INVESTIGATIONAL DRUG BROCHURE
for

POSIPHEN®

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LIST OF ABBREVIATIONS

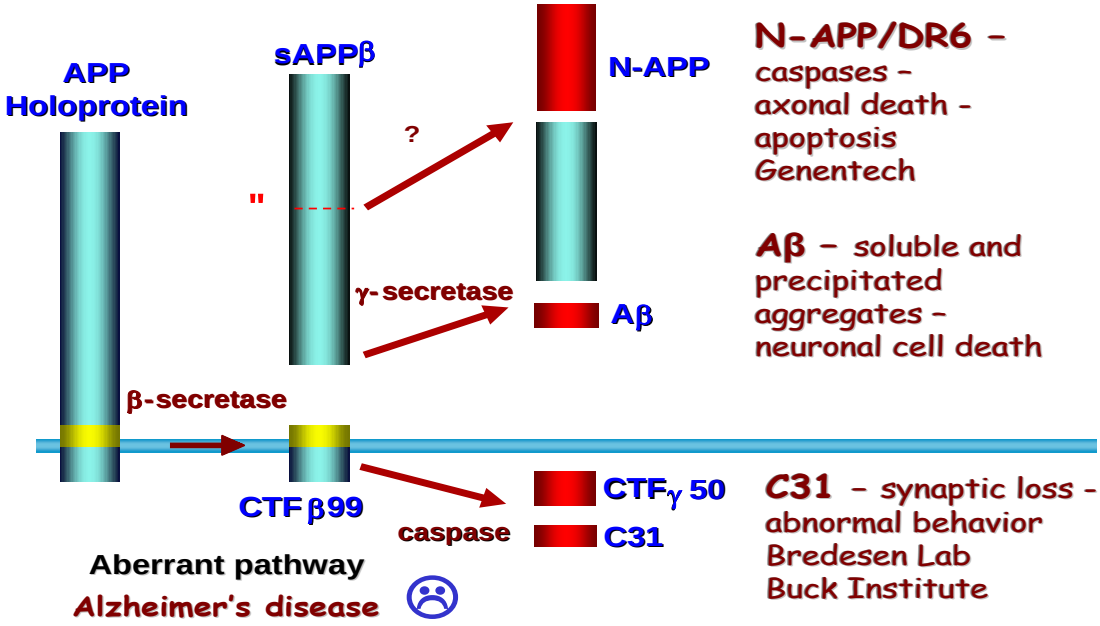
AChE	Acetylcholinesterase
AD	Alzheimer's disease
AE	Adverse Event
APLP-1	Amyloid-like Protein Precursor-1
APP	Amyloid Precursor Protein
βAPP	Beta-Amyloid Precursor Protein
N-APP	Amino Terminal Fragment
Aβ	Beta-Amyloid
AUC	Area under the curve
BChE	Butyrylcholinesterase
BFCN	Basal forebrain cholinergic neurons
BP	Blood pressure
C_{max}	Maximum plasma and CSF concentration
CFR	Code of Federal Regulations
ChE	Cholinesterase
Cl	Chloride
CSF	Cerebrospinal Fluid
DFB	Diisopropylfluorophosphate
ECG	Electrocardiogram
EDTA	Ethylenediaminetetraacetic Acid
FDA	Food and Drug Administration
GFAP	Glial fibrillary acidic protein
GLP	Good Laboratory Practice
HCl	Hydrochloric Acid
HEK	Human Embryonic Kidney Cells
hERG	Human Ether-a-go-go Related Gene
H_2O	Water
HPBL	Human peripheral blood lymphocytes
HPLC	High Pressure Liquid Chromatography
HR	Heart Rate
IC_{50}	Inhibition Coefficient 50%
ICH	International Conference on Harmonisation
IND	Investigational New Drug Application
k_e	Terminal elimination rate constant
LC/MS/MS	Liquid Chromatography/Mass Spectrometry/Mass Spectrometry
LLOQ	Lower limit of quantitation
pM	Picomolar
MAD	Multiple Ascending Dose
MCI	Mild Cognitive Impairment
MedDRA	Medical Dictionary for Regulatory Activities
MMS	methyl methanesulfonate
MS	Mass spectrometry
MTD	Maximum tolerated dose
Na	Sodium
NaOH	Sodium Hydroxide
NGF	Nerve Growth Factor

NIA	National Institute on Aging, under the NIH
NIH	National Institutes of Health
NOAEL	No-observed-adverse-effect-level
PD	Pharmacodynamic
PI	Principal Investigator
PK	Pharmacokinetic
POM	Proof of Mechanism
QID	Four-times-per-day
QT/QTc/QRS/PR/RR	Specified ECG Measurements
RBC	Red Blood Cell
mRNA	Messenger Ribonucleic Acid
SAD	Single Ascending Dose
SAE	Serious Adverse Event
SD	Sprague Dawley rat
sd	Standard Deviation
SP	Senile Plaques
αSYN	Alpha Synuclein
$t_{1/2}$	Half-life
Tmax	Time to Maximum Concentration
UTR	Untranslated Region
Vss	Volume of distribution at steady-state
WB	Whole blood
WBC	White blood cells

1.0 INTRODUCTION

Posiphen, an orally bioavailable small molecule derived via a biochemical synthetic pathway, was discovered at the National Institutes of Aging (NIA). It was selected from a series of structurally-related compounds designed for Aβ and APP specificity with minimal acetylcholinesterase (AChE) inhibitory activity. Posiphen is the chirally pure, positive (+) enantiomer of Phenserine. However, whereas Phenserine is an AChE inhibitor, Posiphen lacks AChE activity; instead, it inhibits the translation of APP and selectively inhibits beta-amyloid (Aβ) and beta-amyloid precursor protein (βAPP) production. This mode of action is postulated to have utility as a disease modifying treatment for Alzheimer's disease (AD).

A major histopathological hallmark of AD is the appearance of senile plaques composed of aggregated forms of Aβ derived from APP by β- and γ-secretases. The assembled forms of Aβ have been causally associated with neurological damage in results from *in vitro* and *in vivo* experimental pharmacology studies and hence represent a target for drug development in AD treatment.



In the case of all three toxic peptides, reducing APP synthesis could be beneficial to the brain, because through the Aβ pathway neurotoxic plaque formation is reduced, and through the

inhibition of toxic N- and C- terminal fragments nerve cell death is inhibited and brain cells are preserved.

Posiphen [(+)-phenserine] as well as its chiral isomer, Phenserine [(-)-phenserine][1], significantly reduce APP and Aβ *in vitro* using human neuroblastoma cell cultures and in animals.[2] The amount of newly synthesized APP was lower (up to 60%) following incubation with Posiphen giving an IC_{50} value of approximately 670 nM. Posiphen and Phenserine both reduce newly synthesized APP without changing APP mRNA levels, resulting in significant reductions of Aβ levels. Recent studies aligning and comparing the 5'-UTRs (untranslated region) of the *APP* gene in man and other species, and comparing these to APP-like sequences have demonstrated a close homology between the human and rodent 5'-UTR of *APP* and a lack of similarity to those of *APLP-1* (amyloid-like protein precursor-1) and *APLP-2*.[3] These results suggest that Posiphen can decrease Aβ levels by post-transcriptional regulation of APP levels and can reduce Aβ without affecting important proteolytic pathways.

In light of the evidence that it is an inhibitor of amyloid precursor protein (APP) synthesis[4], Posiphen is being developed by QR Pharma as a potential disease modifying treatment for AD. Through APP inhibition, Posiphen may halt or slow disease progression by reducing APP levels and accordingly β amyloid (Aβ), the substrate available for formation of toxic oligomers. Evidence in the literature suggests that targeting the accumulation of Aβ, a hydrophobic, neurotoxic self-aggregating 40 to 42 amino acid peptide that accumulates preferentially within senile plaques (SP) in the brain, could change the course of AD[5].

In addition, newer research shows that in the absence of neurotrophic factors, APP is shed from the surface of neuronal cells and processed into an amino terminal fragment (N-APP) that

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[1] Phenserine is an AChE inhibitor which has been studied in Phase III clinical trials for mild to moderate Alzheimer's disease. Posiphen is 100- to 1000-fold less active in inhibiting human AChE or BChE *in vitro* compared to Phenserine.
[2] Shaw *et al.* (2001), Phenserine regulates translation of β-amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. Proceedings of the National Academy of Sciences 98: 7605 – 7610.
[3] Maloney *et al*. (2004), Presence of a "CAGA box" in the *APP* gene unique to amyloid plaque-forming species and absent in all *APLP*-1/2 genes: implications in Alzheimer's disease. FASEB 18: 1288 – 1290.
[4] Lahiri DK, Chen D, Maloney B, Holloway HW, Yu QS et al (2007) JPET 320: 386 – 396.
[5] D.J.Selkoe, Arch Neurol 62, 2005, 192-195.

binds to DR6 receptors and induces nerve cell death[6]. This processing initiates a cascade that leads to the subsequent cleavage of other toxic APP fragments. The Bredesen Lab identified another factor that is cleaved from the C-terminal end of APP (C31) and causes nerve cell degeneration and death in tissue culture cells and in transgenic mice[7].

Posiphen has been shown to reduce APP and/or Aβ production in relevant preclinical in vitro and in vivo studies. The preclinical safety of Posiphen has been established in toxicology studies in rats and dogs for up to 30 days. The clinical safety of Posiphen has also been studied in two Phase I studies in healthy human volunteers, and an exploratory study in patients with amnestic mild cognitive impairment (MCI).

[6] A. Nicolaev et al, Nature, Vol 457, 2009, 981-990
[7] V. Galvan et al. PNAS , Vol 103 , No. 18, 2006, 7130–7135

2.0 PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

Posiphen ((+)-phenserine) is the chirally pure positive (+) enantiomer of (-)-phenserine (Phenserine). Posiphen is an orally bioavailable small molecule derived via a biochemical synthetic pathway. Thus far in the development program, Posiphen as the Tartrate salt has been used in preclinical and clinical studies. Currently, the drug product is prepared as an oral dosage form, in hard gelatin capsules containing Posiphen as the Tartrate salt, without excipients or fillers. Posiphen is stable at room temperature (15-30°C; 59-86°F).

Compound Name: Posiphen® Tartrate [(+)-phenserine]

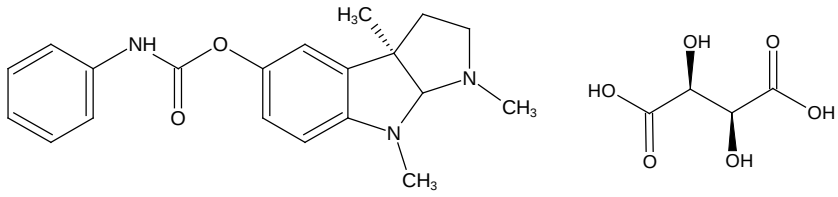
Chemical Name: (3a*R*,8a*S*)-1,2,3,3a,8,8a-hexahydro-1,3a,8-trimethylpyrrolo[2,3-*b*] indol-5-ol phenylcarbamate (ester), (2*R*,3*R*)-dihydroxybutanedioate (salt)

CAS Number: [609800-41-1]

Molecular Formula: $C_{20}H_{23}N_3O_2 \cdot C_4H_6O_6$

Molecular Weight: 487.5

Chemical Structure:



Posiphen . D-Tartrate

Appearance: White to off-white crystalline solid.

Solubility: Highly soluble in H_2O, 0.1N HCl and 0.1N NaOH.

3.0 NON-CLINICAL STUDIES

3.1 Pharmacology

The pharmacological activity of Posiphen [(+)-phenserine] was examined *in vitro* and *in vivo* and was compared to its chiral isomer, Phenserine [(-)-phenserine]. Both Posiphen and Phenserine reduced βAPP and Aβ in these models and although Posiphen appears to be as potent against Aβ as Phenserine, it may offer an advantage relative to manifestations from acetylcholinesterase (AChE) inhibition. Following is a summary of the results of in vitro and in vivo pharmacodynamic (PD) studies.

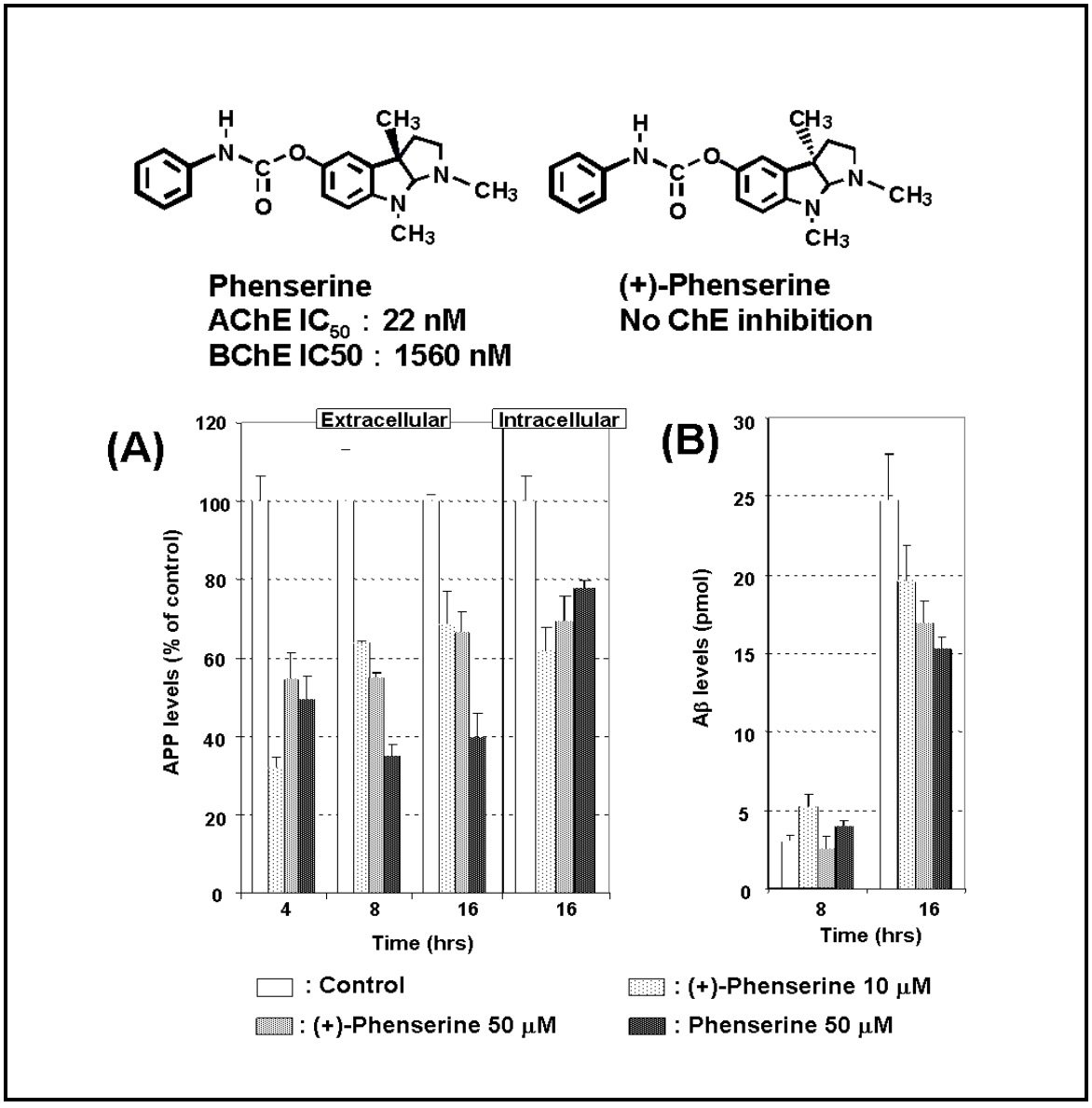
3.1.1 In Vitro Activity

In vitro studies have focused on the characterization of the effects of Posiphen and Phenserine on βAPP and Aβ using neuroblastoma cell cultures. The cholinesterase (ChE) inhibitor, Phenserine, was originally designed and developed as a selective inhibitor of acetylcholinesterase *versus* butyrylcholinesterase, while its chiral isomer, Posiphen was found to have substantially less ChE inhibitory activity. Additionally, both Posiphen and Phenserine were designed with a high but balanced, lipophilicity (log p value = 2.22) to provide a high blood-brain barrier penetrability and brain uptake (10:1 brain: plasma ratio).

It has been reported that Posiphen decreases βAPP levels *in vitro* in a time- and dose-dependent manner, resulting in significant reductions of Aβ without affecting the signaling pathways regulating βAPP processing.[8] The effect of Posiphen on βAPP and Aβ levels in human neuroblastoma cells (SK-N-SH) is shown in **Figure 3.1-1**. Posiphen significantly reduced βAPP and Aβ in a time- and concentration-dependent manner, similar to that of Phenserine. The IC_{50} to lower βAPP was approximately 670 nM (216 ng/mL).

[8] Shaw *et al.* (2001), Phenserine regulates translation of β-amyloid precursor protein mRNA by a putative interleukin-1 responsive element, a target for drug development. Proceedings of the National Academy of Sciences 98: 7605 – 7610.

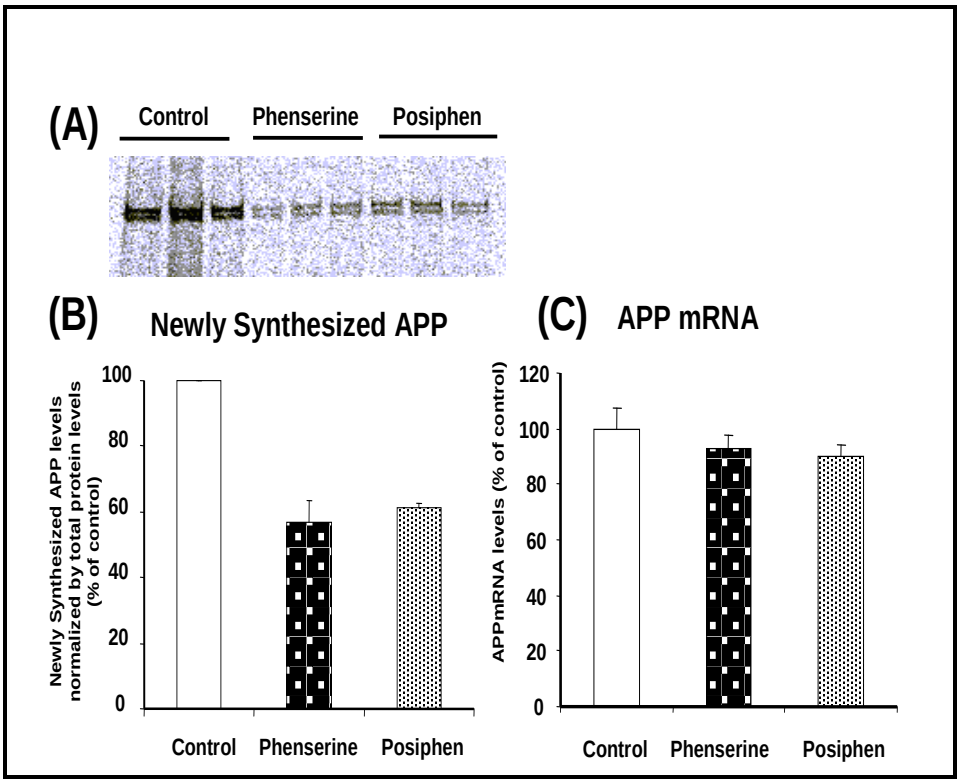
Figure 3.1-1: Effects of Posiphen [(+)-phenserine] on Steady State Levels of βAPP and Aβ in Human Neuroblastoma Cells



SK-N-SH cells were treated with and without Posiphen [(+)phenserine] or Phenserine. The extra- and intracellular βAPP were determined by Western blots (A) and Aβ levels were quantitated by ELISA (B).

