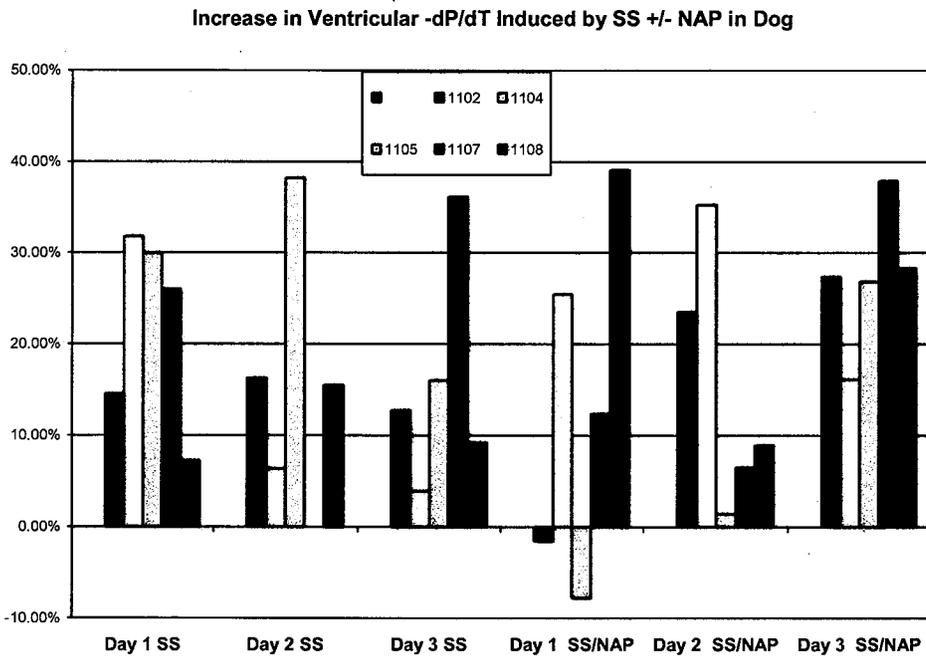
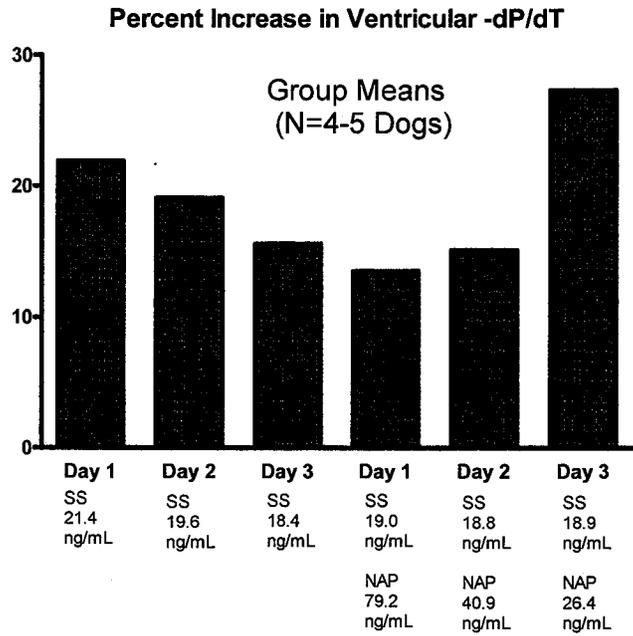


Left Ventricular -dP/dT:

The mean increase in -dP/dT induced by SS was not statistically significantly affected by coadministration of 20 mg/kg IV NAP on Day 1 (p=0.4386), Day 2 (p=0.9855), or Day 3 (p=0.1455).



In Phase III, the mean increase in -dP/dT induced by 200 ug/kg IV SS was not statistically significantly different with coadministration of 20 mg/kg IV NAP (p=0.3097).

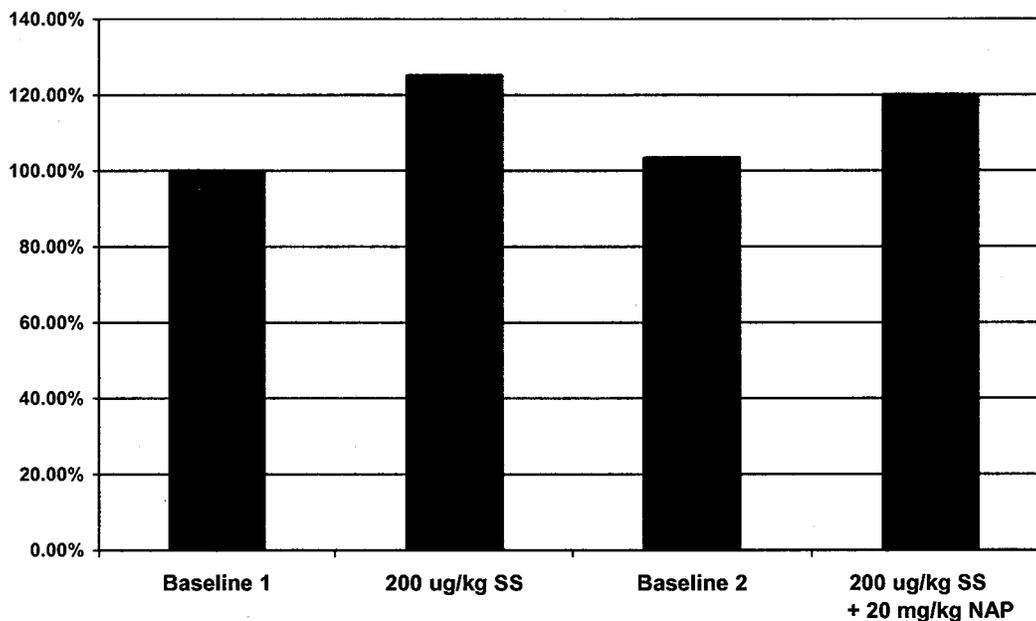
PHASE III  
 Study QCBW 106  
 Table 20  
 Maximum Increases in  
 -dP/dT (mmHg/sec)

Dog ID	200 µg/kg Sumatriptan			20 mg/kg Naproxen + 200 µg/kg Sumatriptan			(Nap+Suma) - Suma
	Baseline [a]	Maximum -dP/dT [b]	Change From Baseline [c]	Baseline [a]	Maximum -dP/dT [b]	Change From Baseline [c]	
1101[d]	.	.	.	.	.	.	.
1102	5464.8	6531.0	1066.2	5034.6	6019.0	984.4	-81.8
1104	2683.8	4130.0	1446.2	2893.0	3749.0	856.0	-590.2
1105[d]	.	.	.	.	.	.	.
1107[d]	.	.	.	.	.	.	.
1108	2610.2	2657.0	46.8	2874.4	2879.0	4.6	-42.2
						Mean	-238.1
						STD	305.6
						95% CI	( -997.2, 521.1)
						p-value	0.3097

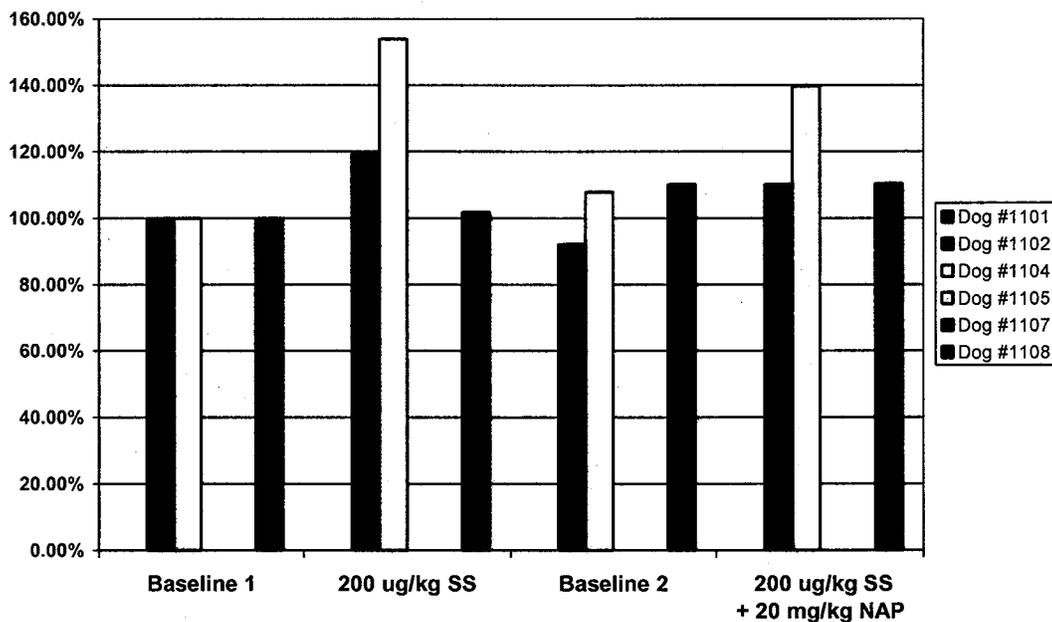
[a] The baseline values were obtained by taking the mean of the data collected during the 5 minute interval immediately preceding sumatriptan (sumatriptan only) or naproxen (naproxen + sumatriptan).  
 [b] Maximum -dP/dT during the first hour after sumatriptan administration.  
 [c] Maximum -dP/dT - Baseline -dP/dT.  
 [d] Period (.) denotes a missing value.

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Mean Change in -dP/dT Induced by SS +/- NAP



Change in -dP/dT Induced by SS +/- NAP



**Deviations from Protocol:**

- The LCX flow probe malfunctioned for Dog #1107 preventing collection of data on blood flow and other parameters dependent upon blood flow during all 3 phases.
- The carotid flow probe malfunctioned for Dog #1104 preventing collection of data on blood flow and other parameters dependent upon blood flow during Phase III only.
- Signal was lost from both LCX and carotid flow probes and catheters, and from the LCX crystals (measuring diameter) in Dog #1105, so no assessments were made in Phase III for this animal.

**Sponsor's Conclusions:**

- **“There were no statistically significant changes to the SS-induced response of the coronary and carotid arteries observed during Phase I as a result of combined administration of naproxen sodium (NAP) with SS during Phase II.”**
- **“There were no statistically significant, biologically relevant changes to the responses of the coronary and carotid arteries as a result of combined administration of NAP with SS,” during Phase III (200 ug/kg IV SS + 20 mg/kg IV NAP).**
- **“In conclusion, co-administration of NAP with SS did not alter the vasoconstrictive effect of SS on the coronary arteries of conscious, chronically instrumented female beagle dogs, nor did it alter any of the other cardiovascular parameters measured in this study.”**

**Reviewer's Conclusions:**

The Sponsor's conclusion that Phases I and II showed no statistically significant changes is technically accurate, based on the paired t-test analyses of the differences between the SS-induced reductions in coronary and carotid artery diameter in the presence and absence of NAP. However, review of the group mean and individual animal data from Day 1 treatments revealed apparent trends toward NAP-related enhancement of SS-induced effects on coronary artery diameter, carotid artery diameter, mean arterial blood pressure (MAP), and coronary artery resistance. The importance of such trends is questionable, though, in the context of the wide inter-individual and intra-individual variation observed and the design flaws noted several paragraphs below.

Four out of six dogs showed substantially (2- to 7-fold) greater SS-induced reductions in LCX coronary artery diameter in the presence (7-10%) vs. the absence (1-4%) of NAP on Day 1. The group average difference (~2-fold) did not reach statistical significance because one dog showed only a small change (~5.0% SS→~5.5% SS/NAP) and one (#1107) showed a change in the opposite direction (~8% SS→~3.5% SS/NAP). However, it is difficult to argue that these data demonstrate a consistent NAP effect when both the intra-individual (day to day) and inter-individual variation are so great.

The mean SS-induced reduction in LCX coronary artery resistance was ~4-fold greater with NAP than without on Day 1, yet this difference was not statistically significant, due to the high inter-individual variability in the SS alone group (from  $\uparrow$ 10% to  $\downarrow$ 23%). In this case, a hint of a possible NAP effect comes from the much lower variability among individuals in the Day 1 SS/NAP group ( $\downarrow$ 17-26% in all 5 dogs).

The mean SS-induced reduction in carotid artery diameter was 2-fold greater with NAP than without on Day 1, yet, again, this difference was not statistically significant, due to the wide variability among individuals in the SS group ( $\downarrow$ 2-24%) and in the SS/NAP group ( $\downarrow$ 7-34%). Variation was also quite wide from day to day within each individual, arguing against the reliability of these data.

The mean SS-induced increase in MAP was ~1.5-fold greater with NAP than without on Day 1, but this difference was not statistically significant, due to variability. One anomalous dog showed an increase in MAP of 29% with SS and -1% with SS/NAP, and two others showed little or no extra increase in MAP with NAP. The two dogs showing the greatest increase in SS-induced MAP increase with NAP (~3-fold), showed similar large increases on Day 3, when NAP levels had decreased 3-fold, and no increases on Day 2. These data suggest that the increases in MAP were due to technical problems, or unknown factors, rather than to the presence of SS/NAP.

**The Sponsor's conclusions regarding Phase III are invalidated by the design flaws described in the third paragraph below.**

Study MT400-T15 was inappropriately designed. The N of 6 dogs was chosen to provide a power of > 80% to detect the mean reduction in external coronary arterial diameter (eCAD) of  $1.37 \mu\text{m} \pm 21$  ( $\downarrow$ 5.3% from baseline) induced by 100  $\mu\text{g}/\text{kg}$  IV SS in conscious dogs (*Carel et al., 2001, Br J Pharmacol 132:1071-1083*). However, the present study was intended to detect a *change* in that level of reduction (by NAP), not just the SS-induced reduction itself. Therefore a larger N would have been needed to provide a power of > 80% to detect an effect of NAP on SS-induced coronary artery vasoconstriction.

It is not clear to this reviewer that the data derived from Days 2 and 3 of Phase II are informative, since having plasma levels of NAP still on board from an injection 24 or 48 hrs before the fresh injection of SS is quite different pharmacologically from the clinical condition of having rapidly rising plasma concentrations of both SS and NAP in the absence of recent prior exposure. Compensatory responses to the prolonged NAP exposure may even interfere with the SS-induced vasoconstriction. If lower doses of NAP are considered desirable to evaluate, they should be administered to a separate group of animals, or to the same animals after a sufficient washout period (at least 5 days, since the half-life of NAP in dogs is ~35-40 hrs). Also, given the variability observed, repeated measures at each dose, with appropriate washout intervals, would improve the reliability of the results.

Most importantly, this study was inappropriately controlled. The changes attributed to SS alone in Phase I were collected by recording the minimum or maximum (depending on whether that parameter had increased or decreased with IV SS treatment in Carel et al. [2001, *Br J Pharmacol* 132:1071-1083]) change from baseline achieved during the one hour period after SS injection. Given the natural variation in these parameters over time, and the lack of a vehicle control for the SS injection, the “SS-induced” changes may not, in fact, be induced by SS at all. A vehicle control for the SS-injection is essential in this sort of min/max change from baseline within 1 hr paradigm. Carel et al. (2001) did not include a vehicle control injection, but they cited previous articles *from their group* demonstrating that “saline administration was devoid of any effect in this experimental setting. Even better, Carel et al. (2001) studied the entire range of the SS dose-response curve, including a dose (0.1 ug/kg) that induced no significant changes in any of the parameters tested. Inclusion of representative traces from the recordings of coronary and carotid artery diameter before and after injection of sumatriptan or frovatriptan also added credibility to the findings of Carel et al. (2001).

Finally, since the half-life of SS in dogs is ~2 hrs, Phase III should have included a washout period of at least 10 hrs (rather than 1 hr) to allow for clearance of the first injection of SS before administration of the second dose of SS (+ NAP). Significant levels of SS were still on board, so the second “baseline” was not a true baseline, and the SS plasma levels were 65% higher than those after the first dose of SS. Therefore, any differences (or lack of differences) seen between the first SS treatment without NAP and the second with NAP may be due to the higher SS plasma levels rather than to the presence of NAP, so no conclusions can be reached. Also, NAP levels were apparently not measured in Phase III, and appropriate vehicle controls for SS and NAP were omitted.

Comparison of Plasma Drug Levels Between Humans and Dogs		
	SS (ng/mL)	NAP (ug/mL)
Migraineurs: Cmax after one Trexima Tablet	40.5 (1.9 hrs)	50.2 (6.2 hrs)
Migraineurs: 4 hrs after one Trexima Tablet	26.5	32.7
Dog Cmax at 80 ug/kg IV SS + 20 mg/kg NAP	19	79.2

(Reviewer's Table)

The comparisons above illustrate that there is no clear margin of safety between plasma levels of SS in dogs exhibiting putatively SS-induced coronary artery vasoconstriction and those in human migraineurs 2-4 hrs after administration of one Trexima tablet. However, as discussed above, the lack of a vehicle control for IV SS in these experiments makes it impossible to know whether the reductions in coronary artery diameter observed were due to SS, the vehicle, the injection procedure, or natural variation.

In regard to NAP plasma levels, even if the Day 1 NAP Cmax values were to be considered a NOEL in dog for exacerbation of SS-induced coronary artery vasoconstriction, this study has established, at best, a ~2-fold margin of safety above mean human NAP plasma levels four hours after one Trexima tablet. In retrospect, the choice of 20 mg/kg IV NAP, based on published dog studies showing that 10-30 mg/kg IV NAP blocked prostaglandin synthesis almost immediately after single IV administration, seems arbitrarily low. A pilot tolerability and TK study would have facilitated a more informed decision.

In summary, wide inter-individual and intra-individual variation observed in most parameters measured in this study made it impossible to draw reliable conclusions from these data regarding the potential of NAP to exacerbate the cardiovascular effects of SS. In addition, numerous serious design flaws, such as the lack of an appropriate control for the IV SS injection, call into question the validity of the study.

Therefore, this reviewer does not concur **with the Sponsor's overall conclusion that "co-administration of NAP with SS did not alter the vasoconstrictive effect of SS on the coronary arteries of conscious, chronically instrumented female beagle dogs, nor did it alter any of the other cardiovascular parameters measured in this study."**

This study should be considered invalid.

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An Investigational Range-Finding Study to Determine Coronary Blood Flow and Resistance Following Intravenous Administration of Sumatriptan Succinate to Anesthetized Beagle Dogs

(POZEN Study #MT400-T17, [REDACTED]; Study # QCBW-0104, Abbreviated Report, Issued 29 JAN 2003; Study initiated 27 SEP 2002; Not GLP, Not QA)

This pilot dog cardiovascular safety study was originally submitted to IND 60,669 #010 20 AUG 2003, and was reviewed by this Reviewer (see Review in DFS).

The intent of this pilot study in 5 anesthetized beagle dogs (1 F, 4 M) was to explore the effects of IV sumatriptan succinate (SS) on coronary arterial blood flow and coronary arterial resistance in preparation for a subsequent study examining the potential for naproxen sodium (NAP) to exacerbate the known vasoconstrictive effects of SS. However, the procedures used in this study failed to result in a reproducible dose-dependent reduction in coronary flow as a function of increasing IV doses of SS, up to 1434 ug/kg, even after sensitizing two dogs with coronary artery stenosis and one dog with beta blocker pre-treatment.

The sponsor was advised to make further attempts to assess the vasoconstrictive potential of the combination of sumatriptan and naproxen in an *in vivo* model by adapting the protocol to include the coronary artery diameter measurement methodology successfully employed in the following publications: Gupta et al., (1995) *Br J Pharmacol* 116(5):2385-2390; Gupta et al., (2000) *Eur J Pharmacol* 398:73-81; and Carel et al., (2001) *Br J Pharmacol* 132:1071-1083. The publications by Gupta et al., described dose-dependent reduction in coronary artery diameter with sumatriptan i.v. 1-300 ug/kg in anesthetized dog preparations. Similarly, Carel et al. demonstrated a significant prolonged and dose-dependent decrease in mean external coronary artery diameter (after a transient *increase* in diameter) with sumatriptan i.v. 0.1-100 ug/kg in a conscious dog preparation.

Pulmonary effects:

No Safety Pharmacology studies examining pulmonary effects were submitted.

Renal effects:

No Safety Pharmacology studies examining renal effects were submitted.

Gastrointestinal effects:

No Safety Pharmacology studies examining gastrointestinal effects were submitted.

Abuse liability:

No Safety Pharmacology studies examining the potential abuse liability were submitted.

Other:

No Safety Pharmacology studies examining other effects were submitted.

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### 2.6.2.5 Pharmacodynamic drug interactions

“No nonclinical drug interaction studies were conducted with the combination of sumatriptan succinate and naproxen sodium.” *(directly from eNDA 21-926, Module 2, Section 2.6, Page 12)*

### 2.6.3 PHARMACOLOGY TABULATED SUMMARY

#### 2.6.3.2 Primary Pharmacodynamics

“No primary pharmacodynamic studies were conducted with the combination of sumatriptan succinate and naproxen sodium as the individual components have already been well characterized.” *(directly from eNDA 21-926, Module 2, Section 2.6, Page 4)*

#### 2.6.3.3 Secondary Pharmacodynamics

“No secondary pharmacodynamic studies were conducted with the combination of sumatriptan succinate and naproxen sodium as the individual components have already been well characterized.” *(directly from eNDA 21-926, Module 2, Section 2.6, Page 4)*

#### 2.6.3.4. Safety Pharmacology

Test Article: Sumatriptan Succinate (SS) <sup>a</sup> Alone or in Combination with Naproxen Sodium (NAP) <sup>b</sup>							
Organ Systems Evaluated	Species /Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings	GLP Compliance	Study Number
Cardiovascular (Coronary Artery Blood Flow and Resistance)	Beagle Dog	Intravenous (IV) Bolus or Infusion (2 minutes)	Escalating SS; 0.7, 71- 72, 712-717, 1424-1434 µg/kg for Group 1; 0.1, 1, 10, 100, 1000 µg/kg for Group 2	1/sex Group 1; 3 males Group 2	Biologically meaningful SS-related changes in coronary arterial blood flow and calculated coronary arterial vascular resistance were not observed after IV administration of SS.	Non-GLP	MT400-T17 <sup>c</sup>
Cardiovascular (Coronary and Carotid Arteries, Reduction in Diameters)	Beagle Dog	Intravenous Bolus or Infusion (1 minute)	Water Vehicle + SS (80 µg/kg) [Phase I] versus NAP (20 mg/kg) + SS (80 µg/kg) [Phase II]; 200 µg/kg SS [Dose 1] versus NAP (20 mg/kg) + 200 µg/kg SS (Dose 2) Phase III <sup>d</sup>	The same 6 female dogs were used for Phases I, II and III	Co-administration of NAP with SS did not significantly alter the vasoconstrictive (decreased diameter) effect of SS alone on the coronary and carotid arteries of conscious chronically instrumented female beagle dogs. There were also no statistically significant, biologically meaningful additive effects of NAP on SS for the other cardiovascular parameters (heart rate, blood pressure, $\pm$ dP/dt, left ventricular pressure, coronary/carotid blood flow and resistance) evaluated in this study.	GLP	MT400-T15 <sup>e</sup>

<sup>a</sup>Sumatriptan succinate doses were calculated as the base. Naproxen sodium doses were calculated as the salt

<sup>b</sup>Phase I (water vehicle followed by SS) and Phase II (NAP followed by SS) were separated by a 1-week washout period.

