ~ 9 months.
- Weight: Males, 8.5–11.7 kg; females, 7.9–10.1 kg.
- Satellite groups used for toxicokinetics or recovery:
- Dosage groups in administered units: See Table XX above.
- Route, form, volume and infusion rate: IT, solution, 0.1 ml/hour.

Drug, lot#, radiolabel, and % purity: Lot #NUX002 [REDACTED]

Formulation/vehicle: Sterile saline for injection ([REDACTED]) or mannitol, 18% [REDACTED]

Observations and times:

- Clinical signs: Twice daily cageside observations for mortality and moribundity, and once daily examinations of skin, hair, eyes, mucous membranes, respiratory system, circulatory system, CNS, somatomotor activity, behavior pattern, occurrence of tremors, convulsions, diarrhea, lethargy, sleep or coma were recorded.
- Body weights: Recorded prior to treatment, twice weekly thereafter and at necropsy.
- Food consumption: Individual food consumption was measured daily.
- Hematology: Blood (0.75 ml in EDTA) was collected for hematology prior to initiation of treatment, on day 15 and on day 43 prior to euthanasia. Blood smear examination included absolute and relative nucleated RBC, band, lymphocyte, monocyte, polymorphonuclear neutrophil, eosinophil and basophil counts; anisocytosis, poikilocytosis, hypochromasia and polychromasia, as well as RBC, WBC and platelet counts, HGB, HCT, MCV, MCH and MCHC.
- Clinical chemistry: Blood (1.0 ml) was collected for clinical chemistry prior to initiation of treatment, on day 15 and on day 43 prior to euthanasia and analyzed with a [REDACTED] Chemistry Analyzer for ALT, AST, ALK, BUN, CRE, CHOL, TPR, ALB, GLOB, ALB/GLOB ratio, GLU, TRG, TBIL, CAL, PHOS, NA, K and CL.
- CSF: Two aliquots (total 3-5 ml) were collected from the cisterna magna at

termination, one being centrifuged, frozen and sent to the sponsor for analysis of ziconotide. The other was processed for cell counts (including differential), total protein

- Gross pathology: Dogs were euthanized under sodium pentobarbital anesthesia and perfused whole body with cold phosphate-buffered saline, followed by 3-4 liters of 4% paraformaldehyde in 0.2M phosphate buffer at ~4°C.

Histopathology: Sections of brain (medulla/pons, cerebellum, cerebral cortex at the level of the basal ganglia, hypothalamus and mid-brain), sciatic nerve and spinal cord (cervical, thoracic and lumbar, including 3 in the immediate vicinity ±2 cm of the catheter tip) underwent special fixation procedures for immunocytochemical processing. Histopathologic evaluation of the tissues listed in the table below was conducted by

Abnormal tissues (masses)	X
Adrenals	X
Aorta	X
Bone marrow (sternum)	X
Brain	X
Cecum	X
Cervix	X
Colon	X
Duodenum	X
Epididymides	X
Esophagus	X
Eyes	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X
Ileum	X
Infusion site	X
Jejunum	X
Kidneys	X
Lachrymal gland	
Larynx	X
Liver	X
Lungs	X
Lymph nodes, cervical	
Lymph nodes, mandibular	X
Lymph nodes, mesenteric	X
Mammary glands	X
Nasal cavity	
Ovaries (and oviducts)	X
Optic nerves	
Pancreas	X
Parathyroids	X
Peripheral nerve	X
Pharynx	X
Pituitary	X
Prostate	X
Rectum	X
Salivary glands	X
Seminal vesicles	

Appears This Way
On Original

Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X
Sternum	X
Stomach	X
Testes	X
Thymus	X
Thyroid	X
Tongue	X
Tonsils	X
Trachea	X
Urinary bladder	X
Uterus	X
Vagina	X

Appears This Way
On Original

- Toxicokinetics: Blood (10 ml in EDTA) was collected for ziconotide analysis prior to initiation of treatment, on day 15 and on day 43 prior to euthanasia.
- Other: a general neurological examination was performed weekly. A physical examination, including rectal body temperature and heart rate, was performed prior to initiation of treatment and prior to euthanasia. Indirect blood pressure recordings were taken prior to the initial treatment, at 1, 2, 4, 8 and 24 hours after the initial treatment and on days 3, 7, 15, 22, 29, 36 and 43.

Results:

- Clinical signs: One low-dose female was found dead on day 38. Otherwise, there appeared to be no ziconotide-related effects on clinical observations because the findings (hunched posture, sensitivity to touch, lethargy, soft feces, swelling of the neck or crown, shaking, twitching) were observed in all groups and considered to be secondary to the catheter placement procedure. Five dogs (vehicle male, mid-dose male and 3 high-dose females) did not complete the infusion due to a break in the infusion line (on days 10, 13, 11, 15 and 23, respectively).
- Body weights: No significant effects were observed.
- Food consumption: No significant effects were observed.
- Hematology: Males receiving ziconotide also showed significant decreases in hemoglobin (13.4%, 15.9% and 14.6%) and in hematocrit (13.5%, 14.9% and 14.3%) in groups 3, 4 and 5, respectively, compared with group 1 values, on day 15, but not on day 43 (and not in females on either day 15 or day 43).
- Clinical chemistry: No ziconotide-related effects were reported. Mid-dose females had significantly elevated sodium on day 15 and low-dose females had significantly elevated calcium on day 43.
- Gross pathology: No ziconotide-related effects were reported. Discoloration or dark foci on the spinal cord and/or brain, foci in the GI tract and dark lymph nodes were observed.
- Histopathology: Immunocytochemical examination of neural tissues was not conducted. Other findings were primarily limited to inflammatory, degenerative and reactive changes at the brain and spinal cord, especially at the infusion site, but were not dose related.
- Toxicokinetics: Analysis of CSF and plasma samples were reported in Study No. 98-5047. The results are presented in the table and accompanying footnotes under

granted, provided that a Syrian Hamster Embryo (SHE) Cell Assay be conducted and the results of this assay proved negative (See Appendix, p. 129, for summary of PTCC minutes). The final study report for this assay was reviewed under IND 45,718 submission N-180 and is reproduced below for the sake of completeness.

Study Title: Clonal Transformation Assay Using Syrian Golden Hamster Embryo (SHE) Cells

Study Number: AA18WM.308. —

Volume #2.047, page #: 224

Test Facility: . —————

Study Date(s): August 11 – September 22, 1999

GLP Compliance: Yes (X) No ()

QA Report: Yes (X) . No ()

Study Type: *In vitro* SHE cell assay

Species/strain: Hamster / Syrian golden , —————

Drug Lot No.: A33294

Drug Purity / Stability / Homogeneity: Peptide content, ———; peptide purity, ———

Doses:

Basis of Dose Selection: Preliminary cytotoxicity assay with a 7-day dosing regimen which resulted in a relative reduction in plating efficiency of 77% at the low dose of 0.05 mg/ml to only 23% at the highest dose evaluated, 1.0 mg/ml (Table 1).

Relation to Clinical Use: Highest concentration tested for SHE cell transformation was 10 times the highest available concentration of the human IT dosage form.

CAC Concurrence: Protocol not submitted to CAC for review.

Route of Administration: Mixed into culture medium, Dulbecco's modified Eagle's medium-LeBoeuf's modification (DMEM-L).

Frequency of Administration: One dose/plate.

Dual Controls Employed: Complete growth medium DMEM-L vehicle (negative control) obtained from Quality Biological and benzo(a)-pyrene (positive control) obtained from —————

Exposure Conditions ($37 \pm 1^\circ\text{C}$ in humid atmosphere containing $10 \pm 0.5\%$ CO_2):

Cytotoxicity assay #1: One 24-hour exposure plus 6 days of culture in DMEM-L.

Cytotoxicity assay #2: Seven days' exposure undisturbed.

Transformation assay: Seven days' exposure undisturbed.

Scoring of cloning efficiency and transformation dishes:

[—————]

(Cells in morphologically transformed colonies frequently are more basophilic than their control counterparts and have increased nuclear/cytoplasmic ratios [Isfort *et al.*, 1994]).

Criteria for test validity:

- Total number of colonies per group for all groups should be >1000.
- Vehicle controls should have 25-45 colonies/plate.

- Minimum of 5 scorable dose levels.
- Transformation frequencies of positive control groups should be significantly greater than vehicle control ($p < 0.05$ by Fisher's Exact Test).

Statistical analysis:

Pairwise comparisons of transformation frequencies between vehicle control group and each treated group were conducted using a one-sided Fisher's Exact Test.

Deviations from Original Protocol:

- After being x-ray irradiated and centrifuged, feeder cells were resuspended in 45 ml of medium instead of 30 ml.
- Before treatment, target cells were incubated for 27 hours, 15 minutes, instead of 24 hours.
- One of 10 dishes from 3 of the 8 dose groups in the first cytotoxicity assay did not receive 4 ml of dosing solution, due to foaming of the dosing solution.
- In the 24-hour cytotoxicity assay, cells were not rinsed twice with 4 ml of HBSS before being re-fed with 8 ml of fresh medium after the 24-hour exposure to test medium.

Results:

TABLE 1: SHE cell transformation assay summary.

Substance/Dose	Total n Scored	MTC	MTF	Colonies, mean/dish	PE (%)	RPE (%)	MT p-value
Vehicle (DMEM-L)	1692	2	0.11	42	26	100	
Benzo(a)pyrene, 5 µg	1373	5	0.30	41	22	85	0.1510
Same, 10 µg	1404	8	0.57	35	22	85	0.0229*
Same, 12.5 µg	1622	11	0.68	35	26	100	0.0093*
Ziconotide 0.05 mg/ml	1245	3	0.24	32	20	77	0.3595
Ziconotide, 0.1 mg/ml	1188	4	0.34	30	19	73	0.1983
Ziconotide 0.25 mg/ml	1411	6	0.43	37	15	58	0.0933
Ziconotide, 0.5 mg/ml	1280	3	0.23	32	13	50	0.3707
Ziconotide 0.75 mg/ml	1210	2	0.17	31	10	38	0.5523
Ziconotide, 1.0 mg/ml	1064	1	0.09	27	6	23	0.6693
Ziconotide, 1.5 mg/ml	Not evaluated due to toxicity						

*Statistically significant difference at the $p < 0.05$ level of confidence (1-tail Fisher's test).
n = Number of colonies per test group. **MTC** = Morphologically transformed colonies.
MTF = Morphological transformation frequency. **PE** = Mean plating efficiency.
RPE = Relative plating efficiency. **MT** = Morphological transformation compared with control.

The positive control caused a dose-related increase in the morphological transformation frequency (MTF) of SHE cells, with the mid and high doses of benzo(a)pyrene being statistically significant. All concentrations of ziconotide evaluated were more cytotoxic than any concentration of the positive control. Although two doses of ziconotide had a larger MTF than the low dose of the positive control, their effects were not statistically significant and ziconotide did not show a dose-related effect.

CONCLUSIONS:

Cytotoxic concentrations of ziconotide were negative in the SHE cell test for morphological transformation. Consequently, according to the recommendations of the PTCC (May 20, 1999), the sponsor does not need to do further non-clinical carcinogenicity studies with ziconotide.

IMMUNOTOXICOLOGY:

Study Title: Immunogenicity of Ziconotide (SNX-111) in Swiss Webster Mice

Study No.: [REDACTED] 291

Volume #2.048, page #002

Site and testing facility: [REDACTED]

Date of study initiation: April 28, 1993

GLP compliance: Yes

QA-Report: Yes (X) No ()

Lot and batch numbers: SNX-111, 8068 SJK006, [REDACTED]; peptide purity [REDACTED]
[REDACTED] peptide content [REDACTED] also contains acetic acid, 12.6%, trifluoroacetic acid, 0.08% and water, 7.9%; vehicle: 0.9% sodium chloride USP [REDACTED]

Methods:

- Species/ strain: Mouse / Swiss Webster [REDACTED]
- Doses employed: 40 µg/mouse, IV (100 µl of a 0.4 mg/ml solution) for group 1, vehicle, IV (100 µl of 0.9% saline) for group 2, and 20 µg/mouse, ID (50 µl of a 0.4 mg/ml solution).
- Route of administration: Intravenous on days 0 and 28; intradermal on day 37.
- Rationale: Twenty mice 5-7 weeks old were quarantined for two weeks and then retested for viral antibodies in serum isolated from blood (>200 µl) obtained from a tail vein. The tests for 16 viruses were negative.
- Number of animals/sex/dosing group: 10 females/group for IV route; all 20 females for intradermal route.
- Endpoints:

[REDACTED]

- Observations and times: On Days 14 and 40, serum was isolated from blood (>200 µl) obtained from a tail vein from the 20 mice and stored at -20°C, until assay for the presence of antibodies to ziconotide by an [REDACTED] ELISA procedure.

Results: The mice showed no behavioral reaction to the ziconotide injections. A temporary slight redness was observed at the injection site in three mice (group 2) which was considered to be clinically insignificant. Sera samples from all of the mice at all sampling times had no detectable levels of antibody to ziconotide.

Summary:

Serum from female Swiss Webster mice collected 14 days after IV ziconotide (40 µg/mouse) and again on day 40 after a second IV injection (40 µg/mouse) on day 28 and 3 days after an intradermal injection (20 µg/mouse) on day 37 was negative for antibodies to ziconotide, as measured by an ELISA assay for measuring titers of anti-ziconotide antibodies.

Study Title: Active Immunization of Rats with SNX-111

Study No.: 95-5020

Volume #2.048, page #176

Site and testing facility: Neurex Corporation, Menlo Park, CA

Date of study initiation: Not indicated (IACUC protocol approved 10/23/1995)

GLP compliance: No

QA-Report: Yes () No (X)

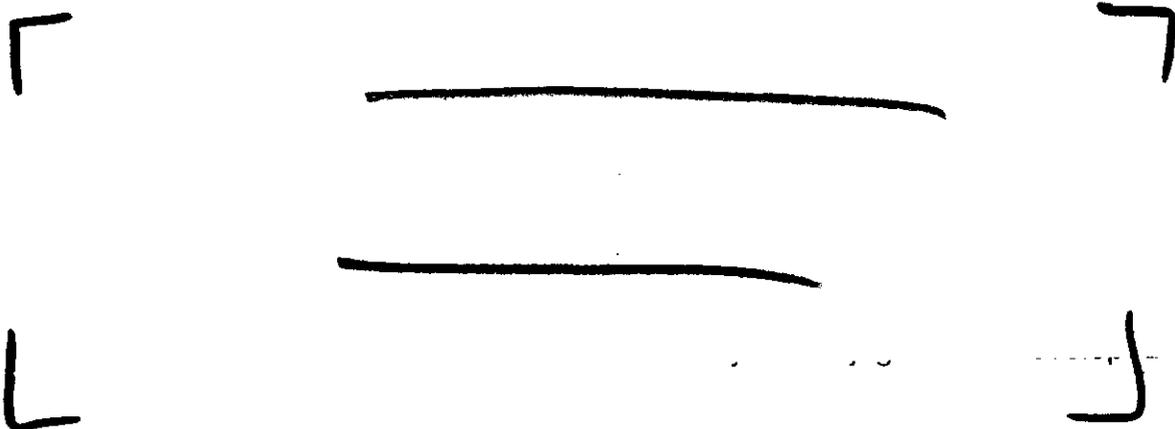
Lot and batch numbers: SNX-111, 8068 SME013, [redacted] peptide purity, [redacted] peptide content 78.4%; also contains acetic acid, 14.4%, trifluoroacetic acid, 0.08% and water, 6.4%; vehicle: 0.9% sodium chloride USP for injection, [redacted]

Methods:

- Species/ strain: Rat / Sprague-Dawley, [redacted]
- Doses employed: Ziconotide in 0.9% saline (n=10 rats) or saline alone (n=10 rats) were mixed 1:1 with Freund's complete adjuvant (FCA) or Freund's incomplete adjuvant (FIA). The dose of ziconotide/rat was 50 µg and administered according to the following treatment schedule:

Study Day	Procedure Performed
1	Collect pre-immune serum; inject 0.5 ml IP and 0.25 ml IM in each rear leg.
21	First booster injection (with FIA): 0.5 ml IP and 0.5 ml SC
28	Collect serum samples.
35	Second booster injection (with FIA): 0.5 ml IP and 0.5 ml SC
42	Collect serum samples.
49	Third booster injection (with FIA): 0.5 ml IP and 0.5 ml SC
56	Collect serum samples.
63	Fourth booster injection (with FIA): 0.5 ml IP and 0.5 ml SC
70	Collect terminal serum samples by cardiac puncture.

- Route of administration: See above treatment schedule.
- Rationale: The purpose of this investigation was to assess the immunogenicity of ziconotide in Sprague-Dawley rats.
- Number of animals/sex/dosing group: 10 males/group.
- Endpoints: The search for antibodies to ziconotide involved use of an [redacted] ELISA procedure. [redacted]



- Observations and times: See table above.
- Results: Sera samples from all of the rats at all sampling times after the beginning of the immunization protocol had no detectable levels of antibody to ziconotide, when subjected to an ELISA assay. However, if Protocol No. 95-5007 for the ELISA assay (Appendix B, pp. 186-191) was followed as the final report indicates, the wrong secondary antibody (anti-rabbit antibody) may have been used for studying rat sera.

Summary:

Serum samples collected from male Sprague-Dawley rats at various times during an immunization procedure involving the injection of ziconotide with Freund's complete adjuvant (IP and IM) or with Freund's incomplete adjuvant (IP and SC) were negative for antibodies to ziconotide, as measured by an ELISA assay protocol that appears to have been meant for measuring rabbit antibodies. Therefore, no valid conclusions can be drawn from these negative results.

Study Title: Antigenicity Study in Guinea Pigs: Systemic Anaphylaxis and Passive Cutaneous Anaphylaxis Reactions

Study No.: 742A-NEU-001-94

Volume #2.048, page #120

Site and testing facility: _____

Date of study initiation: March 9, 1994

GLP compliance: Yes

QA-Report: Yes (X) No ()

Lot and batch numbers: SNX-111, 8068 SJK006, _____ peptide purity _____

_____ peptide content 76.2%; also contains acetic acid, 12.6%, trifluoroacetic acid, 0.08% and water, 7.9%; vehicle: 0.9% sodium chloride USP

Methods:

- Species/strain: Guinea pig / Hartley
- Doses employed: Dose range-finding: 5 and 10 mg/kg, IP; 1 mg/kg, IV
Main study, Induction: 1 and 5 mg/kg, in vehicle \pm 10%

adjuvant (aluminum hydroxide), 3 times/week for 2 weeks.
 Challenge: 1 mg/kg, IV
 A positive control group was treated with 200 µg/kg ovalbumin in adjuvant, IP, and challenged with 10 mg of ovalbumin, IV.

- Route of administration: See above. The guinea pigs were anesthetized with ketamine/xylazine for the IV injections.
- Rationale: For passive cutaneous anaphylaxis testing, naïve animals were injected intradermally on the back with serum diluted 1:10 and 1:100 from the "sensitized" guinea pigs. Eighteen hours later, they were anesthetized with ketamine/xylazine and the test article or ovalbumin (0.3 mg) in 0.5% Evans blue was injected IV. Edema and blue staining at the intradermal injection site indicate a positive reaction.
- Number of animals/sex/dosing group: Two males/group for dose range-finding; 5 males/group in the main study; 10 males in the passive cutaneous anaphylaxis study.
- Endpoints: Symptoms of anaphylaxis were recorded, including restlessness, trembling, rubbing the nose, sneezing and coughing, urination, dyspnea, jumping and rushing, gasping and writhing, convulsion, and death.
- Observations: These were made in the main study immediately after the challenge and at 1, 2, 4 and 24 hours after the challenge.

Results:

Group	Treatment	Dose, mg/kg	Adjuvant	Anaphylaxis Testing, No. of Positive Responses		
				SYSTEMIC	PASSIVE CUTANEOUS	
				No. of Deaths	1:10 dilution	1:100 dilution
1	Vehicle	—	+	0	0	0
2	Ziconotide	5	+	1	1	0
3	Ziconotide	1	+	0	0	0
4	Ziconotide	5	—	1	0	0
5	Ziconotide	1	—	1	0	0
6	Ovalbumin	0.2	+	5	5	5

Deaths occurred 4-16 minutes after the challenge dose. Necropsy indicated an anaphylactic response in all the animals that died. It was observed that all of the animals immunized and anesthetized for administering the challenge dose exhibited prolonged effects of the anesthesia. Among the survivors at 24 hours, one animal immunized with 1 mg/kg with alum showed decreased activity, trembling and low body temperature. The guinea pig that showed a positive passive cutaneous response at the 1:10 dilution of serum received that serum from the same guinea pig that died upon systemic challenge after being treated with the 5 mg/kg doses with adjuvant. The contract laboratory concluded that these results were anomalous, that no definite conclusion can be drawn and recommended that the study be repeated with larger numbers of animals.

Summary:

In studies of systemic anaphylaxis in guinea pigs treated IP with 1 or 5 mg/kg of ziconotide, with or without adjuvant, and passive cutaneous anaphylaxis in guinea pigs treated intradermally with diluted serum from the guinea pigs receiving IP ziconotide, two low dose animals, one treated with adjuvant and one without adjuvant, died of systemic anaphylaxis, whereas only one high-dose (with adjuvant) guinea pig showed systemic anaphylaxis. Only serum (1:10 dilution) from this high-dose animal was positive for passive cutaneous anaphylaxis. In contrast, all 5 guinea pigs immunized with ovalbumin as a positive control demonstrated the expected systemic and passive cutaneous anaphylactic responses.

