CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

022036Orig1s000

PHARMACOLOGY REVIEW(S)

MEMORANDUM

DEPARTMENT OF HEALTH & HUMAN SERVICES
Public Health Service
Food and Drug Administration

Division of Neurology Products (HFD-120)
Center for Drug Evaluation and Research

Date: February 27, 2009

From: Lois M. Freed, Ph.D.
Supervisory Pharmacologist

Subject: NDA 22-036 (Silenor; doxepin HCl); treatment of insomnia

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NDA 22-036 is a 505(b)(2) application (Reference Listed Drugs: Sinequan® capsules [NDA 16-798], Sinequan® oral concentrate [NDA 17-516], Zonalon® cream [NDA 20-126]). The oral dosage forms of doxepin are approved for treatment of depression and anxiety. Because doxepin as a treatment for insomnia is expected to notably increase the number of otherwise healthy patients exposed, the sponsor was asked to conduct the following pivotal nonclinical studies:

- A standard battery of reproductive toxicology studies (fertility and early embryonic development in rat, embryo-fetal development in rat and rabbit, pre- and post-natal development in rat).
- A standard battery of genetic toxicology studies (*in vitro* Ames, *in vitro* chromosomal aberration in mammalian cells or *in vitro* mouse lymphoma tk, *in vivo* micronucleus in rodent).
- Carcinogenicity studies in two species.

The sponsor submitted all but the 2-year carcinogenicity study in rat. The Division agreed to accept the 2-year rat study post-approval if (1) the genetic toxicology battery was negative and if (2) the 26-week oral carcinogenicity study in Tg.rasH2 mouse raised no concerns regarding carcinogenic potential; both conditions were met. The nonclinical studies and published literature submitted by the sponsor have been reviewed by Melissa K. Banks, Ph.D. (Pharmacology/Toxicology Review and Evaluation, 2/25/09). Based on that review, Dr. Banks has concluded that the nonclinical package supports approval of doxepin for treatment of insomnia.

However, Dr. Banks notes the lack of a complete understanding of the in vivo metabolic profile of doxepin in humans. The sponsor did provide published literature suggesting that the in vivo metabolism of doxepin in animals and humans are qualitatively similar. And, one major active circulating metabolite, nordoxepin, was quantitated in the animal

species used in the pivotal studies (i.e., CByB6F1 hybrid mouse, Sprague-Dawley rat, New Zealand White rabbit); plasma exposures (C_{max} and AUC) achieved in these studies provided adequate safety margins compared to plasma exposures in humans at doses up to 6 mg.

As Dr. Banks states, it is unclear if there are additional major circulating metabolites in humans. However, doxepin is approved (since 1969) for use at much higher doses (75-150 mg/day), and the Clinical Pharmacology reviewer has concluded that the human data submitted are sufficient for approval (cf. Clinical Pharmacology/Biopharmaceutics Review, Ju-Ping Lai, Ph.D. 11/6/08).

One clinical concern is the potential for doxepin to prolong the QT interval. From a nonclinical standpoint, the sponsor provided only published literature relevant to this issue. According to Dr. Banks' review, the IC_{50} for doxepin in the *in vitro* hERG assay was reported to be 4.4-6.5 μM (Duncan RS *et al*. *Biochem Pharmacol* 74:425-437, 2007). This is not a strong signal for QT prolongation, but, as Dr. Banks notes, some published literature suggest the possibility of accumulation of doxepin in cardiac tissue (Elonen *et al. Acta Pharmacol et Toxicol* 37:274-281, 1975). In addition, doxepin (a tricyclic antidepressant) has been reported to prolong QT in humans at high doses (Baker B *et al J Clin Psychopharmacol* 17(1):15-21, 1997; Rademacher S *Ann Pharmacotherapy* 39(10):1762, 2005) and produce TdP in humans following overdose (Alter P *et al. Ann Intern Med* 135(5):384-385, 2001).

To more fully investigate doxepin's potential to prolong QT, Dr. Banks recommends that the sponsor assess both doxepin and nordoxepin in the *in vitro* hERG assay. I agree, unless this concern can be dismissed based on clinical data. If needed, the assay should test both doxepin and nordoxepin because (1) the plasma AUC for nordoxepin in humans is up to 2 times that for doxepin at 6 mg (cf. Table 2.7.2.19 in the sponsor's Summary of Clinical Pharmacology Studies) and (2) inter-assay/inter-laboratory variability would preclude direct comparisons between IC_{50} values obtained and those reported for doxepin in published literature.

Recommendations

From a pharmacology/toxicology standpoint, the only deficiency in the NDA is a lack of sufficient data to determine if all major circulating human metabolites have been adequately tested in the pivotal nonclinical studies. However, since the sponsor is not being asked to provide additional human data prior to approval, there is no reason to ask for further evaluation of *in vivo* metabolism in animals at this time.

The nonclinical studies submitted support approval of the NDA.

There is clinical concern that doxepin's potential to prolong the QT interval has not been adequately assessed in humans. If the medical team determines that data from an *in vitro* hERG assay would be important for characterizing this potential, the following wording for the sponsor is recommended.

- In order to further investigate the potential for doxepin to prolong the QT interval, you will need to conduct an in vitro hERG assay on doxepin and nordoxepin, a major circulating metabolite in humans. This study should be conducted in a hERG-expressing mammalian cell line under steady state conditions. Both doxepin and nordoxepin should be tested over a full concentration range. The assay should include both negative and positive controls; the positive control should be tested at a concentration near its IC_{50}.

Labeling Recommendations

Labeling is not being addressed at this time.

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/s/

Lois Freed
2/27/2009 06:15:41 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-036**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**January 31, 2008**
PRODUCT:	**Silenor™ (doxepin HCl)**
INTENDED CLINICAL POPULATION:	**Treatment of Insomnia**
SPONSOR:	**Somaxon**
DOCUMENTS REVIEWED:	**Electronic submission**
REVIEW DIVISION:	**Division of Neurology Drug Products (HFD-120)**
PHARM/TOX REVIEWER:	**Melissa K. Banks, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell G. Katz, M.D.**
PROJECT MANAGER:	**Cathleen Michaloski, M.P.H.**

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The application is approvable from a Pharmacology/Toxicology perspective.

B. Recommendation for nonclinical studies

It was determined that the use of doxepin for the treatment of insomnia would significantly expand the potential treatment population. To support the safety of the low dosage of doxepin HCl (1-6 mg, to be marketed as Silenor) in this 505(b)(2) application, the sponsor conducted assessments of genetic toxicology, reproductive toxicology and carcinogenicity. Detailed information about human metabolism were not requested (data were provided only for doxepin and nordoxepin); therefore, a comprehensive evaluation of the adequacy of the nonclinical coverage for all major human metabolites in the conducted studies was not possible. The sponsor is currently conducting a 2-year lifetime rat carcinogenicity bioassay as a Phase 4 commitment.

In light of the clinical concern for a potential QT signal, it is recommended that the sponsor conduct a hERG assay assessing both doxepin and any major human metabolites (e.g., nordoxepin).

C. Recommendations on labeling

[Note: These recommendations reflect the reviewer's opinion, but have not been subject to internal discussion or external negotiation and may not reflect final labeling.]

----------USE IN SPECIFIC POPULATIONS----------
Pregnancy: **Based on animal data, doxepin may cause fetal harm. (8.1)**

8. Pregnancy

8.1 Pregnancy Category C

There are no adequate and well-controlled studies of doxepin in pregnant women. Silenor should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

Administration of doxepin to pregnant rats and rabbits resulted in adverse effects on offspring development at doses greater than the maximum recommended human dose (MRHD) of 6 mg/day. There appeared to be a negative effect on offspring viability and an increase in low incidence fetal alterations at approximately 75-100x the AUC exposures of nordoxepin and doxepin at the MRHD.

(b) (4)

(b) (4)

(b) (4)

13. NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenesis

(b) (4)

Mutagenesis

(b) (4)

Impairment of Fertility

(b) (4)

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

This 505(b)(2) application for Silenor relied on the Agency's findings of safety for doxepin hydrochloride, marketed as Sinequan®, 75-150 mg and Zonalon®, 5% topical cream. Following review of the nonclinical information available in approved labeling for the reference listed drugs and in light of the expanded population (insomnia), the following studies were requested by the Agency: reproductive toxicology, genetic toxicology, and carcinogenicity studies.

A complete reproductive toxicology assessment was performed, to include: a rat fertility study, rat and rabbit embryofetal studies, and a rat pre- and postnatal study. In the rat fertility study, overall pregnancy indices appeared only slightly affected (e.g., increased copulatory interval and very slightly decreased fertility index at 100 mg/kg); however, adverse effects were observed on sperm (percent motility and percent abnormal) at 100 mg/kg and in uterine examinations (i.e., decreased numbers of corpora lutea, implantation sites and viable embryos, as well as preimplantation loss) at ≥ 30 mg/kg. The overall NOAEL for reproductive parameters was 10 mg/kg/day. The rat embryofetal study demonstrated adverse maternal and developmental effects at ≥ 100 mg/kg; the NOEL for developmental alterations was 30 mg/kg, based on observed developmental delays and total low incidence of fetal alterations. The rabbit embryofetal study demonstrated few clearly drug-related adverse effects; the NOAEL was 30 mg/kg, based on slightly decreased fetal body weights. In the pre-/post-natal study in rats, altered viability, growth and development of the F_1 pups was observed; the NOAEL was 30 mg/kg. Reproductive effects were not observed in the F_1 generation (NOAEL = 100 mg/kg).

The genotoxic potential of doxepin hydrochloride was assessed in a standard battery of genetic toxicology studies (i.e., *in vitro* bacterial reverse mutation assay, *in vitro* chromosomal aberrations assay [HPBL] and *in vivo* rat micronucleus assay); doxepin HCl was not genotoxic.

To address the need for carcinogenicity assessment, the sponsor conducted a 26-week Tg.rasH2 transgenic mouse carcinogenicity assay. By agreement with the Agency following negative results in the genetic toxicology battery, the sponsor was permitted to conduct a transgenic mouse assay (submitted within the application) and a 2-year lifetime rat carcinogenicity assay (to be submitted as a Phase 4 commitment; currently ongoing). The submitted 26-week Tg.rasH2 transgenic mouse carcinogenicity assay demonstrated no drug-related neoplasms.

B. Pharmacologic activity

Doxepin is a dibenzoxepin tricyclic compound which acts as a histamine (H_1) antagonist at low concentrations (IC_{50} approximately 2-7 nM; K_i < 1 nM). Notably, at higher concentrations (presumably more similar to those resulting from the higher clinical doses recommended for treatment of depression and anxiety), doxepin produces dose-dependent pharmacological effects consistent with other tricyclic antidepressants, including: biogenic amine re-uptake inhibition, alpha-adrenergic inhibition and muscarinic inhibition, as well as histamine H_1 antagonism. Doxepin also showed antagonism at the serotonin 5-HT_{2A} receptor at higher concentrations (IC_{50} in the sponsor's studies varied between 20-240 nM). According to published literature, doxepin exhibits some binding at several other sites, including ion channels, and has pharmacodynamic effects ranging from sleep to feeding behavior to pain. Notably, doxepin was identified in one literature report as a potential hERG blocker (IC_{50} of 4.4-6.5 μM; Duncan et al., 2007).

C. Nonclinical safety issues relevant to clinical use

Conducted studies to assess for mutagenic and/or clastogenic, as well as carcinogenic, potential demonstrated no safety concerns. The rat 2-year bioassay to assess for carcinogenic potential is currently ongoing.

Reproductive toxicity studies demonstrated a few concerns for human use. Although overall fertility rates were not drastically altered in rats, there were effects on reproductive parameters in males and females that could suggest a potential effect on fertility in humans. In females, the numbers of corpora lutea and implantations sites were reduced, and pre-implantation losses were increased, at MD and HD. In males, abnormal sperm and decreased sperm motility were observed at HD. Additionally, evidence of adverse effects on fetal viability, growth and development was observed.

A comprehensive determination of the adequacy of the conducted studies to evaluate human safety is contingent upon coverage of all major human metabolites; however, detailed human metabolism data were not provided to

support this 505(b)(2) application. For the nonclinical and clinical studies within this submission, only doxepin and nordoxepin were measured. Metabolites other than nordoxepin are known. It is unclear whether any other metabolites circulate as major metabolites at this low dose of doxepin, but the sponsor's summary suggests that there may be more species than doxepin and nordoxepin that circulate in significant quantity in humans. Only literature information was provided for the nonclinical drug-related species, as well. According to Hobbs (1969), doxepin was absorbed after oral administration and evidence of extensive metabolism was observed in rats and dogs; metabolic pathways included demethylation, N-oxidation, hydroxylation and glucuronide formation. Hobbs also noted that doxepin and its metabolites appeared widely distributed and rapidly excreted in rat; one exception was stated, as doxepin was reported to show a strong affinity for melanin (albeit less than others in the class, such as amitriptyline). The information provided in this 505(b)(2) application, and the evaluation of it, does not address circulating drug-related entities other than doxepin and nordoxepin.

Notably, although the sponsor did not conduct nonclinical studies to address the issue, a literature report was provided which indicated that doxepin has some potential to block I_{Kr} current (IC_{50} of 4.4-6.5μM). Such activity is believed to reflect cardiac toxicity liability, in the form of QT prolongation.

Literature References

Duncan RS, McPate MJ, Ridley JM, Gao Z, James AF, Leishman DJ, et al. (2007) Inhibition of the hERG potassium channel by the tricyclic antidepressant doxepin. Biochem Pharmacol., 74: 425-437.

Hobbs, DC (1969) Distribution and metabolism of doxepin. Biochemical Pharmacology: 18, 1941-1954.

Yan, JH, Hubbard, JW, McKay, G, Korchinski, ED, Midha, KK (2002) Absolute bioavailability and stereoselective pharmacokinetics of Doxepin. Xenobiotica, 32(7): 615-623.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-036
Review number: 1
Sequence number/date/type of submission: SDN000, 1/30/08, Orig NDA
Information to sponsor: Yes ( ) No (X)
Sponsor and/or agent: Somaxon Pharmaceuticals
3721 Valley Centre Drive, Suite 500
San Diego, CA 92130
T: 858-480-0400, F: 858-509-1761
www.somaxon.com

Manufacturer for drug substance: (b) (4)

Type II Drug Master File No. (b) (4)

Bulk product doxepin HCl, USP: (b) (4)

OR

(b) (4)

Reviewer name: **Melissa Banks, Ph.D.**
Division name: **Div. of Neurology Products**
HFD #: **120**
Review completion date: **02/03/09**

Drug:

Trade name: Silenor™
Generic name: doxepin hydrochloride (HCl)
Chemical name: 1-Propanamine, 3-dibenz [b,e]oxepin-11(*6H*)ylidene-*N*,*N*-Dimethylhydrochloride
OR
11-3(-dimethylaminopropylidene)-6,11-dihydrodibenz[b,e]oxepin HCl
OR
N,*N*-Dimethyldibenz[*b*,*e*]oxepin-$\Delta^{11(6H),\gamma}$-propylamine hydrochloride
CAS registry number: 1229-29-4
Molecular formula/molecular weight: $C_{19}H_{21}NO \cdot HCl$; 315.84
(Structure, next page)

Structure: (from sponsor's submission)
isomeric mixture of E- and Z- doxepin

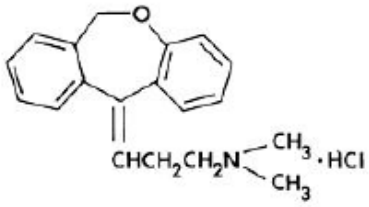


Figure 3.2.S.1.2-1 Doxepin HCl, USP Structure

Relevant INDs/NDAs/DMFs:
505(b)(2) Safety and Efficacy Referenced NDAs:
NDA 016-798 (Sinequan® Capsules, Pfizer), MRHD of 75-150 mg/day
NDA 017-516 (Sinequan® Oral Concentrate, Pfizer)
NDA 020-126 (Zonalon® 5% Cream, Bradley Pharmaceuticals, Inc.)

Drug class: dibenzoxepin tricyclic agent that acts as a histamine (H_1) antagonist, FDA approved as an antidepressant and anxiolytic

Intended clinical population: Treatment of insomnia

Clinical formulation:
The Silenor drug product, with active pharmaceutical ingredient doxepin HCl, is provided as 1 mg, 3 mg or 6 mg tablets.

Route of administration: PO, immediate release tablets

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

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-036 are owned by Somaxon or are data for which Somaxon has obtained a written right of reference. Any information or data necessary for approval of NDA 22-036 that Somaxon does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Somaxon does not own (or from FDA reviews or summaries of a previously approved application) are for descriptive purposes only and are not relied upon for approval of NDA 22-036.

Studies reviewed within this submission:

Pharmacology

SP-D0114 *In Vitro* Pharmacology Study of Doxepin HCl

SP-D0117 *In Vitro* Pharmacology Study of Doxepin HCl and Ritanserin

ADME

SP-D0115 Binding of doxepin to human, rat, rabbit, and mouse plasma proteins using equilibrium dialysis-based method

SP-D0118 ADME-Tox: CYP Inhibition - Study of Doxepin HCl

SP-D0119 *In Vitro* Permeability Study of Doxepin Hydrochloride According to the Biopharmaceutics Classification System (BCS) Guidelines Issued by the United States Food and Drug Administration

Repeat-Dose Toxicity

SP-D0110 28-Day Toxicity and Toxicokinetic Study in CByB6F1 Mice with a Preliminary 5-Day Range-finding Study

SP-D0104 2-Week Oral Dose-Range Finding in Rats (for reproductive toxicity studies below)

SP-D0105 2-week Oral Dose-Range Finding and Toxicity Study in Rabbits (for reproductive toxicity study below)

Carcinogenicity

SP-D0111 26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice

Reproductive and Developmental Toxicity

SP-D0106 Fertility and Early Embryonic Development in the Rat

SP-D0107 Embryo-fetal Development in the Rat

SP-D0108 Embryo-fetal Development in the Rabbit

SP-D0109 Pre- and Postnatal Development in the Rat

Studies not reviewed within this submission:

Single- and Repeat- Dose Toxicity

SP-D0103 Single-Dose Range Finding in the Rat (for Micronucleus assay below) Previously reviewed as part of the Micronucleus assay; see P/T review for I67,162 N048 dated 2/2/07

SP-D0112 13-Week Oral Toxicity Study in the Rat (for carcinogenicity study below) Previously reviewed; see P/T review for I67,162 N057, dated 8/16/07

Genotoxicity (All previously reviewed; see P/T review for I67,162 N048 dated 2/2/07)

In Vitro Studies

SP-D0101 Salmonella and E. coli Mammalian-Microsome Reverse Mutation Assay

SP-D0102 Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes

In Vivo Studies

SP-D0103 In Vivo Rat Micronucleus Assay

Carcinogenicity (Study is ongoing, to be completed as a Phase 4 Commitment)

SP-D0113 2-Year Repeated Dose Oral Carcinogenicity Study in Rats

Notes: SD= single dose, LD= low dose, MD= medium dose, HD= high dose, M= male, F= female, D= day, Wk= week, Mo= month; [ss]= statistically significant, [nss]=not statistically significant, sd= standard deviation, gp=group, conc=concentration; trtmt=treatment

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

According to the sponsor, doxepin is a sleep-promoting, selective histamine H_1 antagonist. It binds with high affinity (K_i <1 nM) to human histamine H_1 receptors, where it functions as an antagonist; this activity is hypothesized to promote sleep initiation and maintenance. The histaminergic system in the central nervous system regulates the circadian sleep-wakefulness cycle, and antagonism of histaminergic neurotransmission, particularly via the H_1 receptor, has been shown in animal models and human studies to decrease wakefulness. The sponsor's study and several literature reports indicated that doxepin has lesser affinity at a number of other receptor, transporter, uptake and enzyme sites, but these sites were not believed to contribute to the pharmacological activity of Silenor at recommended doses. Doxepin appeared to have moderate affinity for 5-HT receptor (2A and 2C), α-adrenergic receptors (1B, 2B, and 2C), muscarinic ACh receptors (1, 2, 3, 4 and 5), and the NE and 5-HT transporters. Similar binding was evident from the literature (e.g., Richelson & Nelson, 1984). The provided literature reported antagonism at another serotonin receptor (5-HT_{1C}; Jenck et al., 1994) and potential for an effect on certain GABA transporters (Nakashita et al., 1997). The sponsor stated that doxepin has no detectable activity at benzodiazepine recognition sites or at other sites on the GABA receptor complex; sponsor-generated data to support this statement do not appear to have been provided, only literature references (e.g., Heal et al., 1992, Wong et al., 1983). Activity at ion channels (e.g., sodium and calcium channels), assessed in *in vitro* assays, was also suggested by a number of articles (e.g., Beauchamp et al., 1993, Pancrazio et al., 1998, Schwaninger et al., 1995); these activities are believed to occur at relatively high doxepin concentrations. Literature references were also provided describing effects on EEG evidence for sleep (e.g., Kamei et al., 1996), feeding (e.g., Orthen-Gambill, 1988, Ookuma et al., 1990) and pain (e.g., Chen et al., 2004, Gerner et al., 2006, Sudoh et al., 2004, Wordliczek et al., 2005).

No new safety pharmacology studies were conducted by the sponsor. Of the several literature reports provided by the sponsor, one recent article identified that doxepin has potential as a hERG channel blocker, with an IC_{50} of 4.4-6.5 μM (i.e., Duncan et al., 2007).

2.6.2.2 Primary pharmacodynamics

Mechanism of action: H_1 receptor antagonist

SP-D0114 *In Vitro* Pharmacology Study of Doxepin HCl

Conducted by (b) (4), Study Number 12129, Final Report (non-GLP, no QA)
(Note: limited screen of 17 receptor/enzyme/transporters; 5-HT_{2B} was not screened)
The sponsor's tabular results are included, following.

Table 2 - 1

IC_{50} Determination: Summary Results

Assay (b) (4) Compound I.D.	Client Compound I.D.	IC_{50} (M)
NE uptake		
12129-1	Doxepin HCl	1.3E-08
DA uptake		
12129-1	Doxepin HCl	4.6E-06
5-HT uptake		
12129-1	Doxepin HCl	2.1E-07

Table 2 - 4

IC_{50} Determination: Summary Results

Assay (b) (4) Compound I.D.	Client Compound I.D.	IC_{50} (M)
H_1 *(h)* (antagonist effect)		
12129-1	Doxepin HCl	7.1E-09

(based on H_1-mediated calcium mobilization)

Table 1 - 1

IC_{50} Determination: Summary Results

Assay (b) (4) Compound I.D.	Client Compound I.D.	IC_{50} (M)	K_i (M)	n_H
α_{1B} *(h)*				
12129-1	Doxepin HCl	4.4E-08	1.2E-08	*1.1*
α_{2A} *(h)*				
12129-1	Doxepin HCl	2.5E-06	1.1E-06	*1.0*
α_{2B} *(h)*				
12129-1	Doxepin HCl	4.2E-08	2.8E-08	*1.0*
α_{2C} *(h)*				
12129-1	Doxepin HCl	3.0E-07	9.6E-08	*0.8*
H_1 *(h)*				
12129-1	Doxepin HCl	2.1E-09	7.8E-10	*1.3*
M_1 *(h)*				
12129-1	Doxepin HCl	2.1E-08	1.8E-08	*0.8*
M_2 *(h)*				
12129-1	Doxepin HCl	3.3E-07	2.3E-07	*1.1*
M_3 *(h)*				
12129-1	Doxepin HCl	3.5E-08	2.5E-08	*0.9*
M_4 *(h)*				
12129-1	Doxepin HCl	3.3E-08	2.0E-08	*1.0*
M_5 *(h)*				
12129-1	Doxepin HCl	1.1E-08	5.6E-09	*1.2*
5-HT_{2A} *(h)*				
12129-1	Doxepin HCl	2.0E-08	1.1E-08	*0.8*
5-HT_{2C} *(h)*				
12129-1	Doxepin HCl	4.3E-07	2.0E-07	*0.9*
NE transporter *(h)*				
12129-1	Doxepin HCl	7.7E-08	5.8E-08	*0.9*
5-HT transporter *(h)*				
12129-1	Doxepin HCl	2.1E-07	9.5E-08	*0.9*

SP-D0117 *In Vitro* Pharmacology Study of Doxepin HCl and Ritanserin
Conducted by (b) (4), Study Number 792501, Final Report (non-GLP, no QA)
(Note: limited screen of antagonist activity at 5-HT_{2A} receptors)
The sponsor's tabular results are included, following.

Table 1 - 1

IC_{50} Determination: Summary Results

Assay (b) (4) Compound I.D.	Client Compound I.D.	IC_{50} (M)	K_B (M)
5-HT_{2A} *(h)* (antagonist effect)			
792501-1	Doxepin HCl	2.4E-07	3.4E-08
792501-2	Ritanserin	4.9E-07	7.1E-08

Table 1 - 3

Reference Antagonist Data

Assay Reference Compound	IC_{50} (M)	K_B (M)
5-HT_{2A} *(h)* (antagonist effect)		
ketanserin	3.3E-08	4.7E-09

As well as identifying activity as defined by in vitro binding assays, the sponsor noted that there are literature articles describing pharmacodynamic effects presumed due to the histamine antagonism, such as EEG evidence for sleep (e.g., Kamei et al., 1996).

2.6.2.3 Secondary pharmacodynamics

In addition to the numerous receptor and transporter sites where doxepin was noted to have some activity (e.g., other histamine receptors [H_2], 5-HT receptors, α-adrenergic receptors, muscarinic ACh receptors, as well as NE and 5-HT transporters), activity at sodium and calcium ion channels was also suggested in *in vitro* assays (e.g., Beauchamp et al., 1993, Pancrazio et al., 1998, Schwaninger et al., 1995). In general, these activities are believed to occur at relatively high doxepin concentrations. Potentially related pharmacodynamic effects on pain (e.g., Chen et al., 2004, Gerner et al., 2006, Sudoh et al., 2004, Wordliczek et al., 2005) have been described.

2.6.2.4 Safety pharmacology

No new safety pharmacology studies were conducted by the sponsor. Several relevant literature reports were provided by the sponsor. Since cardiotoxicity is a known liability for tricyclic antidepressant drugs, of particular note was a recent article that identified doxepin as a hERG channel blocker (recombinant I_{hERG} and native I_{Kr}), with an IC_{50} of 4.4-6.5 μM (i.e., Duncan et al., 2007). The authors concluded that hERG blockade is relevant to doxepin's reported QT prolonging effect in humans at high doses. A few other articles describing *in vitro* and/or *in vivo* investigations of doxepin cardiotoxicity were provided. Although it has been suggested that accumulation of drug in cardiac tissues might be responsible (Elonen et al., 1975), other evidence did not appear to support such a clear relationship (Hobbs, 1969; for brief details, see 2.6.4.4. Distribution section of this review).

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

Limited PK/TK data were provided for doxepin, as this 505(b)(2) application is for lower doses of doxepin than are currently available. The sponsor provided a limited number of studies, and data were provided only for doxepin and nordoxepin in the clinical and nonclinical species. The sponsor provided a number of literature articles addressing the pharmacology and pharmacokinetics of doxepin. The overall conclusion from available information is that doxepin is well absorbed, widely distributed and extensively metabolized by animals and humans. Known metabolic transformations include demethylation, N-oxidation, hydroxylation and conjugation (glucuronide formation); doxepin undergoes extensive Phase I and Phase II metabolism, the most important pathways being demethylation, hydroxylation and glucuronidation. Doxepin is moderately bound to plasma proteins in all species tested. The sponsor believes doxepin metabolism to be qualitatively similar in humans and animals, and shows isomer-specific metabolism (with a predominant role for CYP2D6 in the hydroxylation of trans(E)-doxepin and E-nordoxepin, and the participation of CYPs 2C9, 2C19 and 1A2 in the demethylation of cis-(Z) and E-doxepin). The excretion of doxepin and doxepin metabolites appears to be predominantly in the urine for both animals and humans. Doxepin and nordoxepin exposure in animal species increases with dose and repeated dosing. Early drug metabolism studies with tricyclic doxepin in rats and dogs (see Hobbs, 1969) indicated that,

> "doxepin is well absorbed after oral administration and measurable amounts of doxepin and demethyl doxepin quickly appear in the blood. Although numerous metabolites of doxepin were reportedly observed in liver and in urine, only doxepin and demethyl doxepin are found in the rat brain."

Hobbs (1969) also noted that doxepin and its metabolites were found in, and rapidly cleared from, all tissues examined in rats except for pigmented eye. Doxepin was reported to show an affinity for melanin, as also reflected in *in vitro* studies with beef eyeball melanin, but was less strongly bound than amitriptyline. Another literature article provided (Uhr et al., 2003) identified doxepin and its metabolite d-doxepin as substrates of P-glycoprotein in a mouse P-gp mutant model.

2.6.4.2 Analytical

VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF DOXEPIN IN K2 EDTA RAT PLASMA

Project Number: YGH00016LX, (b) (4)), Final report, GLP (1 exception) and QA

A bioanalytical method was developed and validated for the determination of doxepin in K2 EDTA rat plasma using doxepin-d3 as the internal standard. The validated concentration range for the analysis of doxepin was from 10.0 - 10,000 ng/ml using a 0.05 ml sample.

VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF DOXEPIN IN K2 EDTA RABBIT PLASMA

Project Number: YGH00020LX, (b) (4), Final report, GLP (1 exception) and QA
A bioanalytical method was developed and validated for the determination of doxepin in K2 EDTA rabbit plasma using doxepin-d3 as the internal standard. The validated concentration range for the analysis of doxepin is from 10.0 – 10,000 ng/ml using a 0.05 ml sample.

VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF DOXEPIN AND NORDOXEPIN IN K2 EDTA RAT PLASMA
Project Number: YGH00033LX, CRL(MA), Final report, GLP (1 exception) and QA
A bioanalytical method was developed and validated for the determination of doxepin and nordoxepin in K2 EDTA rat plasma using doxepin-d3 as the internal standard. The validated concentration range is from 1.00 - 1000 ng/ml for doxepin and from 1.00 - 1000 ng/ml for nordoxepin using a 0.05 ml sample.

VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF DOXEPIN AND NORDOXEPIN IN K2 EDTA RABBIT PLASMA
Project Number: YGH00034LX, CRL(MA), Final report, GLP (1 exception) and QA
A bioanalytical method was developed and validated for the determination of doxepin and nordoxepin in K2 EDTA rabbit plasma using doxepin-d3 as the internal standard. The validated concentration range was from 1.00 - 1000 ng/ml for doxepin and from 1.00 - 1000 ng/ml for nordoxepin using a 0.05 ml sample.

VALIDATION OF A HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC-MASS SPECTROMETRIC METHOD FOR THE ANALYSIS OF DOXEPIN AND NORDOXEPIN IN K2 EDTA MOUSE PLASMA
Project Number: YGH00038LX, CRL(MA), Final report, GLP (1 exception) and QA
A bioanalytical method was developed and validated for the determination of doxepin and nordoxepin in K2 EDTA mouse plasma using doxepin-d3 as the internal standard. The validated concentration range was from 1.00 - 1000 ng/ml for doxepin and from 1.00 - 1000 ng/ml for nordoxepin using a 0.05 ml sample.

2.6.4.3 Absorption

SP-D0119 (Study Report 7S0MAP2R2GLPS43): *In Vitro* Permeability Study of Doxepin Hydrochloride According to the Biopharmaceutics Classification System (BCS) Guidelines Issued by the United States Food and Drug Administration
Conducted by (b) (4) Final report, GLP & QA
The average P_{app} value for doxepin hydrochloride was greater than the P_{app} value of pindolol at all three concentrations (1, 10 & 100 µM). See the sponsor's summary Table 1. The recovery of doxepin hydrochloride at all three tested concentrations was relatively low, which was later discovered to result from doxepin accumulating in the cells. When the intracellular amount was included, the mass balance for recoveries was close to 100% for all concentrations; see sponsor's summary Table 2. (Note that A= apical and B=basolateral.) The B-to-A P_{app} to A-to-B P_{app} ratios of doxepin hydrochloride were less

than 3 at all three concentrations; this is evidence that doxepin hydrochloride permeates the Caco-2 membrane by passive diffusion. See the sponsor's summary Table 3. It was concluded that doxepin hydrochloride is a highly permeable substance.

Table 1. A-to-B Permeabilities of Doxepin Hydrochloride and Internal Control Compounds Using Caco-2 Monolayers (Mean ± SD, n = 6)

Nominal Doxepin Hydrochloride Dosing Concentration (µM)		1	10	100
Doxepin Hydrochloride	P_{app} (10^{-6} cm/s)	17.7 ± 1.99	23.2 ± 1.05	37.6 ± 1.19
	Recovery (%)	57.4 ± 4.39	64.5 ± 2.59	77.7 ± 3.13
Pindolol	P_{app} (10^{-6} cm/s)	11.4 ± 1.05	9.10 ± 0.536	12.5 ± 1.02
	Recovery (%)	93.8 ± 2.26	91.0 ± 2.77	96.0 ± 2.86
Atenolol	P_{app} (10^{-6} cm/s)	0.143 ± 0.0654	0.198 ± 0.0462	0.266 ± 0.120
	Recovery (%)	87.7 ± 3.23	89.4 ± 3.52	90.6 ± 2.96

Table 2. Mass Balance Results

Dosing (µM)	Direction	Mean Doxepin HCl Concentration (µM)				Mass Balance (%)
		Measured Dosing	Receiver at 45 min	Donor at 45 min	Lysate	
1	A-to-B	1.08	0.0404	0.512	0.394	95.1%
	B-to-A		0.141	0.829	0.266	89.4%
10	A-to-B	10.9	0.508	5.42	2.74	88.9%
	B-to-A		1.82	9.00	2.19	94.8%
100	A-to-B	105	7.81	59.4	18.0	96.2%
	B-to-A		26.3	90.5	11.8	98.5%

Table 3. B-to-A vs. A-to-B P_{app} Ratios of Doxepin Hydrochloride (Ratios calculated using the mean P_{app} values)

Nominal Doxepin Hydrochloride Dosing Concentration (µM)	1	10	100
A-to-B P_{app} (10^{-6} cm/s)	17.7 ± 1.99	23.2 ± 1.05	37.6 ± 1.19
B-to-A P_{app} (10^{-6} cm/s)	22.6 ± 2.53	27.4 ± 2.56	36.6 ± 6.40
B-to-A P_{app} vs. A-to-B P_{app} Ratio	1.28	1.18	0.974

2.6.4.4 Distribution

SP-D0115 (Study YGH00029): BINDING OF DOXEPIN TO HUMAN, RAT, RABBIT, AND MOUSE PLASMA PROTEINS USING EQUILIBRIUM DIALYSIS-BASED METHOD. Revised Summary Report, [redacted] (b) (4), non-GLP. Doxepin was moderately plasma protein bound (~80-90%) in all species tested; binding to human plasma proteins was generally somewhat lower than the other species tested (~3-6%). See the sponsor's summary table below.