<sup>c</sup>Phase III was conducted in a single day, with about 60 minutes between Dose 1 (SS only) and Dose 2 (NAP followed by SS).

<sup>d</sup>Written summary is found in Section 2.6.2.4.1.

<sup>e</sup>Written summary is found in Section 2.6.2.4.2.

*(directly from eNDA 21-926, Module 2, Section 2.6, Page 13)*

## 2.6.4 PHARMACOKINETICS/TOXICOKINETICS

### 2.6.4.1 Brief summary

**“Nonclinical pharmacokinetic studies were not conducted for the sumatriptan succinate and naproxen sodium combination, as the pharmacokinetic profiles of the individual components have already been well characterized.”** *(directly from eNDA 21-926, Module 2, Section 2.6, Page 16)*

Toxicokinetic information and tables are included within the reviews of each individual study in the Safety Pharmacology and Toxicology sections. APPENDIX 1 contains human PK data.

## 2.6.5 PHARMACOKINETICS TABULATED SUMMARY

**“Nonclinical pharmacokinetic studies were not conducted for the sumatriptan succinate and naproxen sodium combination, as the pharmacokinetic profiles of the individual components have already been well characterized.”** *(directly from eNDA 21-926, Module 2, Section 2.6, Page 17)*

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## 2.6.6 TOXICOLOGY

### 2.6.6.1 Overall toxicology summary

#### General toxicology:

General toxicology studies included two 90-day mouse studies (MT400-T05 and MT400-T19), one 28-day mouse study (MT400-T02), two 28-day rat studies (MT400-T01 and MT400-T04, both without histopathology evaluation), and one 28-day minipig study (MT400-T03, with inaccuracies in dosing solutions of  $\pm 15$ -26%). In all of these studies, groups administered high doses of naproxen sodium (NAP), with or without sumatriptan succinate (SS), demonstrated changes consistent with the known toxicities of non-steroidal anti-inflammatory drugs (NSAIDs): GI inflammation, erosion, ulcer, peritonitis, and/or reactive hyperplasia; changes secondary to the GI changes (mild increases in WBC count, neutrophils, reticulocytes and platelets; increased absolute and relative weights of spleen and/or liver; increased erythroid extramedullary hematopoiesis in spleen and/or liver; mild reductions in RBC count, HGB, and HCT; and/or decreased serum total protein and albumin); and/or renal toxicity (tubular dilatation and/or regeneration). No toxicities were attributed to SS.

In the pivotal 90-day mouse study (MT400-T19), the high dose female (HDF) SS/NAP group (320→210 mg/kg/day SS + 50 mg/kg/day NAP) showed greater GI toxicity than the corresponding HDF NAP alone group (50 mg/kg/day NAP), despite 31% lower NAP AUC in the SS/NAP group. In the same study high dose male (HDM) SS/NAP mice showed greater GI toxicity than HDM NAP alone mice, but this could be because NAP exposure was 37.5% higher in the SS/NAP group. Hence, it appeared that coadministration of SS may have exacerbated the GI toxicity of NAP in the HDF group in this study. Data from the 28-day mouse study, however, which tested higher doses of both drugs and appeared to be a more reliable study, showed greater GI toxicity in females given 75 mg/kg/day NAP alone than when combined with 160, 320, or 500 mg/kg SS. Males in the 28-day mouse study showed no ulcers or erosions at 75 mg/kg/day NAP with or without 500 mg/kg/day SS. The extent of NAP-related toxicity in the presence and absence of SS was not notably different in the rat and minipig studies.

The incidence of NAP-related GI toxicity and associated changes observed in HDM and HDF SS/NAP groups after 90 days of treatment was notably reduced in parallel groups allowed to recover for 4 weeks, suggesting reversibility.

NAP plasma exposures ( $AUC_{0-\infty}$ ) at the highest no observed adverse effect (NOAEL) dose (110/30 mg/kg/day SS/NAP) in mouse study MT400-T19 were 0.32 times (F) and 0.13 times (M) those observed in humans after a single dose of TREXIMA during a migraine. Thus, there is no margin of safety for NAP-induced GI toxicity based on this study.

SS alone was not toxic in mouse study MT400-T19 at 320/(210) mg/kg/day in F, and at 320 mg/kg/day in M, yielding wide margins of safety: 117-fold and 152-fold, respectively, above expected human exposures, based on AUC.

Genetic toxicology:

SS and NAP, alone and in combination, were negative in an *in vitro* bacterial mutagenicity assay and an *in vivo* mouse micronucleus assay. High dose NAP  $\pm$  high dose SS was positive in the CHO cell chromosomal aberrations assay, in the presence or absence of metabolic activation, in association with moderate, but not excessive, cytotoxicity. SS was not genotoxic alone, but there was some evidence to suggest that SS might exacerbate the genotoxicity of NAP.

Carcinogenicity:

No carcinogenicity studies were submitted for Trexima, and none were required, since the components are currently marketed in the U.S. for chronic or chronic/intermittent use, and since the new non-clinical studies conducted with SS/NAP did not yield any findings suggesting increased carcinogenic potential for SS/NAP in combination compared to SS or NAP alone.

Reproductive toxicology:

Reproductive and developmental toxicity studies with SS and NAP included a definitive embryo-fetal development study in pregnant rabbits, and dose-ranging embryo-fetal development studies in rabbit and rat. In the definitive embryo-fetal development study in rabbits, significant reductions in maternal and fetal weights were observed even at the lowest combination dose tested, but significant increases in resorption parameters and specific malformations and variations were only observed in HD NAP groups  $\pm$  SS. The rabbit dose-ranging study showed reductions in maternal body weight gain (BWG) with increasing dose of SS in combination with the high dose of NAP, reduction of fetal BW at HD SS/NAP, increased resorption parameters at HD NAP  $\pm$  SS, and increased gross external malformations at HD NAP + LD and HD SS. The rat dose-ranging study showed reduced maternal BWG and fetal BW with increasing SS dose in the presence of HD NAP, but no treatment-related changes in resorption parameters or fetal gross external malformations or variations.

Taken together, the three embryo-fetal toxicity studies demonstrated that NAP and SS toxicities resulting in reduction maternal and fetal body weights appeared to be additive. In contrast, increases in resorption parameters and incidence of malformations and variations observed in animals treated with HD NAP were not consistently exacerbated by coadministration of HD SS. Also, the teratogenic effects attributed to NAP were only observed at doses well above those that were maternally toxic.

Special toxicology:

No special toxicology studies were submitted.

**2.6.6.1 Single-dose toxicity**

No single-dose toxicity studies were submitted.

### 2.6.6.3 Repeat-dose toxicity

#### A 28-Day Oral Gavage Dose Range-Finding Toxicity Study in Female Gottingen Minipigs with Naproxen Sodium and Sumatriptan Succinate

(POZEN Study #MT400-T03, ██████████ Study #02-3499, Final report completed 24 FEB 2004, GLP (except for endoscopy), QA; Sumatriptan Succinate (SS) Lot #QT0 1004, Purity 99.5%; Naproxen Sodium (NAP) Lot #NPXNAM-127, L002662, Purity 99.2%; SS doses were calculated as sumatriptan base)

##### Methods:

Female Gottingen minipigs (3/group) were given SS and NAP in the following combinations for 28 days via oral gavage using water as the vehicle: 0/0, 10/100, 50/100, 100/100, 150/100, 100/0, 0/100, and 0/125 SS/NAP mg/kg/day). Parameters measured included: viability, clinical observations, eye exam, ECG, body weight (BW), food consumption (FC), toxicokinetics (collected but not performed because the study was considered invalid), clinical pathology (pre-dose and at termination), organ weights (surviving animals), necropsy (all animals), histopathology (all animals), and gastric endoscopy (0/0, 150/100, 0/100, 0/125 SS/NAP in Wks 1 and 3).

##### Results:

Dose analysis for NAP and SS were ~15.5% greater and ~26% lower than targeted concentrations. Severe gastric ulcers were observed in 13/18 minipigs that received NAP (with gastric perforation in 4 of these), associated with decreased RBC parameters, increased neutrophils. Other findings included increased vomiting, increased blood urea nitrogen, extramedullary hematopoiesis in liver and/or spleen, in increased reticulocytes in NAP treated animals, with or without SS. Early deaths included one 100/100 found dead, and one 0/125 and two 0/100 SS/NAP animals sacrificed moribund (signs included lethargy/decreased activity, pale appearance, loss of appetite, slight/moderate BW loss, and severe decreases in RBCs). All 4 early death animals had gastric ulcers, and 3/4 had pale liver at necropsy.

The incidence and severity of gastric ulcers and/or erosions was equivalent in groups 150/100, 0/100, and 0/125 (all 3 animals/group, moderate to marked), but lower in groups 10/100, 50/100, and 100/100 (see Table 3.12.1 below). Only one gastric ulcers/erosions was observed at necropsy in the 100/0 group (SS alone), and this lesion was not confirmed by the histopathological evaluation.

##### Conclusions:

The sponsor concluded that NAP induced GI toxicity consistent with its known effects, and coadministration of SS with NAP did not exacerbate this GI toxicity. However, the sponsor considered the study to be compromised due to the inhomogeneous and inaccurate dosing solutions, survival of only 1-2 animals per group, uninterpretable endoscopic results due to feed in the stomach, and poor quality of most bone marrow smear slides.

**Text-Table 3.12.1. Stomach Ulcers/Erosions at Necropsy / Histopathology**

Dose mg/kg/d	Study Day	RBC x10 <sup>6</sup> /μL	Pig #	Stomach			Other
				Ulcers/erosions/perforation			
SB/NAP		Control* (7.54-8.33)		Necropsy	Histo	P	
10/100	12	5.84 (↓)	2515	-	-		Fracture
	T	4.01 (↓)	2516	2x4; 2x3	(M)		
	T	7.99	2517	0.2x0.5	(S)		
50/100	T	6.41	3515	-	-		
	T	7.88	3516	0.6x0.9	(M)		
	T	7.19	3517	-	-		
100/100	22	NS	4515	-	-		Gavage error
	26	NS	4516	2x5; 1x5	MM)	Yes?	
	T	7.88	4517	-	-		
150/100	T	7.27	5515	2x2; 2x6 1x1; 2x4	MM)		
	T	6.55	5516	1x1.5; 0.5x1 1x5	MM)		
	T	8.32	5517	2 U: 1x1 2 U: 0.5x0.5	MM)		
100/0	T	8.60	6515	2x2.9	-		
	T	8.24	6516	-	-		
	5	NS	6517	-	-		Gavage error
0/100	22	1.09 (↓)	7515	2 U: 0.2x0.5; 1x1.5	MM)	Yes	
	27	1.92 (↓)	7516	2x5 2 U: 0.2x0.5	(M)		
	T	6.79	7517	3x4; 1x0.5 2 U: 1x1	MM)	Yes?	
0/125	T	7.65	8516	2x3	(MM)		
	T	6.88	8517	2x2; 0.5x0.5	(M)		
	18	3.53 (↓)	8518 <sup>§</sup>	1x1.5	(M)	Yes?	

\*: RBC control values at termination. (↓): ↓ RBC ≥ 25% compared to pretest / control values

T: end of study; U: ulcers; P: perforation; (X): focal; (X): multifocal; S: slight; M: moderate; MM: marked  
Yes?: adhesions between mesentery and ulcerated area or between stomach and pancreas at necropsy and plant material in peritoneum by light microscopy would suggest stomach perforation

§: Animal 8515 was replaced by Animal 8518.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 5441)

### A 28-Day Oral Range-Finding Toxicity Study in Rats with Sumatriptan Succinate Combined with Naproxen Sodium

(POZEN Study #MT400-T01, ██████ Study #907-005, Completed 05 MAY 2004, GLP, QA; Sumatriptan Succinate (SS) Lot #QT0 1002, L002711, Purity 99.4%; Naproxen Sodium (NAP) Lot #NPXNAM-126, L002662, Purity 99.5%; SS was calculated as free base)

#### Methods:

Wistar Han (████WI(G1x/BRL/Han) IGS Br rats (10/sex/group main study, 10/sex/group TK) were treated via oral gavage (10 mL/kg) for 28 days with SS/NAP at 0/0, 300/20, 500/20, 750/20, 500/0, and 0/20 mg/kg/day. Evaluations included morbidity, mortality, injury, and availability of food and water (2X/day); detailed clinical exam (daily at ~1 hr postdose); body weight (BW, weekly); food consumption (FC, weekly); ophthalmology exam (predosing and Day 26); hematology, blood chemistry, urinalysis (Day 29); TK (Day 28); macroscopic changes and organ weights (at termination). Microscopic evaluations were not conducted due to termination of the study by the sponsor.

#### Results:

No treatment-related changes were observed in mortality, clinical signs, BW, FC, ophthalmology, urinalysis, necropsy, or organ weights. All groups dosed with 20 mg/kg/day NAP ( $\pm$  SS) showed increased reticulocytes and eosinophils. All treated groups showed slight decreases in erythrocyte parameters compared to controls. SS did not appear to exacerbate the hematology changes seen with NAP alone. ALP was decreased (25-28%) in M at 300/20, 500/20, and 750/20. BUN was increased in F at 300/20 (51%), 500/20 (41%), 750/20 (30%), and 0/20 (28%), and in M (26-27%) at 500/20 and 750/20. Cholesterol was increased in M at 300/20 (25%), 500/20 (43%), and 0/20 (21%). Total protein, albumin, and globulin were lower (6-10%) in M at 0/20, and total protein and albumin were decreased (6-8%) in F at 0/20, 300/20, and 500/20. Blood chemistry changes seen with NAP were not exacerbated by SS.

#### Conclusions:

Mild changes attributed to NAP included increased reticulocytes and eosinophils, decreased erythrocyte parameters, decreased alkaline phosphatase, increased BUN and cholesterol, and decreased total protein, albumin, and globulin. These changes are **consistent with mild regenerative anemia (often seen associated with GI toxicity— inflammation/ulcers/erosions/hemorrhage)** and renal toxicity, both of which are characteristic toxicities of NSAIDs. None of these changes appeared to be exacerbated with administration of increasing dosages of SS. However, in the absence of histopathology results definitive conclusions regarding the toxicity of the combination compared to NAP and SS alone cannot be reached.

**Table 2. Day 28 Toxicokinetic Parameters for Sumatriptan in Rats Following Once Daily Oral Administration of Sumatriptan Succinate and Naproxen Sodium for 28 days**

Group No.	Dose SB/NAP (mg/kg/day)	Male			Female		
		C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-12</sub> (hr·ng/mL)	C <sub>max</sub> (ng/mL)	t <sub>max</sub> (hr)	AUC <sub>0-12</sub> (hr·ng/mL)
2	300/20	7048	4	53444	8602	4	63980
3	500/20	12595	4	96573	13355	2	104582
4	750/20	15175	4	110150	22473	2	147019
5	500/0	16053	2	100434	15694	1	112712

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**Table 3. Day 28 Toxicokinetic Parameters for Naproxen in Rats Following Once Daily Oral Administration of Sumatriptan Succinate and Naproxen Sodium for 28 days**

Group No.	Dose SB/NAP (mg/kg/day)	Male			Female		
		C <sub>max</sub> (µg/mL)	t <sub>max</sub> (hr)	AUC <sub>0-12</sub> (hr·µg/mL)	C <sub>max</sub> (µg/mL)	t <sub>max</sub> (hr)	AUC <sub>0-12</sub> (hr·µg/mL)
2	300/20	39.78	2	315.44	50.75	1	315.39
3	500/20	39.82	4	357.30	39.43	1	356.91
4	750/20	35.92	2	335.14	47.07	4	428.78
6	0/20	59.21	2	401.33	66.91	1	364.16

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 5141)

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**A Second 28-Day Oral Range-Finding Toxicity Study in Rats with Sumatriptan Succinate Combined with Naproxen Sodium**

(POZEN Study #MT400-T04, Study #907-008, Completed 12 MAR 2004, GLP, not QA; Naproxen Sodium (NAP) Lot #NPXNAM-126, L002662, Purity 99.5%)

**Methods:**

Female Wistar Han (WI/GLx/BRL/han)IGS Br rats (N=10 per group, ~7 weeks old at start of dosing) were dosed via oral gavage with naproxen sodium (NAP) at 0, 25, 30, or 35 mg/kg/day in distilled water at 5 mL/kg once daily for 28 days.

Measurements included observations for mortality and morbidity (twice daily), detailed clinical examination (daily 30-90 min postdose), body weight (BW) and food consumption (FC, weekly), ophthalmologic exam (pre-dose and at necropsy), clinical pathology (hematology, clinical chemistry, urinalysis; prior to necropsy), bone marrow smears, macroscopic examination, and organ weights. No microscopic evaluation of tissues was performed.

Phase II of this study was intended to determine the maximum dose of sumatriptan succinate (SS) that could be coadministered with a maximum tolerated dose (MTD) of NAP. However, Phase II was terminated before it was treatment was initiated, following receipt of feedback from the FDA the rat studies would not be required. The planned microscopic analysis and dose solution analysis for Phase I were also eliminated.

**Results:**

Treatment-related findings included dose-related decreases in BW and FC during the first two weeks (significant at 30 and 35 mg/kg/day); increased WBC, neutrophils, eosinophils, platelets, reticulocytes, and mean corpuscular volume ( $\geq 25$  mg/kg/day); reductions in RBCs, hematocrit, hemoglobin, mean corpuscular hemoglobin concentration ( $\geq 25$  mg/kg/day); decreased serum calcium and alkaline phosphatase ( $\geq 30$  mg/kg/day); reductions in serum total protein, albumin, and globulin ( $\geq 25$  mg/kg/day); reduced albumin:globulin ratio (35 mg/kg/day); increased serum cholesterol ( $\geq 30$  mg/kg/day); increased blood urea nitrogen ( $\geq 25$  mg/kg/day); decreased pH of urine; and increased proportion of total granulocytic precursor cells and myeloid:erythroid ratios in the bone marrow smears (dose-related). No treatment-related changes were observed in mortality, clinical signs, or macroscopic findings.

**Conclusions:**

The dose-related changes in hematology and clinical chemistry observed here with NAP are consistent with findings of regenerative anemia associated with gastrointestinal toxicity (inflammation, erosion, and ulceration) and renal toxicity (tubular dilatation and/or regeneration) observed in prior rodent studies with NAP that included microscopic examination.

**A 28-Day Oral Range-Finding Toxicity Study in Mice with Sumatriptan Succinate Combined with Naproxen Sodium**

(POZEN Study #MT400-T02, ██████████ Study #907-006, Completed 14 MAY 2004, GLP, QA; Sumatriptan Succinate (SS) Lot #QT0 1002, L002711, Purity 99.4%; Naproxen Sodium (NAP) Lot #NPXNAM-126, L002662, Purity 99.5%; SS doses were calculated based on sumatriptan free base concentrations, where mg SS = 1.4 X mg sumatriptan base)

**Key Points:**

- Mortality was observed in 6/25 F at 0/75, 4/25 F at 500/75, 16/25 F at 160/150, and 11/25 F at 320/150 mg/kg/day SS/NAP, but only in 0-1/25 M or F in all other groups (0/0, 160/0, 160/75, and 320/75 mg/kg/day SS/NAP).
- NAP-related lesions observed in M and F included inflammation, erosion, and ulceration of the glandular stomach, trace erosions in duodenum and jejunum, and renal tubular dilatation and/or regeneration.
- Neither NAP-induced lesions nor mortality were exacerbated by combined administration with SS in M or F.
- Female mice were much more sensitive than males, even in the 0/75 group, where NAP AUC was lower in F than in M.
- Other changes observed were considered secondary to the GI toxicity induced by NAP, and included regenerative macrocytic hypochromic anemia, increased white blood cell and neutrophils counts, decreased serum albumin, increased total bone marrow erythroid and granulocytic cells, enlarged mesenteric lymph nodes and spleens, increased liver and spleen weights, and extramedullary hematopoiesis of liver and spleen.

**Methods:**

Eight groups of CD-1 (ICR) BR mice (10/sex/group main, 15/sex/group TK) received vehicle (distilled water, group 0/0), SS/NAP (160/75, 320/75, 500/75, 160/150, or 320/150 mg/kg/day), SS alone (160 mg/kg/day, group 160/0), or NAP alone (75 mg/kg/day, group 0/75) via oral gavage at 10 mL/kg once daily for 28 days (except F in groups 160/150 and 320/150, which were terminated early on Day 23 due to high mortality). Evaluations included morbidity, mortality, injury, and availability of food and water (2X/day); detailed clinical exam (daily 30-90 min postdose); body weight (BW, weekly); food consumption (FC, weekly); ophthalmology exam (predosing and at termination); hematology, blood chemistry, urinalysis (at termination); TK (at termination); bone marrow smears (at termination); macroscopic changes, organ weights, and microscopic changes (at death or termination).

To justify the dose levels used in this study (up to 500 mg/kg/day SS and up to 150 mg/kg/day NAP), the sponsor cited the findings from mouse studies described in the FDA supervisory overview of pharmacology and toxicology for SS in NDA 20-132 (IMITREX<sup>®</sup> Tablets) and the sponsor's own 28-day mouse study with NAP (MT 100-T29, submitted to [redacted])

According to the information cited, the acute oral LD<sub>50</sub> in mice was ~1.2 g/kg sumatriptan base. SS appeared to be well-tolerated in mice when given in drinking water at 500 mg/kg/day for several days, at 160 mg/kg/day for 90 days, and at 160 mg/kg/day for 78 weeks. The sponsor acknowledged that the MTD of SS after repeated dosing in mice was not clearly established in these previous studies, and "doses greater than doses used historically were selected for this study to ensure that a maximum dose of SS that can be co-administered with the MTD of NAP is identified." (eNDA 21-926, Module 4, Section 4.2, Page 1119) The 28-day mouse study with NAP cited by the sponsor demonstrated a MTD for NAP of 50 mg/kg/day, due to findings indicative of regenerative anemia associated with mild blood loss at doses ≥ 75 mg/kg/day NAP.