Study Title: Antigenicity Study in Guinea Pigs: Systemic Anaphylaxis

Study No.: 742B-NEU-001-94

Volume #2.048, page #147

Site and testing facility: _____

Date of study initiation: December 5, 1994

GLP compliance: Yes

QA-Report: Yes (X) No ()

Lot and batch numbers: SNX-111, CS122293, _____

peptide purity _____ in 7/93, but post-study analysis was 92.4%, with two unidentified impurities _____

adjuvant: aluminum hydroxide (_____)
vehicle: 2.5% dextrose plus 0.45%
ovalbumin (_____) sodium chloride, pH 4.5.

Methods:

- Species/strain: Guinea pig / Hartley (_____)
- Doses employed: Main study, Induction: 1 and 5 mg/kg, in vehicle \pm 10% adjuvant (aluminum hydroxide, first dose only), 3 times/week for 2 weeks.
Challenge (day 33): 1 mg/kg
A positive control group was treated with 200 μ g/kg ovalbumin in adjuvant, IP, and challenged with 10 mg of ovalbumin, IV.
- Route of administration: See above. The guinea pigs were anesthetized with ketamine/xylazine for the IV injections.
- Rationale:
- Number of animals/sex/dosing group: 15 males/group.
- Endpoints: Symptoms of anaphylaxis were recorded, including restlessness, trembling, rubbing the nose, sneezing and coughing, urination, dyspnea, jumping and rushing, gasping and writhing, convulsion, and death.
- Observations: These were made immediately after the challenge and at 1, 2, 4 and 24 hours after the challenge.

Results:

Group	Treatment	Dose, mg/kg	Adjuvant	No. in Group	Number of Deaths	P from Fisher's Exact Test
1	Vehicle	—	+	15	2	
2	Ziconotide	5	+	14	8	0.021
3	Ziconotide	1	+	15	7	0.109
4	Ziconotide	5	—	15	6	0.215
5	Ziconotide	1	—	15	4	0.651
6	Ovalbumin	0.2	+	15	15	<0.001

One high-dose (with adjuvant) animal was found dead after the first injection in the treatment phase. In the challenge phase, deaths occurred 1-38 minutes after the challenge dose, except the two vehicle controls found dead at 24 hours after the challenge. Necropsy indicated an anaphylactic response in all the animals that died, except the vehicle controls. Findings included purple discoloration of the extremities, skeletal muscle and connective tissue, hyperinflation of the lungs, and air-filled stomachs. This lot of ziconotide was antigenic.

Summary:

In a study of systemic anaphylaxis in guinea pigs treated IP with 1 or 5 mg/kg of ziconotide, with or without adjuvant (aluminum hydroxide), the high-dose (with adjuvant) group had a significantly higher incidence of deaths due to systemic anaphylaxis upon challenge with ziconotide than did the vehicle controls. All 15 guinea pigs immunized with ovalbumin as a positive control demonstrated the expected anaphylactic response to ovalbumin challenge.

Study Title: Passive Cutaneous Anaphylaxis Reaction in Guinea Pigs

Study No.: ■ 745GP-NEU-001-95

Volume #2.048, page #195

Site and testing facility: _____

Date of study initiation: July 14, 1995

GLP compliance: Yes

QA-Report: Yes (X) No ()

Lot and batch numbers: SNX-111, 8068 SME013 _____ peptide purity _____
 _____ peptide content 78.4%; also contains acetic acid, 14.4%, trifluoroacetic acid, 0.08% and water, 6.4%; vehicle: 0.9% sodium chloride USP

Methods:

- Species/strain: Guinea pig / Hartley _____
- Doses employed: Ziconotide: 1 mg/kg, IV
 Evans blue: 0.5 ml of a 1% solution, IV
 Human serum: 50 µl of 5, 10, 20, 40, 80, 160 and 320-fold dilutions in phosphate-buffered saline, intradermal.
- Route of administration: See above. The guinea pigs were anesthetized with

ketamine/xylazine for the IV injections.

- Rationale: For passive cutaneous anaphylaxis testing, naïve animals were injected intradermally on the back with one of the serum dilutions in duplicate from human subjects who had received 24-hour infusions of ziconotide in doses ranging from 0.0003 to 1.0 mg/kg/24 hr, 12-26 months (mean, 20.5 months) before the samples were taken. Nineteen samples were from subjects who had received ziconotide (median infusion rate, 0.01 mg/kg/24 hr) and seven were from subjects who had received placebo infusions. The route of infusion (e.g., IV or IT) was not identified in the study report. Four hours later, the guinea pigs were anesthetized with ketamine/xylazine and ziconotide (1 mg) was injected IV, followed by the IV administration of 0.5 ml of 1% Evans blue.
- Number of animals/sex/dosing group: One male/serum dilution.
- Observations: One hour after the IV challenge dose and Evans blue administration, the guinea pigs were euthanized by CO₂ inhalation, and the bluing region around the intradermal injection sites were measured.
- Endpoints: If the mean major and minor axes of the bluing area was 5 mm or greater, the response was regarded as positive.

Results: One hour after the guinea pigs received the IV challenge of ziconotide and Evans blue, no reactions were detected in any of the animals at any of the serum dilutions. Therefore, this test failed to detect any antibodies to ziconotide in serum from patients acutely treated with ziconotide a year or more before the serum samples were taken.

Summary:

When 50 µl samples of diluted serum (as concentrated as 1:5 dilution) obtained from human patients at least 12 months (mean, 20.5 months) after they had received a 24-hr infusion with placebo or ziconotide (median dose, 0.01 mg/kg, route not identified) were injected intradermally in guinea pigs for the passive cutaneous anaphylaxis test for the presence of antibodies to ziconotide, none of the samples gave a positive reaction.

REPRODUCTIVE TOXICOLOGY:

Study Title: An Intravenous Infusion Male Fertility Study of Ziconotide in the Rat

Study No.: 96428

Volume #2.034, page #002

Conducting laboratory and location: _____

Date of study initiation: February, 1999

GLP compliance: Yes

QA-Report: Yes () No ()

Lot and batch numbers: Ziconotide lot# 8068 A33294 \ _____ ; vehicle (ziconotide diluent, 0.9% saline + 50 µg of L-methionine/ml) lot# NUB001 \ _____

Protocol reviewed by Division, Yes () No ():

Methods:

- Species/strain: Rat / Sprague-Dawley (CD®[SD]IGS BR) from _____
- Doses employed: 0 (vehicle), 1, 3 and 10 mg/kg/day
- Route of administration: Intravenous (continuous infusion) at 2 ml/kg/hr.
- Study Design: Males 83-87 days of age and weighing 325-414 grams at treatment initiation were mated with untreated females 77-98 days of age and weighing 213-274 grams. At least one week prior to treatment initiation, males were implanted with a silastic catheter in the right femoral vein under isoflurane anesthesia. The catheter ran subcutaneously to the nape of the neck, where it was tethered to a jacket worn by the rat that allowed attachment of the catheter to a swivel connected to a syringe and infusion pump outside the cage. Other drug treatments are shown in the table below:

Non-ziconotide treatments	Administration times	Purpose or function
Penlong-XL (0.1 ml) IM	One hour before, and 2 days after surgery	Systemic penicillin-based antibiotic.
Hibitane™	Presurgery	Topical disinfectant
Betadine™	Presurgery	Topical disinfectant
Duratears™	During anesthesia	Eye moisturizer
Penicillin-G sodium	During surgery	Topical antibiotic at site.
Neosporin cream	Daily after surgery	Topical antibiotic at site.

Males received continuous infusions for 28 days prior to mating initiation, during mating and until necropsy (total 58 days, except for 45 and 48 days in two rats).

- Number of animals/sex/dosing group: 27 males/group (including 5/group for TK).
- Parameters and endpoints evaluated: Mortality, signs of ill health, and/or reaction to treatment (twice daily), complete detailed examination (weekly), body weight of males (twice weekly), food consumption (weekly), mating (maximum of 21 days), toxicokinetics (days 1, 28 and 49), gross pathology, uterine examination of dams (day 13 of gestation), organ weights (epididymides, prostate, seminal vesicles and testes), male reproductive assessments (using the left cauda epididymis), motility, spermatozoa count, morphology and histopathology of the right testes in the control and high-dose groups, and histopathology of the seminal vesicles from all groups.
- Statistical evaluations: Group mean (±S.D.) were analyzed by one-way ANOVA and Dunnett's test when the F value was significant (p<0.05). Heterogeneous data were analyzed with Kruskal-Wallis and significant differences between control and test groups was assessed using Dunn's test. The following parameters were calculated: mating index, fertility index, fecundity rate, pre-implantation loss, post-implantation loss, and group means for corpora lutea count, number of implants, number of live and dead embryos, and number of resorptions.

Results:

- Clinical signs: Signs that appeared to occur with a higher incidence in the treated than the vehicle control males are summarized in the table below:

Clinical Sign	Incidence of Sign in Each Group out of N=22/group			
	Vehicle	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
Piloerection	0	4	1	2
Ptosis	1	7	8	14
Swollen, rt. surgical site	1	6	9	3
Feces, soft	0	2	0	4
Penis, protruding	0	1	2	1
Hunched posture	0	0	0	2
Tremors	0	0	0	2

- Mortality: Two rats were euthanized (and replaced) because of problems with the infusion system. One control TK male was found dead on day 23 without prior clinical signs. One high-dose male was found dead on day 28, having shown ptosis, hunched posture, piloerection, head bobbing, tremors, teeth grinding and uncoordination during the first 5 days of treatment and, during days 20-25, it showed skin pallor, piloerection and swelling at the surgical site. A low-dose male was euthanized on day 44 in poor condition, showing skin pallor, piloerection, soft feces, decreased muscle tone, weight loss, limited usage of both hind limbs, hypersensitivity to external stimuli and vocalization. Both of these males had large infusion-site masses upon gross pathological examination.
- Body weight: Body weights of selected time points following the initiation of treatment are shown in the table below. By day 3, all three treatment groups had mean body weights significantly below the vehicle controls. Subsequently, there was an inverse relationship between ziconotide dose and recovery from the initial weight loss or attenuation of weight gain.

Treatment Day of Body Weight Measurement	Mean body weights of male rats infused IV (grams)			
	Vehicle	1 mg/kg/day	3 mg/kg/day	10 mg/kg/day
-2	374	369	371	375
3	392	376*	371**	370**
7	408	391*	391*	396
14	436	414*	420	426
21	456	436	441	448
28	472	449*	456	466
35	483	446**	463*	477
42	496	458**	478	492
49	510	474**	498	495
56	524	481	508	528

*P<0.05 and **p<0.01, compared with the vehicle control group.

- Food consumption: Mean consumption in grams of food per male rat per week was significantly (p<0.01) decreased in low, mid and high dose groups by 13.4%, 14.2% and 20.7%, respectively, compared with the vehicle controls during the first week of treatment. Food consumption during subsequent weeks did not differ among the groups.

• Toxicokinetics:

Group No.	Ziconotide dose		Mean±SD ziconotide concentration, ng/ml*		
	µg/kg/hr	mg/kg/day	Day 1	Day 28	Day 49
2	41.7	1	92.6±3.3	105.1±27.6	153.8±41.5
3	125.0	3	245.2±34.1	276.2±54.4	374.6±189.0
4	416.7	10	651.0±223.24	1374±1111	2460±3136

Assays were conducted by _____

• Fertility in Males

- In-life observations: There were no significant differences among groups in mean days to mating (2.4-2.7 days), mating index (all 100%), fertility index (95.2-100%) and fecundity index (95.2-100%).
- Terminal and Necroscopic evaluations: Epididymal sperm evaluation found no significant differences in cauda epididymis weight (mean range, 0.285-0.294 g), spermatozoa count (mean range, 652.4-724.6 million/g) or in % sperm motility (mean range, 65.9-70.1%).

Group No.	Body Weight (grams)	MEAN ABSOLUTE MALE ORGAN WEIGHT (grams)						
		Testis		Prostate	Epididymis		Seminal vesicle	
		Left	Right		Left	Right	Left	Right
1-Veh.	507.0	1.648	1.661	1.371	0.654	0.648	0.444	0.434
2-Low	470.6**	1.688	1.700	1.532	0.641	0.661	0.327**	0.344**
3-Mid	486.8	1.625	1.635	1.349	0.656	0.651	0.326**	0.327**
4-High	493.7	1.628	1.622	1.225	0.630	0.625	0.303**	0.305**

*Significantly different from control, p<0.01 (Dunnett's test).

Group No.	Body Weight (grams)	MEAN RELATIVE MALE ORGAN WEIGHT (gram %)						
		Testis		Prostate	Epididymis		Seminal vesicle	
		Left	Right		Left	Right	Left	Right
1-Veh.	507.0	0.326	0.328	0.269	0.129	0.128	0.087	0.086
2-Low	470.6**	0.360**	0.363**	0.327**	0.137	0.141**	0.069**	0.073
3-Mid	486.8	0.336	0.337	0.279	0.135	0.134	0.067**	0.068**
4-High	493.7	0.331	0.330	0.248	0.128	0.127	0.061**	0.061**

*Significantly different from control, p<0.01 (Dunnett's test).

• Fertility and Early Embryonic Development in Females:

- In-life observations: All females were untreated.
- Terminal and Necroscopic evaluations: Among the groups, only one female out of 21 mated with a male in the high-dose group failed to be pregnant.
- Embryo-fetal development
 - In-life observations:
 - Terminal and Necroscopic evaluations:
 - Dams: One dam mated with a mid-dose male was excluded from the calculations of group means because it had only 5 corpora lutea, 1 implantation site and 1

Lot and batch numbers: Ziconotide lot# 8068 A33294 _____; vehicle (ziconotide diluent, 0.9% saline + 50 µg of L-methionine/ml) lot numbers NUB001 and NUB002

Methods:

- Species/strain: Rat / Sprague-Dawley (CD@[SD]IGS BR) from _____
- Doses employed: 0 (vehicle), 1, 3 and 10 mg/kg/day
- Route of administration: Intravenous (continuous infusion) at 2 ml/kg/hr.
- Study Design: Females 63-69 days of age and weighing 172-219 grams at treatment initiation were mated with untreated proven-breeder males 86-120 days of age. At least one week prior to treatment initiation, females were implanted with a silastic catheter in the right femoral vein under isoflurane anesthesia. The catheter ran subcutaneously to the nape of the neck, where it was tethered to a jacket worn by the rat that allowed attachment of the catheter to a swivel connected to a syringe and infusion pump outside the cage. Other drug treatments are shown in the table below:

Non-ziconotide treatments	Administration times	Purpose or function
Penlong-XL (0.1 ml) IM	One hour before, and 2 days after surgery	Systemic penicillin-based antibiotic.
Hibitane™	Presurgery	Topical disinfectant
Betadine™	Presurgery	Topical disinfectant
Duratears™	During anesthesia	Eye moisturizer
Penicillin-G sodium	During surgery	Topical antibiotic at site.
Neosporin cream	Daily after surgery	Topical antibiotic at site.

Females received continuous infusions for 14 days prior to mating, during mating and through day 7 of gestation. After completion of dosing, catheters were disconnected, tied off and inserted subcutaneously in the dorsal regions. They were euthanized on day 13 of gestation.

- Number of animals/sex/dosing group: 28 females/group (including 6/group for TK).
- Parameters and endpoints evaluated: Mortality, signs of ill health, and/or reaction to treatment (twice daily), complete detailed examination (weekly), body weight of females (weekly until mating and on gestation days 0, 3, 7, 10 and 13), food consumption (weekly until mating and for gestation periods of days 0-3, 3-7, 7-10 and 10-13), estrus cycles (determined daily for 10 days prior to placement for mating by vaginal smear examination), presence of spermatozoa during mating, toxicokinetics (treatment days 1 and 14 and gestation day 7), gross pathology, uterine examination of dams (day 13 of gestation), organ weights (ovaries weighed separately), and histopathology of the following: abnormalities identified at necropsy, heart (including section of aorta), infusion site (including catheter tip), mammary glands (cervical and inguinal), ovaries, uterus (horn, body and cervix) and vagina.
- Statistical evaluations: Group means (±S.D.) were analyzed by one-way ANOVA

and Dunnett's test when the F value was significant ($p < 0.05$). Heterogenous data were analyzed with Kruskal-Wallis and significant differences between control and test groups was assessed using Dunn's test. The following parameters were calculated: mating index, fertility index, conception rate, pre-implantation loss (both individual litter and group), post-implantation loss (both individual litter and group), and group means for corpora lutea count, number of implants, number of live and dead embryos, and number of resorptions. The uterine data were analyzed statistically with the Kruskal-Wallis test, and where $p < 0.05$ for the "H" value, the Mann-Whitney "U" test was used for analyzing the difference between control and treatments.

Results:

- **Clinical signs:** Several signs related to body surface (thin or stained fur, skin scab or lesion, swelling at the surgical site) were attributed to the infusion procedure. One mid-dose and one high-dose female had hunched posture, decreased muscle tone or ptosis on treatment days 4 and/or 7.

- **Mortality:**

Group #	Circumstance of death	Observations in life	Necropsy findings
1-control	1 TK died at blood collec	No signs.	N/A
2-low dose	1 Euthanized on day 4	↓ activity and tone, ptosis, internal abdominal firmness.	No abnormalities.
3-mid dose	1 Found dead on day 3	No signs.	Dark areas stomach and adrenals. Left adrenal enlarged.
4- hi dose	1 Found dead on day 11	No signs.	No abnormalities.

- **Body weight:**

Study Day	Mean of Group 1		Mean of Group 2		Mean of Group 3		Mean of Group 4	
	Weight	Gain	Weight	Gain	Weight	Gain	Weight	Gain
<i>Premating:</i>								
Day -5	189.3		189.4		185.5		187.3	
Day -1	198.8	9.5	201.4	11.9	193.7	8.4	195.6	8.3
Day 7	216.8	18.0	217.1	15.7	212.8	19.1	218.1	22.5
Day 14	232.8	16.0	223.8	6.7**	222.7	9.9*	227.6	9.1**
Day 21	MATING PERIOD							
Day 28								
<i>Gestation:</i>								
Day 0	244.8		232.2*		225.3**		234.7	
Day 3	260.9	16.1	248.1*	15.9	242.2**	16.9	250.6	15.9
Day 7	276.1	15.2	259.6**	11.4	250.7**	8.5**	261.7*	11.1
Day 10	291.2	15.0	271.5**	12.0	262.4**	11.8	269.5**	7.8**
Day 13	310.7	19.5	294.1*	22.6	284.5**	22.1	291.4**	21.9

Significantly different from control (Group 1) value: * $p < 0.05$, ** $p < 0.01$ (Dunnett's test).

- Food consumption: Consumption was significantly decreased in the low and mid dose groups during the first week of treatment prior to mating, but no significant group differences were observed during gestation.
- Toxicokinetics:

Group No.	Ziconotide dose		Mean±SD ziconotide concentration, ng/ml*		
	µg/kg/hr	mg/kg/day	Day 1	Day 14	Gest. Day 7
2	41.7	1	84.9±22.8	81.0±11.3	87.0±15.8
3	125.0	3	178.0±21.3	198.8±43.8	205.2±46.0
4	416.7	10	570.2±89.8	697.0±74.6	1232±1248

*Assays were conducted by ~~████████████████████~~

One of the high-dose females had the disproportionately high concentration of 3440 ng/ml. The mean of the other four females was 680 ng/ml, which is similar to the mean of 697 ng/ml for the high-dose group when sampled on treatment day 14.

- Fertility and Early Embryonic Development in Females:
 - In-life observations: One high-dose female (out of 21) failed to mate. The mating, fertility and conception indices among groups ranged from 95.5 to 100%.
 - Terminal and Necroscopic evaluations: One female out of 22 mated with a male in each of the low and mid-dose groups failed to be pregnant.

Group No.	Mean Body Weight (grams)	MEAN INDIVIDUAL OVARIAN WEIGHTS			
		Absolute (grams)		Relative (gram %)	
		Left	Right	Left	Right
1-Veh.	300.0	0.057	0.058	0.019	0.019
2-Low	283.8*	0.057	0.054	0.020	0.019
3-Mid	273.4**	0.050	0.056	0.018	0.020
4-High	281.9**	0.050	0.054	0.018	0.019

Significantly different from control, *p<0.05 or **p<0.01 (Dunnett's test).