Study: YGH00029
ASSAY: Equilibrium Dialysis Protein Binding

Plasma Protein Binding Results Summary (Equilibrium Dialysis)

Test Compound	Matrix Species	% Plasma Protein Bound (Plasma-to-Buffer)	Equilibrium Ratio (Plasma-to-Plasma) *	% Recovery on Assay Plate (Plasma-to-Buffer)	Comment
Doxepin	Human Plasma	80.3%	0.99	79.3%	DXPN
Doxepin	Rabbit Plasma	88.5%	1.04	83.7%	DXPN
Doxepin	Rat Plasma	90.8%	1.32	72.6%	DXPN
Doxepin	Mouse Plasma	86.0%	1.02	73.0%	DXPN
Warfarin	Human Plasma	99.2%	3.95	78.7%	WARF

* ER = 1 if equilibrium is achieved

Literature references were provided describing the tissue distribution of doxepin. According to Hobbs (1969), doxepin was widely distributed into tissues after a single oral 10 mg/kg radiolabeled dose. Highest levels were detected in stomach, liver, kidney and lung after 1 hour; few tissues still retained radioactivity at 8 days (eye, liver, kidney, stomach, muscle and skin). Notably, the levels in heart were approximately double those in blood at 4 hours postdose, although levels were still much lower than those in other organs such as stomach, liver and kidney. Another article (Kimura et al., 1972) also suggested that, although other organs demonstrated higher exposures, doxepin levels in heart appeared to be retained longer than in plasma (levels in heart tissue were 3-4 times plasma exposure at 4 hours postdose) after a single 50 mg/kg oral dose in rats. However, Elonen et al. (1975) suggested that doxepin concentrates in cardiac tissue (~41 times plasma) in rabbits; the methods used in this study (i.v. infusion into jugular, blood draw from carotid) may account for the magnitude of the difference. While some sources (e.g., Marshall & Forker, 1982) posit that tricyclics are lipophilic and therefore concentrate in tissues such as heart, the extent to which this is true is unclear from the information provided.

2.6.4.5 Metabolism

The sponsor provided literature as evidence that the metabolic pathways in rats, dogs, and humans appear to be qualitatively similar. The sponsor reported that doxepin appears to be predominantly metabolized by CYP2D6 (E-doxepin ring hydroxylation) as well as CYP2C19 (demethylation) with a lesser involvement of CYP1A2 and CYP2C9 (possibly also CYP 3A4). The proposed metabolism of doxepin is presented in the sponsor's Figure 2.6.4.1, following.

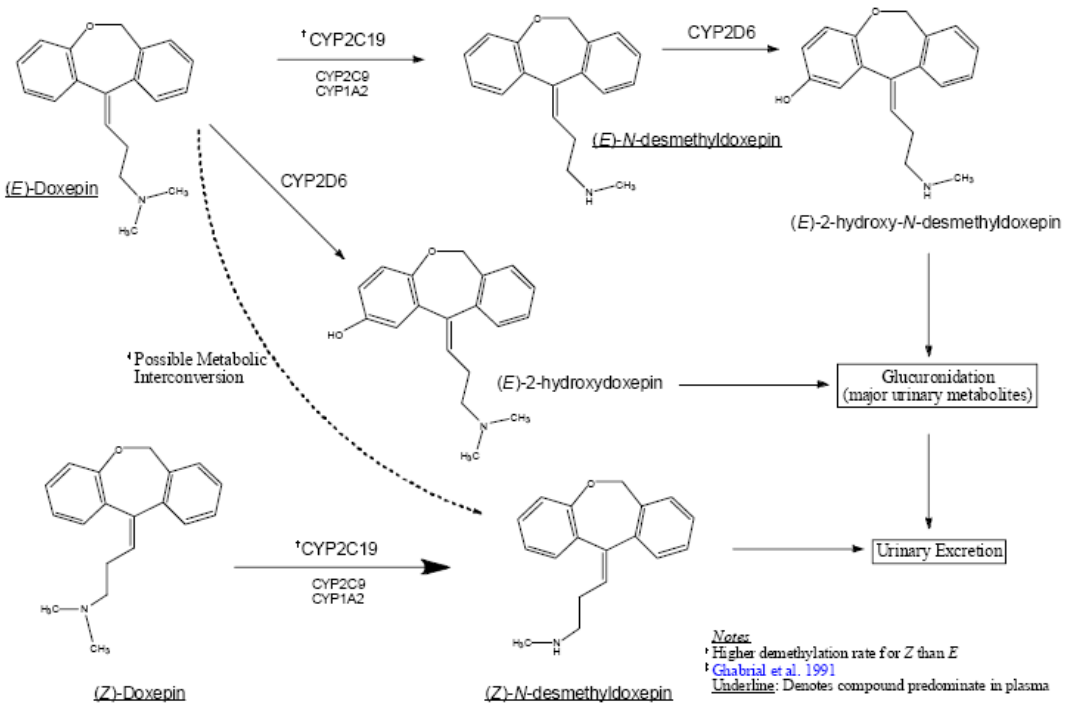


Figure 2.6.4.1 Doxepin Metabolism

In addition to study SP-D0118 (summarized below), the sponsor provided literature sources for additional metabolism information. Several papers (e.g., Harritos et al., 2000, Härtter et al, 2002) demonstrated contributions of P450 enzymes CYP2C19, CYP2C9, CYP1A2 and/or CYP2D6; there was some evidence suggesting that polymorphisms in CYP2D6 might play a role in metabolism and adverse effects (Kirchheiner et al., 2002).

SP-D0118 (Study 792502): ADME-Tox: CYP Inhibition - Study of Doxepin HCl
(b) (4) QA statement without a GLP certification
According to the sponsor, doxepin HCl did not inhibit CYP isoforms except CYP2D6; the sponsor indicated this was "consistent with known literature (for example, Härtter et al. Journal of Clinical Psychopharmacology Vol. 24 p. 568-71, 2004)". See the sponsor's summary data, below. The summary data suggest inhibition of CYP 2D6 and 2C19.

Table 1 - 1

Summary Results

Assay (b) (4) Compound I.D.	Client Compound I.D.	Test Concentration (M)	% Inhibition of Control Values
CYP1A2 Inhibition (recombinant, CEC substrate)			
792502-1	Doxepin HCl	1.0E-05	16
CYP2B6 Inhibition (recombinant, EFC substrate)			
792502-1	Doxepin HCl	1.0E-05	14
CYP2C8 Inhibition (recombinant, DBF substrate)			
792502-1	Doxepin HCl	1.0E-05	23
CYP2C9 Inhibition (recombinant, MFC substrate)			
792502-1	Doxepin HCl	1.0E-05	0
CYP2C19 Inhibition (recombinant, CEC substrate)			
792502-1	Doxepin HCl	1.0E-05	48
CYP2D6 Inhibition (recombinant, MFC substrate)			
792502-1	Doxepin HCl	1.0E-05	49
CYP2E1 Inhibition (recombinant, EC substrate)			
792502-1	Doxepin HCl	1.0E-05	-22
CYP1A2 Inhibition (recombinant, phenacetin substrate)			
792502-1	Doxepin HCl	1.0E-05	30
CYP2C9 Inhibition (recombinant, diclofenac substrate)			
792502-1	Doxepin HCl	1.0E-05	8
CYP2C19 Inhibition (recombinant, omeprazole substrate)			
792502-1	Doxepin HCl	1.0E-05	27
CYP2D6 Inhibition (recombinant, dextromethorphan substrate)			
792502-1	Doxepin HCl	1.0E-05	**64**
CYP3A4 Inhibition (recombinant, testosterone substrate)			
792502-1	Doxepin HCl	1.0E-05	27
CYP3A4 Inhibition (recombinant, midazolam substrate)			
792502-1	Doxepin HCl	1.0E-05	5

Table 1 - 4

IC_{50} Determination: Summary Results

Assay (b) (4) Compound I.D.	Client Compound I.D.	IC_{50} (M)	n_H
CYP2D6 Inhibition (recombinant, dextromethorphan substrate)			
792502-1	Doxepin HCl	6.9E-06	*1.1*

2.6.4.6 Excretion

The sponsor provided only literature to address excretion. Hobbs (1969) discusses several studies, including a study assessing excretion of radioactivity [14C]-label after doxepin was administered at 10 mg/kg PO and IP in rats. Recovery of radioactivity was >80% within 24 hours. By 120 hours, most of the radioactivity (~60%) following both routes of administration was recovered from urine. Analysis of rat urine indicated that the majority of radioactive drug product represented doxepin metabolites, and suggested the presence of doxepin-N-oxide, hydroxydoxepin and hydroxydoxepin glucuronide and low amounts of doxepin, nordoxepin, and didesmethyldoxepin. Hobbs also reported that ~50% of a labeled radioactive dose was excreted in the urine in dog (fecal excretion was not reported). Additionally, a study by Kimura et al. (1972) examined the excretion of doxepin and nordoxepin in the rat. After single 50 mg/kg PO doses of doxepin, little

unchanged doxepin (≤ 5% of administered dose) was recovered in urine or feces over 72 hours. Very little (<1%) unchanged doxepin was detected in bile. The amount of nordoxepin excreted in the urine and feces was less than 1%. Shu et al. (1990) reported the presence of doxepin glucuronide metabolites 3-O-glucuronyldoxepin and 2-O-glucuronyldoxepin in rat bile.

Cited Literature References

Beauchamp G, Lavoie PA, Elie R (1993) Effect of some stereoisomeric tricyclic antidepressants on ^{45}Ca uptake in synaptosomes from rat hippocampus. Psychopharmacology. 110(1- 2): 133-139.

Chen YW, Huang KL, Liu SY, Tzeng JI, Chu KS, Lin MT, et al. (2004) Intrathecal tri-cyclic antidepressants produce spinal anesthesia. Pain, 112: 106-112.

Elonen E, Linnoila M, Lukkari I & Mattila MJ (1975) Concentration of Tricyclic Antidepressants in Plasma, Heart, and Skeletal Muscle after their Intravenous Infusion to Anaesthetized Rabbits. Acta Pharmacol. et Toxicol., 37: 274-281.

Gerner P, Srinivasa V, Zizza AM, Zhuang ZY, Luo S, Zurakowski D, et al. (2006) Doxepin by topical application and intrathecal route in rats. Anesth Analg., 102(1): 283-287.

Haritos VS, Ghabrial H, Ahokas JT & Ching MS (2000) Role of cytochrome P450 2D6 (CYP2D6) in the stereospecific metabolism of E- and Z-doxepin. Pharmacogenetics, 10: 591-603.

Härtter S, Tybring G, Friedberg T, Weigmann H & Hiemke1 C (2002) The N-Demethylation of the Doxepin Isomers Is Mainly Catalyzed by the Polymorphic CYP2C19. Pharmaceutical Research, 19(7): 1034-1037.

Heal D, Cheetharn S,Martin K, Browning J, Luscornbe G & Buckett R (1992) Comparative Pharmacology of Dothiepin, Its Metabolites, and Other Antidepressant Drugs. Drug Development Research, 27: 121-135.

Hobbs DC (1969) Distribution and metabolism of Doxepin. Biochemical Pharmacology, 18: 1941-1954.

Jenck F, Moreau J-L, Mutel V & Martin JR (1994) Brain 5-HT_{1C} Receptors and Antidepressants. Prog. Neuro-Psychopharmacol. & Biol. Psychiat.,18(3): 563-574.

Kamei C, Ichiki C, Izumo T, Yoshida T, Tsujimoto S (1996) Effect of the new antiallergic agent olopatadine on EEG spectral powers in conscious rats. Arzneimittelforschung, 46(8): 789-793.

Kimura Y, Kume M & Kageyama K (1972) Absorption, Distribution, Excretion and Metabolism of Doxepin. Pharmacometrics, 6: 955-71. [translated from the original Japanese]

Kirchheiner J, Meineke I, Müller G, Roots I & Brockmöller J (2002) Contributions of CYP2D6, CYP2C9 and CYP2C19 to the biotransformation of E- and Z-doxepin in healthy volunteers. Pharmacogenetics, 12: 571 -580.

Marshall JB & Forker AD (1982) Cardiovascular effects of tricyclic antidepressant drugs: Therapeutic usage, overdose and management of complications. American Heart Journal, 103(3): 401-414.

Nakashita M, Sasaki K, Sakai N & Saito N (1997) Effects of tricyclic and tetracyclic antidepressants on the three subtypes of GABA transporter. Neuroscience Research, 29: 87–91.

Ookuma K, Sakata T, Fujimoto K. (1990) Evidence for feeding elicited through antihistaminergic effects of tricyclic antidepressants in the rat hypothalamus. Psychopharmacology (Berl), 101(4): 481-485.

Orthen-Gambill, N. (1988) Antihistaminic drugs increase feeding, while histidine suppresses feeding in rats. Pharmacol Biochem Behav., 31(1): 81-86.

Pancrazio JJ, Kamatchi GL, Roscoe AK, Lynch C, 3rd. (1998) Inhibition of neuronal Na+ channels by antidepressant drugs. J Pharmacol Exp Ther., 284(1): 208-214.

Richelson E & Nelson A (1984) Antagonism by Antidepressants of Neurotransmitter Receptors of Normal Human Brain *In Vitro*. J. Pharmacol. And Exp. Therapeu., 230: 94-102.

Schwaninger M, Schofl C, Blume R, Rossig L, Knepel W (1995) Inhibition by antidepressant drugs of cyclic AMP response element-binding protein/cyclic AMP response element-directed gene transcription. Mol Pharmacol., 47(6): 1112-1118.

Shu YZ, Hubbard JW, McKay G, Midha KK (1990) Identification of phenolic doxepin glucuronides from patient urine and rat bile. Drug Metab Dispos., 18(6): 1096-99.

Sudoh Y, Cahoon EE, De Girolami U, Wang GK (2004) Local anesthetic properties of a novel deriviative, N-methyl doxepin, versus doxepin and bupivicaine. Anesth Analg., 98(3): 672-676.

Uhr M, Grauer MT & Holsboer F. (2003) Differential Enhancement of Antidepressant Penetration into the Brain in Mice with abcb1ab (mdr1ab) P-Glycoprotein Gene Disruption. Biol Psychiatry, 54: 840–846.

Wong DT, Bymaster FP, Reid LR & Threlkeld PG (1983) Fluoxetine and two other serotonin uptake inhibitors without affinity for neuronal receptors. Biochemical Pharmacology, 32(7): 1237-1293.

Wordliczek J, Banach M, Labuz D, Przewlocka B. (2005) Intrathecal administration of doxepin attenuated development of formalin-induced pain in rats. J Neural Transm., 112(10); 1321-1329.

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Genetic toxicology:

Genetic toxicology studies are not reviewed here. These studies have been reviewed in detail previously (P/T review dated 2/2/07 for I67,162 submissions N046 & N048, dated 9/15/06 and 2/2/07; finalized study reports were submitted SN054, dated4/2/07). A standard battery of genetic toxicology studies (i.e., *in vitro* bacterial reverse mutation assay, *in vitro* chromosomal aberrations assay (HPBL) and *in vivo* rat micronucleus assay) was conducted and evaluated as negative.

Carcinogenicity:

The 26-week oral carcinogenicity transgenic (Tg.rasH2) mouse protocol was submitted as a special protocol for concurrence by the ExecCAC (SN055, dated 4/26/07), but was denied because the study was already ongoing. The study generally appears adequate (e.g., sensitivity of the assay was demonstrated by the development of pulmonary and splenic tumors in urethane-treated mice), although the health of the animals was somewhat in question due to the atypical nasal cavity findings (inflammation/irritation) across all groups. While nasal, pulmonary and splenic tumors were observed in doxepin-treated animals, there were no statistically significant, dose-related increases in tumors (see independent FDA statistical review dated 6/30/08). The nasal cavity tumors are of note because they are a new finding for the contract laboratory (b) (4)) and this strain of mice. The increased severity of the inflammatory lesion observed combined with the development of neoplasias in doxepin-treated animals was considered drug-related and of potential relevance. The incidence of the nasal neoplasias was not dose-dependent, but these were rare tumors not previously demonstrated in other similar studies (N=4, historical control data). The splenic hemangiosarcomas in males are of note because the incidence rates, though not clearly dose-dependent, exceeded the historical background rate (and range) for (b) (4). However, the ExecCAC indicated that the incidence rates were within those observed in other studies reviewed by the committee. The sponsor considered the development of splenic hemangiosarcomas and nasal adenocarcinomas in doxepin groups "noteworthy" because these tumors were not observed in the concurrent vehicle controls. It was concluded (see ExecCAC minutes dated 11/6/08) that doxepin hydrochloride was not tumorigenic in Tg.rasH2 mice administered the drug daily for 26 weeks, based on: 1) a lack of statistical difference between tumor frequencies in doxepin treatment groups and vehicle controls and 2) the lack of dose- or exposure-dependence for the doxepin group tumors (nasal cavity, lung and spleen).

The protocol for the 2-yr. rat carcinogenicity bioassay was submitted for ExecCAC concurrence (SN057, dated 6/19/07); the ExecCAC meeting was held 7/31/07 and the meeting minutes (dated 8/1/07) were faxed to the sponsor. This study is currently ongoing, being conducted as a Phase 4 commitment.

Reproductive toxicology:
A complete reproductive toxicology assessment was performed: a rat fertility study, rat and rabbit embryofetal studies, and a rat pre- and postnatal study. In the rat fertility study, fertility indices appeared relatively unaffected; however, adverse effects were observed on sperm (percent motility and percent abnormal) and in uterine examinations (i.e., decreased numbers of corpora lutea, implantation sites and viable embryos, as well as increased preimplantation loss) at ≥ 30 mg/kg. The NOAEL for reproductive performance and fertility was 10 mg/kg/day. The rat embryofetal study demonstrated adverse maternal and developmental effects at ≥ 100 mg/kg; the NOEL for developmental alterations was 30 mg/kg, based on developmental delays and total low incidence fetal alterations at higher doses. The rabbit embryofetal study demonstrated a slight decrease in fetal weights; the NOAEL was 30 mg/kg. In the pre- and postnatal study in rats, altered viability, growth and development of the F_1 pups was observed; the NOAEL was 30 mg/kg. No F_1 reproductive effects were observed (NOAEL = 100 mg/kg).

2.6.6.3 Repeat-dose toxicity

Study title: 2-WEEK ORAL DOSE-RANGE FINDING AND TOXICITY STUDY OF DOXEPIN HCL IN RATS
Key study findings:

- MTD_{14d}= 100 mg/kg

Study no.: Sponsor #SP-D0104, 1288-002
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)
Date of study initiation: July 5, 2006
GLP compliance: Yes, pg. 2
QA report: yes (X) no ( ) pg. 7
Drug, lot #, and % purity: doxepin HCl, lot 201402004, 100.4%
Methods

Doses: 0, 10, 30, 100, 300/200 mg/kg
Species/strain: CD® [Crl:CD®(SD)] rats
Number/sex/group or time point (main study): 6/sex/gp
Route, formulation, volume, and infusion rate: PO, gavage, 10 ml/kg, QD 14
Age: ~7 weeks of age
Weight: 152-200 g at randomization

Group Assignments

Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
1	0	6	6
2	10	6	6
3	30	6	6
4	100	6	6
5	300/200[a]	6	6

[a]Animals were dosed at 300 mg/kg/day on Days 1 and 2. Due to test article-related clinical signs, the animals were not dosed on Days 3 and 4, and then were dosed at 200 mg/kg/day on Days 5 to 14.

Details: Individually housed in stainless wire mesh cages *ad lib.* food and water

Observations times & Results:

Mortality: twice daily

One HDM and 1 HDF were found dead on D6. Based on pathology observations, the sponsor considered the deaths possibly test article related; however, an accidental cause of death could not be excluded.

Clinical signs: weekly

Test article-related clinical observations mostly at HD included decreased activity, limb splay, increased urination, and salivation. Impaired limb function, hypersensitivity, and hypothermia were noted in a single HDF (#1055) on D4. One HDM (#1030) had observations of material around the face, discolored hair on the ventral surface, and difficulties breathing on D5-6. These clinical observations were considered related to treatment. These two HD animals were subsequently found dead on D6. Most clinical signs appeared transient (not observed Wk2).

	MALES					FEMALES				
Clinical Observations	Con	LD	MLD	MHD	HD	Con	LD	MLD	MHD	HD
Decreased activity										
Wk 1	-	-	-	-	3	-	-	-	1	4
Wk 2	-	-	-	-	-	-	-	-	-	1
Limbs splayed										
Wk 1	-	-	-	-	2	-	-	-	-	2
Wk 2	-	-	-	-	-	-	-	-	-	-
Urination increased										
Wk 1	-	-	-	-	1	-	-	-	-	2
Wk 2	-	-	-	-	-	-	-	-	-	-
Lacrimation										
Wk 1	-	-	-	-	-	-	-	-	-	2
Wk 2	-	-	-	-	-	-	-	-	-	-
Salivation										
Wk 1	-	-	-	-	-	-	-	-	-	-
Wk 2	-	-	-	-	-	-	-	-	-	1

Body weights: weekly

Dose-related decreases in body weights were demonstrated in males. Decreased body weights were observed in HDM (~20% less than controls) and HDF (~10% less than controls). The differences in body weights were reflected in the mean body weight gains. See the sponsor's tabular summary and figures, below.

Percent Differences in Mean Body Weight Gain[a] (g)					
Males					
Dose Level (mg/kg/day)	0	10	30	100	300/200
Mean Body Weight Gain	123.0[b]	↑6%	↓8%	↓10%	↓52%
[a]Arrows indicate increase or decrease from the control [b]Control value is expressed in grams					

Percent Differences in Mean Body Weight Gain[a] (g)					
Females					
Dose Level (mg/kg/day)	0	10	30	100	300/200
Mean Body Weight Gain	45.2[b]	↓15%	↓9%	↓26%	↓43%
[a]Arrows indicate increase or decrease from the control [b]Control value is expressed in grams					

Figure 1 Mean Body Weight Values – MALE

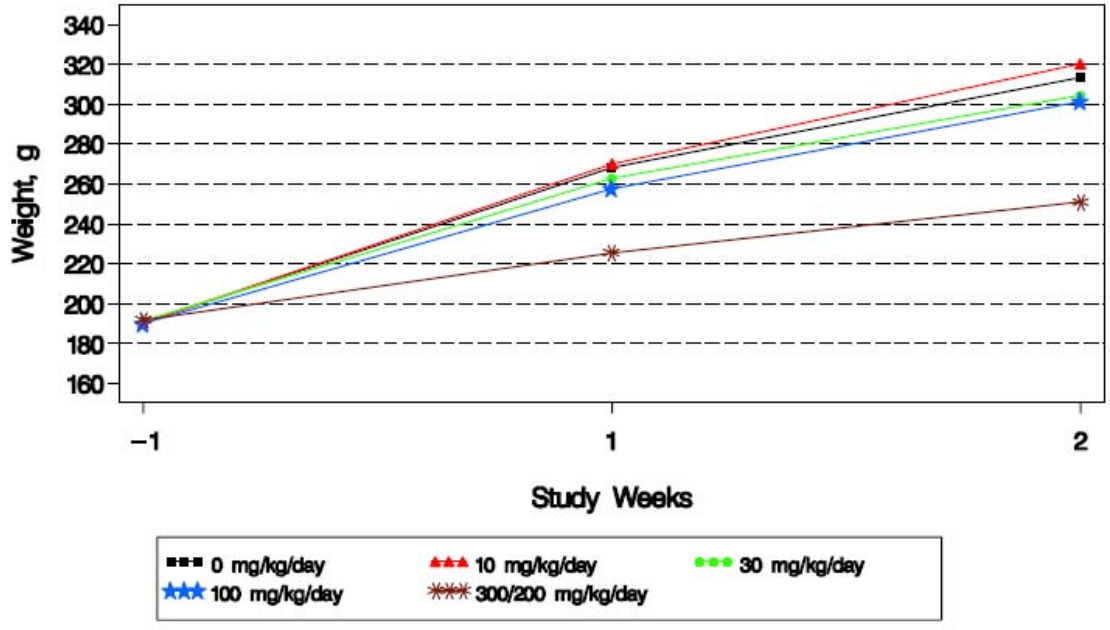
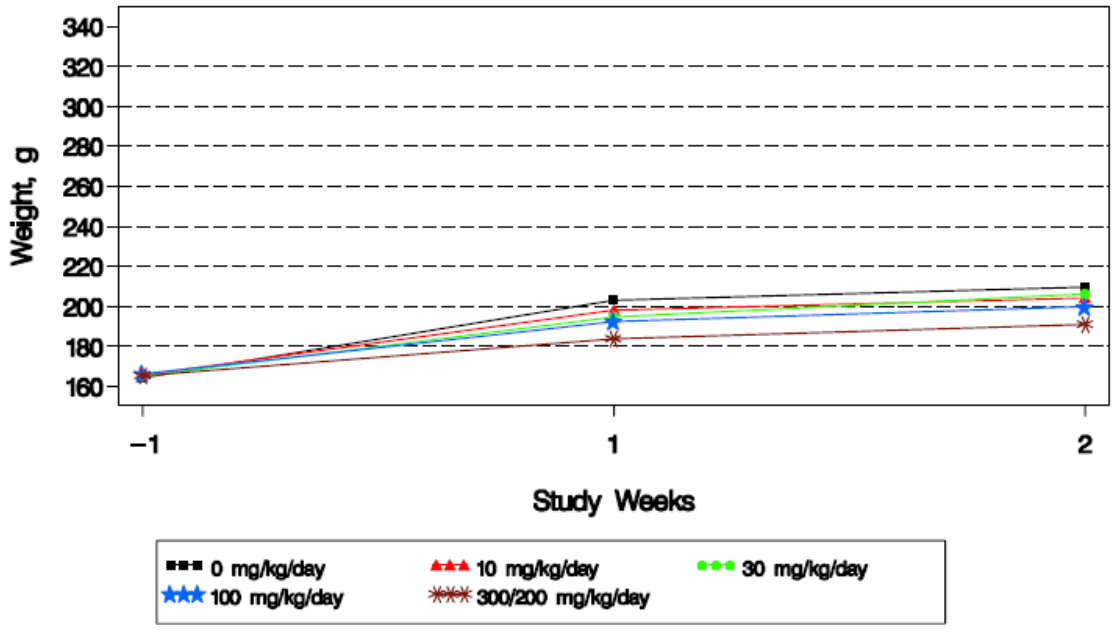


Figure 1A Mean Body Weight Values – FEMALE



Food consumption: weekly

Decreased food consumption was observed in HDM during week 1, in HDF during weeks 1 and 2, and in MHDF in week 2. See the sponsor's tabular data, following.

Percent Differences in Mean Food Consumption[a] (g/animal/day)					
Males					
Dose Level (mg/kg/day)	0	10	30	100	300/200
Mean Food Consumption Week 1	26.31[b]	↑1%	↓1%	↓4%	↓25%
Mean Food Consumption Week 2	27.24[b]	↑6%	0%	↑17%	↓33%
[a]Arrows indicate increase or decrease from the control [b]Control value is expressed in grams/animal/day					

Percent Differences in Mean Food Consumption[a] (g/animal/day)					
Females					
Dose Level (mg/kg/day)	0	10	30	100	300/200
Mean Food Consumption Week 1	19.47[b]	↓9%	↓8%	↓10%	↓23%
Mean Food Consumption Week 2	19.12[b]	↓3%	↓10%	↓14%	↓27%
[a]Arrows indicate increase or decrease from the control [b]Control value is expressed in grams/animal/day					

Hematology: at terminal necropsy
The sponsor reported no drug-related effects on hematology or coagulation parameters. The basophil count was statistically decreased in HDF (0.082, 0.088, 0.082 & 0.054 $10^3/\mu l$ vs. 0.095 in the LD, MLD, MHD and HD VS. controls). APTT appeared slightly increased in HDM (slight increase in mean with large sd; see excerpt from sponsor's summary tables, below).

Table 5 Summary of Coagulation Values - MALE Summary o

Endpoint	Interval of Study	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	300/200 mg/kg/day Mean	SD	N
APTT sec	Terminal	15.08	2.529	6	14.42	1.433	6	15.66	2.391	5	15.32	0.893	16.68	4.797	5
Prothrombin Time sec	Terminal	17.23	0.327	6	17.57	0.418	6	17.52	0.545	5	16.94	0.358	17.12	0.311	5

Clinical chemistry: at terminal necropsy
Glucose appeared increased at MHD and HD (F > M). Cholesterol was also increased at HD. Chloride was very slightly decreased at MHD and HD. Additionally, slight elevations in total bilirubin, alkaline phosphatase (ALP), alanine aminotransferase (ALT), and urea nitrogen were observed in HDF (also in MHDF for BUN). In HDM, total protein and globulins were increased, and AST was decreased.

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Table 6 Summary of Clinical Chemistry Values - FEMALE

Endpoint	Interval of Study	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	Summary of 300/200 mg/kg/day Mean	SD	N
Glucose mg/dL	Terminal	192.2	56.55	6	167.7	64.17	6	158.7	80.22	6	212.7	60.71		305.2[a]	46.01	5
Cholesterol mg/dL	Terminal	59.0	12.25	6	60.5	14.32	6	65.3	20.03	6	73.3	14.81		80.2	10.03	5
Chloride mEq/L	Terminal	102.7	1.21	6	103.8	1.83	6	104.2	1.72	6	100.5	1.64	6	100.4	2.07	5
Total Bilirubin mg/dL	Terminal	0.10	0.000	6	0.10	0.000	6	0.15	0.055	6	0.12	0.041	6	0.18[b]	0.045	5
Alkaline Phosphatase U/L	Terminal	114.3	12.40	6	105.0	23.66	6	116.3	23.85	6	135.0	9.86	6	171.4[b]	50.89	5
ALT U/L	Terminal	30.8	5.15	6	33.8	5.88	6	27.0	4.69	6	30.2	5.98	6	54.8[b]	13.03	5
Urea Nitrogen mg/dL	Terminal	14.7	1.63	6	13.7	1.03	6	15.2	1.72	6	17.8[a]	1.72	6	16.2	2.28	5

Table 6 Summary of Clinical Chemistry Values - MALE

Endpoint	Interval of Study	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	Summary of 300/200 mg/kg/day Mean	SD	N
Glucose mg/dL	Terminal	220.3	45.10	6	192.0	48.23	6	238.5	98.81	6	267.0	43.74	6	288.4	96.86	5
Cholesterol mg/dL	Terminal	46.3	11.78	6	49.7	5.75	6	50.3	11.50	6	55.7	8.89	6	67.4[b]	10.09	5
Chloride mEq/L	Terminal	103.0	1.67	6	102.5	1.87	6	101.8	1.17	6	97.7[b]	1.63	6	100.8	1.30	5
Total Protein g/dL	Terminal	6.28	0.319	6	6.30	0.200	6	6.27	0.163	6	6.65	0.281	6	6.70[a]	0.212	5
Globulin g/dL	Terminal	2.68	0.194	6	2.75	0.207	6	2.70	0.155	6	2.88	0.194	6	3.00[a]	0.158	5
AST U/L	Terminal	77.3	2.88	6	77.8	3.87	6	70.3	1.86	6	72.0	5.40	6	65.6[a]	11.65	5

Gross pathology: at terminal necropsy
The sponsor recorded no drug-related effects. Moderate red pulmonary discoloration was observed in 1HDF (an unscheduled death); this lesion was believed to reflect gavage injury.

Organ weights: at terminal necropsy
The sponsor recorded potentially drug-related effects in the thymus glands of MHD and HD females, and the epididymides, spleens and/or livers of males. Overall, there appeared to be alterations in liver, spleen and pituitary weights. Thymus weights were altered in females, and brain, spleen and reproductive organ weights appeared altered in males. Notably, the body weights of HD animals were decreased (M > F), compared to controls.

As the sponsor indicated, absolute and relative thymus weight reductions were observed in the 1MHDF (26% and 27%, respectively) and HDF (36% and 29%, respectively). In HDF, liver weights appeared slightly increased (particularly relative to body weight), and

pituitary and spleen weights suggested a slight decrease. See excerpts from the sponsor's summary table, below.

Summary of Organ Weight Values - FEMALE
Table 8 Terminal

Endpoint	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	300/200 mg/kg/day Mean	SD	N
Body weight															
g	186	10	6	182	8	6	184	13	6	192	39	6	169	6	5
Liver															
g	7.254	0.522	6	7.486	0.518	6	7.535	0.790	6	7.447	0.874	6	7.648	0.380	5
Liver/BWt %	3.8967	0.1895	6	4.1117	0.1863	6	4.0893	0.2604	6	3.9852	0.7582	6	4.5377	0.3264	5
Pituitary gl															
g	0.0148	0.0009	6	0.0145	0.0025	6	0.0145	0.0017	6	0.0149	0.0012	6	0.0130	0.0008	5
Pituitary gl/BWt %	0.0079	0.0006	6	0.0080	0.0016	6	0.0079	0.0007	6	0.0079	0.0011	6	0.0077	0.0004	5
Pituitary gl/BrWt ratio	0.0084	0.0007	6	0.0081	0.0015	6	0.0085	0.0012	6	0.0086	0.0008	6	0.0075	0.0006	5
Spleen															
g	0.535	0.062	6	0.462	0.039	6	0.501	0.042	6	0.508	0.078	6	0.435[a]	0.063	5
Spleen/BWt %	0.2869	0.0264	6	0.2541	0.0191	6	0.2721	0.0139	6	0.2698	0.0488	6	0.2578	0.0382	5
Thymus gl															
g	0.594	0.098	6	0.490	0.117	6	0.560	0.092	6	0.439[a]	0.089	6	0.379[b]	0.073	5
Thymus gl/BWt %	0.3180	0.0423	6	0.2679	0.0555	6	0.3028	0.0349	6	0.2310[b]	0.0411	6	0.2244[b]	0.0426	5

In males, an apparent dose-related reduction in absolute and relative epididymides weights were observed in LD, MHD and HD males (see the sponsor's table from the pathologist's report, below). Brain and pituitary weights appeared slightly decreased at MHD and HD. In HDM, absolute spleen weights were increased (33%), and there was an increased relative liver-to-body weight ratio (15%, [ss]). An apparent slight decrease in absolute thymus weight was not reflected in thymus-to-body weights.