*Reviewer's Note:*

The sponsor's choice of NAP doses of 75 and 150 mg/kg/day appeared to be reasonable, based on the previous data cited, and turned out to be appropriate. The sponsor's choice of 500 mg/kg/day for the highest dose of SS appeared to be reasonable, based largely on the reported acute oral LD<sub>50</sub> in mice of ~1.2 g/kg. However, since no toxicity was attributed to SS in the group given 500/75 mg/kg/day SS/NAP or the group given 160 mg/kg/day SS alone in the current 28-day mouse study, subsequent mouse studies should have been conducted with doses of SS higher than 500 mg/kg/day. In the in vivo mouse micronucleus study (MT400-T08), single oral doses of SS up to 1500 mg/kg (M) and 1625 mg/kg (F) induced no observable changes in mortality, clinical signs, or body weight when given in combination with 500 mg/kg (M) or 375 mg/kg (F) NAP.

Results:

Summary of Mortality*								
Sex	Dose Level (mg SS/mg NAP/kg/day)							
	0/0	160/75	320/75	500/75	160/150	320/150	160/0	0/75
M	1	1	1	1	0	1	0	0
F	1	1	1	4	16	11	0	6

\*Includes TK and main study animals

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1125)

Female groups 160/150 and 320/150 mg/kg/day SS/NAP were terminated early on Day 23 due to high mortality (16 of 25 and 11 of 25, respectively). Mortality was also increased in F groups 500/75 (4 of 25) and 0/75 (6 of 25). The absence of an increase in mortality in F when SS was increased while holding NAP constant (160/150 → 320/150 and 0/75 → 500/75 mg/kg/day SS/NAP) demonstrated that coadministration of SS did not exacerbate the fatal toxicity of NAP. Treatment-related clinical signs were observed only in F, and included decreased activity, hunched posture, skin cold to touch, pale, red skin in groups 0/75, 160/150, and/or 320/150. BW was decreased in F at 160/150, 320/150, and 160/0 vs. 0/0 on Day 14. FC was decreased in F at 320/150.

Toxicokinetic analysis revealed that NAP exposure was ~1.5-fold higher in F than in M, which may explain the increased toxicity in F vs. M mice. Also, AUC values for NAP were ~1.7-fold higher in F SS/NAP groups (160/75, 320/75, and 500/75) compared to the F NAP alone group (0/75). In M mice, NAP exposure was not increased with SS administration, but neither was it increased in groups 160/150 and 320/150 vs. groups 0/75, 160/75, 320/75 and 500/75, suggesting saturation of absorption. Coadministration of NAP resulted in lower C<sub>max</sub> of SS in M & F, higher AUC of SS in M, and lower AUC of SS in F.

**Table 4. Sumatriptan Toxicokinetic Parameters on Day 28 of Dosing in Male and Female Mice**

Group	Dose (mg/kg) SB/NAP	Males					Females				
		C <sub>max</sub> ng/mL	t <sub>max</sub> hr	AUC <sub>0-12</sub> hr·ng/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg	C <sub>max</sub> ng/mL	t <sub>max</sub> hr	AUC <sub>0-12</sub> hr·ng/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg
2	160/75	3336	4.0	24899	1.3	6.40	3482	2.0	12443	1.6	12.78
3	320/75	9206	0.5	33444	1.4	9.54	7754	2.0	30249	2.1	10.41
4	500/75	10362	1.0	49356	1.7	10.06	13798	0.5	54202	2.3	9.00
5	160/150	2838	2.0	16328	1.5	9.74	NA	NA	NA	NA	NA
6	320/150	7863	1.0	36651	1.5	8.68	NA	NA	NA	NA	NA
7	160/0	4648	1.0	15723	2.5	9.78	6348	1.0	16089	2.1	9.78

NA=Data not available.

**Table 5. Naproxen Toxicokinetic Parameters on Day 28 of Dosing in Male and Female Mice**

Group	Dose (mg/kg) SB/NAP	Males					Females				
		C <sub>max</sub> µg/mL	t <sub>max</sub> hr	AUC <sub>0-12</sub> hr·µg/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg	C <sub>max</sub> µg/mL	t <sub>max</sub> hr	AUC <sub>0-12</sub> hr·µg/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg
2	160/75	60.7	0.5	263	2.5	0.275	74.2	1.0	393	2.3	0.186
3	320/75	80.6	0.5	374	2.0	0.197	71.5	0.5	496	3.9	0.133
4	500/75	50.9	0.5	298	2.5	0.242	101.5	0.5	466	4.1	0.142
5	160/150	74.1	0.5	347	1.8	0.426	NA	NA	NA	NA	NA
6	320/150	77.6	0.5	371	2.2	0.393	NA	NA	NA	NA	NA
8	0/75	86.0	0.5	320	2.2	0.229	90.6	0.5	260	6.3	0.108

NA=Data not available.

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1707)

Treatment-related changes in hematology included decreases in erythrocyte parameters (F at 0/75, 160/75, 320/75, 500/75, 160/150, and 320/150; M at 160/150 and 320/150); and increases in reticulocytes and platelets (F at 160/150 and 320/150) associated with increased MCV and anisocytosis, hypochromasia, and macrocytosis, indicative of regenerative macrocytic hypochromic anemia, correlating to stomach lesions (erosion/ulceration) observed microscopically. Neutrophil and leukocyte counts were increased in F at 0/75, 160/150, and 320/150, and were also correlated to the stomach lesions.

Increases in albumin and A/G were seen in F at 0/75 and 320/150 and ALP was increased in F at 320/150 (no clinical pathology results were available for the F 160/150 group due to the high mortality).

F at 160/150 and 320/150 showed increases in the proportion of total erythroid cells and total granulocytic cells with a resulting decrease in the proportion of lymphoid cells in bone marrow smears. The increased erythroid cells correlated with the increased reticulocytes observed in the hematology evaluation in these F. A mild increase in granulocytic cells was observed in F at 0/75.

Absolute and relative increases were seen in the following organ weights: pituitary (M at 500/75, 0/75, 160/150/ 320/150, and 160/0); thyroid/parathyroid (0/75, 160/0, 160/150, and 320/150); liver (F at 0/75); and spleen (F at 0/75).

Treatment-related macroscopic findings were observed in F only, and included: enlarged mesenteric lymph nodes (160/75, 320/150, 0/75), enlarged spleens (500/75, 160/150, 320/160, 0/75), and peritonitis with an adhesion between liver and glandular stomach (1 F at 500/75).

Treatment-related microscopic findings were observed primarily in the glandular stomach and the kidney. Changes in the glandular stomach included inflammation of the mucosal epithelium (trace to mild in M, trace to moderate in F), erosions (trace in M, trace to severe in F), glandular epithelial hyperplasia (trace to mild, M & F, attributed to a reparative response to injury), and ulcer (mild in 1 M, trace to severe in many F). The frequency and severity of these lesions increased with the dose of NAP, but were not exacerbated by administration of SS.

Renal lesions observed in NAP groups with and without SS included renal cortical tubule dilatation (trace to mild), and renal cortical tubular regeneration (usually trace). These lesions were attributed to NAP, and were not exacerbated by SS.

Other lesions observed in SS/NAP and NAP only groups were considered secondary to the GI lesions caused by NAP, and included: extramedullary hematopoiesis in liver and spleen; peritonitis, hyperplasia of mesenteric lymph nodes; and bone marrow hyperplasia.

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Summary of Test Article-Related Microscopic Findings (Number of Animals Affected*)									
Microscopic Finding	Sex	Dose Level (mg SS/mg NAP/kg/day)							
		0/0	160/75	320/75	500/75	160/150	320/150	160/0	0/75
Number of Animals Examined	M	10	10	10	10	10	10	10	10
	F	10	10	10	10	10	10	10	10
<b>Stomach, glandular</b>									
Erosion	M	0	1	2	0	3	3	0	0
	F	0	1	2	1	6	2	0	2
Inflammation	M	0	0	1	0	2	5	0	0
	F	0	2	2	1	3	2	0	3
Ulcer	M	0	0	0	0	1	0	0	0
	F	0	2	1	0	8	9	0	5
Peritonitis, chronic	M	0	0	0	0	0	0	0	0
	F	0	0	0	2	2	4	0	0
<b>Jejunum</b>									
Erosion (trace)	M	0	0	0	0	0	0	0	0
	F	0	0	0	0	0	1	0	0
<b>Duodenum</b>									
Erosion (trace)	M	0	0	0	0	0	0	0	0
	F	0	0	0	0	1	0	0	0
<b>Kidney</b>									
Tubular dilatation	M	0	0	1	1	0	0	1	0
	F	0	0	0	1	2	2	0	2
Tubular regeneration	M	1	2	1	0	3	2	0	3
	F	0	4	0	0	3	5	1	2

\*Incidences include died on study animals and animals necropsied at study termination.

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1131; Note that the number of animals examined is stated in the table above as 10 per sex per group; this is true for all groups except for groups F 160/150 and F 320/150, in which 17 animals were examined microscopically per group, due to the high mortality and early termination of these groups on Day 23)

Examination of the frequency of erosions/ulcers in this experiment (see table below) clearly demonstrates that coadministration of the highest dose of SS (500 mg/kg/day) with 75 mg/kg/day did not exacerbate the GI toxicity induced by 75 mg/kg/day NAP in M or F. The occurrence of one or two ulcers/erosions in M at 160/75 and 320/75 and not in M at 0/75 mg/kg/day SS/NAP seems likely to be due to variation at this threshold toxic dose of NAP in M rather than to the presence of SS, since no ulcers/erosions were seen in M given 500 mg/kg/day SS with 75 mg/kg/day NAP.

Animals with Ulcers/Erosions in 28-Day Mouse Study (N=10/sex/group)					
SS/NAP mg/kg/day	0/0	0/75	160/75	320/75	500/75
Male mice	0	0	1E	2E	0
Female mice	0	5U, 2E	2U, 1E	1U, 2E	1E

(Reviewer's Table, U=Ulcer, E=Erosion; U and E were mutually exclusive in this experiment)

Conclusions:

Mortality was increased slightly at 75 mg/kg/day NAP and severely at 150 mg/kg/day in F mice, but not in M. All of the treatment-related findings were attributed to NAP, including inflammation, erosion, and ulceration of the glandular stomach, trace erosions in duodenum and jejunum, and renal tubular dilatation and/or regeneration. These NAP-induced lesions were not exacerbated by combined administration with SS. Female mice were much more sensitive than males, even in the 0/75 group, where NAP AUC was lower in F than in M. In SS/NAP groups, the increased toxicity in F vs. M mice may also be at least partially due to their increased exposure to NAP.

Other changes observed were considered secondary to the GI toxicity induced by NAP, and included regenerative macrocytic hypochromic anemia, increased white blood cell and neutrophils counts, decreased serum albumin, increased total bone marrow erythroid and granulocytic cells, enlarged mesenteric lymph nodes and spleens, increased liver and spleen weights, and extramedullary hematopoiesis of liver and spleen.

According to the sponsor, the maximum tolerated dose (MTD) was 500/75 for M, due to stomach erosions and ulcers, and decreased red cell indices (suggestive of GI bleed) at 160/150; and the MTD was 320/75 for F, due to increased mortality and decreased red cell indices at higher doses. However, mortality was increased in F groups 500/75 and 0/75 and ulcers were observed in F groups 0/75, 160/75, and 320/75, suggesting that even 75 mg/kg/day NAP exceeds the MTD in F mice. The NOEL for SS alone was 160 mg/kg/day, and there were no effects attributed to SS in the groups given 500 mg/kg/day SS in combination with 75 mg/kg/day NAP. Therefore, the MTD for SS in mice was not established, and subsequent mouse studies should have been conducted with SS doses higher than 500 mg/kg/day. The NOEL for NAP was not determined, since stomach lesions were observed at the lowest dose examined (75 mg/kg/day NAP).

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Male Histopathology Summary Table:

TISSUE OBSERVATION	0 mg/kg SS		160 mg/kg		320 mg/kg		500 mg/kg		160 mg/kg		320 mg/kg	
	- 0 mg/kg		SS - 75		SS - 75		SS - 75		SS - 150		SS - 150	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
<b>Stomach, Glandular</b>	(0)	(10)	(1)	(9)	(1)	(9)	(0)	(10)	(1)	(9)	(1)	(9)
Within normal limits	0	10	1	7	1	7	0	9	1	5	1	4
Inflammation, chronic,	0	0	0	0	0	0	0	0	0	1	0	1
-trace	0	0	0	0	0	0	0	0	0	1	0	0
-mild	0	0	0	0	0	0	0	0	0	0	0	1
Inflammation, subacute, trace	0	0	0	0	0	1	0	0	0	0	1	0
Inflammation, acute, trace	0	0	0	0	0	0	0	0	0	0	0	1
Erosion, trace	0	0	0	1	0	2	0	0	0	3	0	3
Hyperplasia, epithelial,	0	0	0	2	0	0	0	1	0	0	0	3
-trace	0	0	0	0	0	0	0	0	0	0	0	1
-mild	0	0	0	2	0	0	0	1	0	0	0	2
Inflammation, chronic active, mild	0	0	0	0	0	0	0	0	0	1	0	0
Ulcer, mild	0	0	0	0	0	0	0	0	0	1	0	0

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1247)

TISSUE OBSERVATION	160 mg/kg		0 mg/kg	
	SS - 0		SS - 75 mg/kg	
	DOS	SAC	DOS	SAC
<b>Stomach, Glandular</b>	(0)	(10)	(0)	(10)
Within normal limits	0	10	0	10
Inflammation, chronic,	0	0	0	0
-trace	0	0	0	0
-mild	0	0	0	0
Inflammation, subacute, trace	0	0	0	0
Inflammation, acute, trace	0	0	0	0
Erosion, trace	0	0	0	0
Hyperplasia, epithelial,	0	0	0	0
-trace	0	0	0	0
-mild	0	0	0	0
Inflammation, chronic active, mild	0	0	0	0
Ulcer, mild	0	0	0	0

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1248)

Female Histopathology Summary Table:

TISSUE OBSERVATION	0 mg/kg SS		160 mg/kg		320 mg/kg		500 mg/kg		160 mg/kg		320 mg/kg	
	- 0 mg/kg		SS - 75		SS - 75		SS - 75		SS - 150		SS - 150	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
<b>Stomach, Glandular</b>	(0)	(10)	(0)	(10)	(1)	(9)	(3)	(7)	(7)	(10)	(3)	(14)
Within normal limits	0	10	0	6	1	5	1	6	1	1	0	4
Autolysis too severe for diagnosis	0	0	0	0	0	0	0	0	1	0	0	0
Inflammation, chronic,	0	0	0	0	0	0	0	1	0	0	0	2
-trace	0	0	0	0	0	0	0	0	0	0	0	0
-moderate	0	0	0	0	0	0	0	0	0	0	0	2
Inflammation, subacute,	0	0	0	2	0	2	0	0	1	1	0	0
-trace	0	0	0	2	0	1	0	0	1	1	0	0
-mild	0	0	0	0	0	1	0	0	0	0	0	0
Inflammation, acute, trace	0	0	0	0	0	0	0	0	0	1	0	0
Bacterial colonies,	0	0	0	0	0	0	0	0	1	0	1	1
-trace	0	0	0	0	0	0	0	0	0	0	0	1
-mild	0	0	0	0	0	0	0	1	0	1	0	0
Erosion,	0	0	0	1	0	2	0	1	3	3	0	2
-trace	0	0	0	1	0	1	0	1	0	2	0	0
-mild	0	0	0	0	0	1	0	0	2	1	0	2
-severe	0	0	0	0	0	0	0	0	1	0	0	0

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1261)

TISSUE OBSERVATION	0 mg/kg		(10)		(1)		(9)		(3)		(7)		(10)		(3)		(14)	
	- 0 mg/kg		SS - 75		SS - 75		SS - 75		SS - 75		SS - 150		SS - 150		SS - 150		SS - 150	
	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC	DOS	SAC
<b>Stomach, Glandular (Continued)</b>	(0)	(10)	(0)	(10)	(1)	(9)	(3)	(7)	(7)	(10)	(3)	(14)						
Foreign body, plant material, trace	0	0	0	0	0	0	0	0	0	1	0	0						
Hyperplasia, epithelial, mild	0	0	0	1	0	1	0	0	0	0	0	0						
Inflammation, chronic active,	0	0	0	2	0	1	0	0	0	1	0	0						
-trace	0	0	0	1	0	0	0	0	1	0	0	0						
-mild	0	0	0	1	0	1	0	0	0	5	1	6						
-moderate	0	0	0	0	0	0	0	0	0	1	0	1						
Peritonitis, chronic,	0	0	0	0	0	0	2	0	2	0	2	2						
-trace	0	0	0	0	0	0	1	0	1	0	2	2						
-mild	0	0	0	0	0	0	1	0	0	0	0	0						
-moderate	0	0	0	0	0	0	0	0	1	0	0	0						
Ulcer,	0	0	0	2	0	1	0	0	2	6	1	6						
-trace	0	0	0	1	0	0	0	0	0	0	0	1						
-mild	0	0	0	1	0	1	0	0	1	4	0	3						
-moderate	0	0	0	0	0	0	0	0	2	1	4	0						
-severe	0	0	0	0	0	0	0	0	1	0	0	0						

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1263)

TISSUE OBSERVATION	160 mg/kg SS - 0 mg/kg		0 mg/kg SS - 75 mg/kg NAP	
	DOS	SAC	DOS	SAC
<b>Stomach, Glandular</b>	(0)	(10)	(2)	(8)
Within normal limits	0	10	1	1
Autolysis too severe for diagnosis	0	0	0	0
Inflammation, chronic,	0	0	0	0
-trace	0	0	0	0
-moderate	0	0	0	0
Inflammation, subacute,	0	0	1	2
-trace	0	0	1	2
-mild	0	0	0	0
Inflammation, acute, trace	0	0	0	0
Bacterial colonies,	0	0	0	0
-trace	0	0	0	0
-mild	0	0	0	0
Erosion,	0	0	1	1
-trace	0	0	1	0
-mild	0	0	0	1
-severe	0	0	0	0

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1262)

<b>Stomach, Glandular (Continued)</b>	(0)	(10)	(2)	(8)
Foreign body, plant material, trace	0	0	0	0
Hyperplasia, epithelial, mild	0	0	0	0
Inflammation, chronic active,	0	0	0	4
-trace	0	0	0	1
-mild	0	0	0	0
-moderate	0	0	0	3
Peritonitis, chronic,	0	0	0	0
-trace	0	0	0	0
-mild	0	0	0	0
-moderate	0	0	0	0
Ulcer,	0	0	0	5
-trace	0	0	0	0
-mild	0	0	0	3
-moderate	0	0	0	2
-severe	0	0	0	0

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 1264)

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**A 90-Day Oral Toxicity Study in CD-1 Mice with Sumatriptan Succinate Combined with Naproxen Sodium**

(POZEN Study #MT400-T05, ██████████ Study #907-009, Completed 15 APR 2005, GLP, QA; Sumatriptan Succinate (SS) Lot #QT0 1002, Purity 99.4% and Lot #QT0 1004, Purity 99.5%; Naproxen Sodium (NAP) Lot #NPXNAM, Purity 99.5%; SS doses were calculated based on sumatriptan free base concentrations, where mg SS = 1.4 X mg sumatriptan base)

NOTE: Concerns raised in the interpretation of the results of this study included:

- 1) acceptability of the homogeneity of mixing results in the highest dose combination (320/150 M) in the initial test batch (results were **“less than optimal”**)
- 2) acceptability of dosing formulation concentrations (~14-28% of samples were outside  $\pm 15\%$  of the targeted nominal concentration)
- 3) NAP or SS was detected in plasma samples from animals that were not intended to be exposed to those analytes; the origin of the unintended analytes was suspected to be contamination during handling rather than mis-dosing of animals

**Key Points:**

- Treatment-related mortality was observed in 1/40 (2.5%) M at 210/100, 11/50 (22%) M at 320/150(100), and 3/50 (6%) M at 0/150(100); and in 1/50 (2%) F at 210/50, 8/40 (20%) F at 320/75, and 3/50 (6%) F at 0/50 mg/kg/day SS/NAP.
- Lesions observed in M and F were NAP-related and included GI inflammation, erosion, ulceration, and hyperplasia (mostly in the glandular stomach); and mild renal tubular dilatation.
- Other changes observed were considered secondary to the GI toxicity induced by NAP, and included regenerative macrocytic hypochromic anemia, decreased serum albumin and total protein, myeloid hyperplasia, extramedullary hematopoiesis of liver and spleen, and thymic atrophy/necrosis.
- Recovery groups demonstrated reversibility of almost all changes, with only trace to mild inflammation and/or hyperplasia of glandular stomach observed.
- Direct comparison of the percentage of animals with ulcers/erosions suggests that the GI toxicity of HD NAP alone (31%) was increased with the addition of HD SS (47%), whereas in F the GI toxicity of HD NAP alone (38%) was decreased with the addition of HD SS (27%).
- Mortality rates and GI toxicity were increased in M and decreased in F HD SS/NAP groups compared to the corresponding HD NAP alone groups at the same NAP dosage. However, these differences could be at least partially explained by the differences in NAP exposure in HD NAP groups, which was increased 51% in M with HD SS and decreased 37% in F with HD SS coadministration (based on NAP  $AUC_{0-\infty}$  Day 90).
- The conclusions reached in this study must be interpreted cautiously due to the concerns noted above regarding the accuracy and homogeneity of the dosing solutions.

Methods:

Seven groups of CD-1 (ICR) BR mice (10/sex/group main, 10/sex/group recovery [groups 1, 6, 7, 5 (M), and 4 (F)], 30/sex/group TK) received SS/NAP (M: 0/0, 25/12, 105/50, 210/100, 320/150 [lowered to 320/100 Days 62-91, after drug holiday Days 57-61], 320/0, 0/150 [lowered to 0/100 Days 62-91, after drug holiday Days 57-61]; F: 0/0, 50/12, 110/25, 210/50, 320/75, 210/0, 0/50 mg/kg/day) via oral gavage in distilled water at 10 mL/kg once daily for 90 days.

The high doses of NAP chosen for this study were based largely on the 28-day mouse study (MT400-T02) in which M showed increased erosions, ulcers, and regenerative anemia at 320/150 mg/kg/day SS/NAP, but no changes in mortality or BW; and F showed increased mortality, erosions, ulcers, and regenerative anemia at 75 mg/kg/day NAP with or without SS. The choice of 320 mg/kg/day as the high dose for SS was not explained, other than that it was the dose used in Study MT400-T02 in the groups that showed toxicity when treated with 320/150 (M) or 320/75 (F) mg/kg/day SS/NAP.

Evaluations included morbidity, mortality, injury, and availability of food and water (2X/day); detailed clinical exam (daily 30-90 min postdose); body weight (BW, weekly); food consumption (FC, weekly); hematology, blood chemistry, urinalysis (at termination); TK (at termination); bone marrow smears (at termination); macroscopic changes, organ weights, and microscopic changes (at death or termination).