To further investigate the post-transcriptional or post-translational effects of Posiphen, the rate of βAPP protein synthesis was assessed with and without Posiphen or Phenserine using human neuroblastoma (SHSY-5Y) cells. Newly synthesized βAPP was significantly decreased by 40% following Posiphen or Phenserine treatment (**Figure 3.1-2A and 3.1-2B**) but neither treatment changed βAPP mRNA levels (**Figure 3.1-2C**) or global protein synthesis.

Figure 3.1-2: Effects of Posiphen on Newly Synthesized βAPP Levels in Human Neuroblastoma Cells



After treatment with 10 μM of Posiphen or Phenserine for 16 hrs, newly synthesized βAPP was assessed by ^{35}S-radio labeling for 10 min followed by immunoprecipitation (Ab2072, Abcam) of βAPP protein (A). The newly synthesized βAPP levels (A) were quantitated by densitometric analysis and normalized with newly synthesized total protein by TCA precipitable counts (B). βAPP mRNA levels were assessed by quantitative RT-PCR (C).

These results indicate that Posiphen does not act on transcription, but down-regulates βAPP expression at either the post-transcriptional or post-translational level.

3.1.1.1 Effects on Acetylcholinesterase (AChE) and Butyrylcholinesterase (BChE)

The inhibitory activity of Posiphen (0.3 nM to 10 µM) was assessed *in vitro* using freshly prepared AChE and BChE obtained from human red blood cells (RBCs). Sodium phosphate buffer and physostigmine (0.3 nM to 10 µM) served as the negative and positive controls, respectively. The IC_{50} values were determined from a regression analysis of the plot of the log concentration of the test substance versus percent inhibitory activity.

Following incubation with Posiphen (25 min/37°C), there was minimal inhibition of human AChE or BChE compared to physostigmine or the optical isomer of Posiphen (Phenserine) as presented in **Table 3.1-1**.

Table 3.1-1: IC_{50} Value (nM) of Posiphen on AChE and BChE Activities

Agent	IC_{50} value (nM)	
	AChE	**BChE**
Posiphen tartrate	15660 ± 1600	27890 ± 1415
Physostigmine hemisulfate	15.0	6.7
Phenserine tartrate[a]	22 ± 1.4	1560 ± 45
Donepezil[a]	22 ± 8	4150 ± 1700

For Posiphen tartrate, assays conducted in duplicate on three separate occasions and for physostigmine, the assays were conducted in duplicate once.
[a] For Phenserine tartrate and donepezil, the results presented in this Table are taken from a study of identical design conducted at this same laboratory but on a separate occasion. These data are being here cited for comparative purposes only. In that study, the IC_{50} value for physostigmine was 28.2 ± 2 for inhibition of acetylcholinesterase and 16 ± 3 for inhibition of butyrylcholinesterase.

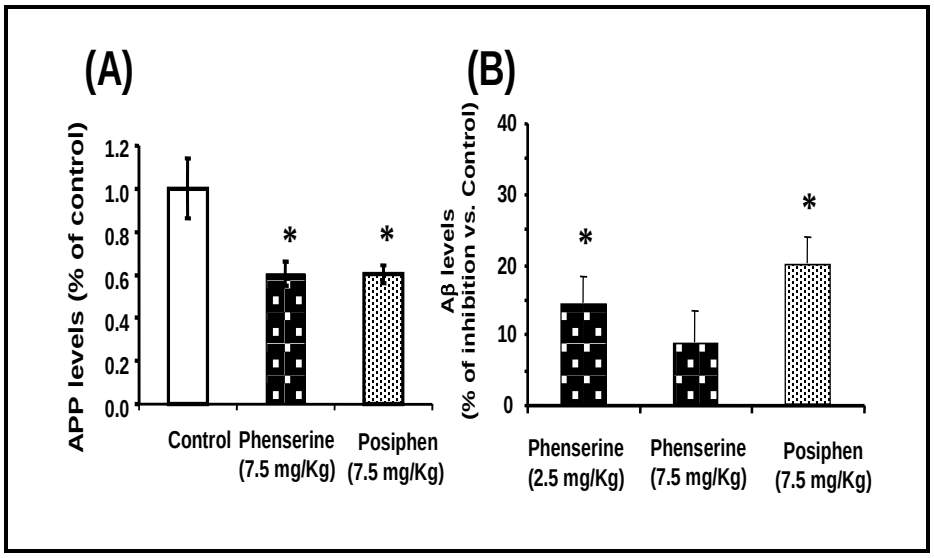
The IC_{50} value for Posiphen in inhibiting human acetylcholinesterase (15660 nM) differs from the earlier published IC_{50} value of 3500 nM.[9] The reason for this difference in IC_{50} values may be related to the chiral purity of Posiphen that was used in the earlier assay.[10] In summary, Posiphen was shown *in vitro* to minimally affect human AChE or BChE activity.

[9] Yu *et al.* (1998), Syntheses and Anticholinesterase Activities of (3a*S*)-*N*1, *N*8-Bisnorphenserine, (3a*S*)-*N*1, *N*8-Bisnorphysostigmine, Their Antipodal Isomers, and Other Potential Metabolites of Phenserine. J. Med. Chem. 41: 2371 – 2379.
[10] In the earlier study, a minute amount of phenserine (with IC_{50} ~24 nM for acetylcholinesterase) may have been present (even as little as 0.1%) and consequently may have dramatically affected the measured activity.

3.1.2 In Vivo Assessments

To further assess the significance of the *in vitro* findings, *in vivo* studies were conducted in normal C57Blk mice. Groups of mice were administered intraperitoneal doses of either Posiphen tartrate or Phenserine tartrate for 21 days. The results showed that Posiphen tartrate at 7.5 mg/kg/day significantly reduced the βAPP by 40% (**Figure 3.1-3**). However, Aβ was reduced to a greater extent by Posiphen than by an equivalent dose of Phenserine.

Figure 3.1-3: Effects of Posiphen on βAPP and Aβ Levels in Mice



Posiphen or Phenserine (2.5 and 7.5 mg/kg) were injected intraperitoneally to C57Blk mice for 21 days. Brain samples were then collected for analysis of βAPP and Aβ levels by Western blot and by ELISA, respectively. The values presented for % of control are given in terms of a ratio = 1.0 (100%).

**p<0.05, compared to the control group*

Posiphen tartrate was sufficiently well tolerated to allow the intraperitoneal dose to be escalated to 75 mg/kg/day for 21 days (compared to only 7.5 mg/kg/day for Phenserine). In another study in which Posiphen tartrate was administered intraperitoneally at doses up to 75 mg/kg/day using a paradigm similar to that in **Figure 3.1-3**, βAPP and Aβ were further reduced by up to 60%[4].

3.1.2.1 Posiphen Lowered APP and Protected Neural Differentiation In Vivo

Human neural stem cells (HNSCs) transplanted into aged rats differentiated into neural cells and reversed age-associated cognitive impairment in these animals[11]. However, HNSCs exposed to high concentrations of APP *in vitro* differentiated mainly into astrocytes, instead of neurons[12]. APP and glial fibrillary acidic protein (GFAP) levels in the hippocampus of APP23 mice were reduced after 14 days treatment with Posiphen (25 mg/kg i.p.). No significant change in APP gene expression was detected, suggesting that Posiphen decreased APP levels by posttranscriptional regulation. When HNSCs were transplanted into Posiphen-treated APP23 mice, followed by an additional 7 days of treatment with Posiphen, the HNSCs migrated and differentiated into neurons in the hippocampus and cortex after 6 weeks. Moreover, Posiphen significantly increased neuronal differentiation of implanted HNSCs in hippocampal and cortical regions of APP23 mice and in the CA1 region of control mice. These results indicated that Posiphen reduced APP protein *in vivo* and increased neuronal differentiation of HNSCs.

3.1.2.2 Posiphen Lowered APP in Mouse Trisomy Ts65Dn In Vivo

In a study of trisomic mice (Ts65Dn, a model for Down Syndrome), Posiphen was shown to reduce APP levels while doubling NGF levels[13]. Down Syndrome (DS), the most common genetic cause of mental retardation, is due to the presence of an extra copy of human chromosome 21 (i.e. trisomy 21). Human chromosome 21 (HSA 21) is estimated to contain over 300 genes. The symmetry between mouse chromosome 16 and human chromosome 21 served as a rationale to engineer mice that contain an extra copy of genes homologous to those on HSA 21. The Ts65Dn mouse has an extra copy of ~ 140 homologous mouse genes, including an extra copy of APP. There was an apparent link between the decrease in nerve growth factor (NGF) transport from hippocampus to basal forebrain cholinergic neurons (BFCNs), and the degeneration of these neurons. The extra copy of the gene for APP causes both disruption of NGF transport and degeneration of BFCNs[14]. Posiphen restored APP levels in TS65Dn mice to normal mouse levels, and reversed the decrease in NGF transport. In the 2N group, compared

[11] Qu T, Brannen C, Kim H, Sugaya K (2001) NeuroReport 12: 1127 – 1132.
[12] Kwak TD, Brannen C, Qu T, Kim H, Dong X, et al. (2006) Stem Cells Dev 15: 381 – 389.
[13] Salehi A, Faizi M, Takimoto R, Valleta J, Danks A, Moblely WC (2008) Poster, ICAD.
[14] Salehi A, Delcroix JD, Belichenko PV, Zhan K, Wu C, Valletta JS et al., (2006) Neuron 51: 1-2.

with saline-treated mice, there were 24 and 16% decreases in APP levels in mice treated with 25 mg/kg or 50 mg/kg Posiphen, respectively. In the Ts65Dn group, there were 10 and 30% reductions in APP levels in mice treated with 25mg/kg or 50mg/kg Posiphen, respectively.

3.2 Safety Pharmacology

A series of safety pharmacology studies were undertaken in standard animal models to detect potential undesirable pharmacological properties from a single dose of Posiphen. These studies evaluated the cardiovascular function (using conscious, unrestrained dogs), respiratory function (using rats), and renal function (using rats). The cardiac electrophysiological properties of Posiphen were also investigated *in vitro* using hERG (human ether-a-go-go related gene) transfected human embryonic kidney cells (HEK 293). The effect of Posiphen tartrate on the central nervous system (using mice, rats, and dogs) is addressed in the Toxicology Section (**Section 3.4**) and is briefly summarized in the concluding remarks for this section.

The selection and design of these studies follows the International Conference on Harmonization (ICH) Guidance for Safety Pharmacology Studies for Human Pharmaceuticals [15] utilizing a core battery of tests along with an *in vitro* assessment of QT prolongation.[16] All studies were conducted in compliance with Good Laboratory Practice (GLP) standards.[17]

3.2.1 Effects on Cardiovascular Function

3.2.1.1 In Vitro hERG (Potassium Channel) Assay

HEK 293 transfected with hERG were exposed to cumulative extracellular concentrations of 0 (vehicle), 12.5, 42.1, 132.9 or 396 ng/mL of Posiphen tartrate (unbound) dissolved in an isotonic buffered solution (pH 7.3) to investigate potential effects on the delayed rectifier I_{Kr} current (*i.e.* potassium channel current) using a whole-cell patch-clamp method (Report 500117-1). E-4031[18] (0.5 μM) served as the positive control.

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[15] ICH Guidance for Industry, S7A Safety Pharmacology Studies for Human Pharmaceuticals
[16] ICH Guidance for Industry, S7B Nonclinical Evaluation of the Potential for Delayed Ventricular Repolarization (QT Interval Prolongation) by Human Pharmaceuticals
[17] US FDA 21 CFR Part 58 (Good Laboratory Practices for Nonclinical Laboratory Studies)
[18] E-4031 = N-(4-((1-(2-(6-methyl-2-pyridinyl)ethyl)-4-piperidinyl)carbonyl)phenyl) methane sulfonamide (CAS # 113558-89-7), a selective hERG channel blocker

Posiphen tartrate at incubation concentrations of 12.5 – 396 ng/mL did not affect maximal (peak) tail current[19] relative to the vehicle control (**Table 3.2-1**). The positive control E-4031 significantly reduced the relative peak tail current of the hERG channel.

Table 3.2-1: Effect of Posiphen Tartrate on Peak Outward Tail Current Amplitude in the hERG (Potassium Channel) Assay

Treatment	Concentration (ng/mL)	No. of Cells	Net Normalized Maximal Tail Current Density [a] (mean ± SD)
Vehicle	0	7	1.000 ± 0.000
Posiphen	12.5	7	0.982 ± 0.060
Posiphen	42.1	7	1.076 ± 0.078
Posiphen	132.9	7	1.211 ± 0.085
Posiphen	396	7	1.026 ± 0.121
E-4031	0.5 μM	6	0.335 ± 0.167*

[a] Peak density amplitude 10 min after application/peak amplitude before application
*p<0.05, compared to the vehicle control (Student's t-test)

In conclusion, exposure to concentrations of up to 396 ng/mL of Posiphen tartrate did not affect the peak tail current of hERG channel. The response of the positive control confirmed the sensitivity of the assay. These results indicate a low potential, if any, for QT prolongation with Posiphen tartrate.

3.2.1.2 In Vivo Study in Conscious Dogs

The effects of Posiphen tartrate on the cardiovascular system were evaluated by telemetry in conscious, unrestrained, instrumented adult male and female beagle dogs (Report AA25847). The study utilized a Latin square design where each dog (n = 3 males and 3 females) was assigned to receive a single oral (gavage) dose of 0, 5, 10 or 20 mg/kg of Posiphen tartrate in distilled water with a minimum washout period of 2 days between successive dosing. Blood pressure (BP), heart rate (HR), and the electrocardiogram (ECG) were monitored for 24 hours after dosing. At the completion of this assessment, 1 animal/sex received a dose of either 5, 10, or 20 mg/kg and blood samples were collected at selected intervals over 24 hours for

[19] The tail current reflects mainly the open channel state and is the appropriate index for hERG channel evaluation.

determination of plasma concentrations of Posiphen using a validated LC/MS/MS assay (LOQ = 0.1 ng/mL).

Peak (C_{max}) and total ($AUC_{0\text{-}24\ h}$) plasma concentrations of Posiphen increased as a function of the administered dose (**Table 3.2-2**). T_{max} was between 0.25 and 1 hour, and a mean $t_{1/2\beta}$ of about 4 hours that was independent of the administered dose (Report 05-4665.01).

Table 3.2-2: Mean Peak (C_{max}) and Total ($AUC_{0\text{-}24h}$) Plasma Concentrations of Posiphen in Beagle Dogs after a Single Oral Dose of Posiphen Tartrate

Metric	**Male/Female Dose (mg/kg)**		
	5	**10**	**20**
C_{max} (ng/mL)	95	505	1305
$AUC_{0\text{-}\infty}$ (ng·h/mL)	109	424	1477
Lower Limit of Quantification (LLOQ) = 0.10 ng/mL			

There were no clinical signs associated with single oral administration of up to 20 mg/kg Posiphen tartrate. A very slight decrease in body weight was observed in females receiving Posiphen tartrate relative to the pre-test value and this correlated with a reduction in food intake in this group.

Posiphen tartrate did not significantly affect cardiovascular parameters (BP and HR), or ECG results after a single oral dose of 5 or 10 mg/kg. At 20 mg/kg, there was a slight statistically significant increase in mean HR (transient) at 30 minutes post-dosing (107 ± 6 b/m compared to the pretest value of 92 ± 5 b/m). A significant increase in HR (13 – 30 b/m) was also noted from 0.5 – 3 hours post-dosing relative to the vehicle control. Posiphen tartrate at 20 mg/kg was also associated with a significant rise in arterial BP (mean, systolic or diastolic) from 2 to 4 hours post-treatment (12 to 21 mmHg increase in mean arterial BP as compared with vehicle). All values returned to their control levels 4 – 6 hours post-dosing. Summaries of the pre-test and post-dosing values for both cardiovascular parameters are presented in **Tables 3.2-3 and 3.2-4**.

Table 3.2-3: Mean Heart Rates (HR) in Beagle Dogs after Oral Administration of Posiphen Tartrate

Posiphen tartrate (mg/kg)	Mean HR (b/m)							
	Time (hours) after Posiphen Tartrate Administration							
	Pre-test	**0.5**	**1**	**2**	**3**	**4**	**6**	**24**
Vehicle	85	86	75	82	65*	76	84	76
5	86	89	79	73	76	79	91	86
10	81	92	76	78	78	77	91	88
20	92	107^{*+++}	95^{+++}	95^{+++}	95^{+++}	85	91	81

N = 6 (3 males and 3 females)
*p<0.05, compared to the pre-test value (pre-test refers to the -2 h to 0 h mean value taken prior to dosing)
$^{+++}$p<0.001, compared to the vehicle control value (1-way ANOVA and Dunnett's test)

Table 3.2-4: Mean Arterial Blood Pressure (BP) in Beagle Dogs after Oral Administration of Posiphen Tartrate

Posiphen tartrate (mg/kg)	Mean BP (mmHg)							
	Time (hours) after Posiphen Tartrate Administration							
	Pre-test	**0.5**	**1**	**2**	**3**	**4**	**6**	**24**
Vehicle	104	94	98	109	101	106	98	103
5	102	94	101	107	105	108	103	104
10	104	101	97	109	111	108	100	103
20	103	102	105	122^{+++}	122^{+++}	118^{+++}	105	104

N = 6 (3 males and 3 females)
$^{+++}$p<0.001, compared to the vehicle control value (1-way ANOVA and Dunnett's test)

The increase in HR with 20 mg/kg was accompanied by a corresponding decrease in the RR and the QT interval durations. However, there was no effect by posiphen tartrate on the QTc interval duration indicating that the compound did not affect ventricular repolarization.[20] Posiphen tartrate at any dose did not affect the PR interval or the QRS complex duration. Taken together, these results indicate that Posiphen tartrate is devoid of any potentially deleterious effect on atrioventricular or ventricular conduction velocity. Exposure to Posiphen tartrate was not associated with induction of any abnormal ECG waveforms or arrhythmias.

