CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-926

PHARMACOLOGY REVIEW(S)

MEMORANDUM

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

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

Date: April 15, 2008

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

Subject: NDA 21-926 (Treximet; sumatriptan/naproxen, Submissions 11 OCT 2007 (Amendment 025) and 11 JAN 2008 (Amendment 026)

The submission dated October 11, 2007 represents the sponsor's Complete Response to the Agency's Approvable letter (August 1, 2007). Briefly, the nonclinical issues were as follows:

- Inconsistency between the negative results for naproxen in an in vitro mouse lymphoma tk assay submitted for Treximet and the positive results for naproxen in in vitro mouse lymphoma tk assays submitted for [redacted]
- The apparent synergistic genotoxicity finding in the in vitro chromosomal aberration assay in CHO cells when naproxen and sumatriptan were tested in combination; neither was positive when tested alone.

Regarding the latter issue, the sponsor was asked to either demonstrate that the in vitro findings were not relevant to the in vivo situation or to assess the genotoxic potential of the combination (and naproxen alone) in vivo in humans.

Four new study reports are included in this submission. These consist of (1) in vitro cell cycle analysis in CHO cells treated with various NSAIDs and Indoles (not including either naproxen or sumatriptan), (2) in vitro cell cycle analyses in CHO cells treated with naproxen sodium and sumatriptan succinate (2 studies) and (3) an open-label, placebo-controlled, parallel group study in healthy volunteers to assess the effects of MT 400 tablets or naproxen sodium on the frequency of chromosomal aberrations in peripheral lymphocytes. These data have been reviewed by David Hawver, Ph.D. (Pharmacology/Toxicology Review, 4/15/08) and the in vivo study in humans has also been reviewed by David Jacobson-Kram, Ph.D. (Associate Director of Pharmacology/Toxicology, IO).

Based on his review, Dr. Hawver has concluded that the sponsor has addressed the nonclinical issues stated in the Agency's AE letter and that the nonclinical package for Treximet is sufficient to support approval.

Nonclinical AE issues

1. Regarding the apparently discrepant findings for naproxen, i.e., the negative results obtained in the in vitro mouse lymphoma tk assay conducted (by GSK) for Treximet and the positive responses in the same assay [redacted] the sponsor provided the following explanations:

(a) Different concentrations of naproxen were used in the studies. "Thus, in the NAP arm of the repeated study (with S9 activation) the highest concentration tested only reduced RTG to 59% and was not excessively cytotoxic. In contrast, the positive findings with NAP in the earlier study (at concentrations of 150 & 300 µg/mL) reduced total growth to 32% and 12%, respectively, indicating significant toxicity, albeit at concentrations which were much lower than those used in the GSK study (1700 µg/mL)".

(b) "At GSK, a 3 hr treatment arm (with and without S9 activation) is the standard study design, whereas [redacted] used a 4 hr treatment arm (with S9 activation) in the earlier study."

(c) "As L5178Y mouse lymphoma cells have relatively short cycling times (~8-10 hrs), the 25% increase in treatment duration and increased NAP exposure likely contributes to the increased cytotoxicity and associated positive findings in the earlier [redacted] study. Moreover, since the repeated GSK mouse lymphoma TK+/- assay...was designed to investigate the genotoxic potentiation of the NAP/SS combination, the assessment of NAP alone was not a primary objective. The repeated GSK mouse lymphoma TK +/- assay was GLP/OECD compliant for the combination (i.e., the primary objective) and the positive controls induced the expected increase in mutant colonies, confirming the validity of this assay. The contribution of inter-laboratory variation, based on cell phenotype, passage number, compound batches, etc., on the contradictory outcome of the two studies also cannot be excluded."

(d) "Finally, false positive *in vitro* findings in general are not uncommon in standard *in vitro* mammalian genotoxicity assays, especially at highly toxic exposure levels, and are considered by many to be unacceptably high..."

• Dr. Hawver concluded that "The sponsor's points are valid, and adequately address this issue". The sponsor has addressed this issue by suggesting potential differences in study conduct that might be responsible for the discrepant results. However, the only specific suggestion provided is that the 25% increase in duration of the short treatment arm and, therefore, exposure to NAP, "likely" contributed to the increased cytotoxicity and positive finding in the [redacted] study. It is not intuitive that simply a 25% longer duration of exposure at <0.1 times the concentration (i.e., 150 vs. 1700 µg/mL) would have resulted in greater cytotoxicity. (Although a 3-hr treatment duration may be standard for GSK, the 4-hr duration used by [redacted] is acceptable. The OECD

guidelines state that the duration of exposure "should be for a suitable period of time (usually three to six hours is effective).") And, NAP was positive at a concentration associated with a RTG of 32%, which is not excessively cytotoxic in this assay. The sponsor provided no data to support this possibility, nor any of the other potential reasons suggested as explanations for the discrepant findings. Therefore, in my opinion the sponsor did not adequately address this issue.

2. Regarding the apparently synergistic genotoxic effect when naproxen and sumatriptan were tested in combination in the in vitro chromosomal aberration assay in CHO cells, the sponsor conducted studies to address both of the recommendations of the Agency: (a) demonstrate that the in vitro result were not clinically relevant or (b) conduct a clinical trial to assess effects of naproxen alone and in combination with sumatriptan on peripheral lymphocytes.

(a) It is the sponsor's position that the reproducible synergistic effect observed in the in vitro chromosomal aberration assay in CHO cells with the combination of naproxen and sumatriptan was due to a synergistic inhibition of DNA synthesis, resulting in induction of chromosomal aberrations at high (i.e., not clinically relevant), excessively cytotoxic concentrations of the combination. To address the relevance of the in vitro data, the sponsor conducted 3 in vitro studies (all non-GLP), reviewed in detail by Dr. Hawver.

In **Study No. V27824**, selected NSAIDs (e.g., diclofenac, ibuprofen) and indoles (tryptamine, serotonin) were tested in CHO cells for their effects on the cell cycle. All NSAIDs tested decreased the % of cells in S phase, while increasing the % of cells in G_1 and G_o phases. Serotonin and tryptamine tended to have the opposite effect. The sponsor stated that the combination of diclofenac and tryptamine resulted in potentiation of DNA synthesis arrest (i.e., increase in % of cells in S phase) and an associated synergistic effect on cytotoxicity.

• Dr. Hawver agreed that the combination of diclofenac and tryptamine resulted in potentiation of DNA synthesis arrest, but not of cytotoxicity. Of note was that neither naproxen nor sumatriptan was tested, and no genotoxicity assessment was conducted. Therefore, in this study it was not possible to correlate effects on cell cycle with effects on chromosomal aberrations. The sponsor did provide a copy of a published study by Reddy *et al.* (Reddy MV *et al. Environ Mole Mutagen* 40:1-17, 2002) that demonstrated that inhibition of DNA synthesis may result in induction of chromosomal aberrations. This study, however, also reported no increase in the chromosomal aberrations in cultured CHO cells treated with tryptamine, even though, at the concentrations tested, tryptamine induced "a strong, dose-dependent inhibition of DNA synthesis...." Therefore, these data would suggest that a "strong" inhibition of DNA synthesis (i.e., cell cycle delay) does not invariably lead to an increase in chromosomal aberrations in this system.

Study No. V27836 assessed in cultured CHO cells the effects of naproxen and sumatriptan alone and in combination on cell cycle; CHO cells were exposed to

test articles for 24 hr treatment duration. Only effects on cell cycle and cytotoxicity were assessed; genotoxicity was not measured. The data indicate that the combination of naproxen and sumatriptan produced a concentration-dependent decrease in the % of cells in S phase, greater than either compound alone.

• Dr. Hawver concluded that the data suggested an additive effect of the combination. Unfortunately, assessment of genotoxicity was not conducted in this study and combinations associated with genotoxicity in the in vitro CHO cell assays previously submitted to NDA 21-926 are not similar to those tested in this study. Therefore, the results of this study do not adequately support the sponsor's proposed mechanism.

Study No. V27862 also assessed in cultured CHO cells the effects of naproxen and sumatriptan alone and in combination on cell cycle; CHO cells were exposed to test articles for 3 and/or 24 hrs as in Study No. V27836. The results of this study were inconclusive since none of the experiments tested naproxen alone, sumatriptan alone, and the combination of naproxen and sumatriptan.

• Dr. Hawver concluded that naproxen, sumatriptan, and the combination reduced the % of cells in S phase, but that the magnitude of the effect of each cannot be compared since they were not all tested in any one experiment. Also, since genotoxicity data were not collected, effects on cell cycle, cytotoxicity, and chromosomal aberrations could not be correlated.

(b) The sponsor conducted an "Open-Label, Placebo-Controlled, Parallel Group Study in Healthy Volunteers..." in order to assess the potential for a synergistic genotoxicity effect of the combination of naproxen and sumatriptan directly in humans. The study (MT400-108) was conducted in a total of 42 non-smoking healthy volunteers. According to Dr. Hawver, potential subjects were screened at baseline for "abnormal cell cycle proliferation, stable chromosomal rearrangements or abnormally high background chromosomal aberration frequencies". MT 400, naproxen sodium (550 mg), or placebo were administered to 5/sex/grp b.i.d. for 7 consecutive days. Blood samples were collected on Day 1 and 24 and 48 hr after the final dose (Day 7); only the 24-hr samples were used for analysis of chromosomal aberrations in peripheral lymphocytes.

No increase in number of cells containing chromosomal aberrations was detected with either MT 400 or naproxen sodium.

• This study report was reviewed by Drs. Hawver and Jacobson-Kram. There is agreement that the study was adequately conducted and negative.

Conclusion

In my opinion, the sponsor did not adequately address the inconsistencies in the in vitro mouse lymphoma assay results for naproxen. Although a number of possible reasons were proposed, no data or other information was provided to demonstrate that any or all of the suggested differences in the conduct of the GSK and the [redacted] assays actually accounted for the discrepant results.

It is also my opinion that, while the sponsor did provide data suggesting that delays in cell cycling may be contributory, the synergistic genotoxic effect of the combination of naproxen and sumatriptan in the in vitro chromosomal aberration assay in CHO cells cannot be completely dismissed based on the data provided by the sponsor. Reasons for this are as following:

- None of the new in vitro studies was designed to demonstrate a correlation between effects on cell cycle and on induction of chromosomal aberrations. The variability between studies is sufficiently large to preclude definitive assessment when each effect is tested in separate studies, particularly when the studies are conducted years apart.
- The data provided by the sponsor in this submission did not demonstrate a clear synergistic effect of naproxen and sumatriptan on cell cycle or a relationship between inhibition of the cell cycle and induction of chromosomal aberrations for these compounds. The sponsor did provide published literature that demonstrated this relationship for some compounds; however, tryptamine was a notable exception.
- The sponsor attempted to demonstrate that the synergistic effect of naproxen and sumatriptan on chromosomal aberrations occurred only at excessively cytotoxic concentrations based on inhibition of population doubling. It is the sponsor's position that for compounds that inhibit the cell cycle, population doubling is a more appropriate parameters for assessing cytotoxicity than, e.g., relative cell growth. The sponsor provided several published articles that support this position. However, it is not clear to me that there is consensus among experts on this issue. In addition, it is unclear from the sponsor's data and discussion what the quantitative relationship is between decreases in population doubling and induction of chromosomal aberrations, i.e., what is the magnitude of the effect on population doubling that would be reasonably expected to result in a clastogenic effect. It is also important to note that the population doubling data were quite variable. For example, in Study No. V27862, inhibition of population doubling (PD) was markedly inconsistent and non-concentration related at combinations of naproxen and sumatriptan ranging from 500/500 to 2000/2000 µg/mL; inhibition of PD was 0% at 1675/1675 and 1745/1745 µg/mL, but 100% at 1710/1710 µg/mL.

There does not appear to be a way to further assess the clinical relevance of the synergistic in vitro genotoxicity results in nonclinical studies, and in vivo measurement of test article effects on circulating lymphocytes is a recognized strategy for assessing

potential clastogenicity in humans. Although the sponsor did not adequately address all the issues in the Agency's AE letter, I believe that the data from the clinical trial demonstrating no genotoxic effects of naproxen either alone or in combination with sumatriptan is sufficient to support approval of the application.

[redacted]

Recommended labeling

I would recommend retaining the labeling conveyed to the sponsor in the AE letter, with the following changes (designed by **bold** and strikethroughs):

[redacted]

The combination of sumatriptan and naproxen sodium was negative in an in vitro mouse lymphoma tk assay in the presence and absence of metabolic activation. However, in separate in vitro mouse lymphoma tk assays, naproxen sodium alone was reproducibly positive in the presence of metabolic activation.

Naproxen sodium alone and in combination with sumatriptan was positive in an in vitro clastogenicity assay in mammalian cells in the presence and absence of metabolic activation. The clastogenic effect for the combination was reproducible within this assay and was greater than observed with naproxen sodium alone. Sumatriptan alone was negative in this assay.

Chromosomal aberrations were not induced in peripheral blood lymphocytes following 7 days of twice daily dosing with Treximet in human volunteers.

In previous studies, sumatriptan alone was not mutagenic in two gene mutation assays (the Ames test and the in vitro Chinese Hamster V79/HGPRT assay) and was not clastogenic in two cytogenetics assays (the *in vitro* human lymphocyte assay and the *in vivo* rat micronucleus assay).

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/s/

Lois Freed
4/15/2008 03:48:08 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-926**
SERIAL NUMBER:	**025 & 026**
DATE RECEIVED BY CENTER:	**11 OCT 2007 (025) & 11 JAN 2008 (026)**
PRODUCT:	**Treximet**
	Sumatriptan/Naproxen combination tablet
INTENDED CLINICAL POPULATION:	**Migraine patients**
SPONSOR:	**POZEN Inc., Chapel Hill, NC**
DOCUMENTS REVIEWED:	**eNDA**
REVIEW DIVISION:	**Division of Neurology Products**
PHARM/TOX REVIEWER:	**David B. Hawver, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Lana Chen**

Date of review submission to Division File System (DFS): 15 APR 2008

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EXECUTIVE SUMMARY

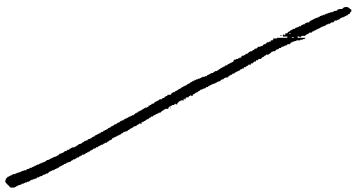
I. Recommendations

A. Recommendation on approvability

The nonclinical package is adequate to support an approval action for NDA 21-926 TREXIMET (sumatriptan succinate/naproxen sodium) Tablets for the acute treatment of migraine.

B. Recommendation for nonclinical studies: None

C. Recommendations on labeling



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The current Complete Response to Approvable Letter (submitted October 11, 2007 at Amendment #025) contained the following items:

- a detailed explanation of the factors that might account for the discrepancy in the mouse lymphoma assay results with naproxen sodium
- three nonclinical studies evaluating the effects of NAP and SS (2 studies) or other NSAIDs and indoles (tryptamine and serotonin) on the cell cycle in CHO cell cultures
- a study of the clastogenic potential of naproxen alone and in combination with sumatriptan in humans (submitted as Amendment #026 on January 11, 2008)

The sponsor has adequately addressed the issues raised in the Approvable Letter of August 1, 2007. The explanation for the discrepancy in the findings for naproxen sodium in the two mouse lymphoma assays included several factors (e.g., treatment period of 4 vs. 3 hrs, higher levels of cytotoxicity) that could reasonably account for the positive results in the earlier study. The nonclinical studies presented a compelling case that the combination of NAP and SS can profoundly disrupt the cell cycle at concentrations well below those inducing cytotoxicity and clastogenicity. This reviewer considers it reasonable to conclude that the profound inhibition of DNA synthesis induced by NAP/SS **may** contribute to the clastogenicity observed at very high concentrations ($\geq$ 7.6 mM NAP; $\geq$ 5.9 mM SS) of this combination in CHO cells (though it is not clear how widely accepted this proposed link is). If the clastogenicity induced by NAP/SS was caused by indirect effects on DNA, then it is reasonable to consider the large safety margins (~30-fold for NAP, ~30,000-fold for SS) between the clastogenic in vitro concentrations and the maximum clinical plasma concentrations in evaluating the risk to patients. The lack of significant increases in the frequency of cells with chromosomal aberrations in peripheral lymphocytes collected from humans treated for 7 days with the maximum recommended daily dose of Treximet (compared to placebo) provides additional assurance that the risk of genotoxicity in humans is reasonable.

B. Pharmacologic activity: No new pharmacologic activity studies were conducted.

C. Nonclinical safety issues relevant to clinical use: None.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

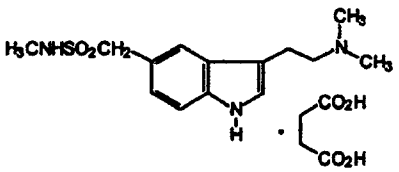
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-926
Review number: 3
Sequence number: 025 & 026
Date of submission: 11 OCT 2007 (025) & 11 JAN 2008 (026)
Type of submission: NDA 505 (b)(2) Resubmission—Complete Response to 01 AUG 2007 Approvable Letter (025); Amendment containing Final Study Report MT400-108
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: POZEN Inc., Chapel Hill, NC
Manufacturer for drug substance:
Sumatriptan Succinate (SS): Glaxo Wellcome Manufacturing Pte Limited, Singapore
Naproxen Sodium (NAP): [redacted]

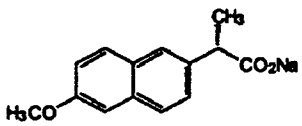
Reviewer name: David B. Hawver, Ph.D.
Division name: Division of Neurology Products
HFD #: 120
Review completion date: 15 APR 2007

Drug:

Trade name: Treximet
Generic name: sumatriptan succinate/naproxen sodium
Code name: MT400
Chemical name:
SS: 3-[2-(dimethylamino)ethyl]-N-methyl-indole-5-methanesulfonamide succinate (1:1)
NAP: (S)-6-methoxy-(alpha)-methyl-2-naphthaleneacetic acid, sodium salt
CAS registry number: 103628-48-4 (sumatriptan succinate)
26159-34-2 (naproxen sodium)
Molecular formula/molecular weight:
sumatriptan succinate: $C_{14}H_{21}N_3O_2S \cdot C_4H_6O_4$ MW 413.5
naproxen sodium: $C_{14}H_{13}NaO_3$ MW 252.25
Structure:



sumatriptan succinate



naproxen sodium

Relevant INDs/NDAs/DMFs:
IND 68,435 MT 400 for migraine, POZEN's current IND for sumatriptan/naproxen combined in one [redacted] tablet; submitted 18 DEC 2003
IND 60,669 MT 400 for migraine, POZEN's initial IND for sumatriptan/naproxen using marketed products in combination; submitted 26 JUL 2000
NDA 20-132 IMITREX® Tablets, sumatriptan succinate for migraine; Glaxo Inc.; approved 01 JUN 1995
NDA 17-581 NAPROSYN® Tablets, naproxen for rheumatoid arthritis, now also for acute pain, ankylosing spondylitis, tendonitis, bursitis, and acute gout; Roche (originally Syntex, Inc.); approved 11 MAR 1976
NDA 18-164 ANAPROX® Tablets, naproxen sodium for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and juvenile arthritis; Roche/Syntex; approved 04 SEP 1980

[redacted]

Drug class:
Sumatriptan succinate is a selective 5-$HT1_D$ receptor agonist.
Naproxen sodium is a nonsteroidal anti-inflammatory drug (NSAID).