- Embryo-fetal Development:
 - In-life observations:
 - Terminal and Necroscopic evaluations:

Dams: The uterine findings are summarized in the table below:

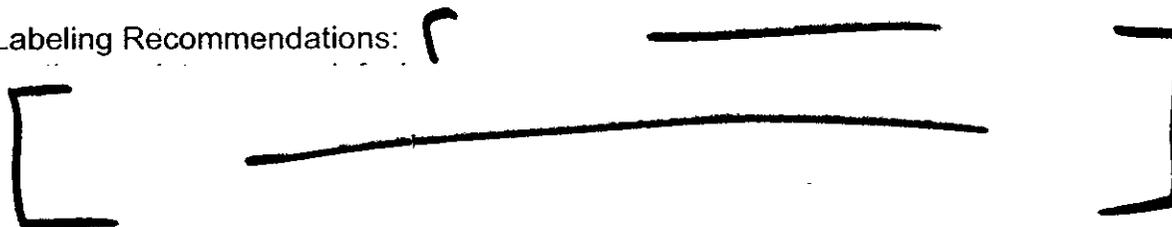
PARAMETER MEASURED	Group mean (±S.D.) for parameter			
	Group 1	Group 2	Group 3	Group 4
Total no. of corpora lutea	16.6 ± 3.2	16.2 ± 2.4	15.2 ± 2.1	14.1 ± 1.9*
No. of implantation sites	15.0 ± 1.9	14.5 ± 1.2	14.3 ± 1.8	12.4 ± 1.8**
No. of live embryos	14.3 ± 2.0	13.9 ± 1.5	13.9 ± 1.7	11.7 ± 1.9**
No. of dead embryos	0	0	0	0
No. of early resorptions	0.7 ± 0.9	0.7 ± 0.9	0.4 ± 0.6	0.7 ± 0.9
No. of early resorptions and dead embryos	0.7 ± 0.9	0.7 ± 0.9	0.4 ± 0.6	0.7 ± 0.9
% Preimplantation loss	8.1 ± 10.3	9.6 ± 8.9	5.4 ± 7.6	11.6 ± 10.2
% Postimplantation loss	4.9 ± 6.6	4.6 ± 6.0	2.5 ± 3.9	5.5 ± 7.6

Significantly different from control, *p<0.01 or **p<0.001 (Mann-Whitney test).

Offspring: Not examined.

- Summary and Evaluation: Female rats receiving ziconotide 1-10 mg/kg/day by continuous intravenous infusion for 14 days prior to mating and through day 7 of gestation showed a decrease in food consumption during week 1 only in the low and mid dose treatment groups and significantly lower body weights in all treatment groups relative to the control. No treatment-related gross pathology was observed. No effects on weight of right or left ovaries, or on histopathology of the heart (including section of aorta), mammary glands (cervical and inguinal), ovaries, uterus (horn, body and cervix) and vagina were reported. Statistically significant decreases in the numbers of corpora lutea, implantation sites and live embryos occurred in the high-dose group. There were no significant effects on fertility indices. Plasma ziconotide did not accumulate in females from day 1 of treatment to day 7 of gestation. The NOAEL for female fertility in this study was 10 mg/kg/day, which corresponded to mean plasma levels of 570, 697 and 1232 (680 without an outlier) ng/ml on treatment days 1 and 14 and gestation day 7, respectively. These levels are only about half those reported in males receiving the same infusion rates of ziconotide. Maternal toxicity (decreased food consumption) was evident at the lowest dose tested (1 mg/kg/day, IV) and the highest dose that had no effect on reproductive performance and early embryonic development was 3 mg/kg/day, IV.

Labeling Recommendations:



Study Title: A Continuous Infusion Teratology Study of SNX-111 in the Rat

Study No.: 95625

Volume #2.037, page #002

Conducting laboratory and location:

Date of study initiation: Not indicated; final study report is dated 9/21/94

GLP compliance: Yes

QA-Report: Yes (X) No ()

Lot and batch numbers: SNX-111, #8068SJK006; 0.9% NaCl for injection, #AP430F0, AP427L9 and AP428H9

Methods:

• Species/strain: Rat / CDR(SD)BR

• Doses employed: 0 (vehicle), 1.5, 4.5 and 15 mg/kg/day.

- Route of administration: Intravenous.
- Study Design: Female rats 86-94 days of age and weighing 227-321 grams at treatment initiation were mated with untreated proven-breeder males. Surgery was performed on Day 0, during which females were implanted with a silastic catheter in the right femoral vein under ether anesthesia. The catheter ran subcutaneously to the nape of the neck, where it was tethered to a jacket worn by the rat that allowed attachment of the catheter to a swivel connected to a syringe and infusion pump outside the cage. All rats were continuously infused with 0.9% saline at the rate of 0.4 ml/hr until initiation of treatment. Other drug treatments related to the surgery are shown in the table below:

Non-ziconotide treatments	Administration times	Purpose or function
Penlong-XL (0.1 ml) IM	One hour before, and 2 days after surgery	Systemic penicillin-based antibiotic.
Hibitane™	Presurgery	Topical disinfectant
Betadine™	Presurgery	Topical disinfectant
Duratears™	During anesthesia	Eye moisturizer
Penicillin-G sodium	During surgery	Topical antibiotic at site.
Neosporin cream	Daily after surgery	Topical antibiotic at site.

Females received continuous intravenous infusions during days 6-15 of gestation and were euthanized on day 20 of gestation.

- Number of animals/sex/dosing group: 30 females (including 4 for pharmacokinetics) each in the vehicle, low- and mid-dose groups and 31 females in the high-dose group (including 5 for pharmacokinetics).
- Parameters and endpoints evaluated: Mortality and comprehensive clinical examination (twice daily during gestation), body weight of females (weekly until mating and on gestation days 0, 3, 6, 9, 12, 15, 18 and 20), food consumption (weekly until mating and for gestation periods of days 0-6, 6-9, 9-12, 12-15, 15-18 and 18-20), gross pathology including infusion site, histopathology of abnormalities identified at necropsy, uterine examination of dams (day 20 of gestation), counting of corpora lutea, fetal weights, fetal sex determination, detailed examinations of fetuses, both external (all) and internal (about half of the fetuses in each litter), and skeletal examination for major malformations, minor visceral or skeletal anomalies, or common skeletal variants.
- Statistical evaluations: Group means (\pm S.D.) were analyzed by one-way ANOVA and Dunnett's test when the F value was significant ($p < 0.05$). Heterogenous data were analyzed with Kruskal-Wallis and significant differences between control and test groups was assessed using Dunn's test. The following parameters were calculated: group mean (S.D.) live litter size, group mean and litter mean fetal weights, pregnancy rate, individual and group litter means for sex ratio (% males), pre-implantation losses, post-implantation losses and group means for corpora lutea count, number of implants, number of live and dead embryos, and number of resorptions. The uterine data and litter mean percentage of common skeletal variants were analyzed statistically with the Kruskal-Wallis test, and where $p < 0.05$

for the "H" value, the Mann-Whitney "U" test was used for analyzing the difference between control and treatments. The overall and individual incidences of litters and fetuses with major malformations and minor anomalies in each test group were compared with the control values using either the chi-square test (with Yate's correction) or Fisher's exact probability test (using cumulative probabilities where appropriate).

Results:

- **Clinical signs:** In addition to external signs related to the surgery, rats in all ziconotide treatment groups showed ptosis, and rats in the mid and high dose groups showed decreased activity, slight tremors of the tail and/or body.
- **Mortality:** There was no treatment-related mortality. One mid-dose female was euthanized on gestation day 13 due to poor condition and was found to have abdominal adhesions and thickening of the wall of various areas of the GI tract. A control female was euthanized following premature delivery on gestation day 13.
- **Body weight:** All groups lost weight for the 3-day period following surgery, and the groups receiving ziconotide had significantly lower body weights than the vehicle controls for the first 3-day period following initiation of ziconotide treatment on day 6.

Gestation	Mean of Group 1		Mean of Group 2		Mean of Group 3		Mean of Group 4	
	Weight	Gain	Weight	Gain	Weight	Gain	Weight	Gain
Day 0	258.3		256.8		253.7		258.7	
Day 3	257.2	-1.0	255.2	-1.7	249.9	-3.8	257.9	-0.8
Day 6	271.1	13.9	267.8	12.7	264.4	13.6	273.3	15.4
Day 9	286.4	15.3	271.6*	3.7**	272.5*	8.7	271.1*	-2.2**
Day 12	305.4	19.0	296.2	24.7	295.3	22.9	302.5	31.4**
Day 15	326.0	20.6	309.6*	13.4	311.6	17.4	323.4	20.9
Day 18	367.9	41.9	353.7	44.1	354.0	42.4	362.3	38.9
Day 20	410.3	42.4	400.3	46.6	397.1	43.2	403.6	41.3

Significantly different from control (Group 1) value: *p<0.05, **p<0.01 (Dunnett's test).

- **Food consumption:** Consumption decreased significantly (p<0.01) in a somewhat dose-related manner (75, 74 and 71% of control) during the first 3-day period following initiation of ziconotide treatment on day 6.

Gestation Days	Mean food consumption (g/rat) of pregnant females with live litters			
	Group 1-control	Group 2-low 1.5	Group 3-mid 4.5	Group 4-hi 15
0-6	135.8	135.8	125.1	128.7
6-9	91.7	69.0**	67.9**	65.0**
9-12	94.6	92.3	87.8	99.9
12-15	99.2	85.2*	87.3	101.2
15-18	101.4	108.3	109.5	116.9**
18-20	70.8	74.6	71.2	72.0

Significantly different from control (Group 1) value: *p<0.05, **p<0.01 (Dunnett's test).

- **Toxicokinetics:** Plasma sample analysis was specified in the protocol, but the samples collected were not analyzed (sponsor's note, Vol. 2.037, page 002).

- Embryo-fetal Development:
 - In-life observations:
 - Terminal and Necroscopic evaluations:

Dams: The uterine findings are summarized in the table below:

PARAMETER MEASURED	Group mean (\pm S.D.) for parameter			
	Group 1	Group 2	Group 3	Group 4
Total no. of corpora lutea	20.3 \pm 3.8	20.1 \pm 3.6	19.6 \pm 3.8	19.6 \pm 3.1
No. of implantation sites	16.5 \pm 1.9	17.1 \pm 1.9	16.9 \pm 1.4	17.0 \pm 1.6
No. of live fetuses	15.3 \pm 1.8	16.0 \pm 2.2	15.8 \pm 1.6	15.4 \pm 2.3
Sex ratio, % male	50.6 \pm 14.3	53.6 \pm 10.4	50.6 \pm 14.5	52.3 \pm 12.0
No. of dead fetuses	0 \pm 0	0 \pm 0	0.0 \pm 0.2	0 \pm 0
No. of early resorptions	1.0 \pm 0.9	1.0 \pm 1.2	0.8 \pm 1.2	1.4 \pm 1.5
No. of middle resorptions	0.0 \pm 0.2	0.0 \pm 0.2	0.1 \pm 0.3	0.1 \pm 0.3
No. of late resorptions	0.1 \pm 0.3	0 \pm 0	0.2 \pm 0.5	0.0 \pm 0.2
Total resorptions	1.1 \pm 1.0	1.0 \pm 1.2	1.1 \pm 1.2	1.5 \pm 1.5
% Preimplantation loss	16.7 \pm 14.6	13.4 \pm 12.8	11.8 \pm 12.7	11.8 \pm 10.9
% Postimplantation loss	6.7 \pm 6.0	6.1 \pm 7.2	6.6 \pm 6.6	9.4 \pm 9.0
Gravid uterus weight, g	89.7 \pm 11.2	92.6 \pm 10.8	91.5 \pm 9.5	88.4 \pm 15.7
Litter mean fetal wts., g	3.83 \pm 0.19	3.78 \pm 0.23	3.77 \pm 0.28	3.65 \pm 0.40

Significantly different from control, * $p < 0.01$ or ** $p < 0.001$ (Mann-Whitney test).

Offspring: Although there was a dose-related tendency for the group litter mean fetal weights to decrease (3.83, 3.78, 3.77 and 3.65 grams) the differences were not statistically significant. The vehicle, low-dose and mid-dose groups each had one fetus in one litter with a major malformation. The high-dose group had 3 fetuses in a total of 2 litters with malformations.

- **Major Malformations:**

- One control fetus had globular heart, stenosis of pulmonary trunk, dilation of ascending aorta, and interventricular septal defect.
- One low-dose fetus had multiple anomalies in the lumbar/sacral vertebrae, including lumbar centrum 4 semi-bipartite, lumbar vertebrae 5 and 6 absent, sacral centrum 1 and 2 absent, and sacral vertebral arches 1 bilateral reduced ossification.
- One mid-dose fetus had multiple fusions and/or anomalies in the thoracic vertebrae.
- The high-dose group had 2 litters with a total of 3 fetuses (which were the smallest in their litters) with malformations, including the following:
 1. One of two sibling females had protrusion of brain through parietal/frontal bones (encephalocele), protrusion of cerebellum through cleft at junction of parietal and frontal bones not covered by skin, an area of hemorrhage surrounding the protruding tissue and left eye absent (anophthalmia).
 2. The other female had transposition of the major vessels and right-sided aorta.

3. A male in another litter had the aortic arch absent, the ductus arteriosus connected to the ascending aorta, the descending aorta arising from the pulmonary trunk, subcutaneous edema over head and thorax (anasarca), right pinna absent (anotia), lower jaw reduced (micrognathia), upper jaw reduced, right eye absent (anophthalmia), anal atresia, digits of forepaws and hind paws shortened (brachydactyly): bilateral, abnormal flexure of hind limbs: bilateral, and a minor anomaly, kinked tail.

- **Minor External and Visceral Anomalies:** Oval lens(es) were observed in one low-dose, one mid-dose and three high-dose fetuses (2 litters), but none were seen in the controls. There appeared to be a dose-related decrease in the number of fetuses/litters with dilated ureter(s), which was statistically significant ($p < 0.05$) for the high-dose group, relative to the controls.

- **Skeletal Anomalies:**

Anomaly Parameter Assessed	Incidence in Litters/fetuses from the Treatment Groups							
	Vehicle controls		1.5 mg/kg/day		4.5 mg/kg/day		15 mg/kg/day	
	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses
No. evaluated	22	337	23	369	25	393	25	386
Total anomalies	21	115	22	129	23	119	24	161*
Skull: Hyoid, Absent	8	13	12	17	9	16	8	11
Skull: Hyoid, ↓ Ossifica.	2	5	1	1	2	2	3	5
Skull: Hyoid, Bipartite	0	0	0	0	1	1	1	1
Skull: " Semi-bipartite	0	0	0	0	1	1	0	0
Skull: Parietal, Irregular ossification	4	10	1	2*	1	1**	2	3*
Skull: Interparietal, Irregular ossification	20	80	18	65	20	74	21	88
Skull: Supraoccipital, Irregular ossification	17	67	19	57	21	66	23	76
Skull: Supraoccipital, Reduced ossification	1	1	0	0	0	0	0	0
VERTEBRAL COLUMN Extra pre-sacral verteb.	1	3	2	5	0	0	1	1
Ossification center on 1 st lumbar vertebra	4	11	8	22	1	1**	4	13
Cervical arches: Absent	0	0	0	0	0	0	1	1
Thoracic arches: absent	0	0	0	0	1	1	0	0
Lumbar centrum: Semi-bipartite	1	1	2	2	1	1	3	4
Lumbar centrum: Bipartite	0	0	0	0	1	1	0	0
Sacral centrum: Absent	0	0	0	0	1	1	0	0
Sacral centrum: ↓ ossifi.	0	0	0	0	1	1	1	1
Caudal vertebrae: Reduced number	0	0	0	0	3	3	4	6*
Caudal centrum: ↓ ossi.	0	0	0	0	0	0	1	1

Sternebral Column: Sternebrae: Fused	0	0	0	0	1	1	0	0
Ribs: Wavy	1	1	1	1	0	0	2	4
Ribs: Absent	0	0	0	0	1	1	0	0
Ribs: Fused	0	0	0	0	1	1	0	0
Ribs with nodule(s)	0	0	0	0	1	1	0	0
" Reduced ossification	3	3	2	3	3	3	4	6
Rudimentary 14 th rib(s)/ ossification center	0	0	2	2	0	0	0	0
Rudimentary 14 th rib(s)	1	4	4	4	0	0	1	1
Ribs Ossification center 13 th thoracic vertebrae	1	1	0	0	1	1	0	0
Pubic bones: ↓ ossifica.	3	4	7	13	5	9	14**	35***
Pubic bones: irregular ossification	3	5	3	5	3	3	2	2
Pubic bones: reduced, unilateral ^a	3	3	4	4	5	6	8	10
Pubic bones: reduced, bilateral ^a	1	1	6	9*	3	3	11**	24***
Pubic bones: Absent	0	0	0	0	1	1	3	11**
Ischial bone(s): Reduced ossification	1	1	2	2	2	2	3	4
Ischial bone(s): Irregular ossification	0	0	1	1	1	1	3	6*
Ischial bone(s): absent	0	0	0	0	0	0	1	1
Iliac bone(s): Irregular ossification	0	0	1	1	0	0	0	0
Limbs: ↓ # of phalanges in hindpaw(s)	1	1	0	0	0	0	0	0

Significantly different from control value, *p<0.05, **p<0.01 and ***p<0.001 (Fisher's Exact Test).

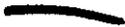
^aNot in sponsor's summary; compiled from individual animal data by reviewer. Litters with both unilateral and bilateral pubic bones reduced: group 1 = 1; group 2 = 3; group 3 = 2; and group 4 = 4.

All groups receiving ziconotide, regardless of dose, showed significant decreases in the incidence of irregular ossification of parietal bone(s) in the skull in fetuses, compared with the concurrent controls. When compared with the historical control database, however, this appears to be an anomalous observation (see table below). The sponsor reported significant increases at the high dose in reduced ossification of pubic bones, in absence of pubic bones, and in irregular ossification of ischial bones. From data supplied for individual fetuses, there also were statistically significant increases in the incidence of reduced pubic bones (bilateral) at both the low and high doses of ziconotide. Application of the Cochran-Armitage Trend Test to the latter skeletal anomaly indicated significance for both litters (p=0.0072) and fetuses (p=0.000007).

Historical control data from 19 studies conducted by the contract laboratory from 1991 to 1997 for the skeletal anomalies in the table above that were significantly affected by ziconotide (except for pubic bones, reduced) are summarized in the table below and compared with the results from ziconotide treatment (regardless of dose):

Anomaly Parameter Assessed	Litters (% incidence)		Fetuses (% incidence)	
	Control ^a	Ziconotide ^b	Control ^a	Ziconotide ^b
Total number evaluated (skeletal)	430	73	5711	1148
Parietal bones: irregular ossification	23 (5.3%)	4 (5.5%)	34 (0.6%)	6 (0.52%)
Caudal vertebrae: reduced number	8 (1.9%)	7 (9.6%)**	8 (0.14%)	9 (0.78%)***
Pubic bones: reduced ossification	92 (21%)	28 (38%)**	144(2.5%)	57 (5.0%)***
Pubic bones: reduced, bilateral	N.P.	20 (27%)	N.P.	36 (3.1%)
Pubic bones: absent	7 (1.6%)	4 (5.5%)	8 (0.14%)	12 (1.0%)***
Ischial bones: irregular ossification	5 (1.2%)	5 (6.8%)**	5 (0.09%)	8 (0.7%)***

Significantly different from control value, *p<0.05, **p<0.01 and ***p<0.001 (Fisher's Exact Test).

^a Historical control data from 19 studies using Sprague-Dawley rats from  N.P.= data not provided.

^b Total from 3 dosage levels of ziconotide.

Comparison of ziconotide treatment to the historical controls indicated significant differences for reduced number of caudal vertebrae in both litters and fetuses, for reduced ossification of pubic bones in both litters and fetuses, for absent pubic bones in fetuses, and for irregular ossification of ischial bones in both litters and fetuses.

Summary and Evaluation: Pregnant rats receiving ziconotide 1.5, 4.5 or 15 mg/kg/day by continuous intravenous infusion from day 6 through day 15 of gestation showed ptosis and, at the mid and high doses, showed decreased activity, slight tremors of the tail and/or body. Significant decreases in weight gain and food consumption occurred in all ziconotide-treated groups during the first 3 days of treatment. No treatment-related gross pathology was observed and no significant changes were observed in the uterine parameters measured. One control fetus had malformations involving the heart and aorta. One low-dose fetus had multiple spinal malformations, including the absence of lumbar vertebrae 5 and 6. One mid-dose fetus had multiple fusions and/or anomalies in the thoracic vertebrae. The high-dose group had 3 fetuses in a total of 2 litters with the following major malformations that were not observed in the control group: One fetus had encephalocele and anophthalmia; one had transposition of the major vessels and right-sided aorta; and one had anotia, anophthalmia, micrognathia, anasarca, anal atresia, abnormal flexure of hindlimbs, brachydactyly, and aortic arch absent. The high-dose fetuses also had a significant increase (40%, p<0.05) in the incidence of skeletal anomalies. Some of these included missing bones (pubic bones in the pelvic girdle and caudal vertebrae) and reduced or irregular bone ossification in the vertebral column, ribs or pelvic girdle. Reduced ossification of various bones could be attributed to decreased food consumption. Absent pubic bones and reduced number of caudal vertebrae were also observed in the mid-dose fetuses, but were not seen in any low-dose or control fetuses. However, a significant dose-related increase in the incidence of abnormally small (reduced) pubic bones, which was statistically significant for litters at the high dose and for fetuses at both the low and high doses, suggests that the low dose is not totally benign in affecting development of the pubic bones. Comparison of ziconotide treatment, regardless of dose, to historical control

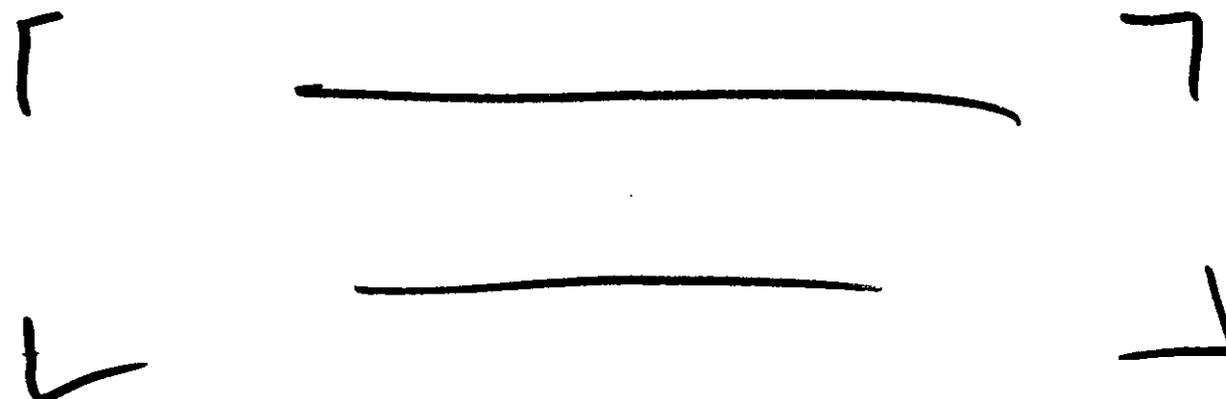
data indicated significant differences for reduced number of caudal vertebrae in both litters and fetuses, for reduced ossification of pubic bones in both litters and fetuses, for absent pubic bones in fetuses, and for irregular ossification of ischial bones in both litters and fetuses. Consequently, ziconotide by the IV route should be considered as teratogenic in rats and should be labeled as Pregnancy Category C, rather than the Pregnancy Category — in the sponsor's draft label.