[20] Using Bazett's correction formula: $QTc = QT/(RR)^{1/2}$ or Fridericia's correction formula: $QTc = QT/(RR)^{1/3}$

A slight increase in individual body temperature (0.4 – 0.8°C, relative to the mean vehicle control value) occurred from 0.5 – 4 hours post-dosing in male and female dogs administered 20 mg/kg.

In summary, Posiphen tartrate administered orally to beagle dogs at 5 or 10 mg/kg did not affect cardiac function, but a dose of 20 mg/kg was associated with a transient increase in HR and BP. All cardiovascular parameters had returned to pre-test control level by 6 hours after dosing. Posiphen tartrate did not affect the ventricular repolarization indicating that the potential risk for QT prolongation is very low.

3.2.2 Effects on Respiratory Function

The effect of Posiphen tartrate on respiratory function was evaluated in conscious male CD® Sprague Dawley (SD) rats (Report 851-006) using a whole body plethysmograph chamber. Each rat (n = 8/group) received a single oral (gavage) dose of 0, 10, 20 or 40 mg/kg of Posiphen tartrate in distilled water. The animals were monitored for 1 hour pre-dose to 4 hours post-dosing for clinical signs and effects on pulmonary function (respiratory rate, tidal volume, and minute volume).

There were no major clinical findings. A minor increase in salivation and the red material around the nose and mouth were occasionally observed at 20 and/or 40 mg/kg. At 40 mg/kg, tremors were noted in one rat and clinical observations of apparent breathing difficulty (possibly orofacial dyskinesia) in 1-2 other males 1-4 hours post-dosing. These latter observations did not correlate with any indices of pulmonary dysfunction and their relationship to treatment is uncertain.

There was no effect on respiratory function (respiratory rate, or minute or tidal volumes) with oral administration of up to 40 mg/kg of Posiphen tartrate over the duration of the study.

3.2.3 Effects on Renal Function

The effect of Posiphen tartrate on renal function was assessed in male CD (SD) rats (10/dose) following a single oral (gavage) dose of 0, 10, 20 or 40 mg/kg in distilled water (Report 851-005). The animals were fasted 18-24 hours prior to an oral loading dose of about 25 mL/kg of physiological saline. Posiphen tartrate was subsequently administered just after the saline load and the animals were placed into individual metabolism cages for the collection of urine at intervals of 0-5 and 6-24 hours. Urine volume was recorded and urinary electrolyte (Na^+,

K^+ and Cl^-) excretion determined. Blood was collected at 24 hours post-dosing for determination of plasma electrolyte levels.

There were no clinical signs associated with single oral administration of up to 40 mg/kg of Posiphen tartrate. There were no statistically significant effects of Posiphen tartrate on water or electrolyte excretion at doses of 10 mg/kg compared to the vehicle control group. At 20 or 40 mg/kg, statistically significant increases in urinary volume, sodium, and/or chloride occurred during the first 5 hours of collection **Table 3.2-5**.

Table 3.2-5: Effect of Oral Administration of Posiphen Tartrate on Cumulative Mean Urine Volume and Electrolyte Excretion in Saline Loaded Rats

Treatment	Dose (mg/kg)	Cumulative Mean Urine Volume (mL)		Cumulative Mean Urine Electrolyte Excretion (mEq/L)					
				Na^+		K^+		Cl^-	
		0-5 h	6-24 h	0-5 h	6-24 h	0-5 h	6-24 h	0-5 h	6-24 h
Control	0	4.20	11.05	85	159	44	175	96	209
Posiphen	10	5.10	11.65	105	136	55	159	103	184
Posiphen	20	5.50	11.45	114**	136	46	172	109	205
Posiphen	40	7.05**	8.80	136**	103**	45	166	122*	178

*p<0.05; **p< 0.01, compared to the control (Dunnett's multiple comparison test)
Electrolyte values were rounded to the nearest whole number

After a single oral dose of 40 mg/kg of Posiphen tartrate to saline loaded rats, urinary volume and urinary electrolyte (Na^+ and Cl^-) excretion were significantly increased during the first 5 hours but were slightly lower compared to the vehicle control group over the succeeding 19 hours. There were no group differences in total urinary output over the full 24 hours post-dose collection period (mean control = 15.25 mL and mean 40 mg/kg = 15.85 mL). Posiphen tartrate produced an initial increase in excretion in urinary volume and electrolytes followed by ion conservation over the subsequent 6-24 hours, such that over 24 hours there was no substantial difference between the control and the 20 or 40 mg/kg treated animals. Total sodium excreted over the 24 hour collection period was similar among all groups (mean control = 46.48 mg and mean 40 mg/kg = 42.50 mg). There were no changes in urinary pH values. There were no differences in water consumption or in plasma electrolytes concentrations between the control and Posiphen treated groups.

These patterns indicated a normal kidney response to pharmacological dosing with Posiphen and showed that Posiphen tartrate does not adversely affect renal function. The no observed adverse effect level (NOAEL) for renal function was established at 40 mg/kg.

3.3 Pharmacokinetics (PK) and Metabolism

LC/MS/MS methods for the measurement of Posiphen in rat (Report 03-1885), dog (Report 03-1890), monkey (Report 04-2100) and human (Report 03-1895) plasma were validated with a lower limit of quantitation (LLOQ) of 0.05 ng/mL for rat plasma, 0.10 ng/mL for dog and monkey plasma and 0.025 ng/mL for human plasma.

3.3.1 Absorption and PK

3.3.1.1 Single Dose Administration in Dogs

The absolute bioavailability of Posiphen was assessed in beagle dogs following a single oral and single intravenous dose of Posiphen tartrate (Report CD05/9559T). In a balanced three-way crossover design, fasted male and female beagle dogs (n = 3/sex) were administered Posiphen tartrate as a single intravenous dose of 2 mg/kg, or a single oral dose of 5 or 10 mg/kg with a minimum washout period of 4 days between doses. Serial blood samples were withdrawn pre-dose, and at 0.25, 0.50, 1, 1.5, 2, 3, 4, 6, 8, 10, 12, 16, and 24 hours after each dose. Plasma concentrations of Posiphen were measured (Report 05-4655) and the PK parameters of the drug were calculated (Report F05-4655.01).

Following oral administration, the drug was rapidly absorbed with mean peak (C_{max}) plasma concentrations of Posiphen attained at approximately 1 hour after both oral doses. The inter-animal variability in the pharmacokinetics of the drug was high. A summary of the PK parameters is shown in **Table 3.3-1**.

The systemic clearance of Posiphen from plasma after the intravenous dose averaged 3.48 ± 0.48 L/kg·h and the steady-state volume of distribution (Vss) averaged 1.84 ± 0.52 L/kg, with both parameters similar in male and female dogs.

The terminal phase half-life ($t_{1/2}$) of the drug was approximately 2 hours for all dosage regimens. There were no apparent gender-related differences in the pharmacokinetics of the drug in dogs in this study.

Table 3.3-1: Pharmacokinetics and Bioavailability of Posiphen in Dogs Following a Single Oral and Intravenous Administration of Posiphen Tartrate

Metric	Route	Dose (mg/kg)	Mean values (± SD)		
			Males (N=3)	Females (N=3)	All dogs (N=6)
C_{max} (ng/mL)	IV	2	822 ± 266 [a]	1156 ± 433 [a]	989 ± 370 [a]
	PO	5	77 ± 96	53 ± 18	65 ± 63
	PO	10	193 ± 94	146 ± 87	169 ± 85
$AUC_{0-\infty}$ (ng·h/mL)	IV	2	387 ± 60	421 ± 64	404 ± 59
	PO	5	58 ± 54	75 ± 47	67 ± 46
	PO	10	315 ± 174	218 ± 112	266 ± 141
$t_{1/2\beta}$ (hr) [b]	IV	2	1.80 ± 0.15	1.78 ± 0.21	1.79 ± 0.17
	PO	5	1.79 ± 0.28	2.04 ±0.49	1.91 ± 0.36
	PO	10	2.46 ± 0.46	2.64 ± 1.04	2.55 ± 0.70
F (%) [c]	IV	2	NA	NA	NA
	PO	5	6.0 ± 5.9	7.6 ± 5.5	6.8 ± 5.1
	PO	10	15.9 ± 7.4	11.0 ± 6.7	13.5 ± 6.9

[a] The C_{max} for the intravenous (IV) dose represent the theoretical value obtained by extrapolation of the data to time zero based on residual analyses of the drug concentrations and a bi-exponential PK model
[b] Harmonic mean terminal phase half-life
[c] Dose-normalized absolute bioavailability

3.3.1.2 Multiple Dose Administration (Toxicokinetics)

A summary of the toxicokinetics from the repeated dose toxicity studies is found in **Table 3.4-9** for rats, and **Table 3.4-15** and **Table 3.4-17** for dogs. Posiphen concentrations in plasma (and associated PK parameters) have been undertaken in the rat and dog toxicology studies (toxicokinetics). Posiphen was measured in plasma samples collected in support of a 1 month oral toxicology study in male and female SD rats and in support of the 7-day and 1 month oral toxicology studies in male and female beagle dogs. Details on the results of these analyses are presented in the Toxicology Section 4.4 and are summarized below:

- Orally administered Posiphen tartrate was rapidly absorbed with peak plasma concentrations of Posiphen attained by the first sampling point of 0.5 hour in the rat and within 0.5-3 hours in the dog.
- In the rat, oral administration of Posiphen tartrate (10, 20, 40 and 80 mg/kg/day) resulted in a dose-related increase in plasma concentrations of unchanged drug. Posiphen concentrations were markedly higher in the plasma of female rats compared to males.
- In the dog, oral administration of Posiphen tartrate (20, 40 and 60 mg/kg/day in the 7 day study, and 5, 10, and 30 mg/kg/day in the 1 month study) resulted in a dose-related increases in the plasma concentrations of Posiphen. Although Posiphen plasma

concentrations were generally higher in the female than in the male dogs, considerable inter-animal variability was observed, and it did not appear likely that there were significant gender difference in peak or total exposure of Posiphen in the dog.

- In both species, there was no evidence of an increase or decrease in plasma concentrations of Posiphen after daily dosing for 28 days versus a single daily dose.

3.3.2 Tissue Distribution - Studies in Mice

The objective of the study (Report 7552-109) was to collect blood and brain from male C57BL/6 mice following administration of daily oral (25, 50, or 75 mg/kg) or intraperitoneal (25 or 50 mg/kg) doses of Posiphen tartrate for 7 or 21 days for subsequent PK/PD analysis. Ninety-five (95) male mice were used in this study. Cageside observations for clinical signs were made approximately 30 minutes after dose administration each day. Animals were sacrificed approximately 2 hours after the final dose administration. Blood was collected and centrifuged to obtain plasma and cellular fraction. Brain was collected and separated into left and right cerebral hemispheres and cerebellum for analysis. The effect of orally administered Posiphen on Aβ42 levels in the mouse brain was examined, and plasma samples were assayed for Posiphen concentrations by LC/MS/MS.

Full body tremors and hypoactivity were observed in the animals dosed orally and intraperitoneally at the 50 and 75 mg/kg dose levels.

The results of analysis for effects of orally administered Posiphen on brain levels of Aβ42 in the mouse are summarized below:

<u>Day 7</u>

- Mean (SD) brain Aβ42 levels were 124.0 (39.4), 81.0 (17.1), and 68.7 (12.3) pg/mg protein in the vehicle, 50, and 75 mg/kg/day groups, respectively. Differences from control were significant in both Posiphen treated groups ($p \leq .05$).
- Relative to vehicle control, Posiphen 50 and 75 mg/kg/day reduced mean brain Aβ42 levels by 34.7 and 44.6%, respectively.

Day 21

- Mean (SD) brain Aβ42 levels were 190.2 (74.2), 90.6 (20.0), 82.0 (22.0), and 75.6 (17.2) pg/mg protein in the vehicle, 25, 50, and 75 mg/kg/day groups, respectively. Differences from control were significant in all three Posiphen treated groups ($p \leq .01$).
- Relative to vehicle control, Posiphen 25, 50, and 75 mg/kg/day reduced mean brain Aβ42 levels by 52.3, 56.9 and 60.2%, respectively.

Published results concluded that in mice, orally administered Posiphen lowered brain Aβ42 levels at plasma concentrations that are attainable with oral dosing in humans.[21]

Mean Posiphen plasma concentrations were lower than maximum Posiphen plasma concentrations in a recent clinical pharmacology study of Posiphen in healthy men and women, suggesting that the therapeutic ratio may also be favorable in humans.

The objective of the study (Report 7552-110) was to collect blood and brain from male C57BL/6 mice following administration of daily oral 2, 4, 8, 16, or 32 mg/kg doses of Posiphen tartrate for 7 or 21 days. One hundred twenty-seven male mice were used in this study; 124 study animals and 3 replacement animals received doses. Observations were made approximately 30 minutes after dose administration each day. No clinical signs were noted at the cageside observations. No significant changes in mean body weights were noted over the course of the study. Animals were sacrificed approximately 2 hours after the final dose administration. Blood and brain samples were collected following sacrifice but samples were not analyzed.

3.3.3 In Vitro Metabolism

3.3.3.1 In Vitro Metabolism in Hepatocytes

The comparative metabolism of Posiphen across species was evaluated *in vitro* in hepatocytes (Report 125-1092-05). Cryo-preserved hepatocytes from SD rats, C57BL/6 mice, beagle dogs, cynomolgus monkeys, and human donors were incubated in the presence of 0.5, 5,

[21] Cullen et al. (2006), Brain Beta-Amyloid (Aβ42) in Mice Treated Orally with Posiphen Tartrate is Significantly Lower than in Vehicle Controls. Poster presented at the 9th International Geneva/Springfield Symposium on Advances in Alzheimer Therapy, Geneva, Switzerland, April 19-22, 2006.

or 50 μM Posiphen tartrate for 1, 4, and 20 hours. The relative formation of metabolites was measured using HPLC with fluorescence and MS analysis. The Posiphen metabolic profiles of each species were qualitatively compared by analysis of parent and metabolite peaks, primarily after incubating 50 μM Posiphen tartrate with hepatocytes for 4 hours.

Posiphen tartrate underwent extensive biotransformation when incubated *in vitro* with primary hepatocytes from all species tested. In incubations from human hepatocytes, 18 metabolites were detected; 14 were identified as demethylated and/or hydroxylated or dehydrogenation products (**Table 3.3-2**).

Table 3.3-2: A Summary of the Detected Metabolites of Posiphen in Cultures of Cryo-preserved Hepatocytes

Human	Metabolic Reaction	Designation of Corresponding Animal Metabolite			
		Monkey	Dog	Rat	Mouse
H-1[a]		MK-1[a]	D-1[a]	R-1[a]	MS-1[a]
H-2[a]		MK-2[a]	D-2[a]	R-2[a]	MS-2[a]
H-3[a]		MK-3[a]	D-3	R-3[a]	MS-3[a]
H-4	Di-demethylation	MK-5	D-5	R-4	MS-17[c]
H-5	Demethylation	MK-6	D-6	R-5	MS-4
H-6	Demethylation	MK-22[c]	D-15[c]	R-21[c]	MS-16[c]
H-7[a]		ND	D-7[a]	R-6[a]	ND
H-8	Hydroxylation	MK-7	D-8	R-7	MS-12[c]
H-9	Demethylation/hydroxylation/oxidation	MK-8	ND	ND	ND
H-10[c]	Demethylation/hydroxylation	MK-11[c]	ND	R-13[c]	MS-7[c]
H-11[c]	Demethylation/hydroxylation	MK-12[c]	ND	R-14[c]	ND
H-12[c]	Demethylation/hydroxylation	MK-13[c]	ND	R-15[c]	MS-8[c]
H-13[c]	Hydroxylation/oxidation	MK-14[c]	D-11[c]	R-16/R-17[c]	MS-9[c]
H-14[c]	Hydroxylation	MK-15[c]	D-12[c]	R-18[b]	MS-10[c]
H-15[b]	Hydroxylation	MK-16[c]	D-13[c]	R-19[c]	MS-11[c]
H-16[c]	Hydroxylation+glucuronide conjugation	ND	ND	ND	ND
H-17[c]	Demethylation/hydroxylation/oxidation	MK-18[c]	ND	R-8	MS-14[c]
H-18[c]	Dehydrogenation	MK-19[c]	ND	ND	ND
ND		MK-4[a]	ND	ND	ND
ND	Demethylation/hydroxylation	MK-9[c]	D-10[c]	R-10[b]	MS-5[c]
ND	Demethylation/hydroxylation	MK-10[c]	ND	R-11[b]	MS-6[c]
ND	Glucuronide conjugation	MK-17[c]	ND	ND	ND
ND	Dehydrogenation	MK-20[c]	ND	ND	ND
ND	Dehydrogenation	MK-21[c]	ND	ND	ND
ND	Demethylation	MK-23[c]	ND	ND	ND
ND		ND	D-4[a]	ND	ND
ND		ND	D-9[a]	R-9[a]	ND
ND	Dihydroxylation	ND	D-14[c]	ND	ND
ND	Demethylation/hydroxylation	ND	ND	R-12[b]	ND
ND	Hydroxylation/oxidation	ND	ND	R-17[c]	ND
ND	Dihydroxylation	ND	ND	R-20[c]	ND
ND	Hydroxylation	ND	ND	ND	MS-13[c]
ND	Dehydrogenation	ND	ND	ND	MS-15[c]

ND, not detected by mass spectral analysis
[a] Detected only with fluorescence
[b] Detected with mass spectral analysis but not identified in chromatograms with fluorescence
[c] Detected only with mass spectral analysis

There was minimal evidence of *in vitro* conjugation. Of these human metabolites, 4 were considered to be major (2 mono-demethylated, 1 hydroxylated, and 1 hydroxylated/oxidative metabolite). Three of these were also major metabolites of Posiphen tartrate in all animal species examined; the fourth major metabolite was found in monkeys > rats > mice > dogs. With the exception of a minor conjugated metabolite, all metabolites identified in human hepatocytes were also formed by hepatocytes in at least one laboratory animal species (**Table 3.3-3**). There was no evidence of any unique Phase 1 human metabolite.