Results:

Dose analyses revealed that 14% of dosing samples for M and 28% of the samples for F were outside the acceptable  $\pm 15\%$  range for suspensions. Also, homogeneity was not achieved for the initial (Wk 1-Wk 8) high dose SS/NAP formulation (NAP: Top -21.5%, Mid +11.3%, Btm -4.2%; SS: Top -30.3%, Mid 0.0%, Btm -13.9%).

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<b>Percent of Nominal Concentration Sumatriptan Succinate - Male</b>					
	25 mg/kg/day Groups 2/9	105 mg/kg/day Groups 3/10	210 mg/kg/day Groups 4/11	320 mg/kg/day Groups 5/12	320 mg/kg/day Groups 6/13
Week 1	142.8 <sup>a</sup>	104.3	95.6	85.3 <sup>a</sup>	95.9
Week 2	124.8	100.3	96.3	96.8	114.6
Week 3	100.4	97.1	103.9	96.9	102.8
Week 4	103.2	100.1	103.0	101.8	97.8
Week 8	97.6	133.8	102.8	99.9	101.3
Week 12	103.6	104.7	97.3	91.9	102.1
Mean	112.1	106.7	99.8	95.4	102.4
Range	97.6 - 142.8	97.1 - 133.8	95.6 - 103.9	91.9 - 101.8	95.9 - 114.6

<sup>a</sup>The mean of the homogeneity samples was used for verification of Week 1 test article concentration.

<b>Percent of Nominal Concentration Sumatriptan Succinate - Female</b>					
	50 mg/kg/day Groups 2/9	110 mg/kg/day Groups 3/10	210 mg/kg/day Groups 4/11	320 mg/kg/day Groups 5/12	320 mg/kg/day Groups 6/13
Week 1	97.6	103.6	98.0	100.8	121.6
Week 2	79.6	96.4	97.9	99.7	82.0
Week 3	105.4	100.0	94.6	124.8	98.9
Week 4	102.4	97.2	98.5	98.1	105.3
Week 8	111.8	113.9	102.0	114.4	109.7
Week 12	108.8	101.3	140.9	101.3	99.5
Mean	100.9	102.1	105.3	106.5	102.8
Range	79.6 - 111.8	96.4 - 113.9	94.6 - 140.9	98.1 - 124.8	82.0 - 121.6

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 2090)

<b>Percent of Nominal Concentration Naproxen - Male</b>					
	12 mg/kg/day Groups 2/9	50 mg/kg/day Groups 3/10	100 mg/kg/day Groups 4/11	150/100 mg/kg/day Groups 5/12	320 mg/kg/day Groups 7/14
Week 1	110.8 <sup>a</sup>	109.0	105.4	95.2 <sup>a</sup>	110.5
Week 2	104.2	110.6	111.5	111.6	107.9
Week 3	112.5	100.8	111.7	107.7	114.9
Week 4	105.0	102.4	108.8	106.0	103.3
Week 8	92.5	131.6	113.2	108.3	108.2
Week 12	112.5	106.8	106.1	68.5	78.1
Mean	106.2	110.2	109.5	99.6	103.8
Range	92.5 - 112.5	100.8 - 131.6	105.4 - 113.2	68.5 - 111.6	78.1 - 114.9

<sup>a</sup>The mean of the homogeneity samples was used for verification of Week 1 test article concentration.

<b>Percent of Nominal Concentration Naproxen - Female</b>					
	12 mg/kg/day Groups 2/9	25 mg/kg/day Groups 3/10	50 mg/kg/day Groups 4/11	75 mg/kg/day Groups 5/12	50 mg/kg/day Groups 7/14
Week 1	112.5	111.6	102.6	105.6	105.0
Week 2	115.8	113.6	115.2	109.5	123.0
Week 3	120.0	108.0	98.2	118.3	107.4
Week 4	107.5	95.6	91.6	87.2	100.2
Week 8	115.0	123.6	107.4	107.2	110.4
Week 12	120.8	107.6	204.8	99.3	108.6
Mean	115.3	110.0	120.0	104.6	109.1
Range	107.5 - 120.8	95.6 - 123.6	91.6 - 204.8	87.2 - 118.3	100.2 - 123.0

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 2091)

Sumatriptan TK Parameters:

		(mg/kg/day) SUMA/NAP										
			C <sub>max</sub> ng/mL	t <sub>max</sub> hr	AUC <sub>0-inf</sub> hr-ng/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg	C <sub>max</sub> ng/mL	t <sub>max</sub> hr	AUC <sub>0-inf</sub> hr-ng/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg
9	M	25/12	1099	1.0	2298	1.1	10.88	770	2.0	2862	1.5	8.74
9	F	50/12	1524	2.0	4181	0.7	11.96	896	2.0	2532	0.8	19.75
10	M	105/50	2605	1.0	8168	1.0	12.86	2549	0.5	7242	0.7	14.50
10	F	110/25	3460	1.0	10390	1.3	10.59	2652	2.0	7627	0.6	14.42
11	M	210/100	4774	0.5	15218	1.3	13.80	4442	2.0	14323	1.1	14.66
11	F	210/50	7766	0.5	22234	1.1	9.45	5057	1.0	13086	0.7	16.05
12	M <sup>a</sup>	320/150 <sup>b</sup>	5675	0.5	27284	1.4	11.73	8595	1.0	27149	2.4	11.79
12	F <sup>c</sup>	320/75	8422	1.0	31325	1.4	10.22	4906	3.0	35837	2.0	8.93
13	M	320/0	8368	1.0	24896	1.9	12.85	13747	2.0	76391	1.2	4.19
13	F	210/0	7929	2.0	28328	1.5	7.41	7672	1.0	16883	1.4	12.44

a. Second blood sample collection for Group 12 males occurred during Week 8.

b. NAP dose was lowered to 100 mg/kg on Day 62.

c. Second blood sample collection for Group 12 females occurred during Week 10.

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 3019)

Naproxen TK Parameters:

Table 3. Naproxen Toxicokinetic Parameters From Composite Profiles on Days 1 and 90 in CD-1 Mice

Group	Sex	Dose (mg/kg/day) SUMA/NAP	Day 1					Day 90				
			C <sub>max</sub> µg/mL	t <sub>max</sub> hr	AUC <sub>0-inf</sub> hr-µg/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg	C <sub>max</sub> µg/mL	t <sub>max</sub> hr	AUC <sub>0-inf</sub> hr-µg/mL	t <sub>1/2</sub> hr	CL/F L/hr/kg
9	M	25/12	36.9	0.5	74	2.3	0.163	28.0	0.5	64	1.0	0.188
9	F	50/12	43.9	1.0	226	2.5	0.053	38.5	0.5	129	2.2	0.093
10	M	105/50	95.4	0.5	270	2.3	0.185	59.3	0.5	178	1.7	0.280
10	F	110/25	69.5	1.0	318	2.6	0.079	46.9	0.5	172	1.9	0.146
11	M	210/100	91.3	0.5	404	1.3	0.248	93.0	0.5	293	2.8	0.341
11	F	210/50	90.8	0.5	420	2.0	0.119	67.3	1.0	254	3.5	0.197
12	M <sup>a</sup>	320/150 <sup>b</sup>	118.4	0.5	621	5.2	0.161	59.9	1.0	511	5.9	0.293
12	F <sup>c</sup>	320/75	119.4	0.5	542	2.7	0.138	64.0	1.0	468	4.7	0.160
14	M	0/150 <sup>b</sup>	113.9	0.5	396	2.2	0.379	98.7	0.5	338	2.2	0.296
14	F	0/50	121.5	0.5	506	1.6	0.099	106.0	0.5	406	2.3	0.123

a. Second blood sample collection for Group 12 males occurred during Week 8.

b. NAP dose was lowered to 100 mg/kg on Day 62.

c. Second blood sample collection for Group 12 females occurred during Week 10.

(reproduced directly from eNDA 19-926, Module 4, Section 4.2, Page 3020)

Mortality (found dead or sacrificed moribund) was observed in 1/40 M at 210/100, 11/50 M at 320/150(100), and 3/50 M at 0/150(100); and in 1/50 F at 210/50, 8/40 F at 320/75, and 3/50 F at 0/50 mg/kg/day SS/NAP. F 320/75 mice were terminated early on Day 65 due to excessive mortality. The M 210/100 mouse that died had mild necrosis of the thymus and spleen, so this death was considered treatment-related. The F 210/50 that died had a gastric ulcer and peritonitis, so this death was also considered treatment-related. The one M death in the TK group at 105/50 was not considered treatment-related since there was no other evidence of toxicity in this group.

Comparison of the mortality rate of 3/50 (6%) for M at 0/150(100) with the rate of 11/50 (22%) for M at 320/150(100) might lead one to conclude that coadministration of SS exacerbated the toxicity of NAP in M. However, examination of the TK tables (see previous page) reveals that NAP exposures were much higher in M at 320/150(100) vs. M at 0/150(100) [AUC<sub>0-∞</sub> ↑57% Day 1, 51% Day 90], which could easily account for the greater mortality observed. A similar argument can be used to explain the lower mortality observed in F at 210/50 (1/50, 2%) compared to F at 0/50 (3/50, 6%), since NAP exposure was much lower in the F at 210/50 (AUC<sub>0-∞</sub> ↓17% Day 1, 37% Day 90).

<b>SUMMARY OF MORTALITY</b>							
<b>Males:</b>							
<b>Dose<sup>a</sup></b>	<b>0 SS/ 0 NAP</b>	<b>25 SS/ 12 NAP</b>	<b>105 SS/ 50 NAP</b>	<b>210 SS/ 100 NAP</b>	<b>320 SS/<sup>b</sup> 0 NAP</b>	<b>320 SS/ 0 NAP</b>	<b>0 SS/<sup>b</sup> 0 NAP</b>
<b>Main + RC</b>	0/20	0/10	0/10	1/10	5/20	0/20	3/20
<b>TK</b>	0/30	0/30	1/30	0/30	6/30	0/30	0/30
<b>Females:</b>							
<b>Dose<sup>a</sup></b>	<b>0 SS/ 0 NAP</b>	<b>50 SS/ 12 NAP</b>	<b>110 SS/ 25 NAP</b>	<b>210 SS/ 50 NAP</b>	<b>320 SS/ 75 NAP</b>	<b>210 SS/ 0 NAP</b>	<b>0 SS/ 50 NAP</b>
<b>Main + RC</b>	0/20	0/10	0/10	1/20	4/10	0/20	3/20
<b>TK</b>	0/30	0/30	0/30	0/30	4/30	0/30	0/30
TK – Toxicokinetic							
RC – Recovery							
<sup>a</sup> Represents mg/kg for both sumatriptan base and NAP.							
<sup>b</sup> 150 mg NAP/kg from Days 1 to 56 and 100 mg NAP/kg from Days 62 to 91.							

Treatment-related clinical observations included pale discolored skin and/or eyes, skin cold to touch, abdomen distended, hunched posture, few/absent feces, difficult/rapid/slow breathing, and decreased activity in HD SS/NAP and NAP groups.

No significant changes in body weight were noted, and sporadic reductions in body weight changes observed in males were not considered toxicologically relevant.

No treatment-related changes in food consumption, ophthalmology exam, or urinalysis were observed.

Non-significant, but possibly treatment-related, decreases in red cell indices (erythrocytes, hemoglobin, hematocrit) were seen in the M 320/150(100) group. Recovery group M 320/150(100) showed red cell indices comparable to controls, indicating reversibility of this effect. Non-significant increases in mean corpuscular volume were noted in M 320/150(100) and M 0/150(100) and F 210/50 and F 0/50 groups. Non-significant decreases in leukocyte, neutrophil, and eosinophil counts were seen in the F 210/50 group and neutrophils were also reduced in the F 110/25 group. [Note: hematology data from the F 320/75 group was not analyzed statistically, since data was only available from 3 animals].

Treatment-related clinical chemistry findings were limited to decreased albumin (in M & F at MD and HD SS/NAP and HD NAP alone groups) that did not reach statistical significance, and the following findings in the HD F SS/NAP group that was sacrificed early: reduced total protein and globulin, and increased cholesterol and glucose. These findings were not seen in recovery animals, and were considered secondary to the GI toxicity observed.

Treatment-related bone marrow findings included lower proportions of lymphoid cells, higher proportions of total granulocytic cells, and a higher myeloid:erythroid ratio in M HD SS/NAP and NAP alone groups, and were considered secondary to the GI toxicity observed.

Treatment-related macroscopic findings included a stomach perforation (M 320/150(100)), enlarged mandibular and/or mesenteric lymph nodes (M: 25/12, 210/100, and 320/150(100)), and enlarged spleen (M 320/150(100); F: 110/25, 210/50, 320/75). Absolute and relative spleen weights were increased in M (210/100, 320/150(100), and 0/150(100)) and F (320/75 and 0/50).

GI erosions and/or ulcers were observed in 2/11 (18%) M at 210/100, 7/15 (47%) M at 320/150(100) and 4/13 (31%) M at 0/150(100); and in 3/11 (27%) F at 210/50, 6/14 (43%) F at 320/75, and 5/13 (38%) of F at 0/50 mg/kg/day SS/NAP. Most of these lesions were in the glandular stomach, with occasional erosions/ulcers in esophagus, non-glandular stomach, duodenum and cecum. Associated GI findings included peritonitis, inflammation and glandular hyperplasia. Recovery groups did not have ulcers or erosions, just minimal inflammation of the submucosa of the stomach, indicating almost complete reversibility of the GI toxicity.

Direct comparison of the percentage of animals with ulcers/erosions suggests that the GI toxicity of HD NAP alone (31%) was increased with the addition of HD SS (47%) in M, whereas in F the GI toxicity of HD NAP alone (38%) was decreased with the addition of HD SS (27%). Similar differences were seen in mortality rates, and both could be at least partially explained by the differences in NAP exposure in HD NAP groups, which was increased 51% in M with HD SS and decreased 37% in F with HD SS coadministration (based on NAP AUC<sub>0-∞</sub> Day 90).

Treatment-related findings in kidney included only mild dilatation of renal cortical tubules in 1/15 M at 320/150(100) and 1/13 F at 0/50.

Microscopic findings considered secondary to the GI lesions included myeloid hyperplasia in bone marrow, increased extramedullary hematopoiesis in spleen and liver, hyperplasia in lymph nodes, and thymic atrophy/necrosis.

Conclusions:

Treatment-related findings were consistent with the known toxicity of Naproxen and other NSAIDs: increased mortality, renal injury (mild dilation of renal cortical tubules), and GI injury (ulcer, erosion, inflammation, peritonitis, and reparative hyperplasia). Findings considered secondary to the GI lesions included regenerative macrocytic hypochromic anemia, decreased serum total protein and albumin, increased extramedullary hematopoiesis in spleen and liver, myeloid hyperplasia in bone marrow, and thymic atrophy. Recovery groups showed only trace to mild inflammation and/or hyperplasia, demonstrating reversibility of the GI lesions.

NOAEL levels were 105/50 mg/kg/day SS/NAP in M (Day 90 NAP  $AUC_{0-\infty} = 178$  ug\*hr/mL) and 110/25 mg/kg/day SS/NAP in F (Day 90 NAP  $AUC_{0-\infty} = 172$  ug\*hr/mL), with no toxicity seen in SS alone groups at 320 mg/kg/day M (Day 90 SS  $AUC_{0-\infty} = 13747$  ng\*hr/mL) and 210 mg/kg/day F (Day 90 SS  $AUC_{0-\infty} = 16883$  ng\*hr/mL).

Mortality rates and GI toxicity were increased in M and decreased in F HD SS/NAP groups compared to the corresponding HD NAP alone groups at the same NAP dosage. However, these differences could be at least partially explained by the differences in NAP exposure in HD NAP groups, which was increased 51% in M with HD SS and decreased 37% in F with HD SS coadministration (based on NAP  $AUC_{0-\infty}$  Day 90).

Since no toxicity was attributed to SS even at the highest doses used in this study (M at 320 and F at 210 mg/kg/day SS alone, M and F at 320 mg/kg/day SS in combination with NAP), higher doses of SS should have been used to assess the potential for NAP to exacerbate the toxicity of SS.

The conclusions reached in this study must be interpreted cautiously due to concerns regarding the accuracy and homogeneity of the dosing solutions.

**90-Day Oral Toxicity Study in CD-1 Mice with Sumatriptan Succinate and Naproxen Sodium as Single Entities and in Combination****Key study findings:**

- GI toxicity (ulcer/erosion/inflammation of glandular stomach) was increased in incidence and severity in female (F) mice with high dose (HD) administration of sumatriptan succinate (SS) and naproxen sodium (NAP) in combination compared with HD administration of NAP alone, despite a 31% lower Day 90 NAP AUC<sub>0-∞</sub> in HDF SS/NAP vs. HDF NAP.
- GI toxicity (ulcer/atrophy/regenerative hyperplasia of glandular stomach, ileum, and duodenum) was increased in HDM SS/NAP but not in HDM NAP alone, though this might be attributable to the 37.5% greater Day 90 NAP AUC<sub>0-∞</sub> in HDM SS/NAP vs. HDM NAP alone.
- There is no adequate safety margin between NAP exposures in MD mice treated with SS/NAP at the NOEL for GI toxicity in mice and expected NAP exposures in humans given one tablet of Trexima during a migraine. However, it is generally recognized that rodents are more sensitive than humans to the GI toxicity of NSAIDs, and NSAID-related GI toxicity in humans is well described in the labels for all marketed NSAIDs and in the proposed label for Trexima.
- Since no SS-related toxicity was observed at the highest doses tested (320 [M] or 320 lowered to 210 Wk 4 [F] mg/kg/day SS alone or with NAP, the potential for NAP to exacerbate the toxicity of SS remains untested.
- A number of unusual procedures and unexpected complications may have compromised the validity of this study.

**Study no.:** █████ Study 04-293; POZEN Study MT400-T19

**Volume #, and page #:** Module 4, Section 4.2.3.2, Pages 3411-4680

**Conducting laboratory and location:** █████

**Date of study initiation:** 25 OCT 2004

**GLP compliance:** Statement signed by █████ Study Director, 15 JUL 2005

**QA report:** yes (X) no ( )

**Drug, lot #, and % purity:** Sumatriptan succinate (SS), Lot #A03L58, 99.2% purity;  
Naproxen sodium (NAP), Lot # A03L263, 99.9% purity

**Methods**

**Doses:** (SS doses were calculated as the free base, using a correction factor of 1.4 to convert from the sodium salt; NAP doses were calculated as sodium salt)  
The rationale for the doses selected was not explained.

**Text Table 2 Study Design<sup>a</sup>**

Study Group	Dose (mg/kg/day) SS/NAP		Group Name	Number of Animals/		
	Male	Female		Core Group <sup>d</sup>	Recovery Group	TK Group <sup>c,d</sup>
1	0/0	0/0	Vehicle Control	20/sex	10/sex	33/sex
2	30/10	30/10	Low	20/sex	--	33/sex
3	100/30	100/30	Mid	20/sex	--	33/sex
4	320/100	320(210)/50 <sup>b</sup>	High	20/sex	10/sex	33/sex
5	320/0	320(210)/0 <sup>b</sup>	High-S	20/sex	--	33/sex
6	0/100	0/50	High-N	20/sex	--	33/sex

<sup>a</sup>The first day of dosing was designated as Day 1.

<sup>b</sup>The dose of sumatriptan was lowered to 210 mg/kg/day at the beginning of Week 4.

<sup>c</sup>Blood was collected from 3 TK mice/sex/group at 0.5, 1, 2, 4 and 12 hours post-dose on the first day of dosing, and prior to dosing and at 0.5, 1, 2, 4 and 12 hours post-dose on Day 90.

<sup>d</sup> Two additional mice/sex/group were treated similarly to the core 20/sex/group animals for possible use as replacement animals (core, recovery or TK). The first 20 surviving mice/sex/group were designated as core animals. For Groups 1 and 4, the surviving mice/sex/group were designated as recovery animals. Mice not assigned for core or recovery were designated for TK evaluation or discarded without necropsy. See Text Table 3 for specific animal reassignment.

**Text Table 3 Replacement Animals Added by Protocol Amendment #1**

Study Group	Dose (mg/kg/day) Sumatriptan/Naproxen		Group Name	Addition of Mice (Assigned to Study for Use)		
	Male	Female		Replacement Initial Dose 11/04/2004	Replacement Initial Dose 11/10/2004	Replacement Initial Dose 11/11/2004
1	0/0	0/0	Vehicle Control	-- <sup>a</sup>	--	--
2	30/10	30/10	Low	--	--	-- <sup>d</sup>
3	100/30	100/30	Mid	--	3 Male- TK	--
4	320/100	320(210)/50 <sup>b</sup>	High	3 Males - Core	1 Male - TK	4 Females - TK
5	320/0	320(210)/0 <sup>b</sup>	High-S	--	-- <sup>c</sup>	2 Females - TK
6	0/100	0/50	High-N	--	3 Males - TK	--

<sup>a</sup> -- None.

<sup>b</sup> The dose of sumatriptan was lowered to 210 mg/kg/day at the beginning of Week 4 (11/15/04).

<sup>c</sup> An extra animal (E-19) died on its study day 3; only clinical and necropsy observations are included in this report

<sup>d</sup> An extra animal (F-6) died on its study day 6; only clinical and necropsy observations are included in this report

Species/strain: CD-1 mice ( XXXXXXXXXX )

Number/sex/group or time point (main study): 20/sex/group (+3 M in High-S)

Route, formulation, volume, and infusion rate: Formulations consisted of homogeneous suspensions of mixed SS and NAP in 0.5% carboxyl methyl cellulose from stock solutions of 32 mg/mL SS and 5 or 10 mg/mL NAP. Mixed final solutions contained 3/1, 10/3, 32/5, and 32/10 mg/mL SS/NAP originally, but after 4 weeks, the top concentration of SS used for females was lowered from 32 to 21 mg/mL. All formulations were delivered at a constant volume of 10 mL/kg via oral gavage.

Satellite groups used for toxicokinetics or recovery: 33/sex/group TK

(3/sex/time point + replacements noted in Text Table 3 above); 10/sex/group Control (CON) and High Dose (HD) 4-week recovery groups.

**Age:** 4-5 weeks old upon receipt 13 OCT 2004

**Weight:** 15-24 g the day after receipt (only a subset was weighed)

**Sampling times:** 90 days (Main Study) and 118 days (Recovery)

**Unique study design or methodology (if any):** Unexpected deaths in 3/18 HDF TK mice (320/50 mg/kg/day SS/NAP) and 2/18 HDF SS (320 mg/kg/day SS) triggered lowering of the top SS dose in F from 320 mg/kg/day to 210 mg/kg/day starting on Day 22 (11/15/04, beginning of Week 4).