Possible Test Article-Related Effects
Male
Terminal

Dose level: mg/kg/day	0	10	30	100	300/200
Number Examined	6	6	6	6	5
Epididymides (g)	0.620	0.522[a] (↓16%)	0.628	0.497[b] (↓20%)	0.463[b] (↓25%)
Epididymides/BWt %	0.2281	0.1873[a] (↓18%)	0.2371	0.1918[a] (↓16%)	0.2105 (↓8%)
Epididymides/BrWt ratio	0.3212	0.2728[a] (↓15%)	0.3319	0.2747[a] (↓14%)	0.2530[b] (↓21%)

BWt - Body Weight
BrWt – Brain Weight
[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Summary of Organ Weight Values - MALE

Table 8 Terminal

Endpoint	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	300/200 mg/kg/day Mean	SD	N
Body weight g	273	8	6	279	11	6	265	14	6	259	10		220[b]	14	5
Brain g	1.926	0.058	6	1.911	0.061	6	1.891	0.040	6	1.809[a]	0.086		1.832	0.072	5
Liver g	11.231	0.644	6	11.789	0.794	6	10.564	1.289	6	10.722	0.734	6	10.458	1.189	5
Liver/BWt %	4.1247	0.2732	6	4.2289	0.2540	6	3.9746	0.3095	6	4.1422	0.2229	6	4.7530[b]	0.4500	5
Pituitary gl g	0.0134	0.0020	6	0.0128	0.0020	6	0.0127	0.0024	6	0.0118	0.0014	6	0.0105	0.0010	5
Pituitary gl/BWt %	0.0049	0.0007	6	0.0046	0.0007	6	0.0048	0.0007	6	0.0046	0.0005	6	0.0048	0.0004	5
Pituitary gl/BrWt ratio	0.0070	0.0012	6	0.0067	0.0010	6	0.0067	0.0012	6	0.0065	0.0008	6	0.0057	0.0004	5
Spleen g	0.801	0.142	6	0.895	0.172	6	0.709	0.150	6	0.653	0.105	6	0.537[a]	0.139	5
Spleen/BWt %	0.2945	0.0542	6	0.3206	0.0597	6	0.2688	0.0504	6	0.2517	0.0359	6	0.2430	0.0554	5
Thymus gl g	0.558	0.152	6	0.711	0.113	6	0.651	0.158	6	0.597	0.082	6	0.448	0.098	5
Thymus gl/BWt %	0.2052	0.0570	6	0.2544	0.0340	6	0.2449	0.0559	6	0.2305	0.0271	6	0.2025	0.0360	5
Epididymides g	0.620	0.103	6	0.522[a]	0.051	6	0.628	0.053	6	0.497[b]	0.050		0.463[b]	0.034	5
Epididymides/BWt %	0.2281	0.0400	6	0.1873[a]	0.0180	6	0.2371	0.0217	6	0.1918[a]	0.0163		0.2105	0.0112	5
Prostate gl g	0.254	0.049	6	0.277	0.090	6	0.271	0.055	6	0.247	0.037	6	0.181	0.066	5
Prostate gl/BWt %	0.0930	0.0176	6	0.0999	0.0353	6	0.1026	0.0219	6	0.0951	0.0113	6	0.0819	0.0279	5

Histopathology: Adequate Battery: yes ( ), no (X)— Not performed.
Peer review: yes ( ), no ( )

Toxicokinetics: predose, and 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose on Day 13
Summary TK data were not provided; doxepin concentrations were provided for each animal at each timepoint. All controls showed doxepin levels below the quantitation limit, as were most levels in most LD and MLD animals. Plasma concentrations generally appeared to increase with increasing dose.

Other:
Dosing formulation analysis demonstrated that mean dosing formulation concentrations were between 94.4 and 108.0% of nominal concentration.

Study title: *SP-D0105: 2-WEEK ORAL DOSE-RANGE FINDING AND TOXICITY STUDY OF DOXEPIN HCL IN RABBITS*

Key study findings:

- Sponsor MTD_{14D}= 30 mg/kg (based on weight loss and mortality at ≥ 200 mg/kg, and clinical signs, including convulsion, at 100 mg/kg in Wk2)
- Note that some mortality occurred at 20, 30 and 100 mg/kg, but was of unclear relationship to drug
- Pilot study to provide dose-ranging information for rabbit embryofetal study

Study no.: 1288-001

Volume #, and page #: Electronic submission, 418 pgs.

Conducting laboratory and location: (b) (4)

Date of study initiation: 7/5/06

GLP compliance: Yes, pg 2

QA report: yes (X) no ( ) pg. 7

Drug, lot #, and % purity: doxepin HCl, lot 201402004, 100.4% in distilled water

Methods

Doses: 0, 10, (20, 25,) 30, 100, (200), & 300 mg/kg/day [Gps added in ( )]

Species/strain: New Zealand White rabbits, Hra:(NZW)SPF albino

Number/sex/group or time point (main study): 3/sex/gp

Route, formulation, volume, and infusion rate: PO, gavage, QD for 14 days (except 200 & 300 mg/kg for 3-4 days), 10 ml/kg

Age: ~7.5 mo.

Weight: 3.13-3.8 kg, at randomization

Unique study design or methodology (if any): (sponsor's table, below)

Group Assignments

Group Number	Dose Level (mg/kg/day)	Number of Animals Male	Female
1	0	3	3
2	10	3	3
3	30	3	3
4	100	3	3
5	300	3	3
6	200[a]	3	3
7	20[b]	3	3
8	25[b]	3	3

[a]The 200 mg/kg/day group was added due to mortality in the 300 mg/kg/day group.
[b]The 20 and 25 mg/kg/day groups were added due to mortality in the 30 and 100 mg/kg/day groups.

Individually housed, SS cage ~125 g/day food; *ad lib* water

Observations times & Results:

Mortality: 2x daily

Fourteen animals were found dead on study. Drug-related deaths were observed at 200 and 300 mg/kg/day, following 1-3 doses. A few mortalities also occurred at 20, 30, and 100 mg/kg/day; however, it was unclear if they were related to drug although a relationship could not be excluded. See the sponsor's summary table, below, for details.

Animals Found Dead on Study

Dose Level (mg/kg/day)	Animal Number	Sex	Day of Death
20	143	F	14
30	107	M	12
30	124	F	7
100	125	F	11
200	131	M	4
200	132	M	3
200	133	M	3
200	134	F	3
200	136	F	3
300	113	M	2
300	114	M	2
300	115	M	4
300	128	F	4
300	130	F	2

Clinical signs: Weekly

Drug-related clinical observations noted at 100, 200, and 300 mg/kg/day included: decreased activity, convulsions, prostration, breathing changes (slow, rapid, shallow, or difficult), tremors (200 mg/kg/day), cold skin, feces few/absent, and salivation (300 mg/kg/day), limb splay (100 and 200 mg/kg/day), and skin discoloration (100 and 300 mg/kg/day). See the reviewer's summary table, next page.

	MALES								FEMALES							
	0	10	20	25	30	100	200	300	0	10	20	25	30	100	200	300
Clonic Convulsions																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	**1**	**2**	*0*	*0*	*0*	*0*	*0*	*0*	**2**	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	**1**	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	**1**	*n/a*	*0*
Tremors																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**	*0*
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*
Decreased activity																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	**2**	**3**	*0*	*0*	*0*	*0*	*0*	*0*	**2**	**3**
Wk 2	*0*	*0*	*0*	*0*	*0*	**3**	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	**2**	*n/a*	*0*
Prostration																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	**1**	**3**	*0*	*0*	*0*	*0*	*0*	*0*	**2**	**2**
Wk 2	*0*	*0*	*0*	*0*	*0*	**3**	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	**2**	*n/a*	*0*
Salivation																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*
Splayed Limbs																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	**1**	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Wk 2	*0*	*0*	*0*	*0*	*0*	**1**	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	**1**	*n/a*	*0*
Cold to Touch																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*
Skin Discolored																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	**1**	n/a	*0*
Breathing rapid																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	**1**	**3**	*0*	*0*	*0*	*0*	*0*	*0*	**1**	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	**3**	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*
Breathing difficult																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*
Breathing shallow																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*
Breathing slow																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	**1**	*n/a*	*0*
Feces few/absent																
Wk 1	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	*0*	**1**
Wk 2	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*n/a*	*0*	*0*	*0*	*0*	*0*	*0*	*n/a*	*0*

Body weights: Weekly

Body weight loss was observed in surviving female animals at 200 and 300 mg/kg/day compared to controls. See the sponsor's summary figures, below.

Figure 1 Mean Body Weight Values – MALE

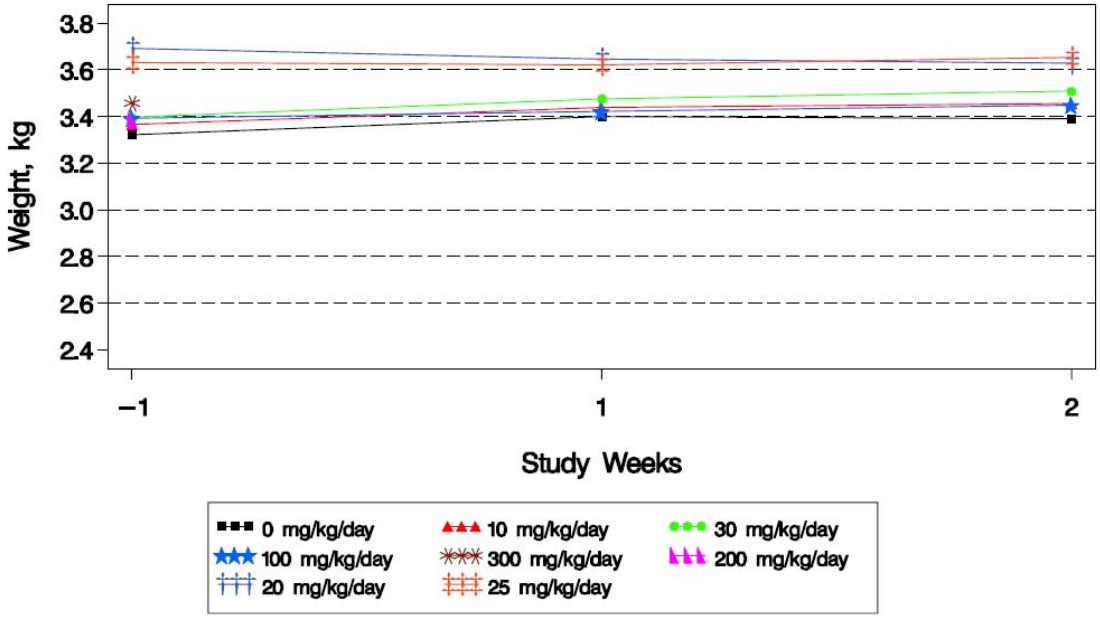
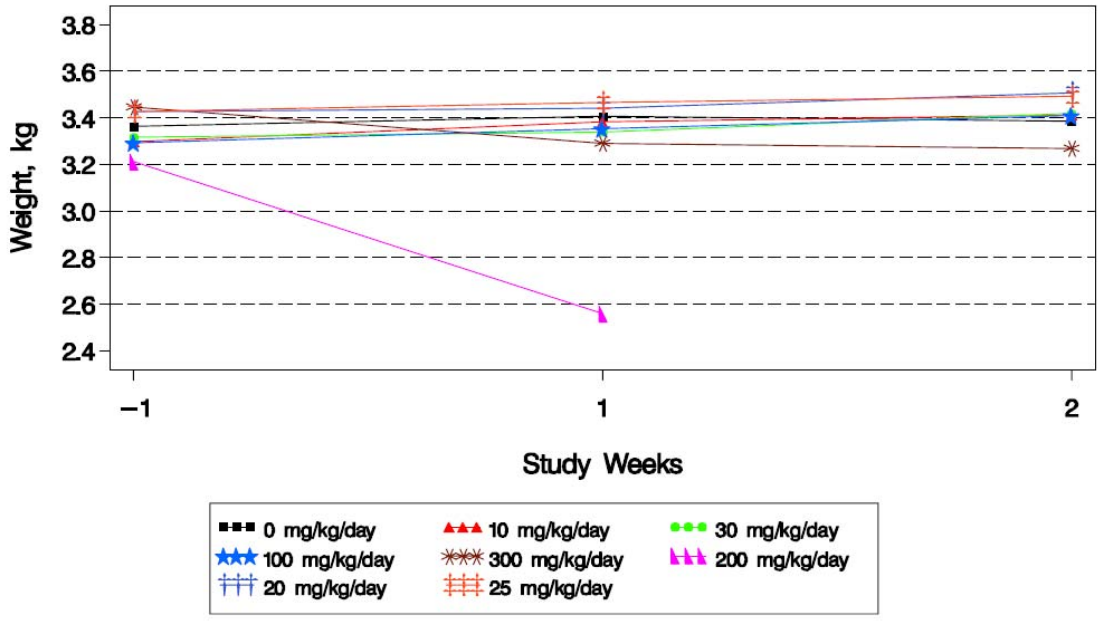


Figure 1A Mean Body Weight Values – FEMALE



Food consumption: Weekly
Decreased food consumption was observed in surviving female animals at 200 and 300 mg/kg/day compared to controls. Males at 100 mg/kg/day had decreased food consumption (9-12%) compared to controls.

Hematology: Terminal necropsy, D14
The sponsor recorded no drug-related differences; notably, the data were highly variable. In females, the data at 30 mg/kg and 100 mg/kg appeared to suggest a biphasic effect. In the 100 mg/kg F, erythrocyte count, hemoglobin, hematocrit, platelet count (each 13-24%) and eosinophils appeared increased (118%); reticulocytes (absolute and percent) appeared slightly decreased (18-33%). In 100 mg/kg M, leukocyte counts (30%) and lymphocyte counts (42%) were reduced, and platelet counts were increased (63%). There appeared to be a biphasic effect on eosinophil counts, reduced at low doses and increased at 100 mg/kg (48%), although data were variable. There were no clear differences in coagulation parameters.

Clinical chemistry: Terminal necropsy, D14
The sponsor recorded no drug-related differences. In the 100 mg/kg F, several parameters appeared increased: calcium (6%), ALP (53%), total protein (10%), albumin (7%) and globulin (19%, dose-related). Also, AST (19%) and creatinine (8%) appeared reduced. In the 100 mg/kg M, several parameters appeared to be increased: ALP (11%), GGT (34%), AST (27%), ALT (27%), and glucose (10%, roughly dose-related).

Gross pathology: Terminal necropsy, D14
The sponsor recorded no drug-related differences.
Only a few findings were recorded in the early mortalities. In the two 300 mg/kg F early decedents, the following were observed: unidentified spleen (1), moderate red discoloration of the lung (1), severe friable fundus of the stomach (1) and moderate hemorrhage of the vagina (1). Mild red fluid in the thoracic cavity, moderate red lung discoloration and mild erosion/ulcer of the stomach fundus were observed in the 100 mg/kg F early decedent. One 30 mg/kg F early decedent showed moderate erosion/ulcer of the stomach pylorus. Red discoloration of the kidneys and clear fluid in the trachea were each observed in one 300 mg/kg M early decedent. Mild clear fluid in the trachea was observed in two 300 mg/kg early decedents (1M, 1F), but was considered a terminal accumulation by the pathologist.

Organ weights: Terminal necropsy, D14
The sponsor recorded no drug-related differences.

Thyroid/parathyroid weight appeared to show a dose-related increase in F (up to 85%); the relative thyroid weights of the single 200 and 300 mg/kg F also appeared increased (130 & 54%, respectively). Liver weight (relative to body; up to 13%) appeared increased in 30 and 100 mg/kg F; the relative liver weight of the single 300 mg/kg F was also increased (25%). Spleen weight (relative to body; 36%) was increased in 100 mg/kg F; the relative spleen weight of the single 300 mg/kg F also appeared increased (39%). Heart weight (relative to body; 13%) appeared decreased in 100 mg/kg F; the relative

heart weight of the single 300 mg/kg F also appeared reduced (12%). Other changes suggested in the single 300 mg/kg F included increased relative kidney weight (15%) and increased relative lung weight (14%).

In 100 mg/kg M, epididymides weight (relative to body; 33%), liver weight (relative to body; 13%) and salivary gland (relative to body; 33%) appeared to be increased. In 30 and 100 mg/kg M, lung weight appeared decreased ~20%.

Histopathology: Adequate Battery: yes ( ), no (X)—explain: Not performed
Peer review: yes ( ), no ( )

Toxicokinetics: Predose & 0.5, 1, 2, 3, 4, 6, 8, 12, and 24 hours postdose on Day 13/14 Plasma concentrations generally appeared to increase with increasing dose. Generally, females showed greater exposure than females.

Summary of Doxepin Plasma Toxicokinetic Parameters

Group	Dosage (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 13								
2	10	M	0[a]	NA	NA	0[a]	NE	NE
		F	19.7	0.5	0.5	4.93	NE	NE
3	30	M	54.9	0.5	3	75.7	102	1.5
		F	67.9	0.5	2	60.3	NE	NE
4	100	M	503	0.5	12	2430	2670	3.3
		F	1150	0.5	24	5970	6050	3.7
Day 14								
7	20	M	20.2	1	2	30.2	NE	NE
		F	68.2	0.5	2	70.6	NE	NE
8	25	M	48.0	0.5	3	71.7	106	1.8
		F	62.3	0.5	3	80.2	110	1.6

NE: Not estimated, due to insufficient characterization of terminal phase
NA: Not applicable
a: All mean concentrations were below the limit of quantitation (BQL)

Other: Days 1 & 14
Dose formulation analysis conducted by (b) (4). was not QAU audited. Homogeneity and stability analysis were not conducted. Dosing analysis indicated that the solutions used were 89.1-107% of the nominal concentrations. Vehicle samples did not contain detectable drug.

Study title: *SP-D0110: 28-Day Repeated-Dose Oral Toxicity and Toxicokinetic Study in CByB6F1 Hybrid Mice With A Preliminary Range-finding Toxicity Study*

Key study findings:

- MTD_{28D}= ≥ 50 mg/kg/day
- $NOAEL_{28D}$= between 25 & 50 mg/kg/day
- CRO suggested Low, Mid and High doses for the 26-week transgenic mouse carcinogenicity study
  - 10, 25 and 50 mg/kg/day
- Alternatively, if four dose groups are indicated, CRO suggested doses for the 26-week carcinogenicity study (2 variations, based on spacing of the doses)
  - 10, 25, 50 and 75 mg/kg/day
  - 10, 20, 40 and 80 mg/kg/day

Study no.: AB37CC.2G3R (b) (4)

Volume #, and page #: Electronic submission, 325 pgs

Conducting laboratory and location: (b) (4)

Date of study initiation: October 2, 2006

GLP compliance: Yes, pg. 2

QA report: yes (X) no ( ) Pgs. 3-4

Drug, lot #, and % purity: Doxepin HCl, lot 3045911, in sterile water for injection, USP

Methods

Doses:
5-Day
0, 10, 25, 50, 100 & 150 mg/kg/day
28-day
0, 10, 25 and 50 mg/kg/day

Species/strain: CByB6F1 hybrid mice, Tg.rasH2 nontransgenic littermates (b) (4)

Number/sex/group (main study):
5-Day
Main: 5/sex/gp
At initiation, ~8 wks of age; 19.8-32.4 g
28-day
Main: 10/sex/gp
Plus TK: 35/sex/gp & 5/sex/gp con
At initiation, ~7-8 wks of age; 18.7-28.7 g

Route, formulation, volume, and infusion rate: PO, QD by oral gavage, 10 ml/kg

Other details: *ad libitum* diet and water
Individually housed in polycarbonate cages

Observations times & Results:

5-Day

Mortality:

There were four Gp6 mortalities; two M were found dead on D2 and D3, two F were found dead on Day 2. No evidence of gavage error was found in the animals found dead. See the sponsor's summary table, below. All other animals survived until terminal sacrifice.

TABLE 1 - SUMMARY OF MORTALITY

MALES

		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	Found Dead	0/5	0/5	0/5	0/5	0/5	1/5
Day 3	Found Dead	0/5	0/5	0/5	0/5	0/5	1/5
Day 6	Terminal Sacrifice	5/5	5/5	5/5	5/5	5/5	3/5

FEMALES

		Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 2	Found Dead	0/5	0/5	0/5	0/5	0/5	2/5
Day 6	Terminal Sacrifice	5/5	5/5	5/5	5/5	5/5	3/5

Note: Represents the number of animals affected / the number of animals started on test.
Statistical analysis (Fisher's Exact Test) did not reveal any significant differences when mortality in Group 6 males was compared to Group 1 males or when mortality in Group 6 females was compared to Group 1 females.

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day
Group 4 - 50 mg/kg/day Group 5 - 100 mg/kg/day Group 6 - 150 mg/kg/day

Clinical signs:

Dose-related clinical observations noted included coma, lethargy, prostration and labored breathing/dyspnea. These signs first appeared on D1 (prostration and labored breathing/dyspnea in Gp4 F, Gp5 and Gp6), D2 (prostration and labored breathing/dyspnea in Gp4M, and coma in Gp4, Gp5 and Gp6) or D4 (lethargy in Gp4 and Gp5) and continued through D5 in most animals. See the sponsor's summary tables below for details. No abnormalities were noted during the detailed hands-on observations in the 5-Day study.

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TABLE 2 – SUMMARY OF CLINICAL OBSERVATION INCIDENCE – CAGESIDE (5-DAY)

Clinical Observations - Clinical Signs by Group

Study : AB37CC.2G3R. (b) (4) 5 Day Range-finding Toxicity Study in CByB6F1 Hybrid

Day numbers relative to Start Date

Sex: Male

	Group 1 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 25 mg/kg/day	Group 4 50 mg/kg/day	Group 5 100 mg/kg/day	Group 6 150 mg/kg/day
Comatose						
Number of Observations	.	.	.	11	13	11
Number of Animals	.	.	.	5*	5*	4*
Days from - to	.	.	.	2 4	2 5	2 5
Lethargic						
Number of Observations	.	.	.	7	7	.
Number of Animals	.	.	.	4*	4*	.
Days from - to	.	.	.	4 5	4 5	.
Prostrate						
Number of Observations	.	.	.	9	19	20
Number of Animals	.	.	.	4*	5*	5*
Days from - to	.	.	.	2 4	1 5	1 5
Labored/Dyspnea						
Number of Observations	.	.	.	4	19	20
Number of Animals	.	.	.	2	5*	5*
Days from - to	.	.	.	2 3	1 5	1 5

* p ≤ 0.05 (Dunnett's T-test) when compared to Group 1.

TABLE 2 – SUMMARY OF CLINICAL OBSERVATION INCIDENCE – CAGESIDE (5-DAY CONTINUED)

Clinical Observations - Clinical Signs by Group

Study : AB37CC.2G3R (b) (4) 5 Day Range-finding Toxicity Study in CByB6F1 Hybrid

Day numbers relative to Start Date

Sex: Female

	Group 1 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 25 mg/kg/day	Group 4 50 mg/kg/day	Group 5 100 mg/kg/day	Group 6 150 mg/kg/day
Comatose						
Number of Observations	.	.	.	8	14	14
Number of Animals	.	.	.	4*	5*	5*
Days from - to	.	.	.	2 3	2 5	2 5
Lethargic						
Number of Observations	.	.	.	9	6	.
Number of Animals	.	.	.	5*	3	.
Days from - to	.	.	.	4 5	4 5	.
Prostrate						
Number of Observations	.	.	.	14	19	19
Number of Animals	.	.	.	5*	5*	5*
Days from - to	.	.	.	1 3	1 5	1 5
Labored/Dyspnea						
Number of Observations	.	.	.	14	19	19
Number of Animals	.	.	.	5*	5*	5*
Days from - to	.	.	.	1 3	1 5	1 5

* p ≤ 0.05 (Dunnett's T-test) when compared to Group 1.

Body weights:

Generally, mean body weights of Gp5 and Gp6 were reduced. Day 5 mean body weights in Gp5 were 11.9% less for males [ss] and 2.5% less for females than corresponding vehicle control group. Day 5 group mean body weights in Gp6 were 10.1% less for males and 8.7% less for females. Body weight gain data demonstrated statistically significant decreases in Gp5 and Gp6. See the sponsor's summary data, below.

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TABLE 4 - SUMMARY OF BODY WEIGHTS (5-DAY)

Body weight (Grams)

Day numbers relative to Start Date

Group Sex		1	5
1m	Mean	27.86	27.22
	S.D.	0.81	0.88
	N	5	5
2m	Mean	29.08	28.20
	S.D.	3.00	2.95
	N	5	5
3m	Mean	28.18	27.46
	S.D.	1.06	0.73
	N	5	5
4m	Mean	27.44	25.68
	S.D.	1.49	2.23
	N	5	5
5m	Mean	26.28	23.98*
	S.D.	1.36	1.08
	N	5	5
6m	Mean	28.20	24.47
	S.D.	2.19	2.06
	N	5	3

* $p \leq 0.05$ (Dunnett's t-test) when compared to Group 1.

Arithmetic Mean Values Presented

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day
Group 4 - 50 mg/kg/day Group 5 - 100 mg/kg/day Group 6 - 150 mg/kg/day

TABLE 4 - SUMMARY OF BODY WEIGHTS (5-DAY CONTINUED)

Body weight (Grams)

Day numbers relative to Start Date

Group Sex		1	5
1f	Mean	21.60	21.02
	S.D.	1.06	1.01
	N	5	5
2f	Mean	22.10	21.96
	S.D.	1.16	1.04
	N	5	5
3f	Mean	21.30	21.20
	S.D.	0.45	0.48
	N	5	5
4f	Mean	21.62	21.22
	S.D.	1.52	1.31
	N	5	5
5f	Mean	22.26	20.50
	S.D.	1.42	1.43
	N	5	5
6f	Mean	21.26	19.20
	S.D.	1.09	0.78
	N	5	3

Statistical analysis (Dunnett's t-test) did not reveal any significant differences when Groups 2-6 were compared to Group 1.

Arithmetic Mean Values Presented

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day
Group 4 - 50 mg/kg/day Group 5 - 100 mg/kg/day Group 6 - 150 mg/kg/day

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TABLE 5 - SUMMARY OF BODY WEIGHT GAINS (5-DAY)

Body Weight Gain (Grams)

Day numbers relative to Start Date

Group	Base Weight Day		Abs Gain From: 1
Sex	1	To:	5
1m	27.86	Mean	-0.64
	0.81	S.D.	0.48
	5	N	5
2m	29.08	Mean	-0.88
	3.00	S.D.	0.42
	5	N	5
3m	28.18	Mean	-0.72
	1.06	S.D.	0.45
	5	N	5
4m	27.44	Mean	-1.76
	1.49	S.D.	1.02
	5	N	5
5m	26.28	Mean	-2.30*
	1.36	S.D.	0.49
	5	N	5
6m	28.20	Mean	-4.27*
	2.19	S.D.	1.70
	5	N	3

* p ≤ 0.05 (Dunnett's t-test) when compared to Group 1.

Abs Gain = absolute body weight gain between base period and end of the analysis period

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day
Group 4 - 50 mg/kg/day Group 5 - 100 mg/kg/day Group 6 - 150 mg/kg/day

TABLE 5 - SUMMARY OF BODY WEIGHT GAINS (5-DAY CONTINUED)

Body Weight Gain (Grams)

Day numbers relative to Start Date

Group	Base Weight Day		Abs Gain From: 1
Sex	1	To:	5
1f	21.60	Mean	-0.58
	1.06	S.D.	0.26
	5	N	5
2f	22.10	Mean	-0.14
	1.16	S.D.	0.42
	5	N	5
3f	21.30	Mean	-0.10
	0.45	S.D.	0.61
	5	N	5
4f	21.62	Mean	-0.40
	1.52	S.D.	0.25
	5	N	5
5f	22.26	Mean	-1.76*
	1.42	S.D.	0.88
	5	N	5
6f	21.26	Mean	-2.40*
	1.09	S.D.	0.87
	5	N	3

* p ≤ 0.05 (Dunnett's t-test) when compared to Group 1.

Abs Gain = absolute body weight gain between base period and end of the analysis period

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day
Group 4 - 50 mg/kg/day Group 5 - 100 mg/kg/day Group 6 - 150 mg/kg/day

Gross pathology, Organ weights, Histopathology & TK: Not performed

28-Day

Mortality: *Twice daily*

All Main and TK Study animals in the 28-Day study survived until terminal or scheduled sacrifice.

Clinical signs: *once daily (within 2 hours after the last animal was dosed)*

Drug-related clinical observations including coma, decreased motor activity, lethargy, prostration and labored breathing/dyspnea were noted during postdose cageside observations in both sexes. The incidence of these clinical signs was statistically significantly increased in MD and HD animals of both sexes when compared to the vehicle control group; MD and HD animals showed no clinical signs after D20. No abnormal detailed hands-on observations were recorded.

TABLE 7 – SUMMARY OF CLINICAL OBSERVATION INCIDENCE – CAGESIDE (28-DAY)

Clinical Observations - Clinical Signs by Group

Study : AB37CC.2G3R(b) (4) - 28-Day Repeated Dose Oral Toxicity and Toxicokinetic

Day numbers relative to Start Date

Sex: Male

	Group 1 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 25 mg/kg/day	Group 4 50 mg/kg/day
Comatose				
Number of Observations	.	.	.	97
Number of Animals	.	.	.	10*
Days from - to	.	.	.	1 12
Decreased Motor Activity				
Number of Observations	.	.	.	8
Number of Animals	.	.	.	8*
Days from - to	.	.	.	13 13
Lethargic				
Number of Observations	.	.	80	69
Number of Animals	.	.	10*	10*
Days from - to	.	.	1 12	8 20
Prostrate				
Number of Observations	.	.	.	97
Number of Animals	.	.	.	10*
Days from - to	.	.	.	1 12
Labored/Dyspnea				
Number of Observations	.	.	.	97
Number of Animals	.	.	.	10*
Days from - to	.	.	.	1 12

* p ≤ 0.05 (Dunnett's t-Test) when compared to Group 1.

TABLE 7 – SUMMARY OF CLINICAL OBSERVATION INCIDENCE – CAGESIDE (28—DAY CONTINUED)

Clinical Observations - Clinical Signs by Group

Study : AB37CC.2G3R (b) (4) - 28-Day Repeated Dose Oral Toxicity and Toxicokinetic

Day numbers relative to Start Date

Sex: Female

	Group 1 0 mg/kg/day	Group 2 10 mg/kg/day	Group 3 25 mg/kg/day	Group 4 50 mg/kg/day
Comatose				
Number of Observations	.	.	.	73
Number of Animals	.	.	.	10*
Days from - to	.	.	.	1 11
Decreased Motor Activity				
Number of Observations	.	.	.	11
Number of Animals	.	.	.	10*
Days from - to	.	.	.	12 13
Lethargic				
Number of Observations	.	.	82	93
Number of Animals	.	.	10*	10*
Days from - to	.	.	1 11	6 20
Prostrate				
Number of Observations	.	.	.	73
Number of Animals	.	.	.	10*
Days from - to	.	.	.	1 11
Labored/Dyspnea				
Number of Observations	.	.	.	73
Number of Animals	.	.	.	10*
Days from - to	.	.	.	1 11

* p ≤ 0.05 (Dunnett's t-Test) when compared to Group 1.

Body weights: *Days 1, 8, 15, 22, 28 (pre-fasted weight) & 29 (terminal fasted weight)*
Mean body weights appeared relatively unaffected; however, the sponsor indicated there was a drug-related trend for slightly reduced body weight gains that was more pronounced in the males. See the sponsor's summary table, below.

TABLE 10 – SUMMARY OF BODY WEIGHT GAIN (28-DAY)

Body Weight Gain (Grams)

Group Sex	Base Weight Day 1	From: To:	Day numbers relative to Start Date 1 8	8 15	15 22	22 28	Abs Gain 1 28
1m	26.00	Mean	0.07	0.00	0.76	-0.34	0.49
	1.87	S.D.	0.72	0.55	0.93	0.31	0.91
	10	N	10	10	10	10	10
2m	26.52	Mean	-0.67	0.12	0.51	-0.32	-0.36
	1.43	S.D.	0.49	0.58	0.39	0.50	1.00
	10	N	10	10	10	10	10
3m	25.88	Mean	-0.47	-0.23	0.40	0.05	-0.25
	1.02	S.D.	0.55	0.76	0.76	0.62	0.79
	10	N	10	10	10	10	10
4m	25.92	Mean	-0.89*	0.49	0.20	-0.31	-0.51
	1.49	S.D.	0.93	0.31	0.51	0.68	0.91
	10	N	10	10	10	10	10
1f	20.82	Mean	-0.01	0.53	0.37	0.58	1.47
	1.07	S.D.	0.87	0.88	0.44	0.47	0.78
	10	N	10	10	10	10	10
2f	20.42	Mean	-0.06	0.99	0.20	0.76	1.89
	0.89	S.D.	0.52	0.46	0.58	0.57	0.83
	10	N	10	10	10	10	10
3f	19.93	Mean	0.19	0.01	-0.06	1.09	1.23
	1.16	S.D.	0.60	0.74	0.69	0.80	1.63
	10	N	10	10	10	10	10
4f	20.46	Mean	0.39	-0.27*	0.20	0.91	1.23
	1.05	S.D.	0.54	0.61	0.60	0.36	0.73
	10	N	10	10	10	10	10

Abs Gain = absolute body weight gain between base period and end of the analysis period

* p ≤ 0.05 (Dunnett's t-test) when compared to Group 1.

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day Group 4 - 50 mg/kg/day

Food consumption: *Days 1, 8, 15, 22 & 28*

There was an apparent drug-related trend for decreased food consumption that was more pronounced in the males. Total food consumption in the HDM was 7.7% less than controls. Total food consumption in HDF did not appear affected, but weekly consumption values indicated a transient decrease (13.5%, 3.4% and 2.8% less than controls in weeks 2, 3 and 4). See the sponsor's summary data, below.

TABLE 11 - SUMMARY OF FOOD CONSUMPTION (28-DAY STUDY)

Day numbers relative to Start Date

Group Sex	From: To:	1 8	8 15	15 22	22 28	Total 1 28
1m	Mean	5.31	4.49	4.47	4.03	124.1
	S.D.	1.25	0.81	1.24	0.93	16.8
	N	10	10	10	10	10
2m	Mean	5.29	4.96	5.33	4.70	116.8
	S.D.	1.98	0.76	1.55	0.88	38.1
	N	8	10	8	8	10
3m	Mean	5.71	4.89	5.12	4.41	116.2
	S.D.	1.37	1.36	1.32	0.72	23.1
	N	8	9	9	8	10
4m	Mean	5.23	4.31	3.88	3.79	114.5
	S.D.	2.16	0.93	1.14	1.08	22.9
	N	10	10	10	9	10
1f	Mean	4.34	4.59	5.05	4.64	92.0
	S.D.	0.39	1.62	1.41	1.04	33.1
	N	6	7	9	7	10
2f	Mean	5.47	5.10	4.45	4.54	125.4*
	S.D.	1.32	1.51	1.03	1.71	24.5
	N	9	10	9	10	10
3f	Mean	5.08	5.67	4.72	3.73	98.4
	S.D.	0.72	1.06	1.26	0.89	31.7
	N	8	7	8	7	10
4f	Mean	4.94	3.97	4.88	4.51	114.7
	S.D.	0.93	1.24	1.18	1.64	19.8
	N	10	9	9	9	10

NOTE: In Groups with N values less then 10, there were animals with invalid food consumption values that were excluded from weekly data as per (b) (4) SOPs See individual food consumption data in Appendix C.