Intended clinical population:
The proposed indication for Treximet Tablets is for the treatment of acute migraine headache with or without aura in adults.

Clinical formulation:
Each Treximet Tablet contains 119 mg sumatriptan succinate (equivalent to 85 mg sumatriptan) and 500 mg naproxen sodium. Inactive ingredients (which are all GRAS for use in oral pharmaceuticals) include: [redacted] (microcrystalline cellulose), croscarmellose sodium, dibasic calcium phosphate, magnesium stearate, microcrystalline cellulose, [redacted], sodium bicarbonate and talc; the aqueous film coat contains sodium carboxymethyl-cellulose, maltodextrin, dextrose monohydrate, titanium dioxide, lecithin and FD&C Blue No. 2.

Route of administration: Oral tablet

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

Data reliance:
Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-926 are owned by POZEN Inc. or are data for which POZEN Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-926 that POZEN Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that POZEN Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-926.

Studies reviewed within this submission:

- Cell Cycle Analysis in CHO Cells Treated with Various NSAIDs and Indoles, Individually and in Combination (Study No. V27824)
- Investigative Study: Cell Cycle Analysis Using Chinese Hamster Ovary Cells Treated with Naproxen Sodium and Sumatriptan Succinate Individually and in Combination (NON-MONITORED STUDY) (Study No. V27862)
- Investigative Study: Cell Cycle Analysis Using Chinese Hamster Ovary Cells Treated with a 1:1 Combination of Naproxen Sodium and Sumatriptan Succinate (NON-MONITORED STUDY) (Study No. V27836)
- Open-Label, Placebo-Controlled, Parallel Group Study in Healthy Volunteers to Evaluate the Effects of MT 400 Tablets or Naproxen Sodium Tablets on Chromosomal Aberrations in Peripheral Blood Lymphocytes (Study MT400-108)

Studies not reviewed within this submission: None.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

No Pharmacology studies were included in this submission.

2.6.2.2 Primary pharmacodynamics

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.3.2 Primary Pharmacodynamics

2.6.3.3 Secondary Pharmacodynamics

2.6.3.4. Safety Pharmacology

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No Pharmacokinetics/Toxicokinetics studies were included in this submission.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

2.6.6.2 Single-dose toxicity

No single-dose toxicity studies were included in this submission.

2.6.6.3 Repeat-dose toxicity

No repeat-dose toxicity studies were included in this submission.

2.6.6.4 Genetic toxicology

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

- Cell Cycle Analysis in CHO Cells Treated with Various NSAIDs and Indoles, Individually and in Combination (Study No. V27824)
- Investigative Study: Cell Cycle Analysis Using Chinese Hamster Ovary Cells Treated with a 1:1 Combination of Naproxen Sodium and Sumatriptan Succinate (NON-MONITORED STUDY) (Study No. V27862)
- Investigative Study: Cell Cycle Analysis Using Chinese Hamster Ovary Cells Treated with Naproxen Sodium and Sumatriptan Succinate Individually and in Combination (NON-MONITORED STUDY) (Study No. V27836)
- Open-Label, Placebo-Controlled, Parallel Group Study in Healthy Volunteers to Evaluate the Effects of MT 400 Tablets or Naproxen Sodium Tablets on Chromosomal Aberrations in Peripheral Blood Lymphocytes (Study MT400-108)

Cell Cycle Analysis in CHO Cells Treated with Various NSAIDs and Indoles, Individually and in Combination (Study No. V27824)

(GSK Study #WD2007/01420-01; Initiated 13 SEP 2006, Completed 11 OCT 2007; conducted by GSK in the United Kingdom; no GLP or QA statement)

The effect of 6 and 24 hour treatments of CHO cells with marketed non-steroidal anti-inflammatory drugs (NSAIDs: diclofenac, ibuprofen, indomethacin, piroxicam, and sulindac) and indoles (tryptamine and serotonin) were evaluated using flow cytometry to assess the % of cells in S phase, G1 phase, and G2/M phase. Additional studies were conducted with diclofenac and tryptamine, alone and in combination, assessing the % of cells in each phase of the cell cycle after 24-hr treatments. Evaluation of genotoxicity by counting micronucleated cells in each culture was planned, but results were not available due to a technical error. Vehicle controls and positive controls (hydroxyurea) were reported to have performed as expected in all assays. *(Note: Data tables were not submitted, so results of controls could not be verified.)*

As shown in the figures below, all of the NSAIDs dose-dependently reduced the percentage of cells in S phase and increased the % of cells in G1 and/or G2/M phase after 6 and/or 24 hrs of treatment. In contrast, treatment with tryptamine induced increases in the % of cells in S phase and decreases in the % of cells in G1 and G2/M phase, and serotonin had very little effect on these parameters.

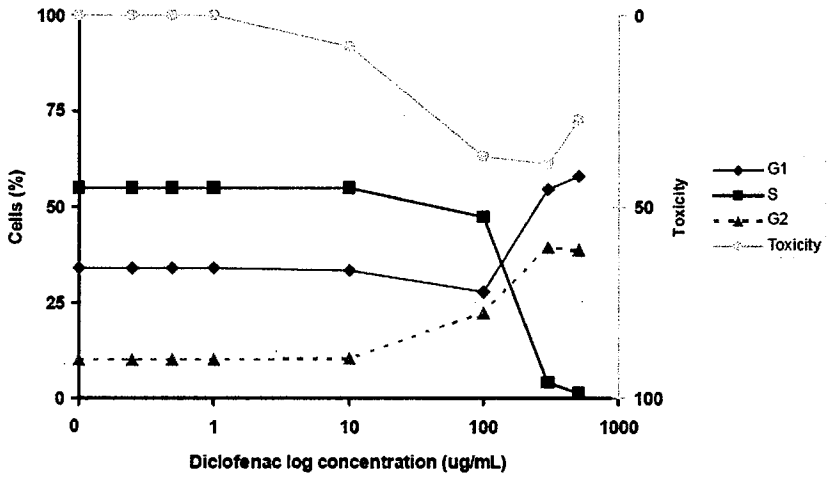
Figure 15 below was very difficult to interpret, due to incomplete labeling and the lack of supporting data tables. However, this reviewer believes that Figure 15 shows that, in the presence of 100 ug/mL tryptamine (upper panels), 50 ug/mL diclofenac treatment for 24 hrs induced an increase to ~90% cells in S phase, while higher concentrations of diclofenac shifted cells away from S phase, and back toward G1 and G2/M phases. At 200 ug/mL diclofenac + 100 ug/mL tryptamine, only ~5% of cells were left in S phase, down from 60% in controls. In contrast, the presence of 300 ug/mL tryptamine (lower panels in Figure 15) altered the effect of diclofenac such that virtually all cells were **"blocked" in S phase at 50, 100, and 200 ug/mL diclofenac.**

The sponsor concluded that treatment with the combination of diclofenac and tryptamine for 24 hrs induced a concentration-dependent potentiation of DNA synthesis arrest **compared with each component alone, associated with "a synergistic increase in cytotoxicity." Diclofenac alone at 50 ug/mL for 24 hrs induced no change in the % of** cells in S phase in CHO cells and was associated with cytotoxicity of ~30% (see Figure 8). Tryptamine alone at 100 ug/mL for 24 hrs induced no change in the % of cells in S phase and was associated with cytotoxicity of ~40% (see Figure 13). The combination of 50 ug/mL diclofenac and 100 ug/mL tryptamine for 24 hrs induced an increase in the % of cells in S phase to ~90% (from the control level of ~50%), and was associated with cytotoxicity of ~70%. Thus, the addition of 50 ug/mL diclofenac clearly appeared to potentiate the ability of 50 ug/mL tryptamine to increase the % of cells in S phase, since even 250 ug/mL tryptamine in the absence of diclofenac only increased the % of cells in S phase to 80% (see Figure 13). The effects on cytotoxicity, however, appeared to be

additive rather than synergistic (30% + 40% = 70%). The addition of 200 ug/mL diclofenac to 100 ug/mL tryptamine led to the opposite effect, a dramatic reduction of the % of cells in S phase and arrest of cells in phases G1 and G2/M. Thus, at this concentration of tryptamine, low concentrations of diclofenac appeared to potentiate **tryptamine's effects, but high concentrations appeared to overwhelm tryptamine's effects.**

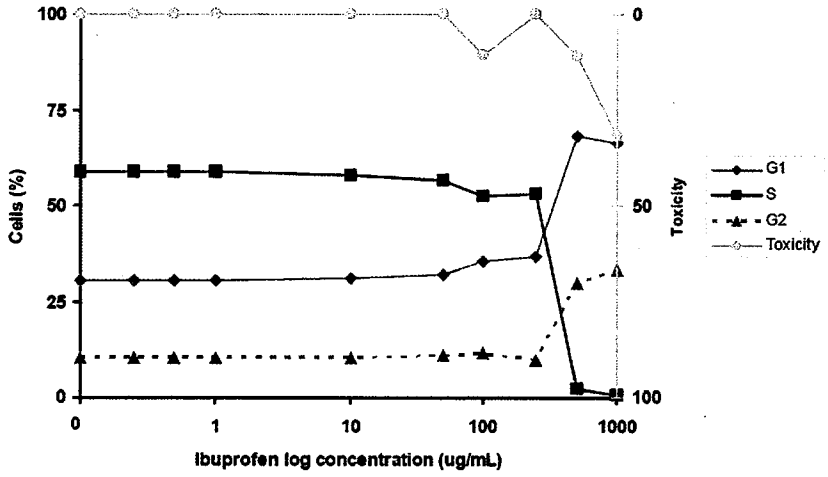
In combination with 300 ug/mL tryptamine, however, all three concentrations of diclofenac tested (50, 100, and 200 ug/mL) potentiated the increase in % of cells in S phase, leading to near complete arrest of the cell cycle in S phase, associated with cytotoxicity of ~70-90%.

Figure 1 Diclofenac 6 Hour



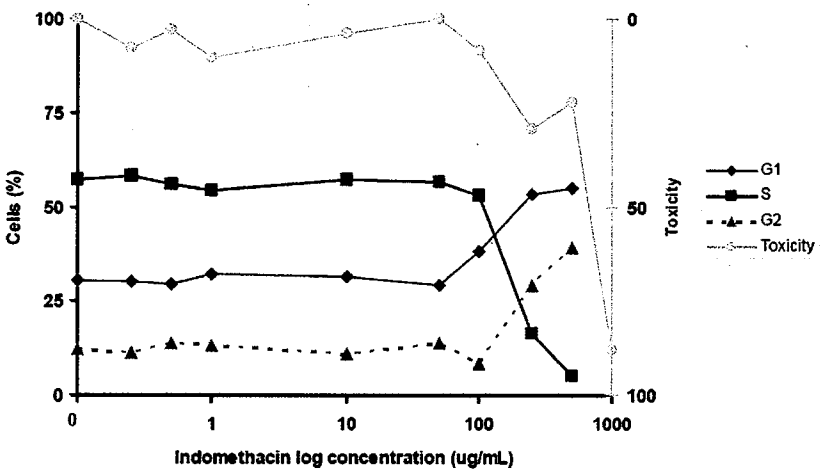
(from Page 12 of Study Report)

Figure 2 Ibuprofen 6 Hour



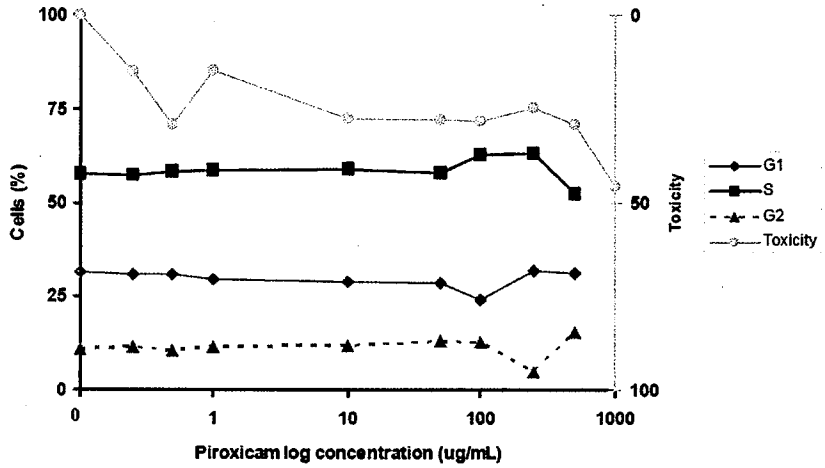
(from Page 12 of Study Report)

Figure 3 Indomethacin 6 Hour



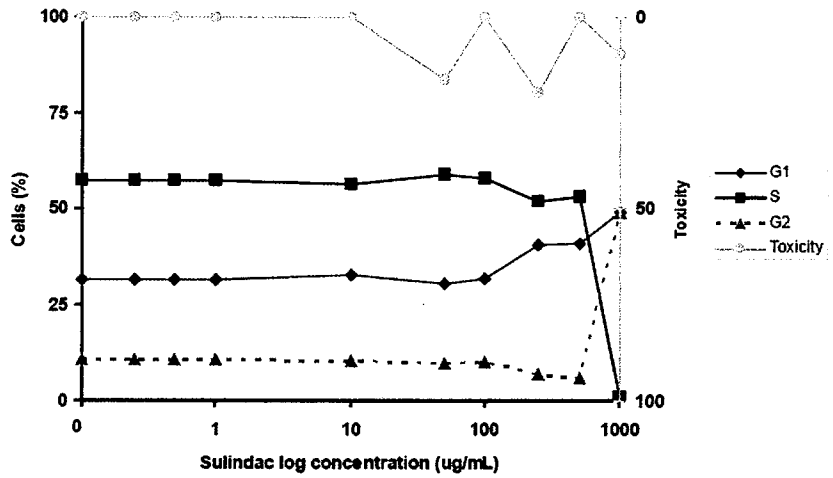
(from Page 13 of Study Report)

Figure 4 Piroxicam 6 Hour



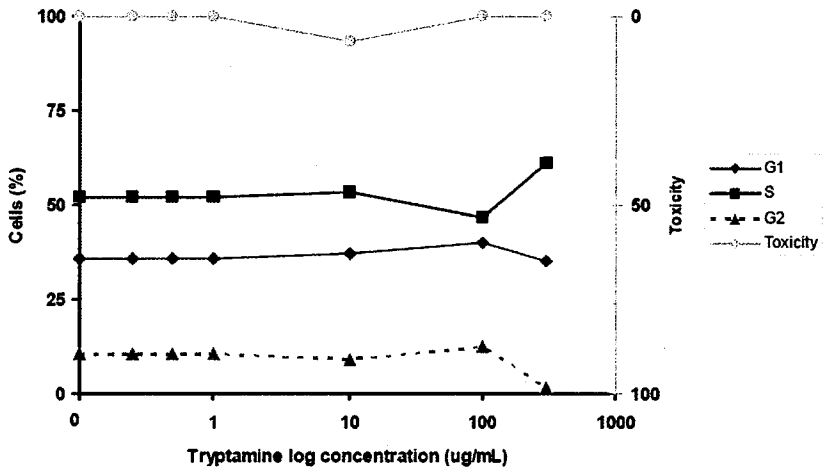
(from Page 13 of Study Report)

Figure 5 Sulindac 6 Hour



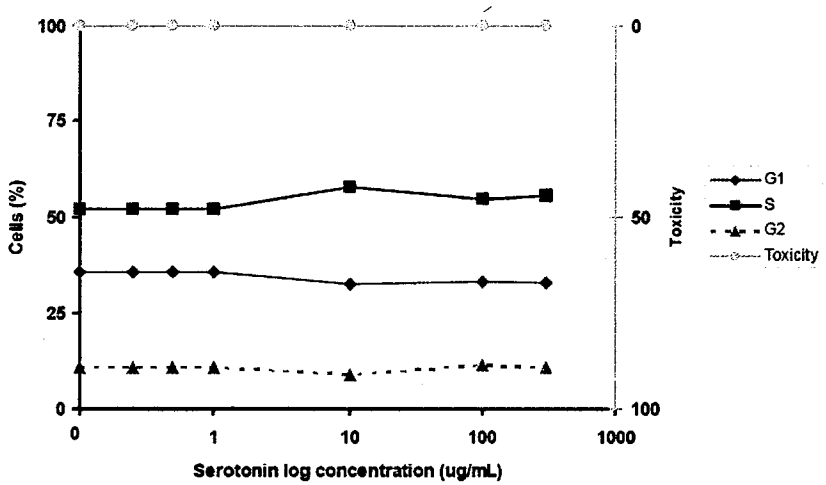
(from Page 14 of Study Report)