**Reviewer's Note:** It is not clear that this dose reduction was justified. The rationale stated in Amendment 1 to the Protocol was very **brief**: "**Based on unexpected deaths (some associated with gavage trauma and others of undetermined cause)...**" TK animals were not examined, and no clear SS-related toxicity was noted in HD SS/NAP or HD SS animals in the main study. Also, replacement animals were added by amendment during Wks 2-3 (see Text Table 3 above) due to the high number of deaths early in the study associated with gavage errors (18 Main Study M and F), and lower urinary tract obstruction (11 Main Study M). Also, it is unusual that animals were assigned to core or recovery groups based on survival rather than random selection (the first 20/sex/group that survived were designated the "**core**" group), and only the first 10/sex/group of the survivors (and those found dead or sacrificed moribund) were necropsied at termination.

**Observations and times:**

**Mortality:** Observed for moribundity and mortality twice daily on weekdays and once on weekends and holidays.

**Clinical signs:** Detailed clinical exam was performed weekly, and cage-side observations were performed for ~1-2 hrs postdose (excluding TK animals).

**Body weights:** Pre-test, and weekly, starting on Day 0 (Day 1 = first day of dosing). Fasted BW was collected prior to terminal sacrifice.

**Food consumption:** Recorded weekly (excluding TK mice).

**Ophthalmoscopy:** "**Indirect funduscopic examinations** were performed by a board-certified veterinary ophthalmologist on all core toxicology mice and those designated as potential replacement animals for the core groups during quarantine (pretest) and at the end of the study (week 13). Recovery animals were not examined since end of **treatment exams did not reveal any adverse effects of treatment.**"

**Toxicokinetics:** "**Blood specimens were collected** from 3 TK animals/sex/time-point; mice were lightly anesthetized with CO<sub>2</sub> and bled via the orbital sinus at designated times (i.e., 0.5, 1, 2, 4, and 12 hours) after the first and last dose (dose 90) administrations. In addition a zero hour time-point was collected prior to administration of the last dose (dose 90). After blood collection, the animals were euthanized (by CO<sub>2</sub> asphyxiation) and **discarded without necropsy.**"

**EKG:** Not performed.

Hematology/Clinical chemistry/Urinalysis:

Clinical Pathology (excluding TK animals): “Blood for clinical chemistry and hematology was collected from the orbital sinus plexus. The mice were fasted for approximately 4 hours and were lightly anesthetized with CO<sub>2</sub> prior to blood collection. For the terminal necropsy, the first 10 core toxicology mice/sex/group were designated for hematology and urine collection, while the remaining 10 mice/sex/group were bled for clinical chemistry parameters. For the recovery necropsy, 5 mice/sex/group were designated for hematology and urine collection, while the remaining 5 mice/sex/group were sampled for clinical chemistry. Urine, voided overnight in metabolism cages, was similarly collected prior to scheduled necropsy. The urine collection tubes were placed on ice during collection to minimize bacterial growth.”

**Text Table 4 Clinical Chemistry (Termination and Recovery)**

Albumin (A)	Chloride	PO <sub>4</sub>
A/G ratio	Cholesterol	Potassium
Alanine aminotransferase	Creatinine	Protein, Total
Alkaline phosphatase	Creatine Kinase	Sodium
Aspartate aminotransferase	Gamma-glutamyl transferase <sup>a</sup>	Triglycerides
Bilirubin, Total	Globulin (G)	
Blood urea nitrogen	Glucose	
Calcium	Lactate dehydrogenase	

<sup>a</sup>GGT values are generally 1 or less. When a negative reading is recorded, a value of 0 or <3 is indicated in the raw data. For reporting purposes, all values of <3 were considered to be zero (0) and were reported as such.

**Text Table 5 Hematology (Termination and Recovery)**

Red blood cell count	Mean corpuscular hemoglobin concentration
White blood cell count	Platelet count
Hemoglobin	Automated differential Leukocyte count
Hematocrit	(absolute and relative)
Mean corpuscular hemoglobin	Automated red cell morphology
Mean corpuscular volume	Reticulocyte count (absolute and relative)

**Text Table 6 Urinalysis (Termination and Recovery)**

Appearance	Blood
Color	pH
Bilirubin	Specific gravity/refractive index <sup>a</sup>
Glucose	Urobilinogen
Ketones	Protein
Leukocytes	Microscopic sediment evaluation <sup>a</sup>
Nitrite	Volume

<sup>a</sup> Values outside the limit of the detector are listed as ≤ or ≥ in the raw data. These values are reported without the symbol for purposes of generating means.

<sup>b</sup> Evaluated/tabulated if the other parameters show evidence of an adverse effect.

**Gross pathology (excluding TK animals):** “The first ten surviving mice/sex/group underwent a complete necropsy; these were the mice designated for hematology and urine collection. In addition, any animal found dead or euthanized moribund received a complete necropsy. Necropsy included examination of the external surface of the body, all orifices, the cranial, thoracic, and peritoneal cavities, and their contents. Mice scheduled for terminal necropsy were fasted for approximately 4 hours and were euthanized using CO<sub>2</sub> asphyxiation. Recovery mice were held for at least 4 weeks after the final dose prior to necropsy. Tissues collected at necropsy are listed below. Femoral bone marrow smears were collected and processed from all core and recovery animals that survived until scheduled termination. A quantitative bone marrow analysis (conducted by a board certified clinical pathologist from [REDACTED]) was conducted for the high dose combination group and the vehicle control group of the main study and recovery animals. Eyes, optic nerves, Harderian glands and lacrimal glands were fixed in Davidson’s solution, and the testes were fixed in Bouin’s solution; all other tissues were fixed in 10% neutral buffered formalin.”

**Organ weights:** See histopath table below for organs weighed. Paired organs were weighed together.

**Histopathology:** Adequate Battery: yes (X); See histopath table below for tissues evaluated. Larynx, cervical lymph nodes, and zymbal gland were not examined.

Peer review: yes (X)

“Tissues required for histopathologic evaluation were trimmed, processed, sectioned, and stained with hematoxylin and eosin in accordance with [REDACTED] Standard Operating Procedures. All protocol required tissues were processed and evaluated for terminal sacrifice animals in Groups 1 and 4, as well as all early death animals. In addition, protocol defined potential target tissues were trimmed, processed, sectioned and stained with hematoxylin and eosin in Groups 2, 3, 5 and 6. The protocol defined potential target tissues included: stomach (glandular and nonglandular), large and small intestine, cecum, colon, bone marrow and kidney. Potential target organs identified after evaluation of the control and high-dose combination groups (spleen, and stomach) were evaluated microscopically for animals in Groups 2, 3, 5 and 6 and in the recovery animals in Groups 1 and 4. The duodenum was also identified as a target and was evaluated in Groups 3, 5 and 6 and recovery Groups 1 and 4. After initial microscopic evaluation of protocol-required tissues from terminal sacrifice animals in Groups 1 and 4, the Sponsor requested microscopic evaluation of additional sections of stomach and duodenum. Based on previous experience of the Sponsor with the test article NAP, a higher frequency of gastrointestinal inflammation, erosions and ulcers was anticipated in this study, primarily at the pyloric junction and in the proximal duodenum.

A concerted effort was made to examine all remaining pyloric tissue. Additional cross sections were obtained for all groups from both terminal and recovery sacrifice animals and selected early death animals (i.e. early deaths without evidence of gavage trauma at necropsy). For the additional evaluation of the stomach, three areas of pyloric mucosa were trimmed, processed and placed into a block. A total of 4 sections (approximate 30 $\mu$  step sections) were obtained from each block; these sections were stained with hematoxylin and eosin and evaluated microscopically. Every attempt was made to have the same number of sections of stomach available for comparison from all recovery and terminal sacrifice animals. Five slides, containing a total of 13 sections of glandular stomach from each animal, were evaluated consecutively for all terminal sacrifice, recovery sacrifice and selected early death animals. For each microscopic finding, the section of gastric mucosa with the most severe change for each diagnosis was recorded.”

“Four additional cross-sections of duodenum were trimmed, processed, placed into a paraffin block, stained with hematoxylin and eosin and evaluated microscopically from select early death animals (those with no evidence of gavage trauma at necropsy) and from terminal animals in Groups 1, 3, 4, 5 and 6; a four sections of duodenum was evaluated for recovery animals in Groups 1 and 4.”

## Results

Dose Formulation Analysis: All samples collected during the dosing period met the criteria of 90-110% of claimed concentration, except for the 32/5 mg/mL SS/NAP for Wk 1, which averaged only 89.1%. All preparations met the criteria for 5% variation of homogeneity.

Toxicokinetics: Coadministration of SS increased the NAP AUC<sub>0-∞</sub> in HDM (↑13.0% Day 1, ↑37.5% Day 90 in 320/100 mg/kg SS/NAP vs. 100 mg/kg NAP). However, Coadministration of SS decreased the NAP AUC<sub>0-∞</sub> in HDF (↓10.9% Day 1 in 320/50 mg/kg SS/NAP vs. 50 mg/kg NAP, ↓31.1% Day 90 in 210/50 mg/kg SS/NAP vs. 50 mg/kg NAP).

NAP C<sub>max</sub> was not appreciably changed in HDM with coadministration of SS, but in HDF NAP C<sub>max</sub> was decreased with SS compared to NAP alone (↓22.2% Day 1 in 320/50 mg/kg SS/NAP vs. 50 mg/kg NAP, ↓18.9% Day 90 in 210/50 mg/kg SS/NAP vs. 50 mg/kg NAP).

SS AUC<sub>0-∞</sub> in HDM was decreased on Day 1 (↓14.0%) but increased on Day 90 (↑35.0%) with 320/100 mg/kg SS/NAP vs. 320 mg/kg SS. No appreciable changes were observed in HDF SS AUC<sub>0-∞</sub> at 320/50 mg/kg SS/NAP vs. 320 mg/kg SS alone (Day 1) or 210/50 mg/kg SS/NAP vs. 210 mg/kg SS alone (Day 90).

SS C<sub>max</sub> was decreased 7-8% in HDM (Day 90) and HDF (Days 1 and 90) with coadministration of HD NAP compared to HD SS alone.

**Text Table 8 Toxicokinetic Parameters of Sumatriptan on Day 1 and Day 90 in CD-1 Mice**

		Sumatriptan								
		Day 1				Day 90				
Sex	PK Parameter	Units SS/NAP g/kg	30/10	100/30	320/100	320/0	30/10	100/30	320/100	320/0
M	Tmax	(hr)	0.5	0.5	0.5	0.5	0.5	0.5	1.0	0.5
	Cmax	(ng/mL)	394	2090	8474	8446	743	2515	9657	10415
	Cmax/Dose	(ng*kg/mg*mL)	13.1	20.9	26.5	26.4	24.8	25.2	30.2	32.5
	AUC(0-inf)	(hr*ng/mL)	1103	6054	30120	35026	1428	8217	39730	29423
	AUC(0-inf)/Dose	(hr*kg*ng/mL/mg)	36.8	60.5	94.1	109	47.6	82.2	124.0	91.9
	t1/2	(hr)	0.796	1.51	1.44	1.23	0.936	2.96	1.42	2.32
	CL/F	(L/hr/kg)	27.2	16.5	10.6	9.14	21.0	12.2	8.05	10.9
		SS/NAP mg/kg	30/10	100/30	320/50	320/0	30/10	100/30	210/50	210/0
F	Tmax	(hr)	0.5	2.0	2.0	0.5	0.5	2.0	2.0	0.5
	Cmax	(ng/mL)	657	3138	6662	7242	804	2001	6860	7451
	Cmax/Dose	(ng*kg/mg*mL)	21.9	31.4	20.8	22.6	26.8	20.0	32.7	35.5
	AUC(0-inf)	(hr*ng/mL)	1633	11889	40944	39651	1310	9380	21093	22591
	AUC(0-inf)/Dose	(hr*kg*ng/mL/mg)	54.4	118.9	128	123.9	43.7	93.8	100.4	108
	t1/2	(hr)	0.886	6.14	1.08	1.12	0.962	1.69	1.98	1.68
	CL/F	(L/hr/kg)	18.4	8.41	7.82	8.07	22.9	10.7	9.96	9.30

**Text Table 9 Toxicokinetic Parameters of Naproxen on Day 1 and Day 90 in CD-1 Mice**

		Naproxen								
		Day 1				Day 90				
Sex	PK Parameter	Units SS/NAP g/kg	30/10	100/30	320/100	0/100	30/10	100/30	320/100	0/100
M	Tmax	(hr)	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
	Cmax	(mcg/mL)	27.0	69.9	104	111	33.0	52.0	103	108
	Cmax/Dose	(mcg*kg/mg*mL)	2.70	2.33	1.04	1.11	3.30	1.73	1.03	1.08
	AUC(0-inf)	(hr*mcg/mL)	71.2	252	522	462	51.9	154	363	264
	AUC(0-inf)/Dose	(hr*kg*mcg/mL/mg)	7.12	8.40	5.22	4.62	5.19	5.13	3.63	2.64
	t1/2	(hr)	1.46	3.48	1.71	1.98	1.41	1.67	1.65	1.12
	CL/F	(L/hr/kg)	0.141	0.119	0.192	0.216	0.193	0.195	0.275	0.378
		SS/NAP mg/kg	30/10	100/30	320/50	0/50	30/10	100/30	210/50	0/50
F	Tmax	(hr)	0.5	0.5	0.5	0.5	0.5	1.0	0.5	0.5
	Cmax	(mcg/mL)	39.3	98	97.3	125	46.6	74.3	99.7	123
	Cmax/Dose	(mcg*kg/mg*mL)	3.93	3.27	1.95	2.50	4.66	2.48	1.99	2.46
	AUC(0-inf)	(hr*mcg/mL)	207	467	541	607	188	369	381	553
	AUC(0-inf)/Dose	(hr*kg*mcg/mL/mg)	20.7	15.6	10.8	12.1	18.8	12.3	7.62	11.1
	t1/2	(hr)	2.34	2.40	2.26	1.51	2.00	1.75	2.23	1.69
	CL/F	(L/hr/kg)	0.0483	0.0643	0.0924	0.0824	0.0532	0.0812	0.1310	0.0904

**Mortality:** No treatment-related mortality was observed. Of 26 animals found dead or sacrificed *in extremis* during the study (18 M, 8 F), 18 were thought to have been victims of gavage trauma, based on microscopic evidence. The remaining 8 early deaths (all males) had clinical, gross, and microscopic signs of urinary tract obstruction (inguinal crust correlated with ulcerative dermatitis, inguinal abscess, inguinal irritation, inguinal sore/scab, discolored/wet inguinal fur, distended penis, urinary bladder dilatation, renal dilatation, and/or hydronephrosis). Three of the 18 mice with gavage trauma also had signs of urinary tract obstruction. The lower urinary tract obstruction was not considered treatment-related because it was not dose-dependent (control males were affected as well), it was not seen in males surviving to scheduled termination, and it has been reported to occur spontaneously in male CD-1 mice (see Woo-Chan Son, 2003). However, the sponsor noted that the incidence rate reported for spontaneously occurring lower urinary tract obstruction (5/1453 mice, or 0.3%) is much lower than that observed in the current study (11/151 M mice, or 7.3%); and the reported age of onset (age 31-40 wks) was much later than in the current study (age 6-18 wks). The sponsor stated that, **“The reason(s) for the earlier onset and high incidence of lower urinary tract obstruction seen in the present study in control and treated males is unknown.”** (*eNDA 21-926, Module 4, Section 4.2, Page 3445*)

**Clinical signs:** No treatment-related clinical signs were noted. All clinical signs reported were attributed to gavage errors and lower urinary tract obstruction as described in **“Mortality”** above.

**Body weights:** No consistent differences were seen in body weight or body weight gain beyond the first week of dosing, which showed slight increases in HD groups vs. controls.

**Food consumption:** Significant increases (8-14%) were observed in HDM SS/NAP vs. CON M Wks 1, 2, 6, 9, and 13; no differences were seen during recovery weeks 14-17.

**Ophthalmoscopy:** No treatment-related differences were noted.

**EKG:** No EKG was performed.

**Hematology:** Statistically significant changes compared to corresponding controls were observed for the following: red blood cell count (↓7.3% in HDM SS/NAP recovery), white blood cell count (↓33% in LDF SS/NAP, ↓40% in MDF SS/NAP, ↓34% in HDF SS), absolute reticulocyte count (↑21% HDM SS/NAP, ↑49% in HDF SS/NAP, ↑10% in HDM SS\*, ↑11% in HDM NAP\*, ↑20% in HDF NAP\*), relative reticulocyte count (↑35% HDM SS/NAP, ↑56% in HDF SS/NAP, ↑12% in HDM SS\*, ↑15% in HDM NAP\*, ↑24% in HDF NAP\* [\*=not statistically significant, but considered treatment related by the sponsor]). Some statistically significant changes in absolute and relative counts of neutrophils, lymphocytes, and monocytes were observed in LDF SS/NAP, MDF SS/NAP, and HDF SS groups, but not in HDF SS/NAP or any male groups, so they were not considered treatment-related.

*Reviewer's Note: Only the increases in reticulocytes appeared to be treatment related, and they were mild and reversible.*

**Clinical chemistry:** Statistically significant changes compared to corresponding controls were observed for the following: serum phosphorous ( $\uparrow$ 26% in HDM SS/NAP,  $\uparrow$ 14% in HDF SS), albumin ( $\downarrow$ 10% in HDM and  $\downarrow$ 15% in HDF SS/NAP), cholesterol ( $\uparrow$ 23% in HDM SS,  $\uparrow$ 27% in HDF NAP), total protein ( $\downarrow$ 8% in HDF SS/NAP), potassium ( $\uparrow$ 14% in HDF SS), alkaline phosphatase ( $\downarrow$ 32% in HDF SS/NAP and  $\downarrow$ 27% in HDF SS/NAP recovery), triglycerides ( $\downarrow$ 27% in HDF SS/NAP recovery), and lactate dehydrogenase ( $\uparrow$ 53% in HDF SS/NAP recovery).

*Reviewer's Note: Serum phosphorous was increased in HDF SS, not decreased as stated in the sponsor's text. However, none of the changes listed above appeared to be toxicologically meaningful.*

**Urinalysis:** No treatment-related differences were noted.

**Organ weights:** Increases were observed in spleen weights in HDM SS/NAP ( $\uparrow$ 25% absolute;  $\uparrow$ 25% organ/body wt;  $\uparrow$ 29% organ/brain wt), in HDF SS/NAP ( $\uparrow$ 28% absolute), and in HDF NAP ( $\uparrow$ 14% absolute). These increases were considered treatment-related even though they did not reach statistical significance.

**Gross pathology:** Twenty-six main study animals died prior to terminal sacrifice. Eighteen early deaths were attributed to gavage trauma (2 CON M, 1 CON F, 2 LDF SS/NAP, 1 MDF SS/NAP, 5 HDM SS/NAP, 2 HDF SS/NAP, 2 HDM SS, 2 HDF SS, and 1 HDM NAP). **"Gavage trauma was indicated** when one of the following was noted: gross evidence of a ruptured esophagus, microscopic evidence of hemorrhage in the adventitia of the esophagus and/or trachea, inflammation of structures of the mediastinum or thoracic cavity, and/or **fracture of the sternum.**" **The remaining eight deaths (in 1 CON M, 3 MDM SS/NAP, 2 HDM SS/NAP, and 2 HDM NAP)** were attributed to lower urinary tract obstruction, which occurs spontaneously in male CD-1 mice (see Woo-Chan Son, 2003). Three males that died early had signs of both lower urinary tract obstruction (gross dilatation of urinary bladder with microscopic correlates) and gavage trauma.

One HDM SS/NAP (#359) that was sacrificed in extremis on Day 83 with evidence of urinary tract obstruction was found to have mild villus atrophy and mild hyperplasia of the mucosa in the proximal duodenum that was considered to be unrelated to the cause of death, but related to drug treatment ("this reparative lesion was likely secondary to a previous erosive process.")

**TABLE V**  
**SELECT EARLY DEATH FINDINGS**

Treatment (mg/kg/day)	Animal number	Days on Test	Conclusions supported by gross and/or microscopic findings
0	011	20	Gavage trauma
0	015	23	Lower urinary tract obstruction; gavage trauma
0	020	75	Lower urinary tract obstruction
100 Sumatriptan 30 Naproxen	246	86	Lower urinary tract obstruction; ulcerative dermatitis
100 Sumatriptan 30 Naproxen	249	62	Lower urinary tract obstruction; ulcerative dermatitis; ascending urogenital tract infection
100 Sumatriptan 30 Naproxen	253	81	Lower urinary tract obstruction; ulcerative dermatitis
320 Sumatriptan 100 Naproxen	351	8	Gavage trauma
320 Sumatriptan 100 Naproxen	352	8	Gavage trauma
320 Sumatriptan 100 Naproxen	355	8	Gavage trauma
320 Sumatriptan 100 Naproxen	355R	49	Lower urinary tract obstruction; ulcerative dermatitis; ascending urogenital tract infection
320 Sumatriptan 100 Naproxen	358	87	Lower urinary tract obstruction; ulcerative dermatitis; ascending urogenital tract infection; gavage trauma
320 Sumatriptan 100 Naproxen	359	83	Lower urinary tract obstruction; ascending urogenital tract infection; ulcerative dermatitis; gavage trauma
320 Sumatriptan 100 Naproxen	364	21	Gavage trauma
320 Sumatriptan	486	7	Gavage trauma
320 Sumatriptan	E19	4	Gavage trauma
100 Naproxen	595	82	Lower urinary tract obstruction; ulcerative dermatitis; ascending urogenital tract infection
100 Naproxen	609	72	Lower urinary tract obstruction; ulcerative dermatitis; ascending urogenital tract infection
100 Naproxen	612	70	Lower urinary tract obstruction; ulcerative dermatitis; ascending urogenital tract infection; gavage trauma
0	033	48	Gavage trauma
30 Sumatriptan 10 Naproxen	159	17	Gavage trauma
30 Sumatriptan 10 Naproxen	F6	6	Gavage trauma
100 Sumatriptan 30 Naproxen	270	69	Gavage trauma
320to210 Sumatriptan 50 Naproxen	387	18	Gavage trauma
320to210 Sumatriptan 50 Naproxen	397	9	Gavage trauma
320to210 Sumatriptan	506	8	Gavage trauma
320to210 Sumatriptan	509	11	Gavage trauma

(Table V above reproduced from Sponsor's 04-293 Vol. III Page 32)

Treatment-related gross findings in mice surviving to scheduled termination included crust on the abdominal skin and an enlarged preputial gland in one HDM NAP; these findings were not evaluated microscopically. Also, another HDM NAP had multiple red foci in stomach correlated with congestion of the mucosa.