Since the plasma samples taken for TK were not analyzed, there is no exposure data for this study, in which the high dose was 15 mg/kg/day, IV. TK data are available for 1, 3, and 10 mg/kg/day IV dose infusions in female rats in other reproductive toxicity studies. The mean plasma concentrations from the 1, 3 and 10 mg/kg/day infusions for three sampling times in female fertility studies were 84, 194 and 649 ng/ml (excluding one outlier at the third sampling time), respectively, indicating good proportionality to dose in the female fertility study. Extrapolating these values to the 1.5, 4.5 and 15 mg/kg/day doses used in this study would result in estimated plasma concentrations of approximately 125, 300 and 1000 ng/ml, respectively. When attempts to measure plasma ziconotide in 102 patients receiving IT infusions were made, only 30 had quantifiable levels of ziconotide. In this subset, the hourly infusion rate ranged from 0.1 to 7.0 µg/hr, which resulted in a mean (± S.D.) plasma ziconotide concentration of 0.137 ± 0.138 ng/ml in humans. Thus, the low, mid and high IV doses (1.5, 4.5 and 15 mg/kg/day) in female rats produced an estimated 900, 2200 and 7300 times this human exposure, respectively.

Labeling Recommendations:

Pregnancy

Teratogenic Effects — Pregnancy Category C



Study Title: A Continuous Infusion Teratology Study of SNX-111 in the Rabbit

Study No.: — 95627

Volume #2.039, page #104

Conducting laboratory and location: _____

Date of study initiation: June 5, 1994 (first day of treatment)

GLP compliance: Yes, except that the protocol included analyses of dose-preparation

samples, plasma samples and amniotic fluid samples, but these analyses were not conducted.

QA-Report: Yes (X) No ()

Lot and batch numbers: SNX-111, lot# 8068SJK006 () peptide purity, () peptide content) and 8068SMC011 () peptide purity, () peptide content); 0.9% sodium chloride for injection, U.S.P, lot# AP434N6, AP435A7 and AP438A1 ()

Methods:

- Species/strain: Rabbit / New Zealand White ()
- Doses employed: 0 (vehicle), 0.2, 1.0 and 5.0 mg/kg/day (continuous infusion)
- Route of administration: Intravenous (femoral vein).
- Study Design: Female rabbits about 5 months of age and weighing 2.8-3.8 kg at treatment initiation were artificially inseminated (Day 0) with pooled semen samples containing at least 1.8×10^8 spermatozoa/ml, approximately one to two weeks following surgery. Surgery was performed under isoflurane anesthesia to implant a silastic catheter in the right femoral vein. The catheter ran subcutaneously to the nape of the neck, where it was tethered to a jacket worn by the rabbit that allowed attachment of the catheter to a swivel connected to a syringe and infusion pump outside the cage. All rabbits were continuously infused with 0.9% saline at the rate of 1.5 ml/hr until initiation of treatment. After completion of dosing, the catheters were disconnected, tied off and the catheter inserted subcutaneously in the dorsal region. Other drug treatments related to preparation of the rabbits for study are shown in the table below:

Non-ziconotide treatments	Administration times	Purpose or function
HCG (50 I.U.) IV	19 days and 2-4 hr before insemination	Luteinization for fertility.
Innovar-Vet® (0.2 mg/kg)	Presurgical prep.	Anesthesia for shaving.
Hibitane™	Presurgery	Topical disinfectant
Isopropanol, 70%	Presurgery	Topical disinfectant
Betadine™	Presurgery	Topical disinfectant
Isoflurane	During surgery	General anesthesia
Duratears™	During anesthesia	Eye moisturizer
Penicillin-G sodium	During surgery	Topical antibiotic at site.
Neo-Sporin cream	Daily after surgery	Topical antibiotic at site.

The rabbits received continuous intravenous infusions from day 6 through day 18 of gestation, except for premature termination due to non-patency of the catheter in one control rabbit (gestation day 10; excluded from analyses), one mid-dose rabbit (gestation day 12; excluded from analyses) and one high-dose rabbit (gestation day 16; included in analyses). The rabbits were euthanized on day 29 of gestation.

- Number of animals/sex/dosing group: 24 females/group (including 4/group for toxicokinetics)

- Parameters and endpoints evaluated: Mortality (twice daily) and comprehensive clinical examination (once daily after surgery and twice daily from start of treatment), signs of abortion (material examined externally and preserved in neutral buffered 10% formalin), body weight (three times during acclimation and on gestation days 0, 6, 9, 12, 15, 18, 24 and 29), food consumption (daily during gestation), gross pathology including infusion site, histopathology of abnormalities identified at necropsy, uterine examination of dams (day 29 of gestation), uterine weight, number and position of live fetuses, dead fetuses, recording of the number of early, middle and late resorptions and empty implantation sites, counting of corpora lutea, fetal weights, fetal sex determination, detailed external and internal examinations of fetuses, examination of the heads by the technique of Wilson in 1/3 of the fetuses, and skeletal examination for major malformations, minor visceral or skeletal anomalies, or common skeletal variants, were conducted.
- Statistical evaluations: Group means (\pm S.D.) were analyzed by one-way ANOVA and Dunnett's test when the F value was significant ($p < 0.05$). Heterogeneous data were analyzed with Kruskal-Wallis and significant differences between control and test groups was assessed using Dunn's test. The following parameters were calculated: group mean (S.D.) live litter size, group mean and litter mean fetal weights, pregnancy rate, individual and group litter means for sex ratio (% males), pre-implantation losses, post-implantation losses and group means for corpora lutea count, number of implants, number of live and dead embryos, and number of resorptions. The uterine data and litter mean percentage of common skeletal variants were analyzed statistically with the Kruskal-Wallis test, and where $p < 0.05$ for the "H" value, the Mann-Whitney "U" test was used for analyzing the difference between control and treatments. The overall and individual incidences of litters and fetuses with major malformations and minor anomalies in each test group were compared with the control values using either the chi-square test or Fisher's exact probability test (using cumulative probabilities where appropriate). Statistical analysis of percentage of common skeletal variants was performed by comparing the litter means percentage incidences of each test group with the control group using the Kruskal-Wallis and Mann-Whitney "U" tests.

Results:

- Clinical signs: Aside from a dose-related incidence of ptosis (1, 2, 3 and 6 out of 20/group), other signs occurring mainly or exclusively in the ziconotide-treated groups, such as fur staining (3, 10, 16, 14), thinness (0, 7, 6 and 7), tremors (0, 0, 1, 1), no feces in tray (0, 12, 17, 19), soft feces (7, 14, 13, 19), liquid feces (0, 4, 6, 6), urine decreased (0, 2, 4, 2) and activity decreased (2, 12, 11, 18) showed little or no dose dependence over the 25-fold range of doses used.

• **Mortality:**

Group #	Circumstance of death	Observations in life	Necropsy findings
1-control	Died gestation day 29	Lying on its side with labored respiration	No abnormalities.*
2-low dose	Died gestation day 26	Abortion signs on days 24 and 25	Dark areas gastric mucosa, pyloric wall thickening and ileal adhesions.*
3-mid dose	Found dead on day 9	Pinnae cold to touch and fur stain on days 7 and 8	Dark fluid in the thoracic cavity.*
4-hi dose	Euthanized in poor condition on day 21	Signs of abortion and avoided hindlimb use	Data not found by reviewer.
4-hi dose	Euthanized in poor condition on day 28	Aborted on gestation days 26-28	Pale areas in liver; discolored kidneys.

*The cause of death of these three females could not be determined.

- **Body weight (kg):** There was a significant and dose-related decrease in body weight during the first weighing (on day 24 of gestation) after termination of the infusion on day 19. On the day of euthanasia the mean body weights of all treatment groups were significantly lower ($p < 0.05$) than that of the controls by about 8-10%.

Gestation	Mean of Group 1		Mean of Group 2		Mean of Group 3		Mean of Group 4	
	Weight	Gain	Weight	Gain	Weight	Gain	Weight	Gain
Day 0	3.28		3.24		3.20		3.21	
Day 6	3.35	0.06	3.30	0.06	3.25	0.05	3.26	0.06
Day 9	3.41	0.06	3.32	0.02	3.35	0.13	3.45	0.19*
Day 12	3.40	-0.01	3.28	-0.04	3.30	-0.05	3.63	0.18**
Day 15	3.51	0.11	3.41	0.13	3.43	0.13	3.74	0.11
Day 18	3.52	0.01	3.43	0.01	3.41	-0.02	3.82	0.09
Day 24	3.66	0.15	3.39*	-0.04	3.31**	-0.10*	3.25**	-0.57**
Day 29	3.78	0.08	3.46*	0.08	3.41*	0.08	3.42*	0.12

Significantly different from control (Group 1) value: * $p < 0.05$, ** $p < 0.01$ (Dunnett's test).

- **Food consumption:** Daily food consumption was significantly decreased, mostly at the $p < 0.01$ level of significance, in all treatment groups after initiation of the infusions (days 6-7) through gestational days 14-15, which appeared inversely related to dose through days 11-12. Significant decreases again occurred following termination of the infusion, but both the magnitude and duration of these were directly related to dose.
- **Toxicokinetics:** Plasma and amniotic samples were taken, but not analyzed.
- **Embryo-fetal development**
 - In-life observations:
 - Terminal and Necroscopic evaluations:
Dams: Few findings were observed in the females examined and were

considered to be either incidental or secondary to the experimental procedures. No histopathological examination was undertaken. The uterine findings are summarized in the table below:

PARAMETER MEASURED	Group no. or group mean (\pm S.D.) for parameter			
	Group 1	Group 2	Group 3	Group 4
No. of pregnant rabbits	24/24	20/24	19/23 ^a	21/24
Pregnancy rate, %	100.00	83.33	82.61 ^a	87.50
No. with total resorption	2	0	3	7
Number aborting	2	2	3	1
Total # of corpora lutea A	11.8 \pm 2.3	12.2 \pm 2.1	11.4 \pm 2.1	10.5 \pm 3.4
Total # of corpora lutea B	12.0 \pm 2.3		11.6 \pm 2.1	11.8 \pm 2.2
# of implantation sites A	7.3 \pm 2.6	8.4 \pm 2.1	7.5 \pm 1.6	6.8 \pm 2.3
# of implantation sites B	8.1 \pm 1.4		7.5 \pm 1.6	7.7 \pm 1.6
No. of live fetuses A	5.8 \pm 3.4	7.3 \pm 2.4	5.4 \pm 3.1	4.1 \pm 3.5
No. of live fetuses B	6.6 \pm 2.8		6.4 \pm 2.1	6.6 \pm 1.4
Sex ratio, % male B	44.0 \pm 24.4	53.1 \pm 19.1	45.3 \pm 21.0	63.7 \pm 11.4
Female fetuses A	3.2 \pm 2.1	3.4 \pm 1.9	2.8 \pm 1.9	1.5 \pm 1.46*
Female fetuses B	3.6 \pm 1.8		3.4 \pm 1.5	2.4 \pm 1.1
No. of dead fetuses A	0	0.1 \pm 0.5	0	0.1 \pm 0.3
No. of dead fetuses B	0		0	0.2 \pm 0.4
No. of early resorptions A	1.3 \pm 0.8	0.4 \pm 0.8	1.6 \pm 2.8	2.1 \pm 2.9
No. of early resorptions B	1.2 \pm 2.2		0.6 \pm 1.5	0.3 \pm 0.7
No. of middle resorptions	0	0	0	0
No. of late resorptions A	0.2 \pm 0.4	0.6 \pm 1.2	0.4 \pm 1.4	0.4 \pm 0.6
No. of late resorptions B	0.2 \pm 0.4		0.5 \pm 1.5	0.6 \pm 0.7
All resorptions A	1.4 \pm 2.0	1.0 \pm 1.3	2.0 \pm 2.9	2.5 \pm 2.7
All resorptions B	1.4 \pm 2.1		1.1 \pm 2.0	0.8 \pm 1.0
% Preimplantation loss A	38.5 \pm 20.6	30.8 \pm 14.4	34.0 \pm 11.0	34.4 \pm 16.6
% Preimplantation loss B	31.8 \pm 10.1		34.7 \pm 11.0	34.1 \pm 11.7
% Postimplantation loss A	29.1 \pm 38.2	12.8 \pm 19.2	27.5 \pm 38.4	45.9 \pm 44.2
% Postimplantation loss B	19.0 \pm 28.3		14.3 \pm 23.0	13.4 \pm 11.9
Gravid uterus weight, g A	381 \pm 197	424 \pm 95	325 \pm 160	261 \pm 217
Gravid uterus weight, g B	433 \pm 146		381 \pm 90	412 \pm 104
Group litter mean fetal wt	44.8 \pm 5.3	39.0 \pm 6.5†	40.9 \pm 3.9	42.2 \pm 4.6

^a Excluding one rabbit whose status was not determined.

A – Including animals with total resorption.

B – Excluding animals with total resorption.

*Significantly different from control (group 1) value, $p < 0.05$ (Mann-Whitney test).

† Significantly different from control (group 1) value, $p < 0.05$ (Dunnett's test); units in grams.

Four of 24 rabbits in the low and mid-dose groups and three in the high-dose group were not pregnant. Among the groups receiving ziconotide, there was a dose-related increase in the number of pregnant rabbits (0, 3, 7) showing total resorption, although 2 controls also had total resorption. Application of the

Cochran-Armitage Trend Test to these data indicated a significant dose-effect relationship ($p < 0.01$).

Offspring: Although there was a tendency for the male, female and total group litter mean fetal weights to decrease the differences were statistically significant ($p < 0.05$) only for the low-dose male fetuses and low-dose total for both sexes. This is not considered to be biologically significant. The vehicle, low-dose and mid-dose groups each had one fetus in one litter with a major malformation.

- **Major Malformations:** The incidence of major malformations was 2 fetuses (2 litters) in the vehicle controls (hydrocephaly, micrognathia and other cranio/facial malformations), 0 in the low dose group, 3 fetuses (3 litters) in the mid-dose group (hydrocephaly with cerebral hemorrhage, microcaudia, abdominal organs enentrated at umbilicus) and 1 fetus in the high-dose group (hydrocephaly).
- **External and Visceral Anomalies:** Anomalies seen in the treated, but not control, fetuses included absence of the innominate artery in one high-dose fetus, and the left subclavian/left carotid arteries arising from the innominate artery in two low-dose (1 litter) and three mid-dose (2 litters) fetuses, missing gall bladder in one low-dose fetus, enlarged kidney and reduction of renal papillae in one mid-dose fetus, dilated ureter in one mid-dose fetus and oval eye lens in one low-dose fetus.
- **Skeletal Anomalies and Common Skeletal Variants:** There appeared to be an inverse relationship between dose and total incidence of skeletal anomalies, such that the high-dose incidence was significantly below the control incidence (13/93 vs. 2/66, $p < 0.05$). There also appeared to be a dose-related decrease in the % of fetuses/litter with a unilateral 13th rib compared with controls, which was not statistically significant. All three ziconotide-treated groups had an increase in the % of fetuses/litter with bilateral 13th rib compared with controls, which was significant ($p < 0.05$) for the low and mid dose groups, but not for the high dose group, as this increase appeared to have an inverse relationship to dose. No skeletal anomalies of any kind (reduced ossification, irregular ossification, reduced or absent) were reported for any bone in the pelvic girdle.

Anomaly Parameter Assessed	Significant differences in skeletal anomalies or common variants							
	Vehicle controls		0.2 mg/kg/day		1.0 mg/kg/day		5.0 mg/kg/day	
	Litters	Fetus	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses
No. evaluated	14	93	14	102	11	70	10	66
Total anomalies	7	13	10	22	5	7	2	2*
Mean \pm S.D.	% Fetuses/litter		% Fetuses/litter		% Fetuses/litter		% Fetuses/litter	
Unilateral 13 th Rib	17.0 \pm 25.3		13.1 \pm 12.8		12.9 \pm 9.2		5.1 \pm 8.3	
Bilateral 13 th Rib	24.3 \pm 21.2		60.8 \pm 20.6 †		53.9 \pm 34.6 †		48.6 \pm 41.0	

*Significantly different from control value, $p < 0.05$ (Fisher's Exact Test).

† Significantly different from control value, $p < 0.05$ (Mann-Whitney Test).

- **Summary and Evaluation:** Pregnant rabbits receiving ziconotide 0.2, 1.0 or 5.0 mg/kg/day by continuous intravenous infusion from day 6 through day 18 of gestation showed ptosis and decreased activity. Significant decreases in food consumption occurred during days 6-15 and again at termination of the infusion, at which time significant weight loss occurred, resulting in significantly lower body weights than the controls on gestation days 24 and 29. No treatment-related gross pathology was observed and no significant changes were observed in the uterine parameters measured. There was, however, a dose-related increase in the number of pregnant rabbits (0, 3, 7) showing total resorption, which was significant ($p < 0.01$) in the trend test. There were no teratogenic effects. The incidences of major malformations included 2 fetuses (2 litters) in the vehicle controls (hydrocephaly, micrognathia and other cranio/facial malformations), 0 in the low dose group, 3 fetuses (3 litters) in the mid-dose group (hydrocephaly with cerebral hemorrhage, microcaudia, abdominal organs everted at umbilicus) and 1 fetus in the high-dose group (hydrocephaly). There were no effects on skeletal or visceral organs. In contrast to the findings of increased skeletal anomalies in rat fetuses with high-dose ziconotide infusion, the high-dose rabbit fetuses had a significant decrease (-85%, $p < 0.05$) in the incidence of skeletal anomalies. All treatment groups had an increase in the % of fetuses/litter with bilateral 13th rib compared with controls, which was significant ($p < 0.05$) for the low and mid dose groups, but not for the high dose group, as this increase appeared to have an inverse relationship to dose. Also unlike the rat study, no skeletal anomalies of any kind (reduced ossification, irregular ossification, reduced or absent) were reported for any bone in the pelvic girdle. Ziconotide was not teratogenic in rabbits at doses up to 5 mg/kg/day, IV.

Labeling Recommendations:

Study Title: A Continuous Intravenous Infusion Pre- and Postnatal Study of Ziconotide in the Rat

Study No.: _____ 96589

Volume #2.040, page #002

Conducting laboratory and location: _____

Date of study initiation: March 22, 1999 (dosing started)

GLP compliance: Yes

QA-Report: Yes (X) No ()

Lot and batch numbers: Ziconotide #8068 A33294 (82.4% peptide content, _____ purity); 0.9% sodium chloride solution + 50 µg/ml L-methionine, #NUB001/NUB002.

Protocol reviewed by Division, Yes (X) No ():

Methods:

- Species/strain: Rat / Sprague-Dawley _____ : CD@[SD]IGS BR) from _____

- Doses employed: 0 (vehicle), 1, 3 and 10 mg/kg/day
- Route of administration: Intravenous (continuous infusion) at 2 ml/kg/hr.
- Study Design: Females 75-79 days of age and weighing 176-283 grams at treatment initiation were mated with untreated proven breeder males 89-145 days of age. At least two weeks prior to treatment initiation, females were implanted with a silastic catheter in the right femoral vein under isoflurane anesthesia. The catheter ran subcutaneously to the nape of the neck, where it was tethered to a jacket worn by the rat that allowed attachment of the catheter to a swivel connected to a syringe and infusion pump outside the cage. Animals were subsequently infused with 0.9% sodium chloride USP at a rate of 0.4 ml/hr until initiation of treatment. Other drug treatments are shown in the table below:

Non-ziconotide treatments	Administration times	Purpose or function
Penlong-XL (0.1 ml) IM	One hour before, and 2 days after surgery	Systemic penicillin-based antibiotic.
Hibitane™	Presurgery	Topical disinfectant
Betadine™	Presurgery	Topical disinfectant
Duratears™	During anesthesia	Eye moisturizer
Penicillin-G sodium	During surgery	Topical antibiotic at site.
Neosporin cream	Daily after surgery	Topical antibiotic at site.