Figure 6 Tryptamine 6 Hour



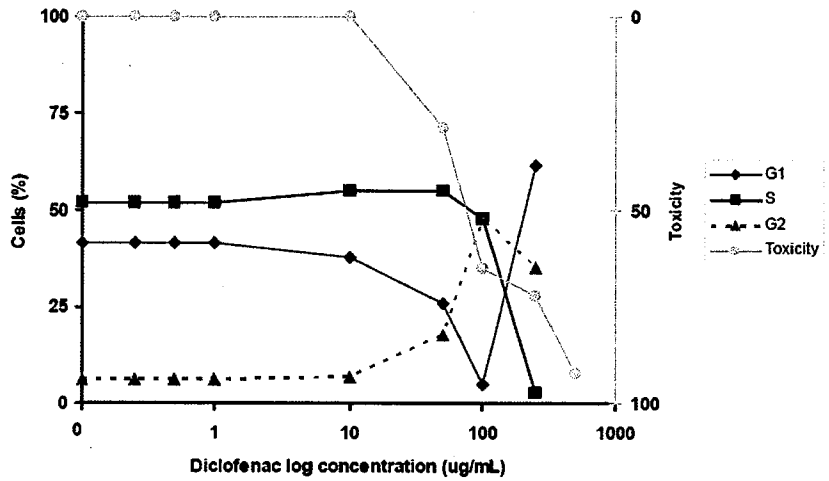
(from Page 14 of Study Report)

Figure 7 Serotonin 6 Hour



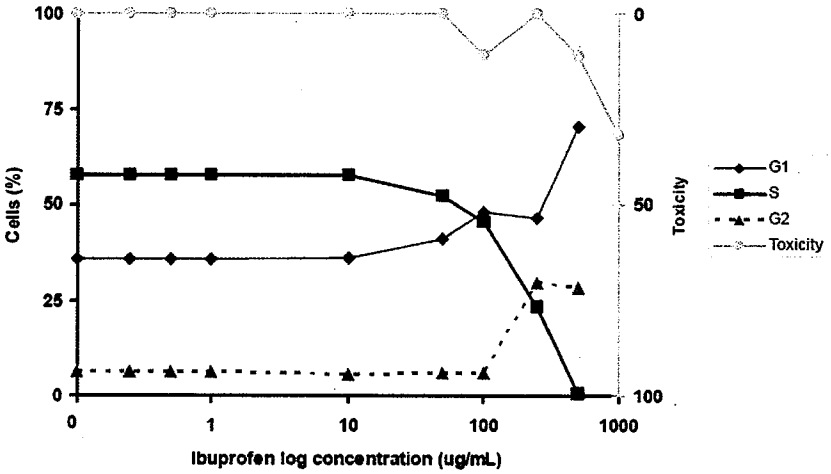
(from Page 15 of Study Report)

Figure 8 Diclofenac 24 Hour



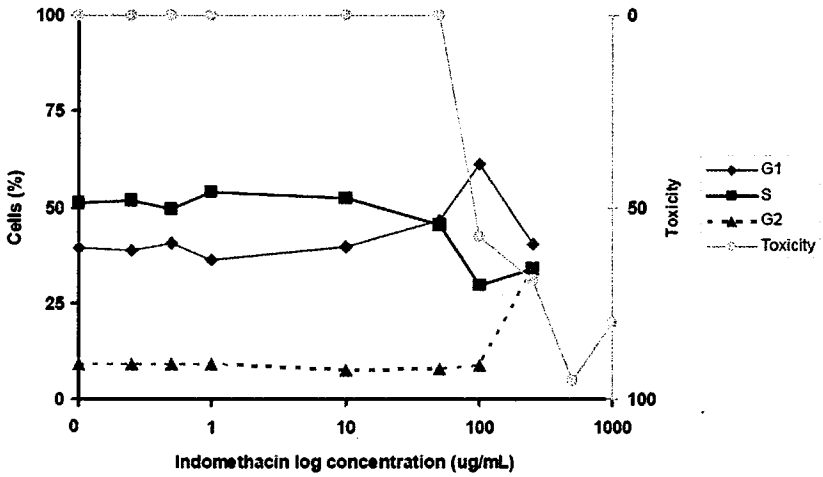
(from Page 15 of Study Report)

Figure 9 Ibuprofen 24 Hour



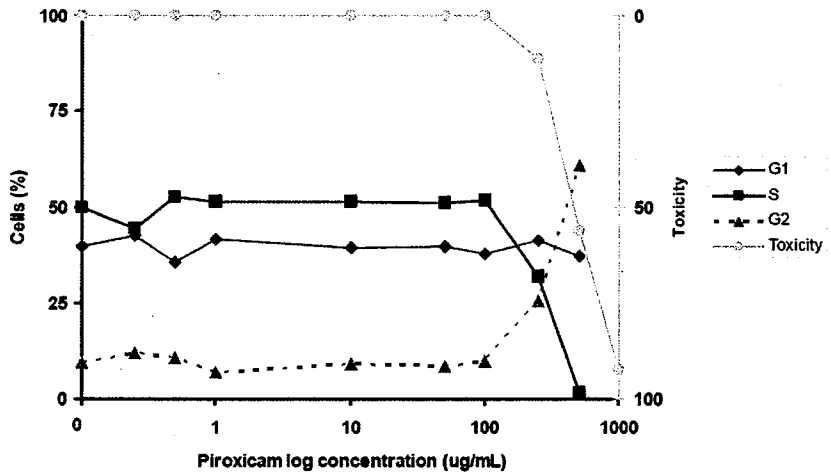
(from Page 16 of Study Report)

Figure 10 Indomethacin 24 Hour



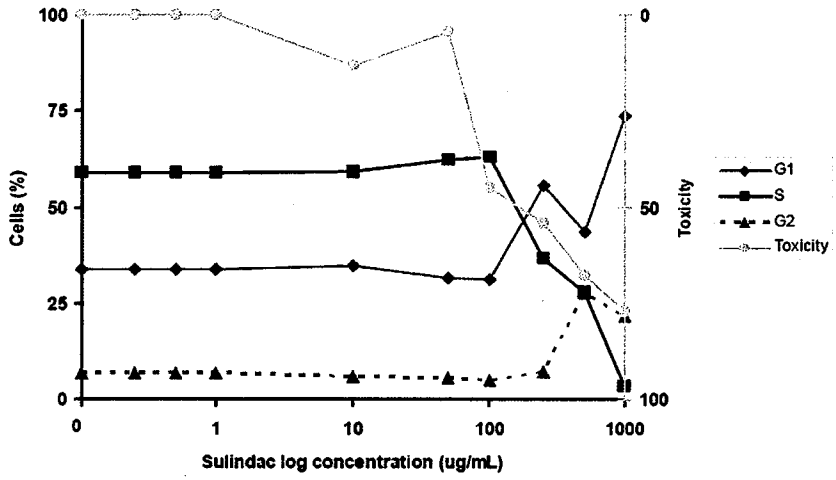
(from Page 16 of Study Report)

Figure 11 Piroxicam 24 Hour



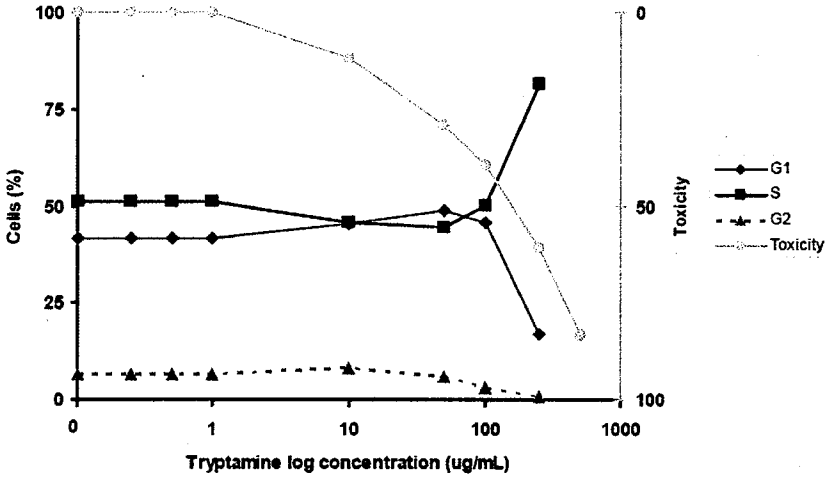
(from Page 17 of Study Report)

Figure 12 Sulindac 24 Hour



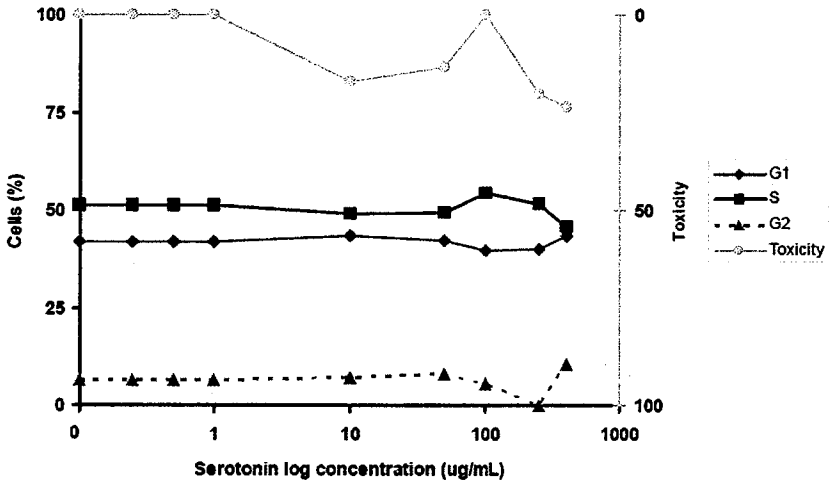
(from Page 17 of Study Report)

Figure 13 Tryptamine 24 Hour



(from Page 18 of Study Report)

Figure 14 Serotonin 24 Hour



(from Page 18 of Study Report)

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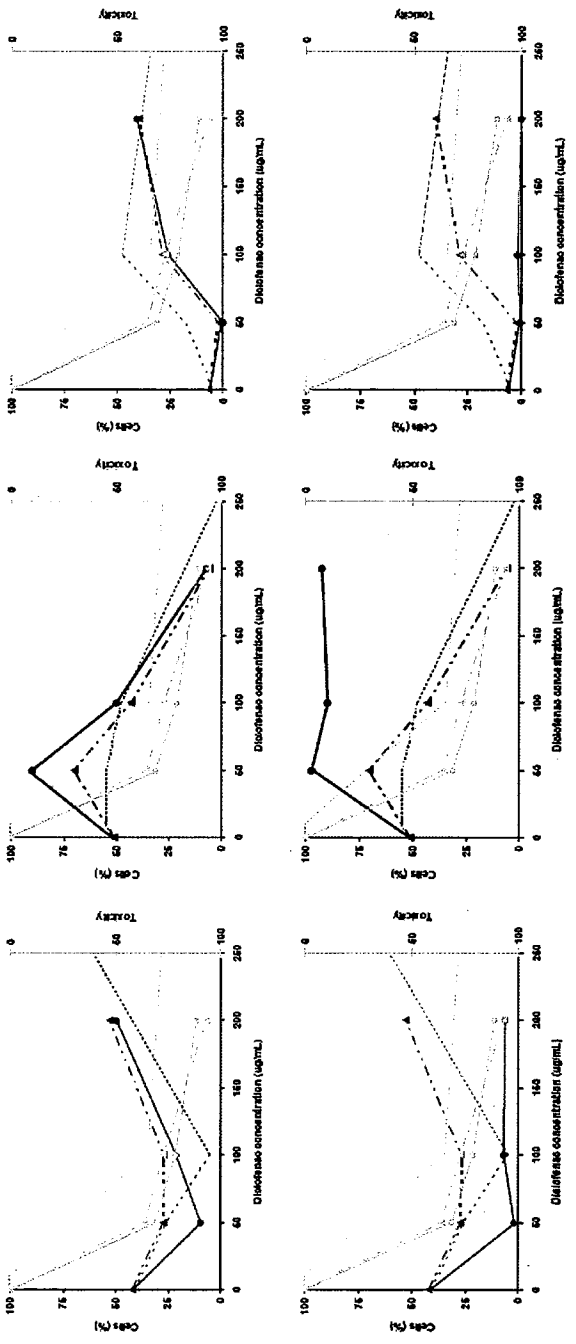
Figure 15 Diclofenac and Tryptamine Combination 24 Hour [1]



1. Various concentrations of diclofenac were treated in combination with tryptamine at 100 ug/mL and 300ug/mL. Light grey line represents toxicity, black line represents % cells in S-phase, dark grey lines represent % cells in G1 or % cells in G2/M. Dashed lines represent individual treatments whereas solid lines represent combination treatments.

Conclusions:
Treatment of CHO cells with NSAIDs (diclofenac, ibuprofen, indomethacin, piroxicam, and sulindac) for 6 or 24 hrs resulted in dose-dependent effects on the cell cycle: the % of cells in S phase was decreased, and the % of cells in G1 and G0/M phases was increased. Tryptamine showed the opposite effect, increasing the % of cells in S phase, and decreasing the % of cells in G1 and G0/M phases. Cytotoxicity was much higher after 24 hrs than after 3 hr treatments with NSAIDs or tryptamine. Serotonin had very little effect on cell cycle parameters or cytotoxicity.

Combination studies with CHO cells demonstrated that the effects of tryptamine on the cell cycle (increase in % of cells in S phase and reduction in the % of cells in G1 and G0/M phases) were potentiated by the presence of 50 ug/mL diclofenac. Co-treatment with 200 ug/mL diclofenac also potentiated the effect of tryptamine at 300 ug/mL, but when tryptamine was present at only 100 ug/mL, this higher dose of diclofenac overwhelmed the tryptamine effect and resulted in decreased % cells in S phase and increased % cells in G1 and G0/M phases, similar to the effect seen with diclofenac alone.

The sponsor claims that the combination of diclofenac and tryptamine also showed **"synergistic" effects on cytotoxicity, but the data submitted do not support this** conclusion.

Investigative Study: Cell Cycle Analysis Using Chinese Hamster Ovary Cells Treated with a 1:1 Combination of Naproxen Sodium and Sumatriptan Succinate (NON-MONITORED STUDY) (Study No. V27862)

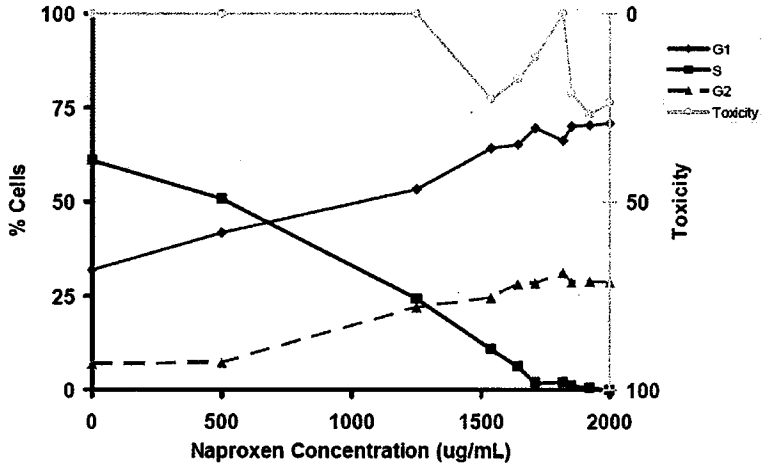
(GSK Study #WD2007/01560/00; Initiated 07 SEP 2007, Completed 03 OCT 2007; conducted by GSK in the United Kingdom; no GLP or QA statement)

The effects of naproxen sodium (NAP), sumatriptan succinate (SS), and 1:1 combinations of NAP and SS on the cell cycle were evaluated after treatment of CHO cells for 3 hrs (and for 24 hrs, on one experiment with the combination) in the absence of a mammalian oxidative metabolizing system. The percentage of cells in G1/G0, S, and G2/M phases were assessed using flow cytometry [redacted]

Data for vehicle controls (water) were said to be "within or close to the acceptable ranges determined from laboratory historical data." Positive controls, aphidicolin and hydroxyurea, were said to have "induced clear unequivocal increases in the % cells accumulating in S-phase." *(Page 8 of Study Report)* The data submitted showed that 24 hrs of treatment with positive controls increased the % of cells in S-phase to 81-85% from vehicle control levels of 66%, and reduced the % of cells in G1/G0 phase to 7-11% from control levels of 27% *(Page 24 of Study Report)*.

Figure 1 below illustrates that treatment of CHO cells with NAP alone resulted in a dramatic dose-dependent reduction in the % of cells in S phase. At 1250 ug/mL, which induced no cytotoxicity, the % of cells in S phase was less than half of that in controls. At concentrations of 1710-2000 ug/mL NAP, the % of cells in S phase was reduced to ≤ 2.08 % (compared to ~61% in cultures treated with the vehicle control), while cytotoxicity was ≤ 27%. Corresponding increases were observed in the % of cells in G1/G0 and G2/M phases.