*p≤ 0.05 (Dunnett's t-test) when compared to Group 1.
Arithmetic Mean Values Presented
Food Consumption Units are g/animal/day. Total = Total consumption for the whole period (g/animal)
Nominal Dose: Group 1 - 0 mg/kg/day Group 2 - 10 mg/kg/day Group 3 - 25 mg/kg/day Group 4 - 50 mg/kg/day

Hematology: *At termination (Day 29), retro-orbital sinus bleed*
Main study animals were fasted overnight, anesthetized with CO_2/O_2 and bled from the retro-orbital sinus for clinical pathology samples on D29. Whole blood samples for hematology from up to 5 animals/sex/group were prepared.

Any changes were considered incidental by the Clinical Pathologist. WBC appeared decreased in MDM (43%) and HDM (47%). Segmented neutrophils (up to 30%), lymphocytes (up to 55%) and monocytes (up to 86%) appeared decreased in MD and HD M. WBC appeared decreased in HDF (22%), but were variable.

Clinical chemistry: *At termination (Day 29), retro-orbital sinus bleed*
Main study animals were fasted overnight, anesthetized with CO_2/O_2 and bled from the retro-orbital sinus for clinical pathology samples on D29. Serum samples for clinical chemistry from up to 5 animals/sex/group were prepared.

Any changes were considered incidental by the Clinical Pathologist. Total bilirubin showed a dose-related slight reduction in HDF (as much as 31%).

Gross pathology: *At termination (Day 29)*
There were no gross lesions in this study.

Organ weights: *At termination (Day 29)*
The sponsor recorded no organ weight changes in this study; there were no statistically significant changes. Absolute and relative adrenal weights appeared to show a dose-

related decrease, with HDM reduced 23% and 20%, respectively). Absolute and relative adrenal weights appeared slightly reduced in HDF (13% and 10%, respectively). Absolute and relative ovary weights appeared slightly reduced in HDF (14% and 12%, respectively).

Histopathology: Adequate Battery: yes (X), no ( )
Peer review: yes ( ), no (X)
Tissues from the vehicle control and HD group were embedded in paraffin and sectioned at ≤6 microns, stained with H&E and evaluated microscopically.

The sponsor recorded no histopathological changes in this study. One HDF showed minimal, focal, granulomatous inflammatory foreign body reaction in the nasal cavity.

Toxicokinetics: *from 3/sex/dose/time point, D28 at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 & 24 hours postdose*
Plasma concentrations for doxepin and nordoxepin were variable. Absorption was rapid, and terminal half-life increased with dose. C_{max} and AUC showed greater than dose-proportional increases. See the sponsor's summary table, below.

Summary of Doxepin and Nordoxepin Plasma Toxicokinetic Parameters on Day 28 of Daily Oral Administration of Doxepin HCl to Mice

Group	Dosage (mg/kg/day)[a]	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Doxepin								
2	10	M	46.3	0.25	4	55.0	58.4	1.0
		F	25.8	0.25	3	33.4	36.8	0.93
3	25	M	165	0.25	12	267	287	5.7
		F	166	0.25	8	174	183	2.4
4	50	M	406	0.25	24	735	743	4.0
		F	464	0.25	24	655	665	7.1
Nordoxepin								
2	10	M	55.0	0.25	6	90.0	92.8	1.1
		F	13.9	0.5	3	19.5	22.8	0.98
3	25	M	234	0.25	12	545	614	6.2
		F	63.1	0.25	8	121	NE	NE
4	50	M	658	0.5	24	3330	3410	4.1
		F	295	0.5	24	751	787	7.8

NE: Not estimated, due to insufficient characterization of terminal phase.
a: Doxepin HCl dosage.

Other:
Doxepin HCl in sterile water was found to be stable for 18 days when stored at 2-8°C, protected from light. For the first dose formulation analysis, the concentrations found for all formulations were 97.8-106.7% of nominal. For the second dose formulation analysis, the concentrations found for all formulations were 98.5-102.0% of nominal. No test article was detected in the vehicle control in either analysis.

Histopathology inventory

Study	D0104	D0105	D0110	D0112
Species	Rat	Rabbit	Mouse CON&HD	Mouse Tg rasH2 All[$]
Adrenals	*	*	X	X*
Aorta			X	X
Bone Marrow smear			X	X
Bone (femur)			X	X
Brain	*	*	X*	X*
Cecum			X	X
Cervix				
Colon			X	X
Duodenum			X	X
Epididymis	*	*	X	X
Esophagus			X	X
Eye			X	X
Fallopian tube				
Gall bladder			X	X
Gross lesions			X	X
Harderian gland			X	X
Heart	*	*	X*	X*
Ileum			X	X
Injection site				
Jejunum			X	X
Kidneys	*	*	X*	X*
Lachrymal gland				
Larynx				
Liver	*	*	X*	X*
Lungs	*	*	X	X
Lymph nodes, cervical				
Lymph nodes mandibular			X	X
Lymph nodes, mesenteric			X	X
Mammary Gland			X	X
Nasal cavity			X	X
Optic nerves				
Ovaries	*	*	X*	X*
Pancreas			X	X
Parathyroid	*	*	X	X
Peripheral nerve				
Pharynx				
Pituitary	*	*	X	X
Prostate	*		X	X
Rectum			X	X
Salivary gland	*	*	X	X
Sciatic nerve			X	X
Seminal vesicles			X	X
Skeletal muscle			X	X

Skin			X	
Spinal cord			X	X
Spleen	*	*	X	X*
Sternum			X	X
Stomach			X	X
Testes	*	*	X*	X*
Thymus	*		X	X
Thyroid	*	*	X	X
Tongue				
Trachea			X	X
Urinary bladder			X	X
Uterus			X	X
Vagina			X	X
Zymbal gland				

X, histopathology performed
*, organ weight obtained

[$]Note: D0111: Only lungs, spleen and gross lesions from urethane-treated positive control

2.6.6.4 Genetic toxicology

Genetic toxicology studies have been reviewed in detail previously (P/T review dated 2/2/07 for I67,162 submissions N046 & N048, dated 9/15/06 and 2/2/07finalized study reports were submitted SN054, dated4/2/07). Also see advice responses to SN048 and SN052, dated 2/12/07.

The 26-week oral carcinogenicity transgenic (Tg.rasH2) mouse protocol was submitted as a special protocol for concurrence by the ExecCAC (SN055, dated 4/26/07), but was denied because the study was already ongoing. The protocol for the 2-yr rat carcinogenicity bioassay was submitted for ExecCAC concurrence (SN057, dated 6/19/07); the ExecCAC meeting was held 7/31/07 and the meeting minutes (dated 8/1/07) are appended to this review.

2.6.6.5 Carcinogenicity

Study title: SP-D0111: 26-WEEK REPEATED DOSE ORAL CARCINOGENICITY STUDY IN Tg.rasH2 MICE

Key study findings:

- **Non-statistically significant increases in nasal cavity, lung and spleen tumors**
- **Nasal lesions and tumors are not a background lesion in this strain**
- **Lung and spleen tumors are spontaneous tumors in this strain**

Adequacy of the carcinogenicity study and appropriateness of the test model:
The model and study were considered adequate. Please see the appended ECAC meeting minutes for the final study review (Appendix 1). The special protocol assessment (protocol and dose selection) was not reviewed for ECAC concurrence because the study was ongoing at the time of submission.

Evaluation of tumor findings:
According to the FDA statistical reviewer (Dr. Rahman, see review dated 6/30/08), no significant positive dose-response relationships in tumor incidence were detected in males or females. Doxepin was not considered tumorigenic.

Study no.:	AB37CC.7G8R (b) (4)
Volume #, and page #:	Electronic submission, 877 pages
Conducting laboratory and location:	(b) (4)
Date of study initiation:	April 16, 2007
GLP compliance:	Yes, pg 2
QA report: yes ( ) no ( )	Pgs. 3-6
Drug, lot #, and % purity:	Doxepin HCl, Lot 3045911, 100.0% (b) (4) E-isomer; (b) (4) Z-isomer In Sterile Water for Injection, USP Grade
CAC concurrence:	Yes; ECAC Meeting held11/4/08 and Minutes dated 11/6/08

Methods

Doses:	0, 25, 50, 75 and 100 mg/kg doxepin HCl
Basis of dose selection:	MTD in 5- & 28-day toxicity study in CByB6F1 Hybrid mice
Positive Control:	Urethane (in sterile saline), 1000 mg/kg
Species/strain:	Tg.rasH2 mice, [CB6F1Jic- TgrasH2@Tac] (hemizygous C57BL/6 x BALB/cBy knock-in mouse carrying the human prototype c-Ha-ras gene with its own promoter/ enhancer) (b) (4)
Number/sex/group (main study):	(see sponsor's summary table, below)

Table 6 – Experimental Design for Carcinogenicity and Toxicokinetics of Doxepin HCl in Mice

Dose Group and Treatment	Number of Animals			
	Main Study (Tg.rasH2)		TK Study (CByB6F1)	
	Male	Female	Male	Female
Group 1 Vehicle Control	25	25	5	5
Group 2 Positive Control, urethane*	25	25	-	-
Group 3 Low dose (25 mg/kg/day)	25	25	35	35
Group 4 Middle dose (50 mg/kg/day)	25	25	35	35
Group 5 Middle High dose (75 mg/kg/day)	25	25	35	35
Group 6 High dose (100 mg/kg/day)	25	25	35	35
Total	150	150	145	145

*The Positive Control animals were administered a total of 3 intraperitoneal injections of urethane (1000 mg/kg) on Days 1, 3, and 5.

Route, formulation, volume:	PO, by oral gavage, vol.= 10 ml/kg
Frequency of dosing:	doxepin QD for 182 days (for urethane POS CON, 3 IP injection)
Satellite groups for toxicokinetics:	CByB6F1 Hybrid mice (Tg.rasH2 non-transgenic littermates) TK: (b) (4)
Age:	Main: 8-9 weeks; TK: 9-10 weeks
Animal housing:	Individually housed on study Polycarbonate cages with Sani-Chip Hardwood bedding (P.J. Murphy Forest Products, Montville, NJ)
Dietary parameters:	*ad libitum* diet & water
Drug stability/homogeneity:	Not performed here- see Other section
Deviations from study protocol:	Study Protocol: 1) animals were single-housed during quarantine because they had been mixed at receipt and had to be genotyped 2) 1HDF was removed from study because she was the incorrect strain 3) labels on tail snip samples were not marked 4) adrenal weight of 1CONM was corrected

5) 1LDTKF had incorrect bleed time listed
6) various housing condition perturbations
Formulation Analysis:
1) stability not assessed within study because previously established
2) Dosing formulation used prior to analysis because initial run failed and was retested

Methodological Notes:
Positive control animals were sacrificed as a group (on D116 and D114 in the males and females, respectively) once signs of toxicity were evident in the majority of animals. This was done to avoid the loss of tissues for histopathologic evaluation due to autolysis. The primary target organs for urethane (used as the positive control article for this study) are lungs and spleen; therefore, the expected urethane-related clinical signs include: rapid and shallow breathing, palpable internal masses, and edema.]

Observation times & Results
Mortality: *Twice daily*
In the main study, early mortality (found dead or sacrificed moribund) was observed in a few control and doxepin-treated animals, and in a number of positive controls. FDA statistical review (see review by Dr. Rahman) indicated that mortality was not significantly increased in doxepin-treated groups and was significantly increased in the urethane-treated groups. Early mortality was observed in 3/25 CONF, 1/25 LDM, 1/25 LDF, 1/25 MDF, 2/25 MHDF, 3/25 HDM. A slight increase in early mortality was suggested in HDM compared to CONM, and appeared to be supported by the early mortalities demonstrated in the HDTK M. In the TK portion of the study, 1/35 MDF (D102), 2/35 MHDM, and 7/35 HDM died early. No increase in mortality was apparent in females. See the sponsor's summary tables 8 and 9, next pages. The cause of death, if known, is also provided for the main study animals. There was no evidence of gavage error in any of the animals that died early.

Table 8 - SUMMARY OF MORTALITY (MAIN STUDY Tg.rasH2 ANIMALS)

MALES

		COD	Group 1	Group 2*	Group 3	Group 4	Group 5	Group 6
Day 6	Found Dead	U	-	1/25	-	-	-	-
Day 63	Found Dead	P	-	1/25	-	-	-	-
Day 71	Found Dead	U	-	-	-	-	-	1/25
Day 75	Found Dead	P	-	1/25	-	-	-	-
Day 84	Found Dead	P	-	2/25	-	-	-	-
Day 96	Found Dead	P	-	1/25	-	-	-	-
Day 98	Found Dead	P	-	1/25	-	-	-	-
Day 103	Found Dead	P	-	1/25	-	-	-	-
Day 109	Found Dead	U	-	-	-	-	-	1/25
Day 114	Found Dead	P	-	1/25	-	-	-	-
Day 115	Found Dead	P	-	1/25	-	-	-	-
Day 141	Found Dead	U	-	-	1/25	-	-	-
Day 155	Found Dead	Hs	-	-	-	-	-	1/25
Day 116	Scheduled Sacrifice	NA	-	15/25	-	-	-	-
Day 183 or 184	Terminal Sacrifice	NA	25/25	-	24/25	25/25	25/25	22/25
	TOTAL:		25/25	25/25	25/25	25/25	25/25	25/25

FEMALES

		COD	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Day 17	Found Dead	U	-	-	-	-	-	1/25
Day 91	Found Dead	U	-	-	1/25	-	-	-
Day 95	Found Dead	P	-	1/25	-	-	-	-
Day 96	Found Dead	P	-	2/25	-	-	-	-
Day 100	Other[1]	NA	-	-	-	-	-	1/25
Day 102	Found Dead	P	-	1/25	-	-	-	-
Day 104	Found Dead	P	-	1/25	-	-	-	-
Day 105	Found Dead	P	-	1/25	-	-	-	-
Day 120	Moribund Sacrifice	Pv	-	-	-	-	-	1/25
Day 125	Found Dead	U	1/25	-	-	-	-	-
Day 126	Found Dead	U	-	-	-	-	1/25	-
Day 156	Found Dead	U	-	-	-	-	1/25	-
Day 174	Found Dead	U	-	-	-	1/25	-	-
Day 176	Found Dead	L	1/25	-	-	-	-	-
Day 182	Found Dead	HeAl	1/25	-	-	-	-	-
Day 114	Scheduled Sacrifice	NA	-	19/25	-	-	-	-
Day 183 or 184	Terminal Sacrifice	NA	22/25	-	24/25	24/25	23/25	22/25
	TOTAL:		25/25	25/25	25/25	25/25	25/25	25/25

[1] Genotyping results revealed that Group 6 female 6276 was the wrong strain, therefore this animal was sacrificed and all associated data was removed from the study.

Note: Represents the number of animals affected / the number of animals started on test.

* $p < 0.05$ (Fisher's Exact Test) compared to Group 1.

COD = Cause of Death NA = Not Applicable

U = Undetermined L = Lymphoma (spleen, liver) HeAl = Hemangiosarcoma (ear), Adenoma, lung

P =Positive Control-related death Hs = Hemangiosarcoma (spleen) Pv = Papilloma (vagina)

Nominal Dose: Group 1 - 0 mg/kg/day Group 2 – Positive Control Group 3 - 25 mg/kg/day

Group 4 - 50 mg/kg/day Group 5 - 75 mg/kg/day Group 6 - 100 mg/kg/day

Table 9 - SUMMARY OF MORTALITY (TK STUDY CByB6F1 ANIMALS)

MALES

		Group 1	Group 3	Group 4	Group 5	Group 6
Day 2	Found Dead	-	-	-	-	1/35
Day 117	Found Dead	-	-	-	-	1/35
Day 123	Found Dead	-	-	-	1/35	-
Day 137	Found Dead	-	-	-	-	1/35
Day 150	Found Dead	-	-	-	1/35	1/35
Day 152	Found Dead	-	-	-	-	1/35
Day 175	Found Dead	-	-	-	-	2/35
Day 177 or 178	Scheduled Sacrifice	5/5	35/35	35/35	33/35	28/35
	TOTAL:	5/5	35/35	35/35	35/35	35/35

FEMALES

		Group 1	Group 3	Group 4	Group 5	Group 6
Day 102	Found Dead	-	-	1/35	-	-
Day 177 or 178	Scheduled Sacrifice	5/5	35/35	34/35	35/35	35/35
	TOTAL:	5/5	35/35	35/35	35/35	35/35

Note: Represents the number of animals affected / the number of animals started on test.
Statistical analysis (Fisher's Exact Test) did not reveal any significant differences when Groups 3 – 6 were compared to Group 1.
COD = Cause of Death
Nominal Dose: Group 1 - 0 mg/kg/day Group 2 – Positive Control Group 3 - 25 mg/kg/day
Group 4 - 50 mg/kg/day Group 5 - 75 mg/kg/day Group 6 - 100 mg/kg/day

Clinical signs: *Weekly within 2 hrs postdose*

Drug-related clinical observations noted during cageside observation included: coma, decreased motor activity and lethargy. Coma was observed only through day 8 at HD, but many of the other signs persisted. Labored breathing/dyspnea was observed during cageside observations in MD, MHD and HD males, but not females; however, rapid and/or shallow breathing was observed in both sexes during hands-on observations. One HDM was observed to show compulsive licking on D1. Convulsions were observed in 1MDM on 2 occasions (D64 & D176). Muscle twitch as observed in 1 HDF on D162. One HDF was observed to show rectal prolapse from D85-D120. Individual animals were noted to be hyperactive or hyper-reactive, but a dose relationship was unclear. See the reviewer's summary table for details regarding incidence and timing.

Cageside Observations

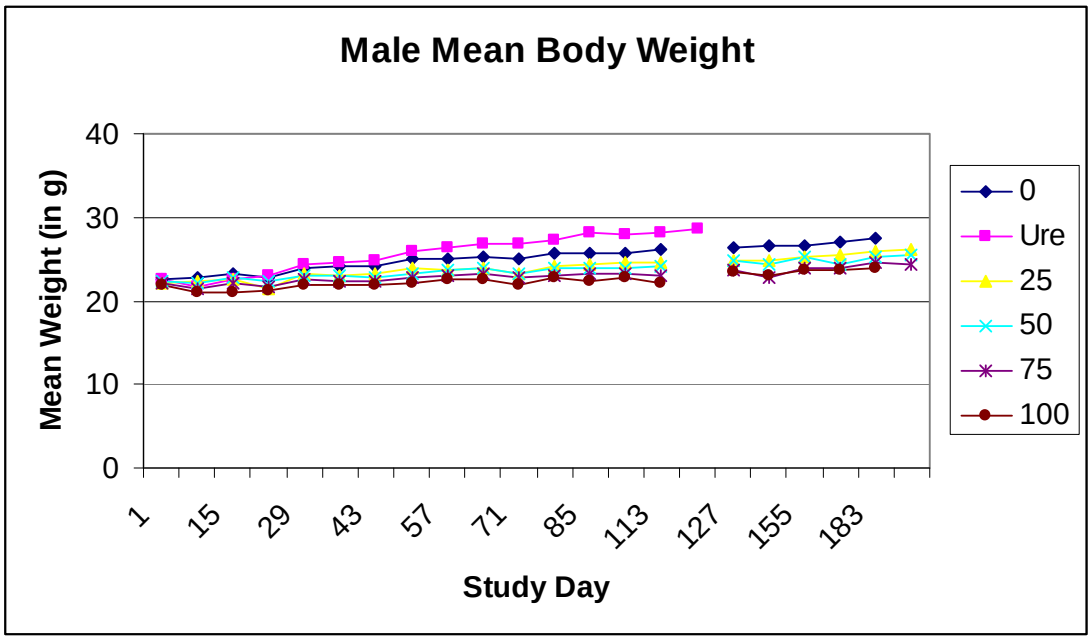
	MALES						FEMALES					
	0	POS	LD	MD	MHD	HD	0	POS	LD	MD	MHD	HD
Comatose												
# obs				23	20	28					8	27
# animals				**23***	**20***	**17***					**8***	**24***
Days from-to				1	1	1 8					1	1 8
Lethargic												
# obs		25		19	141	79		26		68	157	49
# animals		**25***		**14***	**25***	**23***		**25***		**25***	**25***	**19***
Days from-to		1		1 183	1 176	1 176		1 8		1 141	1 141	8 148
Dec Activity												
# obs				1		222					32	296
# animals				**1**		**25***					**13***	**23***
Days from-to				176		36 176					106 134	43 141
Labored / Dyspnea												
# obs				7	4	7						
# animals				**5**	**4**	**7***						
Days from-to				176 183	176	176						
Rapid / Shallow												
# obs			3	12	2	3						
# animals			**2**	**3**	**1**	**3**						
Days from-to			176 183	134 183	176 183	176						

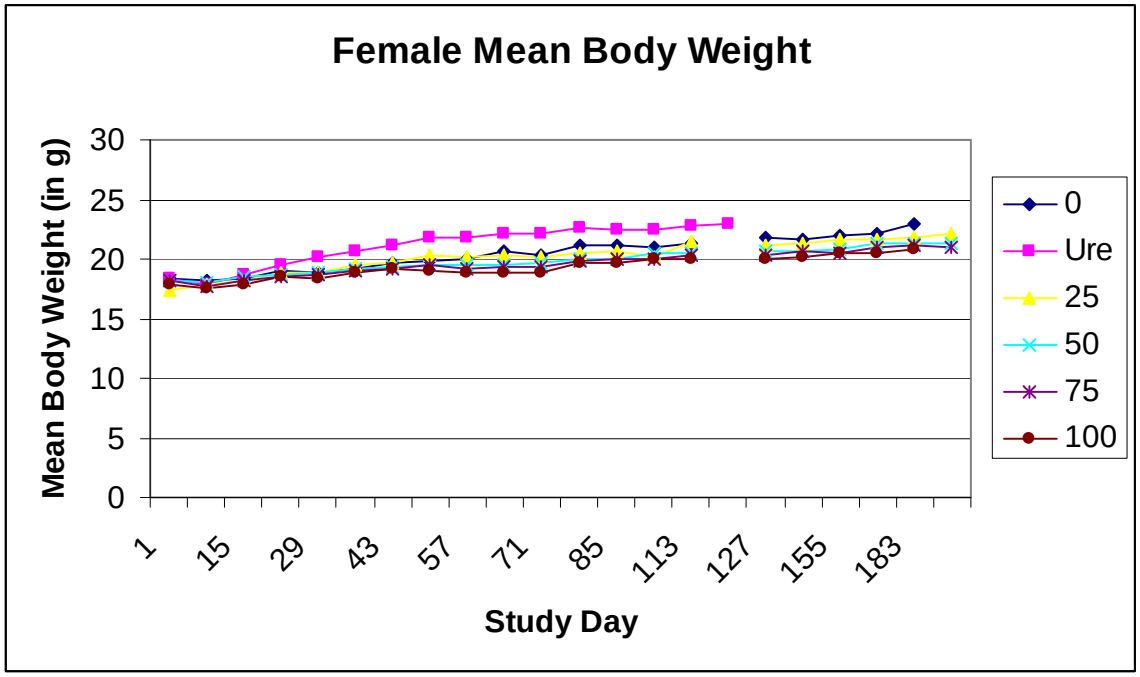
Hands-on Observations

	MALES						FEMALES					
	0	POS	LD	MD	MHD	HD	0	POS	LD	MD	MHD	HD
Rapid & Shallow												
# obs		118	1	20	11	56	16	121	10	26	24	82
# animals		**21***	**1**	**10***	**9***	**21***	**1**	**23***	**2**	**15***	**16***	**20***
Days from-to		78 116	184	64 184	176 184	127 183	78 183	71 114	134 184	183 184	183 184	141 183
Labored / Dyspnea												
# obs		1		3		2		1				
# animals		**1**		**1**		**1**		**1**				
Days from-to		78		176 184		176 183		99				
Mass												
# obs								9				16
# animals								**2**				**1**
Days from-to								78 114				15 120
Discharge												
# obs							1		2	3		22
# animals							**1**		**1**	**1**		**2**
Days from-to							183		183 184	176 184		15 176

* $p < 0.05$, Fisher's Exact test, compared to CON

Body weights: *Weekly through Week 13 and biweekly thereafter*
Group mean body weights were slightly but statistically significantly and dose-dependently reduced in all doxepin-treated groups, compared to controls. Mean body weight reductions were significant in MHDM and HDM beginning week 2, and in LDM and MDM beginning week 6. The decrease in group mean body weights in the LD, MD, MHD and HD male doxepin-treated groups on Day 183 were 6.2%, 8.4%, 10.9% and 13.2% less than the vehicle control. The statistically significant reductions in group mean body weight in females were not as consistent as those observed in males, but were generally reduced after week 10. The HDF, MHDF, MDF and LDF generally showed reductions beginning approximately week 8, week 9, week 10 and week 27 (respectively). The decrease in group mean body weights in the LD, MD, MHD and HD female doxepin-treated groups on Day 183 were 5.1%, 7.4%, 8.1% and 9.3% less than the vehicle control. See the reviewer's figures, below.





Weekly body weight gain in the doxepin-treated groups in both sexes was sporadically statistically significantly lower than the vehicle control group; however, the absolute body weight gain (from Day 1 to Day 183) was statistically significantly and dose-dependently decreased in MD, MHD and HD in both sexes when compared to vehicle controls. LD animals also showed a decrease in absolute body weight gain [nss]. Group mean absolute weight gain decreases ranged from 21.3% to 56.9% less than the vehicle control in doxepin-treated males, and from 5.8% to 38.1% less than the vehicle control in the doxepin-treated females.

Food consumption:
There were few significant differences in weekly group mean food consumption in the doxepin treatment groups compared to the vehicle control group; however, total food consumption (from Day 1 to Day 183) was statistically significantly and dose-dependently decreased in MDM, MHDM, MHDF, HDM and HDF compared to vehicle controls. Although the differences were not statistically significant, total food consumption in the LDM, LDF and MDF were also lower than that of the vehicle control group. Group mean total food consumption decreases ranged from 3.4% to 14.4% lower than the vehicle control group in the doxepin-treated males, and ranged from 5.7% to 20.7% lower than the vehicle control in the doxepin-treated females.

Organ Weight: *Terminal sacrifice*
The sponsor identified no statistically significant differences in absolute or relative organ weights in doxepin-treated groups of either sex, compared to the vehicle control groups. In males, there appeared to be slight reductions in spleen weight (absolute and relative, HD, 23%) and kidney weights (absolute & relative, dose-related, up to 13%). In females, there appeared to be slight reductions in ovary weights (absolute and relative, dose-

related, up to 19%), spleen weights (absolute and relative, ≥MD, up to 22%), kidney weights (absolute and relative, ≥MD, up to 13%) and heart weights (absolute and relative, dose-related, up to 11%).

Gross pathology: *Terminal sacrifice & early mortalities as needed*
In the doxepin-treated groups, nodules or masses were observed in the spleens of 0/25 CONM, 4/25 LDM, 3/25 MHDM, 3/25 HDM and 0/25 CONF, 1/25 LDF and 3/24 HDF. These lesions were considered to be doxepin-related. Individual animals showed nodules in the lung, but the incidence did not appear dose-related. One HDF had a ruptured eye. One HDM and 1 MHDF had a firm white nodule in the stomach. Other gross lesions were noted to occur in individual animals, but did not appear drug-related.

In the urethane-treated positive control group, the expected pulmonary and splenic lesions (i.e., nodules) were noted. Red fluid was observed in multiple body cavities. Thymus was enlarged in 2/25 F. A few skin lesions were noted (e.g., alopecia, nodules or masses), as were a few nodules or masses in the stomach. Other lesions were noted to occur in individual animals, but were not clearly urethane-related.

Histopathology: Peer review: yes ( ), no (X) *Terminal sacrifice*
All tissues collected at necropsy from all groups and selected tissues from the positive control animals (lungs and spleen, and any gross lesions) were embedded, sectioned at ≤6 µm, stained with H&E, and evaluated microscopically.

Non-neoplastic:
In the doxepin-treated animals, a number of histopathological alterations were observed; the majority of these alterations were considered spontaneous or incidental by the pathologist.

Microscopic evaluation demonstrated irritation of the nasal cavities. Although nasal cavity lesions were noted in controls as well as doxepin-treated animals, the nature of the irritation was different. In vehicle control animals, the lesion was diagnosed as "an acute inflammatory lesion of the submucosal glands of minimal intensity." The lesion was described as a few scattered submucosal glands in the nasal cavities of control mice that contained necrotic debris and degenerate neutrophils; the incidence of this lesion was 7/25 and 13/25 in the male and female control mice, respectively. The pathologist considered the alteration a background or spontaneous lesion; notably, the pathologist also stated that nasal cavity lesions have not been previously observed in other examinations of vehicle-treated rasH2 mice at the (b) (4) test facility. The cause of the development of the lesion is unknown. Furthermore, chronic-active inflammatory, hyperplastic and neoplastic lesions were noted in the nasal cavities of the doxepin-treated groups in both sexes; these more severe lesions were not noted in the vehicle control groups. See the sponsor's summary table 25 for details. The pathologist indicated that the term chronic-active was used to describe both the chronicity as well as the acuteness of the lesion. According to the pathologist, "acuteness" was characterized by one of the following features: a) infiltration of degenerate neutrophils in the submucosa, b) erosion of the mucosa, c) accumulation of sero-mucous fluid in the nasal cavity or d) infiltration of necrotic debris, sloughed cells, eosinophilic crystals and/or degenerate neutrophils in

the sero-mucous fluid, and "chronicity" was characterized by one of the several changes, including: a) attenuation of the epithelium, b) squamous metaplasia, c) subsequent hyperplasia of squamous cells or d) hyperplasia of the submucosal glands. The pathologist stated that not all of the features indicative of acuteness or chronicity were present simultaneously. Although the finding did not show a clear dose-response, the sponsor considered the development of inflammation, hyperplasia and metaplasia of the nasal cavities doxepin-related.

Other lesions noted included: minimal proteinosis in the kidney (1MDM, 1MHDM, 1MHDF, 1HDM & 1HDF), hyperplasia of the non-glandular stomach (in several individual animals of doxepin treated groups), moderate atypical histiocytic hyperplasia of the thymus (1LDF, 1MDF, 1MHDF), mild myeloid hyperplasia of the bone marrow (1HDM & 1MHDF) and submucosal vascular proliferation of the urinary bladder (1HDF). Inadequate tissue for assessment of pituitary gland was observed in single animals in many groups.

Neoplastic:
In the doxepin-treated animals, possibly drug-related neoplastic alterations were observed in the nasal cavity, the lung and the spleen.

In addition to the chronic-active inflammation noted in the nasal cavity of doxepin-treated animals, hyperplastic and neoplastic lesions (adenocarcinomas) were noted in the nasal cavities in both sexes that were not noted in the vehicle control groups. Adenocarcinomas were noted in LDM, LDF, MDF and MHDF. See the sponsor's summary table 25, next page, for details. The sponsor hypothesized that the initial local irritation may have led to chronic-active inflammation, subsequently to hyperplasia, and eventually to carcinoma; however, this study was not designed to assess for such a progression. The sponsor considered the development of carcinomas in the nasal cavities noteworthy, as nasal cavity adenocarcinoma did not occur in any vehicle control animal in either sex and is not a spontaneous tumor of Tg.rasH2 mice. However, the sponsor indicated that the development of nasal cavity adenocarcinomas was not "dose- or exposure-related," and that the incidence was not statistically significantly different compared to the vehicle control.

Table 25 - Incidence of Microscopic Nasal Cavity Lesions in rasH2 Mice

	Vehicle	Doxepin 25 mg/kg/day	Doxepin 50 mg/kg/day	Doxepin 75 mg/kg/day	Doxepin 100 mg/kg/day
Males					
Number Examined	25	25	25	25	25
Chronic-Active Inflammation					
Minimal	0	16	14	18	18
Mild	0	0	2	2	6
Moderate	0	0	6	4	0
Submucosal Gland Hyperplasia					
Minimal	0	15	7	8	16
Mild	0	8	16	14	7
Moderate	0	0	1	2	2
Squamous Metaplasia with Hyperplasia					
Minimal	0	21	19	23	23
Mild	0	2	2	1	2
Moderate	0	0	3	0	0
Adenocarcinoma					
	0	2	0	0	0
Females					
Number Examined	25	25	25	25	24
Chronic-Active Inflammation					
Minimal	1	12	14	12	5
Mild	0	1	2	9	9
Moderate	0	0	4	3	8
Submucosal Gland Hyperplasia					
Minimal	0	15	8	6	9
Mild	0	5	10	13	11
Moderate	0	0	4	5	2
Squamous Metaplasia with Hyperplasia					
Minimal	0	13	15	20	19
Mild	0	4	5	3	2
Moderate	0	0	2	1	1
Adenocarcinoma					
	0	1	2	1	0

Note: Multiple adenomas and/or carcinomas were present in the same animal in urethane treated mice
* $p<0.05$ (Fisher's Exact Test) compared to vehicle controls (Group 1).

Doxepin-treated animals were observed to have adenomas and carcinomas of the lung. Pulmonary tumors are spontaneous tumors known to occur in this strain of mouse. The sponsor indicated that statistical analysis revealed no significant increase in incidence in doxepin-treated groups and no relationship to dose or exposure; the FDA statistical reviewer concurred. The incidences of single and/or multiple pulmonary adenomas were similar across the vehicle and doxepin-treated groups. Notably, pulmonary carcinomas

were observed in 1MDM and 1MHDM, but were not noted in vehicle control groups of either sex. Based on the overall low and similar incidence of pulmonary tumors in the vehicle- and doxepin-treated groups, as well as the lack of dose dependence, these tumors were not considered drug-related by the sponsor. See the sponsor's summary table 23, below.