Females received continuous infusions from day 6 of gestation to *post partum* days 21, 22 or 23. After completion of the dosing, the catheters were disconnected, tied off and inserted subcutaneously in the dorsal region. The animals were euthanized on days 21-23.

- Number of animals/sex/dosing group: 30 females (including 6/group for toxicokinetics).
- Parameters and endpoints evaluated:
 - F₀ Generation: Mortality, signs of ill health, and/or reaction to treatment (twice daily), complete detailed examination (once before mating period and on gestation days 3, 6, 9, 12, 15, 18 and 21, and on lactation days 0, 4, 7, 11, 14, 17 and 21), body weight of females (once during acclimation and on gestation days 3, 6, 9, 12, 15, 18 and 20, and *post partum* days 0, 4, 7, 11, 14, 17 and 21), food consumption (days 0-3; 3-6, 6-9, 9-12, 12-15 and 15-18 of gestation), presence of spermatozoa during mating (to determine gestation day 0), toxicokinetics (gestation days 6 and 17 and *post partum* days 4 and 21), time of parturition and number of pups, signs of dystocia, *post partum* maternal behavior, gross pathology, uterine examination of implantation site scars (days 26-28 *post coitum* for non-littering and days 21 or 22 *post partum* for littering dams), and preservation of the following tissues/organs: abnormalities identified at necropsy, infusion site (including catheter tip), mammary glands (thoracic and inguinal), ovaries, uterus (horn, body and cervix) and vagina; prostate, seminal vesicles, and testes were measured/performed.

- **F₁ Generation:** Pups were examined for malformations, viability, sex and individual body weight of live pups (*post partum* day 0), general condition (daily during lactation period); culled to 8/litter (day 4); weighed (days 4, 7, 14 and 21); assessed for pinna detachment (days 1-4), tooth eruption (day 7 on), eye opening (day 12 on), righting reflex (days 2-4), negative geotaxis test (day 8 on), auricular startle response (day 12 on); and examined for gross pathology if not selected to form the F₁ adult generation. Weanlings not selected for breeding were euthanized and given a gross pathological examination. Those selected for the F₁ adult generation (1 male and 1 female from each litter) were examined for general health (twice daily), clinical signs (weekly), body weight (weekly during pre-mating and on gestation days 0, 3, 6, 9, 12, 15, 18 and 20, and *post partum* days 0 and 4 for mated females), vaginal opening (day 26 on), preputial separation (day 34 on), pupillary closure and visual placing responses (day 21), locomotor activity in figure-8 maze (days 35±2 and 60±2), 120 dBA auditory startle habituation (day 55±1), performance in the 'E' water maze (between days 60 and 71) and mating (day ~85 until vaginal lavage positive for spermatozoa). Pregnant females were observed (t.i.d.) from day 20 of gestation for time of parturition (day 0 *post partum*), number of pups/litter and *post partum* maternal behavior. F₁ females were euthanized and necropsied on *post partum* days 4, 5 or 6, with the number of implantation scars being recorded. Counting of implantation scars and examination of mammary tissue was conducted for females with total litter losses prior to weaning. Adult F₁ generation males were euthanized 4-5 weeks after the mating period and subjected to gross pathological examination.
- **F₂ Generation:** On *post partum* day 0, pups were examined for malformations, viability (number live or dead), sex and individual body weight of live pups. General condition was checked daily. Pups with external abnormalities and pups found dead between days 0 and 4 were placed in Bouin's fluid for subsequent external and internal examination using a modified Barrow and Taylor technique. Externally normal pups were euthanized and not further examined on *post partum* days 4, 5 or 6.
- **Statistical evaluations:** Group means (±S.D.) were analyzed by one-way ANOVA and Dunnett's test when the F value was significant (p<0.05). Heterogenous data were analyzed with Kruskal-Wallis and significant differences between control and test groups was assessed using Dunn's test. The following parameters were calculated: body weights and weight gains (F₀ females and F₁ generation), pregnancy rate (F₀ females), mating index, fertility index, conception rate, mean day of mating (F₁ generation), number of live, dead and malformed pups and sex ratio (% male); group mean gestation length, live birth index, number of implantation site scars and gestation index (F₀ and F₁ dams); viability index (F₁ pups), survival index and lactation index (F₁ and F₂ pups); litter mean pup body weight and group litter mean. The mean day of positive response data (F₁ pups and adults) and errors on the water maze were analyzed statistically with the Kruskal-Wallis test, and where p<0.05 for the "H" value, the Mann-Whitney "U" test was used for analyzing the difference between control and treatments. ANOVA with repeated measures was

used for auditory startle and motor activity data analysis, whereas one-way ANOVA and Dunnett's test were used to analyze performance in the water maze.

Results:

Clinical signs: Observed signs in the F₀ females considered to be treatment-related are shown in the following table:

Clinical Sign	Incidence of sign per group			
	Control	1 mg/kg/d	3 mg/kg/d	10 mg/kg/d
Hunched posture ¹	0	0	0	5
Ptosis ¹	0	1	4	7
Piloerection ²	0	7	10	14

¹Count from sponsor's summary.

²Reviewer's count (somewhat greater than sponsor's at all 3 doses).

- Mortality:

Group #	Circumstance of death	Observations in life	Necropsy findings
F ₀ -low dose	2 Dams euthanized.	One total litter loss. One failure to litter.	
F ₀ -mid dose	TK rat found dead after blood sampling on post partum day 21.		
F ₀ -hi dose	5 Dams euthanized	3 Total litter losses. 2 Failures to litter.	

- Body weight: Weight loss occurred during the first 3 days of treatment.

Gestation	Mean of Group 1		Mean of Group 2		Mean of Group 3		Mean of Group 4	
	Weight	Gain	Weight	Gain	Weight	Gain	Weight	Gain
Day 3	252.5		250.3		257.5		254.5	
Day 6	264.6	12.1	263.3	13.0	272.4	14.9	266.7	12.1
Day 9	273.6	9.0	260.3	-3.0**	267.8	-4.6**	266.8	0.2**
Day 12	288.1	14.5	279.2	18.9*	288.5	20.7**	288.6	21.8**
Day 15	305.6	17.5	288.8*	9.7**	299.4	10.8**	299.7	11.0**
Day 18	337.3	31.8	327.5	38.7**	341.6	42.2**	337.3	37.6
Day 20	361.0	23.7	348.1	20.6	357.3	15.8	360.2	23.0
Lactation	Weight	Gain	Weight	Gain	Weight	Gain	Weight	Gain
Day 0	285.5		265.6**		277.6		280.3	
Day 4	299.8	14.3	293.2	27.7*	302.2	24.5	307.4	27.1*
Day 7	309.4	9.5	288.8**	-7.1**	305.4	3.3	304.0	-5.9
Day 11	323.3	13.9	303.1	14.3	312.8	7.3	314.8	8.5
Day 14	332.3	9.0	309.5**	6.4	324.1	11.3	323.0	8.1
Day 17	336.0	3.8	316.5*	7.0	319.6*	-4.5	327.2	4.2
Day 21	320.8	-15.3	301.2**	-15.4	303.8*	-15.8	313.0	-14.1

Significantly different from control (Group 1) value: *p<0.05, **p<0.01 (Dunnett's test).

- Food consumption: Consumption by F₀ dams during gestation decreased significantly (p<0.01) independent of dose (78, 71 and 76% of control) during the first 3-day period following initiation of ziconotide treatment on day 6.

Gestation Days	Mean food consumption (g/rat) of pregnant F ₀ females			
	Group 1-control	Group 2-low 1.0	Group 3-mid 3.0	Group 4-hi 10
0-3	81.5	75.9	78.5	80.0
3-6	80.2	80.5	82.8	82.5
6-9	80.1	62.4**	56.9**	60.5**
9-12	81.8	86.0	86.5	90.0
12-15	86.9	83.7	86.8	85.8
15-18	93.3	90.9	97.5	95.0

Significantly different from control (Group 1) value: **p<0.01 (Dunnett's test).

- Toxicokinetics:

Grp. No.	Ziconotide dose		Mean (± S.D.) ziconotide concentration, ng/ml*			
	µg/kg/h	mg/kg/d	Gestation Day		Post partum Day	
			6	17	4	21
2	41.7	1	87.0 ± 12.2	90.8 ± 16.4	66.9 ± 21.4	82.3 ± 44.7
3	125.0	3	200 ± 48	191 ± 24	204 ± 49	236 ± 25
4	416.7	10	384 ± 252	512 ± 106	448 ± 425	633 ± 158

* Assays were conducted by _____

- Fertility and Early Embryonic Development in F₀ Females:
 - In-life observations: There were no significant differences between treated groups and control in maternal performance. Pregnancy rates ranged from 95.8 to 100%, and the gestation index was 100% for all groups.
- Embryo-fetal development
 - In-life observations: The mean gestation length ranged from 21.6 to 21.9 days, and the mean live birth index ranged from 97.0% (low dose) to 90.4% (high dose). No malformed F₁ pups were seen in any group, and the mean number of dead pups/litter was 0-0.3 (control group, 0.1). The sex ratio ranged from 56.2 (controls) to 43.5 (high dose) % male.
 - Terminal and Necroscopic evaluations:
 - F₀ Dams: The mean number of implant scars ranged from 13.7 (low dose) to 14.0 (control and mid-dose groups).
- Prenatal and postnatal development, including maternal function:
 - In-life observations:
 - Dams: One low-dose F₀ female and three high-dose F₀ females had all of their pups die by day 8 post partum.
 - Offspring:
 - Viability data on the F₁ pups during lactation are summarized in the table below:

PARAMETER MEASURED	F ₁ group mean (±S.D.) for parameter			
	Group 1	Group 2	Group 3	Group 4
Day 4 Viability Index, %	98.2 ± 3.2	96.0 ± 9.6	98.3 ± 3.4	91.6 ± 21.0

Day 7 Survival Index, %	100 ± 0.0	95.7 ± 20.8	99.5 ± 2.6	92.0 ± 26.0
Day 14 Survival Index, %	100 ± 0.0	95.7 ± 20.8	98.8 ± 4.2	90.9 ± 29.4
Day 21 Lactation Index %	100 ± 0.0	95.7 ± 20.8	98.8 ± 4.2	90.9 ± 29.4

The only clinical signs related to dose in the F₁ pups involved thin fur cover in certain areas, the incidence of which is shown in the table below. The body surface areas affected by thin fur cover (including forepaws) were not randomly distributed among pups, but appeared to segregate according to litters.

Area affected	Mean incidence of thin fur cover as a clinical sign in F ₁ rat pups							
	Group 1		Group 2		Group 3		Group 4	
	Male	Female	Male	Female	Male	Female	Male	Female
Total N	178	141	152	153	173	137	132	158
cranium/ muzzle	0	0	4	4	4	4	7	7
intercapsular dorsal thora- cic/lumbar	1	1	4	4	6	4	8	8

Body weights of pups from the high-dose F₀ dams tended to be lower than the controls, and were significantly lower ($p < 0.05$) for the males and combined sexes on lactation day 14, as shown in the table below:

DAY POST PARTUM	F ₁ group litter mean (±S.D.) for pup body weight			
	Group 1	Group 2	Group 3	Group 4
Day 0	6.1±0.5	5.9±0.5	6.3±0.4	5.8±0.5
Day 4	8.7±0.8	8.6±1.0	9.1±1.0	8.5±1.1
Day 7	13.8±1.5	13.3±1.7	13.5±1.9	12.7±2.2
Day 14	28.8±3.6	26.2±3.4	26.3±4.4	25.7±4.2*
Day 21	47.3±5.4	44.9±5.4	44.3±7.1	43.0±7.2

Significantly different from control (Group 1) value: * $p < 0.05$ (Dunnett's test).

For the F₁ rats chosen for mating, body weights of high-dose females were slightly lower from weaning until they were paired for mating and also during the gestation period. Body weights of the males in the mid and high dose groups were slightly lower after weaning, but were similar to the controls by week 5. Body weight gains of high-dose males were significantly ($p < 0.05$) higher than the controls during weeks 4-5 and 8-9. Body weight gains of the treated females were similar during the pre-mating and gestation periods, except for a significantly ($p < 0.05$) lower weight gain of the high-dose group during gestation days 18-20.

Developmental assessments indicated that the mean day of development for pinna detachment, righting reflex, negative geotaxis, tooth eruption, auricular startle and eye opening of the F₁ pups were unaffected by ziconotide treatment. All rats in all groups showed normal visual placing and pupillary closure. The

mean day of development of vaginal opening for the females (31.9-32.6 days) and preputial separation for the males (43.6-44.1 days) was similar for the F₁ generations from the control and ziconotide-treated groups. Measures of F₁ parental and maternal performance are summarized in the table below:

PARAMETER MEASURED	Group mean (±S.D.) for parameter			
	Group 1	Group 2	Group 3	Group 4
No. placed for mating	23	22	24	20
Mating index, %	95.7	81.8	95.8	95.0
Fertility index, %	82.6	72.7	95.8	90.0
Conception rate, %	86.4	88.9	100	94.7
Gestation index, %	100	100	100	100
Mean live pups at birth	14.6 ± 2.1	14.7 ± 3.4	15.1 ± 1.7	13.4 ± 4.5
Mean dead pups at birth	0.21 ± 0.42	0.31 ± 0.70	0.52 ± 0.99	0.39 ± 0.85
Mean (±SD) % male pups	48.8 ± 12.3	49.5 ± 13.3	45.3 ± 15.0	50.1 ± 17.2
Mean no. implant scars	16.1 ± 1.7	16.6 ± 3.4	16.3 ± 2.3	14.8 ± 4.4
Live birth index, %	91.0 ± 10.1	88.3 ± 8.8	93.4 ± 7.3	87.9 ± 13.8

Viability data on the F₂ pups are summarized in the table below:

PARAMETER MEASURED	F ₂ group mean (±S.D.) for parameter			
	Group 1	Group 2	Group 3	Group 4
Day 4 Viability Index	99.0 ± 2.5	99.1 ± 2.5	93.9 ± 19.8 ^a	98.3 ± 3.2 ^a
No. of pups found dead, days 0-4	4	5	36 (23 ^a)	23 (8 ^a)
No. of litters with pups found dead, days 0-4	4	3	8 (7 ^a)	8 (7 ^a)

^a Excluding 1 litter each in the mid-dose (13 pups) and high-dose (15 pups) groups that died from a water sipper mishap.

In calculating the day 4 viability index, the sponsor excluded 1 litter each in the mid-dose (13 pups) and high-dose (15 pups) groups, which apparently died from "a technical error with the water sippers" on day 2. The pathology report for those dying on day 2 indicated "no abnormal findings," so it is not clear whether the pups drowned or died by another mechanism. However, 3 of the female pups from the high-dose litter were found dead on day 0 with various stages of internal autolysis. One other F₁ dam in the mid-dose group had lost 13/14 pups by day 4, contributing to the lower viability index for that group.

- Terminal and Necroscopic evaluations:

F₁ Adults: No gross pathological findings in the F₁ generation adults were reported to be related to ziconotide treatment of their F₀ dams.

F₂ Offspring: One male pup from the mid-dose group that was found dead on *post partum* day 0 had major malformations, including absence of anus and tail and subcutaneous edema over the entire body. Internally, the intestine of this

pup was obstructed and the colon was severely dilated in the pelvic region. These findings were considered by the sponsor to be incidental in nature. Four pups from another dam showed bilateral reduction (3) or absence (1) of the renal papilla. One pup from a third dam in the mid-dose group had missing left kidney, ureter and adrenal gland.

Summary and Evaluation:

Pregnant rats receiving the continuous intravenous infusion of ziconotide (1, 3 or 10 mg/kg/day) from day 6 of gestation to *post partum* days 21, 22 or 23 showed a dose-related increase in the incidence of piloerection and ptosis, and hunched posture was observed in the high-dose group. Food consumption by F₀ dams during gestation decreased significantly (p<0.01) at all doses during the first 3-day period of ziconotide treatment and decreased body weight gains were evident in all treatment groups at various time segments during both gestation and lactation. One low-dose F₀ female and three high-dose F₀ females had all of their pups die by day 8 *post partum*. The only clinical, developmental, or reproductive sign related to dose in the F₁ pups involved thin fur cover in certain areas. All other F₁ indices were normal, except for slightly lower weights in the high-dose group at birth, during lactation and post weaning. The mid- and high-dose F₁ females had about twice as many litters with pups dying during days 0-4 *post partum* as did the control and low-dose groups, but the Day 4 viability index for the F₂ pups was not significantly affected by ziconotide. The mid-dose group had one pup with major malformations (missing anus and tail), one pup with missing left kidney, ureter and adrenal gland, and four pups in another litter with bilateral reduction/absence of the renal papilla. Sponsor considers the NOAEL for maternal toxicity to be <1 mg/kg/day, IV, and the NOAEL for the F₁ generation to be 3 mg/kg/day.

Labeling Recommendations:



GENETIC TOXICOLOGY:

Study Title: Mutagenicity test on SNX-111 in the Salmonella/mammalian-microsome

_____ Mutation Assay (Ames Test) Preincubation Method

Study No.: _____ 15766-0-420

Study Type: *In vitro*

Volume #2.047, page #4

Conducting laboratory and location: _____

Date of study initiation/completion: 7/27/1993 – 10/18/1993

GLP compliance: Yes

QA-Reports: Yes (X) No ()

over the mean vehicle control in mean revertants/plate for at least one strain, accompanied by a dose-response relationship is required. For strains TA1535, TA1537 and TA1538, at least a 3-fold increase over the mean vehicle control in mean revertants/plate for at least one strain, accompanied by a dose-response relationship is required.

Results:

- Study validity: A second experiment was conducted with tester strain TA1537 because in the first experiment, the mean vehicle control value for tester strain TA1537 was outside the acceptable range, both in the absence and presence of S9.

- Study outcome:

Test Article	S9	Mean (\pm S.D. or range of means) Number of Revertants/Plate					
		TA98	TA100	TA1535	TA1537		TA1538
		1 st Expt.	1 st Expt.	1 st Expt.	1 st Exp	2 nd Expt.	1 st Expt.
Vehicle Control	-	23 \pm 3	84 \pm 13	12 \pm 4	58 \pm 10	5 \pm 1	15 \pm 5
Vehicle Control	+	26 \pm 6	90 \pm 4	10 \pm 4	49 \pm 2	9 \pm 5	11 \pm 1
Range for SNX	-	(14-20)	(82-85)	(10-15)		(4-6)	(8-12)
Range for SNX	+	(21-27)	(88-105)	(10-13)		(8-12)	(12-14)
Positive Control	-	295 \pm 24	665 \pm 67	622 \pm 20		1105 \pm 151	381 \pm 24
Positive Control	+	987 \pm 87	982 \pm 67	126 \pm 4		138 \pm 10	1106 \pm 41

Summary:

When tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538 in the absence or presence of metabolic activation (S9) at concentrations ranging from 3.14 to 100 μ g/ml, ziconotide was negative for mutagenicity under all test conditions. the highest concentration tested was not chosen on the basis of cytotoxicity.

Study Title: Bacterial Reverse Mutation Assay

Study No.: G97BK

Study Type: *In vitro*

Volume #2.047, page #48

Conducting laboratory and location: _____

Date of study initiation/completion: 7/29/1997 – 11/19/1997

GLP compliance: Yes

QA-Reports: Yes (X) No ()

Drug Lot Number: 8068 V07465, _____ peptide purity _____ peptide content 81.32%; also contains acetic acid, 14.5%, trifluoroacetic acid, 0.01% and water, 3.4%. S9 batch prepared 4/28/97 and stored \leq -70°C until used.

Study endpoint: Induction of reverse mutations at the histidine locus in the genome of specific *Salmonella typhimurium* tester strains or at the tryptophan locus of *Escherichia coli* testor strain _____

Methodology:

Strains/Species/Cell line: *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain _____

- Dose Selection Criteria:
 - Basis of dose selection: Range finding studies in which the maximum dose tested was 5000 µg/plate.
 - Range finding studies: Ziconotide was soluble at 50 mg/ml and 100 µl/plate of this concentration did not cause either precipitation or cytotoxicity.
- Test Agent Stability: Dosing preparation solutions at concentrations of _____
- Metabolic Activation System: S9 fraction from liver of rats treated with Aroclor 1254 (500 mg/kg, IP, 5 days prior to euthanasia).
- Controls:
 - Vehicle: 0.9% Saline \ _____
 - Negative Controls: 0.5 ml of 100 mM phosphate buffer substituted for S9 fraction _____
 - Positive Controls: In the absence of S9, 2-nitrofluorene _____ 1.0 µg/plate, for strain TA98; sodium azide _____ 1.0 µg/plate, for strains TA100 and TA1535; 9-aminoacridine _____ 275 µg/plate, for strain TA1537; and methyl methanesulfonate _____ 1000 µg/plate for *E. coli* strain _____. In the presence of S9, 2-aminoanthracene _____, 1.0 µg/plate, for all strains except 10 µg/plate for *E. coli* strain _____
- Exposure conditions:
 - Incubation and sampling times: Tester strain, test agent and S9 or _____ were added to 2.0 ml of molten selective top agar at 45±2°C. After vortexing, the mixture was overlaid onto the surface of 25 ml of minimal bottom agar. After the overlay had solidified, the plates were inverted and incubated for approximately 48-72 hours at 37±2°C.
 - Doses used in definitive study: 89, 266, 887, 2660 and 5000 µg/plate (corrected for purity and peptide content of the ziconotide lot).
 - Study design: Test article dilutions were prepared immediately before use. Each plate was dosed with a volume of 100 µl.
- Analysis:
 - No. plates/replicates analyzed: 3 plates/dose
 - Counting method: Number of revertant colonies per plate were counted either entirely by automated colony counter or entirely by hand.
 - Cytotoxic endpoints: Background lawn was evaluated for evidence of cytotoxicity
 - Genetic toxicity endpoints/results: Count of revertant colonies of tester strains.
 - Statistical methods: The mean and standard deviation for each replicate plating was calculated.
- Other: A minimum of 3 non-cytotoxic dose levels were required to evaluate assay

data.