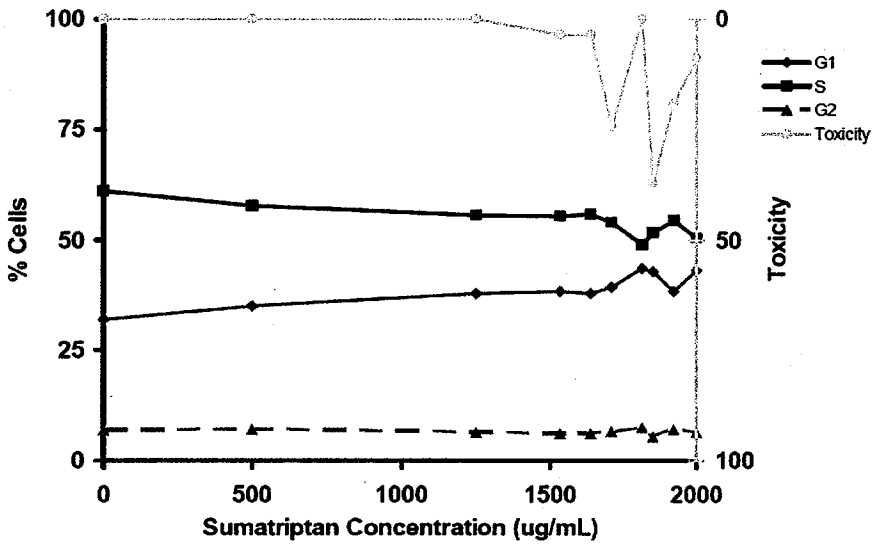
Figure 1 Naproxen 3 Hour



(from Page 15 of Study Report)

Figure 2 below illustrates that treatment of CHO cells with SS alone resulted in a much smaller dose-dependent reduction in the % of cells in S phase, corresponding with slight increases in the % of cells in G1/G0 phase (but not in the % of cells in G2/M phase).

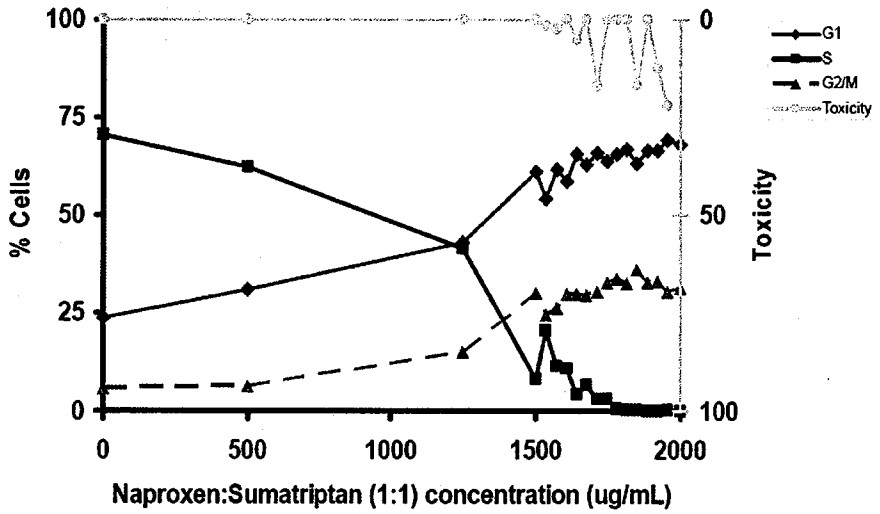
Figure 2 Sumatriptan 3 Hour



(from Page 16 of Study Report)

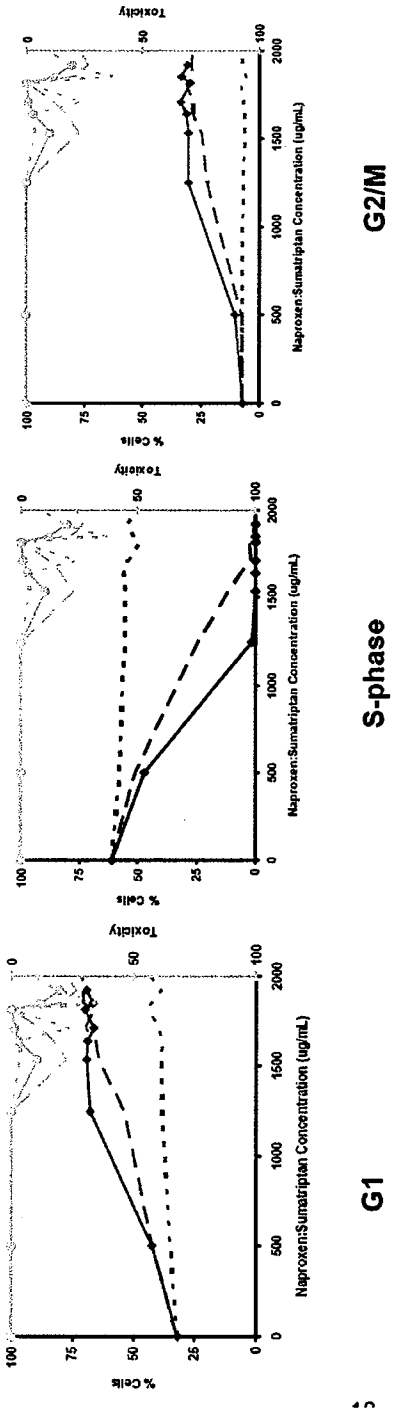
Figures 3, 4, and 5 below illustrate that the NAP/SS combination dose-dependently reduced the % of cells in S phase and in increased the % of cells in G1/0 and G2/M phases, in both 3 hr and 24 hr incubations. In two 3-hr experiments, the effect of NAP/SS on these parameters was observed even at 1250/1250 ug/mL, a dose at which little or no cytotoxicity was observed. The cytotoxicity observed with NAP alone and with SS/NAP combinations was much higher in cultures incubated for 24 hrs than for 3 hrs. The % of cells in S phase was maximally reduced to near 0% at 1000/1000 ug/mL NAP/SS after 24 hrs of treatment, associated with cytotoxicity of 64% (36% relative survival, 91.4% inhibition of population doubling). *(Note: firm conclusions cannot be drawn regarding the comparison of the results of the 24-hr 1:1 NAP/SS treatment (Figure 5) with those of each component alone because the NAP and SS alone results presented were generated in previous experiments [Study No. V2786])*

Figure 3 Experiment 1 1:1 Combination Naproxen:Sumatriptan 3 Hour Treatment



(from Page 17 of Study Report; Figures 4 & 5 below are from pages 18 & 19 of Study Report, respectively)

Figure 4 Experiment 2 1:1 Combination Naproxen:Sumatriptan 3 Hour Treatment

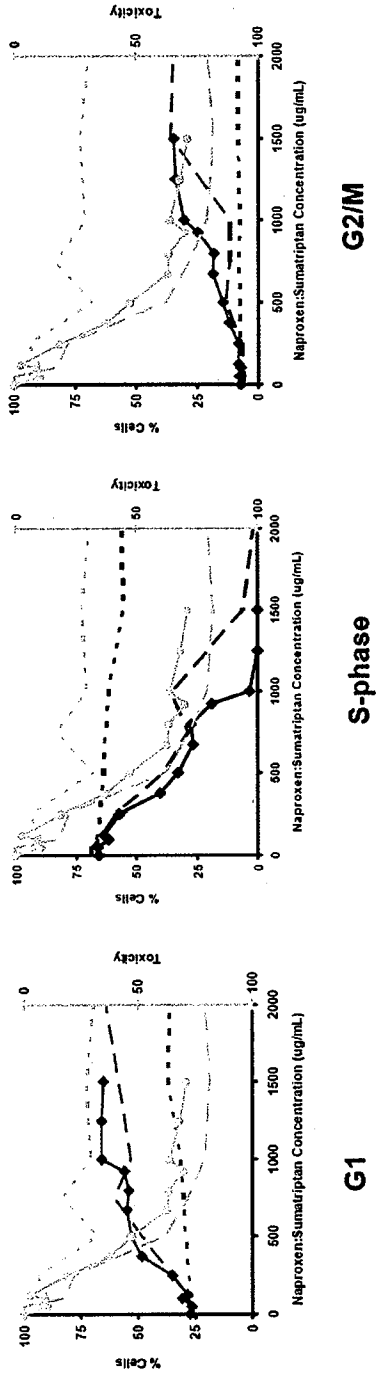


Cell cycle analysis was performed in CHO cells after exposure to various concentrations of naproxen (1:1) sumatriptan treatments for 3 hours.

Light grey lines: Toxicity; Black lines: % cells in S-phase, Drak grey lines: % cells in G1 or G2/M.

Solid lines represent results for naproxen (1:1) sumatriptan; dashed lines naproxen alone and dotted lines sumatriptan alone.

Figure 5 1:1 Combination Naproxen:Sumatriptan 24 Hour Treatment



Cell cycle analysis was performed in CHO cells after exposure to various concentrations of naproxen (1:1) sumatriptan treatments for 24 hours.

Light grey lines: Toxicity; Black lines: % cells in S-phase, Drak grey lines: % cells in G1 or G2/M.

Solid lines represent results for naproxen (1:1) sumatriptan; dashed lines naproxen alone and dotted lines sumatriptan alone. This data was generated previously [WD2007/01476/00].

Conclusions:
Treatment of CHO cells for 3 hrs with NAP alone at 500-2000 ug/mL dose-dependently reduced the % of cells in S phase and increased the % of cells in G1/G0 and G2/M phases. Near maximal effects on these parameters were achieved at 1640 ug/mL and above, at which cytotoxicity ranged from 0-27% (73-100% relative survival, 66-100% inhibition of population doubling).

Treatment of CHO cells for 3 hrs with SS alone at 500-2000 ug/mL resulted in very slight reduction in the % of cells in S phase and very slight increase in the % of cells in G1/G0 phase. Cytotoxicity of 0-37% (63-100% relative survival, 25-100% inhibition of population doubling) was observed at 1640-2000 ug/mL SS.

The combination of NAP/SS induced near maximal reduction in the % of cells in S phase and increases in the % of cells in G1/G0 and G2/M phases at 1500/1500 ug/mL in one 3-hr assay, with little or no effects on cytotoxicity or inhibition of population doubling. Near maximal effects on cell cycle parameters were observed at 1000/1000 ug/mL NAP/SS in the 24-hr assay, associated with cytotoxicity of 64% (36% relative survival, 91.4% inhibition of population doubling). No conclusions can be drawn regarding the **potentiation of NAP's effects by SS, since NAP and SS were not evaluated separately** and in combination in the same assay.

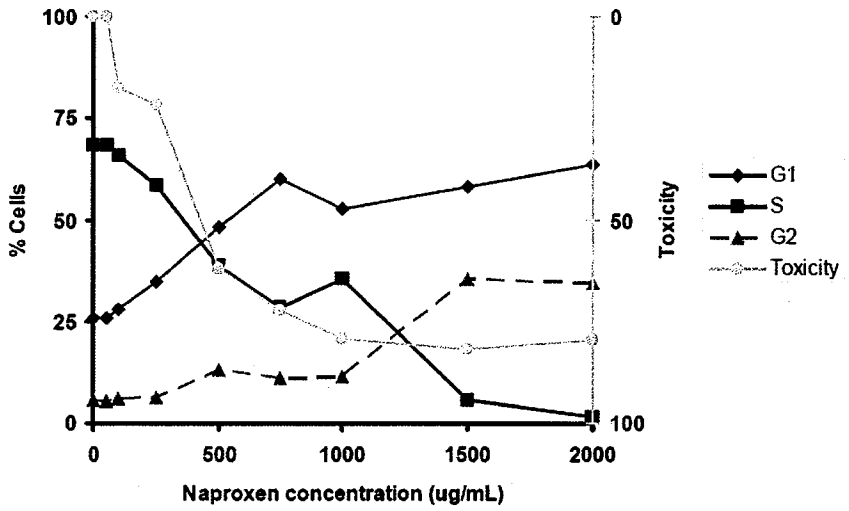
Investigative Study: Cell Cycle Analysis Using Chinese Hamster Ovary Cells Treated with Naproxen Sodium and Sumatriptan Succinate Individually and in Combination (NON-MONITORED STUDY) (Study No. V27836)

(GSK Study #WD2007/01476/00; Initiated 31 AUG 2007, Completed 05 SEP 2007; conducted by GSK in the United Kingdom; no GLP or QA statement)

Chinese hamster ovary (CHO) cell cultures were treated for 24 hrs with sumatriptan succinate (SS) or naproxen sodium (NAP), or with the two drugs in combination (NAP/SS), in the absence of a mammalian oxidative metabolizing system. Following the 24-incubation with drug(s), the % of cells in S phase, G1 phase, and G2/M phase was determined by flow cytometry. Data for vehicle (water) and positive controls (aphidicolin and hydroxyurea) were reported to be consistent with a valid cell cycle analysis assay.

Figure 1 below illustrates that NAP dose-dependently reduced the % of CHO cells in S phase, while increasing the % of cells in G1 and G2/M phases, and increasing cytotoxicity to a plateau of ~80% (~20% relative survival, 100% inhibition of population doubling) at 1000 ug/mL and above.

Figure 1 Naproxen 24 Hour

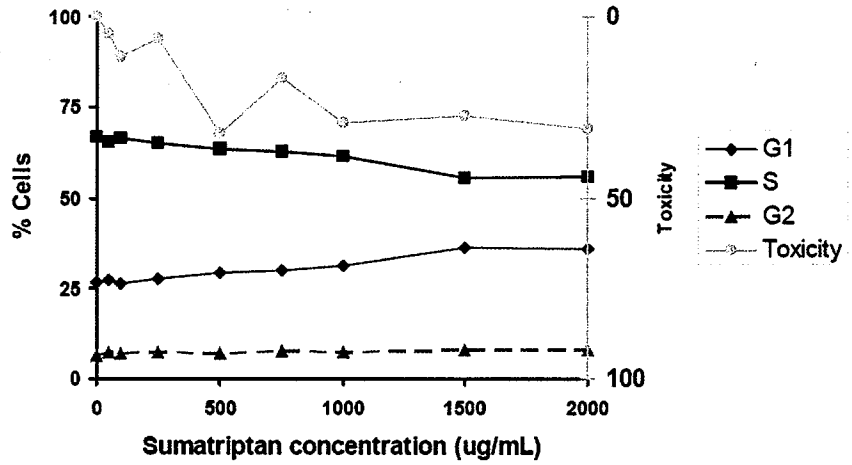


(from Page 12 of Study Report)

Figures 2 and 3 below illustrate that SS induced a much less dramatic dose-dependent reduction in the % of cells in S phase and increase in the % of cells in G1 phase, with only a very slight increase observed in the % of cells in G2/M phase at 3000 ug/mL and above. The dose-dependent cytotoxicity observed with SS was also much less than with

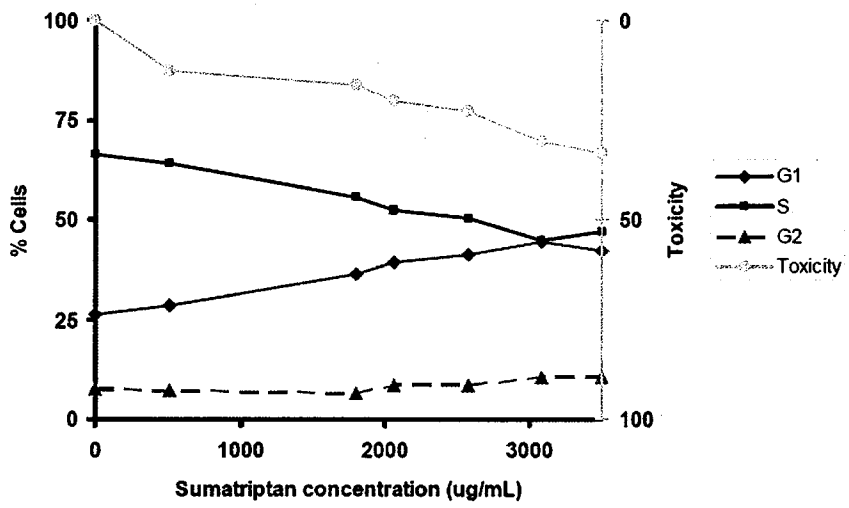
NAP, reaching a maximum of only 33.4% (66.4% relative survival, 27.3% inhibition of population doubling) at 3500 ug/mL.

Figure 2 Sumatriptan 24 Hour (Experiment 1)



(from Page 13 of Study Report)

Figure 3 Sumatriptan 24 Hour (Experiment 2)



(from Page 13 of Study Report)

Table 4 and Figure 4 below illustrate that the presence of 500 ug/mL SS (top panel) did not substantially alter the dose-dependent effects of 100-500 ug/mL NAP, but 1750 ug/mL SS (middle panel) slightly increased the effects of NAP on the % of cells in S phase and G1 phase and on cytotoxicity. The addition of 3000 ug/mL SS (lower panel) did not appear to increase the effects of NAP on the % of cells in S and G1 phases much more than did 1750 ug/mL, but potentiated the dose-dependent effects of NAP on cytotoxicity and on the % of cells in G2/M phase (especially at 400 and 500 ug/mL). Maximal cytotoxicity of 81.5% (18.5% relative survival, 100% inhibition of population doubling) was observed at 500 ug/mL NAP + 3000 ug/mL SS. Maximal cytotoxicity of 67.5% (32.5% relative survival, 90.9% inhibition of population doubling) was observed at 500 ug/mL NAP + 1750 ug/mL SS. Maximal cytotoxicity of 68.6% (31.4% relative survival, 93.6% inhibition of population doubling) was observed at 500 ug/mL NAP + 500 ug/mL SS.