Gross findings considered unrelated to treatment included dilatation of bladder or kidney (in 4 CON M, 3 LDM SS/NAP, 4 MDM SS/NAP, 4 HDM SS/NAP, 5 HDM SS, and 5 HDM NAP). Dilatation of the kidney was always correlated with hydronephrosis.

One CON F had an abscess in skeletal muscle and thoracic cavity, enlarged spleen, extramedullary myeloid hematopoiesis, enlarged bronchial and lumbar lymph nodes, lymphofollicular hyperplasia and/or medullary plasmacytosis. One HDF SS/NAP and 1 HDF NAP had enlarged spleens correlated with extramedullary erythroid hematopoiesis.

No treatment-related gross observations were noted in HD SS/NAP recovery animals.

**Histopathology:** Treatment related findings were observed in the GI tract in HDM SS/NAP, HDF SS/NAP and HDF NAP. In HDF SS/NAP glandular stomach showed increased incidence and severity of acute inflammation (minimal-mild, 6/10 vs. 2/10 minimal in CON F), pyloric hyperplasia (5/10, minimal-moderate), and gastric erosions (2/10, mild) and/or ulcerations (1/10, mild) [or thickened submucosa with serosal inflammation (2/10), suggesting a nearby section might have shown an ulcer or erosion]. In the HDF SS/NAP recovery group, 1/10 had a mild ulcer in the glandular stomach. One HDM SS/NAP had a mild ulcer in the glandular stomach with associated inflammation, another had moderate villous atrophy of ileum, and (as mentioned previously) one Early Sacrifice HDM SS/NAP had mild regenerative mucosal hyperplasia in the duodenum suggestive of prior erosions. One HDF NAP had a minimal erosion of the glandular mucosa with associated acute inflammation.

One MDM and one MDF SS/NAP had mild erosions of the nonglandular stomach with associated acute inflammation and hyperplasia of the epithelium, but these were not considered treatment-related since similar changes were seen in 1/10 CON F recovery mice and have been reported to be related to the gavage procedure in mice (Leininger et al., 1999, Chapter 4 “Oral cavity, esophagus and stomach.” In: Pathology of the mouse. Ed. Maronpot RR, Cache River Press, p34).

Upper GI toxicity is an expected effect of NAP, a member of the class of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs). However, the greater incidence and severity of glandular stomach lesions in the HDF SS/NAP group compared to the HDF NAP alone group is puzzling, especially considering the toxicokinetic results demonstrating that exposure (AUC<sub>0-∞</sub> Day 90) was 31% lower in the SS/NAP vs. the NAP alone group. Also unexpected, the HDM SS/NAP group showed much less GI toxicity than the HDF SS/NAP group despite having similar NAP exposures on Days 1 and 90.

**Table 11 Summary of Test Article-Related Effects in the Glandular Stomach of Female Mice (Incidence [Severity])<sup>a</sup>**

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
SS/NAP (mg/kg)	0/0	30/10	100/30	320(210)/50	320(210)/0	0/50
Microscopic Finding						
Ulcer	0	0	0	1	0	0
Erosion	0	0	0	2 <sup>b</sup>	0	1
Inflammation, acute, glandular	2 (0.2)	3 (0.3)	4 (0.4)	6 (1.10)	2 (0.3)	2 (0.2)
Hyperplasia, pylorus	0	0	0	5 (1.10)	0	1 (0.2)

a. N=10/group

b. Two additional Group 4 females (animal numbers 388 and 390) had a thickened submucosa with serosal inflammation, suggesting the plane of section was adjacent to an additional ulcer/erosion.

(Sponsor’s Table. Note that severity scores are means of the 10 animals per group, where 0=no findings, 1=minimal, 2=mild, 3=moderate, and 4=marked)

The sponsor described the gastric lesions in **this study** as “**very subtle,**” as evidenced by their low incidence and minimal severity; very mild changes in RBC parameters, reticulocytes, and platelets; slight decreases in total protein and albumin; slight increases in cholesterol and phosphorous; slight increases in absolute and relative spleen weights; and slight increases in the incidence and severity of splenic extramedullary hematopoiesis (min-mod).

Kidney hydronephrosis was noted in 26 M (4 CON, 3 LD SS/NAP, 4 MD SS/NAP, 5 HD SS/NAP, 5 HD SS, and 5 HD NAP), but was not considered treatment-related due to the lack of dose-dependence.

Minimal inflammation of liver was observed in 4/10 HDM SS/NAP and 0/10 CON M, but was not considered treatment-related because similar findings were observed in 7/10 CON F and 5/10 HDF SS/NAP and it is stated to be a “**common spontaneous change in this mouse strain.**”

The only treatment-related microscopic finding in recovery animals was an ulcer in the glandular stomach in 1/10 HDF SS/NAP, suggesting that most changes were reversible.

#### Sponsor's Conclusions

Direct treatment-related toxicity was typical of the known toxicity of NAP (GI inflammation, erosions, ulcers and hemorrhage, and regenerative anemia. Indirect compensatory changes observed included mild increases in WBC count, neutrophils, reticulocytes and platelets; increased absolute and relative spleen weights (correlated with increased erythroid extramedullary hematopoiesis); mild reductions in RBC count, HGB, and HCT; decreased serum total protein and albumin; and increased serum phosphorous and cholesterol.

The HDM SS/NAP group showed lower SS exposure (M), and higher NAP exposure than the corresponding HDM SS and HDM NAP groups, respectively, whereas the HDF SS/NAP group showed similar SS exposure and lower NAP exposure compared to the HDF SS and HDF NAP groups, respectively.

Clinical signs were observed only in unscheduled death animals and were attributed to gavage error and/or lower urinary tract obstruction rather than to drug treatment. Changes in body weight and food consumption were minor and transient.

Findings persisting into the recovery period included decreased RBC parameters (HDM SS/NAP) and ulcer in the glandular stomach (1/10 HDF SS/NAP).

The NOAEL was considered to be MD SS/NAP (100/30 mg/kg/day) for M and F CD-1 mice in this 90-day toxicity study due to observations of treatment-related toxicity in the glandular stomach (erosions, ulcers, inflammation, and glandular hyperplasia) in the HD SS/NAP groups (320/100 mg/kg/day M; 320[210]/50 mg/kg/day F). Exposures associated with the NOAELs were 8217 and 9380 ng\*hr/mL SS and 154 and 369 ug\*hr/mL NAP (Day 90 AUC<sub>0-∞</sub>, M, F).

*Reviewer's Notes:*

The sponsor does not discuss the possibility that the increased GI toxicity observed in HDF SS/NAP vs. HDF NAP (despite a 31% lower NAP AUC in SS/NAP vs. NAP) might be due to an unexpected potentiation of NAP GI toxicity by SS. Similarly, HDM SS/NAP showed increased GI toxicity compared to HDM NAP (though in this case a 37.5% higher NAP AUC might account for the difference). In the absence of other explanations, the exacerbation of NAP-induced GI toxicity by SS must be considered as a real possibility. Below is a table of Cmax and AUC values to allow comparison of exposures between mice at the effect and no-effect doses and expected human exposures at the maximum recommended dose:

**Sumatriptan Exposure Ratios**

Species	Dose	Cmax (ng/mL)	Cmax Ratio	AUC <sub>0-∞</sub> (ng*hr/mL)	AUC <sub>0-∞</sub> Ratio	Treatment-Related GI Toxicity?
Humans (Study MT400-101*)	1 tablet	74.9	--	270	--	--
HDF SS/NAP 90-Day Mouse	320(210)/ 50 mg/kg	6860	92	21093	78	Ulcer/erosion (4/10, 1/10 rec)
NOEL SS/NAP 90-Day Mouse	100/30 mg/kg	2001	27	9380	35	No
HDF SS 90-Day Mouse	320(210) mg/kg	7451	99	22591	84	No
HDM SS/NAP 90-Day Mouse	320/100 mg/kg	9657	129	39730	193	Ulcer/atrophy/ hyperplasia (3/10)
NOEL SS/NAP 90-Day Mouse	100/30 mg/kg	2515	34	8217	30	No
HDM SS 90-Day Mouse	320 mg/kg	10415	139	29423	109	No

**Naproxen Exposure Ratios**

Species	Dose	Cmax (ug/mL)	Cmax Ratio	AUC <sub>0-∞</sub> (ug*hr/mL)	AUC <sub>0-∞</sub> Ratio	Treatment-Related GI Toxicity?
Humans (Study MT400-101*)	1 tablet	69.7	--	1548	--	--
HDF SS/NAP 90-Day Mouse	320(210)/ 50 mg/kg	99.7	1.4	381	0.25	Ulcer/erosion (4/10, 1/10 rec)
NOEL SS/NAP 90-Day Mouse	100/30 mg/kg	74.3	1.1	369	0.24	No
HDF NAP 90-Day Mouse	50 mg/kg	123	1.8	553	0.36	Erosion (1/10)
HDM SS/NAP 90-Day Mouse	320/100 mg/kg	103	1.5	363	0.23	Ulcer/atrophy/ hyperplasia (3/10)
NOEL SS/NAP 90-Day Mouse	100/30 mg/kg	52	0.75	154	0.10	No
HDM NAP 90-Day Mouse	100 mg/kg	108	1.5	264	0.17	No

(\*Human Values are Geometric Means (N=8) from Clinical Study Report MT400-101)  
(Mouse values are from Day 90; "rec" = 4 week recovery group; Reviewer's Tables)

From the tables above, it is clear that there is no adequate safety margin between NAP exposures in MD mice treated with SS/NAP at the NOEL for GI toxicity in mice and expected NAP exposures in humans given one tablet of Trexima during a migraine. However, it is generally recognized that rodents are more sensitive than humans to the GI toxicity of NSAIDs, and NSAID-related GI toxicity in humans is well described in the labels for all marketed NSAIDs and in the proposed label for Trexima.

The likelihood that expected exposures to SS after one tablet of Trexima may exacerbate the expected GI toxicity of NAP is not clear from this study. The SS exposures in MDF and MDM mice treated with SS/NAP at the NOEL for GI toxicity were ~50-fold above expected human exposures, suggesting that a wide margin of safety exists, and levels of SS in humans will be much too low to exacerbate NAP-induced GI toxicity (assuming that SS did, in fact, exacerbate the NAP effect in HDF SS/NAP mice). However, a threshold for the putative exacerbation of NAP toxicity by SS has not been established, since both NAP and SS were reduced simultaneously in stepping down from the HD SS/NAP to the MD SS/NAP doses.

- Since no SS-related toxicity was observed at the highest doses tested (320 [M] or 320 lowered to 210 Wk 4 [F] mg/kg/day SS alone or with NAP, the potential for NAP to exacerbate the toxicity of SS remains untested.

The results of this study are somewhat questionable considering that several unusual circumstances occurred:

1. Unexplained occurrence of lower urinary tract obstruction in control and treated male mice at a much higher incidence rate (11/151) and much earlier age of onset than the previously reported spontaneous occurrence in this strain.
2. Gavage errors in 18/280 mice.
3. Replacement animals were added two and a half weeks after the initiation of treatment.
4. The high dose of SS was lowered from 320 to 210 mg/kg/day in F at the beginning of Wk 4, though SS-related toxicity was not observed.
5. The first 20 surviving animals per sex **per group were assigned to the "core"** (main study) group, rather than having random assignments to core, recovery, and TK groups prior to initiation of treatment.
6. The first 10 surviving animals per sex per group were necropsied (in addition to mice found dead or sacrificed moribund).

Also, the low level of toxicity induced by HD NAP alone in this study made it difficult to assess the potential exacerbation of this effect by SS. No treatment-related mortality was reported in this study.

**TABLE IIIA (TERMINAL SACRIFICE)  
SUMMARY OF TREATMENT-RELATED LESIONS**

ORGAN - lesion		Group					
		1	2	3	4	5	6
<b>STOMACH</b>							
- Inflammation, acute, glandular stomach	M	1/10 (0.10)*	1/10 (0.10)	3/10 (0.30)	2/10 (0.30)	2/10 (0.20)	3/10 (0.30)
	F	2/10 (0.20)	3/10 (0.30)	4/10 (0.40)	6/10 (1.10)	2/10 (0.30)	2/10 (0.20)
- Hyperplasia, pylorus	M	0/10	0/10	0/10	0/10	0/10	0/10
	F	0/10	0/10	0/10	5/10 (1.10)	0/10	1/10 (0.20)
- Erosion, glandular stomach	M	0/10	0/10	0/10	0/10	0/10	0/10
	F	0/10	0/10	0/10	2/10 (0.40)	0/10	1/10 (0.10)
- Ulcer, glandular stomach	M	0/10	0/10	0/10	1/10 (0.20)	0/10	0/10
	F	0/10	0/10	0/10	1/10 (0.20)	0/10	0/10

**TABLE IIIB (TERMINAL SACRIFICE)  
SUMMARY OF EQUIVOCAL LESIONS**

ORGAN - lesion		Group					
		1	2	3	4	5	6
<b>SPLEEN</b>							
- Erythroid extramedullary hematopoiesis	M	2/10 (0.20)*	4/10 (0.50)	2/10 (0.20)	5/10 (0.60)	4/10 (0.50)	4/10 (0.40)
	F	1/10 (0.10)	1/10 (0.10)	2/10 (0.20)	5/10 (0.80)	3/10 (0.40)	3/10 (0.40)

**TABLE IIIC (RECOVERY SACRIFICE)  
SUMMARY OF TREATMENT-RELATED LESIONS**

ORGAN - lesion		Group	
		1	4
<b>STOMACH</b>			
- Ulcer, glandular stomach	M	0/10	0/9
	F	0/10	1/10 (0.20)*

\* Incidence (mean group severity score)

Group 1 = 0 mg/kg/day (vehicle control)

Group 2 = 30 mg/kg/day Sumatriptan Succinate + 10 mg/kg/day Naproxen Sodium

Group 3 = 100 mg/kg/day Sumatriptan Succinate + 30 mg/kg/day Naproxen Sodium

Group 4 = 320♂, 320/210♀\*\* mg/kg/day Sumatriptan Succinate + 100♂, 50♀ mg/kg/day Naproxen Sodium

Group 5 = 320♂, 320/210♀\*\* mg/kg/day Sumatriptan Succinate + 0 mg/kg/day Naproxen Sodium

Group 6 = 0 mg/kg/day Sumatriptan Succinate + 100♂, 50♀ mg/kg/day Naproxen Sodium

\*\*dose for females changed from 320 mg/kg/day to 210 mg/kg/day on 11/15/04

(Table IIIA above reproduced from Sponsor's 04-293 Vol. III, Page 23)

**Histopathology inventory**

Study	T19			
Species	mouse			
Adrenals	X*			
Aorta	X			
Bone Marrow smear	X			
Bone (femur)	X			
Brain	X*			
Cecum	X			
Cervix	X			
Colon	X			
Duodenum	X			
Epididymis	X*			
Esophagus	X			
Eye	X			
Fallopian tube	X			
Gall bladder	X			
Gross lesions	X			
Harderian gland	X			
Heart	X*			
Ileum	X			
Injection site				
Jejunum	X			
Kidneys	X*			
Lachrymal gland	X			
Larynx				
Liver	X*			
Lungs	X*			
Lymph nodes, cervical				
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X			
Mammary Gland	X			
Nasal cavity				
Optic nerves	X			
Ovaries	X*			
Pancreas	X			
Parathyroid				
Peripheral nerve				
Pharynx	X			
Pituitary	X*			
Prostate	X*			
Rectum	X			
Salivary gland	X			
Sciatic nerve	X			
Seminal vesicles	X			
Skeletal muscle	X			

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

X, histopathology performed  
\*, organ weight obtained

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#### **2.6.6.4 Genetic toxicology**

The following genetic toxicology studies were submitted and are reviewed in this section:

- A bacterial reverse mutations test
- An in vivo mouse micronucleus test
- A CHO chromosomal aberrations test
- A CHO BWL chromosomal aberrations test

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**Evaluation of a Test Article in the *Salmonella typhimurium*/*Escherichia coli* Plate Incorporation/Preincubation Mutation Assay in the Presence and Absence of Induced Rat Liver S-9****Key findings:**

SS and NAP alone up to 5000 ug/plate, or in combination 1:1 at up to 2500/2500 ug/plate were negative for induction of reverse mutations in all bacterial strains tested.

**Study no.:** POZEN Study MT400-T06, [REDACTED] Study #0735/0736-2140

**Volume #, and page #:** eNDA 21-926, Module 4, Section 4.2, Page 5999

**Conducting laboratory and location:** [REDACTED]

**Date of study initiation:** 04 MAR 2002

**GLP compliance:** Compliance statement signed 26 SEP 2003 by Study Director.

**QA reports:** yes (X) QA statement signed 26 SEP 2003 by QA Unit Manager.

**Drug, lot #, and % purity:** Sumatriptan Succinate (SS) Lot #QT0 1002, Purity 99.4%; Naproxen Sodium (NAP) Lot #NPXNAM-126, Purity 99.5%) (Note: SS concentrations stated refer to the amount of sumatriptan base present).

**Methods**Strains/species/cell line:

*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 were obtained originally from [REDACTED] *Escherichia coli* strain WP2 uvrA was obtained from [REDACTED]

Doses used in definitive study B-1 and Confirmatory Study B-2:

SS/NAP combinations: 25/25, 50/50, 100/100, 250/250, and 500/500 ug/plate

NAP alone: 50, 200, and 1000 ug/plate

SS alone (base): 50, 200, and 1000 ug/plate

Doses used in definitive study B-3 (65.25 hrs) and B-4 (67 hrs):

SS/NAP combinations: 156.5/156.5, 312.5/312.5, 625/625, 1250/1250, and 2500/2500 ug/plate

NAP alone: 313, 1250, and 5000 ug/plate

SS alone (base): 313, 1250, and 5000 ug/plate

Basis of dose selection:

Doses used in the definitive Mutation Assay were selected based on the results of the Range Finding Test, in which strains *S. typhimurium* TA100 and *E. coli* WP2 *uvrA* were tested with and without rat liver S9 activation at concentrations of SS/NAP up to 1000/1000, NAP alone up to 5000, and SS alone up to 5000 ug/plate. Background lawn toxicity was not significant in any of the plates. Relative cloning efficiencies were only reduced significantly in plates with high doses of NAP alone. The number of revertants was slightly reduced in some or all the plates in each test group. The highest combination concentration of 1000/1000 SS/NAP ug/plate was not used for the definitive study B-1 or confirmatory study B-2 because it would have required 200 uL of dosing solution, which was considered too great a dilution. However, lack of toxicity in the highest concentration plates assayed in B-2 necessitated repeating the assay using more concentrated stock solutions of SS and NAP to allow higher concentrations to be tested in Repeat Definitive Mutation Assays B-3 and B-4 (B-3 TA100 plates were lost due to contamination).

Negative controls: Sterile, deionized, distilled water was the solvent control.

Positive controls:

All positive controls listed below were dissolved in DMSO, except for MMS, which was dissolved in sterile, deionized, distilled water.

<u>Strain</u>	<u>S-9</u>	<u>Chemical</u>	<u>Concentration (µg/plate)</u>
TA98	-	2-NF (2-Nitrofluorene)	5.0
TA98	+	2-AA (2-Aminoanthracene)	1.25
TA100	-	NaAz (Sodium Azide)	1.0
TA100	+	2-AA (2-Aminoanthracene)	1.25
TA1535	-	NaAz (Sodium Azide)	1.0
TA1535	+	2-AA (2-Aminoanthracene)	1.25
TA1537	-	9-AA (9-Aminoacridine)	50
TA1537	+	2-AA (2-Aminoanthracene)	1.25
WP2 <i>uvrA</i>	-	MMS (Methyl Methanesulfonate)	4000
WP2 <i>uvrA</i>	+	2-AA (2-Aminoanthracene)	10

(reproduced from eNDA 21-926, Module 4, Section 4.2, Page 6009)

<u>Chemical</u>	<u>Source</u>	<u>CAS No.</u>	<u>Lot No.</u>	<u>Storage Conditions</u>	<u>Expiration Date</u>
2-AA		613-13-8	39H0945	1-5°C	09-16-04
9-AA		52417-22-8	03024JR	1-5°C	09-10-06
2-NF		607-57-8	BY01073EV	1-5°C	04-06-04
NaAz		26628-22-8	110H0269	1-5°C	04-06-04
MMS		66-27-3	15526AO	1-5°C	09-10-06

(reproduced from eNDA 21-926, Module 4, Section 4.2, Page 6010)

Incubation and sampling times:

Plates were incubated at  $37 \pm 1^\circ\text{C}$  for ~66 hrs, then assayed for the presence of precipitate, background lawn toxicity, and colony counts of revertants.

**Results**Study validity

All criteria for a valid study were met. Triplicate plates were counted using an automatic colony counter ( [REDACTED] ). Solvent control culture reversion frequencies fell within the appropriate ranges, and positive control cultures had mean reversion frequencies  $\geq 3$  times the mean reversion frequencies of the corresponding solvent control plates.

Study outcome:

Strains TA98 and TA100 exhibited mean reversion frequencies that were less than twice that of the mean reversion frequencies of the corresponding solvent controls, and strains TA1535, TA1537, and WP2 uvrA exhibited mean reversion frequencies that were less than three times that of the mean reversion frequencies of the corresponding solvent controls.

Under the conditions of this study, SS and NAP alone up to 5000 ug/plate, or in combination 1:1 at up to 2500/2500 ug/plate were negative in the Salmonella typhimurium/Escherichia coli Plate Incorporation/Preincubation Mutation Assay.

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**TABLE 16**  
**SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY**  
**MUTATION ASSAY RESULTS - WITH S-9 ACTIVATION**

SPONSOR: POZEN, Inc.  
 EXPERIMENT NO.: B-3  
 TEST ARTICLE: Sumatriptan Succinate

STUDY NO.: 0735/0736-2140  
 SOLVENT: Water  
 CONC. IN: µg/plate

S. typhimurium		Average No. of Revertants Per Plate				
		Positive Control	Solvent Control	Concentration per plate		
				313	1250	5000
STRAIN: TA98 DATE PLATED: 04/05/02 CELLS SEEDED: 1.040E+08	REVERTANTS	967	27	27	37	29
	STD. DEV.	67	4	1	5	6
	LAWN	NL	NL	NL	NL	NL
STRAIN: TA100 DATE PLATED: 04/05/02 CELLS SEEDED: 1.212E+08	REVERTANTS	NA	NA	NA	NA	NA
	STD. DEV.	NA	NA	NA	NA	NA
	LAWN	NA	NA	NA	NA	NA
STRAIN: TA1535 DATE PLATED: 04/05/02 CELLS SEEDED: 1.128E+08	REVERTANTS	126	9	9	14	13
	STD. DEV.	21	3	3	5	4
	LAWN	NL	NL	NL	NL	NL
STRAIN: TA1537 DATE PLATED: 04/05/02 CELLS SEEDED: 7.420E+07	REVERTANTS	85	8	10	9	9
	STD. DEV.	9	1	3	0	4
	LAWN	NL	NL	NL	NL	NL
PRECIPITATE		NP	NP	NP	NP	NP
E. coli		Positive Control	Solvent Control	Concentration per plate		
				313	1250	5000
STRAIN: WP2uvrA DATE PLATED: 04/05/02 CELLS SEEDED: 1.416E+08	REVERTANTS	122	14	13	14	16
	STD. DEV.	3	2	4	5	5
	LAWN	NL	NL	NL	NL	NL
PRECIPITATE		NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NA = Not applicable. Plates lost due to contamination.