- Criteria for Positive Results: For strains TA98, TA100 and WP2 *uvrA*, at least a 2-fold increase over the mean vehicle control in mean revertants/plate for at least one strain, accompanied by a dose-response relationship for at least two concentrations is required. For strains TA1535 and TA1537, at least a 3-fold increase over the mean vehicle control in mean revertants/plate for at least one strain, accompanied by a dose-response relationship for at least two concentrations is required.

Results:

- Study validity: All criteria for a valid study were met.

- Study outcome:

Test Article	S9	Mean (±S.D. or range of means) Number of Revertants/Plate				
		TA98	TA100 ^a	TA1535	TA1537	WP2 <i>uvrA</i>
Vehicle Control	-	17 ± 7	131 ± 7	12 ± 4	6 ± 3	22 ± 5
Vehicle Control	+	25 ± 6	158 ± 6	15 ± 1	7 ± 3	19 ± 4
Range for SNX	-	(14-21)	(122-159)	(9-13)	(4-9)	(18-20)
Range for SNX	+	(20-29)	(143-155)	(11-15)	(9-11)	(16-21)
Positive Control	-	206 ± 30	825 ± 26	580 ± 42	1276 ± 322	161 ± 13
Positive Control	+	633 ± 30	857 ± 66	103 ± 1	100 ± 12	634 ± 33

^a Counted by machine; all other strains were counted manually.

Summary:

When tested in *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and *E. coli* strain _____ in the absence or presence of metabolic activation (S9) at doses ranging from 89 to 5000 µg/plate, ziconotide was negative for mutagenicity under all test conditions.

Study Title: Mutagenicity Test of SNX-111 in the L5178yTK+/- Mouse Lymphoma Forward Mutation Assay with an Independent Repeat

Study No.: 15766-0431R

Study Type: *In vitro*

Volume #2.047, page #103

Conducting laboratory and location: _____

Date of study completion: 10/13/1993

GLP compliance: Yes

QA-Reports: Yes (X) No ()

Drug Lot Number: 8068 SJK006 _____ peptide purity _____ peptide content 76.2%; also contains acetic acid, 12.6%, trifluoroacetic acid, 0.08% and water, 7.9%. S9 was obtained commercially (Batch #0434, _____) and stored at about -80°C until used.

Study endpoint: Induction of forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line as assessed by colony growth in the presence of 5-trifluorothymidine.

Methodology:

Strains/Species/Cell line: Mouse lymphoma L5178Y TK+/- cell line

Dose Selection Criteria:

- Basis of dose selection: The maximum dose tested was approximately 100-1000X greater than the projected therapeutic plasma level of ziconotide.
- Range finding studies: No study to determine cytotoxicity was reported.
- Test Agent Stability: Stability in the incubation media was not tested.
- Controls:
 - Vehicle: 0.9% Saline
 - Negative Controls: 0.5 ml of 100 mM phosphate buffer substituted for S9 fraction.
 - Positive Controls: In the absence of S9, methyl methanesulfonate 10 or 15 nl/ml, was used. In the presence of S9, 3-methylcholanthrene 2.0 or 4.0 µg/ml, was used.
- Exposure conditions:
 - Incubation and sampling times: Treatment was started by adding 10 ml of the dosing medium to 6×10^6 cells. The dosed tubes were closed, vortexed and placed in an orbital shaker incubator (80 ± 10 orbits/minute) for 4 hours at approximately 37°C. The cells were washed twice, resuspended in 20 ml of growth medium and returned to the orbital shaker incubator as closed-tube cultures. After an expression period of two days, a sample of 3×10^6 cells was suspended in selection medium and distributed into three 100 mm dishes. The cloning efficiency was determined by serially diluting the cells and seeding each of three dishes with approximately _____ in cloning medium. After 10-14 days of incubation in a 95:5 air:CO₂ atmosphere in a humidified incubator maintained at a temperature of approximately 37°C, the colonies were counted.
 - Doses used in definitive study: 3.13, 6.25, 12.5, 25.0, 50.0, 75.0 and 100 µg/ml (corrected for purity and peptide content of the ziconotide lot).
 - Study design: The primary stock was prepared and filter sterilized. Serial dilutions were made in order to dose each plate at a volume of 50 µl.
- Analysis:
 - No. plates/replicates analyzed: 3 plates/dose
 - Counting method: _____ colony counter fitted with a _____ potentiometer for discrimination of colony size (detection limit, _____).
 - Cytotoxic endpoints: Cloning efficiency = [(total viable colony count)/(number of cells seeded)] X 100; Relative growth = (relative suspension growth X relative cloning efficiency)/100.
 - Genetic toxicity endpoints/results: Count of mutant colonies.

- Statistical methods: The mean and standard deviation for each replicate plating was calculated.
- Other: A minimum of 3 non-cytotoxic dose levels were required to evaluate mutation assay data.
- Criteria for Positive Results: Mutant frequency ≥ 2 times the concurrent background mutant frequency of the vehicle control cultures.

Results:

- Study validity: All criteria for a valid study were met.
- Study outcome:

Parameter measured or calculated	Range of Observations for Parameter					
	Without S9 Metabolic Activation			With S9 Metabolic Activation		
	Vehicle	+ Control	Ziconotide	Vehicle	+ Control	Ziconotide
Tot. mutant colonies	178-217	708-792	146-206	197-311	665-746	157-224
Total viable colonies	497-555	191-271	398-484	469-568	238-259	364-473
Relative Growth (%)	100	14.7-30.0	74.8-108.1	100	10.8-15.9	61.5-80.3
Mutant frequency (10^{-6} units)	71.6-78.9	584.5-741.4	68.9-85.1	96.4-109.5	558.8-576.1	70.6-100.0

+ Positive control: methyl methanesulfonate (-S9) or 3-methylcholanthrene (+S9).

Summary:

When tested *in vitro* in the mouse lymphoma L5178Y TK+/- cell line forward mutation assay in the absence or presence of metabolic activation (S9) at doses ranging from 3.13 to 100 $\mu\text{g/ml}$, ziconotide was negative for mutagenicity under all test conditions.

Study Title: In Vitro Mammalian Cell Gene Mutation Test

Study No.: G97BK84.702

Study Type: *In vitro*

Volume #2.047, page #145

Conducting laboratory and location: _____

Date of study initiation/completion: _____

GLP compliance: Yes

QA-Reports: Yes (X) No ()

Drug Lot Number: 8068 V07465 _____; peptide purity _____, peptide content 81.32%; also contains acetic acid, 14.5%, trifluoroacetic acid, 0.01% and water, 3.4%. S9 batch prepared 4/28/97 and stored $\leq -70^{\circ}\text{C}$ until used.

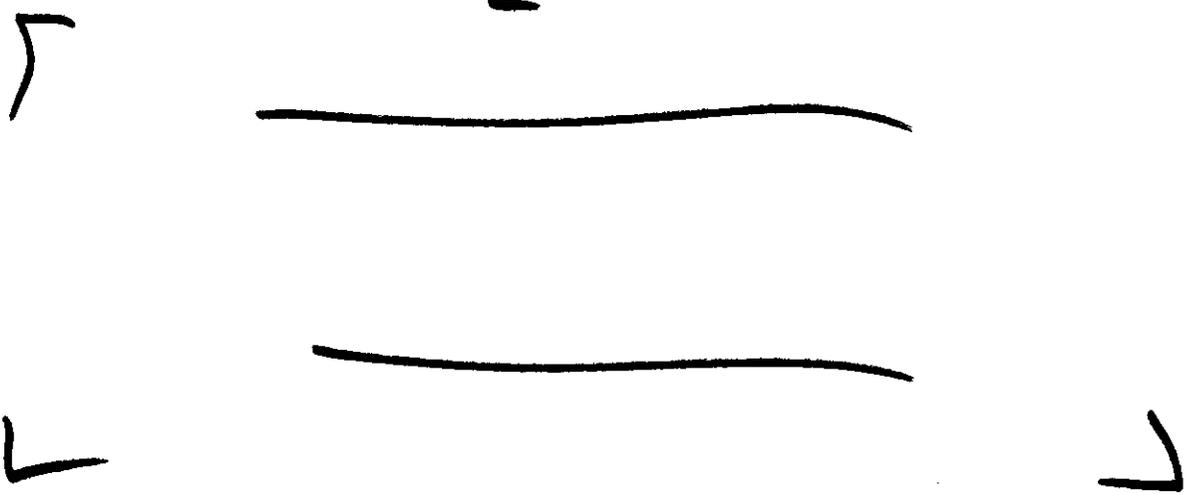
Study endpoint: Induction of forward mutations at the thymidine kinase (TK) locus in the mouse lymphoma L5178Y cell line as assessed by colony growth in the presence of 5-trifluorothymidine.

Methodology: _____

Strains/Species/Cell line: Mouse lymphoma L5178Y TK+/- cell line (_____)

Dose Selection Criteria:

- Basis of dose selection: Range finding studies in which the maximum concentration tested was 5000 µg/ml.
- Range finding studies: Ziconotide was soluble at 50 mg/ml and 100 µl/plate and this concentration did not cause precipitation. Substantial toxicity, i.e., suspension growth ≤50% of the vehicle control was observed only at 5000 µg/ml in the presence of S9.
- Test Agent Stability: Aliquots of dosing solutions were analyzed for concentration.
- Controls:
 - Vehicle: 0.9% Saline _____
 - Negative Controls: 0.5 ml of 100 mM phosphate buffer substituted for S9 fraction _____
 - Positive Controls: In the absence of S9, methyl methanesulfonate _____, 10 or 20 µg/ml, was used. In the presence of S9, 7,12-dimethylbenz(a)anthracene _____, 2.5 or 4.0 µg/ml, was used.
- Exposure conditions:
 - Incubation and sampling times: [_____]



- Doses used in definitive study: 50, 150, 500, 1500 and 5000 µg/ml in the absence of S9 and 500, 1000, 2000, 3000 and 4000 µg/ml in the presence of S9 (corrected for purity and peptide content of the ziconotide lot).
- Study design: Each lot of cells was tested and found to be free of mycoplasma contamination. Duplicate cultures of cells were exposed to a minimum of 8 concentrations of test article as well as positive and negative (solvent) controls.
- Analysis:
 - No. plates/replicates analyzed: 3 plates/dose ± _____
 - Counting method: _____ colony counter fitted with a _____ potentiometer for discrimination of colony size (detection limit, _____)
 - Cytotoxic endpoints: Cloning efficiency = [(total viable colony count)/(number of cells seeded)] X 100; % Total growth = (% suspension growth X % cloning

efficiency)/100.

- Genetic toxicity endpoints/results: Count of mutant colonies.
- Statistical methods: The mean and standard deviation for each replicate plating was calculated.
- Other: A minimum of 4 analyzable dose levels were required to evaluate mutation assay data.
- Criteria for Positive Results: Increase in mutant frequency is concentration-related and one or more dose level with 10% or greater total growth exhibits mutant frequencies of ≥ 100 mutants per 10^6 clonable cells over the background level.

Results:

- Study validity: All criteria for a valid study were met.
- Study outcome:

Parameter measured or calculated	Range of Means of Observations for Parameter					
	Without S9 Metabolic Activation			With S9 Metabolic Activation		
	Vehicle	+ Control	Ziconotide	Vehicle	+ Control	Ziconotide
Tot. mutant colonies	35-44	199-203	20-36	42	163-186	33-41 ^a
Total viable colonies	167-195	52-117	140-214	146-148	70-121	98-184
Total Growth (%)	---	13-48	79-114	---	20-62	7-129
Mutant frequency/ 10^6 surviving cells	36-53	347-764	29-44	57	270-530	38-50

+ Positive control: methyl methanesulfonate, 10 or 20 $\mu\text{g/ml}$ (-S9) or 7,12-dimethylbenz(a)anthracene, 2.5 or 4.0 $\mu\text{g/ml}$ (+S9).

^a Does not include 4000 $\mu\text{g/ml}$ concentration, which was too toxic to count (total growth <10%).

Summary:

When tested in the mouse lymphoma forward mutation assay at doses ranging from 50 to 5000 $\mu\text{g/ml}$ in the absence of metabolic activation (S9) and doses ranging from 500 to 4000 $\mu\text{g/ml}$, ziconotide was negative for mutagenicity under all test conditions.

Study Title: Mutagenicity Test on SNX-111 In Vivo Mouse Micronucleus Assay

Study No.: 15766-0455

Study Type: *In vivo*

Volume #2.047, page #191

Conducting laboratory and location: _____

Date of study initiation/completion: experiment, 8/31/1993 – 10/25/1993

GLP compliance: Yes

QA-Reports: Yes (X) No ()

Drug Lot Number: 8068 SJK006 _____; peptide purity _____, peptide content 76.2%; also contains acetic acid, 12.6%, trifluoroacetic acid, 0.08% and water, 7.9%.

Study endpoint: Induction of micronuclei in bone marrow polychromatic erythrocytes of CD-1 (ICR) mice.

Methodology:

Strains/Species/Cell line: CD-1 Mouse ([redacted])

Dose Selection Criteria:

- Basis of dose selection: Range finding study, in which all doses produced tremors, but of faster onset with 8-32 mg/kg, more severe and longer acting (at least 17 hr) with 16-32 mg/kg.
- Range finding studies: Doses of 4.0, 8.0, 16, 24 and 32 mg/kg, IV (corrected for peptide content and purity) were tested in 3 mice [redacted] per sex per dose, weighing 25.1-29.3 g (males) or 23.8-26.3 g (females) and about 8 weeks of age.
- Test Agent Stability: Stability testing of the dosing solutions was not reported.
- Controls:
 - Vehicle: 0.9% Saline for injection, U.S.P. ([redacted]), 10 ml/kg
 - Negative Controls: Vehicle
 - Positive Controls: Cyclophosphamide, 80 mg/kg, PO
- Exposure conditions:
 - Mice ([redacted]) weighing 28.4-34.0 g (males) and 23.4-28.9 g (females) were given a single IV injection and euthanized approximately 24, 48 or 72 hours later for the test article, and 24 hours after injection for the vehicle-control and positive-control mice.
 - Doses used in definitive study: 0.32, 3.2 and 32 mg/kg, IV (corrected for [redacted] purity and peptide content of the ziconotide lot) were administered to 5 mice/sex per group, with a secondary high dose group (32 mg/kg) of 10 mice/sex.
 - Study design: Mice (5/sex/group) were euthanized by CO₂ and marrow was aspirated from the femoral bones, mixed with ~0.1 ml fetal calf serum to form a suspension. The cells were then air-dried on slides, fixed in methanol, and stained in May-Grunwald solution followed by Giemsa.
- Analysis:
 - No. plates/replicates analyzed: One thousand polychromatic erythrocytes per mouse were scored for micronuclei.
 - Counting method: Method of Schmid (1976) which counts cells, not individual micronuclei, as there may be more than one micronucleus in some cells.
 - Cytotoxic endpoints: Criteria not described.
 - Genetic toxicity endpoints/results: Percent micronucleated cells significantly above the vehicle control. Normal frequency in this mouse strain is 0.0 to 0.4%.
 - Statistical methods: ANOVA of the square root arcsine transformation, followed by [redacted] range test with adjustment for multiple comparisons at each harvest time to determine which dose groups, if any, were significantly different ($p < 0.05$) from the vehicle control.
- Criteria for Positive Results: Statistically significant, dose-related increase in micronucleated polychromatic erythrocytes, or the detection of a reproducible and statistically significant positive response for at least one dose level.

Results:

- Study validity: Six males and 1 female from the high dose group were found dead shortly after injection and another female was found dead about 5 hours later, indicating that the high dose was above the maximum tolerated dose. The positive control induced significant increases in micronucleated polychromatic erythrocytes in both sexes.

Study outcome:

Treatment	Dose, mg/kg	Harvest time, hr	Mean % Micronucleated PCEs			Mean PCE:NCE ratio	
			Males	Females	Total	Males	Females
Vehicle	0	24	0.06	0.18	0.12	0.89	0.52
Cyclophos.	80	24	4.58*	3.60*	4.09*	0.65	0.66
Ziconotide	0.32	24	0.04	0.08	0.06	0.53	0.76
		48	0.06	0.06	0.06	1.18	0.91
		72	0.02	0.06	0.04	0.48	0.45
	3.2	24	0.06	0.14	0.10	0.85	0.62
		48	0.12	0.12	0.12	0.74	0.78
		72	0.04	0.04	0.04	0.85	0.84
	32	24	0.08	0.08	0.08	0.48	0.50
		48	0.10	0.10	0.10	0.62	0.63
		72	0.02	0.08	0.05	0.82	0.46

* Significantly greater than the corresponding vehicle control, p<0.05.

Summary:

When tested *in vivo* in the mouse micronucleus test at doses of 0.32, 3.2 and 32 mg/kg, IV, ziconotide was negative at all doses and all times of marrow harvesting after injection (24, 48 and 72 hours), whereas the positive control drug, cyclophosphamide (80 mg/kg, PO), caused a significant increase in micronucleated polychromatic erythrocytes (4.09 ± 0.44% versus vehicle control 0.12 ± 0.04%) at 24 hours.

SPECIAL TOXICOLOGY STUDIES:

General Comments: Doses in the following report are not corrected for peptide content of the ziconotide used. Although the report refers to several figures (up to #12), no figures were provided in this reviewer's copy of the report.

Study Title: Histopathology of the ω-Conopeptide Ziconotide (SNX-111) after Repeated Intrathecal Injections in the Rat

Study No.: 7-14-95

Volume #2.048, page #223

Conducting laboratory and location: _____

Date of study initiation: Not indicated (Final Report dated 7/14/1995)

GLP compliance: No

QA-Report: Yes () No (X)

Methods: Male Sprague-Dawley rats _____ were fitted with a lumbar IT catheter (PE-10, stretched) by the cisternal approach under halothane anesthesia five days before receiving the first IT injection. All doses were given in a total volume of 10 μ l, followed by a 10- μ l saline flush. Four injections were given at 24-hour intervals, and the rats were euthanized 48 hours after the last injection by pentobarbital anesthesia and thoracotomy. Animals were perfused with 10% formalin and the spinal column from cervical to lower sacral level was removed as a block. For light microscopic examination, each spinal column was sectioned into thoracic (above catheter tip), lumbar to lower thoracic (including catheter tip) and sacral (below catheter tip) blocks without removal of the catheter, and 8-10 μ m sections from each block were stained with hematoxylin and eosin. Sections were examined by four pathologists (3 veterinary and one M.D.-Ph.D.) for the following:

- Position of catheter (dorsal, lateral, ventral) and presence of fibrosis and inflammation around the catheter (graded 0, +, ++ or +++).
- Type of inflammatory cells and degree of inflammation in the arachnoid and subarachnoid space (graded 0, +, ++ or +++); absence (0) or presence (+) of vasculitis (inflamed leptomeningeal vessels).
- Thickening of dura and presence of inflammatory cells.
- Absence (0) or presence (+) of inflammatory cells at the nerve roots.
- Abnormalities of white and gray matter (e.g., inflammatory infiltrates, vacuolization).

Clinical signs assessed during treatment included placing/stepping reflex, righting and ambulation, catalepsy and allodynia.

Dosing: Six rats per group received 0, 1, 3 or 10 μ g of ziconotide/day, IT, for 4 days.

Drug, lot#, and % purity: SNX-111, CS-108292, _____ peptide content.

Observations and times: Body weight and behavior were assessed daily from days 1-6.

Results: One low-dose rat was found dead on day 2, one high-dose rat was found dead on day 4 and another high-dose rat died on day 6. ANOVA with repeated measures indicated significant ($p < 0.05$) weight loss in the ziconotide-treated groups, although there were no significant differences among groups at the individual time points.

Ziconotide produced several behavioral alterations in a dose-dependent manner, including whole body shaking, rigidity, coordination problems, circling behavior and serpentine-like movements of the tail. Three principal findings, inflammation, tissue degeneration and periaxonal dilation/ vacuolization, were indicated uniformly by all pathologists.

Inflammation. The most notable histopathological finding was fibrosis and inflammation of varying degrees in the area around the catheter, regardless of treatment with vehicle or ziconotide. Presence of a mixture of mono- and polynuclear cells and lymphocytes typical of chronic inflammation were suggestive of a non-suppurative meningitis. Two of the pathologists noted the tendency for a slight increase in the severity of the reaction in the mid and high dose groups than in the saline and low dose groups, but a third pathologist (the M.D.-Ph.D.) failed to obtain statistical significance of the scores when applying the Jonckheere non-parametric test. However, when the Jonckheere test was applied to the sum of the scores for inflammation in the leptomeninges in the

sections above and below the catheter sites, there was significant difference ($p < 0.05$) among groups. In this summation of the two sites, the number of rats per group with a total inflammation score of 3 or more (maximum possible = 6) were as follows: vehicle, 1; low dose, 2, mid dose, 2, and high dose, 5.