Table 23 - Incidence of Pulmonary Tumors in rasH2 Mice

	Vehicle	Urethane 1000 mg/kg/day	Doxepin 25 mg/kg/day	Doxepin 50 mg/kg/day	Doxepin 75 mg/kg/day	Doxepin 100 mg/kg/day
Males						
Number Examined	25	25	25	25	25	25
Adenoma Single	3	0	3	3	4	0
Adenoma Multiple	1	24	0	1	0	0
Carcinoma	0	8	0	1	1	0
Number of Males with at Least 1 Type of Lung Tumor	4	25*	3	5	5	0
Females						
Number Examined	25	25	25	25	25	24
Adenoma Single	3	0	1	2	1	0
Adenoma Multiple	0	25	0	0	0	0
Carcinoma	0	24	0	0	0	0
Number of Females with at Least 1 Type of Lung Tumor	3	25*	1	2	1	0
Both Sexes Combined						
Number of Animals with at Least One Type of Tumor	7	50	4	7	6	0

Note: Multiple adenomas and/or carcinomas were present in the same animal in urethane treated mice
* $p<0.05$ (Fisher's Exact Test) compared to vehicle controls (Group 1).

Splenic hemangiosarcomas were observed in a few animals in most doxepin-treated groups (see the sponsor's summary table 24, following). The sponsor stated that although splenic hemangiosarcomas were not observed in this study in the vehicle control group of either sex, previous studies conducted with these mice using similar designs at the (b) (4) test facility have demonstrated splenic hemangiosarcomas in approximately 3% of male and 5% of female controls. Notably, and as discussed by the FDA statistical reviewer, the incidences observed in this study ranged from 0-16% in the doxepin-treated males and from 0-8% in doxepin-treated females. However, the sponsor's statistical analysis revealed no significant increase in incidence in the doxepin-treated groups compared to controls, and no relationship to dose. The FDA statistical reviewer concurred.

Table 24 - Incidence of Splenic Hemangiosarcoma Tumors in rasH2 Mice

	Vehicle	Urethane 1000 mg/kg/day	Doxepin 25 mg/kg/day	Doxepin 50 mg/kg/day	Doxepin 75 mg/kg/day	Doxepin 100 mg/kg/day
Males						
Number Examined	25	25	25	25	25	25
Hemangiosarcoma	0	23*	4	0	3	3
Females						
Number Examined	25	25	25	25	25	24
Hemangiosarcoma	0	23*	2	1	0	2

Note: Multiple adenomas and/or carcinomas were present in the same animal in urethane treated mice
* $p<0.05$ (Fisher's Exact Test) compared to vehicle controls (Group 1).

As expected, the urethane-treated positive control mice of both sexes had statistically significantly higher incidences of pulmonary tumors (i.e., multiple adenomas and carcinomas) and splenic hemangiosarcomas when compared with the vehicle control group. Lung tumors were observed in 25/25 mice of both sexes and splenic hemangiosarcomas were observed in 23/25 animals of both sexes. Squamous cell carcinoma of the spleen (1M) and squamous cell carcinoma of the stomach (3M & 2F) were also observed was observed. Carcinoma of the nose was observed in 1M.

Toxicokinetics: *Wk26 on D177/178 at 0, 0.25, 0.5, 1, 2, 3, 4, 6, 8, 12 & 24 hrs postdose;3/sex/dose/time*
Animals were bled from the retro-orbital sinus; plasma was shipped overnight on dry ice. Plasma concentrations for doxepin and nordoxepin were variable (i.e., standard deviations were large). See the sponsor's summary tables, below.

Summary of Doxepin and Nordoxepin Data

Group	Dosage[a] (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Doxepin								
3	25	M	162	0.25	12	265	269	2.3
		F	93.9	0.25	8	103	107	2.2
4	50	M	426	0.25	12	866	905	4.5
		F	179	0.5	12	251	262	3.2
5	75	M	531	0.5	24	1210	1330	10.8
		F	450	0.5	24	635	649	5.3
6	100	M	525	0.5	24	1670	1840	9.0
		F	460	0.5	12	903	922	2.0
Nordoxepin								
3	25	M	272	0.25	12	535	547	2.4
		F	145	0.25	8	120	124	2.2
4	50	M	591	0.25	24	1900	1940	4.7
		F	209	0.5	12	424	455	3.3
5	75	M	1160	0.5	24	5660	6720	10.4
		F	818	0.5	24	2200	2230	3.5
6	100	M	1230	0.5	24	12000	14700	9.7
		F	1040	0.5	24	4060	4080	3.0

a: Daily dosage of Doxepin HCl.

Table 7 Ratios of Toxicokinetic Parameters (Nordoxepin:Doxepin) on Week 26 During Daily Oral (Gavage) Administration of Doxepin HCl to Mice

Parameter[a]	25 mg/kg/day (Group 3)		50 mg/kg/day (Group 4)		75 mg/kg/day (Group 5)		100 mg/kg/day (Group 6)	
	Males	Females	Males	Females	Males	Females	Males	Females
C_{max}	1.68	1.54	1.39	1.17	2.18	1.82	2.34	2.26
AUC_{last}	2.02	1.17	2.19	1.69	4.68	3.46	7.19	4.50

a: Ratios are based on mass.

Other:
All dosing vials were stored at 2-8°C. Fresh dosing formulations were prepared weekly throughout the course of the study. All formulations used for dosing were found to be within ±10% of their target concentrations (ranging from 94.8-109.7%), and used within the established stability time period. At concentrations of 1 and 5 mg/ml, doxepin HCl in sterile water was found to be stable for at least 18 days when stored at 2-8°C protected from light. Additionally, another study found doxepin HCl in sterile water to be stable for 15 days at room temperature and refrigerated for doses up to 119 mg/ml. No test article was detected in the vehicle control tested on any date.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: *SP-D0106: STUDY FOR EFFECTS ON FERTILITY AND EARLY EMBRYONIC DEVELOPMENT TO IMPLANTATION IN RATS FOLLOWING ORAL ADMINISTRATION OF DOXEPIN HCl*

Key study findings:

- NOAEL for general toxicity is 30 mg/kg/day
- Overall NOEL for reproductive performance and fertility is 10 mg/kg/day
- Although fertility indices appeared relatively unaffected (except that the copulatory interval was increased at HD); however,
  - Percent sperm motility was decreased and percent abnormal sperm was increased at HD (apparent effect at MD was due to one outlier)
  - Uterine examinations showed adverse effects at MD and/or HD, for numbers of corpora lutea, implantations, viable embryos, and litter size

Study no.:	1288-005
Volume #, and page #:	Electronic submission, 523 pgs.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	September 1, 2006
GLP compliance:	Yes, pg. 2
QA reports: yes (X) no ( )	pg. 8
Drug, lot #, and % purity:	doxepin HCl, Lot 3045911, 100.0% E-isomer= (b) (4); Z-isomer= (b) (4) in distilled water, prepared weekly

Methods

Doses:	0, 10, 30 & 100 mg/kg/day
Species/strain:	Sprague-Dawley rats, CD®[Crl:CD®(SD)] (b) (4) ~6 wks of age & 167-221 g at randomization
Number/sex/group:	25/sex/gp
Route, formulation, volume, and infusion rate:	PO, QD by gavage, 10 ml/kg, Males treated 28D prior to pairing to euthanasia Females treated 14D prior to pairing to GD7
Satellite groups used for toxicokinetics:	12/sex/gp
Study design:	Both sexes treated

Other (significant protocol deviations):

- On several occasions during the study, the detailed clinical observations were conducted outside the protocol-specified window.

- On several occasions during the study, several animals were dosed outside the protocol-specified window of ± 2 hours from the Day 1 dose time.
- On several occasions during the study, estrous cycle determination was not conducted by 10:00 A.M.
- Several blood samples for plasma analyses were collected outside allowable time window.
- A final body weight for one female at 100 mg/kg/day (animal number 3243) was inadvertently not recorded at the time of necropsy.

Results

Mortality: *Twice daily*

Three animals died during the study period (1conM, 1MDM, 1HDM). No macroscopic alterations were noted at necropsy; however, treatment-related clinical findings (i.e., decreased activity and/or low carriage/posture) were observed in the MD and HD animals. The sponsor did not consider the mortalities drug-related, due to the control death and the lack of dose response. All remaining males and females in the main study and TK groups survived to terminal euthanasia.

Clinical signs: *Daily, approximately 1 hr postdose*

Several drug-related, dose-dependent clinical findings were observed in HD males and females, including: decreased activity, salivation, ataxia, circling, low and/or high carriage/posture, lacrimation, splayed limbs, dilated pupils, skin cold to touch, and breathing abnormalities (audible breathing and/or rales). A few of these observations were also seen at MD, but at a much lower incidence. Notably, a few findings were seen with a reverse dose-dependency (e.g., aggressive behavior and hypersensitive to touch in M). See the reviewer's table, next page, for a brief summary.

	MALES				FEMALES			
	0	LD	MD	HD	0	LD	MD	HD
Decreased Activity								
# obs/ # animals	0/0	0/0	25/7	955/25	0/0	0/0	21/10	428/25
Ataxia								
# obs/ # animals	0/0	0/0	0/0	6/2				
Behavior aggressive								
# obs/ # animals	7/2	13/2	0/0	0/0				
Hypersensitive to touch								
# obs/ # animals	34/4	23/2	6/1	0/0	0/0	0/0	0/0	5/1
Righting Reflex Impaired								
# obs/ # animals	0/0	0/0	0/0	1/1				
Circling, Counterclockwise								
# obs/ # animals					0/0	0/0	0/0	1/1
Salivation								
# obs/ # animals	0/0	0/0	0/0	208/23	0/0	0/0	5/3	79/16
Lacrimation								
# obs/ # animals	0/0	0/0	6/5	51/18 54/19	0/0	1/1	18/7	245/23 248/23
Carriage Low								
# obs/ # animals	0/0	0/0	53/11	792/25	0/0	0/0	19/12	328/24
Hindlimbs splayed								
# obs/ # animals	0/0	0/0	5/1	26/5	0/0	0/0	0/0 3/1	24/10
Posture hunched								
# obs/ # animals	0/0	0/0	1/1	1/1	0/0	0/0	30/9	15/5
Skin Cold to Touch								
# obs/ # animals	0/0	0/0	0/0	16/6	0/0	0/0	3/1	19/7
Pupil(s) dilated								
# obs/ # animals	0/0	0/0	0/0	30/10				
Breathing audible								
# obs/ # animals	0/0	0/0	0/0	2/2				
Rales								
# obs/ # animals	0/0	0/0	1/1	6/5	0/0	0/0	0/0	1/1

Body weight: *1st dose & twice weekly throughout cohabitation; Also mated F on GD 0, 4, 7, 10 &13*
A dose-related tendency for reduced body weight and body weight gain was observed; both body weight and body weight gain were reduced at HD. Mean body weights in the HDM were significantly lower (18%) than controls beginning on Day 7 and throughout the treatment period. Likewise, body weight gain was reduced throughout the treatment period ([ss] at several intervals, compared to controls). Mean body weights in HDF showed a tendency to be lower during the pre-mating and gestation period (5% [ss] at several intervals, compared to controls). Lower body weights and body weight gains were observed during the pre-mating and early gestation period.

Table 4 **Summary of Body Weight Values - MALE**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Values g													
Premating	1	198.5	5.60	25	198.3	6.76	25	196.3	5.63	25	198.2	6.31	25
	3	214.2	5.42	25	213.8	6.91	25	211.7	5.96	25	210.8	7.85	25
	7	249.1	7.97	25	248.5	9.79	25	244.3	7.71	25	235.9[b]	11.30	25
	10	278.2	10.57	25	277.0	12.94	25	271.2	11.69	25	263.2[b]	14.84	25
	14	304.4	14.97	25	296.4	17.33	25	294.2	16.16	25	287.4[b]	18.27	25
	17	325.0	20.05	25	320.6	19.39	25	314.8	19.19	25	305.7[b]	21.26	25
	21	347.0	24.20	25	342.2	23.55	25	337.7	22.81	25	325.9[b]	26.00	24
	24	364.6	27.84	25	355.8	27.59	25	355.9	24.44	25	341.5[a]	29.06	24
	28	385.9	30.87	25	378.5	28.30	25	375.1	26.17	25	361.3[a]	31.70	24
Pairing	31	394.8	32.12	25	385.2	31.53	25	379.9	25.62	25	363.7[b]	34.52	24
	35	412.6	33.70	25	404.2	34.73	25	400.1	26.61	25	380.4[b]	34.85	24
	38	427.5	37.92	25	419.4	35.77	25	408.3	27.49	25	384.9[b]	35.95	24
	42	445.2	43.13	25	434.5	36.26	25	423.2	28.13	25	401.6[b]	37.09	24
	45	461.4	44.36	24	447.6	38.17	25	438.2	28.48	25	408.9[b]	37.24	24
Postmating	49	472.1	46.02	24	457.0	38.47	25	447.2	29.92	24	419.0[b]	37.71	24
	52	480.7	47.46	24	465.6	40.95	25	455.4	30.00	24	424.9[b]	39.96	24
	56	485.3	51.24	16	484.5	43.84	17	473.3	35.07	16	440.6[a]	43.44	16
	59	529.3	51.40	3	501.0	36.17	4	497.0	16.52	3	436.0[a]	17.32	3

Table 7 **Summary of Body Weight Change Values - MALE**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Change Values g													
Premating	1-3	15.6	2.63	25	15.5	3.29	25	15.4	3.06	25	12.6[a]	5.37	25
	3-7	35.0	4.66	25	34.7	4.01	25	32.6	3.74	25	25.1[b]	7.23	25
	7-10	29.1	4.60	25	28.5	4.39	25	26.9	5.62	25	27.4	6.22	25
	10-14	26.2	5.62	25	19.4[b]	10.55	25	23.0	5.97	25	24.2	6.07	25
	14-17	20.6	7.12	25	24.2	9.01	25	20.6	5.99	25	18.3	7.74	25
	17-21	22.0	5.70	25	21.6	7.47	25	22.9	6.73	25	19.9	9.08	24
	21-24	17.6	5.50	25	13.6	6.98	25	18.2	4.44	25	15.6	6.57	24
	24-28	21.3	5.12	25	22.7	7.10	25	19.2	12.50	25	19.8	6.27	24
	1-28	187.3	30.78	25	180.2	26.62	25	178.8	24.27	25	162.9[b]	28.43	24
Pairing	28-31	8.9	7.31	25	6.8	6.26	25	4.8	8.82	25	2.4[b]	7.84	24
	31-35	17.9	7.45	25	18.9	7.00	25	20.2	7.21	25	16.8	6.90	24
	35-38	14.9	7.01	25	15.3	3.78	25	8.2[b]	5.39	25	4.5[b]	5.44	24
	38-42	17.6	7.83	25	15.1	4.65	25	14.9	5.92	25	16.7	7.36	24
	42-45	15.6	4.71	24	13.0	6.46	25	14.9	4.55	25	7.3[b]	5.32	24
	45-49	10.7	10.64	24	9.4	9.95	25	9.9	6.30	24	10.0	5.80	24
Postmating	49-52	8.5	7.63	24	8.6	8.85	25	8.3	5.66	24	5.9	4.89	24
	52-56	13.2	6.83	16	11.9	9.97	17	14.1	4.91	16	10.8	5.44	16
	56-59	14.7	8.96	3	9.3	5.74	4	7.3	5.03	3	0.0	7.21	3

Table 5 **Summary of Premating Body Weight Values - FEMALE**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Values g													
	1	191.0	5.80	25	189.8	6.65	25	188.9	5.51	25	189.7	4.49	25
	4	197.4	7.11	25	195.6	6.19	25	195.2	7.06	25	193.0[a]	4.98	25
	8	205.4	7.11	25	202.6	8.43	25	202.6	10.92	25	199.0[a]	7.68	25
	11	211.4	8.65	25	208.2	10.39	25	207.9	9.34	25	206.7	8.34	25
	15	217.6	10.95	25	215.7	11.71	25	213.8	10.41	25	211.5	8.31	25

Table 6 **Summary of Gestation Body Weight Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Values g													
	0	224.0	10.35	22	221.0	15.21	25	214.8[a]	11.26	23	215.6	11.14	20
	4	249.9	12.59	22	246.8	13.32	25	243.1	11.13	23	238.2[b]	11.13	20
	7	264.0	13.87	22	259.3	13.53	25	254.7[a]	10.59	23	245.0[b]	11.23	20
	10	278.0	14.27	22	271.8	13.58	25	269.3	12.99	23	260.8[b]	12.35	20
	13	295.6	15.01	22	289.1	15.19	25	287.7	11.63	23	281.5[b]	12.82	20

Table 8 **Summary of Premating Body Weight Change Values - FEMALE**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Change Values g													
	1-4	6.4	4.05	25	5.8	5.07	25	6.2	5.80	25	3.3	4.33	25
	4-8	8.0	3.49	25	7.0	3.96	25	7.5	5.29	25	6.0	5.35	25
	8-11	6.0	4.92	25	5.6	5.82	25	5.3	5.01	25	7.7	5.44	25
	11-15	6.2	4.41	25	7.5	3.85	25	5.8	3.53	25	4.8	3.35	25
	1-15	26.6	7.99	25	25.8	9.76	25	24.8	8.39	25	21.8	6.89	25

Table 9 **Summary of Gestation Body Weight Change Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Change Values g													
	0-4	25.8	5.48	22	25.8	8.07	25	28.3	6.87	23	22.7	6.65	20
	4-7	14.2	4.63	22	12.5	4.86	25	11.6	3.89	23	6.8[b]	6.30	20
	7-10	14.0	6.39	22	12.6	4.23	25	14.5	7.97	23	15.8	4.79	20
	10-13	17.5	5.07	22	17.2	4.50	25	18.4	5.64	23	20.7	4.96	20
	0-7	40.0	7.76	22	38.3	8.93	25	40.0	5.88	23	29.5[b]	8.04	20
	7-13	31.5	6.56	22	29.8	5.27	25	33.0	7.00	23	36.5[a]	6.46	20
	0-13	71.5	10.21	22	68.1	8.57	25	72.9	10.22	23	65.9	10.70	20

Food consumption: *Weekly*

Food consumption (FC) in males (Weeks 1-2 and 3-4) and females (during gestation) was reduced at HD, compared to controls [ss]. The reduced food consumption was consistent with the lower body weight and body weight gain seen at this dose level and was considered related to treatment with doxepin. Food consumption during the pre-mating (males and females) and gestation period at 10 and 30 mg/kg/day was similar to controls and unaffected by treatment. See sponsor's Table 10 for male summary FC data and Tables 11 and 12 for female summary FC data.

Table 10 **Summary of Food Consumption Values - MALE**

Endpoint	Study Interval (Week)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Food Consumption Values g/animal/day													
Premating	1-2	26.9	1.59	25	26.9	2.01	25	26.8	2.27	25	25.4[a]	1.97	25
	2-3	28.4	2.32	25	28.4	2.26	25	28.3	3.23	25	26.9	2.22	25
	3-4	30.3	3.65	25	29.9	2.89	25	29.4	3.01	24	27.8[a]	3.56	24
	4-5	31.7	4.53	25	30.9	3.26	25	30.8	3.47	22	29.2	3.23	24

Table 11 **Summary of Premating Food Consumption Values - FEMALE**

Endpoint	Study Interval (Week)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Food Consumption Values g/animal/day													
	1-2	19.2	3.49	25	18.9	1.37	25	18.8	2.42	23	17.7	1.28	25
	2-3	19.8	2.45	25	18.3	2.79	24	19.5	2.08	25	21.0	2.84	25

Table 12 **Summary of Gestation Food Consumption Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Food Consumption Values g/animal/day													
	0-4	23.7	2.28	22	23.0	2.24	25	23.0	1.69	23	20.8[b]	1.64	20
	4-7	25.4	2.73	22	24.9	2.80	25	24.4	2.08	23	21.4[b]	2.48	20
	7-10	24.6	2.44	22	23.3	2.11	25	23.8	2.08	23	23.4	2.09	20
	10-13	25.9	1.89	22	25.0	2.24	25	25.0	1.16	23	25.1	2.41	20
	0-7	24.4	2.27	22	23.8	2.38	25	23.6	1.72	23	21.0[b]	1.81	20
	7-13	25.3	1.93	22	24.1	1.76	25	24.4	1.26	23	24.2	1.68	20
	0-13	24.8	1.90	22	23.9	1.88	25	24.0	1.16	23	22.5[b]	1.55	20

Toxicokinetics: *0, 0.5, 1, 2, 3, 4, 6, 8, 12, & 24 hrs postdose on GD7 (F) & at termination (M)*
Dose formulation concentrations were found to be 92.7-100.6% of nominal. Doxepin and nordoxepin plasma concentrations were measured in male (Day 58) and female (GD 7) rats following daily oral (gavage) administration. A protocol deviation noted that the storage stability of doxepin and nordoxepin in rat plasma at -70°C was ongoing; that report does not appear to have been submitted.

Plasma concentrations of doxepin and nordoxepin after dosing at 10 mg/kg/day were below the limit of quantitation (BQL) in a majority of samples. Concentrations of doxepin and nordoxepin were quantifiable in both sexes; the length of time that concentrations were quantifiable increased with dose, up to 24-hr postdose at 100 mg/kg/day. Terminal half-lives could not be estimated in some cases (see sponsor's summary table below), due to insufficient characterization of the terminal phases of the mean concentration-time curves. The increases in doxepin and nordoxepin C_{max} and AUC_{last} were generally greater than dose-proportional. See the sponsor's summary table below for details.

Summary of Doxepin Plasma Toxicokinetic Parameters in Rats

Group	Dosage (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 58[a]								
6	10	M	2.60	0.5	0.5	0.650	NE	NE
7	30	M	14.8	1	8	44.4	NE	NE
8	100	M	115	3	24	1010	1020	3.0
GD 7[b]								
2,6	10	F	5.91	0.5	4	7.31	NE	NE
3,7	30	F	46.0	0.5	6	64.4	72.5	2.2
4,8	100	F	157	0.5	24	1160	1170	3.3

NE: Not estimated, due to insufficient characterization of terminal phase
a: Doxepin was administered daily starting 28 days prior to mating
b: Doxepin was administered daily starting 14 days prior to mating

Summary of Nordoxepin Plasma Toxicokinetic Parameters in Rats

Group	Dosage[a] (mg/kg/day)	Gender	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 58[b]								
6	10	M	8.82	0.5	4	6.83	NE	NE
7	30	M	68.0	2	8	239	NE	NE
8	100	M	605	1	24	5500	5510	2.3
GD 7[c]								
2,6	10	F	10.8	0.5	4	10.7	NE	NE
3,7	30	F	57.2	0.5	6	67.7	79.2	3.2
4,8	100	F	242	0.5	12	1230	NE	NE

NE: Not estimated, due to insufficient characterization of terminal phase
a: Dosage of doxepin
b: Doxepin was administered daily starting 28 days prior to mating
c: Doxepin was administered daily starting 14 days prior to mating

Necropsy: *GD13*
No treatment-related macroscopic observations were seen in males or females, but some reproductive organ weights were altered in HD animals.

Mean body weights were dose-dependently reduced in MDM (5.3% vs. control, [nss]) and HDM (18% vs. control, [ss]). Absolute and relative prostate weight was decreased in the HDM (25% and 14%, respectively, [ss]). Absolute cauda epididymis and epididymides weights were slightly decreased in HDM (6-7%, [ss]), but were likely related to the reduced body weights. Seminal vesicle weights generally appeared slightly increased in treated males (up to 13%, [ss] only in LDM), except for absolute weight in HDM (-5%). Absolute testes weight was comparable to control.

Summary of Organ Weight Values - MALE

Table 15 Terminal

	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight g	490	48	24	475	45	25	464	33	24	427[b]	41	24
Cauda, epididymis, rt g	0.281	0.021	24	0.288	0.031	25	0.276	0.036	24	0.261[a]	0.021	24
Cauda, epididymis, rt/BWt %	0.0579	0.0071	24	0.0612	0.0085	25	0.0599	0.0086	24	0.0616	0.0072	24
Epididymides g	1.253	0.082	24	1.286	0.104	25	1.238	0.113	24	1.180[a]	0.056	24
Epididymides/BWt %	0.2575	0.0227	24	0.2728	0.0295	25	0.2682	0.0285	24	0.2784[a]	0.0233	24
Prostate gl g	0.724	0.157	24	0.665	0.143	25	0.659	0.092	24	0.544[b]	0.065	24
Prostate gl/BWt %	0.1495	0.0386	24	0.1410	0.0322	25	0.1429	0.0231	24	0.1285[a]	0.0188	24
Sem. ves. w/ coag. gl g	1.906	0.185	24	2.102[a]	0.332	25	1.989	0.296	24	1.819	0.246	24
Sem. ves. w/ coag. gl/BWt %	0.3941	0.0620	24	0.4479[a]	0.0912	25	0.4307	0.0687	24	0.4289	0.0606	24
Testes g	3.446	0.236	24	3.470	0.333	25	3.550	0.413	24	3.457	0.280	24
Testes/BWt %	0.7097	0.0758	24	0.7354	0.0862	25	0.7684[a]	0.0941	24	0.8140[b]	0.0692	24

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Mean body weight was decreased in HDF (~5%, [ss]). Absolute and relative ovary weights were dose-dependently and statistically-significantly decreased in treated females. Absolute ovary weights were decreased by 13%, 14% and 17%, respectively; relative ovary weights were decreased 11%, 11% and 13%, respectively. Absolute and relative uterine weights were dose-dependently reduced in MDF (13% & 10%, respectively, [nss]) and HDF (19% & 15%, respectively, [ss]).

Summary of Organ Weight Values - FEMALE

Table 16 Terminal

	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Body weight g	289	15	22	282	16	25	280	12	23	274[b]	14	19
Ovaries g	0.125	0.021	22	0.109[b]	0.015	25	0.108[b]	0.014	23	0.104[b]	0.019	20
Ovaries/BWt %	0.0435	0.0082	22	0.0388[a]	0.0051	25	0.0386[a]	0.0053	23	0.0380[a]	0.0072	19
Uterus w/ cervix g	8.711	1.803	22	8.458	1.141	25	7.619	2.181	23	7.099[b]	1.628	20
Uterus w/ cervix/BWt %	3.0231	0.6323	22	2.9970	0.3829	25	2.7134	0.7739	23	2.5844	0.6475	19

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.): Although fertility and fecundity indices showed few clear drug-related effects, the copulatory interval was increased at HD (55%; for reference, the upper range of the historical controls is 4.8%). There were reproductive effects in males. Sperm concentration appeared unaffected by doxepin. However, percent sperm motility was decreased at HD (8%; range 44-99%), compared to controls (range 75-99%); at MD, the mean was not affected due to the exclusion of one animal with 0% motility (that MDM also showed 84% abnormal sperm). Increases in abnormal sperm were also apparent at MD and HD (83% with the inclusion of the outlier noted above & 74%) compared to controls; however, exclusion of the outlier at MD yielded an average of 3.91, which was similar to LD and within one SD of the control average. Abnormal sperm percentages were generally increased at HD (4/24 HD were >10% compared to 0/24 controls). Values for percent sperm motility and percent abnormal sperm were outside of the historical control range. Mean estrous cycle length and number of estrous cycles appeared unaffected. See the sponsor's summary tables below for details.

Table 18 **Summary of Reproductive and Fertility Parameters**

Endpoint	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
No. Females on Study	25	25	25	25
No. Females Paired	25	25	25	25
No. Females Mated	25	25	24	24
No. Pregnant	24	25	24	23
Female Mating Index	100.0	100.0	96.0	96.0
Female Fertility Index	96.0	100.0	96.0	92.0
Female Fecundity Index	96.0	100.0	100.0	95.8
No. Males on Study	25	25	25	24
No. Males Paired	25	25	25	24
No. Males Mated	25	25	24	23
No. Males Impregnating a Female	24	25	24	23
Male Mating Index	100.0	100.0	96.0	95.8
Male Fertility Index	96.0	100.0	96.0	95.8
Male Fecundity Index	96.0	100.0	100.0	100.0
Females with Confirmed Mating Day	23	25	23	21
Copulatory Interval (Days)				
Mean	2.9	3.2	2.6	4.5
SD	2.44	2.36	1.59	3.83
N	23	25	23	21

N - Number of measures used to calculate mean
SD - Standard Deviation

Table 20 Summary of Sperm Evaluation

	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Sperm Motility Percent Motility	88.7	7.23	24	86.1	10.65	25	88.7	7.76	23	81.8	13.18	24
Total Sperm Concentration per Cauda Epididymis x 10^8	2.716	0.4644	24	2.785	0.4153	25	2.728	0.6805	24	2.501	0.3126	24
Sperm Concentration per gram Cauda Epididymis x 10^8	9.675	1.5264	24	9.661	1.0071	25	9.715	2.4188	24	9.571	0.7370	24
Percent Abnormal	3.31	1.955	24	4.08	2.605	25	7.27	16.531	24	5.77	3.710	24

Table 17 Summary of Premating Estrous Cycling

	0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Mean Cycle Length (Days)	4.6	1.48	24	5.0	1.27	24	4.6	0.74	24	4.9	1.03	23
No. of Cycles (Count)	2.2	0.82	24	2.0	0.72	24	2.0	0.72	24	2.1	0.51	23

During uterine examinations on GD13, 1 control, 1 MDF and 2 HDF were determined to be not pregnant. A number of females were pregnant but without confirmation of the day of mating (2, 0, 1 and 3 at control, LD, MD and HD); data from these pregnancies were not included in the GD13 analyses, which yielded 22, 25, 23 and 20 evaluable pregnancies. The numbers of corpora lutea, implantation sites and viable embryos were decreased at MD and HD, often statistically significantly and outside the historical control range. Pre-implantation loss was increased at MD and HD. Litter size also appeared slightly decreased at MD and HD. Although possibly related to the apparent adverse effect on the early viability of the embryos, post-implantation loss appeared reduced at HD. See the sponsor's summary tables below for details.

Table 19 Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
No. Females on Study	25	25	25	25
No. Not Pregnant	1	0	1	2
No. Pregnant	24	25	24	23
No. Died Pregnant	0	0	0	0
No. Abortions	0	0	0	0
No. Early Deliveries	0	0	0	0
No. Females with All Resorptions	0	0	0	0
No. Females with Viable Embryos Day 13 Gestation	22	25	23	20
No. Females with No Confirmed Mating Date	2	0	1	3

Corpora Lutea					
No. per Animal	Mean	16.5	16.3	14.6[b]	14.0[b]
	SD	2.02	2.51	2.13	1.70
	N	22	25	23	20
Implantation Sites					
No. per Animal	Mean	15.2	14.8	13.4	12.1[b]
	SD	2.99	1.52	3.83	2.56
	N	22	25	23	20
Preimplantation Loss					
% per animal	Mean	8.16	8.14	10.00	13.31
	SD	14.153	9.330	20.273	16.668
	N	22	25	23	20
Viable Embryos					
No. per Animal	Mean	14.2	14.2	12.7	11.6[a]
	SD	3.38	1.82	4.03	2.54
	N	22	25	23	20
Postimplantation Loss					
% Implants per Animal	Mean	7.90	4.76	7.77	3.62
	SD	8.707	4.846	12.088	5.090
	N	22	25	23	20
Litter Size					
No. per Animal	Mean	14.2	14.2	12.7	11.6
	SD	3.38	1.82	4.03	2.54
	N	22	25	23	20
Resorptions: Total					
No. per Animal	Mean	1.0	0.7	0.7	0.5[a]
	SD	1.00	0.69	0.81	0.60
	N	22	25	23	20

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

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Embryofetal development

Study title: *SP-D0107: STUDY FOR EFFECTS OF DOXEPIN HCl ON EMBRYO-FETAL DEVELOPMENT IN RATS*

Key study findings:

- $NOEL_{maternal}$= 30 mg/kg (based on clinical signs & maternal body weight reduction)
- $NOEL_{developmental}$= 30 mg/kg (based on developmental delay and total low incidence alterations)

Study no.:	1288-003
Volume #, and page #:	Electronic submission, 503 pgs.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/1/06
GLP compliance:	Yes
QA reports: yes (X) no ( )	
Drug, lot #, and % purity:	doxepin hydrochloride, lot 3045911, 100.9% E-isomer: (b) (4), Z-isomer: (b) (4) Vehicle: water distilled from deionized tap water at (b) (4)

Methods

Doses:	0, 30, 100, & 150 mg/kg/day
Species/strain:	time-mated F Sprague-Dawley, CD® [Crl:CD® (SD)]
Number/sex/group:	25/gp
Route, formulation, volume, and infusion rate:	PO, gavage, 10ml/kg QD for GD6-GD17
Satellite groups used for toxicokinetics:	four groups of 12/group
Other parameters:	Necropsy on GD20 ½ fetuses/litter-visceral exam ½ fetuses/litter-skeletal exam *ad libitum* Lab Meal® (Certified Rodent Meal #5002, PMI Nutrition International, Inc.) *ad libitum* tap water Individually housed in suspended, stainless steel, wire-mesh type cages
Notes:	On GD6, prior to dosing, 3TK controls were replaced due to weight loss

Results

Mortality (dams): *2x Daily*

Four animals (1LD main study and 3TK animals) died during the course of the study. The LD-main study (GD9) and one MD-TK (GD11) animal that died during the study were observed to have a perforation of the esophagus, suggesting dosing error. Additionally, two HD-TK animals died on GD8 and GD15; there were no remarkable necropsy findings in one and the other was "inadvertently" not necropsied, so any relationship to drug is unclear. All other animals survived to termination.

Clinical signs (dams): *Daily, ~ 1 hr postdose*

Clinical signs were observed at MD and HD; these included: decreased activity, limb splay, low (body) carriage, salivation, lacrimation, yellow discoloration of the abdominal/anogenital/ventral surface regions, hypothermia and various forms of abnormal respiration. At HD, mydriasis, impaired righting reflex, righting reflex absent, and clonic convulsions were also observed. Clinical signs generally exhibited a dose-related pattern, with respect to incidence and also for persistence of decreased activity. See excerpts from the sponsor's summary table, below.