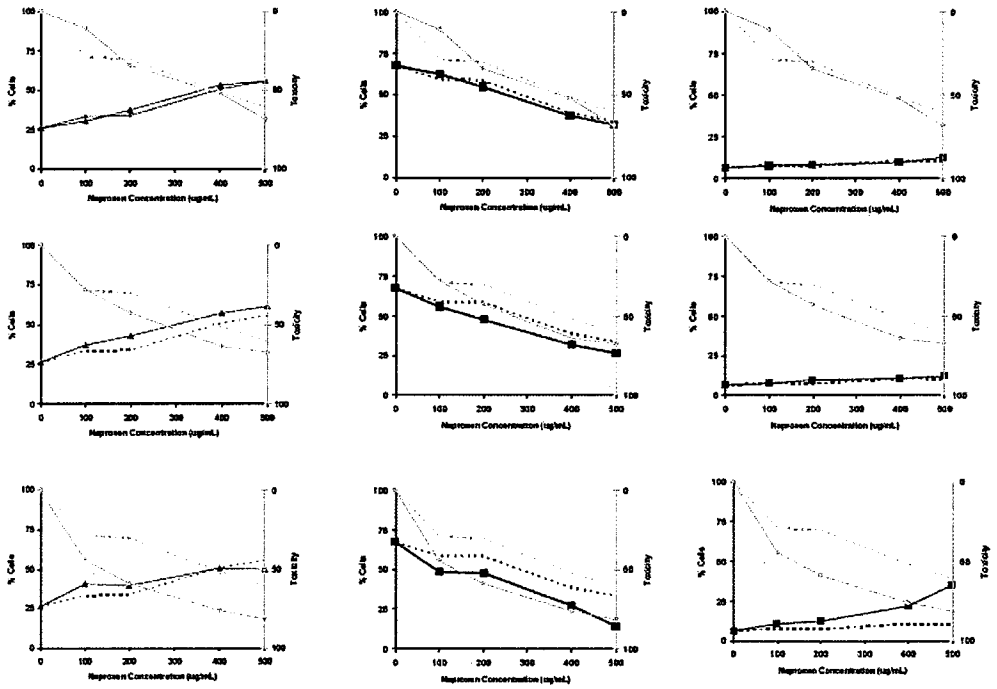
Table 4 Combination Experiment

24HR

	Concentration ug/mL	Compound	Relative Survival (%)	Toxicity	Inhibition PD %	Total No. of "single cell" events	Total No. of cells analysed	No. of events in G1/G0 phase	% Cells in G1/G0 phase	No. of events in S phase	% Cells in S phase phase	No. of events in G2/M phase
	BrdU control	Water	N/A	N/A	N/A	20000	29158	Used to set S phase gate				
	Water	Water	100	0	0	20000	28879	5246	26.23	13262	66.31	1466
	Water	Water	100	0	0	20000	24581	4986	24.93	13800	69.00	1194
	Water	Water	100	0	0	20000	27744	5400	27.00	13342	66.71	1228
Mean	0		100	0	0	20000	27068	5211	26.05	13468	67.34	1296
	100	Naproxen	71.3	28.7	27.13	20000	24343	6638	33.19	11793	58.97	1551
	200	Naproxen	69.6	30.4	29.13	20000	21439	6812	34.06	11670	58.35	1501
	400	Naproxen	48.4	51.6	58.65	20000	20814	10143	50.71	7743	38.71	2108
	500	Naproxen	39.2	60.8	75.74	20000	22038	11170	55.85	6751	33.76	2065
	500	Sumatriptan	70.3	29.7	28.36	20000	27383	5567	27.83	13293	66.47	1116
	1750	Sumatriptan	79	21	18.81	20000	23642	8640	43.20	10423	52.12	918
	3000	Sumatriptan	72.4	27.6	25.88	20000	22621	11016	55.08	7909	39.55	1065
	100/500	NP/SS	89.2	10.8	8.97	20000	25712	6077	30.39	12431	62.16	1472
	200/500	NP/SS	65.6	34.4	33.92	20000	31216	7526	37.63	10863	54.32	1592
	400/500	NP/SS	47.8	52.2	59.57	20000	29127	10641	53.21	7444	37.22	1899
	500/500	NP/SS	31.4	68.6	93.64	20000	32585	11128	55.64	6355	31.77	2490
	100/1750	NP/SS	71.8	28.2	26.66	20000	26381	7409	37.05	11096	55.48	1470
	200/1750	NP/SS	57.1	42.9	45.11	20000	26449	8524	42.62	9533	47.66	1920
	400/1750	NP/SS	36	64	82.49	20000	24442	11477	57.38	6340	31.7	2147
	500/1750	NP/SS	32.5	67.5	90.93	20000	23783	12301	61.5	5305	26.52	2375
	100/3000	NP/SS	55.7	44.3	47.23	20000	24109	8153	40.77	9739	48.7	2092
	200/3000	NP/SS	41.1	58.9	71.79	20000	26642	7882	39.41	9603	48.02	2474
	400/3000	NP/SS	23.8	76.2	100	20000	28783	10130	50.65	5466	27.33	4334
	500/3000	NP/SS	18.5	81.5	100	20000	30864	9955	49.78	2893	14.46	7067
	Aphidicolin 0.4	AC	33.9	66.1	87.37	20000	49468	3318	16.59	16067	80.34	543
	Hydroxyurea 10	HU	46.5	53.5	61.89	20000	22998	3650	18.25	15354	76.77	945
	Untreated	0	97	3	2.19	20000	27240	5479	27.4	13085	65.42	1422
	Untreated	0	105	0	0	20000	27669	5868	29.34	12572	62.86	1536

(from Page 18 of Study Report)

Figure 4 Naproxen and Sumatriptan Combination 24 Hour



G1 **S-phase** **G2/M**

Various concentrations of NAP were treated in combination with SS at 500, 1750 and 3000 ug/mL. Light grey line represents toxicity, black line represents % cells in S-phase, dark grey lines represent % cells in G1 or % cells in G2/M. Dashed lines represent individual treatments whereas solid lines represent combination treatments.

(from Page 14 of Study Report)

Conclusions:
Treatment of CHO cells for 24 hrs with NAP alone dose-dependently reduced the % of cells in S phase and increased the % of cells in G1 phase and G2/M phase, while increasing cytotoxicity to a plateau of ~80% at 1000 ug/mL and above. Similar treatments with SS showed comparatively modest dose-dependent reduction of the % of cells in S phase and increase in G1 phase and (slightly) G2/M phase, associated with cytotoxicity of ~30% at 3500 ug/mL SS. The presence of 3000 ug/mL SS during the 24-hr treatment increased the dose-dependent effects of NAP on the % of cells in S phase (at 100-500 ug/mL NAP), on the % of cells in G2/M phase (at 400 and 500 ug/mL NAP), and on cytotoxicity (at 100-500 ug/mL NAP). The presence of 1750 ug/mL SS slightly increased the effects of NAP on the % of cells in S phase and G1 phase and on cytotoxicity at 200-500 ug/mL NAP, but 500 ug/mL SS had very little effect on the dose-dependent changes induced by NAP at 100-500 ug/mL.

The increased reduction in the % of cells in S phase induced by the combination of NAP and SS generally appeared to be additive.

Open-Label, Placebo-Controlled, Parallel Group Study in Healthy Volunteers to Evaluate the Effects of MT 400 Tablets or Naproxen Sodium Tablets on Chromosomal Aberrations in Peripheral Blood Lymphocytes (Study MT400-108)

(Initiated 26 OCT 2007, Completed 10 NOV 2007; conducted by POZEN, Inc., Chapel Hill, NC; GCP statement present; QA statement not present, but study report was signed by the Vice President for QA)

Forty-two non-smoking healthy volunteers aged 19-35 (22 F, 20 M) were enrolled in the study after exclusion of potential subjects with abnormal cell cycle proliferation, stable chromosomal rearrangements or abnormally high background chromosomal aberration frequencies. Subjects were split into two equal cohorts to be initiated one week apart. Subjects received a single tablet or capsule of either MT 400, naproxen sodium (550 mg), or placebo, followed ~2 hrs later by a second identical tablet or capsule, each day for 7 days. Each dose was taken with ~240 mL of water. Blood samples were collected for evaluation of chromosomal aberrations prior to initiation of dosing on Day 1, and 24 and 48 hrs after the second dose on Day 7, and shipped to [redacted] at 2-8 °C the same day for analysis within 72 hrs of collection. Assessments were not conducted on the samples collected 48 hrs after the final dose, in agreement with the FDA letter of 20 NOV 2007 stating that the 24 hr time point alone would suffice. Dosing occurred in the morning, after food, and standardized beverages, meals, and snacks were provided at appropriate times during the in-patient study. Ten subjects were selected for the primary analysis (5 M, 5 F) per treatment arm. Blood samples from Day 7 were taken at 1 and 5 hrs after the second dose and analyzed for concentration of the test compounds to confirm dosing. Safety parameters measured included vital signs (Days 0-9), ECG (Day 9), Clinical Lab Tests (Day 9), Adverse Event assessment (Days 1-9), and Serious AE assessment (Days 0-9).

Analysis of chromosomal aberrations was document in a study report issued 08 JAN 2008 by [redacted], entitled "Measurement of chromosome aberrations in human peripheral blood lymphocytes prior to and following dosing as detailed in Protocol Number MT400-108 of POZEN Inc." [redacted] Reference Number 2934/1; GSK Document Number WD2007/0208/00; POZEN Study Number MT400-108) and included as APPENDIX 1 to the clinical study report. A statement of GLP was said to be not required, though "the laboratory procedures satisfied the current requirements of the UK and OECD GLP regulations and, applicable references of the ICH GCP consolidated guideline adopted in the EU by CPMP, July 1996, issued as CPMP/ICH/135/95." A Quality Assurance statement for the [redacted] study was provided, signed 08 JAN 2008.

The pre-dose, Day 8 (24 hr postdose), and Day 9 (48 hr postdose) whole blood samples were cultured for 48 and/or 72 hrs, and processed to slides for determination of baseline peripheral blood lymphocyte chromosome aberration frequency, average generation time (AGT), and mitotic index for each subject (Note: Day 9 samples cultured for 48 and 72 hrs were analyzed for AGT only). After slide analysis of the 72-hr cultures from all pre-dose samples for AGT verified that the out-of-range values (outside AGT = 13 ± 1.5 hr)

were not associated with either treatment or cohort, 29 subjects were randomly selected for analysis of slides from Day 8 (24 hr post-dose) sample cultures (5 M & 5 F for placebo and MT 400 groups; 4 M & 5 F for the naproxen sodium group). The reason for the 4 M in the naproxen sodium group instead of the planned 5 M was that 3 of the 7 M subjects assigned to this group were excluded based on having AGT outside the accepted range of 13 ± 1.5 hr in slides prepared from 72-hr whole blood cultures of their pre-dose samples.

Two hundred metaphase lymphocytes were analyzed per subject for chromosome aberrations. Positive control slides (prepared from human peripheral lymphocytes treated with 4-Nitroquinoline 1-oxide [NQO] in a previous GLP study) were coded and inserted randomly with the experimental slides to confirm the ability of the analysts to identify positive responses. After completion of the chromosome aberration analysis, the frequency of aberrant cells per subject was categorized into those with structural aberrations including gaps and those with structural aberrations excluding gaps. Statistical analysis was performed on group mean values for pre-dose and 24-hr post-dose groups first to compare M and F, and then to compare the proportion of aberrant cells (excluding gaps) in placebo vs. MT 400 and in placebo vs. naproxen sodium groups.

Results:
The summary table below shows the results of the analysis for chromosome aberrations for the various treatment groups (Placebo, Naproxen sodium, MT 400, and positive control) at the two time points analyzed (Pre-dose on Day 1, and 24-hrs post-dose on Day 8). The high frequency of aberrant cells observed in the positive control slides (20.3% including gaps, 18.7% excluding gaps) confirmed that the analysts performing the study were able to identify positive responses. Data for both sexes were pooled within each treatment group after statistical analysis verified that there were no significant differences between M and F group mean frequencies of cells containing chromosome aberrations (excluding gaps) in any of the pre-dose or post-dose treatment groups.

No statistically significant increases in the group mean frequency of cells containing structural chromosome aberrations (excluding gaps) were observed when comparing either MT 400 or naproxen-sodium treatment groups to placebo groups, either pre-dose or after 7 days of twice-daily treatment. Group mean frequencies of cells with structural chromosome aberrations (excluding gaps) for all treatment groups, at both time points, fell within the historical range of observed values (0-5% M, 0-4% F) for standard in vitro chromosome aberration assays using human peripheral lymphocytes for the laboratory that conducted the study.

Table 7: Tabulation Summary Table

Treatment	Number of Subjects [1,2]	Pre-dose Sample			24 hour post Day 7 dose (Day 8) Sample		
		Group Mean % Aberrant Cells (incl. gaps)	Group Mean % Aberrant Cells (excl. gaps) [3]	Group Mean Mitotic Index (%)	Group Mean % Aberrant Cells (incl. gaps)	Group Mean % Aberrant Cells (excl. gaps) [3]	Group Mean Mitotic Index (%)
Placebo	5M / 5F	2.95	1.75	5.35	1.90	0.90	4.29
Naproxen sodium	4M[4] / 5F	2.50	1.06	4.58	2.50	1.17	4.27
MT 400	5M / 5F	2.70	1.25	5.64	1.70	0.95	4.85
4-Nitroquinoline 1-oxide[5]	N/A	N/A	N/A	N/A	20.3	18.7	N/A

1. 200 metaphases analysed per subject
2. M = male; F = female
3. % of cells with at least one aberration but excluding those with gaps only.
4. Data from only 4 male volunteers is presented as 3 male volunteers (from a group of 7) were excluded from the analysis in accordance with the exclusion criteria laid out in the Phase Plan.
5. Positive control slide (slides taken from previous GLP in vitro chromosome aberration studies), 187 cells analysed.

Source: Attachment 1

(from Page 38 of Study Report MT400-108)

Medication was administered in the presence of study staff, and mouth checks were performed to ensure that drug was swallowed. Bioanalysis of blood samples collected at 1 and 5 hrs post-dose on Day 7 confirmed the presence of NAP in 28 subjects and SS in 14 subjects, and neither NAP nor SS in 14 subjects.

No serious adverse events were observed. Twelve subjects (29%) reported at least one adverse event: 9/14 (64%) of the MT 400 group, 3/14 (21%) of the naproxen sodium group, and 0/14 (0%) of the placebo group. All but two of the adverse events (nausea and vomiting) were mild. No subjects withdrew from the study due to adverse events. *(See Table 9 below for listing of adverse events)*

Table 9: Clinical Adverse Events Reported by More than One Subject (Greater than 10%) in Any Treatment Group – Safety Population

System Organ Class Adverse Event	MT 400 N=14	Naproxen sodium N=14	Placebo N = 14
	n (%)	n (%)	n (%)
Subjects with at least one adverse event	9 (64)	3 (21)	0
Gastrointestinal Disorders			
Any event	4 (29)	2 (14)	0
Nausea	3 (21)	1 (7)	0
Dyspepsia	1 (7)	2 (14)	0
Nervous System Disorders			
Any event	4 (29)	2 (14)	0
Somnolence	2 (14)	1 (7)	0
General Disorders			
Any event	3 (21)	1 (7)	0
Chest Discomfort	3 (21)	1 (7)	0

Source: Section 14.3.1, Appendix 16.2.11

(from Page 41 of Study Report MT400-108)

Mean values for all clinical laboratory parameters were similar among the placebo, MT 400, and naproxen sodium groups, and no clinically significant changes were observed in post-dose compared to pre-dose values. No clinically significant changes in physical exam, vital sign, or ECG findings were reported.

Conclusions:

Treatment of healthy male and female subjects (N = 9-10/dose group) with one tablet of MT 400 or one tablet of naproxen sodium twice daily for seven days did not significantly increase the frequency of structural chromosome aberrations (excluding gaps) in peripheral blood lymphocytes collected 24 hours after the final dose, compared to placebo treatment.

The study appeared to be adequately performed.

2.6.6.5 Carcinogenicity

No carcinogenicity studies were included in this submission.

2.6.6.6 Reproductive and developmental toxicology

No reproductive and developmental toxicology studies were included in this submission.

2.6.6.7 Local tolerance

No local tolerance studies were included in this submission.

2.6.6.8 Special toxicology studies

No special toxicology studies were included in this submission.

2.6.6.9 Discussion and Conclusions

See Overall Conclusions and Recommendations.

2.6.6.10 Tables and Figures

Tables and Figures were included within the text.

2.6.7 TOXICOLOGY TABULATED SUMMARY

SPONSOR'S RESPONSE TO ISSUES IN 01 AUG 2007 APPROVABLE LETTER

(FDA comments from the 01 AUG 2007 Approvable Letter appear in bold italics, followed by the sponsor's response. Reviewer's comments appear in non-bold italics.)

We acknowledge that you have performed, as we had requested in our Approvable letter of June 8, 2006, a repeat in vitro chromosomal aberration assay in CHO cells, as well as an in vitro mouse lymphoma tk assay (MLA). We further acknowledge that the MLA was negative for sumatriptan and naproxen alone and in combination, up to the highest concentrations tested. We do note, however, that the results for naproxen alone in this study are at odds with the positive findings in the presence of metabolic activation, at lower concentrations, obtained in an earlier MLA conducted to support [redacted] [redacted] The reasons for these discrepant findings are not clear, and we ask that you address this issue.

The primary objective of the additional mouse lymphoma TK+/- assay reported in the January 31, 2007 Full Response, Amendment #16 (GSK Doc. WD2006/03038/00) with naproxen sodium (NAP) and sumatriptan succinate (SS) individually and in combination (1:1 ratio), was to further investigate potential synergistic genotoxic effects of the combination in an alternative *in vitro* assay (also capable of the detection of clastogenicity as an endpoint of genetic damage). The NAP/SS (1:1) concentrations employed were selected to comply with current guidelines on cytotoxicity (10-20% relative total growth (RTG)) and investigate concentration-related responses and, in particular, any potentiation of genotoxic activity. The discrepancies in the outcome of the repeated study compared with the earlier mouse lymphoma TK+/- assays (MT100-T26, T32) are due to one (or a combination) of the following explanations:

A. The highest concentration of each component tested individually was limited to those producing the required levels of cytotoxicity in the combination treatment with NAP/SS i.e., 1700 ug/mL for NAP. Thus, in the NAP arm of the repeated study (with S9 activation) the highest concentration tested only reduced RTG to 59% and was not excessively cytotoxic. In contrast, the positive findings with NAP in the earlier study (at concentrations of 150 & 300 ug/mL) reduced total growth to 32% and 12%, respectively, indicating significant toxicity, albeit at concentrations which were much lower than those used in the GSK study (1700 ug/mL).
B. **There were differences in duration of the "short treatment arms" of the mouse lymphoma TK+/- assay** conducted at GSK [redacted] At GSK, a 3 hr treatment arm (with and without S9 activation) is the standard study design, whereas [redacted] used a 4 hr treatment arm (with S9 activation) in the earlier study.
C. As L5178Y mouse lymphoma cells have relatively short cycling times (~8-10 hrs), the 25% increase in treatment duration and increased NAP exposure likely contributes to the increased cytotoxicity and associated positive findings in the earlier [redacted] study. Moreover, since the repeated GSK mouse lymphoma TK+/- assay (WD2006/03038/00) was designed to investigate the genotoxic potentiation of the NAP/SS combination, the assessment of NAP alone was not a primary objective. The repeated GSK mouse lymphoma TK+/- assay was GLP/OECD compliant for the combination (i.e., the primary objective) and the positive controls induced the expected increase in mutant colonies, confirming the validity of this assay. The contribution of inter-laboratory variation, based on cell phenotype, passage number, compound batches, etc., on the contradictory outcome of the two studies also cannot be excluded.
D. Finally, false positive *in vitro* findings in general are not uncommon in standard *in vitro* mammalian genotoxicity assays, especially at highly toxic exposure levels, and are considered by many to be unacceptably high [Kirkland et al., 2005, 2007; Thybaud et al., 2007a, b].