Degeneration. Changes characterized as Wallerian degeneration or dark neurons were observed in virtually all sections containing catheters and were thought to be due to compression of the cord by the catheter and not dependent upon the presence or concentration of ziconotide. Inflammatory infiltrates in the perivascular spaces within the spinal cord were found in one control and 3 high-dose rats. Although cellular infiltrates in gray matter were not specifically noted by the veterinary pathologists, the M.D.-Ph.D. noted one case in each of the low and mid dose groups and two cases in the high dose group. In addition, one of the high-dose cases also showed marked inflammation around the central canal.

Vacuolization. The M.D.-Ph.D. noted the following incidence of vacuolization in white matter among the treatment groups: saline, 0/6 rats; low dose, 2/5 rats; mid dose, 2/6 rats; and high dose, 3/6 rats. The veterinary pathologists described variable-sized patches in the white matter in several sections as periaxonal dilation or dilated axon sheaths (not associated with demyelination), but these were reported for both vehicle and ziconotide-treated groups.

Summary:

Histopathological studies of the spinal columns of rats given 0, 1, 3 or 10 μg of ziconotide/day, IT, for 4 days and euthanized on day 6 showed several abnormalities (inflammation, tissue degeneration, and periaxonal dilation/vacuolization) that were largely attributed to a local reaction to the chronic IT catheter and/or volume delivery through it. The only dose-related observations were for inflammation scores for the leptomeninges in the sections above and below the catheter sites ($p < 0.05$), cellular infiltrates in gray matter, and vacuolization in white matter. Since, for these three observations, the low-dose group incidence was above the controls, the NOAEL should be considered to be $< 1 \mu\text{g}/\text{day}$.

Study Title: A Study of the Combined Effects of Histamine H₁ and H₂ Receptor Blockers on the Toxicity of SNX-111 in Rats

Study No.: 360-92

Volume #2.048, page #73

Conducting laboratory and location

Date of study initiation: December 4, 1992

GLP compliance: Yes

QA-Report: Yes (X) No ()

Methods: Four groups of rats, each consisting of 5/sex, were surgically implanted with catheters in a femoral vein and femoral artery. They were given a 24-hour IV infusion of saline (Groups 1 and 2) or ziconotide (Groups 3 and 4) after pretreatment with an IV

bolus dose of saline (Groups 1 and 3) or a mixture of the H₂ receptor antagonist famotidine (0.1 mg/kg) and the H₁ receptor antagonist chlorpheniramine (5 mg/kg; Groups 2 and 4). The arterial catheter was used for direct mean arterial blood pressure monitoring at baseline (mean of 3 measurements at 5-minute intervals prior to the start of dosing), at 5 minutes after the bolus injection, and at 2, 5, 10, 15, 30, 45 minutes, as well as at 1, 1½, 2, 2½, 3, 3½, 4 and 24 hours after the start of the infusion, and at 4 hours post-infusion. Clinical signs were recorded at least hourly until the end of the work day and twice daily until termination. No postmortem examination was performed. Dosing: see "Methods" above.

Drug, lot# and % purity: SNX-111, 8068 SJJ004, peptide purity, _____, peptide content, 79.8%, acetic acid, 10.2%, trifluoroacetic acid, 0.05% and water, 6.69%; 0.9% sodium chloride for injection U.S.P., 65354 WS, 65361 WS and 70-154-NA _____
 _____ and AP 380A2 _____; sterile water for injection, 61-008-NA
 _____ : famotidine _____ 0913V, _____ chlorpheniramine
 _____ 2 CD 1 _____

Results: Two of the female rats receiving ziconotide, one of which was pretreated with the antihistamines, were euthanized in poor condition. Clinical signs associated with ziconotide administration included body tremors, ptosis, muscular jerks and increased tonus, and decreased activity. ANOVA of blood pressure data at each time point indicated significant differences among the four groups at 2, 5, 10, 15, and 30 minutes after the start of the infusion, but not at 45 minutes to 4 hours after the start of the infusion. The decreases in blood pressure caused by ziconotide were maximal or near maximal at 5 minutes, when mean (±S.D.) pressures were 122.2±11.6, 128.1±14.5, 96.0±17.8 (-21%) and 89.9±24.0 (-30%) mm Hg for Groups 1, 2, 3, and 4, respectively. Thus, the pressure was approximately 21% less for group 3 than for group 1 and was approximately 30% less for group 4 than for group 2 at 5 minutes. At 24 hours after the start of the infusions and at 4 hours following their termination, ANOVA indicated significant differences among the four groups, with the ziconotide-treated groups having mean blood pressures 21% and 20% below the controls at 24 and 28 hours, respectively, without antihistamine pretreatment and 17% and 11% below the controls at 24 and 48 hours, respectively, with antihistamine pretreatment.

Summary:

In rats given a 24-hour IV infusion of saline or ziconotide (20 mg/kg) after pretreatment with an IV bolus dose of saline or a mixture of famotidine (0.1 mg/kg) and chlorpheniramine (5 mg/kg), no significant effect of the antihistamine treatment was reported for the significant decreases in mean blood pressure resulting at 2-30 minutes and again at 24 hours after the initiation of ziconotide treatment and 4 hours after termination of the infusion.

OVERALL SUMMARY AND EVALUATION:

Introduction: Ziconotide is a new molecular entity consisting of a linear 25-amino acid peptide with three intramolecular disulfide bonds (refer to page 1 for structure). It is a synthetic version of ω -conotoxin MVIIA, a naturally-occurring peptide found in a fish-eating marine snail (*Conus magus*), which potently blocks N-type voltage-dependent calcium ion channels in neurons at sub-nanomolar concentrations. It is thought to produce analgesia by inhibiting the spinal release of substance P and calcitonin gene-related peptide from primary afferent terminals involved in neuronal pain-signaling pathways. The proposed product will be available as a solution in 5-ml or 10-ml vials, containing 0.1 mg of ziconotide and 0.05 mg of L-methionine per ml of sterile 0.9% saline. If desired, it can be diluted with sterile saline for use in implantable pumps for continuous IT infusion. _____ the management of severe chronic pain in whom intraspinal therapy is warranted. The sponsor's maximum recommended clinical dose is an IT infusion rate of 2.4 $\mu\text{g/hr}$ in a 50-kg patient.

Efficacy:

Efficacy studies of antinociceptive activity after IT administration have been conducted (mostly in rats) using the formalin test; suppression of mechanical allodynia and/or heat hypersensitivity following tight spinal nerve ligation, in an incisional pain model, or UV burn model of inflammatory pain; the hot-plate test; the tail-immersion test; the paw-pressure test; carrageenan plus kaolin-induced knee joint inflammation, and a thermally-evoked skin-twitch test (in dogs). In general, ziconotide exhibits antinociceptive activity in a variety of acute and chronic inflammatory pain models in which NSAIDS and/or morphine are also antinociceptive. In pain models, such as the hot-plate and tail-withdrawal tests, that separate weak analgesics (NSAIDS) from strong analgesics (e.g., morphine), there is not a good separation of antinociceptive activity and the elicitation of shaking behavior by IT ziconotide, both of which occur at around 0.3 $\mu\text{g/rat}$ by bolus injection or 0.1 $\mu\text{g/hr/rat}$ by continuous infusion. Thus, there is some question from the non-clinical efficacy studies regarding whether ziconotide will be effective in treating severe pain in humans without simultaneously causing adverse effects.

Safety Evaluation:

- **Neurological:** When mice were given an acute IT injection of ziconotide, a low dose (0.03 μg) "evoked serpentine tail movements and intermittent shaking, predominantly involving the hind legs and tail. Higher doses produced whole-body shaking, which increased in severity and duration as the dose was increased." Similar dose-dependent effects on motor behavior were observed in rats over the dosage range of 0.1-10 $\mu\text{g/rat}$ for the acute IT administration of ziconotide. Acute IV injection in dogs (0.1 mg/kg) produced severe vomiting, scleral vasodilation, facial erythema, whole body trembling, laryngeal spasms, decreased muscle tone and sedation. Acute IT injection of 10 μg (~1 $\mu\text{g/kg}$) in dogs resulted in whole body trembling, panting, decreased arousal and activity. Continuous IT infusions of

ziconotide of 100, 300, 600 or 1200 ng/kg/hr in dogs (approximately 1.3–16 times the maximum recommended human dose) caused increased body temperature, ataxia, tremors and/or hyperactivity, with onset and severity related to dose and sex, females being more sensitive than males. At least half of the ziconotide-treated dogs exhibited decreased or no pupillary light reflex and some exhibited dilated pupils.

- **Cardiovascular:** Although ziconotide causes a dose-related decrease in systemic blood pressure of rats after its IV administration by either bolus ($ED_{50} = 0.48$ mg/kg; $E_{max} = -70$ mm Hg) or 24-hour infusion, a decrease that was not affected by antihistamines (famotidine plus chlorpheniramine), no significant effects on systemic blood pressure were observed up to 90 minutes after the acute IT injection of rats with ziconotide in doses up to 10 μ g/rat. Acute IV bolus in dogs (0.1 mg/kg) produced hypotension and tachycardia lasting 8 hours and decreased respiration for 3 hours. Acute IT injection of 10 μ g (~1 μ g/kg) in dogs resulted in decreases in both blood pressure and heart rate. EKG studies in dogs given continuous IT infusions of ziconotide of 100, 300, 600 or 1200 ng/kg/hr (approximately 1.3–16 times the maximum recommended human dose) showed an increase in the incidence of 2° A-V block in groups receiving 600 or 1200 ng/kg/hr accompanied by increases in respiratory sinus arrhythmia in all ziconotide-treated groups.
- **Pulmonary:** Ziconotide (0.1 μ g/rat, IT bolus) had no respiratory depressant effects alone and did not exacerbate the respiratory depressant effects of subcutaneous morphine in rats during exposure to a 10% CO₂ respiratory stimulus. Rather, ziconotide appeared to attenuate the maximum and duration of the respiratory depressant effect of an acute 10 mg/kg SC dose of morphine over a 2-hour period. The IT administration of ziconotide (0.1 μ g/rat) prior to morphine on Day 7 to rats treated twice daily with morphine (10 mg/kg, SC) did not depress the respiratory response to a 10% CO₂ respiratory stimulus in the morphine-tolerant rats, indicating that IT ziconotide did not reverse the state of tolerance to morphine-induced respiratory depression. Acute IT injection of 10 μ g in dogs decreased respiratory rate.
- **Gastrointestinal (GI):** The ED_{50} of ziconotide for inhibiting GI transit of a charcoal meal in mice after acute IT administration was estimated to be 0.19 μ g. Higher acute IT doses of 1 and 10 μ g were required to inhibit GI transit in rats, whereas the IT administration of a NOEL dose for GI transit inhibition (0.3 μ g/rat) caused approximately a 4-fold shift to the left in the log dose-response curve for morphine administered SC, indicating potentiation. In the electrically-stimulated guinea pig ileum longitudinal muscle preparation, the IC_{50} for inhibition of contractions at a stimulation frequency of 0.1 Hz was estimated to be 13 nM. In comparison, the mean plasma concentration of ziconotide in chronic pain patients receiving an average of 6.72 μ g/day by continuous IT infusion has been estimated to be <0.2 ng/ml (<0.08 nM), i.e., two orders of magnitude lower than the IC_{50} in the guinea pig ileum, indicating lack of peripheral effect of IT ziconotide, but there could be a centrally mediated effect, as indicated by the *in vivo* GI transit studies.
- **Abuse liability:** In a standard battery of *in vivo* studies (mouse p-phenylquinone abdominal constriction and tail-flick tests, suppression of morphine withdrawal signs

in monkeys) and *in vitro* studies (receptor binding assays, electrically stimulated mouse vas deferens), ziconotide was evaluated for dependence and abuse liability within the analgesic drug testing program of the Drug Evaluation Committee, which operates under the auspices of the College on Problems of Drug Dependence, and it was concluded that ziconotide does not have physical dependence potential or abuse liability of the opioid type.

ADME/Pharmacokinetics/Toxicokinetics: The pharmacokinetics profile of IV and IT ziconotide in plasma and cerebrospinal fluid was studied in rats, dogs and monkeys.

- *Intrathecal compared with IV:* In dogs administered ziconotide by IT bolus at 0.69 µg/kg, IT 48-hour infusion at 0.069 and 0.35 µg/kg/hr and IV bolus at 0.1 mg/kg, maximum plasma levels were [REDACTED] respectively at 0.5, 34.67, 25.60 and 0.03 hours respectively. In comparison, the mean CSF C_{max} levels were 7687, 474.82, 2072.40 and 17.39 ng/ml respectively at 0.09, 17.6, 14.4 and 0.75 hours. These results show a high lumbar CSF to plasma ratio, [REDACTED] at peak CSF concentrations after the IT bolus (0.5 hours) and [REDACTED] at 8 hours after continuous IT administration of 0.35 µg/kg/hr. The plasma to lumbar CSF ratios after IV injection were [REDACTED] at 0 minutes, 3:1 at 45 minutes and 1:1 at 8 hours. Lumbar CSF and plasma AUC values were 3445 and 1.53 ng·hr/ml respectively after the IT bolus injection at 0.01 mg. Distribution of ziconotide out of the CSF into the general circulation was rapid with a decline in ratio to [REDACTED] at 8 hours after the bolus and to [REDACTED] at 24 hours after the continuous infusion. Little ziconotide passed into the CSF after IV bolus injection, resulting in bioavailability to the CSF of 0.01%. In humans, an IT infusion rate of 2.4 µg/hr usually yields plasma concentrations less than [REDACTED].
- *IV administration:* Pharmacokinetic studies in rats and monkeys using continuous IV infusion for 24 hours did not observe any significant differences in PK parameters between males and females, and dose proportionality was observed for both steady-state and AUC values in both species.

Distribution of ziconotide through the spinal meninges was studied *in vitro* using tissues obtained from monkeys. Diffusion across the intact meninges including dura, arachnoid and pia was non-linear, showing two phases. The early phase from 0-2 hours was consistent with hydrophilicity of the drug, and the late phase from 3-5 hours suggested transport system activity. Low affinity binding was observed in isolated pia-arachnoid and dura membranes, also suggesting the presence of a molecular transport system.

Distribution of ziconotide into the brain was studied after IV injection in rats. Ziconotide concentration in brain was 0.003-0.006% of the injected dose (0.4 mg) at 3-20 minutes and 0.0003-0.0006% at 4 hours. The overall maximum brain concentration of parent drug was 0.005% per gram of tissue. When infused directly into the hippocampus, ziconotide diffusion in brain tissue was less than 1 mm at 2 hours. The degradation products were products of proteolysis and were similar after IV administration and direct administration into the brain.

Plasma protein binding: Ziconotide showed a higher maximum of percent binding to human plasma protein (89.8%) than rats (55.9%), dogs (61.8%) and monkeys (73.4%). Binding was not saturable at clinically relevant concentrations and usually increased with increasing concentration. Partitioning into cellular components was comparable across species and was independent of drug concentration, with ratios of 0.24-0.31, 0.38-0.43, 0.34-0.38 and 0.35-0.40 in rats, dogs, monkeys and humans respectively.

Metabolism: Ziconotide metabolism was studied after intravenous and intrahippocampal administration in rat brain and plasma. Ziconotide, a 25 amino acid peptide, was cleaved at multiple sites via multiple peptidases. Metabolism was similar in brain and plasma after intrahippocampal infusion and IV administration. It is hypothesized that the peptide degradation involves cleavage of hydrophobic and basic residues by endoproteases followed by digestion of the amino and carboxy terminals by exopeptidases.

Elimination: Elimination of ziconotide given IV was characterized by two components with a short initial half-life of 0.5-0.9 hours in rats and 0.8-1.1 hours in monkeys, and a long terminal half-life of 4.9-5.6 hours in rats and 5.5-8.9 hours in monkeys. Clearance out of the CSF was faster with a terminal half-life of 1.6 hours after IV bolus and 1.8 hours after IT bolus injections. There were no effects of sex or dose on clearance. Ziconotide clearance was 99% at 3 hours in rats and 6 hours in monkeys after the end of the continuous infusion. The elimination of ziconotide after IT administration in dogs was characterized by an initial rapid distribution into the systemic circulation (initial CSF half-life 0.4 hour) followed by movement away from the injection site by bulk CSF flow (terminal half-life 1.8 hours). The terminal half-life in plasma after IT injection was similar at 1.75 hours and after IV injection was 0.75 hour.

Toxicology: Ziconotide was evaluated in single- and repeated-dose IV toxicity studies in rats and cynomolgus monkeys, in single- and repeated-dose IT toxicity studies in rats and dogs, and in reproductive toxicity studies in rats and rabbits using IV infusion.

Acute toxicity:

IV administration: In rats, continuous IV administration of ziconotide at 10 and 40 mg/kg over 24 hours was associated with a dose-related increase in mortality and tremors beginning during the infusion and decreasing in incidence during the subsequent two days after the end of the infusion. In dogs, bolus IV injection of ziconotide at 0.1 mg/kg induced vomiting, scleral vasodilation, facial erythema, whole body trembling, laryngeal spasms, decreased muscle tone and sedation from 0-5 minutes after the injection and profound decreases in mean, diastolic and systolic blood pressure with tachycardia for 8 hours after the injection. These effects were associated with a C_{max} of 639 ng/ml and AUC of 267 ng-hr/ml. Monkeys given a continuous IV infusion of ziconotide at 11.2 and 25 mg/kg/day for 24 hours showed hind limb and whole body tremors associated with plasma levels <1600 ng/ml, lasting up to three days. Decreased blood pressure was observed in a high dose monkey during the first hour of infusion and vomiting was seen in one high dose monkey at 3 hours after the start of the infusion.

IT administration: In dogs, after IT bolus injection of 10 µg, whole body trembling, panting, decreased arousal and activity and decreased blood pressure, heart rate and respiratory rate were observed. No deaths occurred at this dose, which caused lumbar and plasma C_{max} s of _____, respectively (14,500-fold difference), and AUCs of approximately 3.5 µg•hr/ml and 1.5 ng•hr/ml, respectively (2250-fold difference).

Subacute toxicity: In dogs, elevations of body temperature (1.3 – 2.5°C) were produced during continuous IT infusion of 1 µg/hr for 48 hours, followed by 5 µg/hr for 48 hours, doses that resulted in peak plasma levels of _____, respectively. Four to 8 hours after the start of 1 µg/hr, all 5 dogs exhibited trembling, ataxia, decreased arousal and activity. At 5 µg/hr, a prominent decrease in blood pressure and concurrent tachycardia were observed (Study No. TY-96-019). No effects were seen on blood pressure or ECG in another study (No. 2-P55) with dogs (2 male and 2 female) using 48-hr IT infusions ranging from 0.005 to 0.48 µg/kg/hr. Three of the 4 dogs were euthanized after 24 hours of high dose administration due to moribund condition. The IT maximum tolerated dose (MTD) in dogs was considered to be 0.048 µg/kg/hr (~11.5 µg/day or 23 µg/m²/day).

Subchronic toxicity:

28-Day IT rat toxicity study (reviewed by _____): In a 28 day study with rats (16-20/sex/group), #5509-M009-94, an osmotic minipump was used for IT delivery of ziconotide at rates of 15, 150 or 1500 ng/kg/hr or mannitol or buffered saline controls. The minipumps were replaced on Day 14. A set of 10 rats/sex/group was used and tested in the Functional Observational Battery (FOB) and terminal blood and CSF was collected for clinical pathology examinations. A subgroup 5/sex/group underwent whole-body perfusion and tissues were saved for neuropathologic evaluation. Other subsets were used for neurochemical analysis and immunocytochemistry. The high dose (HD 1500 ng/kg/hr) and mid-dose (MD 150 ng/kg/hr) exhibited slight to moderate tremors and stiff tails. Alopecia and chromodacryorrhea (colored discharge from the eyes) were observed in all treatment groups, but were more frequent in MD and HD groups. Abnormal gaits were seen in animals of all groups but more frequently in the MD and HD groups. This was primarily due to spinal cord compression by the catheters, but ziconotide treatments may have increased the insult, although this was not seen histopathologically. The locomotor activity was higher in the HD group on Day 14, but not Days 7 or 28. No findings in necropsy or changes clinical pathology were treatment related. There were no significant treatment related changes in brain area examined for choline acetyltransferase activity, dopamine or its metabolite dihydroxyphenylacetic acid, gamma-aminobutyric acid or glutamic acid levels. Hippocampal serotonin was elevated in MD and HD animals and hippocampal norepinephrine was elevated in all treatment groups relative to control. No treatment related neuropathology was observed, and this indicated that the behavioral changes in the mid-dose and high-dose groups was due to pharmacological action of ziconotide, rather than neurotoxic lesions.