Table 1 Summary of Gestation Clinical Findings[+]

Observation	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
Number of Animals Alive at Start of Interval	25	25	25	25
Behavior/Activity				
Activity decreased	0/0	0/0	230/25	305/25
Convulsions - clonic	0/0	0/0	0/0	1/1
Righting reflex impaired	0/0	0/0	0/0	7/3
Righting reflex lost	0/0	0/0	0/0	6/2
Salivation	0/0	0/0	38/10	149/21
External Appearance				
Carriage low	0/0	0/0	143/18	186/21
Lacrimation, Eye/left	0/0	0/0	112/18	142/20
Lacrimation, Eye/right	0/0	0/0	112/18	143/20
Limbs splayed, Forelimb/left	0/0	0/0	2/2	52/14
Limbs splayed, Forelimb/right	0/0	0/0	2/2	52/14
Limbs splayed, Hind limb/left	0/0	0/0	2/2	77/17
Limbs splayed, Hind limb/right	0/0	0/0	2/2	77/17
Material around eyes, Red, Eye/left	0/0	0/0	0/0	1/1
Material around eyes, Red, Eye/right	0/0	0/0	0/0	5/2
Material around mouth, Red	0/0	0/0	6/2	58/9
Material around mouth, Yellow	0/0	0/0	0/0	6/1
Material around nose, Red	0/0	0/0	0/0	12/3
Posture hunched	0/0	0/0	0/0	2/2
Eye/Ocular				
Pupil dilated, Eye/left	0/0	0/0	0/0	13/3
Pupil dilated, Eye/right	0/0	0/0	0/0	13/3
Pelage/Skin				
Hair discolored, Black, Anogenital region	0/0	0/0	0/0	3/1
Hair discolored, Brown, Abdominal region	0/0	0/0	0/0	4/3
Hair discolored, Brown, Anogenital region	0/0	0/0	0/0	2/1
Hair discolored, Brown, Ventral surface	0/0	0/0	0/0	0/0
Hair discolored, Red, Anogenital region	0/0	0/0	0/0	3/1
Hair discolored, Yellow, Abdominal region	0/0	0/0	0/0	9/3
Hair discolored, Yellow, Anogenital region	0/0	0/0	26/5	84/13
Hair discolored, Yellow, Ventral surface	0/0	0/0	8/1	21/2
Skin cold to touch	0/0	0/0	3/1	23/4
Respiration				
Breathing audible	0/0	0/0	1/1	10/3
Breathing difficult	0/0	0/0	0/0	4/2
Breathing shallow	0/0	0/0	0/0	5/1
Breathing slow	0/0	0/0	0/0	1/1
Rales	0/0	0/0	0/0	1/1

[+]Number of times observed/Total number of animals affected

Body weight (dams): *GD 0, 6, 9, 12, 15, 18, & 20*

There were dose-related decreases in gestation body weight (11 & 16% on GD18, compared to controls) and gestation body weight gain at MD and HD. See the sponsor's summary data, below.

Table 2 **Summary of Gestation Body Weight Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	150 mg/kg/day Mean	SD	N
Body Weight Values g													
	0	207.7	15.00	25	209.2	15.99	25	209.0	14.51	25	209.2	15.13	24
	6	246.7	19.21	25	245.0	18.80	25	245.5	16.05	25	250.5	17.72	24
	9	259.4	19.02	25	253.8	18.12	25	242.3[b]	15.31	25	244.5[a]	17.60	24
	12	278.8	19.51	25	273.8	17.94	24	257.7[b]	16.12	25	253.7[b]	19.52	24
	15	300.2	22.58	25	294.1	17.26	24	271.7[b]	17.19	25	267.3[b]	20.68	24
	18	337.2	25.23	25	330.9	18.79	24	299.7[b]	18.10	25	284.5[b]	24.13	24
	20	366.7	28.86	25	360.6	19.76	24	332.1[b]	21.28	25	313.1[b]	28.33	24

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Table 3 **Summary of Gestation Body Weight Change Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	150 mg/kg/day Mean	SD	N
Body Weight Change Values g													
	0-6	39.0	8.59	25	35.7	7.09	25	36.5	4.05	25	41.3	7.03	24
	6-9	12.8	7.64	25	8.8	4.74	25	-3.2[b]	9.77	25	-6.0[b]	7.68	24
	9-12	19.4	6.22	25	18.6	6.04	24	15.4	5.48	25	9.2[b]	9.34	24
	12-15	21.4	6.42	25	20.3	5.48	24	14.0[b]	4.98	25	13.6[b]	6.79	24
	15-18	36.9	7.34	25	36.8	5.79	24	28.0[b]	7.68	25	17.2[b]	13.67	24
	18-20	29.5	7.07	25	29.7	5.62	24	32.4	6.87	25	28.7	11.15	24
	6-18	90.5	13.66	25	84.6	9.33	24	54.2[b]	15.53	25	34.0[b]	19.08	24
	6-20	120.0	15.37	25	114.3	10.24	24	86.6[b]	15.28	25	62.7[b]	22.92	24
	0-20	159.0	19.88	25	150.5	13.12	24	123.1[b]	15.76	25	103.9[b]	24.50	24

N - Number of measures used to calculate mean
SD - Standard Deviation
[b]Significantly different from control; (p<0.01)

Table 7 **Summary of Gravid Uterine Weight and Adjusted Body Weight/Body Weight Change Values**

Endpoint	0 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	150 mg/kg/day Mean	SD	N
Gravid Uterine Weight, g	70.7	11.43	25	71.0	8.69	24	64.1	10.06	25	60.0[b]	9.20	24
Final Body Weight, g	366.7	28.86	25	360.6	19.76	24	332.1	21.28	25	313.1	28.33	24
Adjusted Final Body Weight, g	296.0	22.30	25	289.6	17.24	24	268.0[b]	16.35	25	253.2[b]	21.22	24
Adjusted Weight Change from Day 0, g	88.3	13.78	25	79.5	9.93	24	59.0[b]	11.04	25	44.0[b]	18.08	24

N - Number of measures used to calculate mean
SD - Standard Deviation
[b]Significantly different from control; (p<0.01)

Food consumption (dams): *GD 0, 6, 9, 12, 15, 18, & 20*

There were mild dose-related decreases in food consumption at MD and HD (up to ~25%). See the sponsor's summary table, below.

Table 4 **Summary of Gestation Food Consumption Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N	150 mg/kg/day Mean	SD	N
Food Consumption Values g/animal/day													
	0-6	19.4	1.91	25	18.8	1.84	25	18.9	1.55	24	19.7	1.44	24
	6-9	21.4	3.28	25	20.6	2.19	25	18.4[b]	4.49	25	17.1[b]	2.74	24
	9-12	23.2	2.56	25	21.8	3.39	24	20.8[a]	3.17	25	18.2[b]	2.90	24
	12-15	24.5	2.89	25	25.3	3.04	24	21.8	7.07	25	19.4[b]	3.87	24
	15-18	26.9	2.60	25	26.6	2.31	24	22.5[b]	3.16	25	17.4[b]	3.89	24
	18-20	27.1	5.77	25	26.4	1.91	24	25.5	2.34	25	22.9[b]	3.21	23
	6-18	24.0	2.18	25	23.6	1.95	24	20.9[b]	3.47	25	18.0[b]	2.45	24
	6-20	24.4	2.27	25	24.0	1.83	24	21.5[b]	3.01	25	18.7[b]	2.32	24
	0-20	22.9	2.08	25	22.5	1.64	24	20.8[b]	2.61	25	19.0[b]	1.87	24

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

Toxicokinetics: *GD6 & 17; predose & 0.5, 1, 2, 3, 4, 6, 8, 12, & 24 hours postdose, via tail vein*
Dose formulation concentration analyses ranged from 97.3-102.0% of nominal concentrations.

Following daily oral administration to female rats on Day 6 through Day 17 of gestation, doxepin was absorbed rapidly (T_{max} 0.5-1 hr). See sponsor's summary table for PK data, below. Mean plasma concentrations generally declined with time, but with some irregularities, and were quantifiable through 8-24 hours (t_{last}). Plasma C_{max}, AUC and apparent terminal half-life estimates generally showed dose-related increases. Some accumulation with repeated dosing was suggested.

Summary of Doxepin Plasma Toxicokinetic Parameters in Female Rats

Group	Dosage (mg/kg/day)[a]	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 6							
6	30	32.4	1	8	84.8	93.4	3.0
7	100	257	0.5	12	992	1070	3.1
8	150	496	0.5	24	3080	NE	NE
Day 17							
6	30	87.9	1	8	143	152	2.9
7	100	330	0.5	24	2340	2400	4.1
8	150	532	0.5	24	4930	5730	8.5

NE: Not estimated, due to insufficient characterization of terminal phase.
a: Daily dosage from Day 6 through Day 17 of gestation.

T_{max} for nordoxepin was also relatively rapid, ranging from 0.5-2 hours. See sponsor's summary table for PK data, below. The mean terminal half-life generally appeared to increase with dose and repeated dosing, and nordoxepin was quantifiable through 8-24 hours (t_{last}). Plasma C_{max} and AUC generally showed dose-related increases (sometimes greater than dose-proportional), and accumulation with repeated dosing was suggested.

Summary of Nordoxepin Plasma Toxicokinetic Parameters in Female Rats

Group	Dosage (mg/kg/day)[a]	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 6							
6	30	42.3	1	8	85.2	88.7	2.3
7	100	272	1	12	930	964	2.2
8	150	327	0.5	24	1970	NE	NE
Day 17							
6	30	95.3	1	8	145	NE	NE
7	100	406	0.5	24	3070	3110	3.5
8	150	641	2	24	8350	9290	6.7

NE: Not estimated, due to insufficient characterization of terminal phase.
a: Doxepin dosage, administered daily from Day 6 through Day 17 of gestation.

Terminal and necroscopic evaluations:C-section data:
Generally, the pregnancy measures were similar among groups. One HD dam was observed not pregnant. See the sponsor's summary data, following.

Table 6 Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint		0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
Corpora Lutea					
No. per Animal	Mean	13.5	14.0	14.4	14.0
	SD	2.29	2.46	2.91	1.71
	N	25	24	25	24
Implantation Sites					
No. per Animal	Mean	12.2	12.6	12.4	12.9
	SD	1.53	1.61	1.78	1.67
	N	25	24	25	24
Preimplantation Loss					
% per animal	Mean	8.66	8.80	11.71	7.73
	SD	10.311	9.497	14.398	8.479
	N	25	24	25	24
Viable Fetuses					
No. per Animal	Mean	11.3	12.0	11.7	12.3
	SD	1.93	1.55	1.93	1.55
	N	25	24	25	24
Fetal Sex Ratio					
% Males per animal	Mean	46.2	50.2	46.6	52.7
	SD	11.03	14.34	11.01	14.29
	N	25	24	25	24
Postimplantation Loss					
% Implants per Animal	Mean	7.83	4.76	5.86	4.55
	SD	9.087	6.384	8.016	6.311
	N	25	24	25	24
Nonviable Fetuses					
No. per Animal	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
	N	25	24	25	24
Litter Size					
No. per Animal	Mean	11.3	12.0	11.7	12.3
	SD	1.93	1.55	1.93	1.55
	N	25	24	25	24
Resorptions: Early + Late					
No. per Animal	Mean	0.9	0.6	0.7	0.6
	SD	1.00	0.82	1.02	0.88
	N	25	24	25	24
Resorptions: Early					
No. per Animal	Mean	0.8	0.6	0.7	0.6
	SD	1.00	0.82	0.99	0.83
	N	25	24	25	24
Resorptions: Late					
No. per Animal	Mean	0.1	0.0	0.0	0.0
	SD	0.33	0.00	0.20	0.20
	N	25	24	25	24

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

Offspring (malformations, variations, etc.):
Mean fetal body weights (male, female, and sexes combined) showed a dose-related decrease (12- 25%) at MD and HD. See the sponsor's summary data, below.

Table 8 **Summary of Fetal Body Weight Values, g**

			0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
Fetal Weight						
	Males	Mean	4.20 (4.18)	4.03 (4.04)	3.64 (3.63)[b]	3.18 (3.19)[b]
		SD	0.299	0.213	0.308	0.404
		N	25	24	25	24
	Females	Mean	3.99 (3.97)	3.87 (3.88)	3.51 (3.51)[b]	3.04 (3.06)[b]
		SD	0.241	0.221	0.257	0.410
		N	25	24	25	24
	Males + Females	Mean	4.08 (4.07)	3.96 (3.96)	3.57 (3.57)[b]	3.12 (3.13)[b]
		SD	0.252	0.204	0.264	0.399
		N	25	24	25	24

SD - Standard Deviation ( ) - Least Square Mean
N - Number of measures used to calculate mean [b]Significantly different from control; (p<0.01)

The sponsor reported no drug-related fetal external, visceral, or skeletal malformations; however, a few external, visceral and/or skeletal malformations occurred at low, but seemingly dose-related, incidences in the drug-treated groups (total malformation counts of 0, 2, 5 & 8 in control, LD, MD and HD). These alterations were observed in 0/0, 1/1, 3/3, and 5/3 fetuses/litter in the control, LD, MD and HD groups; see excerpts from the sponsor's summary malformation results, below). This yields 0, 0.3, 1.0 and 1.7 percent of fetuses and 0, 4.2, 12 and 12.5 percent of litters affected in the control, LD, MD and HD groups.

Table 12 **Summary of External Malformations and Developmental Variations**

	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
No. Litters Evaluated	25	24	25	24
No. Fetuses Evaluated	282	287	292	295
Total Malformations				
No. Litters (%)	0 (0.0)	0 (0.0)	1 (4.0)	2 (8.3)
No. Fetuses (%)[1]	0 (0.0)	0 (0.0)	1 (0.3)	2 (0.7)
Total Variations				
No. Litters (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)

Table 13 **Summary of Visceral Malformations and Developmental Variations**

	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
No. Litters Evaluated	25	24	25	24
No. Fetuses Evaluated	139	145	145	148
Total Malformations				
No. Litters (%)	0 (0.0)	1 (4.2)	2 (8.0)	0 (0.0)
No. Fetuses (%)[1]	0 (0.0)	1 (0.7)	2 (1.4)	0 (0.0)
Total Variations				
No. Litters (%)	2 (8.0)	1 (4.2)	5 (20.0)	1 (4.2)
No. Fetuses (%)[1]	3 (2.2)	1 (0.7)	5 (3.4)	1 (0.7)

Table 14 **Summary of Skeletal Malformations and Developmental Variations**

	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
No. Litters Evaluated	25	24	25	24
No. Fetuses Evaluated	143	142	147	147
Total Malformations				
No. Litters	0 (0.0)	0 (0.0)	0 (0.0)	3 (12.5)
No. Fetuses(%)[1]	0 (0.0)	0 (0.0)	0 (0.0)	4 (2.7)
Total Variations				
No. Litters	18 (72.0)	17 (70.8)	25 (100.0)[b]	22 (91.7)
No. Fetuses(%)[1]	38 (26.6)	26 (18.3)	70 (47.6)	89 (60.5)

No. - Number
[1]Not statistically analyzed
[b]Significantly different from control; (p<0.01)

For further detail on the nature of the malformations, see excerpts from the sponsor's individual summary results, below. In addition, several skeletal variations were observed at MD and HD (i.e., various bones, especially bones of the skull and the sternebrae, incompletely or not ossified). The delay in ossification was sometimes noted to be statistically significant (hyoid and sternebrae) in comparison to controls and/or was outside of the historical control range. The sponsor indicated that the delay in ossification was consistent with the lower fetal body weights observed at these dose levels; therefore, there appeared to be a developmental delay at MD and HD. The sponsor suggested that the developmental delay may be at related to maternal toxicity (as demonstrated by reduced gestational body weights), in part.

Table 9 **Summary of Individual Fetal External Observations**

Observation	Classification	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
No. Litters Evaluated		25	24	25	24
No. Fetuses Evaluated		282	287	292	295
Body					
Umbilicus, Omphalocele	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.3)	1 (0.3)
Head					
Jaw(s), Micrognathia	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)
Placenta					
Entire, Larger than normal	P				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.3)

M - Malformation
P - Pathological
[1]Not statistically analyzed
No. - Number

Table 10 **Summary of Individual Fetal Visceral Observations**

Observation	Classification	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
No. Litters Evaluated		25	24	25	24
No. Fetuses Evaluated		139	145	145	148

Head					
Eye(s), Microphthalmia	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
Thoracic cavity					
Aortic arch, Retroesophageal	M				
No. Litters (%)		0 (0.0)	1 (4.2)	1 (4.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.7)	1 (0.7)	0 (0.0)
Aortic arch, Right sided	M				
No. Litters (%)		0 (0.0)	1 (4.2)	1 (4.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.7)	1 (0.7)	0 (0.0)
Innominate artery, Absent	V				
No. Litters (%)		0 (0.0)	1 (4.2)	1 (4.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.7)	1 (0.7)	0 (0.0)
Subclavian artery, Malpositioned	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
Thyroid, Smaller than normal	V				
No. Litters (%)		1 (4.0)	0 (0.0)	4 (16.0)	1 (4.2)
No. Fetuses (%)[1]		1 (0.7)	0 (0.0)	4 (2.8)	1 (0.7)

M - Malformation
V - Variation
[1]Not statistically analyzed
No. - Number

Table 11 Summary of Individual Fetal Skeletal Observations

Observation	Classification	0 mg/kg/day	30 mg/kg/day	100 mg/kg/day	150 mg/kg/day
No. Litters Evaluated		25	24	25	24
No. Fetuses Evaluated		143	142	147	147
Forelimb(s)					
Humerus, Bent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Pectoral girdle					
Scapula, Bent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	2 (1.4)
Pelvic girdle					
Ischium, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	4 (16.7)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	10 (6.8)
Pubis, Not ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	2 (8.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	3 (2.0)
Rib(s)					
Rib(s), Absent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Rib(s), Bent	V				
No. Litters (%)		1 (4.0)	1 (4.2)	1 (4.0)	3 (12.5)
No. Fetuses (%)[1]		1 (0.7)	1 (0.7)	2 (1.4)	6 (4.1)
Rib(s), Rudimentary	V				
No. Litters (%)		8 (32.0)	6 (25.0)	2 (8.0)	4 (16.7)
No. Fetuses (%)[1]		16 (11.2)	7 (4.9)	6 (4.1)	6 (4.1)

Rib(s), Smaller than normal	V				
No. Litters (%)		1 (4.0)	0 (0.0)	1 (4.0)	5 (20.8)
No. Fetuses (%)[1]		1 (0.7)	0 (0.0)	3 (2.0)	9 (6.1)
Sacral vertebra(e)					
Neural arch(es), Incompletely ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Skull					
Hyoid, Bent	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Hyoid, Not ossified	V				
No. Litters (%)		4 (16.0)	5 (20.8)	12 (48.0)[a]	11 (45.8)[a]
No. Fetuses (%)[1]		4 (2.8)	5 (3.5)	28 (19.0)	24 (16.3)
Interparietal bone, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	3 (12.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	5 (3.4)	1 (0.7)
Jugal, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.2)	0 (0.0)	4 (16.7)
No. Fetuses (%)[1]		0 (0.0)	1 (0.7)	0 (0.0)	5 (3.4)
Mandible, Fused	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Mandible, Smaller than normal	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Parietal bone, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	3 (12.0)	3 (12.5)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	5 (3.4)	3 (2.0)
Squamosal, Incompletely ossified	V				
No. Litters (%)		2 (8.0)	1 (4.2)	4 (16.0)	3 (12.5)
No. Fetuses (%)[1]		2 (1.4)	1 (0.7)	7 (4.8)	4 (2.7)
Supra occipital bone, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	4 (16.0)	2 (8.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	4 (2.7)	2 (1.4)
Sternum					
Sternebra(e), Misaligned	V				
No. Litters (%)		1 (4.0)	1 (4.2)	4 (16.0)	3 (12.5)
No. Fetuses (%)[1]		1 (0.7)	1 (0.7)	4 (2.7)	4 (2.7)
Sternebra(e), Not ossified	V				
No. Litters (%)		12 (48.0)	8 (33.3)	19 (76.0)	20 (83.3)[a]
No. Fetuses (%)[1]		16 (11.2)	10 (7.0)	42 (28.6)	71 (48.3)
Thoracic vertebra(e)					
Centra, Bipartite	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
One or more, Absent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.2)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)

M - Malformation
V - Variation

[1]Not statistically analyzed
No. - Number
[a]Significantly different from control; ($p<0.05$)

Study title: *SP-D0108: STUDY FOR EFFECTS OF DOXEPIN HCl ON EMBRYO-FETAL DEVELOPMENT IN NEW ZEALAND WHITE RABBITS*

Key study findings:

- No MTD in this study (maximum tested= 60 mg/kg); however 100 mg/kg and 200 mg/kg did not appear to be tolerated in a 2-week dose-ranging study in rabbits
- $NOEL_{maternal}$= 60 mg/kg
- $NOEL_{developmental}$= 30 mg/kg, based on slightly decreased fetal body weights at 60 mg/kg

Study no.:	1288-004
Volume #, and page #:	Electronic submission, 517 pgs.
Conducting laboratory and location:	(b) (4)
Date of study initiation:	9/1/06
GLP compliance:	Yes, pg 2
QA reports: yes (X) no ( )	pg 7 (alternate signature)
Drug, lot #, and % purity:	doxepin hydrochloride, lot 3045911, 100.9% E-isomer: (b) (4) Z-isomer: (b) (4) Vehicle: distilled deionized water

Methods

Doses:	0, 10, 30, and 60 mg/kg/day
Species/strain:	time-mated female New Zealand White rabbits, [Hra:(NZW)SPF] (b) (4) 5.5-6.5 mo at arrival (GD0)
Number/sex/group:	Main- 23/gp
Route, formulation, volume, and infusion rate:	PO, gavage, 4 ml/kg/day, QD from GD6 to GD18
Satellite groups used for toxicokinetics:	four groups of 4/group
Other parameters:	Necropsy on GD29: External, visceral, & skeletal exams on all fetuses 170g/animal/day Lab Diet (Certified Rabbit Diet® #5322, PMI Nutrition International, Inc.) *ad libitum* tap water Individually housed in suspended, stainless steel cages
Notes:	5 rabbits were replaced on GD6 prior to dosing (2 control, 1LD, 1HD, 1MD-TK)

Results

Mortality (dams): *2x daily*

One MD female was found dead on GD11 (#250, no clinical or macroscopic findings) and one HD-TK animal was found dead on GD14 (#307, necropsy findings suggested injury during blood collection -i.e. hemorrhage in the ventral neck area). The sponsor considered these deaths unrelated to drug. All other animals survived to termination.

Clinical signs (dams): *Daily, approximately 30 to 60 minutes postdose*

The sponsor recorded no drug-related clinical signs. Few/absent feces was recorded in 3 LD (4 instances) and 5 HD does (11 instances). Audible respiration was observed in 1 HD animal on 2 occasions.

Body weight (dams): *GD 0, 6, 10, 13, 16, 19, 21, 25 & 29*

There was no clear effect on body weight. Gestation body weight changes were slightly reduced in the period GD19-21 only at MD and HD. See the sponsor's summary data, below.

Table 3 **Summary of Gestation Body Weight Change Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	60 mg/kg/day Mean	SD	N
Body Weight Change Values kg													
	0-6	0.157	0.1106	22	0.158	0.0763	22	0.144	0.1040	22	0.137	0.0716	23
	6-10	0.027	0.0516	22	0.027	0.0297	22	0.032	0.0397	22	0.013	0.0649	23
	10-13	0.039	0.0451	22	0.042	0.0424	22	0.058	0.0417	21	0.058	0.0491	23
	13-16	0.069	0.0356	22	0.054	0.0458	22	0.068	0.0443	21	0.063	0.0530	23
	16-19	0.032	0.0511	22	0.041	0.0375	22	0.047	0.0545	21	0.012	0.0647	23
	19-21	0.038	0.0428	22	0.030	0.0428	22	-0.018[b]	0.0426	21	-0.028[b]	0.0486	23
	21-25	0.048	0.0580	22	0.040	0.0392	22	0.063	0.0571	21	0.051	0.0790	23
	25-29	0.048	0.0473	22	0.054	0.0708	22	0.072	0.0814	21	0.079	0.0710	23
	6-19	0.166	0.0941	22	0.163	0.0740	22	0.203	0.1008	21	0.147	0.1226	23
	19-29	0.134	0.1042	22	0.125	0.0913	22	0.118	0.1008	21	0.102	0.1241	23
	0-29	0.457	0.1412	22	0.446	0.1460	22	0.465	0.1736	21	0.386	0.1766	23

N - Number of measures used to calculate mean
SD - Standard Deviation
[b]Significantly different from control; (p<0.01)

Food consumption (dams): *Recorded daily, reported GD 0, 6, 10, 13, 16, 19, 21, 25 & 29*

Food consumption was generally slightly reduced in the HD group, and was occasionally reduced in the MD group.

Table 4 **Summary of Gestation Food Consumption Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	60 mg/kg/day Mean	SD	N
Food Consumption Values g/animal/day													
	0-6	134.5	12.54	22	128.7	12.34	22	129.5	13.33	22	133.0	12.79	23
	6-10	151.6	14.92	22	153.4	10.52	22	150.0	13.24	22	144.5	20.51	22
	10-13	140.2	24.58	22	130.8	25.24	22	136.0	28.10	21	124.7	35.68	23
	13-16	123.4	34.86	22	119.7	31.72	22	122.6	35.01	21	105.3	40.16	23
	16-19	144.5	26.49	21	140.2	21.71	22	135.3	28.83	21	117.4[b]	35.40	23
	19-21	147.6	26.61	22	129.9	30.81	22	104.7[b]	40.82	21	93.8[b]	37.27	23
	21-25	121.1	30.07	22	115.7	33.18	22	108.2	35.09	21	99.4	37.82	23
	25-29	97.4	32.22	22	97.7	29.58	21	92.1	31.20	21	98.2	35.74	23
	6-19	140.7	21.95	22	137.3	17.23	22	137.0	22.69	21	123.2[a]	29.64	23
	19-29	116.9	25.12	22	111.9	26.96	22	101.0	25.04	21	97.9	29.72	23
	0-29	131.3	16.45	22	126.8	14.80	22	123.0	18.22	21	116.5[a]	19.56	23

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Toxicokinetics: *GD6 & GD18 at predose, 0.5, 1, 2, 3, 4, 6, 8, 12 & 24 hours postdose via jugular vein*
Dose formulations concentration analyses ranged from 94.5% to 100.2%.

Doxepin was absorbed rapidly (median t_{max}~ 0.5 hr). There was variability in PK parameters for individuals; the range for T_{max} was 0.5-6 hr. Plasma concentrations declined with in an apparent multi-phasic manner, and were generally measurable through median times of 5-6 hours (t_{last}) at LD and 12 hours at MD and HD. The range for t_{last} was 4-24 hr (with longer values at higher doses). Mean terminal half-life estimates increased slightly with repeated dosing. Doxepin mean C_{max} and mean AUC_{last} each increased with dosage on Day 6 and Day 18. C_{max} and AUC increases were generally less than dose proportional. C_{max} and AUC tended to be lower with repeated dosing at LD and MD; however, C_{max} and AUC suggested the potential for accumulation of doxepin with repeated dosing at HD.

Summary of Mean[a] Doxepin Plasma Toxicokinetic Parameters in Female Rabbits

Group	Dosage (mg/kg/day)[b]	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 6							
6	10	43.6	0.5	6	53.1	65.1[c]	1.9[c]
7	30	92.5	0.5	12	168	210[c]	3.6[c]
8	60	109	0.5	12	342	353	3.1
Day 18							
6	10	24.1	0.5	5	34.5	38.3	2.2
7	30	77.2	0.5	12	125	134	4.1
8	60	431[c]	0.5[c]	12[c]	1290[c]	1320[c]	2.9[c]

a: Median for t_{max} and t_{last}; n=4.
b: Daily dosage from Day 6 through Day 18 of gestation.
c: n=3.

The C_{max} for nordoxepin was observed at median times ranging from 0.5 to 2 hours (T_{max}). The range for T_{max} was 0.5-6 hr. Plasma concentrations generally declined over time, and were measurable through median times (t_{last}) of 8 hours at LD and 12 hours at MD and HD. The range for t_{last} was 4-24. Mean terminal half-life estimates for nordoxepin ranged from 1.6-2.7 hours on Day 6 and from 1.3-2.4 hours on Day 18. Nordoxepin mean C_{max} and mean AUC_{last} generally increased with increases in dose, and tended to be greater than dose-proportional. Mean C_{max} and mean AUC_{last} were generally greater with repeated dosing, suggesting some potential for accumulation of nordoxepin with repeated dosing of doxepin.

Summary of Mean[a] Nordoxepin Plasma Toxicokinetic Parameters in Female Rabbits

Group	Dosage (mg/kg/day)[b]	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
Day 6							
6	10	24.6	2	8	65.2	75.8[c]	1.7[c]
7	30	388	0.75	12	528	436[d]	2.7[d]
8	60	485	0.5	12	971	976	1.6
Day 18							
6	10	54.4	0.75	8	104	107	1.3
7	30	256	1.5	12	756	763	1.8
8	60	704[d]	0.5[d]	12[d]	1690[d]	1720[d]	2.4[d]

a: Median for t_{max} and t_{last}; n=4
b: Doxepin dosage, administered daily from Day 6 through Day 18 of gestation
c: n=2
d: n=3

Terminal and necroscopic evaluations: C-section data: *GD29*
Minimal accentuated lobulation of the liver was observed in 1 of 22 MD does. (The uterus and cervix of 1LD doe was not available for examination.) Pregnancy and uterine parameters appeared unchanged (see the sponsor's summary Table 6, below).

Table 6 Summary of Maternal and Developmental Observations at Uterine Examination

Endpoint	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
No. Females on Study	23	23	23	23
No. Not Pregnant	1	1	1	0
No. Pregnant	22	22	22	23
Pregnancy Index Percent	95.7	95.7	95.7	100.0
No. Died Pregnant	0	0	1	0
No. Abortions	0	0	0	0
No. Early Deliveries	0	0	0	0
No. Females with All Resorptions	0	0	0	0
No. Females with Viable Fetuses Day 29 Gestation	22	22	21	23

Corpora Lutea					
No. per Animal	Mean	10.1	9.6	10.7	9.8
	SD	1.46	1.82	1.59	1.40
	N	22	22	21	23
Implantation Sites					
No. per Animal	Mean	9.0	8.3	9.8	9.5
	SD	1.89	2.01	1.70	1.44
	N	22	22	21	23
Preimplantation Loss					
% per animal	Mean	10.73	12.24	8.78	3.43
	SD	13.250	18.110	8.897	6.498
	N	22	22	21	23
Viable Fetuses					
No. per Animal	Mean	8.4	7.9	9.5	9.0
	SD	2.13	1.88	1.75	1.60
	N	22	22	21	23
Fetal Sex Ratio					
% Males per animal	Mean	44.3	54.0	47.8	47.0
	SD	16.67	15.48	17.85	19.89
	N	22	22	21	23
Postimplantation Loss					
% Implants per Animal	Mean	7.24	4.96	2.50	4.84
	SD	13.919	8.427	4.645	9.513
	N	22	22	21	23
Nonviable Fetuses					
No. per Animal	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
	N	22	22	21	23
Litter Size					
No. per Animal	Mean	8.4	7.9	9.5	9.0
	SD	2.13	1.88	1.75	1.60
	N	22	22	21	23
Resorptions: Early + Late					
No. per Animal	Mean	0.6	0.5	0.2	0.5
	SD	1.09	0.74	0.44	0.95
	N	22	22	21	23
Resorptions: Early					
No. per Animal	Mean	0.4	0.3	0.1	0.4
	SD	0.79	0.65	0.30	0.78
	N	22	22	21	23
Resorptions: Late					
No. per Animal	Mean	0.3	0.1	0.1	0.1
	SD	0.88	0.35	0.36	0.29
	N	22	22	21	23

No. - Number

Offspring (malformations, variations, etc.): *GD29*

Fetal body weights appeared very slightly reduced at MD and HD (<10%); however, litter sizes were larger on average in these groups (8.4, 7.9, 9.5 and 9.0 fetuses per litter in control, LD, MD and HD does). Reduced average fetal body weights did not appear to strictly correlate with larger litter sizes. There was one outlier litter (with two runts) in the MD group; when that litter was excluded, the mean weights for males, females and combined were 39.09 g, 38.16 g and 38.57 g. The combined average weight at HD (38.13g) was statistically significant and slightly lower than the historical control range (38.14 - 45.28 g) at that laboratory. See sponsor's summary Table 8.

Table 8 **Summary of Fetal Body Weight Values, g**

			0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
Fetal Weight						
	Males	Mean	41.97 (41.52)	41.11 (39.82)	38.54 (39.80)	38.24 (38.77)
		SD	5.952	5.520	3.673	3.673
		N	22	22	21	22
	Females	Mean	40.90 (40.44)	40.75 (39.71)	37.82 (38.94)	37.98 (38.36)
		SD	5.464	5.460	4.261	3.417
		N	22	21	21	23
	Males + Females	Mean	41.39 (40.97)	41.24 (40.00)	38.08 (39.32)	38.13 (38.59)[a]
		SD	5.042	5.384	3.614	2.531
		N	22	22	21	23

SD - Standard Deviation ( ) - Least square mean [a]Significantly different from control; (p<0.05
N - Number of measures used to calculate mean

The sponsor reported no drug-related fetal malformations or variations, although a number of fetal external malformations and/or variations were observed in the treated groups. A number of the malformations found in the treated groups were also observed in the control group. The total incidences of malformations appeared increased at MD, but appeared similar to control at HD (no. of fetuses and no. of litters affected; see sponsor's Table 15, below). The total numbers of recorded malformations were 16, 8, 31 and 15 in the control, LD, MD and HD groups. However, a dose relationship was lacking; the lower incidence in the HD group could not be accounted for by a decrease in pups or an increase in early failures (i.e., embryonic or fetal deaths). See excerpts from the sponsor's summary Tables 9, 10 and 11, following. There were a couple of findings potentially of note. Although occurring in 1LD and 2MD rabbit fetuses, umbilical omphalocele was also seen in the rat embryofetal study (1MD fetus and 1HD fetus). There was some evidence of under-ossification, although this was not as clear as in the rat embryofetal study.