Table 3.3-3: The Abundance of Posiphen Metabolites in Cultures of Cryo-preserved Hepatocytes

	Area Absorbance					Abundance (% Observed Metabolites)				
Peak	Human	Monkey	Dog	Rat	Mouse	Human	Monkey	Dog	Rat	Mouse
H-4	52.1	281.9	11.1	171.4	201.1	1.29	7.12	0.566	4.02	4.27
H-5	1144	837.9	363.7	1659.2	2688.8	28.3	21.2	18.6	38.9	57.1
H-6	1055.9	1404.6	237.3	1229.3	1229	26.1	35.5	12.1	28.9	26.1
H-8	927.1	1013.5	1319.4	1018.2	479.7	22.9	25.6	67.3	23.9	10.2
H-9	45.1	11.4	0	0	0	1.12	0.288	0.000	0.000	0.000
H-10	9.6	21.2	0	8	5.9	0.238	0.535	0.000	0.188	0.125
H-11	44.3	8.8	0	13.8	0	1.10	0.222	0.000	0.324	0.000
H-12	26.6	14	0	4.7	3.3	0.658	0.354	0.000	0.110	0.0701
H-13	493.9	118.4	7.8	63.2	32.5	12.2	2.99	0.398	1.48	0.691
H-14	3.1	4.4	16.7	12.3	10.1	0.0767	0.111	0.852	0.289	0.215
H-15	86.4	83.9	4.3	50.6	46.9	2.14	2.12	0.219	1.19	0.997
H-16	7.4	0	0	0	0	0.183	0.000	0.000	0.000	0.000
H-17	63.2	107	0	29.6	8.9	1.56	2.70	0.000	0.695	0.189
H-18	82.5	52.7	0	0	0	2.04	1.33	0.000	0.000	0.000
Total	4,041	3,960	1,960	4,260	4,706	100	100	100	100	100

It was concluded that the general profile of Posiphen metabolism *in vitro* was qualitatively similar across the species studied. Based on the metabolite profile, either the dog or the monkey would be an appropriate large animal species for the toxicological evaluation of Posiphen tartrate.

3.3.3.2 In Vitro Metabolism in Human Whole Blood

The primary objective of the study (Report WJA-06-01) was to determine the stability of Posiphen in K_3EDTA human whole blood (WB) containing 0, 1 or 10 μM

diisopropylfluorophosphate (DFP) at 4°C and 25°C. The secondary objective of this study was to obtain preliminary information on the partitioning of Posiphen into human RBCs.

Whole Blood Stability: The stability of Posiphen in K_3EDTA human WB was examined at two concentrations (6.00 and 0.300 ng/mL) and at two temperatures (4°C and 25°C). In these experiments, fresh human WB samples (25 mL each) containing 0, 1, or 10 µM DFP were fortified with 6.00 and 0.300 ng/mL Posiphen and incubated at 4°C for 0 and 30 minutes, and at 25°C for 30 minutes.

Plasma samples obtained from WB fortified with 6.00 and 0.300 ng/mL Posiphen and incubated at 4°C for 0 minutes all had mean experimentally determined concentrations greater than the theoretical WB concentration. The mean plasma concentrations ranged from 102.2% to 106.3% of the theoretical WB concentration for the 6.00 ng/mL samples, and from 109.0% to 110.0% of the theoretical WB concentration for the 0.300 ng/mL samples. The experimentally determined concentrations of these samples appeared to be independent of the DFP concentration. These data suggest that Posiphen is not uniformly distributed in WB, but appears to be distributed to a lesser extent into RBC as the experimentally determined concentration of Posiphen in plasma was higher than the theoretical concentration in WB. The mean plasma concentrations of Posiphen for the 6.00 ng/mL WB samples held at 4°C for 30 minutes were approximately 1% to 5% lower than the corresponding 0 minute samples. The mean plasma concentrations of Posiphen in the 6.00 ng/mL samples held at 25°C for 30 minutes were approximately 6% to 1.1% higher than the corresponding 0 minute samples. These data collectively indicate that Posiphen at a concentration of 6.00 ng/mL is stable in human WB containing 0, 1, or 10 µM DFP. Thus, it can be concluded that the changes in Posiphen concentration over the 30 minutes equilibration period are not related to enzymatic hydrolysis by choline esterases.

Distribution into Red Blood Cells: In order to obtain an estimate of the extent of distribution of Posiphen into RBC, the experimentally determined mean concentration of Posiphen in fortified plasma (0 time, 4°C) was divided by the experimentally determined concentration of Posiphen in plasma from fortified WB (0 time, 4°C). The calculations were done for each concentration of DFP used (0, 1, and 10 µM DFP). The values were lower than those calculated by using the theoretical concentrations of Posiphen in the fortified plasma samples (0.300 and 6.00 ng/mL, respectively) because the experimentally determined mean

Posiphen concentrations for the fortified plasma samples were approximately 19% and 13% lower than the theoretical concentrations for the 0.300 and 6.00 ng/mL samples, respectively. Since WB contains approximately 45% hematocrit, if there was no distribution of Posiphen into RBCs then the plasma isolated from the fortified WB would have WB to plasma concentration ratios of approximately 0.55. The actual mean ratios for the WB samples fortified at 0.300 ng/mL ranged from 0.711 to 0.762, and the actual values for the WB samples fortified at 6.00 ng/mL ranged from 0.824 to 0.849 for WB containing 0, 1, or 10 μM DFP at the 0 minute time point. Comparison of the mean WB to plasma ratios for plasma isolated from WB after equilibration for 30 minutes at 4°C or at 25°C indicated that the ratios were similar. Thus there appears to be some distribution of Posiphen into RBCs but the extent of distribution is modest.

3.4 Toxicology

The preclinical safety profile of Posiphen tartrate was determined from single oral and intravenous toxicity studies conducted in mice, rats and dogs, and from repeated dose oral dose toxicity studies for up to 30 days in rats and dogs. Posiphen tartrate was administered to rodents by gavage as a solution dissolved in deionized water, intravenously to rodents as a solution dissolved in 0.9% sodium chloride for injection, and to dogs in a capsule containing neat drug substance. Studies were also undertaken to assess the genotoxic potential of Posiphen tartrate *in vitro*. The toxicology studies conducted with Posiphen tartrate are summarized in **Tables 3.4-1** to **3.4-3**. More detailed descriptions of the individual study results are provided in the following pages. The doses presented in these tables are expressed in terms of Posiphen tartrate. All studies were conducted in accordance with GLP.

Table 3.4-1: Summary of Single Dose Toxicity Studies with Posiphen Tartrate

Species and Strain	Route	Administered Dose (mg/kg)	NOAEL (mg/kg)		MTD (mg/kg)		MLD (mg/kg)	
			M	F	M	F	M	F
Mouse/CD-1	PO	20, 40, 80, 160	40	40	80	80	>160	>160
Mouse/CD-1	IV	1, 5, 10, 20	10	20	20	>20	>20	>20
Rat/CD	PO	10, 20, 40, 80	20	40	40	80	>80	>80
Rat/CD	IV	1, 5, 10, 20	10	10	20	20	>20	>20
Dog/Beagle	PO	10, 20, 40, 50, 60, 80, 100	20	20	60	60	100	100

M = Male
F = Female
NOAEL = No Observed Adverse Effect Level
MTD = Maximum Tolerated Dose
MLD = Minimal Lethal Dose

Table 3.4-2: Summary of Repeated Oral Dose Toxicity Studies Conducted with Posiphen Tartrate

Species	N/sex/dose	Administered Dose (mg/kg/day)	Dosing Duration (Weeks)	NOAEL (mg/kg)		MTD (mg/kg)	
				M	F	M	F
Rat/CD	10	10, 20, 40, 80	4	10	20	40	80
Dog/Beagle	1 - 2	20, 40, 80/60	1	20	20	40	40
Dog/Beagle	3 - 5	5, 10, 30	4	10	10	30	30

M = Male
F = Female
NOAEL = No Observed Adverse Effect Level
MTD = Maximum Tolerated Dose

Table 3.4-3: Summary of Genotoxicity Studies Conducted with Posiphen Tartrate

Assay Type	Concentration Range Tested (µg/mL or plate)	Results
In vitro		
Bacterial Reverse Mutation (Ames test)	1.5 – 5000	Negative ± metabolic activation (S9)
Mammalian Cell Chromosomal Aberration (HPBL)	6.25 – 100 or 200	Negative ± metabolic activation (S9)

The results from these studies showed, in brief, that repeated oral administration of Posiphen tartrate for up to 30 days to rats at doses of 20 mg/kg/day or to dogs at 30 mg/kg/day was associated with central nervous system and gastrointestinal effects. The incidence and severity of the clinical findings (twitching or tremors, ataxia) was dose-dependent and reversible. In rats, a slight anemia (associated with reticulocytosis) was noted at 80 mg/kg but the relationship of this finding to drug administration was unclear. No gross or histopathological findings were associated with repeated oral dose administration of Posiphen tartrate administration of up to 40 or 80 mg/kg/day to male or female rats, respectively, or to male or female dogs at 10 mg/kg/day. Microscopic findings in dogs administered 30 mg/kg/day were unremarkable except for an erosion/ulcer with subacute inflammation in one male possibly related to Posiphen tartrate administration. The NOAEL was 10 and 20 mg/kg/day in male and female rats, respectively, and 10 mg/kg/day in male and female dogs based on tremors observed with higher doses.

3.4.1 Single Dose Toxicity Studies

3.4.1.1 Single Oral Dose Toxicity Study in Mice

The acute toxicity of Posiphen tartrate following a single oral dose was investigated in male and female CD-1® (ICR) BR mice (5/sex/dose) (Report WIL-421012). The mice were approximately 9 weeks of age and weighed from 30.1 to 35.0 g for males and from 23.7 to 26.9 g for females at initiation of dosing. Posiphen tartrate was administered to the mice (fasted) as a single oral (gavage) dose of 20, 40, 80, or 160 mg/kg dissolved in deionized water. The mice were subsequently monitored for 14 days. A similar group received the vehicle and served as a negative control. The mice were monitored for clinical signs, food consumption, and body weight changes prior to and for 14 days after dosing. At terminal sacrifice all mice underwent complete macroscopic examinations.

No deaths occurred in any group. Clinical signs related to Posiphen tartrate administration were noted at 160 mg/kg in both sexes and included transient episodes of twitching,[22] impaired equilibrium, impaired muscle coordination, labored respiration,

[22] Twitching was defined as muscle fasciculation, characterized by involuntary fine movements of a localized area of muscles and/or involuntary muscle contractions.

intermittent tremors, clear ocular discharge, and yellow material on various body surfaces. These occurred within the first hour of dosing and persisted 2-6 hours post-dosing. Twitching and yellow material on various body surfaces were also noted in the 80 mg/kg group (both sexes). There were no other treatment-related clinical findings noted for any mouse on the day following dosing or during the remaining 14-day observation period.

The maximum tolerated dose (MTD) for administration of Posiphen tartrate to male and female mice as a single oral (gavage) dose was 80 mg/kg (**Table 3.4-4**).

Table 3.4-4: Summary of Findings after a Single Oral Dose of Posiphen Tartrate to CD-1® (ICR) Mice

Species	Sex	N/dose	Administered Dose (mg/kg)	MTD (mg/kg)	Findings
CD-1® (ICR) Mice	M	5	20, 40, 80, 160	80	**160 mg/kg (M/F)**: twitching, intermittent tremors, impaired equilibrium and muscle coordination, dyspnea, ocular discharge, yellow material on various body surfaces **80 mg/kg (M/F)**: twitching; yellow material on various body surfaces
	F	5	20, 40, 80, 160	80	

There were no effects on body weight or food consumption. Macroscopic findings at terminal sacrifice were unremarkable. The NOAEL for both sexes was 40 mg/kg.

3.4.1.2 Single Intravenous Dose Toxicity Study in Mice

The toxicity of posiphen tartrate following a single intravenous dose was investigated in male and female CD-1® (ICR) BR mice (5/sex/dose) (Report WIL-421011). The mice were approximately 7 weeks of age and weighed from 26.9 – 31.1 g for males and from 22.3 – 27.7 g for females at initiation of dosing. Posiphen tartrate was administered to fasted mice as a single intravenous (tail vein) dose of 1, 5, 10, or 20 mg/kg dissolved in 0.9% sodium chloride for injection. The mice subsequently monitored for 14 days. A similar group received the vehicle and served as a negative control. The mice were monitored for clinical signs, food consumption, and body weight changes prior to and for 14 days after dosing. At terminal sacrifice all mice underwent complete macroscopic examinations.

No deaths occurred in any group during the study. There were no effects at the site of injection. Clinical signs with intravenous administration of Posiphen tartrate were noted only in 1-2 males at 20 mg/kg and included Straub tail, intermittent convulsions, and impaired

equilibrium. These clinical observations occurred shortly after dosing and resolved within the 1st hour. There were no other treatment-related clinical findings noted for any mouse on the day following dosing or during the 14-day recovery period.

The acute non-lethal MTD for administration of Posiphen tartrate to males as a single intravenous dose was 20 mg/kg and for females was greater than 20 mg/kg in the absence of any clinical signs noted (**Table 3.4-5**).

Table 3.4-5: Summary of Findings after a Single Intravenous Dose of Posiphen Tartrate to CD-1® (ICR) Mice

Species	Sex	N/dose	Administered Dose (mg/kg)	MTD (mg/kg)	Findings
CD-1® (ICR) Mice	M	5	1, 5, 10, 20	20	**20 mg/kg (M)**: Straub tail, convulsions and impaired equilibrium
	F	5	1, 5, 10, 20	>20	

There was no effect of Posiphen tartrate on body weight or food consumption. Macroscopic findings at terminal sacrifice were unremarkable. The NOAEL was 10 mg/kg for males and 20 mg/kg for females.

3.4.1.3 Single Oral Dose Toxicity Study in Rats

The acute toxicity of Posiphen tartrate following a single oral dose was investigated in male and female CD® (SD) rats (5/sex/dose) (Report WIL-421014). The rats were approximately 7 weeks of age and weighed from 236-273 g for males and from 149-183 g for females at initiation of dosing. Posiphen tartrate was administered to fasted rats as a single oral (gavage) dose of 10, 20, 40, or 80 mg/kg dissolved in deionized water and the animals subsequently monitored for 14 days. A similar group received the vehicle and served as a negative control. The rats were monitored for clinical signs, food consumption, and body weight changes prior to and for 14 days after dosing. At terminal sacrifice all rats underwent complete macroscopic examinations.

No deaths occurred in any group during the study. Clinical signs were limited to twitching observed at ≥40 mg/kg in males and at 80 mg/kg in females. This occurred immediately after dosing for males and 2 hours after dosing for females. These signs persisted for up to 6 hours post-dosing but were absent by 24 hours. There were no other findings noted for any treated rat during the 14-day observation period.

The MTD for a single oral dose of Posiphen tartrate was 40 mg/kg in male rats and 80 mg/kg in female rats (**Table 3.4-6**).

Table 3.4-6: Summary of Findings after a Single Oral Dose of Posiphen Tartrate to CD® (SD) Rats

Species	Sex	N/dose	Administered Dose (mg/kg)	MTD (mg/kg)	Findings
CD® (SD) Rats	M	5	10, 20, 40, 80	40	**80 mg/kg (M/F)**: twitching **40 mg/kg (M)**: twitching
	F	5	10, 20, 40, 80	80	

The mean body weight of males administered 40 and 80 mg/kg of Posiphen tartrate increased during the 1st week post-dose but this increase was not considered to be of toxicological significance. There were no differences in food consumption between groups and macroscopic examinations were unremarkable. The NOAEL was 20 mg/kg for males and 40 mg/kg for females.

3.4.1.4 Single Intravenous Dose Toxicity Study in Rats

The toxicity of Posiphen tartrate following a single intravenous dose was investigated in male and female CD® (SD) rats (5/sex/dose) (Report WIL-421013). The rats were approximately 7 weeks of age and weighed from 226 to 254 g for males and from 143 to 181 g for females at initiation of dosing. Posiphen tartrate was administered to fasted rats as a single intravenous (tail vein) dose of 1, 5, 10, or 20 mg/kg dissolved in 0.9% sodium chloride for injection and the animals subsequently monitored for 14 days. A similar group received the vehicle and served as a negative control. The rats were monitored for clinical signs, food consumption, and body weight changes prior to and for 14 days after dosing. At terminal sacrifice all rats underwent complete macroscopic examinations.

No deaths occurred in any group during the study. There were no effects observed at the site of injection. Clinical signs with intravenous administration of Posiphen tartrate were limited to males and/or females that received 20 mg/kg and included twitching, hyperactivity followed by hypoactivity, Straub tail, clear/foamy material around the mouth, impaired equilibrium and convulsions and gasping (in 1 male only). Most clinical observations occurred within the 1st hour of dosing and were resolved within 1-2 hours post-dosing. There were no treatment-related clinical findings noted for any rat at 24 hours after dosing or during the subsequent 14-day recovery period.

There were no effects of posiphen tartrate on body weight or food consumption. Macroscopic findings at terminal sacrifice were unremarkable. The NOAEL for both sexes was 10 mg/kg. The acute non-lethal MTD for a single intravenous administration of Posiphen tartrate to male and female rats was 20 mg/kg, the highest dose tested (**Table 3.4-7**).

Table 3.4-7: Summary of Findings after a Single Intravenous Dose of Posiphen Tartrate to CD® (SD) Rats

Species	Sex	N/dose	Administered Dose (mg/kg)	MTD (mg/kg)	Findings
CD® (SD) Rats	M	5	1, 5, 10, 20	20	**20 mg/kg (M/F)**: twitching, Straub tail, hyperactivity followed by hypoactivity, clear/foamy material around the mouth, impaired equilibrium, convulsions, and gasping
	F	5	1, 5, 10, 20	20	

3.4.1.5 Single Oral Toxicity Study in Dogs

The MTD following a single oral dose of Posiphen tartrate was determined in male and female beagle dogs (2/sex) (Report 851-003). The dogs were 5-6 months of age and weighed 7.89 and 8.51 kg for males and 7.02 and 7.19 kg for females. The dogs were administered a single oral (capsule) dose of 10 mg/kg of Posiphen tartrate on Day 1, followed by a 2-day interval period before escalating to the next dose. Dose levels were increased approximately every 3 days accordingly to 20, 40, 50, 60, 80, and 100 mg/kg. Clinical observations and body weight changes were monitored and the stopping dose was based on overt clinical signs of toxicity (designated as the MTD). The dogs were not sacrificed at the conclusion of the study but macroscopic and microscopic examinations were undertaken on the dog that died on test.

One male that received 100 mg/kg/day died on test following convulsions that occurred over 3 hours. Microscopic examination of tissues from this animal showed intestinal ulceration and minimal Kupffer cell hypertrophy/hyperplasia and minimal individual hepatocyte necrosis. The 100 mg/kg dose was considered beyond the MTD.

With increasing doses of Posiphen tartrate there was an increase in the incidence, severity, and duration of adverse clinical signs that were primarily related to the central nervous system and the gastrointestinal tract (**Table 3.4-8**).