Reviewer's Comment:
The sponsor's points are valid, and adequately address this issue.

Of far greater concern, however, is the finding of a synergistic effect in the in vitro chromosomal aberration assay in CHO cells. Specifically, in this study, sumatriptan and naproxen alone were negative, both in the presence and absence of metabolic activation; however, the combination produced a concentration-related increase in the percentage of cells with aberrations, both with and without metabolic activation.

Cytotoxicity was expressed as reductions in mitotic index (% Mitotic Inhibition) and cell count (% Reduction in Cell Count), as well as in population doubling (% Population Doubling Inhibition). Current guidelines (OECD, ICH) indicate that % reduction in cell count is the most appropriate measure of cytotoxicity for this assay. Population doubling has been proposed as an alternative measure (Greenwood SK et al. Environ Mole Mutagen 43:36-44, 2004); however, it has not been accepted as a more valid or more appropriate measure of cytotoxicity and should not be used to dismiss the positive responses observed.

Section 15 of the OECD guidelines (OECD 473 *In vitro* mammalian chromosome aberration test) states *"Cytotoxicity should be determined with and without metabolic activation in the main experiment using an appropriate indication of cell integrity and growth, such as degree of confluency, viable cell counts, or mitotic index. It may be useful to determine cytotoxicity and solubility in a preliminary experiment."*

Since population doubling (PD) is a measure of the degree of confluency, PD was used in the repeat *in vitro* CHO assay [January 31, 2007 Full Response, Amendment #16; GSK Doc. WD2006/03218/00] to provide a scientifically appropriate estimate of cytotoxicity. It is recognized that testing at excessively toxic concentrations often leads to artifactual positive results and subsequent misinterpretations of the data (Kirkland, 1992; Galloway, 2000, Jacobson-Kram and Jacobs, 2005; FDA, 2006). As such, the accurate assessment of cytotoxicity is critical for assay interpretation. Cytotoxicity in cultured mammalian cells is a function of the methods used to estimate it [Fellows and O'Donovan, 2007]. Although PD is clearly an appropriate index (see next section below), the association between DNA synthesis inhibition, clastogenicity and cytotoxicity is evident whichever cytotoxicity index is used (PD, MI, or cell counts) and is a consequence of alterations in cell cycle that would not occur at concentrations seen in patients under conditions of clinical use.

Reviewer's Comment:
This reviewer acknowledges that the issue of positive genotoxicity findings only at "excessively toxic" test article concentrations is currently an active area of research and debate. However, there is not yet consensus regarding the definition of "excessively toxic," and the current (1997) OECD guideline for the in vitro mammalian chromosome aberration test suggests that "the highest concentration should show a significant reduction in degree of confluency, cell count or mitotic index (all greater than 50%)." While population doubling (PD) may eventually be considered an appropriate measure of cytotoxicity, it is not equivalent to "degree of confluency," as suggested by the sponsor, though it may be related to confluency. Greenwood et al., 2004, in their paper promoting PD as a better method to assess cytotoxicity/cytostasis, stated, "We have also pointed out that using final cell counts and/or confluence as a percentage of control to assess toxicity can underestimate toxicity..."

In the absence of metabolic activation (S9), significant increases in the % of cells with chromosomal aberrations were obtained at concentrations of naproxen and sumatriptan in combination associated with 50-68% reductions in cell count. This degree of cytotoxicity is consistent with that recommended for the highest concentrations in this assay (ICH, OECD guidelines). In the presence of S9, increases in the % of cells with chromosomal aberrations were obtained at concentrations associated with only 2-52% decreases in cell count. It is notable that naproxen (at 2500 µg/mL) was negative in the presence of S9, whereas the combination of naproxen and sumatriptan (at 1745/1745 µg/mL) was positive, at the same degree of cytotoxicity (42% reduction in cell count); therefore, the positive response with the combination cannot be explained by a greater cytotoxic effect.

In our view, these findings cannot be dismissed, for the following reasons:

(a) Positive findings in the repeat in vitro CHO assay were not associated with excessive cytotoxicity and, as noted above, naproxen alone at a concentration producing a similar degree of cytotoxicity (as measured by reduction in cell count) was negative.

(b) Although it is true that the other in vitro and the in vivo genetic toxicology assays were negative, there is no apparent basis for dismissing a reproducible positive signal in one component of the standard battery of genetic toxicology assays based solely on negative findings in other assays comprising the battery.

Comparisons of the cytotoxicity of NAP, SS and the NAP/SS combination based on PD and cell counts in CHO cells are shown in Figure 1. The levels of cytotoxicity determined by inhibition of PD clearly diverge from those determined by cell counts with increasing concentrations and are significantly elevated for the NAP/SS combination (both in the absence and presence of S9 activation), at the highest concentrations tested. This is also true for NAP in the presence of S9 activation, but not in the absence of S9. In contrast, for SS the levels of cytotoxicity are very similar using either method (PD or cell counts), independent of S9-activation.

Positive findings in the repeat *in vitro* CHO assay were associated with excessive cytotoxicity. The cytotoxicity of the NAP/SS combination in CHO cells based on PD and cell counts along with those concentrations resulting in statistically significant ($p<0.05$) increases in chromosomal aberration (CA), both in the absence and presence of S9, are shown in Figure 2. Using the PD metric, which we believe to be the most appropriate given the new findings regarding cell cycle disturbances with the NAP/SS combination (see next section), the concentrations of NAP/SS that induce CA in the CHO cell assay (highlighted in red circles) can not be separated from those that induce excessive cytotoxicity. If cell counts are used, the level of cytotoxicity is within the guideline prescribed limits i.e., 50-60% reductions in cell count (ICH, OECD guidelines), but they clearly approach the uppermost guideline limit, and do not account for the cytotoxic (cell killing) and cytostatic effects (in this case, S-phase inhibition) induced by the combination in CHO cells at high concentrations. However, and perhaps more importantly, the NAP/SS combination did not induce CA at moderate levels of cytotoxicity (40-50% by PD and 30-40% by cell counts). This is important **because the IWGT expert panel (including the FDA's David Jacobson-Kram) concluded that genotoxicants** producing positive clastogenicity results at high levels of toxicity, but negative at moderate levels, and also negative in other tests examining the same endpoint (i.e., the MLA), are of "low or no concern" and should not trigger additional testing (Thybaud *et al.*, 2007a).

As per the Agency's suggestion, we have re-evaluated the conduct of the *in vitro* chromosomal aberration assay and found that the apparent synergistic effect is a result of assay conditions that can only exist *in vitro*. NSAIDs, including NAP have been reported to reduce cell proliferation, induce G1 arrest and inhibit DNA synthesis (S-phase) in various cell lines (Klein, 1975; Shiff, 1996; Brooks, 2003) indicating a potential to disturb the cell cycle *in vitro*. Studies on the genotoxicity of naturally occurring indoles show **that 3-methylindole and melatonin induce CA in CHO cells, but only at cytotoxic concentrations (1–10** mM) associated with marked inhibition of DNA synthesis. Interestingly, concurrent studies with serotonin and tryptamine showed that neither compound induced CA despite producing significant inhibition of DNA synthesis and slight to moderate cytotoxicity [Reddy et al., 2002]. Thus, both NSAIDs and indole compounds have the potential to inhibit DNA synthesis and disturb the cell cycle in CHO cells, particularly at high treatment concentrations. This is particularly important as increases in clastogenicity via indirect genotoxic effects are associated with cell cycle perturbances at highly cytotoxic concentrations in CHO cells. Moreover, rodent cell lines are particularly sensitive to cytotoxicity related chromosomal damage (compared with human cells) because of relaxed cell cycle regulation and the sensitivity of CHO cells therefore make them more susceptible to artifactual increases in chromosomal damage [Hilliard et al., 2007; Kirkland et al., 2007].

GSK has recently investigated the effect of the NSAID NAP and the indole SS, both in isolation and in combination, on the cell cycle of CHO cells *in vitro*. When CHO cells were treated with NAP and SS in a 1:1 combination [GSK Doc. WD2007/01560/00], there was concentration-dependent potentiation of NAP-induced DNA synthesis inhibition by SS following both 3 hour and 24 hour treatments (Figure 3). DNA

synthesis inhibition was absolute in CHO cells after 3 hours treatment with NAP/SS (1:1) at concentrations of 1270 ug/mL or more, as evidenced by a total loss of cells in S-phase. Importantly, the concentrations resulting in total DNA synthesis inhibition were significantly below those associated with the induction of structural chromosomal aberrations in CHO cells treated with the 1:1 combination (i.e. >1710 ug/mL). Moreover, the inhibition of DNA synthesis clearly preceded the effect on cell counts. This is consistent with the interpretation that the cytotoxic/clastogenic effects observed with high concentrations of the combination are the result of cell cycle perturbances (e.g., DNA synthesis inhibition and cell cycle arrest) in CHO cells, and that DNA synthesis inhibition occurs prior to the clastogenic/cytotoxic effects observed at higher concentrations and/or later time points.

In comparison, treatment of CHO cells for 3 hours with naproxen alone induced concentration-dependent G1 and G2/M arrest and extensive DNA synthesis inhibition, consistent with previous observations with NSAIDs. Again, the DNA synthesis inhibition preceded the extensive cytotoxicity observed in later 24-hour cultures. In contrast, sumatriptan induced only mild cell cycle effects on G1 and DNA synthesis inhibition, and no effect on G2/M following either 3 or 24-hour treatments (Figure 4).

Previous investigations by GSK indicated that the cell cycle effects seen in CHO cells with NSAIDs are a class effect [GSK Doc. WD2007/01420/01]. All the NSAIDs tested (NAP, diclofenac, ibuprofen, indomethacin, piroxicam and sulindac) induced concentration-dependent G1 (except for piroxicam) and G2/M arrest and extensive DNA synthesis inhibition (Figure 5) and this was associated with significant cytotoxicity in CHO cells (determined by cell counts). In contrast, the indole SS, induced only mild G1 and G2/M arrest and DNA synthesis inhibition at the highest concentrations tested (Figure 6). Tryptamine caused an accumulation of cells in S-phase, but only at the highest concentration tested, whereas serotonin had no effect on the cell cycle of CHO cells (although exposures were limited by solubility in DMSO). These new studies confirmed that NSAIDs and some indoles disturb the cell cycle of CHO cells *in vitro*.

When CHO cells were treated with the combination of NAP/SS there was concentration-dependent potentiation of DNA synthesis inhibition, evidenced by a further ~28% decrease in the number of cells in S-phase at the maximum concentration tested compared with each component alone, and this was associated with a synergistic increase in cytotoxicity [GSK Doc. WD2007/01476/00 and figures therein]. Similarly, treatment of CHO cells with diclofenac (NSAID) and tryptamine (indole) resulted in concentration-dependent potentiation of DNA synthesis arrest (approx. 90% of cells accumulated in S-phase), compared with each component alone, and this was also associated with a synergistic increase in cytotoxicity. These new data provide evidence that high *in vitro* concentrations of NSAID/indole combinations synergistically disrupt the cell cycle of CHO cells and increase cytotoxicity.

It is well-known that chromosome aberrations are often associated with cytotoxicity and DNA synthesis inhibition [Galloway et al., 1998; Hilliard et al., 1998] and that chromosome aberrations can be induced *in vitro* by compounds that do not directly damage DNA (NAP, SS and the combination of NAP/SS were negative in the Ames test [MT400-T06] and mouse lymphoma TK+/- assay [GSK Doc. WD2006/03038/00] and do not trigger any DEREK structural alerts for genotoxicity). It is also recognised that these chromosome aberrations may occur under conditions that would not be relevant *in vivo* [Kirkland and Müller, 2000].

Additionally, CHO cells uniquely continue to undergo cellular growth during DNA synthesis inhibition and the degree of aberrant growth is directly related to the degree of subsequent cytotoxicity and cell death [Kung et al., 1993], implying that the mechanisms for clastogenicity and cytotoxicity are intrinsically linked in CHO cells. Given that NSAID/indole combinations potentiate the inhibition of DNA synthesis, this in turn accounts for the cytotoxicity/clastogenicity effects observed with high concentrations of the NAP/SS combination. Under these conditions, the inhibition of PD is considered the most appropriate metric for cytotoxicity in the repeat *in vitro* CHO assay [WD2006/03218/00] since PDs more appropriately account for cytotoxic (cell killing) and cytostatic effects (in this case, S-phase inhibition and G2/M arrest) compared with cell counts alone. Equally, the proposed mechanism described above would also account for the concentration-dependent decrease observed in mitotic inhibition.

In summary, we have considered the underlying mechanisms likely to be responsible for the clastogenicity observed with the NAP/SS combination in CHO cells compared with treatment with NAP alone. These new data (Section 4.2.3.3.1) demonstrate that NSAID/indole combinations, and naproxen sodium/ sumatriptan succinate (NAP/SS) specifically, potentiate the inhibition of DNA synthesis by naproxen, which in turn accounts for the cytotoxic/clastogenic effects observed with high concentrations of the NAP/SS combination in CHO cells. As such, they represent an artifactual observation as the increase in clastogenicity was the result of an indirect genotoxic effect associated with cell cycle perturbance at highly cytotoxic concentrations in CHO cells *in vitro*. Since the NAP/SS cell cycle study demonstrated potentiation of DNA synthesis inhibition in CHO cells, POZEN and GSK conclude that the therapeutic use of sumatriptan/naproxen sodium tablets would not represent a genotoxic or a carcinogenic risk to humans.

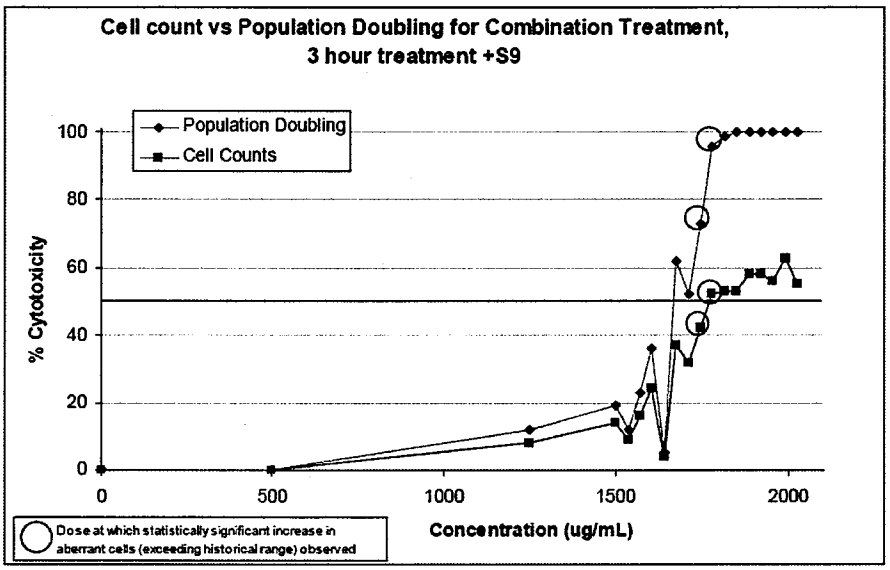
Reviewer's Comment:
The sponsor stated that "Positive findings in the repeat in vitro CHO assay were associated with excessive cytotoxicity," apparently as defined by induction of greater than 60% inhibition of PD (see Sponsor's Figure 2 below). In the CHO cell chromosome aberration assay WD2006/03218/00 (submitted in NDA21-926 #016), the % of cells with structural aberrations (excluding gaps) was significantly increased at 1815/1815 ug/mL NAP/SS (in the absence of S9 activation), associated with 11% inhibition of Mitotic Index (MI), 50% reduction in cell count, and 65% inhibition of PD; and at 1745/1745 ug/mL NAP/SS (in the presence of S9), associated with 26% inhibition of MI, 42% reduction in cell count, and 73% inhibition of PD. Therefore, if inhibition of PD greater than 60% is to be considered the definition of "excessive cytotoxicity," then the study should be considered to be negative for clastogenicity. However, as stated previously, PD has not yet been accepted as a more valid or more appropriate measure of cytotoxicity for this assay.