Table 15 **Summary of External, Visceral, and Skeletal Malformations**

	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
No. Litters Evaluated	22	22	21	23
No. Fetuses Evaluated	185	173	200	207
Total Malformations				
No. Litters (%)	6 (27.3)	5 (22.7)	8 (38.1)	5 (21.7)
No. Fetuses (%)[1]	8 (4.3)	5 (2.9)	17 (8.5)	5 (2.4)

Table 9 **Summary of Individual Fetal External Observations**

Observation	Classification	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
No. Litters Evaluated		22	22	21	23
No. Fetuses Evaluated		185	173	200	207
Body					
Umbilicus, Omphalocele	M				
No. Litters (%)		0 (0.0)	1 (4.5)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	2 (1.0)	0 (0.0)
Forelimb(s)					
Forepaw, Abnormal flexure	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)

M - Malformation
V - Variation
[1]Not statistically analyzed
No. - Number

Table 10 **Summary of Individual Fetal Visceral Observations**

Observation	Classification	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
No. Litters Evaluated		22	22	21	23
No. Fetuses Evaluated		185	173	200	207
Abdominal cavity					
Gallbladder, Absent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Liver, Abnormal lobulation	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Liver, Discolored	P				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Spleen, Smaller than normal	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Head					
Eye(s), Hemorrhagic	P				
No. Litters (%)		1 (4.5)	0 (0.0)	2 (9.5)	2 (8.7)
No. Fetuses (%)[1]		2 (1.1)	0 (0.0)	2 (1.0)	2 (1.0)
Thoracic cavity					
Common carotid artery, Arising from innominate artei	V				
No. Litters (%)		0 (0.0)	3 (13.6)	3 (14.3)	2 (8.7)
No. Fetuses (%)[1]		0 (0.0)	4 (2.3)	5 (2.5)	3 (1.4)
Left lung, Smaller than normal	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Pulmonary trunk, Constricted	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Pulmonary trunk, Dilated	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Right lung, Smaller than normal	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Subclavian artery, Extra	V				
No. Litters (%)		0 (0.0)	2 (9.1)	0 (0.0)	3 (13.0)
No. Fetuses (%)[1]		0 (0.0)	2 (1.2)	0 (0.0)	3 (1.4)
Subclavian artery, Retroesophageal	V				
No. Litters (%)		1 (4.5)	0 (0.0)	0 (0.0)	2 (8.7)
No. Fetuses (%)[1]		1 (0.5)	0 (0.0)	0 (0.0)	2 (1.0)

M - Malformation
V - Variation
P - Pathological
[1]Not statistically analyzed
No. - Number

Table 11 Summary of Individual Fetal Skeletal Observations

Observation	Classification	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	60 mg/kg/day
No. Litters Evaluated		22	22	21	23
No. Fetuses Evaluated		185	173	200	207
Caudal vertebra(e)					
One or more, Misaligned	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	2 (1.0)
Cervical vertebra(e)					
Centra, Misaligned	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)
Centra, Misshapen	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Centra, Not ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Neural arch(es), Additional ossification center	V				
No. Litters (%)		0 (0.0)	1 (4.5)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	1 (0.5)	0 (0.0)
Neural arch(es), Misshapen	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	2 (1.0)	0 (0.0)
Forelimb(s)					
Humerus, Bent	M				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Hind limb(s)					
Talus, Not ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	2 (1.0)	1 (0.5)
Pectoral girdle					
Clavicle, Bent	M				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Scapula, Bent	M				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Spine of scapula, Misshapen	M				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Rib(s)					
Rib(s), Absent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Rib(s), Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Rib(s), Misshapen	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Rib(s), Rudimentary	V				
No. Litters (%)		17 (77.3)	20 (90.9)	16 (76.2)	22 (95.7)
No. Fetuses (%)[1]		50 (27.0)	45 (26.0)	51 (25.5)	52 (25.1)

Rib(s), Unilateral full rib	V				
No. Litters (%)		11 (50.0)	15 (68.2)	13 (61.9)	15 (65.2)
No. Fetuses (%)[1]		16 (8.6)	26 (15.0)	25 (12.5)	23 (11.1)
Skull					
Frontal bone, Additional ossification center	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Frontal bone, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Hyoid arch, Bent	V				
No. Litters (%)		4 (18.2)	4 (18.2)	3 (14.3)	9 (39.1)
No. Fetuses (%)[1]		7 (3.8)	6 (3.5)	3 (1.5)	9 (4.3)
Hyoid arch, Not ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Hyoid body, Not ossified	V				
No. Litters (%)		0 (0.0)	2 (9.1)	1 (4.8)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	2 (1.2)	6 (3.0)	1 (0.5)
Interparietal bone, Bipartite	V				
No. Litters (%)		0 (0.0)	1 (4.5)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	1 (0.5)	0 (0.0)
Interparietal bone, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Interparietal bone, Misshapen	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Jugal, Bipartite	V				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Jugal, Fused	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	3 (1.5)	0 (0.0)
Jugal, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Maxilla, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Nasal bone, Abnormal suture line	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Parietal bone, Additional ossification center	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	2 (8.7)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	4 (1.9)
Parietal bone, Additional suture line	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)

Tympanic ring, Incompletely ossified	V				
No. Litters (%)		0 (0.0)	1 (4.5)	0 (0.0)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Sternum					
Entire, Not ossified	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Sternebra(e), Absent	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Sternebra(e), Additional ossification center	V				
No. Litters (%)		1 (4.5)	1 (4.5)	2 (9.5)	2 (8.7)
No. Fetuses (%)[1]		1 (0.5)	1 (0.6)	2 (1.0)	2 (1.0)
Sternebra(e), Fused	M				
No. Litters (%)		1 (4.5)	3 (13.6)	4 (19.0)	2 (8.7)
No. Fetuses (%)[1]		2 (1.1)	3 (1.7)	8 (4.0)	2 (1.0)
Sternebra(e), Misaligned	V				
No. Litters (%)		0 (0.0)	0 (0.0)	2 (9.5)	4 (17.4)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	2 (1.0)	4 (1.9)
Sternebra(e), Sternoschisis	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Thoracic vertebra(e)					
Centra, Absent	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Centra, Fused	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Centra, Malpositioned	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Centra, Misaligned	V				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Neural arch(es), Fused	M				
No. Litters (%)		0 (0.0)	0 (0.0)	1 (4.8)	0 (0.0)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	1 (0.5)	0 (0.0)
Neural arch(es), Misaligned	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)
Neural arch(es), Misshapen	M				
No. Litters (%)		0 (0.0)	0 (0.0)	0 (0.0)	1 (4.3)
No. Fetuses (%)[1]		0 (0.0)	0 (0.0)	0 (0.0)	1 (0.5)

M - Malformation
V - Variation

[1]Not statistically analyzed
No. - Number

Prenatal and postnatal development

Study title: *SP-D0109: STUDY FOR TOXIC EFFECTS ON PRE- AND POSTNATAL DEVELOPMENT, INCLUDING MATERNAL FUNCTION IN RATS FOLLOWING ORAL ADMINISTRATION OF DOXEPIN HCl*

Key study findings:

- NOAEL for maternal toxicity, and growth and development of the F_1 pups= 30 mg/kg/day
- No clear effects on behavioral assessments & reproductive performance of the F_1 generation at 100 mg/kg/day

Study no.: 1288-006
Volume #, and page #: Electronic submission.
Conducting laboratory and location: (b) (4)

Date of study initiation: 9/1/06
GLP compliance: Yes
QA reports: yes (X) no ( )
Drug, lot #, and % purity: Doxepin HCl, lot 3045911, 100.9%, E: (b) (4), Z: (b) (4) in distilled water

Methods

Doses: 0, 10, 30 & 100 mg/kg

Species/strain: time-mated F Sprague-Dawley rats CD® [Crl:CD®(SD)] (b) (4) 8-10 wks at arrival (GD0)

Number/sex/group: See sponsor's table, below.

Group Assignments

Group Number	Dose Level (mg/kg/day)	Number of Time-mated Female Rats
Main Study		
1	0	25
2	10	25
3	30	25
4	100	25
Toxicokinetic		
5	0	12
6	10	12
7	30	12
8	100	12

Route, formulation, volume, and infusion rate: PO, gavage, QD
On GD6 through LD20
Formulations ±5% nominal

Satellite groups used for toxicokinetics: See sponsor's table above
Study design-Parameters and endpoints evaluated: See sponsor's table below

Endpoint
Parental In-life Data
Premating Body Weights (F_1)
Gestation Body Weights (P, F_1)
Gestation Body Weight Changes (P, F_1)
Gestation Food Consumption (P)
Lactation Body Weights (P)
Lactation Body Weight Changes (P)
Lactation Food Consumption (P)
Fertility Indices
Gestation Length (P)
Copulatory Interval (F_1)
Male Mating Index (F_1)
Female Mating Index (F_1)
Male Fertility Index (F_1)
Female Fertility Index (F_1)
Male Fecundity Index (F_1)
Female Fecundity Index (F_1)
Gestation Index (P)
Uterine Exam
Number Implantations/dam (P, F_1)
Litter Size (F_1)
Viable Embryos (F_1)
Nonviable Embryos (F_1)
Number Resorptions/dam (F_1)
% Preimplantation Loss (F_1)
% Postimplantation Loss (F_1)
F_1 Litter (Pup) Findings
Litter Size
Viable Pups
Pup Sex Ratio (% viable males/litter)
Stillborn Pups
Stillborn Index
Pup Weights
Pup Survival (days 0-4 precull and 4 postcull-21)
Developmental Indices
Pinna Detachment
Eye Opening
Preputial Separation
Vaginal Opening
Behavioral Tests
Static Righting Reflex
Air Drop Righting Reflex
Auditory Response
Cliff Aversion
Motor activity (basic movements, fine movements, rearing, and distance)
Passive avoidance
Number of trials (Passive animals only; Trials = 3, 4, and 5 only)
Incidence of animals passing (Passive vs. Non-passive)
Non-responsive animals

Results

F_0 in-life:

Mortality

One MDF (D16) and 2 HDF (D25 and D37) died during the study. The MDF did not show premonitory clinical signs and had been experiencing normal weight gain before being found dead. Marked body weight loss was noted on D30-33 in the HDF found dead on D37. This HDF showed decreased activity and pupillary dilatation

that were not believed related to the death. However, clinical findings of respiratory distress in both animals and eye closure in one were seen just prior to death and were considered suggestive of dosing trauma by the sponsor. Gross evidence of dosing trauma was not observed at necropsy. All remaining females in the main study and TK groups survived to termination.

Clinical signs

Several treatment-related clinical signs were observed at MD and HD, including: decreased activity, salivation and dilated pupils. See the sponsor's summary tables for details.

Table 1 **Summary of P Gestation Clinical Findings[+]**

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	25	25	25	25
Behavior/Activity				
Activity decreased	0/0	0/0	4/3	380/25
Salivation	0/0	0/0	0/0	24/12
External Appearance				
Material around mouth, Red	0/0	0/0	0/0	2/1
Eye/Ocular				
Pupil dilated, Eye/left	0/0	0/0	0/0	11/7
Pupil dilated, Eye/right	0/0	0/0	0/0	7/5
Pelage/Skin				
Hair discolored, Red, Anogenital region	0/0	0/0	0/0	1/1
Hair sparse, Forelimb/right	0/0	3/1	0/0	0/0
Skin discolored, Blue, Abdominal region	0/0	0/0	0/0	1/1
Unkempt appearance	0/0	0/0	0/0	1/1
Respiration				
Breathing audible	0/0	0/0	0/0	6/1

Table 2 **Summary of P Lactation Clinical Findings[+]**

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	25	24	23	24
Behavior/Activity				
Activity decreased	0/0	0/0	1/1	206/22
Salivation	0/0	0/0	0/0	2/1
External Appearance				
Discharge, Red, Eye/left	0/0	6/1	0/0	0/0
Material around eyes, Red, Eye/left	0/0	1/1	0/0	0/0
Material around nose, Red	0/0	1/1	0/0	0/0
Thin	0/0	0/0	0/0	2/1
Eye/Ocular				
Eyelid partially/completely closed, Eye/left	0/0	0/0	0/0	2/1
Eyelid partially/completely closed, Eye/right	0/0	0/0	0/0	2/1
Pelage/Skin				
Hair sparse, Forefoot/left	13/1	28/4	20/2	0/0
Hair sparse, Forefoot/right	13/1	27/3	20/2	0/0
Hair sparse, Forelimb/left	0/0	14/1	12/1	19/2
Hair sparse, Forelimb/right	0/0	35/2	12/1	19/2
Respiration				
Breathing audible	0/0	0/0	0/0	1/1
Breathing difficult	0/0	0/0	0/0	1/1
Breathing slow	0/0	0/0	0/0	1/1

[+]Number of times observed/Total number of animals affected

Body weight and Food Consumption

Mean body weight in HDF was significantly reduced during gestation and throughout lactation, when compared to controls. In HDF, body weight gain during gestation was also significantly reduced in comparison to controls; however, body weight gain

during lactation was similar to or exceeded that of the controls (48.8 g vs. 42.3 g, respectively) for the entire lactation period (LD 0-21). Lower body weights (gestation and lactation) and body weight gain (gestation) correlated with a decrease in food consumption. See excerpts from the sponsor's summary tables for details.

Table 3 **Summary of P Gestation Body Weight Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD
Body Weight Values g												
	0	212.0	17.05	25	211.8	16.99	24	210.5	16.46	25	213.1	16.52
	6	253.2	20.79	25	253.5	18.59	24	250.6	19.31	25	256.2	19.62
	10	271.8	21.01	25	274.3	19.62	24	268.4	19.28	25	260.8	16.55
	14	298.6	23.51	25	300.3	23.41	24	292.7	21.46	25	281.4[a]	17.12
	17	326.9	26.29	25	329.8	24.99	24	319.5	26.31	24	303.0[b]	19.69
	20	373.9	30.29	25	376.1	28.34	24	367.4	39.27	24	340.0[b]	26.55

Table 4 **Summary of P Lactation Body Weight Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD
Body Weight Values g												
	0	285.4	23.81	25	283.4	21.15	24	273.6	18.68	23	253.3[b]	15.53
	4	297.3	20.72	25	298.5	19.27	24	290.1	18.28	23	269.5[b]	16.12
	7	311.3	22.28	25	314.2	23.34	24	303.7	19.98	23	284.3[b]	15.17
	10	324.3	25.19	25	324.1	21.22	24	313.3	20.64	23	291.4[b]	29.00
	14	333.8	19.97	25	337.9	20.96	24	323.1	15.35	23	306.7[b]	19.03
	17	335.4	20.75	25	340.8	20.28	24	326.6	17.49	23	305.6[b]	27.64
	21	327.7	24.21	25	327.5	22.55	24	317.7	17.46	23	302.0[b]	21.90

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Table 7 **Summary of P Gestation Food Consumption Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD
Food Consumption Values g/animal/day												
	0-6	19.7	2.18	25	19.4	1.77	24	19.2	1.95	25	19.5	1.45
	6-10	21.9	1.68	25	22.3	2.26	24	21.6	2.01	25	19.7[b]	1.96
	10-14	24.4	1.94	25	24.8	2.50	24	23.9	1.90	25	22.4[b]	1.94
	14-17	25.8	1.88	25	26.3	2.61	24	25.7	2.29	24	23.0[b]	2.22
	17-20	27.2	2.25	25	27.6	3.00	24	26.3	3.07	24	22.2[b]	2.32
	6-20	24.6	1.72	25	25.0	2.39	24	24.2	1.98	24	21.7[b]	1.50
	0-20	23.1	1.60	25	23.3	2.12	24	22.7	1.84	24	21.1[b]	1.18

Table 8 **Summary of P Lactation Food Consumption Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD
Food Consumption Values g/animal/day												
	0-4	29.9	4.47	25	31.2	6.75	23	30.5	5.69	23	29.3	6.12
	4-7	42.7	4.12	25	44.1	5.36	24	41.0	5.12	23	40.3	4.58
	7-10	47.5	10.87	25	51.0	5.37	24	47.4	7.36	23	44.2	6.94
	10-14	55.1	6.22	25	60.4[a]	5.82	24	56.3	8.30	23	50.5	7.80
	14-17	64.4	7.70	25	64.3	4.64	23	60.2	10.30	23	54.6[b]	10.70
	17-21	72.6	7.48	25	74.5	6.18	24	70.1	10.10	23	69.6	10.63
	0-21	52.1	4.11	25	54.7	4.65	24	51.1	6.72	23	48.4	6.20

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; (p<0.05)
[b]Significantly different from control; (p<0.01)

Toxicokinetics

Doxepin and nordoxepin plasma concentrations were measured on GD6 and LD20 following daily oral (gavage) administration of doxepin to female rats. In general, plasma concentrations for both doxepin and nordoxepin were variable. Doxepin plasma C_{max} and AUC_{last} increased greater than dose-proportionally on GD 6 and LD 20. Nordoxepin plasma C_{max} and AUC_{last} increased greater than dose-proportionally

on GD 6 and LD 20. Nordoxepin levels on LD20 were greater than on GD6, which suggested the potential for accumulation of nordoxepin with repeated dosing. See the sponsor's summary tables, below.

Summary of Doxepin Plasma Toxicokinetic Parameters in Female Rats

Group	Dosage (mg/kg/day)[a]	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
GD6							
6	10	2.63	2	3	5.03	NE	NE
7	30	11.4	1	6	28.4	35.9	3.0
8	100	261	2	12	896	913	2.3
LD20							
6	10	1.97	1	2	2.12	NE	NE
7	30	30.8	1	6	66.2	71.5	1.9
8	100	243	0.5	12	941	952	1.5

NE: Not estimated, due to insufficient characterization of terminal phase
a: Daily dosage from GD6 through LD20

Summary of Nordoxepin Plasma Toxicokinetic Parameters in Female Rats

Group	Dosage (mg/kg/day)[a]	C_{max} (ng/mL)	t_{max} (h)	t_{last} (h)	AUC_{last} (ng·h/mL)	AUC (ng·h/mL)	$t_{1/2}$ (h)
GD6							
6	10	0[b]	NA	NA	0[b]	NE	NE
7	30	9.11	1	4	19.5	21.0	0.88
8	100	186	2	12	671	677	1.9
LD20							
6	10	12.8	0.5	0.5	3.20	NE	NE
7	30	49.0	1	6	81.9	85.4	1.5
8	100	389	0.5	12	1190	1190	1.2

NE: Not estimated, due to insufficient characterization of terminal phase
NA: Not applicable
a: Doxepin dosage, administered daily from GD6 through LD20
b: All mean concentrations were below the quantitation limit

F_0 necropsy:

Maternal gross pathology

Necropsies were performed on all pregnant females. On GD25, a necropsy of all females that failed to deliver was performed; uteri that appeared nongravid were examined for implantations. On LD21, the dams were subjected to a necropsy and the number of uterine implantation scars was recorded. Implantation summary data are presented in the excerpts of sponsor's table 10, below. The sponsor reported no drug-related macroscopic findings. One HDF showed a severe vaginal obstruction.

Parturition Data

Fertility indices appeared unaffected. Gestation length was significantly longer (22.6 days) in the HDF compared to controls (21.8 days); this is outside of the historical control range for this laboratory (max. 22.5 days). Dams delivering litters numbered 25, 24, 23, and 24 in the control, LD, MD and HD groups, respectively. The mean number of pups (live plus dead)/litter on LD0 in the treated groups ranged from 11.1-11.9, which was slightly less than control (12.4). Stillborn indices in the HD group were approximately doubled, compared to controls. See excerpts from the sponsor's summary table for details.

Table 10 Summary of P Natural Delivery and Litter Data

Endpoint		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
No. Females on Study	N	25	25	25	24
No. Females Pregnant	N	25	24	25	24
Females Delivering Litters[1]	N	25	24	23	24
	%	100.0	96.0	92.0	100.0
With Stillborn Pups[1]	N	4	2	2	5
	%	0.16	0.08	0.09	0.21
With All Stillborn[1]	N	0	0	0	0
	%	0.00	0.00	0.00	0.00
Gestation Length (Days)	Mean	21.8	21.9	22.0	22.6[b]
	SD	0.47	0.34	0.30	0.58
	N	25	24	23	24
No. of Pups at Day 0 (Total Pups Born/Litter)	Mean	12.4	11.7	11.9	11.1
	SD	1.98	1.81	2.78	3.59
	N	25	24	23	24
Liveborn/Litter	Mean	12.1	11.6	11.8	10.5
	SD	2.17	1.86	2.72	3.74
	N	25	24	23	24
No. of Pups at Day 0 cont. Stillborn/Litter	Mean	0.3	0.1	0.1	0.6
	SD	0.74	0.28	0.29	1.38
	N	25	24	23	24
Gestation Index	%	100.0	96.0	92.0	96.0
	N	25	24	23	24
Stillborn Index	Mean %/Litter	2.34	0.82	0.62	5.18
	SD	6.151	2.883	2.074	11.994
	N	25	24	23	24
Total Implantation Scars/Litter	Mean	12.9	12.4	12.4	12.3
	SD	1.88	1.77	2.76	2.99
	N	25	24	23	24

SD - Standard Deviation
N - Number of measures used to calculate mean
No. - Number
[1]Not statistically analyzed
[b]Significantly different from control; (p<0.01)

F_1 Pup Survival and Sex Ratios

The viability index (mean % pups surviving LD 0-4, pre-cull) at HD was significantly lower (76.57%) than controls (98.76%); the value was also outside the historical control range of the laboratory (92.43%-99.47%). The viability index appeared slightly reduced at MD (96.59%). The lactation index (mean % pups surviving LD4/post-cull through LD21) in the HD group (93.66%) appeared reduced compared to controls (99.06%). F_1 pup sex ratios (% male pups/litter) showed slightly increased percentages of male pups in the HD group; this apparent increase was relatively

consistent across days and exceeded the historical control ranges (maximums of 51.7-53.67%). See excerpts from the sponsor's summary tables for details.

Table 10 Summary of P Natural Delivery and Litter Data

Endpoint		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
No. Live Pups/Litter					
Day 4 (Preculling)	Mean	11.9	11.5	11.5	10.3
	SD	2.18	1.77	2.79	2.83
	N	25	24	23	20
Day 4 (Postculling)	Mean	8.0	8.0	7.7	7.6
	SD	0.45	0.20	1.33	1.10
	N	25	24	23	20
Day 7	Mean	7.9	8.0	7.6	7.5
	SD	0.40	0.20	1.31	1.51
	N	25	24	23	20
Day 14	Mean	7.9	8.0	7.6	7.5
	SD	0.44	0.20	1.31	1.17
	N	25	24	23	19
Day 21	Mean	7.9	7.9	7.6	7.5
	SD	0.44	0.28	1.31	1.17
	N	25	24	23	19
Pup Survival Indices					
Viability Index	Mean %/Litter	98.76	99.08	96.59	76.57[b]
	SD	3.563	3.251	8.032	32.562
	N	25	24	23	23
Lactation Index	Mean %/Litter	99.06	99.48	98.97	93.66
	SD	3.275	2.552	3.408	22.427
	N	25	24	23	20
Sex Ratio (% Males per Animal)					
Pups Day 0	Mean %/Litter	44.15	53.85	51.05	51.35
	SD	14.242	12.999	14.474	17.689
	N	25	24	23	23
Pups Day 4 (Preculling)	Mean %/Litter	43.59	53.90[a]	51.44	55.01[a]
	SD	13.701	13.109	13.435	16.565
	N	25	24	23	20
Pups Day 4 (Postculling)	Mean %/Litter	47.22	51.19	51.39	53.55
	SD	8.448	7.697	5.907	13.476
	N	25	24	23	20
Pups Day 21	Mean %/Litter	46.71	51.49	51.32	54.56[a]
	SD	8.307	7.787	6.001	14.487
	N	25	24	23	19

SD - Standard Deviation
N - Number of measures used to calculate mean

[a]Significantly different from control; ($p<0.05$)
[b]Significantly different from control; ($p<0.01$)

F_1 Physical Development:

Clinical signs

During LD0-LD21, drug-related signs were noted, including: decreased activity, skin cold to touch, and pale or blue skin. The frequencies of these signs were evident in the MD and HD groups, attributable to 3 or 4 litters in particular. There was also an increased incidence of slow/difficult breathing in the MD group. The signs were generally seen only on LD0 in the MD group; signs were most frequent on LD0, but occasionally were also seen on LD4, LD7, LD14 and/or LD21, in the HD group. See the sponsor's summary Table 11.

Table 11 **Summary of F_1 Preweaning Pup Clinical Findings[+]**

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	300	278	270	234
Animals with No Abnormalities Detected	296	273	254	190
Behavior/Activity				
Activity decreased	1/1	1/1	6/6	5/5
External Appearance				
Emaciated	0/0	0/0	1/1	1/1
Limb function impaired, Hind limb/left	0/0	0/0	1/1	0/0
Limb function impaired, Hind limb/right	0/0	0/0	1/1	0/0
Swelling, Thoracic region	0/0	0/0	0/0	2/2
Eye/Ocular				
Eye not evident, Eye/left	0/0	1/1	0/0	0/0
Pelage/Skin				
Hair sparse, Entire body	1/1	0/0	0/0	0/0
Nodule, 1-5 mm, Abdominal region	0/0	0/0	0/0	2/1
Scabbed area, Nose/muzzle	0/0	1/1	0/0	0/0
Scabbed area, Shoulder/left	0/0	0/0	1/1	0/0
Skin cold to touch	1/1	1/1	13/13	42/41
Skin discolored, Blue, Abdominal region	0/0	0/0	0/0	1/1
Skin discolored, Blue, Dorsal surface	0/0	0/0	1/1	0/0
Skin discolored, Blue, Entire body	1/1	1/1	8/8	0/0
Skin discolored, Blue, Sacral region	0/0	0/0	1/1	0/0
Skin discolored, Blue, Ventral surface	0/0	0/0	1/1	0/0
Skin discolored, Pale, Entire body	1/1	0/0	0/0	7/7
Respiration				
Breathing difficult	0/0	0/0	8/8	1/1
Breathing slow	0/0	0/0	5/5	0/0

+ - Number of times observed/Total number of animals affected

Body weight

There appeared to be a transient reduction in mean body weights at HD. On LD0 and LD4, significantly lower (9-12%) mean F_1 pup body weights (by sex and combined) were observed at HD, compared to controls. Mean body weights in HD pups were also ~6% lower [nss] than control on LD7. Mean body weights were similar to controls after LD7. See the sponsor's summary table for details.

Table 13 **Summary of F_1 Preweaning Pup Body Weight Values, g**

Study Interval	Sex		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Day 0						
	Males	Mean	6.86 (6.90)	7.01 (6.98)	6.66 (6.65)	6.07 (6.08)[b]
		SD	0.588	0.513	0.420	0.591
		N	25	24	23	22
	Females	Mean	6.47 (6.53)	6.69 (6.67)	6.29 (6.30)	5.80 (5.76)[b]
		SD	0.520	0.527	0.413	0.670
		N	25	24	23	22
	Males + Females	Mean	6.65 (6.70)	6.86 (6.83)	6.47 (6.48)	5.97 (5.93)[b]
		SD	0.551	0.494	0.388	0.620
		N	25	24	23	23
Day 4 Preculling						
	Males	Mean	10.83 (10.93)	11.00 (10.92)	10.34 (10.32)	9.78 (9.78)[b]
		SD	1.078	1.219	0.896	1.602
		N	25	24	23	20
	Females	Mean	10.26 (10.35)	10.46 (10.39)	9.88 (9.86)	9.14 (9.14)[b]
		SD	1.024	1.145	0.816	1.523
		N	25	24	23	20
	Males + Females	Mean	10.50 (10.59)	10.74 (10.67)	10.12 (10.10)	9.52 (9.52)[b]
		SD	1.031	1.138	0.835	1.519
		N	25	24	23	20

Day 4 Postculling						
	Males	Mean	10.79 (10.88)	11.02 (10.94)	10.39 (10.37)	9.77 (9.77)[b]
		SD	1.093	1.207	0.934	1.625
		N	25	24	23	20
	Females	Mean	10.32 (10.40)	10.52 (10.44)	9.85 (9.83)	9.20 (9.20)[b]
		SD	1.010	1.106	0.841	1.579
		N	25	24	23	20
	Males + Females	Mean	10.53 (10.62)	10.77 (10.70)	10.14 (10.12)	9.52 (9.52)[b]
		SD	1.023	1.114	0.846	1.574
		N	25	24	23	20
Day 7						
	Males	Mean	16.27 (16.31)	16.46 (16.43)	15.52 (15.51)	15.25 (15.25)
		SD	1.706	1.851	1.637	2.056
		N	25	24	23	20
	Females	Mean	15.73 (15.79)	15.74 (15.69)	14.85 (14.84)	14.72 (14.72)
		SD	1.379	1.934	1.423	2.295
		N	25	24	23	20
	Males + Females	Mean	15.98 (16.02)	16.09 (16.06)	15.21 (15.20)	15.08 (15.08)
		SD	1.460	1.813	1.446	2.065
		N	25	24	23	20
Day 14						
	Males	Mean	29.67 (29.67)	30.72 (30.73)	30.51 (30.51)	30.05 (30.05)
		SD	3.934	4.162	3.812	3.216
		N	25	24	23	19
	Females	Mean	29.01 (29.03)	30.34 (30.33)	29.64 (29.63)	29.16 (29.16)
		SD	3.211	3.892	3.734	3.409
		N	25	24	23	19
	Males + Females	Mean	29.31 (29.32)	30.51 (30.50)	30.11 (30.11)	29.80 (29.80)
		SD	3.359	3.817	3.567	3.197
		N	25	24	23	19
Day 21						
	Males	Mean	49.37 (49.40)	50.48 (50.45)	50.06 (50.05)	50.11 (50.12)
		SD	5.951	6.503	5.936	5.907
		N	25	24	23	19
	Females	Mean	48.02 (48.09)	49.18 (49.13)	48.36 (48.35)	47.92 (47.93)
		SD	4.934	6.336	5.528	6.360
		N	25	24	23	19
	Males + Females	Mean	48.64 (48.69)	49.86 (49.81)	49.25 (49.23)	49.46 (49.47)
		SD	5.076	6.175	5.430	5.987
		N	25	24	23	19

SD - Standard Deviation ( ) - Least square mean [b] Significantly different from control; (p<0.01)
N - Number of measures used to calculate mean

Macroscopic pathology

Necropsy was performed on all F_1 pups not selected to continue on study. The sponsor recorded no treatment-related macroscopic observations in F_1 pups (stillborn, died while on study, culled on LD4, and LD28 scheduled euthanasia) at necropsy. Of the stillborn pups (2 controlM, 1MDM, 11HDM, 5 controlF, 2LDF, 1MDF and 3HDF), the tissues of 2 controlM, 5 controlF, 2LDF, 1MDF and 1HDF pups were within normal limits but the tissues of the 1MDM, 2HDF and 11HDM pups were too autolyzed to examine. Of the pups that died on study (4, 1, 4 and 25 M and 0, 2, 2 and 26 F in the control, LD, MD and HD groups), 1HDF showed microphthalmia but most tissues were too autolyzed or cannibalized to examine. The external appearances of the LD4 culled pups (37, 52, 47 & 30 M and 63, 32, 42 & 23 F in the control, LD, MD and HD groups, respectively) were not remarkable. On LD28, a broken fibula and a broken tibia were each noted in 1HDM.

Physical Development Landmarks

Data for F_1 pup reflex, sensory, and developmental indices were recorded during lactation, and were generally found to be similar to controls. Beginning on LD2, each pup was tested or observed for static righting reflex (complete righting response

within a 15-sec, retested daily until the criterion met) and pinna detachment (unfolding of the pinna, observed daily until detachment was complete). Beginning on LD11 prior to eye opening, each pup was tested for cliff aversion (observed to perceive depth by moving away from the edge, retested daily until criterion met). Beginning on LD13, each pup was observed for eye opening (considered complete when both eyes were fully open, observed daily until both eyes were opened). Beginning on LD16, each pup was tested for air drop righting reflex (able to turn over in the air and land upright on all four legs when dropped from a height of approximately 30 cm, retested on a daily basis until the response was observed). On LD21 only, after weaning was complete, each pup was given a neurological evaluation (parameters evaluated were comparable to those outlined by Irwin, 1968). Each pup was evaluated at PND22 for auditory response; each pup was observed for movement of the ears in response to a sound emitted from a Galton whistle (3 trials).

The mean age to criteria for static righting reflex, pinna detachment, cliff aversion, eye opening, air drop righting reflex, and auditory response appeared unaffected by doxepin treatment. Air drop righting reflex was slightly delayed (16.5 days vs. 16.1 days) in the HD group, compared to controls. Although not apparent from the mean data, the sponsor appeared to have excluded data from several animals in the HD group on a number of occasions; the reasons for the omissions are unclear. The sponsor' s data for pinna detachment (control and HD) are presented, following the data summary table, for demonstration. Pup neurological evaluations (observations, as outlined by Irwin, 1968) appeared unaffected; urination was the only sign recorded in a few animals across groups. See the sponsor's summary Table 16, following.

Table 16 **Summary of F_1 Physical Development**

Endpoint		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Static Righting Reflex (Days)	Mean	2.5	2.4	2.5	2.4
	SD	0.29	0.31	0.28	0.30
	N	25	24	23	20
Pinna Detachment (Days)	Mean	2.5	2.3	2.4	2.4
	SD	0.47	0.48	0.34	0.49
	N	25	24	23	20
Cliff Aversion (Days)	Mean	11.0	11.0	11.0	11.0
	SD	0.03	0.00	0.00	0.00
	N	25	24	23	20
Eye Opening (Days)	Mean	14.9	15.0	14.9	14.5
	SD	0.64	0.86	0.75	1.00
	N	25	24	23	19
Air Drop Righting Reflex (Days)	Mean	16.1	16.3	16.3	16.5 [a]
	SD	0.16	0.40	0.27	0.47
	N	25	24	23	19
Auditory Response					
Percent Pups Passing/Dam	Mean	100.0	100.0	100.0	100.0
	SD	0.00	0.00	0.00	0.00
	N	25	24	23	19

SD - Standard Deviation
N - Number of measures used to calculate mean
[a]Statistically different from control; ($p<0.05$)

Individual F_1 Physical Development (Pinna Detachment), day of age

Group, Animal Number	No. of Pups	Pup Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14
0 mg/kg/day															
201	8	2	3	2	3	2	2	3	3						
202	8	2	2	2	2	2	2	2	2						
203	8	2	2	2	2	2	2	2	2						
204	8	3	3	3	3	3	2	3	3						
205	8	2	2	2	2	2	3	2	2						
206	8	3	3	3	3	3	3	3	3						
207	8	3	2	2	2	3	3	2	2						
208	8	2	2	2	2	2	2	2	2						
209	8	4	4	4	3	3	3	3	3						
210	8	3	3	3	3	3	3	3	3						
211	8	3	3	3	3	3	3	3	3						
212	8	4	3	4	3	4	3	3	4						
213	8	2	2	2	2	2	2	2	3						
214	8	3	3	3	3	3	3	3	3						
215	8	2	2	2	2	3	2	2	2						
216	8	3	2	2	2	2	2	2	2						
217	8	2	3	2	2	2	3	2	2						
218	8	3	3	2	3	2	3	3	2						
219	8	2	2	2	2	2	2	2	2						
220	7	2	2	2	2	2	2	2							
221	8	3	3	3	3	3	3	3	3						
222	8	3	3	3	3	3	2	3	2						
223[r]	8	2	2	2	2	2	2	3	2						
224	8	3	3	2	2	2	2	2	2						
225	8	3	2	3	2	2	3	4	3						

Negative number - Did not pass test
Positive number - Did pass test
[r] Replacement animal

Individual F_1 Physical Development (Pinna Detachment), day of age

Group, Animal Number	No. of Pups	Pup Number 1	2	3	4	5	6	7	8	9	10	11	12	13	14
100 mg/kg/day															
276[r]	8	2	2	2	2	2	2	2	2						
277	8	3	3	3	3	3	3	3	3						
278	8	3	3	3	2	3	3	3	2						
279	8	2	2	2	2	2	2	2	2						
280	8	3	2	3	3	NA	2	2	2	2					
281	4	2	2	2	2										
284	8	3	3	4	3	4	NA	3	4	4[2]					
285	8	2	2	2	2	2	2	2	2						
286	7	2	2	2	2	2	2	3							
287	8	3	2	2	3	2	2	2	3						
288	8	4	3	3	NA	3	3	3	NA	3[2]	NA	4[2]			
290	10	3	3	3	3	2	3	3	3	4[1]	NA	4[1]			
291	5	2	2	2	2	2									
293	8	2	2	NA	2	2	2	2	2	2					
295	9	4	3	NA	4	NA	3	NA	3	3	NA	NA	4[2]	2	4
296	8	3	2	2	3	2	3	3	2						
297	8	2	2	2	2	2	2	2	2						
298	8	3	3	2	3	3	2	2	3						
299	8	2	2	2	2	2	2	2	2						
300	8	2	2	2	2	2	2	2	2						

Negative number - Did not pass test
Positive number - Did pass test
NA- Not Applicable/Not Available
[r] Replacement animal
[1]Excluded from summary statistics, extra animal
[2]Excluded from summary statistics, not tested previous day

Selection of F_1 pups

Pups were separated by sex on LD21, and remained together as littermates, by sex, until 28 days of age. A minimum of one male and one female pup from each litter in each group was randomly selected to continue on study for assessment of sexual maturation, and behavioral and reproductive performance, for a maximum of 25 males and 25 females per group. If there were less than 25 litters in a group, additional pups were chosen from randomly selected litters from the same group. On PND28, the selected F1 rats (25 males and 25 females per group) were individually weighed and housed. All rats were examined for mortality, clinical observations and

body weights (weekly until termination in M or until positive evidence of copulation was observed in F); during gestation, F body weights were recorded on GD0, 7, 10 and 13.