There were no apparent gender differences in response. At 40 mg/kg, ataxia lasted for up to 4 hours after dosing and tremors persisted for 7 hours. At 80 mg/kg/day, the dogs experienced

tremors but did not develop convulsions, but two of the dogs at this dose were unable to stand. The MTD was considered to be 60 mg/kg, and the NOAEL was 20 mg/kg/day.

Table 3.4-8: Summary of Notable Findings after a Single Oral Dose of Posiphen Tartrate to Beagle Dogs

Species	Sex	N	Administered Dose (mg/kg)	MTD (mg/kg)	Findings (in one or more dogs)
Beagle Dogs	M	2	10, 20, 40, 50, 60, 80, 100	60	**10 mg/kg**: Lacrimation **20 mg/kg**: + salivation, soft feces **40 mg/kg**: + ataxia, tremors, excessive licking, emesis, injected sclera **50 mg/kg**: + skin cold to touch, hypoactivity, excessive licking, watery feces, discolored red skin, and coughing **60 mg/kg**: + impaired limb function **80 mg/kg**: + unable to stand **100 mg/kg**: + convulsions, prostration, slow gum refill time, dilated pupils and diminished pupil reflex; intestinal ulceration, single cell hepatic necrosis and Kupffer cell hypertrophy and hyperplasia in the 1 male that died on test at this dose
	F	2	10, 20, 40, 50, 60, 80, 100	60	
+: Finding(s) observed with the previous dose					

3.4.2 Repeated Oral Dose Toxicity Studies

3.4.2.1 Repeated 4-Week Oral Dose Toxicity Study in Rats

CD® (SD) rats (10/sex/dose) were administered a single oral (gavage) dose of Posiphen tartrate once daily for 4 weeks at 10, 20, or 40 mg/kg/day for males and 20, 40, or 80 mg/kg/day for females (Report WIL-421015). A similar group received the vehicle (deionized water) and served as a negative control. Additional satellite groups of rats (12/sex/dose) administered Posiphen tartrate at the cited doses were used for toxicokinetic assessments. The main study animals were about 7 weeks of age and weighed 209.4 to 255.8 g for males and 149.1 to 184.8 g for females.

All animals were monitored for clinical signs, and changes in body weights and food consumption. Ophthalmologic examinations were undertaken prior to initiation of dosing and during Week 3. Clinical laboratory assessments (hematology, clinical biochemistry, and urinalysis) were assessed immediately prior to terminal sacrifice. All animals underwent complete macroscopic examinations of the major organ systems and selective organs were

weighted. Tissues (including sternum bone marrow smears for M/E ratios) were processed for microscopic examinations. Blood samples were collected from the satellite group (3/sex/timepoint) on Days 0 (first day of dosing) and 27 at 0, 0.5, 1, 2, 4, 8, 12 and 24 hours post-dosing for the determination of plasma concentrations of Posiphen using a validated LC/MS/MS method (LLOQ = 0.05 ng/mL).

Peak (C_{max}) and total ($AUC_{0\text{-}24h}$) exposures to Posiphen increased in proportion to the administered dose (**Table 3.4-9**). $AUC_{0\text{-}24h}$ and C_{max} values remained relatively constant between Day 0 (first day of dosing) and Day 27. Peak and total exposure were higher in females than in males at equivalent doses. The apparent plasma elimination $t_{1/2}$ of Posiphen was approximately 1-2 hours and was independent of gender. Apparent systemic clearance was approximately 5-fold higher for males than for females. There was no indication of accumulation or increased metabolic activity with repeated dosing.

Table 3.4-9: Mean Peak (Cmax) and Total (AUC0-24h) Plasma Concentrations of Posiphen in Male and Female CD (SD) Rats following Oral Dosing of Posiphen Tartrate for 4 Weeks

Sample Day	Males (mg/kg/day)			Females (mg/kg/day)		
	10	**20**	**40**	**20**	**40**	**80**
	C_{max} (ng/mL)			**C_{max}** (ng/mL)		
0	100	297	595	630	2100	3170
27	111	266	451	840	2190	3110
	$AUC_{0\text{-}24\ h}$ (ng·h/mL)			**$AUC_{0\text{-}24\ h}$** (ng·h/mL)		
0	202	507	1066	1440	4883	10971
27	214	509	882	2037	5223	12351

Lower Limit of Quantification (LLOQ) = 0.05 ng/mL
Note: AUC on Day 0 = $AUC_{0\text{-}\infty}$

There were no deaths during the study. Clinical signs were limited to muscle twitching that occurred in males at 20 mg/kg/day (beginning on Day 14) and 40 mg/kg/day (beginning on Day 0) and in females at 80 mg/kg/day (beginning on Day 11). This twitching generally persisted through Day 26 for the males and Day 17 for the females. A summary of these results is presented in **Table 3.4-10**.

Table 3.4-10: Incidence of Twitching in Male and Female CD (SD) Rats During Oral Dosing of Posiphen Tartrate for 4 Weeks

Incidence of Twitching (Number of Occurrences/Number of Animals)								
	Posiphen (mg/kg/day)							
	Male				Female			
	0	10	20	40	0	20	40	80
No. of Rats	10	10	10	10	10	10	10	10
1 Hour Post-Dosing								
Total	0/0	0/0	2/2	86/10	0/0	0/0	0/0	4/4
Study Day 0	0/0	0/0	0/0	8/8	0/0	0/0	0/0	0/0
Study Day 1	0/0	0/0	0/0	5/5	0/0	0/0	0/0	0/0
Study Days 2-6	0/0	0/0	0/0	17/9	0/0	0/0	0/0	0/0
Study Days 7-13	0/0	0/0	0/0	17/6	0/0	0/0	0/0	1/1
Study Days 14-20	0/0	0/0	2/2	17/8	0/0	0/0	0/0	3/3
Study Days 21-26	0/0	0/0	0/0	22/9	0/0	0/0	0/0	0/0
Unscheduled Observation								
Study Day 0	0/0	0/0	0/0	3/3	0/0	0/0	0/0	0/0
Detailed Physical Examination								
Study Days 21-26	0/0	0/0	0/0	4/4	0/0	0/0	0/0	0/0

Excessive chewing was observed in 3 females that received 80 mg/kg/day (Days 16 and/or 19) at 1 hour post-dosing. No other treatment-related clinical signs were noted. Male body weights in the 40 mg/kg/day group were slightly lower (-5.4%) than the control group and correlated with a slight reduction in food consumption. There were no changes in body weight or food consumption in females or in the lower dosed males. Ophthalmologic examinations were unremarkable.

There was a lower mean hemoglobin concentration in females at 80 mg/kg/day, and an increase in reticulocyte counts in females at 40 and 80 mg/kg/day at terminal sacrifice (**Table 3.4-11**). A lower mean corpuscular hemoglobin was also noted in females at 80 mg/kg/day. These changes were suggestive of an effect of Posiphen tartrate on erythropoiesis, with a regenerative response that was manifested as an increase in reticulocytes. However, histological examination of the bone marrow did not reveal any correlate to the observed hematological differences.

Table 3.4-11: Mean Reticulocyte Count, Hemoglobin, and MCH Values in Male and Female CD (SD) Rats Administered Posiphen Tartrate by Gavage for 4 Weeks

Hematology Parameter	Posiphen Tartrate (mg/kg/day) Males				Posiphen Tartrate (mg/kg/day) Females			
	0	**10**	**20**	**40**	**0**	**20**	**40**	**80**
Reticulocytes ($x10^3/\mu L$)	184.3	194.7	187.6	208.1	156.1	161.4	203.3*	285.0**
Hemoglobin (g/dL)	15.1	14.8	14.5	14.6	15.1	14.8	14.5	14.0*
MCH (pg)	19.0	19.4	19.1	19.1	19.5	19.1	19.4	19.0*
WBC ($x10^3/\mu L$)	8.29	10.22	9.46	8.85	5.67	7.12	7.60	8.07*
Lymphocytes ($x10^3/\mu L$)	7.09	8.57	8.26	7.51	4.89	6.23	6.32	7.16*
*p<0.05 and **p<0.01, compared to the control group (Dunnett's test)								

Although the absolute number of WBC and lymphocytes in the high dose female group were slightly increased relative to the controls, these differences occurred only in one gender (females), were minimal, and fell within the historical control for this species and were not considered to represent a treatment-related change.

Clinical serum biochemistry parameters were similar between treated and control groups. There were no treatment-related changes in serum liver enzymes or serum proteins.

There were no Posiphen-related urinalysis findings. Lower mean urine pH values were observed in males that received 20 or 40 mg/kg/day compared to the control group (**Table 3.4-12**). However, these changes were small in magnitude, occurred only in male rats, and were within the range of the historical control data at the testing laboratory. This finding was not considered to be toxicologically significant.

Table 3.4-12: Mean Urinary pH Values for Male and Female CD (SD) Rats after Administration of Posiphen Tartrate by Gavage for 4 Weeks

Urinalysis Parameter	Posiphen Tartrate (mg/kg/day) Males				Posiphen Tartrate (mg/kg/day) Females			
	0	**10**	**20**	**40**	**0**	**20**	**40**	**80**
Number Examined	(10)	(10)	(10)	(10)	(10)	(10)	(10)	(10)
pH	6.6	6.4	6.0**	6.0**	5.9	5.8	5.7	5.9
**p<0.01, compared to the control group (Dunnett's test)								

There were no treatment-related macroscopic findings. The only organ weight changes noted were lower absolute thymic and thyroid/parathyroid weights in the male at 20 mg/kg/day group and a higher relative liver weight in females at 40 and 80 mg/kg/day (**Table 3.4-13**). However, there were no histological correlates and these changes were not considered biologically significant.

Table 3.4-13: Mean Absolute and Relative Thymus and Thyroid/Parathyroid Weights in Male and Female CD (SD) Rats Administered Posiphen Tartrate by Gavage for 4 Weeks

Organ	Posiphen Tartrate (mg/kg/day) Males				Posiphen Tartrate (mg/kg/day) Females			
	0	**10**	**20**	**40**	**0**	**20**	**40**	**80**
Liver								
Absolute (g)	10.85	10.76	10.69	10.71	6.64	6.78	7.26	7.35
Relative (% BW)	2.987	2.976	3.040	3.092	3.163	3.259	3.538**	3.479*
Thymus								
Absolute (g)	0.563	0.556	0.433*	0.490	0.449	0.384	0.403	0.397
Relative (% BW)	0.154	0.154	0.124	0.142	0.214	0.184	0.198	0.189
Thyroid/Parathyroid								
Absolute (g)	0.024	0.021	0.020*	0.21	0.016	0.016	0.016	0.017
Relative (% BW)	0.007	0.006	0.006	0.006	0.007	0.008	0.008	0.008
*p<0.05 and **p<0.01, when compared to controls (Dunnett's test)								

Microscopic examinations were unremarkable. All findings observed were consistent with normal background lesions in clinically normal rats of the age and strain used on this study and these were considered spontaneous and/or incidental in nature and unrelated to Posiphen administration.

A summary of the findings in male and female CD (SD) rats with dosing of Posiphen tartrate for 4 weeks is provided in **Table 3.4-14**. The NOAEL for Posiphen tartrate in males was 10 mg/kg/day and in females was 20 mg/kg/day.

Table 3.4-14: Summary of Findings in Male and Female CD (SD) Rats Administered Posiphen Tartrate by Gavage for 4 Weeks

Parameter		Posiphen Tartrate (mg/kg/day)			
		10	**20**	**40**	**80**
Mortality	**M**	0/10	0/10	0/10	-
	F	-	0/10	0/10	0/10
Clinical Signs		No Change	Tremors (M: ≥20 mg/kg/day; F: 80 mg/kg/day)		
					Excessive Chewing (F)
Body Weights		No Change		↓ (M)	
Food Intake		No Change		↓ (M)	
Hematology		No Change		↑ Reticulocytes; ↓ hemoglobin, ↓ MCH (F)	
Biochemistry		No Change			
Urinalysis		No Change			
Ophthalmology		No Change			
Myelogram		No Change			
Organ Weights		No Change			
Macroscopic		No Change			
Microscopic		No Change			
Toxicokinetics: Posiphen $AUC_{0\text{-}24\ h}$ (ng·h/mL) **– Day 27**					
Males		214	509	882	-
Females		-	2037	5223	12351

3.4.2.2 Repeated 1-Week Oral Dose Toxicity Study in Dogs

The MTD to repeated oral dosing of Posiphen tartrate for 7 days was investigated in male and female beagle dogs (Report 851-003). The dogs (initially 1/sex/dose) were administered a single oral (capsule) dose of 0 (control), 20, 40 or 80/60 mg/kg/day daily for up to 7 consecutive days. Each dog was monitored for morbidity/mortality, clinical signs, and body weight and food consumption changes. Blood samples were collected on Day 1 (first day of dosing) and Day 7 at 0, 0.5, 1, 2, 4, 8 and 24 hours after dosing for determination of plasma concentrations of Posiphen using a validated LC-MS/MS assay (LLOQ = 0.1 ng/mL). The animals were not sacrificed at the conclusion of the study.

Peak (C_{max}) and total ($AUC_{0\text{-}\tau}$) exposures to Posiphen generally increased in a manner that was greater than proportional to the increase in the administered dose but the values were quite variable (**Table 3.4-15**) (Report 05-4660.01). The apparent plasma elimination $t_{1/2}$ of Posiphen was approximately 2-4 hours and was independent of gender.

Table 3.4-15: Individual Peak (Cmax) and Total (AUC0-τ) Plasma Concentrations of Posiphen in Male and Female Beagle Dogs with Oral Dosing of Posiphen Tartrate for 7 Days

Sample Day	Males (mg/kg/day)			Females (mg/kg/day)		
	20	**40**	**80/60**	**20**	**40**	**80/60**
	C_{max} (ng/mL)			**C_{max}** (ng/mL)		
1	791	343	5890[a]	602	1760	9270[a]
7	144	675	1790	1150	1100	1235
	$AUC_{0-\tau\ h}$ (ng·h/mL)			**$AUC_{0-\tau\ h}$** (ng·h/mL)		
1	842	986	7995[a]	809	3417	13096[a]
7	402	1466	6647	1103	2403	4279

[a] Note: AUC on Day 0 was at a dose of 80 mg/kg/day and on Day 7 at 60 mg/kg/day

Lower Limit of Quantification (LLOQ) = 0.10 ng/mL

There were no deaths during the study. One female that received 80 mg/kg on the 1st day of dosing developed convulsions and consequently the dose level for this group was lowered to 60 mg/kg/day on the following day. Because this same animal subsequently developed convulsions at 60 mg/kg/day, two additional animals (1 male and 1 female) were added to this group.

A summary of the incidence of clinical signs is given in **Table 3.4-16**. Both females and one of the males developed convulsions at 60 mg/kg/day throughout the dosing phase. Additional clinical signs in this group included hypoactivity, ataxia, twitching, vocalization, excessive licking, lacrimation, salivation, tremors, emesis, mydriasis, and enteric symptoms (few/absent, discolored, mucoid, soft, and watery feces).

Mean body weights were decreased in males (-12 to -14%) and females (-10 to -13%) receiving 80/60 mg/kg/day of Posiphen tartrate. Decreases in mean food consumption were also noted in both males (-14%) and females (-20%) administered 40 mg/kg/day and in males (-63 to 68%) and females (-49 to -60%) administered 80/60 mg/kg, as compared to controls.

At 40 mg/kg/day, hypoactivity, ataxia, excessive licking, lacrimation, salivation, tremors, emesis, and enteric symptoms were apparent. At 20 mg/kg/day, clinical signs were limited to lacrimation, salivation, and enteric findings (discolored, mucoid, soft or watery feces).

Table 3.4-16: Summary of Clinical Observations following Oral Administrations of Posiphen Tartrate to Beagle Dogs for up to 7 Days

Clinical Observation	Posiphen (mg/kg/day)							
	Male				Female			
	0	**20**	**40**	**60[a]**	**0**	**20**	**40**	**60[a]**
Convulsions/Seizures (post dosing)	-	-	-	4/1[b]	-	-	-	8/2
Prostration	-	-	-	4/2	-	-	-	9/2
Unable to Stand After Dosing	-	-	-	1/1	-	-	-	1/1
Pupils Dilated	-	-	-	4/1	-	-	-	8/2
Coughing	-	-	-	2/1	-	-	-	4/2
Vocalization	-	-	-	2/1	-	-	-	5/2
Tremors	-	-	7/1	12/2	-	-	7/1	12/2
Twitching	-	-	-	1/1	-	-	-	2/2
Ataxia	-	-	3/1	5/2	-	-	4/1	8/2
Activity Decreased	-	-	1/1	11/2	-	-	4/1	10/2
Licking Excessively	-	-	6/1	12/2	-	-	4/1	9/2
Lacrimation	-	-	7/1	5/1	-	7/1	6/1	3/1
Emesis	-	-	2/1	8/2	-	2/1	-	4/2
Sclera Injected	-	-	2/1	1/1	-	-	-	-
Salivation	-	7/1	7/1	12/2	-	6/1	7/1	12/2
Feces Watery	-	1/1	4/1	3/1	-	1/1	3/1	1/1
Feces Mucoid	1/1	4/1	6/1	12/2	-	5/1	7/1	6/2
Feces Soft	1/1	5/1	7/1	12/2	-	6/1	7/1	8/2

[a] Was dosed at 80 mg/kg on Day 1 but was reduced to 60 mg/kg as of Day 2

[b] Number of occurrences/Number of dogs with observation

The most severe clinical sign that developed was convulsions at 80/60 mg/kg/day with episodes lasting from 20 seconds to 3 minutes. Tremors persisted in the 60 mg/kg/day group for up to 10 hours and in the 40 mg/kg/day group up to 9 hours.

The MTD with repeated oral dosing of Posiphen tartrate for up to 7 days in the dog was 40 mg/kg/day. The NOAEL was considered to be 20 mg/kg/day. For the 4 week study in dogs, the highest dose selected was 30 mg/kg/day in anticipation of tremors and gastrointestinal distress that may adversely affect the intent of study with a longer duration of dosing at higher doses.

3.4.2.3 Repeated 4-Week Oral Dose Toxicity Study in Dogs

Adult male and female beagle dogs (3/sex/dose) were administered 0, 5, 10, or 30 mg/kg/day of Posiphen tartrate orally (capsule) once daily for 30 days (Report 851-004). Additional groups of 2/sex were included in the control and 30 mg/kg/day groups for a 1 month treatment-free (recovery) period. Serial observations included clinical signs (daily), physical and neurological examinations (weekly), body weights, and food consumption. Ophthalmologic examinations, electrocardiography (1 hour post-dosing), clinical laboratory assessments (hematology, coagulation, and clinical biochemistry), and urinalysis were assessed prior to initiation of the study and during Week 4 of dosing and Week 8 of recovery. Blood samples were also collected on Days 1 and 28 at 0.25, 0.5, 1, 1.5, 2, 4, 8 and 24 hours after dosing for determination of plasma concentrations of Posiphen tartrate using a validated LC-MS/MS method (LLOQ = 0.1 ng/mL). RBC pellets were also retained for future assessment of RBC ChE activity. All animals were terminally sacrificed and underwent complete macroscopic examinations. Selected organs were weighed; all tissues were taken and processed for microscopic examinations (excluding bone marrow smears).