NP = No precipitate.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6049)

**TABLE 17**  
**SALMONELLA TYPHIMURIUM/ESCHERICHIA COLI PLATE INCORPORATION MUTATION ASSAY**  
**MUTATION ASSAY RESULTS - WITHOUT ACTIVATION**

SPONSOR: POZEN, Inc.  
 EXPERIMENT NO.: B-3  
 TEST ARTICLE: Naproxen Sodium/  
 Sumatriptan Succinate(Base)

STUDY NO.: 0735/0736-2140  
 SOLVENT: Water  
 CONC. IN: µg/plate

		Average No. of Revertants Per Plate						
		Positive Control	Solvent Control	Concentration per plate				
				a	b	c	d	e
<b>S. typhimurium</b>								
STRAIN: TA98	REVERTANTS	617	23	19	15	17	19	17
DATE PLATED: 04/05/02	STD. DEV.	46	8	2	3	2	7	3
CELLS SEEDDED: 1.040E+08	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA100	REVERTANTS	NA	NA	NA	NA	NA	NA	NA
DATE PLATED: 04/05/02	STD. DEV.	NA	NA	NA	NA	NA	NA	NA
CELLS SEEDDED: 1.212E+08	LAWN	NA	NA	NA	NA	NA	NA	NA
	PRECIPITATE	NA	NA	NA	NA	NA	NA	NA
STRAIN: TA1535	REVERTANTS	228	9	11	13	8	11	11
DATE PLATED: 04/05/02	STD. DEV.	15	2	4	1	5	3	2
CELLS SEEDDED: 1.128E+08	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
STRAIN: TA1537	REVERTANTS	174	11	6	7	10	9	5
DATE PLATED: 04/05/02	STD. DEV.	30	4	3	2	3	1	1
CELLS SEEDDED: 7.420E+07	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP
<b>E. coli</b>								
STRAIN: WP2LMA	REVERTANTS	363	14	16	15	16	13	13
DATE PLATED: 04/05/02	STD. DEV.	9	4	1	3	3	3	3
CELLS SEEDDED: 1.418E+08	LAWN	NL	NL	NL	NL	NL	NL	NL
	PRECIPITATE	NP	NP	NP	NP	NP	NP	NP

NL = Normal, healthy microcolony lawn.

NA = Not applicable. Plates lost due to contamination.

NP = No precipitate.

- a. 156.5/156.5 µg/plate NAP/SS(base)
- b. 312.5/312.5 µg/plate NAP/SS(base)
- c. 625/625 µg/plate NAP/SS(base)
- d. 1250/1250 µg/plate NAP/SS(base)
- e. 2500/2500 µg/plate NAP/SS(base)

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6050)









**In Vivo Test for Chemical Induction of Micronucleated Polychromatic Erythrocytes in Mouse Bone Marrow Cells**

**Key findings:**

Sumatriptan Succinate and Naproxen Sodium, given via oral gavage alone and in combination, were negative for induction of micronucleated polychromatic erythrocytes in mouse bone marrow cells.

**Study no.:** POZEN Study #MT400-T08, Study #0735/0736-1521

**Volume #, and page #:** eNDA 21-926, Module 4, Section 4.2, Page 6441

**Conducting laboratory and location:**

**Date of study initiation:** 07 MAR 2002

**GLP compliance:** GLP compliance statement signed 26 SEP 2003 by Study Director.

**QA reports:** yes (X) no ( ) QA statement signed 26 SEP 2003 by QA Unit Manager.

**Drug, lot #, and % purity:** Naproxen Sodium (NAP) Lot #NPXNAM-126, Purity 99.5%; Sumatriptan Succinate (SS) Lot #QT0 1002, Purity 99.4%, calculated as the base using a correction factor of 1.4)

**Methods**

Strains/species/cell line:

CD-1 M and F mice were obtained from Harlan Sprague Dawley, Inc., and were ~7 weeks old at the start of treatment.

Doses used in definitive study B1: (5/sex/group)

M: SS/NAP 300/500, 750/500, 1500/500 mg/kg;

NAP 500 mg/kg

SS 1500 mg/kg

F: SS/NAP 425/375, 875/375, 1625/375 mg/kg;

NAP 375 mg/kg

SS 1625 mg/kg

Basis of dose selection:

Range Finding Tests A1, A2, and A3 were conducted in 3/sex/group via oral gavage administration of single doses of NAP alone (A1: 100, 250, 500, 750, and 1000 mg/kg), SS/NAP mixed (A2: 75/500, 300/500, 750/500, and 1500/500 mg/kg), or NAP immediately followed by SS (A3: 75/500, 300/500, 750/500, and 1500/500 mg/kg). Observations continued for 3 days following dosing, and body weight (BW) was checked on Days 1 and 4.

Mortality was observed in 1/3 F at 500 mg/kg NAP, 1/3 M at 750 mg/kg NAP, and 2/3 F at 1000 mg/kg NAP. BW was reduced 12.9% in M at 750 mg/kg and 10.7% in F at 500 mg/kg. Clinical signs included ataxia in the F that died at 1000 mg/kg, and inactivity in all M and F at 750 and 1000 mg/kg. On the basis of these toxicities, high doses of NAP selected for further studies were 500 mg/kg for M and 375 mg/kg for F.

No mortality, clinical signs or reductions in BW of greater than 10% were observed in Tests A2 or A3.

Negative controls:

The vehicle control was sterile, deionized, distilled water.

Positive controls:

The positive control was cyclophosphamide (CP, from ██████████), administered at 80 mg/kg (8.0 mg/mL x 10 mL/kg) by oral gavage.

Incubation and sampling times:

Mice were dosed via oral gavage (10 mL/kg total volume) vehicle, CP, NAP alone, SS alone, or with NAP followed immediately by SS and then sacrificed by CO<sub>2</sub> asphyxiation at ~24, 48, and 72 hrs after dose administration (5/sex/group/harvest time, except CP groups were only harvested at 24 hrs). Satellite groups of 3 M/group were dosed for TK analysis one hour after dosing.

## Results

Study validity

Bone marrow from femurs was isolated, washed and prepared on slides to be scored “blind” for the number of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) among 200 erythrocytes (PCE + NCE) per animal, and the number of micronucleated polychromatic erythrocytes (MPCE) for 2000 PCE per animal. The dose range-finding studies provide evidence that the maximum tolerated dose of NAP was examined, and the high doses of 1500 (M) and 1625 mg/kg (F) were not toxic but were reasonably close to the recommended limit dose of 2000 mg/kg. Analysis of dosing solutions indicated NAP concentrations were within  $\pm 11.1\%$  of targeted nominal concentrations and SS concentrations were within  $\pm 6.9\%$  of targeted nominal concentrations. In TK M, plasma NAP was 60-189 ug/mL, SS was 4.4-16.1 ug/mL.

All criteria for a valid assay were met:

- The average number of MPCE per 2000 did not exceed 10.
- The positive control increased the average number of MPCE per 2000 PCE significantly above the vehicle control average value.
- At least five animals from each sex were alive at the time of sacrifice for each dose level.

Study outcome:

Criteria were met for a negative response: none of the test doses showed a statistically significant increase in the number of MPCE when compared to the vehicle control.

No reduction of more than 20% of vehicle control in the percentage of PCE was observed at any dose level, indicating that the test articles were not cytotoxic in this assay.

There was a slight trend toward increased MPCE in HD SS alone in M and F, but this did not reach statistical significance and was within the range of historical controls, and mean values were lower when the same concentration of SS was tested with HD NAP.

**TABLE 21**  
**SUMMARY OF MICRONUCLEUS ASSAY RESULTS**  
**Mean Percent PCE and Incidence of**  
**MPCs in Bone Marrow of Male Mice**

Study No.: 07350736-1021 Vehicle: Water (10 mL/kg)

Time (hours)	Dose (mg/kg)	Cell Counts		PERCENT PCE	Change in %PCE***	MPCE for 2000 PCE
		PCE	NCE			
24	Vehicle	114	96	58.9	-	0.0
24	500/300 <sup>a</sup>	115	85	57.4	0.9 %	0.4
24	500/750 <sup>b</sup>	122	78	61.2	7.6 %	0.2
24	500/1500 <sup>b</sup>	116	84	57.8	1.6 %	0.2
24	500 <sup>c</sup>	120	90	59.9	5.3 %	0.6
24	1500 <sup>d</sup>	118	81	59.5	-0.7 %	0.6
24	CP	109	91	54.4	-4.4 %	64.6 **
48	Vehicle	130	84	67.8	-	0.4
48	500/300 <sup>a</sup>	114	86	58.9	-19.1 %	0.8
48	500/750 <sup>b</sup>	124	76	62.0	-8.6 %	0.6
48	500/1500 <sup>b</sup>	130	70	65.0	-4.1 %	0.4
48	500 <sup>c</sup>	127	73	63.7	-8.0 %	1.2
48	1500 <sup>d</sup>	116	84	58.0	-8.9 %	1.2
72	Vehicle	132	88	66.2	-	0.6
72	500/300 <sup>a</sup>	115	85	57.7	-12.6 %	0.8
72	500/750 <sup>b</sup>	126	74	63.0	-4.8 %	0.8
72	500/1500 <sup>b</sup>	127	73	63.7	-3.8 %	0.4
72	500 <sup>c</sup>	132	68	66.2	0.0 %	0.8
72	1500 <sup>d</sup>	117	83	58.4	-11.6 %	1.6

NOTE: Five animals were used per group. CP was used as positive control and was dosed at 80 mg/kg.

\*\* Statistically significant response.

\*\*\* Change of Percent PCE in comparison with concurrent vehicle, calculated by the following formula:

$$\frac{\text{Percent PCE for Test Dose} - \text{Percent PCE for vehicle}}{\text{Percent PCE for vehicle}} \times 100$$

<sup>a</sup> Naproxen Sodium was dosed first at 5.0 mL/kg immediately followed by Sumatriptan Succinate at 5.0 mL/kg (total of 10 mL/kg per animal). The Vehicle group was dosed at 10 mL/kg.

<sup>b</sup> The dose level was expressed as mg/kg of Naproxen Sodium / mg/kg of Sumatriptan Succinate.

<sup>c</sup> The dose level was expressed as mg/kg of Naproxen Sodium and the group was dosed at 5.0 mL/kg.

<sup>d</sup> The dose level was expressed as mg/kg of Sumatriptan Succinate and was dosed at 5.0 mL/kg.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6842)

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**TABLE 22**  
**SUMMARY OF MICRONUCLEUS ASSAY RESULTS**  
 Mean Percent PCE and Incidence of  
 NPCEs in Bone Marrow of Female Mice

Study No.: 67269736-1621

Vehicle: Water (10 mL/kg)

Time (hours)	Dose (mg/kg)	Cell Counts		PERCENT PCE	Change in %PCE***	MPCE for 2000 PCE
		PCE	NCE			
24	Vehicle	116	84	57.8	-	0.4
24	375/425 <sup>a</sup>	117	83	58.4	1.0 %	0.0
24	375/675 <sup>b</sup>	127	73	63.6	10.0 %	0.6
24	375/1625 <sup>b</sup>	122	78	61.2	5.9 %	0.4
24	375 <sup>c</sup>	124	78	62.1	7.4 %	0.4
24	1625 <sup>d</sup>	110	80	55.2	-4.5 %	0.2
24	CP	111	89	55.6	-3.8 %	0.2 <sup>**</sup>
48	Vehicle	122	78	60.8	-	1.0
48	375/425 <sup>a</sup>	124	78	62.0	2.0 %	0.6
48	375/675 <sup>b</sup>	134	68	67.1	10.4 %	1.0
48	375/1625 <sup>b</sup>	142	58	71.1	16.9 %	0.8
48	375 <sup>c</sup>	129	71	64.5	6.1 %	0.4
48	1625 <sup>d</sup>	131	69	65.7	8.1 %	1.2
72	Vehicle	122	78	61.1	-	0.8
72	375/425 <sup>a</sup>	124	78	62.0	1.5 %	0.6
72	375/675 <sup>b</sup>	130	70	65.0	6.4 %	0.6
72	375/1625 <sup>b</sup>	147	63	73.3	20.0 %	0.6
72	375 <sup>c</sup>	132	68	65.8	7.7 %	0.4
72	1625 <sup>d</sup>	129	71	64.6	5.7 %	1.2

NOTE: Five animals were used per group. CP was used as positive control and was dosed at 80 mg/kg.

\*\* Statistically significant response.

\*\*\* Change of Percent PCE in comparison with concurrent vehicle, calculated by the following formula:

$$\frac{\text{Percent PCE for Test Dose} - \text{Percent PCE for vehicle}}{\text{Percent PCE for vehicle}} \times 100$$

<sup>a</sup> Naproxen Sodium was dosed first at 5.0 mL/kg immediately followed by Sumatriptan Succinate at 5.0 mL/kg (total of 10 mL/kg per animal). The Vehicle group was dosed at 10 mL/kg.

<sup>b</sup> The dose level was expressed as mg/kg of Naproxen Sodium / mg/kg of Sumatriptan Succinate.

<sup>c</sup> The dose level was expressed as mg/kg of Naproxen Sodium and the group was dosed at 5.0 mL/kg.

<sup>d</sup> The dose level was expressed as mg/kg of Sumatriptan Succinate and was dosed at 5.0 mL/kg.

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**Test for Chemical Induction of Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO) Cells With and Without Metabolic Activation****Key findings:**

- The combination of SS and NAP showed greater induction of chromosomal aberrations (CA) than NAP alone, in the presence or absence of S9 metabolic activation:
  - NAP alone at 5000 ug/mL -S9 (4.5% of cells with CA); RMI = 37%
  - SS/NAP at 2500/2500 ug/mL -S9 (10% of cells with CA); RMI = 29%
  - NAP alone at 2500 ug/mL +S9 (4.0% of cells with CA); RMI = 29%
  - SS/NAP at 2000/2000 ug/mL +S9 (11% of cells with CA); RMI = 20%
- SS alone at up to 5000 ug/mL ± S9 did not show increased CA.

**Study no.:** POZEN Study #MT400-T07, [REDACTED] Study #0735/0736-3110

**Volume #, and page #:** eNDA 21-926, Module 4, Section 4.2, Page 6217

**Conducting laboratory and location:** [REDACTED]

**Date of study initiation:** 25 APR 2002

**GLP compliance:** Yes, statement signed 26 SEP 2003 by the Study Director.

**QA reports:** yes (X) no ( ), statement signed by QA Unit Director 26 SEP 2003

**Drug, lot #, and % purity:** Naproxen Sodium (NAP) Lot # NPXNAM-126, Purity 99.5%; Sumatriptan Succinate (SS) Lot #QT0 1002, Purity 99.4%. SS doses were calculated as the free base, using a conversion factor of 1.4)

**Methods**Strains/species/cell line:

Clone CHO-W-B1 used in this study originated at [REDACTED] and was obtained by [REDACTED]

[REDACTED], in 1988. The doubling time of this cell line is ~12 hrs, and its modal chromosome number is 21.

Doses used in definitive studies (combinations were tested in a separate study):

-S9: SS or NAP alone at 500, 1000, 2500, and 5000 ug/mL

SS/NAP together at 250/250, 500/500, 1250/1250, and 2500/2500 ug/mL

+S9: NAP alone at 5, 50, 500, and 2500 ug/mL

SS alone at 5, 50, 500, and 5000 ug/mL

SS/NAP together at 250/250, 500/500, 1250/1250, and 2000/2000 ug/mL

Basis of dose selection:

In range-finding tests, duplicate cultures seeded 24 hrs prior to treatment were incubated with the test agents (SS or NAP alone at 5, 10, 50, 100, 500, 1000, 2500, and 5000 ug/mL; and SS/NAP in combination at 12.5/12.5, 50/50, 125/125, 250/250, 500/500, 1250/1250, and 2500/2500 ug/mL) for 3 hrs, rinsed and incubated for 15 more hrs in complete medium, with 0.1 ug/mL Colcemid<sup>®</sup> present during the final 2 hrs before harvesting.

NAP showed toxicity at 2500 ug/mL (RMI=38%) and 5000 ug/mL (RMI=0%) in the presence of S9, so 2500 ug/mL was chosen as the top dose of NAP in the definitive assay with S9 and 5000 ug/mL without S9.

SS did not show toxicity (RCG or RMI <50%), so the top doses used in the definitive assay were 5000 ug/mL SS with or without S9.

SS/NAP showed toxicity at 2500/2500 ug/mL (RMI=38% -S9 and 1% +S9) only, so top doses selected for the definitive assay were 2500/2500 -S9 and 2000/2000 +S9.

Negative controls:

Water was used as the solvent control.

Positive controls:

Mitomycin C (MMC, ██████████) was used as the positive control in the absence of metabolic activation at 1.25 or 1.5 mg/mL stock solution in sterile, deionized, distilled water, diluted to final concentrations of 0.4 and 0.8 ug/mL in the definitive assay and 0.2 and 0.4 ug/mL for the confirmatory assay.

Cyclophosphamide (CP, ██████████) was used as the positive control in the presence of metabolic activation at 80 ug/mL stock solution in sterile, deionized, distilled water, diluted to final concentrations of 7.5 and 12.5 ug/mL.

Incubation and sampling times:

Duplicate cultures were prepared and treated as described above, with harvesting at 18 hrs after the beginning of treatment, processing to determine the Relative Cell Growth (RCG) and Relative Mitotic Index (RMI), and the top three concentrations without excessive toxicity were scored for chromosome aberrations (100 metaphases from each of the duplicate flasks, providing 200 per concentration level). Numbers of polyploidy and endoreduplicated cells per total of 100 dividing cells was also scored for each culture.

In a confirmatory assay, performed only for the SS alone, since cultures with NAP had shown a positive response in the initial definitive assay, the treatment period was extended from 3 hrs to 18 hrs (without S9 activation) at 125, 250, 500, 1000, 2500, and 5000 ug/mL.

## **Results**

Study validity

Analysis of dosing solutions indicated that most concentrations were within  $\pm 10\%$  of the targeted concentration; however, some ranged from -1.8 to +20% of target for SS, and from +4.9 to +38% for NAP. Since the errors resulted mostly in greater test article concentrations than targeted, the results and conclusions of the study are not much affected.

Duplicate cultures showed consistent positive effects in cultures with the highest concentrations of NAP.

Criteria for a valid assay were met:

- The percentage of cells with aberrations in the solvent controls did not exceed 4%.
- At least 25% of cells scored in the positive controls showed one or more chromosome aberrations.
- At least one of the test concentrations scored showed ~50% reduction in RCG and/or RMI, or no toxicity was observed at the highest concentration allowable.

Study outcome:

Criteria for a positive response (dose-response trend and a statistically significant increase in chromosomal aberration (CA) frequency over that of the solvent controls) were met under the following conditions:

- NAP alone at 5000 ug/mL -S9 (4.5% of cells with CA); RMI = 37%; RCG = 49%
- SS/NAP at 2500/2500 ug/mL -S9 (10% of cells with CA); RMI=29%; RCG=41%
- SS/NAP at 2000/2000 ug/mL +S9 (11% of cells with CA); RMI=20%;RCG=54%

NAP alone at 2500 ug/mL +S9 showed increased CA (4.0%) [RMI = 29%; RCG = 76%]; the sponsor considered this to be positive, even though statistical significance was not reached compared with the solvent control (1.5% CA), because the solvent control value was higher than normally seen.

The combination of SS and NAP showed greater induction of chromosomal aberrations than NAP alone, in the presence or absence of S9 metabolic activation, despite 50% lower NAP concentrations. These data suggest that HD SS may exacerbate the genotoxicity of NAP. There is some possibility that the differences could be related to the higher cytotoxicity in the SS/NAP cultures compared to the NAP alone cultures, but the cytotoxicity differences are relatively small compared to the >2-fold increase in CA frequency.

SS alone did not show increased CA in the definitive (3 hr treatment ±S9) or in the confirmatory assay (18 hr treatment -S9).

Sponsor's Conclusions:

The sponsor concludes that NAP was clastogenic with and without SS and in the presence and absence of metabolic activation, but makes the following three points regarding the demonstrated clastogenic effects:

1. they occurred only at the highest concentration tested for NAP and for SS/NAP
2. they were accompanied by marked cytotoxicity (>50% RMI)
3. they appeared to be threshold effects, since no clastogenicity was observed at any concentration where the RMI was <50%.

The sponsor speculates that the increases in chromosome aberrations observed in this study are likely to be associated with alterations in cell division and cytotoxicity as has been reported for other agents that are nonmutagenic and noncarcinogenic but have been shown to produce double strand breaks to DNA at cytotoxic concentrations.

This reviewer does not consider the Sponsor's **argument to be compelling, since the** toxicity observed in cultures considered positive for clastogenicity was not excessive: while mean RMI ranged from 37 to 20%, mean relative cell growth (RCG) was only decreased to 76-41% of solvent control values (see tables on next page). The agency **acknowledges that "at very low survival levels in mammalian cells, mechanisms other than direct genotoxicity per se can lead to "positive" results that are related to cytotoxicity and not genotoxicity (e.g., events associated with apoptosis, endonuclease release from lysosomes, etc.). Such events are likely to occur once a certain concentration threshold is reached for a toxic compound,"** (see ICH S2A [1996] "Guideline for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals"). However, it is also true that "Some genotoxic carcinogens are not detectable in in vitro genotoxicity assays unless the concentrations tested induce some degree of cytotoxicity," and, "The desired level of toxicity for in vitro cytogenetic tests using cell lines should be greater than 50 percent reduction in **cell number or culture confluency,**" (*ibid*). Since the RCG values associated with positive clastogenicity findings were 76-41%, approximately equal to the desired level of toxicity of 50% for this assay, these data suggest that naproxen may have a direct genotoxic effect on mammalian cells at high concentrations.