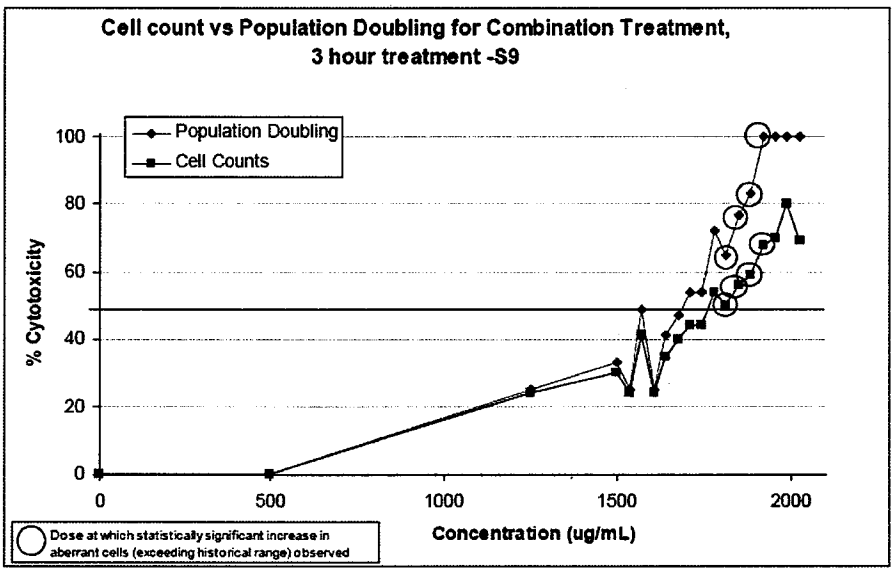
The sponsor demonstrated that NAP alone, SS alone, and the NAP/SS combination dose-dependently reduced the % of CHO cells in S phase (equivalent to inhibition of DNA synthesis in this assay) after 3 or 24 hrs of treatment; the % of cells in S phase was reduced from 61% in controls to near zero after 3 hrs at 1250/1250 ug/mL NAP/SS in one experiment, associated with 96.9% relative survival and 22.7% inhibition of PD (see Table 4, page 23 of Study Report V27862, NDA 21-926 #025). In a separate study (Study V2836), NAP/SS reduced the % of CHO cells in S phase more than NAP or SS alone after a 24 hr treatment. Whether this additive effect is considered "potentiation," "synergism," or something else, is not that important. Clearly, the combination of NAP and SS can profoundly perturb the cell cycle at concentrations well below those inducing cytotoxicity and clastogenicity.

The sponsor argued that the clastogenicity induced by high concentrations of NAP/SS were likely to be indirectly caused by mechanisms related to cytotoxicity and/or DNA synthesis inhibition rather than to direct effects on DNA. In support of this argument, the sponsor cited publications by Galloway et al., 1998, and Hilliard et al., 1998, reporting that some clastogenic compounds that did not directly damage DNA were associated with cytotoxicity and/or DNA synthesis inhibition. The sponsor also cited Kung et al., 1993, suggesting that CHO cells may be especially vulnerable to DNA synthesis inhibition-related clastogenicity because "they continue to undergo cellular growth during DNA synthesis inhibition and the degree of aberrant growth is directly related to the degree of subsequent cytotoxicity and death."

*This reviewer considers it reasonable to conclude that the profound effects of NAP/SS on the cell cycle **may** contribute to the clastogenicity observed at very high concentrations (≥ 7.6 mM NAP; ≥ 5.9 mM SS) of this combination in CHO cells. However, it is not clear that this proposed link between effects on the cell cycle and chromosome aberrations is widely accepted in the field. Also, the sponsor's argument would have been stronger if a direct correlation between effects on the cell cycle and induction of chromosomal aberrations had been demonstrated in the same experiment.*

Figure 2. The cytotoxicity of Naproxen:Sumatriptan (1:1) in CHO cells based on PD and cell counts.





(c) We acknowledge that sumatriptan was negative in carcinogenicity studies in mouse (78-week) and rat (104-week) and that naproxen was negative in a 2-year carcinogenicity study in rats (8-24 mg/kg/day) and, in combination with metoclopramide, in a 26-week p53 transgenic mouse assay (50 mg/kg). However, none of these studies tested the combination of sumatriptan and naproxen. In our opinion, rather than lessening the concern, it is the lack of a signal for carcinogenicity in these studies that heightens the concern regarding a possible synergistic effect of the combination of sumatriptan and naproxen. (It is of note that, due to the sensitivity of the rodent to the gastrointestinal effects of NSAIDs, naproxen could not be evaluated in any of the carcinogenicity studies at more than a fraction of clinically relevant doses or plasma exposures.)

The results of this study raise the possibility that the combination may be carcinogenic. We believe that you must adequately address this concern prior to the application being approved. We acknowledge that, were the application to be approved, the typical patient would not administer the drug daily; however, acute migraine treatments can be administered frequently, and for many years.

For this reason, we consider an adequate assessment of carcinogenicity critical prior to the approval of any acute migraine treatment.

The *in vitro* assay for structural chromosome aberrations in mammalian cells is a cytogenetic test for DNA damage (clastogenicity), which is often conducted as part of the genotoxicity test battery designed to assess the potential genotoxic hazard of pharmaceutical drug candidates. However, an appreciation of whether DNA is the primary target of attack by a test compound is fundamental to interpreting results from this assay for risk assessment in humans. When a compound or its metabolites interact with DNA directly, i.e., by covalent binding or intercalation, then it may be considered a DNA reactive clastogen. In other words, the mechanism of action is primary DNA damage. However, if the main target of a compound is not DNA, but rather the processes involved in DNA metabolism, then this is considered secondary DNA damage. This may occur, for example, through nucleoside pool imbalance, incorporation of abnormal DNA precursors (nucleoside analogues), or inhibition of enzymes that are essential for normal DNA synthesis, processing, and repair or cell cycle control (Galloway et al., 1998; Greenwood et al., 2004; Kirkland et al., 2007; Thybaud et al., 2007a, b).

For non-DNA reactive (e.g., Ames negative) compounds, positive genotoxic responses in the *in vitro* mammalian cell chromosome aberration assay occurring only at highly cytotoxic or high treatment concentrations follow a threshold relationship [Kirkland and Müller, 2000]. As such, a threshold relationship for the combination of NAP/SS is supported by the effect on cell cycle observed at high treatment concentrations (i.e., NAP/SS induces S-phase inhibition). The Cmax values for naproxen and **sumatriptan from a single therapeutic dose of TREXIMA™ are 57.9 ug/mL and 56.0 ng/mL, respectively,** which are significantly below the cytotoxic concentrations associated with the indirect clastogenic response in CHO cells *in vitro* (1710/1710 ug/mL NAP/SS). This represents an overage in terms of human Cmax of ~30 fold for naproxen (1710/57.9) and ~30,000 fold for sumatriptan (1710/0.0560) and thus ~30,000 fold for the NAP/SS combination. Moreover, the human plasma levels of the combination indicate a therapeutic ratio of NAP 1000:1 SS; i.e., the ratio of sumatriptan to naproxen is several orders of magnitude below those observed to elicit the high concentration potentiation of naproxen associated DNA synthesis inhibition and resultant cytotoxic/clastogenic effects *in vitro* described in the previous section. Indeed, a NAP/SS ratio of 1:1 would not be clinically feasible with the proposed therapeutic doses of NAP/SS in humans.

For DNA reactive substances, the use of safety margins in the context of genotoxicity is not appropriate. However, the NAP/SS combination has been adequately shown to be non-DNA reactive (Ames and MLA negative), but rather, affects intracellular processes (e.g., DNA synthesis inhibition and cell cycle arrest) which follow a threshold response and thus the use of safety margins is appropriate, and is well-accepted in the genetic toxicology literature [Kirkland 2000; Galloway 1998].

In addition, the occurrence of CA in CHO cells is not a reliable predictor of carcinogenicity. Recent evidence suggests that rodent cell lines, and in particular Chinese hamster cells, produce more positive

results in the *in vitro* structural chromosome aberrations assay compared with human lymphocytes [Hilliard et al., 2007; Kirkland et al., 2007]. It is also known that there are significant differences between mammalian cell lines in response to the inhibition of DNA synthesis, and that CHO cells are more susceptible to the cytotoxic effects associated with cell cycle disturbances compared with human cells [Kung et al., 1990a, b, and 1993; Schimke et al., 1991]. This can be attributed to the more stringent cell cycle checkpoint controls in human cells (particularly in cells with a functional p53 protein, such as primary lymphocytes) which prevent damaged cells reaching mitosis. This may also account for the increased sensitivity of rodent cells in cytogenetic assays compared with human cells [Hilliard et al., 2007; Kung et al., 1990a, b and 1993; Kirkland et al., 2007]. As such, there is concern that rodent cells may be oversensitive in terms of hazard characterisation of potential genotoxic carcinogens. Consistent with this are the conclusions of [Kirkland et al., 2005, 2006] **who demonstrated that 75–95% of non-carcinogens** were positive in one or more of the standard *in vitro* genotoxicity assays and that the false positive rate was highest in mammalian cell tests such as the chromosome aberration assay in Chinese hamster cells. These data, subsequently confirmed by Matthews et al., [2006 a, b] using FDA and EPA databases, demonstrate that positive results from *in vitro* mammalian genotoxicity assays do not accurately predict rodent carcinogenicity or human carcinogenic risk.

Finally, positive results should not be considered in isolation, and a weight-of-evidence approach considering all pertinent data is the preferred approach [Jacobson-Kram and Jacobs 2005, FDA 2006; Thybaud et al., 2007a; Kirkland et al., 2007]. As part of this weight-of-evidence approach, information on the mode of action, kinetics, and the extent of human exposure is useful for risk assessment [Thybaud et al., 2007b]. Therefore, based on the scientific rational described above and taking all of the genotoxicity data for the NAP/SS combination into consideration, together with the intended human clinical exposure, the weight-of-evidence indicates that the combination of NAP/SS does not represent a biologically relevant genotoxic risk to humans. Furthermore, given the poor concordance of the *in vitro* mammalian genotoxicity assays with respect to predicting carcinogenic risk, particularly when used in isolation, and based on all of the arguments presented above, we conclude that the therapeutic use of NAP/SS does not represent a carcinogenic risk to patients.

Reviewer's Comment:
This reviewer considers it reasonable to conclude that, if the NAP/SS-induced clastogenicity resulted from non-direct DNA damage, then the large safety margins substantially reduce the risk to humans. However, once again, this reviewer believes that stronger evidence could have been provided (e.g., demonstration that DNA damage correlated in time and dose with the effects on the cell cycle).

It appears to us unlikely that conducting additional in vitro or in vivo genetic toxicology studies would provide data that could be used to adequately address our concern about the positive finding in the in vitro CHO cell assays. It is also unlikely that lifetime carcinogenicity studies or shorter-term studies in transgenic animals (e.g., p53, TgHras2) would provide meaningful data, specifically because of the sensitivity of rodents to naproxen. It might be possible, however, to conduct a study in humans to assess the clastogenic potential of naproxen alone and in combination with sumatriptan. A number of studies have been published on the evaluation of clastogenic and/or mutagenic effects in circulating lymphocytes in various populations (e.g., smokers, industrial workers, military personnel). Studies have also been conducted in patients on therapeutic doses of various medications. For example, Saxena and Ahuja (Saxena R, Ahuja YR. Hum Genet 62(3):198-200, 1982) reported a significant increase in patients treated with thioridazine for 4 weeks. Ahuja et al. (Ahuja YR et al. Arzneimittelforschung 34(6):699-701, 1984) reported increases in chromosomal aberrations in patients on therapeutic doses of haloperidol. More recently, studies have been conducted to assess the effects of therapeutic doses of methylphenidate on circulating lymphocytes in children (El-Zein et al. Cancer Lett 230(2):284-291, 2005; Walitz S et al. Environ Health Perspect 115:936-940, 2007). Although we admit that the interpretation of a positive finding in such a study is not entirely clear, we do believe that the results of such a study would provide useful additional information that would affect our decision about the approvability of this combination.

In lieu of conducting such a clinical trial, you could also re-evaluate the conduct of the in vitro chromosomal aberration assays to investigate, for example, whether or not the apparent synergistic effect is an artifact of assay conditions.

Our investigations described above have demonstrated that an artifact involving cell cycle distortions with the NAP/SS combination does exist and accounts for the increased cytotoxicity and clastogenicity observed in CHO cells. Furthermore, these *in vitro* effects were only seen at high concentrations and with NAP/SS ratios that would not be clinically feasible with the proposed human therapeutic dose of NAP/SS. Given the additional preclinical data together with the attached specific responses to comments in the August 1, 2007 **Approvable letter, we believe we have addressed the Agency's concerns on this issue and that a cytogenetic** evaluation of NAP/SS in humans is not required.

Reviewer's Comment:
The proposed study of the clastogenic potential of naproxen alone and in combination with sumatriptan in humans has been completed, and it was negative. While the nonclinical evidence presented suggests that the positive findings in the CHO chromosome aberration assay may be due to additive or synergistic effects of NAP and SS on the cell cycle (indirectly leading to DNA damage), this reviewer does not consider that the evidence has shown the clastogenicity to be "an artifact of assay" conditions.

Sponsor's Summary and Conclusions:

- Differences in interpretation of data between the Agency and sponsor have been identified with regard to genotoxicity studies conducted with naproxen alone, sumatriptan alone and naproxen/sumatriptan in combination. The Agency is concerned that the data suggest a potential for a synergistic genotoxic effect of the combination. The sponsor believes that the observed effect is explained by alterations in cell cycle that would not occur at concentrations or NAP/SS ratios occurring in patients under conditions of clinical use.
- Explanations as to the reason for these different interpretations, including new data to further support **our interpretation, are provided in this Complete Response to the Agency's August 1, 2007 Approvable** letter on this topic.
- These new data (Section 4.2.3.3.1) demonstrate that NSAID/indole combinations, and naproxen sodium/sumatriptan succinate (NAP/SS) specifically, potentiate the inhibition of DNA synthesis by naproxen, which in turn accounts for the cytotoxic/clastogenic effects observed with high concentrations of the NAP/SS combination in CHO cells. As such, they represent an artifactual observation as the increase in clastogenicity is the result of an indirect genotoxic effect associated with cell cycle perturbance (DNA synthesis inhibition) at highly cytotoxic concentrations in CHO cells *in vitro*. The concentrations capable of producing this indirect effect do not occur in humans exposed to clinical (or higher) doses *in vivo*.
- The CHO cell line is particularly sensitive to cytotoxicity related chromosomal damage because of relaxed cell cycle regulation and historically has been a poor predictor of rodent carcinogenicity and human carcinogenic risk.
- We have evaluated cytotoxicity using both population doubling (PD) and mitotic index (MI) as indices of toxicities. We believe that the inhibition of population doubling (PD) method is the most appropriate method for assessing cytotoxicity in the repeat *in vitro* CHO assay, since PDs more appropriately account for cytotoxic (cell killing) and cytostatic effects (in this case, S-phase inhibition) compared with cell counts alone. However, the proposed mechanism described above would also account for the concentration-dependent decrease observed in mitotic inhibition equally well.
- Regardless of how cytotoxicity is measured, these effects only occur at concentrations that are unachievable in humans after acute or chronic exposure (Cmax: safety margins of 30,000x for the NAP/SS combination).
- The NAP/SS combination has been adequately shown to be non-DNA reactive (Ames and MLA negative), but rather, affects intracellular processes (e.g., DNA synthesis inhibition and cell cycle arrest), which follow a threshold response. Thus, the use of safety margins is appropriate, and is well-accepted in the genetic toxicology literature.

- Based on this new evidence indicating that the NAP/SS combination induces cell cycle disturbances leading to exaggerated CHO cell cytotoxicity and subsequent appearance of CHO cell chromosomal aberrations *in vitro*, we conclude that the therapeutic use of sumatriptan 85 mg/naproxen sodium 500 mg as Trexima would not represent a genotoxic or a carcinogenic risk to humans.

OVERALL SUMMARY AND RECOMMENDATIONS:

In the original NDA submission, an in vitro chromosomal aberration assay in CHO cells (Study MT400/T07, #0735/0736-3110) demonstrated greater clastogenic effects with the combination of naproxen and sumatriptan compared with naproxen alone, raising the concern that the two compounds together may have carcinogenic effects not observed with either drug alone. The clastogenic effects were observed only at concentrations producing substantial cytotoxicity, making the biological significance of the effects unclear.

In the approvable letter dated 08 JUN 2006, the sponsor was asked to attempt to clarify this issue by repeating the chromosomal aberrations assay testing concentrations of the 1:1 NAP/SS combination between those exhibiting minimal toxicity (1250/1250 ug/mL) and those inducing substantial toxicity (2500/2500 ug/mL without S9 activation, and 2000/2000 ug/mL with S9 activation), and by conducting an in vitro mouse lymphoma tk assay testing naproxen and sumatriptan alone and in combination.

Amendment #016, the complete response to Approvable Letter, was submitted on January 31, 2007, contained final reports from the requested genotoxicity studies with NAP and SS alone and in combination at 1:1

In the new assay for chromosomal aberrations in CHO cells, the frequency of cells with structural aberrations was increased dose-dependently by NAP/SS at concentrations that reduced the total cell count by 50-68% in the absence of S9 metabolic activation (1815/1815 to 1920/1920 ug/mL), and by 42-52% in the presence of S9 (1745/1745 to 1780/1780 ug/mL). Neither NAP nor SS alone at the same concentrations resulted in significant clastogenicity. NAP alone was also negative at the highest concentration tested, 2500 ug/mL, which is equivalent to ~10.9 mM, exceeding the recommended maximum concentration of 10 mM (Note: these calculations are based on MW = 230.266 for naproxen free acid, and 295.406 for sumatriptan free base). These data demonstrated a synergistic clastogenic effect of the combination of the two drugs at concentrations greater than or equal to ~7.6 mM NAP and ~5.9 mM SS, associated with reductions in cell number of $\geq$ 42%. NAP and SS also had synergistic effects on cytotoxicity at these concentrations. The sponsor argued that using reduction in cell count underestimated the cytotoxicity, and that this assay would have been considered negative if inhibition of population doublings (PD) were used instead.