Systemic exposure to Posiphen (**Table 3.4-17**) was dose related (Report 05-4685.01). Peak plasma concentrations generally occurred between 0.33 and 1.5 hours after administration. Plasma concentrations of Posiphen were detected up to 8 hours in the two lower doses, and through 24 hours at the high dose. The terminal phase $t_{1/2}$ ranged from 1-4 hours, and was similar on days 1 and 28. There was no evidence of drug accumulation, inhibition or gender differences in total ($AUC_{0\text{-}24\ h}$) exposure.

There were no unscheduled deaths during the study. Clinical signs associated with oral administration of Posiphen tartrate were limited to male and female dogs that received 30 mg/kg/day and included tremors, ataxia, salivation, excessive licking, lacrimation, and soft/mucoid/watery feces. The tremors and abnormal feces continued during the study but the ataxia, salivation and excessive licking abated with continued dosing. There were no significant effects on body weights but body weight changes were slightly lower in males receiving 30 mg/kg/day at the end of the 4 weeks, consistent with the lower food consumption for this group. Ophthalmoscopic examinations were unremarkable.

Table 3.4-17: Mean Plasma C_{max} and AUC_{0-24} Values of Posiphen after Oral Dosing of Posiphen Tartrate for 4 Weeks to Beagle Dogs

Day	Posiphen Tartrate (mg/kg/day)					
	Males			Females		
	5	10	30	5	10	30
	C_{max} (ng/mL)			**C_{max}** (ng/mL)		
1	30	144	974	43	221	1263
28	53	141	858	92	281	1385
	AUC (ng·h/mL)[a]			**AUC** (ng·h/mL)[a]		
1	51	203	1309	41	218	1593
28	57	196	1448	168	282	1910

[a]AUC from zero to infinity after the first dose, and form time zero to 24 hr on Day 28.
Lower Limit of Quantification (LLOQ) = 0.10 ng/mL

Posiphen tartrate at 30 mg/kg/day was associated with a mild slowing of the HR and a lengthening of the PR interval but HR did not fall below normal in any animal and bradycardia was not noted. There were no biologically relevant changes in the QRS complex or the QTc interval **(Table 3.4-18)**.

Table 3.4-18: Mean Heart Rates and Selected ECG Values in Beagle Dogs 1-Hour after Oral Administration of Posiphen Tartrate – Week 4

Cardiovascular Parameter	Posiphen Tartrate (mg/kg/day)							
	Males				Females			
	0	5	10	30	0	5	10	30
Heart Rate (b/m)	127	128	124	108	135	148	116	125
PR interval (sec)	0.095	0.113	0.105	0.111	0.106	0.115	0.107	0.121
QTc (sec) [a]	0.232	0.236	0.235	0.240	0.236	0.234	0.238	0.239

[a] Correction factor QTc = QT-0.087 (RR-1000) Van der Walter formula

Oral administration of Posiphen tartrate at 5 or 10 mg/kg/day did not cause qualitative abnormalities or quantitative changes that are considered clinically important.

There were no changes in BP, HR, respiration, or physical/neurological observations in Posiphen-treated animals relative to the controls during the weekly examinations. There was a minimal but statistically significant lower mean BT (-0.6°C) in females administered 30 mg/kg/day but not in females at lower doses or males at any dose. The relationship of these changes to Posiphen tartrate treatment is not clear.

Clinical pathology (hematology, clinical biochemical, and urinalysis parameters) were comparable between groups, apart from a higher serum cholesterol value in males and females that received 30 mg/kg/day (**Table 3.4-19**).

Table 3.4-19: Mean Serum Cholesterol Values in Beagle Dogs after Oral Dosing of Posiphen Tartrate – Week 4

Serum Chemistry Value	Posiphen Tartrate (mg/kg/day)							
	Males				Females			
	0	5	10	30	0	5	10	30
Cholesterol (mg/dL)	144	169	165	201*	135	154	158	178*
*p<0.05, compared to the control value (pair-wise comparison) Values rounded to the nearest whole number								

Macroscopic examinations were unremarkable. There were no treatment-related differences in absolute or relative organ weights compared to the control group.

Microscopic examinations in the Posiphen treated groups at the end of dosing were unremarkable relative to the control group, apart from an erosion/ulcer with associated subacute inflammation noted in one male that received 30 mg/kg/day. This finding may possibly be related to oral administration of Posiphen tartrate. Microscopic changes noted in these dogs were of the type commonly seen in dogs of this strain and age. There were no differences in the microscopic findings between the dosed and control dogs at the end of the recovery period.

A summary of findings with Posiphen tartrate is presented in **Table 3.4-20**. The NOAEL in dogs administered Posiphen tartrate by capsule for 4 weeks was considered to be 10 mg/kg/day.

Table 3.4-20: Summary of Findings in Beagle Dogs Orally Administered Posiphen Tartrate by Capsule for up to 4 Weeks – End of Dosing Phase

Toxicology Parameter		**Posiphen Tartrate (mg/kg/day)**		
		5	**10**	**30**
Mortality	M	0/3	0/3	0/3
	F	0/3	0/3	0/3
Clinical Signs		No Change		Tremors, ataxia, salivation, excessive licking, lacrimation, soft/mucoid/watery stools
Body Weights		No Change		
Body Weight Change		No Change		Decrease in Males
Food Intake		No Change		Decrease in Males
Hematology		No Change		
Biochemistry				Increase in Cholesterol
Urinalysis		No Change		
Ophthalmology		No Change		
ECG		No Change		Decrease in HR, increase in PR interval
Physical/neurological		No Change		
Blood Pressure		No Change		
Heart Rate		No Change		
Respiration		No Change		
Body Temperature		No Change		Decrease in Females
Organ Weights		No Change		
Macroscopic		No Change		
Microscopic		No Change		Ulcer/erosion/subacute inflammation in 1 M
Toxicokinetics - Posiphen $AUC_{0\text{-}24\ h}$ (ng·h/mL) **– Day 28**				
Males		57	196	1448
Females		168	282	1910

3.4.3 Genotoxicity Studies

3.4.3.1 Bacterial Reverse Mutation Assays

The mutagenic potential of Posiphen tartrate was investigated in the bacterial reverse mutation assay (plate incorporation method) using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia coli* tester strain WP2 *uvr*A in the presence or absence of metabolic activation (Aroclor 1254-induced rat liver S9) (Report AB04WS.503.BTL).

An initial assay was performed with Posiphen tartrate at incubation concentrations of 1.5 to 5000 µg/plate (expressed as the free base) dissolved in distilled water (vehicle) to define the concentration-response curve and associated cytotoxicity. No positive mutagenic response was

noted for any tester strain at any concentration with or without S9. No precipitate was observed. Toxicity was observed at 5000 µg/plate in all strains (±S9) but not at 1500 µg/plate.

In the definitive (confirmatory) study, Posiphen tartrate was incubated at concentrations of 50, 150, 500, 1500, 1800, and 5000 µg/plate (expressed as the free base). Distilled water served as the negative control and sodium azide [NaN_3], 9-aminoacridine [9-AA], 2-nitrofluorene [2-NF], methyl methanesulfonate [MMS], and 2-aminoanthracene [2-AA] served as positive controls.

No increase in revertant colony numbers was observed over the control at any concentration of Posiphen tartrate in the presence or absence of S9 in any tester strain of *Salmonella typhimurium* or *Escherichia coli* (**Table 3.4-21**).

Table 3.4-21: Mean Number of Revertants/Plate with Posiphen Tartrate in the Presence or Absence of Metabolic Activation

Posiphen Tartrate (µg/plate)	Mean Revertants/Plate (Confirmatory Test)									
	TA98		TA100		TA1535		TA1537		WP2 *uvr*A	
	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9	–S9	+S9
0	23	38	141	153	16	15	5	4	18	25
50	20	25	152	143	20	12	4	9	21	23
150	17	38	143	141	21	15	8	9	26	23
500	19	49	167	152	21	15	9	8	21	25
1500	30	49	173	172	39	14	8	11	20	24
1800	29	39	175	180	20	14	7	11	15	17
5000	0	2	0	7	0	0	0	1	1	4
Positive Controls										
NaN_3	-	-	533	-	414	-	-	-	-	-
9-AA	-	-	-	-	-	-	719	-	-	-
2-NF	145	-	-	-	-	-	-	-	-	-
MMS	-	-	-	-	-	-	-	-	92	-
2-AA	-	967	-	1288	-	155	-	129	-	771
N = 3 plates per concentration Criteria for a positive response in TA1535 and TA1537 were 3x vehicle control -: Not done; the positive controls are unique to the specific tester strain										

Toxicity was observed at 5000 µg/plate with or without S9, but not at lower concentrations. The response of the tester strains to the negative (vehicle) and positive controls were within the expected historical range and confirmed the sensitivity of the assay. In summary, Posiphen tartrate was not mutagenic in the bacterial reverse mutation assay.

3.4.3.2 Mammalian Chromosomal Aberration Test

The clastogenic potential of Posiphen tartrate was evaluated using cultured human peripheral blood lymphocytes (HPBL) continuously exposed to concentrations of drug substance for either 4 hours with or without metabolic activation (Aroclor 1253-induced rat liver S9) or 20 hours without metabolic activation (Report AB04WS.341.BTL).

A preliminary assay was performed to establish the concentration range for the cytogenetics assay. In this assay, cell viability (mitotic index) was determined following 4 hours (±S9) or 20 hours (–S9) of exposure to cultures containing concentrations of 0.4875 to 4875 µg/mL of Posiphen tartrate (expressed as the free base) dissolved in deionized water (pH of highest incubated concentration was 7.0). Substantial toxicity (at least 50% reduction in the mitotic index relative to the solvent control) was observed with 4 hour exposure at concentrations of ≥ 146.2 µg/mL (100% at 1462 µg/mL). With 20 hour exposure, substantial toxicity was encountered at 48.75 µg/mL or more.

In the definitive assay, duplicate concentrations of Posiphen tartrate (expressed as the free base) were incubated for either 4 hours at 6.25, 12.5, 25, 50, 100, 150, 175, and 200 µg/mL (±S9) or 20 hours at 6.25, 12.5, 25, 35, 50, 75, and 100 µg/mL (–S9). The cells were harvested at 20 hours, and 100 cells/culture plate were analyzed for structural and numerical aberrations. [23] Mitomycin C [MMC] and cyclophosphamide [CP] served as positive controls in the absence and presence of S9, respectively.

Posiphen tartrate did not significantly increase numerical or structural aberrations above that of the solvent control at any concentration. Selected values covering the range of concentrations tested are provided in (**Table 3.4-22**). No precipitation was observed at any concentration with any exposure. The response of these mammalian cells to the negative (vehicle) and positive controls confirmed the sensitivity of the assay method.

Posiphen tartrate did not induce structural or numerical chromosomal aberrations in HPBL in the presence or absence of metabolic activation.

[23] The highest dose which caused at least 50% toxicity (as measured by mitotic inhibition) and two lower dose levels were analyzed.

Table 3.4-22: Mean Number of Chromosomal Aberrations in HPBL after 4 or 20 Hours of Exposure to Posiphen Tartrate in the Presence or Absence of Metabolic Activation

Posiphen Tartrate (μg/mL)	Mitotic Index (%)	Numerical Aberrations (%)	Structural Aberrations (%)
	4 h Exposure -S9		
0	100.0	0.5	0.0
50	87.8	0.0	0.0
100	80.0	0.0	0.0
175	45.5	0.0	0.0
MMC (0.6)	47.8	1.0	16.0**
	4 h Exposure +S9		
0	100.0	0.5	0.0
50	79.2	0.5	0.0
100	68.1	0.5	0.0
150	45.8	0.0	0.0
CP (20)	51.4	0.0	14.0**
	20 h Exposure –S9		
0	100.0	0.0	1.0
12.5	84.4	0.0	0.0
25	82.2	0.0	0.0
50	46.7	1.0	0.0
MMC (0.3)	48.9	0.0	15.0**

Concentrations expressed as Posiphen
**p<0.01, compared to the vehicle control (Fisher's exact test)

4.0 CLINICAL STUDIES

To-date, a total of 101 subjects have received Posiphen tartrate administered orally. Three separate Phase 1 clinical protocols (AX-PO-101, AX-PO-102, and QR-12001) provided safety, tolerability, and PK data for Posiphen in humans following oral administration[24].

Initially, Posiphen's safety was assessed in healthy volunteers in a single ascending dose study and then a multiple ascending dose study (SAD and MAD, respectively). Thereafter, using a well-tolerated dose from the former investigations, an early proof of mechanism (POM) study was conducted in patients with mild cognitive impairment (MCI), wherein time-dependent plasma and cerebrospinal fluid (CSF) samples were obtained prior to and following 10 days of Posiphen administration. This permitted analysis of drug-induced changes in CSF levels of secreted APPα and APPβ, Aβ42, Tau (total and phosphorylated) and inflammatory markers. Additionally, human brain levels of Posiphen and metabolites were estimated from measured plasma and CSF samples of MCI patients.

A tabular summary of the clinical studies conducted with Posiphen is provided in **Table 4.1-1**.

[24] Maccecchini et al. Posiphen as a candidate drug to lower CSF amyloid precursor protein, amyloid-b peptide and s levels: target engagement, tolerability and pharmacokinetics in humans. J Neurol Neurosurg Psychiatry 2012;83:pg.894-902

Table 4.1-1: Summary of Posiphen Tartrate Phase I Clinical Studies

Study Number **Investigator** **Number of Centers** **Associated IND**	**Objective(s) of Study**	**Study Design** **Diagnosis of Subjects**	**Test Product(s)** **Dose** **Timing** **Frequency** **Route**	**Number of Subjects** **Gender** **Age**	**Evaluations**	**Results**
AX-PO-101 Alan K. Copa, Pharm.D. PRACS Institute Ltd. East Grand Forks, MN Single Center IND 72,654	Evaluate the safety and tolerability of Posiphen tartrate given as single ascending doses to healthy men and women; determine the plasma PK profile of Posiphen following single oral doses of Posiphen tartrate in healthy men and women. Determine, if possible, the protein binding of Posiphen tartrate and evaluate the effect of Posiphen tartrate on *ex vivo* erythrocyte acetylcholinesterase activity.	Randomized, double-blind, placebo-controlled, single-dose, ascending, sequential cohort study. Healthy male and female subjects.	Posiphen tartrate 10, 20, 40, 80, and 160 mg, single dose, po Placebo Matching capsules	Posiphen tartrate *n* = 60 30 Males 30 Females Placebo *n* = 12 6 Males 6 Females 18–40 years	PK: Plasma samples PD: RBCs and plasma for assay of cholinesterase collected but not analyzed Safety: Adverse events, physical exams, vital signs, 12-lead ECGs, and clinical laboratory tests (serum chemistry, hematology, urinalysis)	PK: The mean T_{max} for Posiphen ranged from 1.3 to 1.6 h. Posiphen was cleared biphasically from the central compartment with a mean terminal half-life of approximately 4.0 h, independent of dose, for dose-levels where terminal half-life could be measured reliably. Systemic availability of Posiphen was non-linear, with plasma concentrations increasing disproportionately with increasing dose. The apparent volume of distribution was large, suggesting either relatively low drug absorption or extensive metabolism or partitioning out of the central compartment. Total body clearance of drug was large, suggestive of multiple pathways of elimination, possibly including active renal transport and/or extensive metabolic transformation. In the absence of quantitative renal excretion data, this could not be confirmed. Alternatively, poor oral absorption could contribute significantly to both large apparent volume of distribution and total body clearance. Safety: Single doses of Posiphen tartrate were well tolerated by healthy male and female volunteers at doses from 10 to 80 mg. A single dose of 160 mg was associated with an increased incidence of nausea and vomiting which resulted in the decision not to test higher doses.

Study Number **Investigator** **Number of Centers** **Associated IND**	**Objective(s) of Study**	**Study Design** **Diagnosis of Subjects**	**Test Product(s)** **Dose** **Timing** **Frequency** **Route**	**Number of Subjects** **Gender** **Age**	**Evaluations**	**Results**
AX-PO-102 Thomas P. Cariveau, M.D. PRACS Institute Ltd. East Grand Forks, MN Single Center IND 72,654	Evaluate the safety and tolerability of Posiphen tartrate given to healthy men and women as multiple doses, up to four times a day, for seven to ten days; determine the plasma PK profile of Posiphen following single and multiple oral doses of Posiphen tartrate in healthy men and women; evaluate the effect of Posiphen tartrate on *ex vivo* plasma butyrylcholinesterase activity and whole blood acetylcholinesterase activity as well as plasma levels of β-amyloid-related immunoreactivity.	Randomized, double-blind, placebo-controlled, ascending, multiple-dose, three-period study. Healthy male and female subjects.	Posiphen tartrate 20, 40, and 60 mg, up to QID, po Placebo Matching capsules	Posiphen tartrate *n* = 36 18 Males 18 Females Placebo *n* = 12 6 Males 6 Females 18–39 years	PK: Plasma and urine samples PD: Plasma samples for AChE and BChE activity and β-amyloid-related immunoreactivity Safety: Adverse events, physical exams, vital signs, 12-lead ECGs, and clinical laboratory tests (serum chemistry, hematology, urinalysis)	PK: Orally administered Posiphen tartrate achieved maximum plasma concentrations approximately 1.2–1.4 h post dose for all dose groups, and was eliminated biphasically. The terminal half-life in the 60-mg QID treatment group was 5.23 ± 1.24 h after the first dose and 4.74 ± 0.91 h after repeat dosing. Systemic availability of Posiphen tartrate increased disproportionately with dose over the 20–60 mg range. The QID regimen employed in this study resulted in no accumulation of Posiphen. PD: Doses of Posiphen tartrate employed in this study caused weak, transient inhibition of AChE at times near T_{max} and even less inhibition of BChE, followed during the 12-h monitoring period, by comparable increases in enzymatic activity, which was maximal 12 h post dose. The magnitude of enzyme inhibition was not strictly related to either dose or plasma concentration of Posiphen. Safety: Administration of Posiphen at doses of 20–60 mg QID appears to be free from significant safety concerns.