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**TABLE 3-c  
CHROMOSOME ABERRATION ASSAY IN CHO CELLS  
RCG - DEFINITIVE ASSAY**

TEST ARTICLE: Combination of Naproxen Sodium, USP and Sumatriptan Succinate  
SPONSOR: POZEN, INC. SOLVENT: Water STUDY NO.: 0735/0736-3110 TRIAL NO.: B1

WITHOUT ACTIVATION				WITH ACTIVATION			
Test Article Conc. (µg/mL)**	No. of Cells per Flask	Mean No. of Cells X 10 <sup>6</sup>	RCG*	Test Article Conc. (µg/mL)**	No. of Cells per Flask	Mean No. of Cells X 10 <sup>6</sup>	RCG*
Solvent A	1.47			Solvent A	1.27		
Solvent B	1.52	1.50	100%	Solvent B	1.14	1.21	100%
500 A	1.35			500 A	1.30		
500 B	1.40	1.48	99%	500 B	1.16	1.28	102%
1000 A	1.21			1000 A	1.06		
1000 B	0.96	1.10	73%	1000 B	1.22	1.14	84%
2500 A	1.15			2500 A	1.03		
2500 B	1.08	1.12	76%	2500 B	0.78	0.81	79%
5000 A	0.80			4000 A	0.89		
5000 B	0.81	0.81	41%	4000 B	0.80	0.86	84%

\*RCG = Relative Cell Growth =  $\frac{\text{No. of Cells in the Test Flask}}{\text{No. of Cells in the Solvent Flask}} \times 100$

\*\*These concentrations contained 50% of Naproxen Sodium, USP and 50% of Sumatriptan Succinate.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6262)

**TABLE 3-c  
CHROMOSOME ABERRATION ASSAY IN CHO CELLS  
RCG - DEFINITIVE ASSAY**

*Best Possible Copy*

TEST ARTICLE: Naproxen Sodium, USP  
SPONSOR: POZEN, INC. SOLVENT: Water STUDY NO.: 0735-3110 TRIAL NO.: B1 & B2

WITHOUT ACTIVATION				WITH ACTIVATION			
Test Article Conc. (µg/mL)	No. of Cells per Flask	Mean No. of Cells X 10 <sup>6</sup>	RCG*	Test Article Conc. (µg/mL)	No. of Cells per Flask	Mean No. of Cells X 10 <sup>6</sup>	RCG*
Solvent A	1.62			Solvent A	1.13		
Solvent B	1.62	1.62	100%	Solvent B	1.05	1.08	100%
500 A	1.73			5.0 A	1.20		
500 B	1.51	1.62	100%	5.0 B	1.05	1.13	104%
1000 A	1.48			50 A	1.16		
1000 B	1.43	1.48	80%	50 B	1.06	1.12	103%
2500 A	1.17			500 A	1.13		
2500 B	1.23	1.20	74%	500 B	1.02	1.08	98%
5000 A	0.81			2500 A	0.74		
5000 B	0.79	0.80	48%	2500 B	0.62	0.63	79%

\*RCG = Relative Cell Growth =  $\frac{\text{No. of Cells in the Test Flask}}{\text{No. of Cells in the Solvent Flask}} \times 100$

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6258)



**TABLE 3-b**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**CHROMOSOME ABERRATIONS - CONFIRMATORY ASSAY**

TEST ARTICLE: Sumatriptan Succinate  
SPONSOR: FOZIN, INC.  
SOLVENT: Water

TREATMENT TIME: 3 Hours  
HARVEST TIME: 18 Hours

STUDY NO: 0736-3110  
TRIAL NO: B3  
METABOLIC ACTIVATION: Yes ( ) No (X)

TREATMENT AND CONC. (µg/mL)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS															NO. OF ABS. PER CELL	% CELLS WITH ABS.	P-VALUE IN CHI-SQUARE**	
		NOT COMPUTED				Chromatid Type					Chromosome Type									
		W	SB	% S	% SB	Sh	Id	Tr	Gr	Or	Id	Cl	Sh	D	R	DM				PS
Solvent A	100			0	0													0.00	0.0	
Solvent B	100			0	0													0.00	0.0	
Solvent A+B	200			0.0	0.0													0.000	0.0	
125 A	100	1		0	0													0.01	1.0	
125 B	100			0	0								1					0.01	1.0	
125 A+B	200	1		0.0	0.0								1		1			0.010	1.0	=0.9085
500 A	100			0	0													0.00	0.0	
500 B	100			0	0													0.00	0.0	
500 A+B	200			0.0	0.0													0.000	0.0	=Solvent
5000 A	100			0	0													0.00	0.0	
5000 B	100			0	0								1					0.01	1.0	
5000 A+B	200			0.0	0.0								1					0.005	0.5	=0.5368
MMC 0.2 A	100	1		0	0	8	6	6	4	11		2	8			1	2	0.66	31.0	
MMC 0.2 B	100			0	0	9	4	4	8	2	4	5	8					0.42	29.0	
MMC 0.2 A+B	200	1		0.0	0.0	17	10	10	10	13	4	7	16			1	2	0.540	30.0	<0.0001

MMC = Mitomycin-C

\* sd = 10 aberrations in calculations.

\*\*Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In the Chi-square test, MMC was compared to historical data for negative (untreated) control (0.57%) since the solvent for MMC was water and concurrent value was 0%.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6270; note that the Treatment Time of 3 hrs at the top of this table is wrong—the treatment time for the Confirmatory Assay was 18 hrs.)

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**TABLE 3-a**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY**

TEST ARTICLE: Naproxen Sodium, USP  
SPONSOR: FOZIN, INC.  
SOLVENT: Water

TREATMENT TIME: 3 Hours  
HARVEST TIME: 18 Hours

STUDY NO: 0736-0110  
TRIAL NO: B1  
METABOLIC ACTIVATION: Yes ( ) No (X)

TREATMENT AND CONC. (µg/mL)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS															NO. OF ABS. PER CELL	% CELLS WITH ABS.	P-VALUE IN CHI-SQUARE**	
		NOT COMPUTED				Chromatid Type					Chromosome Type									
		W	SB	% S	% SB	Sh	Id	Tr	Gr	Or	Id	Cl	Sh	D	R	DM				PS
Solvent A	100			1	0													0.00	0.0	
Solvent B	100			1	0													0.00	0.0	
Solvent A+B	200			1.0	0.0													0.000	0.0	
1000 A	100			0	0													0.00	0.0	
1000 B	100			1	0													0.00	0.0	
1000 A+B	200			0.5	0.0													0.000	0.0	=Solvent
2500 A	100			0	0													0.00	0.0	
2500 B	100			0	0	1												0.01	1.0	
2500 A+B	200			0.0	0.0	1												0.005	0.5	=0.5368
5000 A	100	2		1	0			2	2				5					0.09	6.0	
5000 B	100	1		1	0				1		1		1					0.03	3.0	
5000 A+B	200	3		1.0	0.0			2	3		1		6					0.060	4.5	=0.0280
MMC 0.8 A	100	4		0	0	8	5	10	5	10	1	2	5	1	1		1	0.58	30.0	
MMC 0.8 B	100			0	0	6	4	2	5	9	1	2	5	1			1	0.45	28.0	
MMC 0.8 A+B	200	4		0.0	0.0	14	9	12	10	19	2	4	10	2	1		2	0.515	29.0	<0.0001

MMC = Mitomycin-C

Trend test: P<0.001

\* sd = 10 aberrations in calculations.

\*\*Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In the Chi-square test, MMC, 2500 and 5000 µg/mL was compared to historical data for negative (untreated) control (0.57%) since the solvent for MMC, 2500 µg/mL was water and concurrent value was 0%.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6260)

**TABLE 4-a**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY**

TEST ARTICLE: Naproxen Sodium, USP  
SPONSOR: POZEN, INC.  
SOLVENT: Water

TREATMENT TIME: 3 Hours  
HARVEST TIME: 18 Hours

STUDY NO: 0733-3110  
TRIAL NO: B2  
METABOLIC ACTIVATION: Yes (X) No ( )

TREATMENT AND CONC. (µg/mL)	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS																	NO. OF ABE. PER CELL	% CELLS WITH ABE.	P-VALUE IN CHI-SQUARE**				
		NOT COMPUTED				Chromosomal Type							Chromosome Type			Other									
		W	SE	% a	% b	St	hb	tr	qr	id	cl	sb	d	r	dm	ps	af								
Solvent A	100	1		1	0	1			1												0.02	2.0			
Solvent B	100	2		2	0									1								0.01	1.0		
Solvent A+B	200	3		1.5	0.0	1			1					1								0.015	1.5		
50 A	100			0	0																	0.00	0.0		
50 B	100			1	0																	0.00	0.0		
50 A+B	200			0.5	0.0																	0.000	0.0	<Solvent	
500 A	100			1	0					1												0.01	1.0		
500 B	100			2	0																	0.00	0.0		
500 A+B	200			1.5	0.0					1												0.005	0.5	<Solvent	
2500 A	100			1	0	1	1	2						3								0.07	5.0		
2500 B	100			1	0			2			1		1									0.04	3.0		
2500 A+B	200			1.0	0.0	1	1	3	2		1		4									0.055	4.0	=0.2213	
CP7.5 A	100	1		0	0	7	11	9	5	7	9	6	9				1	1	2			0.85	32.0		
CP7.5 B	100			0	0	3	7	6	10	5	7	2	8						2	3			0.80	30.0	
CP7.5 A+B	200	1		0.0	0.0	10	18	15	15	12	16	8	17				1	3	5			0.825	31.0	<0.0001	

CP=Cyclophosphamide

\* n = 10 aberrations in calculations.

\*\*Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6261)

**TABLE 5-c**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY**

TEST ARTICLE: Combination of Naproxen Sodium, USP & Sumatriptan Succinate  
SPONSOR: POZEN, INC.  
SOLVENT: Water

TREATMENT TIME: 3 Hours  
HARVEST TIME: 18 Hours

STUDY NO: 0736/0736-0119  
TRIAL NO: B1  
METABOLIC ACTIVATION: Yes ( ) No (X)

TREATMENT AND CONC. (µg/mL)***	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS																	NO. OF ABE. PER CELL	% CELLS WITH ABE.	P-VALUE IN CHI-SQUARE**				
		NOT COMPUTED				Chromosomal Type							Chromosome Type			Other									
		W	SE	% a	% b	St	hb	tr	qr	id	cl	sb	d	r	dm	ps	af								
Solvent A	100			0	0																		0.00	0.0	
Solvent B	100			0	0	1																	0.01	1.0	
Solvent A+B	200			0.0	0.0	1																	0.005	0.5	
1000 A	100			0	0																		0.00	0.0	
1000 B	100			0	1																		0.00	0.0	
1000 A+B	200			0.0	0.5																		0.000	0.0	<Solvent
2500 A	100			0	0																		0.00	0.0	
2500 B	100			0	0																		0.00	0.0	
2500 A+B	200			0.0	0.0																		0.000	0.0	<Solvent
5000 A	100			2	0	0	2		2	1		1	1	2									0.09	6.0	
5000 B	100			2	0	0	2	1	4	7	3		1	6		1							0.25	14.0	
5000 A+B	200			4	0.0	0.0	4	1	6	8	3	1	2	8		1							0.170	10.0	<0.0001
MMC 0.4 A	100			0	0	8	3	3	8	4	4	4	8										0.42	27.0	
MMC 0.4 B	100	1		0	0	5	2	6	8	2	4	3	5										0.35	27.0	
MMC 0.4 A+B	200	1		0.0	0.0	13	5	9	16	6	8	7	13										0.385	27.0	<0.0001

MMC = Mitomycin-C

\* n = 10 aberrations in calculations.

\*\*Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

\*\*\*These concentrations contained 50% of Naproxen Sodium, USP and 50% of Sumatriptan Succinate.

Trend test: P<0.001

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6275)

**TABLE 6-c**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY**

TEST ARTICLE: Combination of Naproxen Sodium, USP & Salsalicylic Succinate  
 TREATMENT TIME: 3 Hours  
 SPONSOR: FOZEN, INC.  
 HARVEST TIME: 18 Hours  
 SOLVENT: Water

STUDY NO.: 0728/0726-0110  
 TRIAL NO.: B1  
 METABOLIC ACTIVATION: Yes (Q) No ( )

TREATMENT AND CONC. (µg/mL) <sup>***</sup>	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS																		NO. OF ABN. PER CELL	% CELLS WITH ABN.	P-VALUE IN CHI-SQUARE <sup>***</sup>		
		NOT COMPUTED						Chromatid Type						Chromosome Type										
		%		%		%		Simple			Complex			Simple			Complex						Other	
		ab	af	bc	bd	ce	cd	ab	bc	d	e	f	g	h	i	j	k	l	m				n	
Solvent A	100			0	0																	0.00	0.0	
Solvent B	100			3	0																	0.00	0.0	
Solvent A+B	200			1.5	0.0																	0.000	0.0	
1000 A	100			2	0																	0.00	0.0	
1000 B	100			1	0																	0.00	0.0	
1000 A+B	200			1.5	0.0																	0.000	0.0	=Solvent
2500 A	100			1	0																	0.00	0.0	
2500 B	100			1	0																	0.00	0.0	
2500 A+B	200			1.0	0.0																	0.000	0.0	=Solvent
4000 A	100		2	0	0	2		1	4		4	3	3									0.17	12.0	
4000 B	100		2	0	0	1			3	2	2	3	3									0.14	10.0	
4000 A+B	200		4	0.0	0.0	3		1	7	2	6	6	6									0.155	11.0	<0.0001
CP 7.5 A	100	3		0	0	6	5	13	9	5	13	3	2	1						1		0.67	30.0	
CP 7.5 B	100			0	0	7	3	4	8	7	7	4	11						1		3	0.82	30.0	
CP 7.5 A+B	200	3		0.0	0.0	13	8	17	17	12	20	7	13	1					1		4	0.745	30.0	<0.0001

CP = Cyclophosphamide

Trend test: P<0.001

\* ad = 10 aberrations in calculations.

\*\*Statistical analysis was performed on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In the Chi-square test, CP & 4000µg/mL was compared to historical data for negative (untreated) control (0.07%) since the solvent for CP & 4000µg/mL was water and concurrent value was 0%.

\*\*\*These concentrations contained 50% of Naproxen Sodium, USP and 50% of Salsalicylic Succinate.

(reproduced directly from eNDA 21-926, Module 4, Section 4.2, Page 6276)

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**Non-GLP Test for Chemical Induction of Chromosome Aberrations in Cultured Chinese Hamster Ovary (CHO BWL) Cells With and Without Metabolic Activation**

(POZEN Study #MT400-T16, Study #1501-3110, Summary report 29 SEP 2003; eNDA 21-926, Module 4, Section 4.2, Page 6420; Naproxen Sodium (NAP) Lot # NPXNAM-126, Purity 99.5%; Sumatriptan Succinate (SS) Lot #QT0 1002, Purity 99.4%.)

The purpose of this investigation was to test the hypothesis that the positive clastogenic results obtained in POZEN Study MT400-T07 with high concentrations of NAP and SS/NAP might have occurred because direct addition of the test articles to the culture media covering the cells produced transient locally high concentrations of test article. In this new assay, flasks of CHO cells were treated with SS/NAP 2500/2500 ug/mL  $\pm$ S9 by direct addition of the drugs (as in MT400-T07) or by mixing the drugs thoroughly with fresh medium before replacing to old culture medium to start the treatment (indirect addition). Other procedures and positive and negative controls were similar to those used in MT400-T07. Cultures treated with SS/NAP at 2500/2500 ug/mL +S9 had excessive toxicity, and could not be scored (RMI = 0-2%).

**Results:**

Treatment A: Direct addition of SS/NAP at 2500/2500 ug/mL -S9: 25% of cells had Chromosomal Aberrations; RMI = 24%; RCG = 44%.

Treatment B: Indirect addition of SS/NAP at 2500/2500 ug/mL -S9: 28% of cells had Chromosomal Aberrations; RMI = 33%; RCG = 47%.

**Conclusions:**

The method of drug delivery to the flasks made no difference, as clastogenicity was comparable to the positive control in both cultures treated with SS/NAP at 2500/2500 ug/mL. Once again, the clastogenic effect was observed only in cultures that had significant, but not excessive, toxicity.

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**TABLE 6**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**RCG - DEFINITIVE ASSAY**

TEST ARTICLE: Combination of Naproxen Sodium, USP and Sumatriptan Succinate  
SPONSOR: Pozen, INC. SOLVENT: Water

STUDY NO.: 1501-5110  
TRIAL NO.: B3

WITHOUT ACTIVATION				WITH ACTIVATION			
Test Article Conc. (µg/mL) <sup>**</sup>	No. of Cells per Flask	Mean No. of Cells X 10 <sup>6</sup>	RCG <sup>*</sup>	Test Article Conc. (µg/mL)	No. of Cells per Flask	Mean No. of Cells X 10 <sup>6</sup>	RCG <sup>*</sup>
Untreated	1.75	1.75	100%	Untreated	1.36	1.36	100%
5000A	0.77	0.77	44%	5000A	0.65	0.65	48%
5000B	0.83	0.83	47%	5000B	0.60	0.60	44%

\*RCG = Relative Cell Growth =  $\frac{\text{No. of Cells in the Test Flask}}{\text{No. of Cells in the Solvent Flask}} \times 100$

\*\*These concentrations contained 2500µg/mL of Naproxen Sodium, USP and 2500µg/mL of Sumatriptan Succinate.

A: The test article was added directly in the flask with cells.

B: The test article was added in the flask with medium and then the medium with test article was transferred to the flask with cells.

(eNDA 21-926, Module 4, Section 4.2, Page 6430)

**TABLE 7**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**MITOTIC INDEX - DEFINITIVE ASSAY**

SPONSOR: POZEN, INC.

SOLVENT: WATER

STUDY NO.: 1501-3110

TRIAL NO.: B3

TEST ARTICLE: Combination of Naproxen Sodium and Sumatriptan Succinate

Without Activation - Treatment: 3 Hours Harvest: 18 Hours					With Activation - Treatment: 3 Hours Harvest: 18 Hours				
Test Article Concentration (µg/mL) <sup>*</sup>	Tube No.	No. of Dividing Cells/500	Mean Mitotic Index (MI)	Relative Mitotic Index (RMI)	Test Article Concentration (µg/mL)	Tube No.	No. of Dividing Cells/500	Mean Mitotic Index (MI)	Relative Mitotic Index (RMI)
Untreated	90	54	10.8	100%	Untreated	9	64	12.8	100%
5000 A	13	13	2.6	24%	5000 A	66	1	0.2	2%
5000 B	15	18	3.6	33%	5000 B	48	0	0	0
MMC 0.4	36	45	9.0	83%	CP 7.5	2	14	2.8	22%

MMC=Mitomycin-C

CP=Cyclophosphamide

The positive controls were compared to Untreated since the solvent for MMC and CP was water.

MI =  $\frac{\text{No. of dividing cells scored from 500 cells}}{5}$

RMI =  $\frac{\text{Test Dose MI}}{\text{Solvent Control MI}} \times 100$

\*\*These concentrations contained 2500µg/mL of Naproxen Sodium, USP and 2500µg/mL of Sumatriptan Succinate.

A: The test article was added directly in the flask with cells.

B: The test article was added in the flask with medium and then the medium with test article was transferred to the flask with cells.

(eNDA 21-926, Module 4, Section 4.2, Page 6431)

**TABLE 8**  
**CHROMOSOME ABERRATION ASSAY IN CHO CELLS**  
**CHROMOSOME ABERRATIONS - DEFINITIVE ASSAY**

TEST ARTICLE: Combination of Naproxen Sodium, USP and Sumatriptan Succinate

STUDY NO.: 1501-3110

SPONSOR: POZEN, INC.

TREATMENT TIME: 3 Hours

TRIAL NO.: B3

SOLVENT: water

HARVEST TIME: 18 Hours

METABOLIC ACTIVATION: Yes ( ) No (X)

TREATMENT AND CONC. (µg/mL) <sup>***</sup>	CELLS Scored	NUMBER AND TYPE OF ABERRATIONS															NO. OF ABS. PER CELL	% CELLS WITH ABS.	P-VALUE IN CHI-SQUARE**	
		NOT COMPUTED				Chromatid Type						Chromosome Type			Others					
		tg	sg	%*	%pp	Simple		Complex				Simple		Complex						
						sb	isb	tr	qr	cr	id	ci	sb			d				r
Solvent	100	1	0	0														0.00	0.0	
5000 A	100		0	0	3	1	11	8	2	4	1	3						0.33	25.0	<0.0001
5000 B	100	2	2	0	1	7	2	11	8	2	3	4	4				1	0.42	28.0	<0.0001
MMC 0.4	100		0	0	5	1	11	7	4		1	1						0.30	26.0	<0.0001

\* sd = 10 aberrations in calculations.

\*\*Statistical analyses done on the % cells with aberrations. The results are considered significant if p-value is ≤ 0.05.

In Chi-square test, MMC, 5000µg/mL(A & B) were compared to historical Untreated control data (0.57%) since the solvent for MMC, 5000µg/mL(A & B) was water and concurrent value is 0%.

\*\*\*These concentrations contained 2500µg/mL of Naproxen Sodium, USP and 2500µg/mL of Sumatriptan Succinate.

A: The test article was added directly in the flask with cells.

B: The test article was added in the flask with medium and then the medium with test article was transferred to the flask with cells.

(eNDA 21-926, Module 4, Section 4.2, Page 6432)

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### 2.6.6.5 Carcinogenicity

No carcinogenicity studies were submitted. According to the minutes of the Pre-IND Meeting of 28 FEB 2002 for IND 68,436, the Division agreed that no carcinogenicity studies would be required to support an NDA for this combination product, provided that the new non-clinical studies planned for the combination did not yield unexpected findings bearing on the carcinogenic potential for MT400.

The new non-clinical studies conducted with SS/NAP did not yield any findings suggesting increased carcinogenic potential for MT400 compared to SS or NAP alone.

The labeling for Imitrex describes a 104-week rat carcinogenicity study at up to 160 mg/kg/day SS (~15 times the maximum recommended single human oral dose [MRHD] of 100 mg on a mg/m<sup>2</sup> basis dose); and a 78-week mouse carcinogenicity study at up to 160 mg/kg/day SS via drinking water (average exposures were ~40X those at the MRHD). Neither study showed a treatment-related increase in tumors.

The labeling for Anaprox describes a 2-year rat carcinogenicity study at up to 24 mg/kg/day NAP in which no evidence of tumorigenicity was found. Maximum exposures in rat plasma were 0.28 times the systemic exposure at the MRHD.

In addition, the sponsor of the current NDA conducted a 2-year rat carcinogenicity study in which a comparator group was treated with a maximum tolerated oral dose of NAP. Plasma exposures in males and females averaged 0.1-0.3 times the systemic exposure to NAP in humans given one Trexima tablet, [REDACTED]

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