28-Day IT dog toxicity study: Dogs given continuous IT infusions of ziconotide of 100, 300, 600 or 1200 ng/kg/hr displayed motor abnormalities (ataxia, tremors and/or hyperactivity) with onset and severity related to dose and females being more sensitive than males. Two dogs each at 600 or 1200 ng/kg/hr were euthanized in poor condition. At least half of the ziconotide-treated dogs exhibited decreased or no pupillary light reflex and some exhibited dilated pupils. Two dogs per treatment group also displayed a decreased flexor reflex during the first week, whereas one (at 600 ng/kg/hr) showed an increased flexor reflex on days 7 and 14. Appetite and body weight were maintained by supplementing treated groups with canned food. Dose-related decreases in albumin/globulin ratio, increases in BUN and increases in serum potassium were observed. In females only, dose-related increases in number of segmented neutrophils and activated partial thromboplastin time were observed. A dose-related increase in the incidence of reports of a small prostate in the male dogs (5/5 at 600 and 1/1 at 1200 ng/kg/hr) was not seen in the one recovery male treated with 600 ng/kg/hr. Adverse microscopic findings of inflammation/infection in both control and treated groups were considered to be secondary to mechanical damage. Compression of the spinal cord was often associated with degeneration of nerve fibers characterized by myelin sheath dilation, axonal swelling and/or axonal debris with or without macrophages surrounded by a myelin sheath. Purulent meningitis occurred at and above the infusion site in some dogs, including the brain of one control dog. Mixed cell infiltration observed in the brain and various spinal levels was thought to be an extension of the meningeal inflammation and not drug-related. The contract lab concluded 300 ng/kg/hr to be the NOAEL dose.

The sponsor revised the NOAEL to ≥ 180 ng/kg/hr (from 300) following analysis of the dosing solutions. Considering, however, that only one dog receiving the target rate of 300 ng/kg/hr failed to show signs of ataxia, tremors and/or hyperactivity, and that two dogs receiving the next highest target rate of 600 ng/kg/hr required humane euthanasia before the midpoint of the intended infusion duration because of toxicity, it is this reviewer's opinion that the NOAEL in this study was the target dosing rate of 100 ng/kg/hr, IT (but analyzed at 26-34% of target dose). On a body surface area basis, this is approximately equivalent to $2.0 \mu\text{g}/\text{m}^2/\text{hr}$, which is slightly greater than the maximum recommended human dose of $1.5 \mu\text{g}/\text{m}^2/\text{hr}$ [$(2.4 \mu\text{g}/\text{patient}/\text{hr})/(1.6 \text{m}^2/\text{patient})$].

42-Day IT dog toxicity study: This study involving the continuous infusion of ziconotide (0.1, 1.0 and $10 \mu\text{g}/\text{dog}/\text{day}$, IT) was found to contain unreliable results because of several major deficiencies, including the lack of establishment that the high-dose dogs received anywhere near the maximum tolerated dose (refer to p. 58 for details). Thus, the 28-day studies in rats and dogs are considered to be the most reliable sources of toxicological information.

Carcinogenicity/SHE cell assay: Sponsor requested a waiver from conducting standard 2-year carcinogenicity studies in rodents, citing technical difficulties with maintaining an IT catheter for extended periods of time. After discussion with the PTCC, this waiver was granted, provided that ziconotide was negative in the SHE cell assay, an assay that has proven useful as an alternative test for assessing carcinogenic potential. Cytotoxic concentrations of ziconotide were negative in the SHE cell test for morphological transformation, whereas the positive control substance benzo(a)pyrene caused a dose-related increase in the morphological transformation frequency of SHE cells, with the mid and high doses of benzo(a)pyrene being statistically significant.

Immunotoxicology: There aren't any preclinical studies to address the question of immunogenicity of long-term IT administration of ziconotide. One mouse study that employed two IV and one SC injection of ziconotide did not detect antibody formation within 40 days. A rat study using IP or IM administration with Freund's complete adjuvant or IP and SC administration with Freund's incomplete adjuvant was inconclusive because of a questionable assay for detecting the presence of antibodies. Ziconotide was antigenic in guinea pigs in studies of systemic anaphylaxis when injected IP (5 mg/kg) with adjuvant (aluminum hydroxide). When 50 µl samples of diluted serum (as concentrated as 1:5 dilution) obtained from human patients at least 12 months (mean, 20.5 months) after they had received a 24-hr infusion with placebo or ziconotide (median dose, 0.01 mg/kg, route not identified) were injected intradermally in guinea pigs for the passive cutaneous anaphylaxis test for the presence of antibodies to ziconotide, none of the samples gave a positive reaction. Ziconotide is a weak sensitizer, based upon a positive response in the systemic anaphylaxis test in guinea pigs.

Reproductive toxicity studies: All of the reproductive toxicity studies were conducted using continuous IV infusion as the route of administration in order to maximize systemic exposure. However, it should be noted that there is no human data on plasma concentrations of ziconotide resulting from the sponsor's maximum recommended IT infusion rate of 2.4 µg/hr for a 50-kg patient.

Fertility (Segment I). Male rats receiving ziconotide 1, 3, or 10 mg/kg/day by continuous IV infusion for 58 days, including 28 days prior to mating, showed a dose-related ptosis, a dose-related decrease in food consumption during week 1 only, and an inverse relationship between dose and body weight gain. Significant ($p < 0.01$) dose-related decreases in mean absolute (73.6-79.2%, 73.4-75.3% and 68.2-70.3% of control) and relative weights of the left and right seminal vesicles were observed. There were no significant effects on fertility indices, uterine parameters (mean number of corpora lutea, implantation sites, live embryos, dead embryos, early resorptions, and % preimplantation and postimplantation losses) or sperm evaluation. Plasma ziconotide accumulations from day 1 to day 49 of +66%, +53% and +278% occurred at the low, mid and high doses, respectively. The NOAEL for male fertility was 10 mg/kg/day (approximately 1700 times the recommended maximum daily clinical IT dose of 2.4

µg/hr on a mg/m² body surface area basis), which corresponded to mean plasma levels of 651, 1374 and 2460 ng/ml on days 1, 28 and 49, respectively.

Female rats receiving ziconotide 1, 3, or 10 mg/kg/day by continuous intravenous infusion for 14 days prior to mating and through day 7 of gestation showed a decrease in food consumption during week 1 only in the low and mid dose treatment groups and significantly lower body weights in all treatment groups relative to the control. Mortality was not dose-related, and no treatment-related gross pathology was observed. No effects on weight of right or left ovaries, or on histopathology of the heart (including section of aorta), mammary glands (cervical and inguinal), ovaries, uterus (horn, body and cervix) and vagina were reported. Small, but statistically significant, decreases in the numbers of corpora lutea, implantation sites and live embryos occurred in the high-dose group. There were no significant effects on fertility indices. Plasma ziconotide did not accumulate in females from day 1 of treatment to day 7 of gestation. The NOAEL for female fertility was 10 mg/kg/day (approximately 1700 times the recommended maximum daily clinical IT dose of 2.4 µg/hr on a mg/m² body surface area basis), which corresponded to mean plasma levels of 570, 697 and 1232 (680 without an outlier) ng/ml on treatment days 1 and 14 and gestation day 7, respectively. The NOAEL for maternal toxicity is below the low dose (1 mg/kg/day, IV) and the NOAEL for reproductive performance and early embryonic development is 3 mg/kg/day, IV (approximately 500 times the recommended maximum daily clinical IT dose of 2.4 µg/hr on a mg/m² body surface area basis).

Teratogenicity (Segment II):

A. Rats

Anomaly Parameter Assessed	Significant differences in incidence of skeletal anomalies from controls							
	Vehicle controls		1.5 mg/kg/day		4.5 mg/kg/day		15 mg/kg/day	
	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses	Litters	Fetuses
No. evaluated	22	337	23	369	25	393	25	386
Total anomalies	21	115	22	129	23	119	24	161*
Skull: parietal, irreg. ossification	4	10	1	2*	1	1**	2	3*
Reduced no. of caudal vertebrae	0	0	0	0	3	3	4	6*
Pubic bones: ↓ ossification	3	4	7	13	5	9	14**	35***
Pubic bones: reduced bilateral	1	1	6	9*	3	3	11**	24***
Pubic bones: absent	0	0	0	0	1	1	3	11**
Ischial bone(s): irreg. ossification	0	0	1	1	1	1	3	6*

Significantly different from control value, *p<0.05, **p<0.01 and ***p<0.001 (Fisher's Exact Test).

Pregnant rats receiving ziconotide 1.5, 4.5 or 15 mg/kg/day by continuous IV infusion from day 6 through day 15 of gestation showed ptosis and, at the mid and high doses, showed decreased activity, slight tremors of the tail and/or body. Significant decreases

in weight gain and food consumption occurred in all ziconotide-treated groups during the first 3 days of treatment. No treatment-related gross pathology was observed and no significant changes were observed in the uterine parameters measured. One control fetus had malformations involving the heart and aorta. One low-dose fetus multiple spinal malformations, including the absence of lumbar vertebrae 5 and 6. One mid-dose fetus had multiple fusions and/or anomalies in the thoracic vertebrae. The high-dose group had 3 fetuses in a total of 2 litters with the following major malformations that were not observed in the control group: One of two sibling females had encephalocele, protrusion of cerebellum through cleft at junction of parietal and frontal bones not covered by skin, an area of hemorrhage surrounding the protruding tissue and anophthalmia. The other female had transposition of the major vessels and right-sided aorta. A male in another litter had the aortic arch absent, the ductus arteriosus connected to the ascending aorta; the descending aorta arising from the pulmonary trunk; anasarca, anotia, micrognathia, anophthalmia, anal atresia, bilateral brachydactyly, bilateral abnormal flexure of hind limbs, and a minor anomaly, kinked tail.

The high-dose fetuses also had a significant increase (40%, $p < 0.05$) in the incidence of skeletal anomalies. Some of these included missing bones (pubic bones in the pelvic girdle and caudal vertebrae) and reduced or irregular bone ossification in the vertebral column, ribs or pelvic girdle (see table above for statistically significant findings). Reduced ossification of various bones could be attributed to decreased food consumption. Absent pubic bones and reduced number of caudal vertebrae were also observed in at the mid-dose, but were not seen in any low-dose or control fetuses. However, a significant dose-related increase in the incidence of abnormally small (reduced) pubic bones, which was statistically significant for litters at the high dose and for fetuses at both the low and high doses, suggests that the low dose is not totally benign in affecting development of the pubic bones. Comparison of ziconotide treatment, regardless of dose, to historical control data indicated significant differences for reduced number of caudal vertebrae in both litters and fetuses, for reduced ossification of pubic bones in both litters and fetuses, for absent pubic bones in fetuses, and for irregular ossification of ischial bones in both litters and fetuses. Most of the historical control data, however, is for rats not subjected to the surgical procedures that were undergone by rats in this study. Nonetheless, ziconotide by the IV route should be considered as teratogenic in rats (personal communication) and should be labeled as Pregnancy Category C, rather than the Pregnancy Category ~~_____~~ in the sponsor's draft label.

B. Rabbits: Pregnant rabbits receiving ziconotide 0.2, 1.0 or 5.0 mg/kg/day by continuous intravenous infusion from day 6 through day 18 of gestation showed ptosis and decreased activity. Significant decreases in food consumption occurred during days 6-15 and again at termination of the infusion, at which time significant weight loss occurred, resulting in significantly lower body weights than the controls on gestation days 24 and 29. No treatment-related gross pathology was observed and no significant changes were observed in the uterine parameters measured. There was a dose-

related increase in the number of pregnant rabbits (0, 3, 7) showing total resorption, compared with 2 in the control group. Total group litter mean fetal weights were decreased (39.0, 40.9 and 42.2 grams) from the control value (44.8), which although statistically significant for the low dose, was not dose-related and thus not likely biologically significant. Major malformations were not dose-related. There were 2 fetuses (2 litters) with major malformations in the vehicle control group, 0 in the low dose group, 3 fetuses (3 litters) in the mid-dose group and 1 fetus in the high-dose group. In contrast to the findings of increased skeletal anomalies in rat fetuses with high-dose ziconotide infusion, the high-dose rabbit fetuses had a significant decrease (-85%, $p < 0.05$) in the incidence of skeletal anomalies. All treatment groups had an increase in the % of fetuses/litter with bilateral 13th rib compared with controls, which was significant ($p < 0.05$) for the low and mid dose groups, but not for the high dose group, as this increase appeared to have an inverse relationship to dose. Also unlike the rat study, no skeletal anomalies of any kind (reduced ossification, irregular ossification, reduced or absent) were reported for any bone in the pelvic girdle. Ziconotide was not teratogenic in rabbits at doses up to 5 mg/kg/day, IV (60 mg/m²/day). No TK samples from rabbits were analyzed.

Peri- and postnatal (Segment III). Pregnant rats receiving the continuous IV infusion of ziconotide (1, 3 or 10 mg/kg/day) from day 6 of gestation to *post partum* days 21, 22 or 23 showed a dose-related increase in the incidence of piloerection and ptosis, and hunched posture was observed in the high-dose group. Food consumption by F₀ dams during gestation decreased significantly ($p < 0.01$) at all doses during the first 3-day period of ziconotide treatment and decreased body weight gains were evident in all treatment groups at various time segments during both gestation and lactation. One low-dose F₀ female and three high-dose F₀ females had all of their pups die by day 8 *post partum*. The only clinical, developmental, or reproductive sign related to dose in the F₁ pups involved thin fur cover in certain areas. All other F₁ indices were normal, except for slightly lower weights in the high-dose group at birth, during lactation and post weaning. The mid- and high-dose F₁ females had about twice as many litters with pups dying during days 0-4 *post partum* as did the control and low-dose groups, but the Day 4 viability index for the F₂ pups was not significantly affected by ziconotide. The mid-dose group had one pup with major malformations (missing anus and tail), one pup with missing left kidney, ureter and adrenal gland, and four pups in another litter with bilateral reduction/absence of the renal papilla. Sponsor considers the NOAEL for maternal toxicity to be < 1 mg/kg/day, IV, and the NOAEL for the F₁ generation to be 3 mg/kg/day (approximately 500 times the recommended maximum daily clinical IT dose of 2.4 µg/hr on a mg/m² body surface area basis). The latter corresponds to mean plasma ziconotide concentrations of approximately 200 ng/ml, which is at least 1000 times the mean plasma concentrations (< 0.2 ng/mL) measured in chronic pain patients receiving continuous IT infusion of ziconotide.

Genotoxicity studies: Ziconotide was negative without and with S9 metabolic activation in two Ames tests, one conducted with *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, at a dose up to 65 µg/plate, and the other conducted

with *S. typhimurium* strains TA98, TA100, TA1535 and TA1537, as well as *E. coli* strain WP2 *uvrA*, at a dose up to 5000 µg/plate. Ziconotide was also negative without and with S9 metabolic activation when tested *in vitro* in the mouse lymphoma L5178Y TK+/- cell line forward mutation assay at doses up to 100 µg/ml in one assay and 4000 (+S9) or 5000 (-S9) µg/ml in a second assay. When tested *in vivo* in the mouse micronucleus test at doses of 0.32, 3.2 and 32 mg/kg, IV, ziconotide was negative at all doses and all times of marrow harvesting after injection (24, 48 and 72 hours). It is concluded that ziconotide is not mutagenic.

Special toxicology studies: Histopathological studies of the spinal columns of rats given 0, 1, 3 or 10 µg of ziconotide/day, IT, for 4 days and euthanized on day 6 showed several abnormalities (inflammation, tissue degeneration, and periaxonal dilation/vacuolization) that were largely attributed to a local reaction to the chronic IT catheter and/or volume delivery through it. The only dose-related observations were for inflammation scores for the leptomeninges in the sections above and below the catheter sites ($p < 0.05$), cellular infiltrates in gray matter, and vacuolization in white matter. Since, for these three observations, the low-dose group incidence was above the controls, the NOAEL should be considered as < 1 µg/day.

In rats given a single 24-hour IV infusion of saline or ziconotide (20 mg/kg), after pretreatment with an IV bolus dose of saline or a mixture of famotidine (0.1 mg/kg) and chlorpheniramine (5 mg/kg), no significant effect of the antihistamine treatment was reported for the significant decreases in mean blood pressure resulting at 2-30 minutes and again at 24 hours after the initiation of ziconotide treatment and 4 hours after termination of the infusion.

In response to an Agency request, sponsor conducted a retrospective analysis of their data on blood glucose in non-clinical studies of ziconotide given by the IV, IT, or epidural routes and communicated adverse effects involving blood glucose that have been reported from their clinical trials. In most of these studies, blood glucose was not measured until near the end of a 14-day or 28-day continuous infusion, and in those studies showing statistically significant decreases in fasting blood glucose, the decreases did not appear to be dose-related. In rats, the NOAEL of continuous infusion for significant decrease in blood glucose was 0.42 mg/kg/hr by the IV route (for 14 days) and < 0.015 µg/kg/hr by the IT route (for 28 days). In dogs, the NOAEL of continuous infusion for significant decrease in blood glucose was < 0.1 µg/kg/hr by the IT route (for 28 days) and 1.2 µg/kg/hr by the epidural route (for 14 days). In monkeys, the NOAEL of continuous infusion for significant hypoglycemia was 0.21 mg/kg/hr by the IV route (for 14 days). Because of the time points at which blood glucose was measured, none of the above studies rule out a possible hypoglycemic response to large bolus IT doses, as has been observed with morphine in mice (e.g., Brase *et al.*, 1991). The largest decrease from baseline blood glucose (-43%) was observed in male cynomolgus monkeys receiving the upper-middle dose of ziconotide (0.47 mg/kg/hour) by IV infusion (for 14 days). The clinical experience with ziconotide (up to

"Pregnancy Category C" section:

•

[REDACTED]

[REDACTED]

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[REDACTED]

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[REDACTED]

L

J

RECOMMENDATIONS:

Internal comments: The pharmacological and toxicological profiles demonstrated by non-clinical studies have shown efficacy and reasonable safety of ziconotide that can be labeled for human use. Approval of this NDA is recommended from the non-clinical pharm/tox perspective. Before the NDA can be approved, however, the label should be amended as described above (see revision under "Draft Letter Content for Sponsor" below). The non-clinical efficacy and safety studies of ziconotide administered by the IT route

[REDACTED]

ziconotide will be

patients for whom opioid treatment is inadequate.

As is the case with

[REDACTED]

[REDACTED]

with the use of ziconotide, because its mechanism of action is not limited to neuronal pain-transmission pathways. Possible advantages of ziconotide over opioid therapy include a decrease in the development of tolerance to the analgesic effect, a lack of cross-tolerance to opioid drugs, and better efficacy against neuropathic pain.

[REDACTED]

include the lack of an antagonist to treat overdose-related adverse effects, possible adverse events related to the IT route of administration that are independent of drug action (e.g., infection), and less efficacy than opioid drugs for the treatment of severe pain, at least in opioid-nontolerant patients. Ziconotide

[REDACTED]

when used in conjunction with

opioid drugs.

External Recommendations (to sponsor): Review of the rat teratogenicity study has led to the conclusion that when administered by continuous IV infusion, ziconotide is teratogenic in rats. Consequently, ziconotide should be classified under Pregnancy Category C. When exposure levels in terms of plasma concentration or AUC are not known for the dose(s) studied, the standard mode for expressing animal dosage as a multiple of the maximum recommended daily human dose is to compare animal and human doses on a mg/m² body surface area basis, using 1.62 m² as the reference body surface area for 60 kg human. The following draft revision of the labeling is recommended:

Draft Letter Content for Sponsor (letter sent May 25):

Carcinogenesis, Mutagenesis, Impairment of Fertility

[Redacted]

**Pregnancy
Teratogenic Effects — Pregnancy Category C**

[Redacted]

[REDACTED]

L

J

No adequate and well-controlled studies have been conducted in pregnant women. Because animal studies are not always predictive of human response, Trade Name should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

David A. Brase
David A. Brase, Ph.D. (reviewer)

June 2, 2000
Date completed

Dou Huey Jean
Dou Huey Jean, Ph.D. (team leader)

June 2, 2002
Date completed

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APPENDIX

PTCC AGENDA May 20,1999

Attending. Paul Andrews, Jasti Choudary, William Link for Al DeFelice, Jim Farrelly, Lois Freed for Glenna Fitzgerald, Stephen Hundley for Ken Hastings, Abby Jacobs, Lucy Jean, David Brase, Monte Scheinbaum, Alex Jordan, Bob Osterberg, Chuck Resnick, Nakissa Sadrieh, Ron Steigerwalt, Joe Sun, Mark Vogel, Conrad Chen for Andrea Weir, Chuck Anello, Frank Sistare, Mike Skelly for C.T. Viswanathan, Mercedes Serabian for Dave Green, Leslie Wheelock, Dorrie Balimann, Joe Contrera, Joe DeGeorge, and Adele Seifried

1. Other business.
2. The need of carcinogenicity studies for Ziconotide, a synthetic peptide to be used intrathecally for chronic pain in patients - David Brase

The _____ intractable pain not alleviated by systemic opiates or conventional therapies. It seems particularly effective with neuropathic pain. It is meant for long-term use.

- a) Are carcinogenicity studies required of ziconotide, a peptide drug to be administered intrathecally?

The committee decided that a standard 2-year rodent study with intrathecal administration would not be technically feasible and commented that a positive finding would probably only affect labeling, rather than approval, of the drug.

- b) If so, what would be a possible and relevant means for conducting such studies in rodents?

N/A

- c) If not, are there other types of studies including alternative models that can be conducted to assess the carcinogenic potential of a peptide drug to be administered intrathecally?

The committee recommended that since this study cannot be done intrathecally, other alternative methods to evaluate a carcinogenic potential should be conducted. The *in vitro* SHE cell transformation was recommended as a first step, with TG.AC as a possible follow up test, if the SHE cell assay turns out positive.

IDA 21-060

cc: NDA 21-060
HFD-170/Division file
HFD-170/D. Brase
HFD-170/L. Governale