Study Number **Investigator** **Number of Centers** **Associated IND**	**Objective(s) of Study**	**Study Design** **Diagnosis of Subjects**	**Test Product(s)** **Dose** **Timing** **Frequency** **Route**	**Number of Subjects** **Gender** **Age**	**Evaluations**	**Results**
QR-12001 Mark T. Leibowitz, MD CEDRA Clinical Research, LLC 2455 N.E. Loop 410, Suite 150 San Antonio, TX 78217 Single Center IND 72,654	**Primary**: To assess levels of APP, Aβ40, and Aβ42 in plasma and CSF. To determine the PK of Posiphen® and its metabolites in plasma and CSF. **Secondary**: To assess levels of N-APP, T-tau, P-tau, NGF, BDNF, IL1B, and S-100B in plasma/serum and CSF. To determine the safety and tolerability of a 10-day treatment period with Posiphen®.	Open-label, two-stage trial. 10-day treatment period with Posiphen® in subjects with amnestic MCI.	Stage 1: Subjects will be dosed orally with Posiphen 60 mg QID (240 mg/day) for 10 days. Stage 2: Larger sample size, dosing with Posiphen orally at either 60 mg QID (240 mg/day) or 40 mg QID (160 mg/day) for 10 days.	<u>Posiphen tartrate</u> *n* = 5 3 Males 2 Females 55–80 years	PK: Plasma and CSF PD: APP, Aβ40, Aβ42, N-APP, T-tau, P-tau, NGF, BDNF, IL1B, and S-100B Safety: Adverse events, physical exams, vital signs, 12-lead ECGs, and clinical laboratory tests (serum chemistry, hematology, urinalysis)	This study measures changes in biomarkers associated with Posiphen administration in CSF and plasma of humans to provide proof of mechanism (POM). PK: Calculated PK parameters for Posiphen in plasma of MCI patients were similar to those in healthy volunteers (SAD and MAD studies). Posiphen mean Tmax was 1.3-1.6 h, mean terminal half-life 4.0-5.5 h, apparent volume of distribution: 2171±339 l and total body clearance 310±72 l/h. The QID regimen resulted in some accumulation of Posiphen in plasma and accumulation of Posiphen/metabolites in CSF. PD: Drug-induced differences in CSF biomarkers for MCI patients were determined by comparing the predrug and postdrug biomarker levels at each time point within the same subject to control for both circadian changes and intersubject variability. The majority of the biomarkers were analyzed by two different techniques within two independent institutions to cross-validate the data. In all cases, the direction of change was the same, with lowering of APP and tau biomarkers to levels present in healthy volunteers. Posiphen's actions on CSF inflammation markers also showed significant lowering of pro-inflammatory, C3 and microglial activation markers, MCP-1 and YKL-40. By contrast, sCD14 and factor H were unaffected by Posiphen. Safety: Administration of Posiphen at doses of 60 mg QID yielded a safety profile similar to that observed in the healthy volunteer studies. [NOTE: Stage 2 was not performed due to lack of funding.]
ECG = Electrocardiogram; PD = Pharmacodynamic; PK = Pharmacokinetic; po = Oral; QID = Four times a day						

4.1 Phase I Studies in Healthy Volunteers

4.1.1 A Single Ascending Dose (SAD) Study of the Pharmacokinetics and Tolerability of Posiphen Tartrate in Healthy Men and Women (Protocol AX-PO-101)

Using a typical design for a first-in-man Phase I study, progressively increasing single doses of Posiphen tartrate were administered to healthy normal male and female volunteers, using a double-blind, randomized, placebo-controlled design. Six groups of 10 subjects (5 males and 5 females) received Posiphen tartrate at each dose level, while one male and one female subject per dose level served as placebo controls.

Subjects were monitored for safety and tolerability, with collection of blood and urine samples for 24 hours in order to determine the pharmacokinetics of the drug. Escalating doses of Posiphen studied were 10, 20, 40, 80, and 160 mg. Limiting side effects observed following the 160 mg dose resulted in curtailment of the study without administration of the 240 mg dose. In addition, an unusually high incidence of adverse events (AEs) in both the active treatment and placebo subjects at the 20 mg dose prompted a retest of this dose in a second group of 12 subjects before escalation to the 40 mg dose.

A total of 36 men and 36 women age range 18 to 40 years, 6 of each per treatment group, were recruited from the general population and enrolled. Sixty subjects received Posiphen (10 subjects per dose level for 10, 40, 80, and 160 mg; 20 subjects for the 20 mg dose level) and 12 subjects received placebo (2 subjects per dose level for 10, 40, 80, and 160 mg; 4 subjects for the 20 mg dose level). All 72 subjects who enrolled in the study completed per protocol.

Plasma Concentrations: Observed or derived PK parameters were based upon data from a total of 60 subjects receiving Posiphen (10 subjects per dose level for 10, 40, 80, and 160 mg; 20 subjects for the 20 mg dose level). Following oral administration, peak concentration was achieved rapidly, with mean observed T_{max} between 1.3 and 1.6 hours for both males and females at all doses. C_{max} increased disproportionately with increasing dose, as did the various measures of AUC. Differences in mean observed C_{max} and AUC between males and females at each dose appeared to be related to body weight rather than gender differences.

Posiphen exhibited biphasic elimination after oral administration and for doses with well-defined plasma profiles (40–160 mg), the mean terminal $t_{1/2}$ was approximately 4.0 hours. At lower doses, the apparent mean $t_{1/2}$ was somewhat shorter, probably due to earlier decline in plasma concentration to below the detection limit and resultant incorporation of portions of the penultimate elimination phase in calculations of the terminal elimination rate constant, k_e. There were no sex-related differences in mean values for k_e or $t_{1/2}$.

Dose Proportionality: Both mean AUC and C_{max} increased more than linearly with serial increases in dose level, indicating that systemic exposure was more than dose-proportional.

PK Results: PK data from Study AX-PO-101 are presented in **Table 4.2-1**.

Table 4.2-1: Mean* Pharmacokinetic Parameters for Posiphen by Dose After Single Oral Administration of Posiphen Tartrate – Male and Female Subjects Combined Statistics (Equal Number of Males and Females/Cohort) - AX-PO-101

Parameter	**10 mg** ***n* = 10**	**20 mg** ***n* = 20**	**40 mg** ***n* = 10**	**80 mg** ***n* = 10**	**160 mg** ***n* = 10**
C_{max} (ng/mL)	3.75 ± 3.67	13.8 ± 7.6	42.5 ± 11.0	120 ± 40	384 ± 159
T_{max} (h)	1.25 ± 0.26	1.33 ± 0.37	1.45 ± 0.37	1.45 ± 0.64	1.55 ± 0.72
$AUC_{0\text{-}12}$ (ng·h/mL)	6.82 ± 8.63	23.4 ± 13.4	94.6 ± 35.6	310 ± 95	1220 ± 406
C_{last} (ng/mL)	0.0378 ± 0.0090	0.0415 ± 0.0236	0.0478 ± 0.0240	0.148 ± 0.061	0.934 ± 0.664
T_{last} (h)	7.70 ± 3.65	11.5 ± 3.0	20.3 ± 4.5	22.8 ± 3.6	24.5 ± 0.0
$AUC_{0\text{-}t}$ (ng·h/mL)	6.82 ± 8.72	23.5 ± 13.5	95.5 ± 36.1	315 ± 98	1258 ± 429
$AUC_{0\text{-}\infty}$ (ng·h/mL)	7.42 ± 9.13[a]	24.9 ± 14.0[b]	95.7 ± 36.1	316 ± 98	1264 ± 432
k_e (h^{-1})	0.535 ± 0.217[a]	0.369 ± 0.209[b]	0.183 ± 0.031	0.179 ± 0.041	0.197 ± 0.056
$t_{1/2}$ (h)	1.56 ± 0.78[a]	2.68 ± 2.03[b]	3.90 ± 0.73	4.04 ± 0.87	3.76 ± 1.01
V_z/F (L)	5650 ± 6947[a]	2269 ± 975[b]	1827 ± 678	1095 ± 366	522 ± 216
Cl/F (L/h)	3545 ± 4620[a]	743 ± 428[b]	326 ± 112	192 ± 63	97.6 ± 34.9
$AUC_{0\text{-}t}$ / $AUC_{0\text{-}\infty}$	0.971 ± 0.031[a]	0.994 ± 0.004[b]	0.997 ± 0.002	0.997 ± 0.002	0.996 ± 0.004

* Mean ± S.D.
[a] = n = 9
[b] = n = 17

Results of PK analysis are summarized below:

- The systemic availability of Posiphen increased disproportionately with increasing dose over the range 10–160 mg.
- The time to apparent maximum plasma concentration of Posiphen, approximately 1.3–1.6 hours post dose, was independent of dose and comparable for both sexes.
- The apparent volume of distribution and clearance of Posiphen (uncorrected for F, the fraction of dose absorbed) decreased with increasing dose, consistent with corresponding increases in AUC.

  Calculated values for V_z/F were much larger then V_c, the volume of the central compartment (approximately 0.09–0.1 L/Kg). Based on results of nonclinical PK studies, it is likely that Posiphen is not particularly well absorbed after oral administration (i.e., $F<<1.0$), but large values of V_z/F relative to V_c may also reflect extensive partitioning of absorbed Posiphen out of the central compartment. Even after the 160-mg dose, the mean apparent volume of distribution was approximately 65-fold greater than V_c.
- Posiphen was cleared from the central compartment in a biphasic manner, and for doses with well-defined, complete plasma profiles (40–160 mg), exhibited a dose-independent $t_{1/2}$ of approximately 4 hours in both males and females.
- Apparent sex-related differences in AUC values and C_{max} for the fixed doses of Posiphen used in this study may be explained by differences in body weight of males vs. females (i.e., these distinctions are no longer significantly different between males and females when considering doses on a mg/Kg basis).

4.1.2 A Three Period Study of the Pharmacokinetics, Pharmacodynamics and Tolerability of Posiphen Tartrate Following Multiple Oral Doses in Healthy Men and Women (Protocol AX-PO-102)

This study was a randomized, double-blind, placebo-controlled, safety, tolerance, pharmacokinetics and pharmacodynamics study in which 6 male and 6 female subjects in each of three successive groups were administered one of three serially increasing, multiple dose regimens of Posiphen tartrate, and 2 males and 2 females in each treatment group received placebo. Safety was monitored throughout and blood and urine samples were collected in order to determine the PK and PD effects of the drug. Escalating doses of Posiphen studied were 20, 40, and 60 mg administered as a single dose on the first and last day and QID during the intervening days. The first two treatments were administered for 7 days, and the third for 10 days.

A total of 24 men and 24 women age range 18 to 39 years, 8 per sex per treatment group, were recruited from the general population, enrolled, and randomized to either Posiphen or placebo in a 3:1 ratio. All subjects were included in the safety population. Thirty-six subjects received Posiphen (12 subjects per dose level for 20, 40, and 60 mg) and 12 subjects received placebo. All but two participants completed per protocol. One subject was discontinued by the principal investigator due to AEs after receiving eight 60 mg doses of Posiphen. Another subject withdrew voluntarily after receiving thirteen 60 mg doses of Posiphen.

Plasma Concentrations: Observed or derived PK parameters were based upon data from a total of 36 subjects receiving Posiphen (12 subjects per dose level for 20, 40, and 60 mg). Following oral administration, peak concentration was achieved rapidly, with mean observed T_{max} between 1.2 and 1.4 hours for the combined male and female population at all doses. C_{max} increased disproportionately with increasing dose, as did the various measures of AUC, but there was no evidence of clinically significant systemic accumulation of drug after multiple dosing. Differences in mean observed C_{max} and AUC between males and females at each dose appeared to be related to body weight.

Posiphen exhibited biphasic elimination after oral administration. For the 40 and 60 mg doses, with fully defined plasma profiles, the mean terminal $t_{1/2}$ values after Dose 1 were 3.80 ± 0.88 and 5.23 ± 1.24 hours, respectively. The terminal $t_{1/2}$ values were

essentially unchanged at 3.53 ± 1.03 and 4.74 ± 0.91 hours, respectively, after repeat dosing. For the 20 mg dose, the early decline in plasma concentrations to below the detection limit precluded full definition of the terminal elimination phase. As a consequence, the calculated apparent mean terminal $t_{1/2}$ was somewhat shorter, due to overlap of available data points into the initial elimination phase. There were no sex-related differences in mean values for terminal elimination rate constant, k_e, or for $t_{1/2}$.

Dose Proportionality: On Day 1 and following the final dose, both mean AUC and mean C_{max} increased more than linearly as a function of dose level, indicating that systemic exposure was more than dose-proportional.

Effects of Repeat Dosing: Trough plasma concentrations (C_{min}) were measured prior to the third daily dose on Days 4 and 6 for the 20 and 40 mg Posiphen groups, and on Days 7 and 9 for the 60 mg group. For each dose group, the mean values were essentially identical on both days, reflecting achievement of steady state prior to determination of full PK profiles following the final dose, on Day 7 or 10, respectively. Based on comparisons of mean C_{max} and AUC values in each treatment group, four times a day (QID) administration produced little change between the first and last dose of Posiphen. Thus, for the multiple dose regimen employed in this study, the extent of systemic accumulation of Posiphen at steady state was negligible.

PK Results: PK data from Study AX-PO-102 are presented in Table 4.2-2. Results of the PK analysis are summarized below:

- As reflected in both C_{max} and AUC values, the systemic availability of Posiphen increased disproportionately with increasing dose over the range 20–60 mg.
- At all dose levels, there was no appreciable accumulation of Posiphen after repeat dosing.
- The time to apparent maximum plasma concentration of Posiphen, approximately 1.2–1.4 hours post-dose, was independent of dose and sex and did not change after repeat administration of Posiphen.
- The apparent volume of distribution and whole-body clearance of Posiphen (uncorrected for F, the fraction of dose absorbed) decreased with increasing dose, consistent with the inverse relationship between V_z/F and AUC.

- Posiphen was cleared from the central compartment in a biphasic manner. For the combined male and female population in the 60-mg QID treatment group, the only dose level with well-defined, complete plasma profiles, Posiphen exhibited a mean terminal $t_{1/2}$ of 5.23 ± 1.24 hours after the first dose and was essentially unchanged at 4.74 ± 0.91 hours after repeat dosing.
- Apparent sex-related differences in AUC values and C_{max} for the fixed doses of Posiphen used in this study may be explained by differences in body weight of males vs. females (i.e., these distinctions are no longer significantly different between males and females when doses are expressed on a mg/kg basis).

PD Results: In Study AX-PO-102, blood samples for determination of AChE and BChE activity, and Aβ related immunoreactivity were collected at time points generally corresponding to sampling times for Posiphen pharmacokinetics. Results of PD analysis are summarized below:

- At doses used in this study, inhibition of AChE was maximal but weak at or near T_{max}, and exhibited a small enhancement of activity relative to placebo beyond 5 hours post-dose.
- The differences between the Posiphen and placebo groups with respect to BChE changes were smaller than those for AChE and there was no clear dose-related or temporal pattern suggesting a significant effect in this group of healthy subjects.

Table 4.2-2: Mean* Pharmacokinetic Parameters for Posiphen by Dose After Repeat-Dose Oral Administration of Posiphen Tartrate – Male and Female Subjects Combined Statistics (Equal Number of Males and Females/Cohort) - AX-PO-102

Parameter	**20 mg (*n* = 12)**		**40 mg (*n* = 12)**		**60 mg (*n* = 10)**	
	Day 1	**Day 7**	**Day 1**	**Day 7**	**Day 1**	**Day 10**
C_{max} (ng/mL)	12.7 ± 7.2	9.55 ± 3.85	64.6 ± 23.1	52.7 ± 17.6	103 ± 31	88.0 ± 29.2[b]
T_{max} (h)	1.38 ± 0.43	1.29 ± 0.26	1.21 ± 0.26	1.38 ± 0.38	1.33 ± 0.33	1.40 ± 0.32[b]
AUC_{0-5} (ng·h/mL)	22.3 ± 14.0	16.2 ± 6.9	133 ± 56	113 ± 42	219 ± 76	223 ± 88[b]
C_{last} (ng/mL)	0.0518 ± 0.0412	0.0734 ± 0.0671	0.0525 ± 0.0292	0.149 ± 0.101	0.137 ± 0.093	0.303 ± 0.202[b]
T_{last} (h)	11.2 ± 3.0	10.8 ± 2.4	21.3 ± 3.9	20.0 ± 5.9	24.0 ± 0.0	24.0 ± 0.0[b]
AUC_{0-t} (ng·h/mL)	23.2 ± 14.6	17.1 ± 7.4	141 ± 59	129 ± 55	240 ± 91	275 ± 123[b]
$AUC_{0-\infty}$ (ng·h/mL)	24.2 ± 15.1[a]	17.4 ± 7.7	144 ± 61[a]	134 ± 56[a]	237 ± 94[a]	277 ± 124[b]
k_e (h^{-1})	0.351 ± 0.172[a]	0.294 ± 0.073	0.194 ± 0.056[a]	0.217 ± 0.079[a]	0.140 ± 0.034[a]	0.150 ± 0.024[b]
$t_{1/2}$ (h)	2.76 ± 1.93[a]	2.51 ± 0.69	3.80 ± 0.88[a]	3.53 ± 1.03[a]	5.23 ± 1.24[a]	4.74 ± 0.91[b]
V_z/F (L)	4047 ± 2592[a]	4870 ± 2801	1870 ± 1078[a]	1661 ± 605[a]	2197 ± 986[a]	1741 ± 689[b]
Cl/F (L/h)	1462 ± 1620[a]	1388 ± 664	351 ± 204[a]	346 ± 135[a]	291 ± 119[a]	253 ± 97[b]
AUC_{0-t} / $AUC_{0-\infty}$	0.991 ± 0.007[a]	0.984 ± 0.015	0.997 ± 0.002[a]	0.995 ± 0.003[a]	0.996 ± 0.003[a]	0.993 ± 0.004[b]

* Mean ± SD
[a] = n = 11
[b] = n = 10

4.2 Exploratory Studies in Patients

4.2.1 An Open-Label, Two-Stage Study to Evaluate the Pharmacokinetics and Pharmacodynamics of Posiphen® in Plasma and Cerebrospinal Fluid (CSF) after a 10-Day Treatment Period in Subjects with Amnestic Mild Cognitive Impairment (MCI) (Protocol QR-12001)

This study was designed as an open-label, 2-stage study in which 5 healthy male and female MCI subjects were to be enrolled in Stage 1, and dosed with Posiphen 60 mg QID for 10 days. The design of Stage 2 was identical to the design of Stage 1 except for the number of subjects, the doses of Posiphen (to include 40 mg QID), and the measurements of PK and PD (which may be refined based on the results from Stage 1)[25].