Post-weaning Clinical Signs and Mean Body Weight

There were no drug-related clinical signs post-weaning (PND28). Mean body weights did not appear to differ significantly between groups on LD28.

Table 14 Summary of F_1 Postweaning Pup Body Weight Values, g

Study Interval	Sex		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Day 28						
	Males	Mean	91.15 (91.17)	89.50 (89.48)	91.58 (91.58)	88.23 (88.23)
		SD	9.103	10.303	8.877	9.339
		N	25	24	23	19
	Females	Mean	84.37 (84.39)	84.45 (84.43)	83.76 (83.75)	81.57 (81.57)
		SD	7.665	9.342	8.131	8.055
		N	25	24	23	19
	Males + Females	Mean	87.49 (87.49)	86.94 (86.94)	87.76 (87.76)	85.54 (85.55)
		SD	7.959	9.239	7.951	8.529
		N	25	24	23	19

Sexual Maturation Landmarks

Pups selected to continue on study for behavioral and reproductive assessment were observed daily beginning on PND28 (F) or PND35 (M) for the presence of vaginal opening or preputial separation, respectively. Age and weight at sexual maturation (vaginal opening or preputial separation) were similar among all groups. See sponsor's Table 17 for details.

Table 17 Summary of F_1 Sexual Maturation

Endpoint		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Vaginal Opening (Days)	Mean	33.6	32.8	33.0	32.8
	SD	2.1	1.51	1.46	1.62
	No. of Pups Passing	25	25	25	25
Body Weight on Day Passed Vaginal Opening, g	Mean	120.3	116.4	117.1	112.5
	SD	19.68	10.82	13.57	11.83
	No. of Pups	25	25	25	25
Preputial Separation (Days)	Mean	44.4	44.1	44.3	44.5
	SD	2.33	2.39	3.03	2.95
	No. of Pups Passing	25	25	25	25
Body Weight on Day Passed Preputial Separation, g	Mean	235.4	232.8	235.0	227.7
	SD	22.32	17.8	26.63	25.72
	No. of Pups	25	25	25	25

No. - Number
SD - Standard Deviation

F_1 Behavioral Evaluation:

Motor Activity

Motor activity was assessed in each pup selected to continue on study. On approximately PND35 (±2), basic movements, fine movements, rearing, and total distance were used to evaluate effects on motor activity. These measures were evaluated over a 20-minute testing interval, segregated into 5-minute units. Motor activity in the treated pups (male and female) was generally comparable to controls.

Table 18 **Summary of F_1 Behavioral Observations (Motor Activity) - MALE**

		0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Study Interval (Minutes)	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Basic Movement (count)	0-5	1435.8	206.23	25	1501.7	212.17	25	1569.6	312.20	25	1407.2	363.14	25
	5-10	933.7	229.28	25	1074.2	256.79	25	1143.1[a]	290.32	25	975.5	389.13	25
	10-15	690.6	236.01	25	835.8	230.46	25	835.6	329.82	25	709.4	316.56	25
	15-20	666.8	362.51	25	685.5	370.67	25	676.8	396.14	25	424.7	307.91	25
	0-20	3726.8	815.69	25	4097.1	836.96	25	4225.0	1111.06	25	3516.8	1122.62	25
Fine Movement (count)	0-5	1072.3	141.00	25	1127.1	140.54	25	1166.9	216.70	25	1037.4	250.99	25
	5-10	746.8	156.62	25	846.7	182.31	25	900.8[a]	200.48	25	765.5	281.97	25
	10-15	572.2	171.54	25	682.6	172.54	25	683.2	245.30	25	580.2	241.09	25
	15-20	546.6	255.48	25	556.8	253.79	25	550.3	286.53	25	356.4[a]	242.17	25
	0-20	2937.9	564.29	25	3213.2	581.87	25	3301.1	794.41	25	2739.5	830.14	25
Rearing (count)	0-5	76.1	11.57	25	79.2	14.13	25	76.8	16.09	25	70.6	18.81	25
	5-10	62.6	14.21	25	64.6	15.71	25	70.3	14.99	25	61.3	24.10	25
	10-15	49.1	18.80	25	55.6	14.59	25	59.9	19.56	25	51.0	24.93	25
	15-20	43.7	21.42	25	49.1	19.88	25	44.6	22.89	25	31.4	24.02	25
	0-20	231.5	53.33	25	248.6	53.73	25	251.6	60.98	25	214.3	75.96	25
Total Distance (cm)	0-5	2408.9	310.31	25	2503.8	323.32	25	2610.7	467.52	25	2388.2	606.76	25
	5-10	1588.4	382.26	25	1789.6	397.41	25	1890.7	431.51	25	1639.8	642.09	25
	10-15	1175.6	388.37	25	1401.7	369.72	25	1403.0	537.30	25	1198.4	532.17	25
	15-20	1128.4	607.64	25	1149.1	621.01	25	1132.0	649.75	25	730.3	528.85	25
	0-20	6301.4	1333.42	25	6844.2	1335.57	25	7036.4	1724.99	25	5956.6	1870.38	25

Table 19 **Summary of F_1 Behavioral Observations (Motor Activity) - FEMALE**

		0 mg/kg/day			10 mg/kg/day			30 mg/kg/day			100 mg/kg/day		
Endpoint	Study Interval (Minutes)	Mean	SD	N	Mean	SD	N	Mean	SD	N	Mean	SD	N
Basic Movement (count)	0-5	1483.0	383.52	25	1614.4	234.73	25	1653.7	305.23	25	1431.4	233.70	25
	5-10	1068.3	438.47	25	1227.5	242.23	25	1178.6	299.06	25	1049.2	271.64	25
	10-15	830.8	355.05	25	824.2	229.61	25	884.2	353.02	25	705.0	338.91	25
	15-20	562.3	330.20	25	729.0	202.62	25	537.0	284.16	25	612.4	270.19	25
	0-20	3944.4	1342.67	25	4395.0	698.79	25	4253.5	999.47	25	3798.2	869.04	25
Fine Movement (count)	0-5	1087.0	264.39	25	1173.9	163.99	25	1193.7	216.18	25	1034.5	158.91	25
	5-10	819.7	317.39	25	934.7	174.21	25	894.6	210.28	25	799.2	182.65	25
	10-15	652.7	263.60	25	651.5	160.42	25	690.4	262.55	25	566.4	234.81	25
	15-20	454.4	248.29	25	579.3	150.75	25	439.6	217.95	25	492.6	200.42	25
	0-20	3013.9	976.15	25	3339.4	501.65	25	3218.2	740.14	25	2892.8	597.89	25
Rearing (count)	0-5	63.4	15.95	25	73.4[b]	10.79	25	73.7[b]	10.14	25	66.1	7.38	25
	5-10	56.3	20.40	25	64.3	9.58	25	61.0	12.08	25	55.7	10.95	25
	10-15	47.0	19.71	25	51.1	13.09	25	48.7	17.37	25	40.0	15.71	25
	15-20	35.2	18.01	25	45.4	14.55	25	34.4	17.06	25	32.9	13.83	25
	0-20	201.9	66.25	25	234.1[a]	35.10	25	217.9	44.00	25	194.7	31.73	25
Total Distance (cm)	0-5	2494.3	633.62	25	2721.2	367.89	25	2756.0	467.42	25	2445.0	370.68	25
	5-10	1794.8	730.47	25	2057.2	361.83	25	1977.0	486.72	25	1779.7	441.33	25
	10-15	1399.2	588.73	25	1382.6	370.40	25	1483.4	575.65	25	1191.4	557.91	25
	15-20	955.4	553.55	25	1244.0	353.37	25	911.6	477.03	25	1041.4	446.09	25
	0-20	6643.6	2247.88	25	7405.0	1078.08	25	7128.0	1605.67	25	6457.5	1381.70	25

Learning and Memory

Learning and memory were evaluated on pups selected to continue on study using the step-through passive avoidance test. Testing was initiated between PND81-83, and were evaluated for a maximum of five trials on the day of testing. The test was conducted in a fully automated, computerized system consisting of light and dark components separated by a mechanical door. In each trial, animals moving to the dark compartment were shocked. Animals were considered to have learned the appropriate response (i.e. not to leave the light compartment) if they did not pass into the dark compartment for two consecutive 3 minute trials. Generally, passive avoidance testing appeared unaffected by treatment with doxepin HCl. The number of males recorded as non-passive appeared increased in the HD group, but similar results were not found in females. The number of trials to criterion may have been slightly increased in HDF, but the results were variable.

Table 20 Summary of F_1 Behavioral Observations (Passive Avoidance) - MALE

Endpoint	0 mg/kg/day Frequency	10 mg/kg/day Frequency	30 mg/kg/day Frequency	100 mg/kg/day Frequency
Number of animals tested^	25	25	25	25
Non-responsive animals	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Passive or non-passive				
Passive	24 (96.0%)	21 (84.0%)	23 (92.0%)	20 (80.0%)
Non-passive	1 (4.0%)	4 (16.0%)	2 (8.0%)	5 (20.0%)
Number of trials(passive animals only)				
3	15 (62.5%)	12 (57.1%)	7 (30.4%)	11 (55.0%)
4	6 (25.0%)	7 (33.3%)	11 (47.8%)	7 (35.0%)
5	3 (12.5%)	2 (9.5%)	5 (21.7%)	2 (10.0%)

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Table 21 Summary of F_1 Behavioral Observations (Passive Avoidance) - FEMALE

Endpoint	0 mg/kg/day Frequency	10 mg/kg/day Frequency	30 mg/kg/day Frequency	100 mg/kg/day Frequency
Number of animals tested^	25	25	25	25
Non-responsive animals	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Passive or non-passive				
Passive	16 (64.0%)	17 (68.0%)	13 (52.0%)	20 (80.0%)
Non-passive	9 (36.0%)	8 (32.0%)	12 (48.0%)	5 (20.0%)
Number of trials(passive animals only)				
3	8 (50.0%)	6 (35.3%)	5 (38.5%)	6 (30.0%)
4	5 (31.3%)	7 (41.2%)	5 (38.5%)	7 (35.0%)
5	3 (18.8%)	4 (23.5%)	3 (23.1%)	7 (35.0%)

F_1 Reproduction:

At or after PND80 and completion of the step-through passive avoidance testing, males and females of the same treatment group were placed together in the cage of the male at a ratio of 1:1 for mating (for 20 days). The day on which evidence of mating (sperm and/or vaginal plug) was observed was designated as GD0. When evidence of mating was observed, the female was removed from the cage and individually housed. Females with no positive evidence of mating were removed from the cage at the end of the mating period and individually housed. Thirteen days after the last day of cohabitation, any unmated females or females with no evidence of mating but determined to be pregnant (based on appearance and weight gain) were subjected to necropsy.

Mortality and Clinical Observations

All F_1 animals selected to continue on study survived to terminal euthanasia. Most clinical observations among pups from the F_1 treated and control groups during the pre-mating, mating, and gestation phase were similar (e.g., sparse hair in several regions); however a few notable differences were observed. Malocclusion was noted in 3 HDM pups, compared to 1 control M pup. A hole in the palate was observed in 1 HDM pup, as was swelling of the nose/muzzle.

Table 22 **Summary of F_1 Clinical Findings[+] - MALE**

Observation	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Alive at Start of Interval	25	25	25	25
Excretion				
Material in pan/bedding, Red	1/1	0/0	0/0	2/2
External Appearance				
Malocclusion	7/1	0/0	2/1	15/3
Material around eyes, Black, Eye/left	1/1	0/0	0/0	1/1
Material around eyes, Black, Eye/right	0/0	0/0	0/0	2/1
Material around eyes, Red, Eye/left	6/1	0/0	2/1	12/4
Material around eyes, Red, Eye/right	3/1	0/0	0/0	8/2
Material around mouth, Red	1/1	0/0	0/0	0/0
Material around nose, Red	3/1	0/0	0/0	0/0
Palate hole	0/0	0/0	0/0	2/1
Swelling, Nose/muzzle	0/0	0/0	0/0	4/1

[+]Number of times observed/Total number of animals affected

Body weight

Mean body weights and body weight gain during the pre-mating, mating, and gestation phases were relatively unaffected by treatment; few statistically significant differences were found. Mean body weight in HDM was approximately 7% lower than that of controlM at the beginning of the pre-mating phase and approximately 5% lower than that of controlM at the end of the post-mating period. See sponsor's summary Table 25 below. In the pre-mating period, mean body weight of the HDF was approximately 7% less than that of controlF at the beginning and was approximately 13% less than controlF by the end. Mean body weights in HDF during gestation were 3-5% lower than those of controls; mean body weight change over GD0-GD13 was reduced ~8%. See sponsor's summary Tables 26 and 27, below.

Table 25 **Summary of F_1 Body Weight Values - MALE**

Endpoint	Study Interval (Week)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD	N
Body Weight Values g													
Premating	1	131.9	17.42	25	126.8	18.98	25	130.2	18.75	25	122.2	13.22	25
	2	195.3	23.24	25	192.5	24.64	25	196.4	24.15	25	183.9	15.96	25
	3	254.0	25.75	25	254.0	29.21	25	254.4	26.29	25	241.8	20.18	25
	4	319.7	28.45	25	321.6	31.57	25	318.6	32.46	25	303.3	24.18	25
	5	368.4	32.61	25	372.6	34.10	25	366.4	33.67	25	345.2[a]	30.68	25
	6	398.8	36.34	25	409.8	35.33	25	399.0	38.53	25	378.4	37.01	25
	7	434.0	39.50	25	447.9	37.58	25	434.0	40.01	25	410.1	43.99	25
	8	459.9	42.42	25	478.6	41.46	25	461.4	43.18	25	436.7	44.96	25
Pairing	9	470.9	42.78	25	490.4	45.72	25	473.2	46.32	25	444.6	47.77	25
	10	489.4	43.87	25	513.5	47.20	25	493.6	46.15	25	468.8	50.70	25
	11	509.1	47.03	25	536.4	49.55	25	511.4	48.59	25	487.8	54.33	25
Postmating	12	529.4	48.86	25	559.4	50.78	25	535.9	50.99	25	505.2	58.71	25

N - Number of measures used to calculate mean
SD - Standard Deviation
[a]Significantly different from control; ($p<0.05$)

Table 26 **Summary of F_1 Premating Body Weight Values - FEMALE**

Endpoint	Study Interval (Week)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD
Body Weight Values g												
	1	115.3	13.37	25	115.7	10.29	25	113.9	13.36	25	107.5	8.81
	2	157.1	15.65	25	162.4	11.75	25	155.7	14.76	25	150.7	11.34
	3	182.0	15.05	25	189.9	13.36	25	182.3	15.64	25	176.8	13.96
	4	207.2	18.32	25	217.2	15.18	25	210.2	18.70	25	204.1	17.01
	5	229.3	19.87	25	239.2	17.22	25	229.6	21.84	25	221.4	19.48
	6	242.7	21.65	25	258.2[a]	19.99	25	245.4	24.27	25	239.7	23.71
	7	258.4	25.47	25	273.1	20.23	25	259.2	26.45	25	251.9	24.79
	8	269.0	22.68	25	283.2	22.71	25	273.1	25.75	25	262.0	26.27
	9	268.7	26.52	18	292.9[a]	23.17	22	279.0	25.98	22	264.6	28.05
	10	314.0	69.30	2	300.6	22.93	7	303.8	32.68	5	272.5	23.33

Table 27 **Summary of F_1 Gestation Body Weight Values**

Endpoint	Study Interval (Day)	0 mg/kg/day Mean	SD	N	10 mg/kg/day Mean	SD	N	30 mg/kg/day Mean	SD	N	100 mg/kg/day Mean	SD
Body Weight Values g												
	0	271.8	30.32	23	290.1	26.52	19	273.2	26.05	21	265.0	26.75
	7	310.9	30.42	23	329.1	32.43	19	309.6	31.22	21	300.6	32.41
	10	326.1	31.90	23	340.9	32.57	19	323.1	33.54	21	311.9	32.77
	13	339.3	31.69	23	355.7	32.36	19	340.4	34.78	21	326.8	32.32

N - Number of measures used to calculate mean [a]Significantly different from control; ($p<0.05$)
SD - Standard Deviation

Macroscopic Pathology

The sponsor recorded no treatment-related macroscopic observations in F_1 animals. At relatively low incidences, small male reproductive organs (epididymides and/or testes) were noted. Pelvic dilatation was noted in the F_1 males. See sponsor's Table 31 for details. No dose-related changes were apparent in females.

Table 31 **Summary of F_1 Macroscopic Observations - MALE**

Tissue / Observation	Severity	Terminal 0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
Number of Animals Examined		25	25	25	25
all tissues					
within normal limits		23	21	22	22
epididymides					
small		0	0	0	2
	- mild	0	0	0	1
	- moderate	0	0	0	1
kidneys					
dilatation, pelvic		0	3	3	1
	- minimal	0	1	1	0
	- mild	0	1	2	1
	- moderate	0	1	0	0
testes					
enlarged		1	1	0	0
	- minimal	0	1	0	0
	- mild	1	0	0	0
small		1	0	0	2
	- mild	1	0	0	1
	- moderate	0	0	0	1

Reproductive Performance

Reproductive performance and fertility of the F_1 animals did not appear to be affected. See the sponsor's summary Table 29, below.

Table 29 Summary of F_1 Reproductive and Fertility Parameters				
Endpoint	0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
No. Females on Study	25	25	25	25
No. Females Paired	25	25	25	25
No. Females Mated	25	24	25	24
No. Pregnant	24	22	25	23
Female Mating Index	100.0	96.0	100.0	96.0
Female Fertility Index	96.0	88.0	100.0	92.0
Female Fecundity Index	96.0	91.7	100.0	95.8
No. Males on Study	25	25	25	25
No. Males Paired	25	25	25	25
No. Males Mated	25	24	25	24
No. Males Impregnating a Female	24	22	25	23
Male Mating Index	100.0	96.0	100.0	96.0
Male Fertility Index	96.0	88.0	100.0	92.0
Male Fecundity Index	96.0	91.7	100.0	95.8
Females with Confirmed Mating Day	24	21	21	23
Copulatory Interval (Days)				
Mean	3.1	4.0	3.5	2.6
SD	2.90	3.41	2.40	1.41
N	24	21	21	23

N - Number of measures used to calculate mean
SD - Standard Deviation

F_2 Findings:

Uterine Examinations (GD13)

There were 23, 19, 21, and 22 pregnant females in the control, LD, MD and HD groups, respectively. Additionally, one, three, four, and one females in the control, 10, 30, and 100 mg/kg/day groups, respectively, were pregnant but the day of mating was not confirmed; data from these pregnancies were not included in GD 13 analyses. Uterine parameters (number of corpora lutea, implantation sites, viable embryos, early and late resorptions, and pre- and post-implantation loss) were not clearly altered by treatment. See sponsor's summary Table 30 for details.

Table 30 **Summary of F_1 Maternal and Developmental Observations at Uterine Examination**

Endpoint		0 mg/kg/day	10 mg/kg/day	30 mg/kg/day	100 mg/kg/day
No. Females on Study		25	25	25	25
No. Not Pregnant		1	3	0	2
No. Pregnant		24	22	25	23
Pregnancy Index Percent		96.0	91.7	100.0	95.8
No. Died Pregnant		0	0	0	0
No. Abortions		0	0	0	0
No. Early Deliveries		0	0	0	0
No. Females with All Resorptions		0	0	0	0
No. Females Pregnant with No Confirmed Mating Date		1	3	4	1
No. Females with Viable Embryos Day 13 Gestation		23	19	21	22
Corpora Lutea					
No. per Animal	Mean	17.3	18.5	17.9	17.3
	SD	3.24	1.95	2.65	3.15
	N	23	19	21	22
Implantation Sites					
No. per Animal	Mean	15.7	16.7	16.1	15.4
	SD	1.92	1.83	1.67	2.08
	N	23	19	21	22
Preimplantation Loss					
% per animal	Mean	8.24	9.35	9.00	9.65
	SD	9.413	8.169	8.718	13.206
	N	23	19	21	22
Viable Embryos					
No. per Animal	Mean	14.8	16.0	15.3	14.2
	SD	2.61	1.89	2.31	2.20
	N	23	19	21	22
Postimplantation Loss					
% Implants per Animal	Mean	6.13	4.07	4.96	7.47
	SD	8.788	4.868	8.247	6.769
	N	23	19	21	22
Nonviable Embryos					
No. per Animal	Mean	0.0	0.0	0.0	0.0
	SD	0.00	0.00	0.00	0.00
	N	23	19	21	22
Litter Size					
No. per Animal	Mean	14.8	16.0	15.3	14.2
	SD	2.61	1.89	2.31	2.20
	N	23	19	21	22
Resorptions: Total					
No. per Animal	Mean	0.9	0.7	0.8	1.1
	SD	1.14	0.82	1.22	1.04
	N	23	19	21	22

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

2.6.7 TOXICOLOGY TABULATED SUMMARY

(from the sponsor's submission, Module 2.4 Nonclinical Overview pg. 4 & 17)

Table 2.4.1.1 Supporting Nonclinical Documentation for Doxepin

Topic	Supporting Information	Source of Information	Source
Primary Pharmacology	Receptor binding and functional in vitro studies; *in vivo* feeding and EEG studies	Literature and Somaxon-conducted studies	Section 2.6.2.2, Reports SP-D0114, SP-D0117
Secondary Pharmacology	*In vitro* and *in vivo* experiments examining effects on ion channels, gene transcription, pain responses	Literature reports	Section 2.6.2.3
Safety Pharmacology	Gastrointestinal, Central Nervous, Respiratory, Cardiovascular systems (*in vitro* and *in vivo*)	Literature	Section 2.6.2.4
Pharmacokinetics and Metabolism	Absorption	Literature	Section 2.6.4.3
	Distribution	Literature and Somaxon-conducted studies	Section 2.6.4.4, Report SP-D0115
	Metabolism and Excretion (*in vitro* and *in vivo*);	Literature and Somaxon-conducted study	Sections 2.6.4.5, 2.6.4.6; Report SP-D0118
	Toxicokinetics (supporting new toxicity studies)	Somaxon-conducted Studies	Reports SP-D0103, SP-D0105, SP-D0106, SP-D0107, SP-D0108,SP-D0109, SP-D0110
Toxicity	Single-Dose	Literature	Section 2.6.6.2, Report SP-D0103
	Repeat-Dose (range-finding studies)	Literature and Somaxon-conducted Studies	Section 2.6.6.3, Reports SP-D0104, SP-D0105, SP-D0110, SP-D0112
	Genotoxicity studies (Reverse Mutation, Chromosomal Aberration, Micronucleus)	Literature and Somaxon-conducted Studies	Section 2.6.6.4, Reports SP-D0101, SP-D0102, SP-D0103
	Developmental Toxicity Studies (Fertility and early embryonic development, Embryo-fetal development, Prenatal and postnatal development)	Somaxon-conducted Studies	Section 2.6.6.6, Reports SP-D0106, SP-D0107, SP-D0108, SP-D0109
	Carcinogenicity	Somaxon-conducted Studies	SP-D0111, SP-D0113 (in progress)
	Other	Literature	Section 2.6.6.8

Table 2.4.4.1 Overview of the Doxepin Toxicology Program

Study Topic	Study Number	Concentration or Dose	Tabulated Summary
Single-Dose Toxicity			
Single-Dose Range Finding in the Rat (for Micronucleus assay below)	SP-D0103	100, 200, 400, 800 mg/kg	2.6.7.9
Repeat-Dose Toxicity			
28-Day Toxicity and Toxicokinetic Study in CByB6F1 Mice with a Preliminary 5-Day Rangefinding Study	SP-D0110	10, 25, 50, 100, 150 mg/kg/day (5-day); 10, 25, 50 mg/kg/day (28-day)	2.6.7.6
2-Week Oral Dose-Range Finding in Rats (for reproductive toxicity studies below)	SP-D0104	10, 30, 100, 300/200 mg/kg/day	2.6.7.6
13-Week Oral Toxicity Study in the Rat (for carcinogenicity study below)	SP-D0112	10, 25, 50, 100 mg/kg/day	2.6.7.6
2-week Oral Dose-Range Finding and Toxicity Study in Rabbits (for reproductive toxicity study below)	SP-D0105	10, 20, 25, 30, 100, 200 and 300 mg/kg/day	2.6.7.6
Genotoxicity			
In Vitro Studies			
Salmonella and E. coli Mammalian-Microsome Reverse Mutation Assay	SP-D0101	10.0-5000 µg/ plate (with S9 mix); 3.33- 5000 µg/plate (w/o S9 mix)	2.6.7.8A
Chromosomal Aberrations in Cultured Human Peripheral Blood Lymphocytes	SP-D0102	15.0- 45.0 µg/mL (w/o metabolic activation) ; 80.0- 150 µg/mL (w/ metabolic activation)	2.6.7.8B
In Vivo Studies			
In Vivo Rat Micronucleus Assay	SP-D0103	50, 100, 200 mg/kg	2.6.7.9
Carcinogenicity			
26-Week Repeated Dose Oral Carcinogenicity Study in Tg.rasH2 Mice	SP-D0111	25, 50, 75, 100 mg/kg/day	2.6.7.10
2-Year Repeated Dose Oral Carcinogenicity Study in Rats	SP-D0113	15, 30, 75 mg/kg/day	Study ongoing
Reproductive and Developmental Toxicity			
Fertility and Early Embryonic Development in the Rat	SP-D0106	10, 30, 100 mg/kg/day	2.6.7.12
Embryo-fetal Development in the Rat	SP-D0107	30, 100, 150 mg/kg/day	2.6.7.13A
Embryo-fetal Development in the Rabbit	SP-D0108	10, 30, 60 mg/kg/day	2.6.7.13B
Pre- and Postnatal Development in the Rat	SP-D0109	10, 30, 100 mg/kg/day	2.6.7.14

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Please see the Executive Summary.

APPENDIX/ATTACHMENTS

Appx. A: ECAC Meeting Minutes- Results of a 26-Week Transgenic Mouse Carcinogenicity Assay
Appx. B: ECAC Meeting Minutes- 2-year Rat Carcinogenicity Bioassay Protocol

Appendix A: ECAC Meeting Minutes- Results of a 26-Week Transgenic Mouse Carcinogenicity Assay

Executive CAC
Date of Meeting: November 4, 2008

Committee:	David Jacobson-Kram, Ph.D., D.A.B.T., OND IO, Chair Abby Jacobs, Ph.D., OND IO, Member Paul Brown, Ph.D., OND IO, Member Barbara Hill, Ph.D., DDDP, Alternate Member Lois M. Freed, Ph.D., DNP, Supervisory Pharmacologist Melissa K. Banks, Ph.D., DNP, Presenting Reviewer
Coordinator:	Sam Habet, R.Ph., Ph.D., OND IO, Senior Clinical Pharmacologist/ Science Policy Analyst (Detail)
Author of Draft:	Melissa K. Banks, Ph.D.
NDA #:	22-036
Date of Submission:	January 30, 2008
Drug Name:	Silenor™, doxepin hydrochloride
Sponsor:	Somaxon Pharmaceuticals

The following information reflects a brief summary of the Committee discussion and conclusions:

Doxepin is a tricyclic compound exerting histamine (H_1) receptor antagonism, which is currently being developed as a sedative-hypnotic; it is FDA approved as an antidepressant and anxiolytic (as Sinequan®) and for the treatment of atopic dermatitis & lichen simplex chronicus (as Zonalon®). Based on results of an *in vitro* bacterial reverse mutation assay, an *in vitro* chromosomal aberrations assay (HPBL) and an *in vivo* rat micronucleus assay, doxepin is not genotoxic. To evaluate the potential for carcinogenicity, the sponsor performed a 26-week transgenic mouse assay in Tg.rasH2 mice; Executive CAC concurrence on the doses used in the study was not requested prior to initiation of the study.

Mouse Carcinogenicity Study
Doxepin was administered orally (by gavage) at doses of 0 (vehicle: water for injection), 25, 50, 75 and 100 mg/kg in male and female transgenic Tg.rasH2 mice for 26 weeks. Survival rate was not significantly affected, although mortality rate was slightly increased in high dose males. A slight but statistically significant and dose-related decrease in mean body weights was observed. At the high dose, mean body weight was reduced by 9-13% compared to controls. The high dose appeared to be an MTD in males and females, based on body weight and clinical signs; data from previous studies indicate that higher doses were not tolerated. Histopathological evaluation of a full battery of tissues was performed on all control and doxepin-treated groups. Neoplasms were detected in the nasal cavity (adenocarcinomas), lung (adenomas and carcinomas) and spleen (hemangiosarcomas), but not in a dose-related manner. The sponsor considered the occurrence of nasal cavity and splenic tumors to be "noteworthy", but concluded that

doxepin was not tumorigenic. Urethane-treated positive controls were used to verify the sensitivity of the assay; the expected increases in pulmonary and splenic neoplasms were observed.

Executive CAC Conclusions

The Committee concurred that the study was adequate and that there were no drug-related neoplasms.

David Jacobson-Kram, Ph.D., D.A.B.T.
Chair, Executive CAC

cc:\
/Division File, DNP
/LFreed, DNP
/MBanks/Reviewer, DNP
/CMichaloski/CSO/PM, DNP
/DJacobson-Kram/OND, IO
/SHabet/OND IO

Appx. B: ECAC Meeting Minutes- 2-year Rat Carcinogenicity Bioassay Protocol

Executive CAC
Date of Meeting: July 31, 2007

Committee: Abby Jacobs, Ph.D., OND IO, Acting Chair
Joseph Contrera, Ph.D., OPS, Member
Anne Pilaro, Ph.D., DBOP, Alternate Member
Ed Fisher, Ph.D., DNP, Acting Supervisory Pharmacologist
Melissa Banks, Ph.D., DNP, Presenting Reviewer

Author of Draft: Melissa Banks, Ph.D., DNP:

The following information reflects a brief summary of the Committee discussion and its recommendations.

The Committee did not address the sponsor's proposed statistical evaluation for the 2-yr carcinogenicity bioassays, as this does not affect the sponsor's ability to initiate the bioassays. The sponsor may seek guidance on the statistical evaluation of bioassay results from agency staff separately. Data files should be submitted electronically following the CDER/CBER Guidance for Industry, Providing Regulatory Submission in Electronic Format – Human Pharmaceutical Product Applications and Related Submissions Using the eCTD Specifications (April 2006).

IND #67,162
Drug Name: doxepin HCl, proposed Silenor™
Sponsor: Somaxon Pharmaceuticals, Inc.

Doxepin HCl is a tricyclic dibenzoxepin with anti-histaminergic properties (also anti-cholinergic and anti-serotonergic properties). It is currently approved at recommended doses from 75 mg up to a maximum of 300 mg/day (~5 mg/kg/day) for treatment of depressive disorders and short-term management of moderate pruritis in adult patients with atopic dermatitis or lichen simplex chronicus (as Sinequan® capsules/liquid and Zonalon® cream, respectively). The current patient population is limited in comparison to the potential population under IND #67,162 for treatment of insomnia. The daily maximum clinical dose of doxepin for the treatment of insomnia is anticipated to be 6 mg/day, which is equivalent to 0.1 mg/kg for a 60 kg subject. The sponsor is seeking agency concurrence on dose selection for a 2-year oral gavage carcinogenicity study in Sprague-Dawley rats. Data from a 13-week oral gavage toxicity study in Sprague-Dawley rats was used as the basis for dose selection. Doxepin was negative in a standard battery of genetic toxicology studies (in vitro Ames and chromosome aberration [in HBPL] assays, and an in vivo rat micronucleus assay).

Rat Carcinogenicity Study Protocol and Dose Selection
The sponsor proposed doses of (b) (4) and 75 mg/kg/day for both male and female Sprague Dawley rats. The sponsor based their dose selection on MTD and AUC, citing decreased body weight gain at 100 mg/kg in their supporting 13-week toxicity study in rats and adequate AUC safety margins to expected human plasma exposures, covering the range from nearly therapeutic concentrations to large multiples of human plasma concentrations.

Executive CAC Recommendations and Conclusions:

Rat:
The Committee concurred with the sponsor's proposed high dose, but not the lower doses. The Committee recommended oral (gavage) doses of 0, 15, 30, and 75 mg/kg/day for both males and females. The recommended high dose was based on MTD from the supporting 13-week toxicity study in rats (decreased body weight gain), but the mid and low doses were adjusted to provide a broader range of doses. The Committee noted that TK analysis (to include quantitation of parent compound and all major human circulating metabolites) should be conducted only in satellite groups of animals. Hematology parameters do not need to be assessed.

Abigail Jacobs, Ph.D.
Acting Chair, Executive CAC

cc:\
/Division File, DNP
/Lois Freed/Supervisor, DNP
/Melissa Banks/Reviewer, DNP
/Cathleen Michaloski/CSO/PM, DNP
/ASeifried, OND IO

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

/s/

Melissa Banks
2/25/2009 04:54:42 PM
PHARMACOLOGIST

Lois Freed
2/25/2009 05:28:41 PM
PHARMACOLOGIST