Subjects were male or postmenopausal females, between 55 and 80 years of age, with self-reported memory complaints that were corroborated by spouse or companion or caregiver as appropriate, and memory difficulties as measured on neuropsychological tests. MCI was determined according to Petersen's criteria[26] with a Mini Mental Status Examination score ≥24, cut-off score on the logical memory II delayed paragraph recall subtest of the Wechsler Memory Scale Revised, Clinical Dementia Rating of 0.5 with a memory box score of 0.5 or 1.0.

This study measured PK in plasma and CSF, and PD changes in biomarkers associated with Posiphen administration in CSF and plasma of subjects with MCI, to provide proof of mechanism (POM). CSF and plasma were obtained over a 12 hour period prior to and following 10 days of dosing with Posiphen.

Posiphen administration for 10 days had a statistically significant effect on lowering secreted APP α and β as well as total-tau and phosphorylated-tau levels. All measurements were conducted by two independent labs with different assay conditions and different antibodies. The data obtained for APP and tau was comparable and reproducible[27].

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[25] Stage 2 of Protocol QR-12001 was not conducted due to lack of funding.

[26] Petersen RC. Mild cognitive impairment as a diagnostic entity. J Intern Med 2004;256:183-94

[27] Measurement of Aβ42 levels were compromised due to an unknown handling factor that caused Aβ42 to precipitate in a number of CSF samples at Day 11. No samples precipitated at Day 0. A comparison of precipitated and un-precipitated samples at Day 11 with Day 0, showed precipitated samples to be lower by about 95%, whereas un-precipitated samples did not show a decrease in Aβ42. Exclusion of the precipitated samples resulted in Aβ42 values that did not seem to differ from the Day 0 Aβ42 values.

PK Results: Calculated pharmacokinetic parameters for Posiphen in plasma of MCI patients were similar to those in healthy volunteers (SAD and MAD studies). Posiphen mean T_{max} was 1.3-1.6 h, mean $t_{1/2}$ 4.0-5.5 h, apparent volume of distribution: 2171±339 l and total body clearance 310±72 l/h. The QID regimen resulted in some accumulation of Posiphen in plasma and accumulation of Posiphen/metabolites in CSF.

Extrapolated brain levels for MCI patients were determined by applying the rat plasma/CSF/brain data to the human plasma and CSF data. This estimate predicts Posiphen brain levels approaching 1 ug/g or approximately 3.5µM for a dose of 4x60 mg/day.

PD Results: Drug-induced differences in CSF biomarkers for MCI patients were determined by comparing the predrug and postdrug biomarker levels at each time point within the same subject to control for both circadian changes and intersubject variability. The majority of the biomarkers were analyzed by two different techniques within two independent institutions to cross-validate the data. In all cases, the direction of change was the same, with lowering of APP and tau biomarkers to levels present in healthy volunteers. Posiphen's actions on CSF inflammation markers also showed significant lowering of pro-inflammatory, C3 and microglial activation markers, MCP-1 and YKL-40. By contrast, sCD14 and factor H were unaffected by Posiphen.

4.3 Overview of Efficacy

Not applicable.

4.4 Overview of Safety

Posiphen tartrate has been evaluated for safety and tolerability in humans when administered orally. A summary of the available safety data from these studies is provided below.

4.4.1 A Single, Ascending Dose Study of the Pharmacokinetics and Tolerability of Posiphen Tartrate in Healthy Men and Women (Protocol AX-PO-101)

The pattern of AEs was similar to that seen in typical studies in healthy normal volunteers, with an overall incidence of 33.3% among placebo-treated subjects and 35% (31.7%, treatment-related) for all Posiphen treatment groups combined. There was a tendency, but no definitive pattern of increased incidence of AEs with increasing dose of Posiphen. Most AEs were of short duration, mild or moderate in severity, resolved without medical intervention, and occurred in one or a few subjects. Only two subjects

experienced severe AEs, including symptoms associated with orthostatic hypotension (1 placebo and 1 Posiphen 20-mg subject).

There were no serious adverse events (SAEs) and no one was withdrawn from the study as a result of an AE.

There were no clinically significant changes associated with Posiphen in physical examination findings, vital signs, ECG parameters, or clinical laboratory tests. One clinical laboratory parameter for which a pattern of dose-related changes was suggested was a modest decrease in total serum protein. The incidence of this change was comparable between the placebo group and all Posiphen groups except the highest dose group (160 mg). The difference was due primarily to a single subject in the 160 mg dose group, so its significance was considered uncertain. Serum glucose levels were elevated in 80% of subjects in the 10 mg dose group on Day 2 but not on Day 6. This effect was not observed at any other time in any other treatment group and the cause was undetermined.

Most AEs in this blinded study were considered by the investigator to be at least possibly related to study treatment. Among those receiving Posiphen, the proportion of subjects with any AE increased at the 160-mg dose (70%), but overall incidence among Posiphen subjects (35%) was comparable to that for the placebo group (33.3%). Severe AEs were reported for 2 subjects in the 20 mg treatment group (nausea, dizziness and orthostatic hypotension) and one who received placebo (lightheadedness, fainting, orthostatic hypotension, and tachycardia). Mild and moderate AEs occurred with similar prevalence in both the placebo group (n = 12) and the entire Posiphen population (n = 60), at 25.0 and 33.3%, respectively.

Adverse events that occurred in more than one subject in the Posiphen group, the placebo group, or in the entire cohort (corresponding to at least 10–15% of at least one population), are summarized by dose in **Table 4.4-1** for both male and female subjects combined.

Table 4.4-1: Summary of Adverse Events in More Than One Subject Per Treatment Group (Males and Females Combined) Study AX-PO-101

Body System Adverse Event[a] (Preferred Term)	**Number (%) of Patients in Treatment Group (*n* = 72)**						
	10 mg (*n* = 10)	**20 mg (*n* = 20)**	**40 mg (*n*= 10)**	**80 mg (*n* = 10)**	**160 mg (*n* = 10)**	**All Posiphen (*n* = 60)**	**Placebo (*n* = 12)**
All AEs, mild	2 (20.0)	4 (20.0)	1 (10.0)	3 (30.0)	3 (30.0)	13 (21.7)	2 (16.7)
All AEs, moderate	1 (10.0)	2 (10.0)	0 (0)	0 (0)	4 (40.0)	7 (11.7)	1 (8.3)
All AEs, severe	0 (0)	1 (5.0)	0 (0)	0 (0)	0 (0)	1 (1.7)	1 (8.3)
Gastrointestinal Disorders							
Nausea	0 (0)	2 (10.0)[b]	0 (0)	0 (0)	4 (40.0)	6 (10.0)	0 (0)
Vomiting	0 (0)	0 (0)	0 (0)	0 (0)	3 (30.0)	3 (5.0)	0 (0)
General Disorders and Administration Site Conditions							
Feeling hot	0 (0)	2 (10.0)	0 (0)	0 (0)	0 (0)	2 (3.3)	0 (0)
Investigations							
Heart rate increased	2 (20.0)	1 (5.0)	0 (0)	0 (0)	0 (0)	3 (5.0)	2 (16.7)[b]
Nervous System Disorders							
Dizziness	1 (10.0)	4 (20.0)[b]	1 (10.0)	3 (30.0)	4 (40.0)	13 (21.7)[b]	3 (25.0)[b]
Fainting[c]	1 (10.0)	1 (5.0)	0 (0)	0 (0)	0 (0)	2 (3.3)	1 (8.3)
Vascular Disorders							
Orthostatic hypotension	0 (0.0)	1 (5.0)[b]	0 (0)	0 (0)	0 (0)	1 (1.7)[b]	1 (8.3)[b]

[a] = A subject may have experienced more than one AE in more than one category at more than one severity, each counted as a separate event.
[b] = Includes at least one AE reported as severe.
[c] = *Dizziness* is the preferred MedDRA term, which encompasses descriptive terms *fainting*, *dizzy*, and *lightheadedness.*

Although not always regarded by the investigator as AEs, dizziness/fainting and orthostatic hypotension were the most frequently observed safety observations during the course of this study. Orthostatic hypotension was observed with increasing frequency as the Posiphen dose increased from 40 to 160 mg but also occurred with comparable frequency in placebo subjects. Some subjects experienced orthostatic effects at multiple measurement points post dose, but there was no apparent correlation between time of occurrence and plasma concentration of Posiphen.

Overall, single doses of 10–80 mg of Posiphen were well tolerated. Collectively, mild and moderately severe related AEs occurred with greater frequency as the Posiphen dose was increased, with a higher incidence for the Posiphen treatment population as a whole than placebo. An increased incidence of nausea and vomiting was observed following the 160 mg dose. The incidence of AEs at this dose was deemed dose-limiting, prompting study termination without evaluation of the 240 mg dose.

4.4.2 A Three Period Study of the Pharmacokinetics, Pharmacodynamics and Tolerability of Posiphen Tartrate Following Multiple Oral Doses in Healthy Men and Women (Protocol AX-PO-102)

The pattern of AEs was generally typical of healthy normal volunteers in Phase I studies, with an overall incidence of 50% among placebo-treated subjects and 41.6% (38.9%, treatment-related) for all Posiphen treatment groups combined. There was a tendency, but no definitive pattern of increased incidence of AEs with increasing dose of Posiphen. No AEs were considered severe by the investigator.

There were no SAEs or deaths in this study. One female subject receiving Posiphen 60 mg was discontinued on Day 3 as a result of AEs (dizziness, “feeling warm,” nausea and vomiting) which resolved without further medical intervention.

There were no clinically significant changes associated with Posiphen in physical examination findings, vital signs, ECG parameters, or clinical laboratory findings. An unexplained decrease in serum total protein (but not albumin) was noted post treatment in all treatment groups. This phenomenon, while not deemed clinically significant by the investigator, had also been observed in the prior Phase I clinical trial, AX-PO-101.

Subjects receiving Posiphen 20 mg had the highest overall incidence of AEs. Most AEs were mild (30 AEs reported for Posiphen and 14 for placebo) and a few were moderate in severity (7 for Posiphen, 2 for placebo). There were no severe AEs recorded for any subject in either the Posiphen or placebo groups. With the small number of subjects, it was difficult to project trends for incidence of AEs, with the possible exception of dizziness, nausea and vomiting, the overall pattern and prevalence of mild and moderately severe AEs seemed similar for Posiphen and placebo.

Adverse events that occurred in more than one subject are summarized by treatment group in **Table 4.4-2** for both male and female subjects combined.

Table 4.4-2: Summary of Adverse Events in More Than One Subject Per Treatment Group (Males and Females Combined) Study AX-PO-102

Body System **Adverse Event[a]** **(Preferred Term)**	**Number (%) of Patients in Treatment Group (*n* = 48)**				
	20 mg **(*n* = 12)**	**40 mg** **(*n* = 12)**	**60 mg** **(*n* = 12)**	**All Posiphen** **(*n* = 36)**	**Placebo** **(*n* = 12)**
All AEs, mild	6 (50.0)	3 (25.0)	3 (25.0)	12 (33.3)	4 (33.3)
All AEs, moderate	2 (16.7)	0 (0)	1 (8.3)	3 (8.3)	2 (16.7)
All AEs, severe	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gastrointestinal Disorders					
Abdominal pain, upper	1 (8.3)	0 (0)	0 (0)	1 (2.8)	1 (8.3)
Constipation	2 (16.7)	1 (8.3)	0 (0)	3 (8.3)	3 (25.0)
Nausea	1 (8.3)	0 (0)	2 (16.7)	3 (8.3)	1 (8.3)
Vomiting	0 (0)	0 (0)	3 (25.0)	3 (8.3)	0 (0)
General Disorders and Administration Site Conditions					
Feeling hot	0 (0)	0 (0)	1 (8.3)	1 (2.8)	1 (8.3)
Nervous System Disorders					
Abnormal dreams	3 (25.0)	0 (0)	0 (0)	3 (8.3)	2 (16.7)
Dizziness	2 (16.7)	2 (16.7)	3 (25.0)	7 (19.4)	1 (8.3)
Headache	2 (16.7)	3 (25.0)	1 (8.3)	6 (16.7)	2 (16.7)

[a] = A subject may have experienced AEs in more than one category. Multiple AEs with the same preferred term are counted once for each subject, at the maximum reported severity.

Overall, Posiphen tartrate, administered orally in doses of 20, 40, and 60 mg QID was well tolerated. Mild and moderately severe treatment-related AEs occurred with greatest frequency in the Posiphen 20 mg group, with an incidence only slightly greater than that for placebo. Overall, the proportion of Posiphen subjects reporting mild and moderately severe AEs was lower than that for placebo. There were no severe, treatment-related AEs.

Aside from nausea and vomiting, which are well-known responses to treatment with ChE inhibitors, the only consistent pattern of AEs entailed dizziness/fainting, headache, and reduction in total serum protein. These effects were seen to varying degrees at all doses of Posiphen and also in the placebo group. For other ChE inhibitors, nausea and vomiting upon initiation of treatment have been reduced in both frequency and severity by initial dose titration, a strategy that may work for Posiphen, as well.

4.4.3 An Open-Label, Two-Stage Study to Evaluate the Pharmacokinetics and Pharmacodynamics of Posiphen® in Plasma and Cerebrospinal Fluid (CSF) after a 10-Day Treatment Period in Subjects with Amnestic Mild Cognitive Impairment (MCI) (Protocol QR-12001)

A total of 5 subjects were enrolled in Stage 1, and 4 subjects completed the scheduled procedures. Subjects were monitored for any adverse events from the beginning of confinement until discharge. A total of 19 treatment emergent AEs were reported by 5 of the 5 subjects over the course of the study. Thirteen (13) of the 19 AEs were mild and 6 were moderate. Five (5) of the AEs were probably related to the study drug, 10 were possibly related, and the remaining 4 were unrelated. Subject 503 withdrew consent due to adverse events of anorexia, nausea, headache, vivid dreams and vomiting prior to the Day 2, 1700 hour dose.

Posiphen (60 mg QID x 10 days) in MCI subjects showed a similar safety profile as found in the healthy volunteer studies. The most commonly reported AEs were headache/intermittent headache (n=7) and nausea/intermittent nausea (n=4).

Adverse events that occurred in more than one subject are summarized by treatment group in **Table 4.4-3** for both male and female subjects combined.

Table 4.4-3: Summary of Adverse Events in More Than One Subject Per Treatment Group (Males and Females Combined) Study QR-12001

Body System **Adverse Event[a]** **(Preferred Term)**	**Number (%) of Patients in Treatment Group** **60 mg QID** **(*n* = 5)**
All AEs, moderate	0 (0) 1*
All AEs, severe	0 (0)
Gastrointestinal Disorders	
Nausea	1 (20.0) 1*
Vomiting	0 (0) 1*
Nervous System Disorders	
Dizziness	1 (20.0)
Headache	5 (100.0)
General Disorders and Administration Site Conditions	
General pain	1 (20.0)
Other	5 (100.0)

[a] = A subject may have experienced AEs in more than one category. Multiple AEs with the same preferred term are counted once for each subject, at the maximum reported severity.

* = One subject had leg cramps and was nauseous during the catheterization (predrug). The subject vomited and dropped out after the second and before the third 60mg dose on Day 1.

5.0 SUMMARY OF DATA AND GUIDANCE FOR THE INVESTIGATOR

Clinical studies with single and repeated daily oral administration of Posiphen tartrate to healthy volunteers showed Posiphen to be well tolerated up to single doses of 80 mg or QID doses of 60 mg. A single dose of 160 mg was associated with an increased incidence of nausea and vomiting which resulted in the decision not to test higher doses. A similar safety profile was observed in the study of Posiphen (60mg QID x 10 days) in MCI patients.

Aside from nausea and vomiting, which are well-known responses to treatment with ChE inhibitors, the only consistent pattern of AEs entailed dizziness/fainting, headache, and reduction in total serum protein. These effects were seen to varying degrees at all doses of Posiphen and also in the placebo group. There was a tendency, but no definitive pattern of increased incidence of AEs with increasing dose of Posiphen. There have been no SAEs reported in any clinical study with Posiphen to-date.

Preclinical pharmacology and toxicology studies with repeated daily oral administration of Posiphen tartrate for up to 30 days in mice, rats and dogs showed effects limited to the central and peripheral nervous systems and gastrointestinal tract. Dose limiting toxicities were seen at 30 mg/kg/day and above and included ataxia and tremors/twitching. Additional effects in animals included salivation, lacrimation, excessive licking or chewing, slight anemia with reticulocytosis and emesis. Full body tremors and hypoactivity were observed in mice dosed orally and intraperitoneally at the 50 and 75 mg/kg/day dose levels. Tolerance to some of these occurred with continued dosing. The signs/symptoms noted at these high doses of Posiphen tartrate may be related to cholinergic manifestations.

A study conducted to analyze the effect of orally administered Posiphen on Aβ levels in the mouse brain, revealed that Posiphen lowered Aβ levels at plasma concentrations that are attainable with oral dosing in humans.

Posiphen tartrate did not adversely affect the QT (or QTc) interval *in vitro* or *in vivo*. In dogs, a transient increase in BP, HR, and body temperature occurred after a single oral dose of 20 mg/kg; however, HR and body temperature were slightly lower in dogs following repeated oral dosing at 30 mg/kg/day for 4 weeks. Given the minimal changes observed in these parameters and their transient nature, it is unclear whether these divergent results represent a possible clinical risk. No treatment-related changes were

noted in organ weights, or in macroscopic or microscopic examinations in either species with the exception of a possibly related erosion/ulcer in the ileum in one male dog that received 30 mg/kg/day for 4 weeks.

Posiphen tartrate was not mutagenic or clastogenic as assessed by *in vitro* assays. There were no effects on the reproductive organs associated with 4 week exposure to Posiphen tartrate in male or female rats or dogs. However, definitive reproductive and developmental toxicity studies have not been conducted. Therefore, caution should be taken for women that will participate in the initial Phase I clinical development program.

The clinical investigator should advise all potential subjects and patients of the possibility of unexpected side effects and carefully evaluate each person exposed to Posiphen tartrate for possible adverse experiences.

Because strategies for the management of overdose are continually evolving, it is advisable to contact a poison control center to determine the latest recommendations for the management of an overdose of any drug. Although Posiphen is a weak ChE, based upon overdose information published in prescribing information for related products, overdosage with ChE inhibitors can result in cholinergic crisis characterized by severe nausea, vomiting, salivation, sweating, bradycardia, hypotension, respiratory depression, collapse and convulsions. Increasing muscle weakness is a possibility and may result in death if respiratory muscles are involved. Tertiary anticholinergics such as atropine may be used as an antidote for overdosage. Intravenous atropine sulfate titrated to effect is recommended. Atypical responses in BP and HR have been reported with other cholinomimetics when coadministered with quaternary anticholinergics such as glycopyrrolate. It is not known whether dialysis (hemodialysis, peritoneal dialysis, or hemofiltration) is an effective treatment for overdose.