In the new in vitro mouse lymphoma assay, the 1:1 NAP/SS combination was not genotoxic at concentrations up to those inducing cytotoxicity within (or close to) the **desired range of 10-20% Relative Total Growth (1450/1450 ug/mL after 3 hrs –S9; 1700/1700 ug/mL after 3 hrs +S9; and 400/400 ug/mL after 24 hrs –S9).**

The original NDA submission for Treximet included negative results for a valid bacterial reverse mutation assay (up to 2500 ug/plate NAP/SS) and for a valid in vivo mouse micronucleus assay (up to an MTD of 500/1500 mg/kg NAP/SS (M) or 375/1625 mg/kg

(F)). Negative carcinogenicity studies are described in the current labeling for SS (Imitrex; rat and mouse) and NAP (Anaprox; rat). In addition, the current sponsor conducted a two-year rat study to support [redacted] that demonstrated no increases in neoplasms in rats receiving NAP alone at the MTD of 8 mg/kg/day. Finally, a 26-week carcinogenicity study in $p53^{+/-}$ mice was negative in mice given 50 mg/kg/day NAP in combination with 50 or 1.6 mg/kg/day metoclopramide (see [redacted]

In the Approvable Letter of August 1, 2007, the sponsor was asked:

1. to explain why NAP alone was not genotoxic in the new mouse lymphoma assay at up to 1700 ug/mL (+S9), when a previous mouse lymphoma assay had shown dose-dependent increases in mutation frequency at 50, 150, and 300 ug/mL NAP alone after 4-hr treatments with S9;
2. to conduct a study of the clastogenic potential of naproxen alone and in combination with sumatriptan in humans; or to provide compelling evidence that the apparent synergistic clastogenic effect of NAP and SS in CHO cell cultures was an artifact of assay conditions

The current Complete Response to Approvable Letter (submitted October 11, 2007 at Amendment #025) contained the following items:

- a detailed explanation of the factors that might account for the discrepancy in the mouse lymphoma assay results with naproxen sodium
- three nonclinical studies evaluating the effects of NAP and SS (2 studies) or other NSAIDs and indoles (tryptamine and serotonin) on the cell cycle in CHO cell cultures
- a study of the clastogenic potential of naproxen alone and in combination with sumatriptan in humans (submitted as Amendment #026 on January 11, 2008)

The sponsor explained that the positive findings with NAP in the earlier [redacted] mouse lymphoma forward mutation at 150 and 300 ug/mL occurred were associated with greater cytotoxicity (relative total growth, RTG, was reduced to 32% and 12%, respectively) compared with that observed at 1700 ug/mL NAP in the new GSK study (RTG was reduced to 59%). Other differences in the two studies conducted in different laboratories that might account for the discrepancy in the results include a longer treatment time (4 hrs in the [redacted] study vs. 3 hrs in the GSK study), cell phenotype, passage number, or batch of test article. These arguments seem reasonable to this reviewer.

GSK Study #WD2007/01420-01 (V27824) demonstrated that treatment of CHO cells with NSAIDs (diclofenac, ibuprofen, indomethacin, piroxicam, and sulindac) for 6 or 24 hrs resulted in dose-dependent effects on the cell cycle: the % of cells in S phase was decreased, and the % of cells in G1 and G0/M phases was increased. Tryptamine showed the opposite effect, increasing the % of cells in S phase, and decreasing the % of cells in G1 and G0/M phases. Serotonin had very little effect on cell cycle parameters or cytotoxicity. Co-treatment with 50 ug/mL diclofenac potentiated the effects of 100 and 300 ug/mL tryptamine. Diclofenac at 200 ug/mL potentiated the effects of 300 ug/mL

tryptamine, but induced effects similar to those with diclofenac alone when combined with 100 ug/mL tryptamine. The sponsor included this study to illustrate that other NSAIDs had similar effects on the cell cycle to those of NAP, and that the combination of an NSAID (diclofenac) with an indole (tryptamine) could lead to enhanced disruption of the cell cycle compared with either drug alone, as seen with NAP and SS (also an indole).

GSK Study #WD2007/01560/00 (V27862) demonstrated that NAP alone had dramatic dose-dependent effects on the cell cycle of CHO cell cultures; near maximal reductions in the % of cells in S phase and increases in the % of cells in G1/G0 and G2/M phases were achieved at 1640 ug/mL NAP in the 3-hr assay. SS alone produced much smaller effects in the same direction. In combination at 1:1, NAP/SS at 1250/1250 ug/mL reduced the % of cells in S phase to near zero in a 3-hr assay, with little or no effects on cytotoxicity or inhibition of population doubling. Near maximal effects on cell cycle parameters were observed at 1000/1000 ug/mL NAP/SS in a 24-hr assay, associated with cytotoxicity of 64% (36% relative survival, 91.4% inhibition of population doubling). The extent of the **proposed potentiation of NAP's effects by SS could not be determined in this study,** because NAP and SS were not evaluated alone and together in the same experiments. Effects on chromosomal aberrations were not included.

GSK Study #WD2007/01476/00 (V27836) demonstrated that treatment of CHO cells for 24 hrs with NAP alone dose-dependently reduced the % of cells in S phase and increased the % of cells in G1 phase and G2/M phase, while increasing cytotoxicity to a plateau of ~80% at 1000 ug/mL and above. Similar treatments with SS showed comparatively modest dose-dependent reduction of the % of cells in S phase and increase in G1 phase and (slightly) G2/M phase, associated with cytotoxicity of ~30% at 3500 ug/mL SS. The presence of 3000 ug/mL SS during the 24-hr treatment increased the dose-dependent effects of NAP on the % of cells in S phase (at 100-500 ug/mL NAP), on the % of cells in G2/M phase (at 400 and 500 ug/mL NAP), and on cytotoxicity (at 100-500 ug/mL NAP). The presence of 1750 ug/mL SS slightly increased the effects of NAP on the % of cells in S phase and G1 phase and on cytotoxicity at 200-500 ug/mL NAP, but 500 ug/mL SS had very little effect on the dose-dependent changes induced by NAP at 100-500 ug/mL. The increased reduction in the % of cells in S phase induced by the combination of NAP and SS generally appeared to be additive.

POZEN Study MT400-108 demonstrated that treatment of healthy male and female subjects (N = 9-10/dose group) with one tablet of MT 400 or one tablet of naproxen sodium twice daily for seven days did not significantly increase the frequency of structural chromosome aberrations (excluding gaps) in peripheral blood lymphocytes collected 24 hours after the final dose, compared to placebo treatment. The study appeared to be adequately performed.

Reviewer's Conclusions:
The sponsor has adequately addressed the issues raised in the Approvable Letter of August 1, 2007. The explanation for the discrepancy in the findings for naproxen sodium in the two mouse lymphoma assays included several factors (e.g., treatment period of 4

vs. 3 hrs, higher levels of cytotoxicity) that could reasonably account for the positive results in the earlier study. The nonclinical studies presented a compelling case that the combination of NAP and SS can profoundly disrupt the cell cycle at concentrations well below those inducing cytotoxicity and clastogenicity. This reviewer considers it reasonable to conclude that the profound inhibition of DNA synthesis induced by NAP/SS **may** contribute to the clastogenicity observed at very high concentrations (≥ 7.6 mM NAP; ≥ 5.9 mM SS) of this combination in CHO cells (though it is not clear how widely accepted this proposed link is). If the clastogenicity induced by NAP/SS was caused by indirect effects on DNA, then it is reasonable to consider the large safety margins (~30-fold for NAP, ~30,000-fold for SS) between the clastogenic in vitro concentrations and the maximum clinical plasma concentrations in evaluating the risk to patients. The lack of significant increases in the frequency of cells with chromosomal aberrations in peripheral lymphocytes collected from humans treated for 7 days with the maximum recommended daily dose of Treximet (compared to placebo) provides additional assurance that the risk of genotoxicity in humans is reasonable.

Recommendations:

The nonclinical package is adequate to support an approval action for NDA 21-926 TREXIMET (sumatriptan succinate/naproxen sodium) Tablets for the acute treatment of migraine.

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/s/

David Hawver
4/15/2008 03:37:32 PM
PHARMACOLOGIST

Lois Freed
4/15/2008 03:41:27 PM
PHARMACOLOGIST
Please see comments in separate memo.

MEMORANDUM

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

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

Date: August 1, 2007

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

Subject: NDA 21-926 (TRADENAME; sumatriptan/naproxen), Submission 016/31 JAN 2007

The sponsor's response to the Agency's Approvable (AP) Letter (dated: 6/8/06) was provided in Submission 016/31 JAN 2007.

The primary concern conveyed in the AP letter (and discussed in my memo of June 9, 2006) was the possibility that sumatriptan and naproxen in combination would have increased carcinogenic potential compared to that of each compound alone. The relevant section of the AP letter is provided below:

> 1. The results of the in vitro chromosomal aberration assay in Chinese Hamster Ovary (CHO) cells (Study MT400/T07, #0735/0736-3110) demonstrated clastogenic effects of naproxen alone and in combination with sumatriptan. The magnitude of the clastogenic effect was greater with the combination of naproxen and sumatriptan than with naproxen alone, both in the absence and presence of metabolic activation. (Sumatriptan was negative in this assay.) These results raise the concern that naproxen and sumatriptan in combination may have carcinogenic effects not observed with either drug alone. However, since the clastogenic effects were observed only at concentrations producing substantial cytotoxicity, the biological significance of these effects is unclear, but cannot be dismissed. Therefore, you need to conduct the following additional studies:
>
> > a. a repeat in vitro chromosomal aberration assay in CHO cells testing concentrations between those exhibiting minimal or no cytotoxicity (i.e., 1250/1250 µg/mL naproxen/sumatriptan) and those resulting in substantial cytotoxicity (i.e., 2500/2500 and 2000/2000 naproxen/sumatriptan in the absence and presence of metabolic activation, respectively).
> >
> > b. an in vitro mouse lymphoma tk assay (with colony sizing) testing naproxen and sumatriptan alone and in combination.
>
> The results of these studies will determine the need for additional nonclinical studies.

In response, the sponsor provided reports for the following studies: (a) an in vitro chromosomal aberration assay of sumatriptan and naproxen, alone and in combination (1:1) and (b) an in vitro mouse lymphoma tk assay of sumatriptan and naproxen, alone and in combination (1:1). These data have been reviewed by David B. Hawver, Ph.D. (Pharmacology/Toxicology Review NDA 21-926, July 30, 2007). Based on his review, Dr. Hawver has concluded that the sponsor has

adequately addressed the concerns raised in the AP letter and that the "nonclinical package is adequate to support an approval action..." for this application.

I do not concur with Dr. Hawver's conclusions. It is my opinion that the issue regarding the carcinogenic potential of the combination of sumatriptan and naproxen has not been adequately resolved.

• The findings from the in vitro chromosomal aberration assay in CHO cells provided in the original NDA submission are provided below (sponsor's table):

Table (1) Relative Mitotic Indices and % Cells with Aberrations (- S9): 3 Hour Treatment with 18 Hour Harvest

NAP/SS[a] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	NAP[c] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	SS[d] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]
Solvent	100	0.0	Solvent	100	0.0	Solvent	100	0.0
250/250	160		500	97		500	119	
500/500	151	0.0	1000	91	0.0	1000	134	0.0
1250/1250	153	0.0	2500	108	0.5	2500	94	0.0
2500/2500	29	10.0**	5000	37	4.5*	5000	110	0.5
MMC 0.4[e]	81	27.0**	MMC 0.4[e]	69		MMC 0.4[e]	69	
MMC 0.8[e]	39		MMC 0.8[e]	66	29.0**	MMC 0.8[e]	66	29.0**

a. Naproxen sodium / Sumatriptan (dose calculated as the base)
b. % of cells with chromosome aberrations
c. Naproxen sodium
d. Sumatriptan (dose calculated as the base)
e. MMC - positive control Mitomycin C

** $p \leq 0.0001$
* $p = 0.028$

Table (2). Relative Mitotic Indices and % Cells with Aberrations (+ S9): 3 Hour Treatment with 18 Hour Harvest

NAP/SS[a] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	NAP[c] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]	SS[d] Concentration (µg/mL)	RMI (%)	% Cells with CA[b]
Solvent	100	0.0	Solvent	100	1.5	Solvent	100	1.5
250/250	85		5	116		5	91	
500/500	91	0.0	50	88	0.0	50	130	0.0
1250/1250	84	0.0	500	99	0.5	500	119	0.0
2000/2000	20	11.0**	2500	29	4.0[f]	5000	100	0.0
CP 7.5[e]	10	30.0**	CP 7.5[e]	14	31.0**	CP 7.5[e]	14	31.0**
CP 12.5[e]	13		CP 12.5[e]	11		CP 12.5[e]	11	

a. Naproxen sodium/Sumatriptan (dose calculated as the base)
b. % of cells with chromosome aberrations
c. Naproxen sodium
d. Sumatriptan (dose calculated as the base)
e. CP – positive control Cyclophosphamide
f. Value was not statistically different from solvent control due to the high % of cells with aberrations in the solvent control group

** $p \leq 0.0001$

The data in these tables illustrate that (1) sumatriptan (SS) alone is negative, both in the absence (-S9) and presence (+S9) of metabolic activation, (2) naproxen (NAP) alone is positive, both in the absence and presence of S9, and (2) the combination of sumatriptan and naproxen (NAP/SS) is positive, both in the absence and presence of S9; in addition, the magnitude of the effect is notably higher with the combination than with naproxen alone.

In these assays, cytotoxicity was expressed as a decrease in the mitotic index (RMI, or relative mitoxic index), although a more direct index of cytotoxicity for in vitro assays is a reduction in

cell number or culture confluency. (Current ICH and OECD guidelines specify that the highest concentration tested in this assay should result in a decreased of >50% in mitotic index, cell number, or culture confluency, unless otherwise limited by solubility, etc.) As evident from Tables 1 and 2 above, the positive responses, both for the combination and for naproxen alone, were associated with notable cytotoxicity, i.e., 20-29 and 29-37% RMI, respectively. Therefore, the sponsor was asked (in the AP letter) to further investigate concentrations between those exhibiting no and substantial cytotoxicity in a repeat in vitro chromosomal aberration assay, and to conduct an in vitro mouse lymphoma tk assay of sumatriptan and naproxen, alone and in combination.

• The results of the repeat in vitro chromosomal aberration assay in CHO cells are presented in the following tables (from Dr. Hawver's review):

Treatment (3 hr Incubation Without S9)	Dose Level (ug/mL)	% Mitotic Inhibition	% Reduction In Cell Count	% Population Doubling Inhibition	% of Cells w/ Structural Aberrations (excluding gaps)
Purified Water	0	-	0	0	0.75
NAP/SS	1710/1710	0	44	54	1.50
NAP/SS	1815/1815	11	50	65	7.50*
NAP/SS	1850/1850	0	56	77	11.00*
NAP/SS	1885/1885	0	59	83	18.50*
NAP/SS	1920/1920	61	68	100	37.09*
NAP	1920	0	30	33	1.50
NAP	2500	0	33	37	2.50
SS	1920	0	24	25	1.00
4-NQO	0.3	ND	ND	ND	24.00*

* Statistically significant: p<0.001

Treatment (3 hr Incubation With S9)	Dose Level (ug/mL)	% Mitotic Inhibition	% Reduction In Cell Count	% Population Doubling Inhibition	% of Cells w/ Aberrations (excluding gaps)
Purified Water	0	-	0	0	1.19
NAP/SS	1640/1640	0	4	5	2.00
NAP/SS	1710/1710	2	32	52	3.50#
NAP/SS	1745/1745	26	42	73	9.50*
NAP/SS	1780/1780	57	52	96	22.50*
NAP	1780	0	31	49	1.00
NAP	2500	0	42	73	3.50
SS	1780	0	0	0	1.00
CPA	12.5	ND	ND	ND	70.50*

* Statistically significant: p<0.001 #Statistically significant: p< 0.05, but within historical control range.

The data in these tables indicate that (1) sumatriptan and naproxen alone were negative, both in the absence (-S9) and presence (+S9) of metabolic activation and (2) sumatriptan and naproxen in combination produced a concentration-related increased in % of cells with chromosomal aberrations, both in the absence and presence of S9.

Cytotoxicity was expressed as reductions in mitotic index (% Mitotic Inhibition) and cell count (% Reductions in Cell Count), as well as in population doubling (% Population Doubling Inhibition). As noted by Dr. Hawver, current guidelines (OECD, ICH) indicate that % reduction in cell count is the most appropriate measure of cytotoxicity for this assay. Population doubling has been proposed as an alternative measure (Greenwood SK et al. *Environ Mole Mutagen* 43:36-44, 2004); however, it has not been accepted as a more valid or more appropriate measure of cytotoxicity and should not be used to dismiss the positive responses observed (Martha Moore, Ph.D., personal communication).

In the absence of S9, significant increases in the % of cells with chromosomal aberrations were obtained at concentrations of naproxen and sumatriptan in combination (NAP/SS) associated with 50-68% reductions in cell count. This degree of cytotoxicity is consistent with that recommended for the highest concentrations in this assay (ICH, OECD guidelines). In the presence of S9, increases in the % of cells with chromosomal aberrations were obtained at concentrations associated with only 32-52% decreases in cell count. It is notable that naproxen (at 2500 μg/mL) was negative in the presence of S9, whereas the combination of naproxen and sumatriptan (at 1745/1745 μg/mL) was positive, at the same degree of cytotoxicity (42% reduction in cell count); therefore, the positive response with the combination cannot be explained by a greater cytotoxic effect.

• The sponsor also conducted an in vitro mouse lymphoma tk assay (MLA) of sumatriptan and naproxen, alone and in combination; sumatriptan and naproxen, alone and in combination, were negative. As Dr. Hawver notes, the naproxen data are inconsistent with MLA studies conducted to support [redacted]). The reason(s) for the inconsistent results are not apparent since, as Dr. Hawver notes, the concentrations of naproxen tested in the current assay were higher than those used in the MLA assays for [redacted] (1780-2500 and 150-300 μg/mL, respectively).

It is Dr. Hawver's opinion that, although this repeat study confirms the previous finding of a synergistic clastogenic effect of sumatriptan and naproxen, no additional nonclinical studies are needed based on the following:

(a) the positive effects "...occur at moderate levels of cytotoxicity (≥42% reduction in cell number) and at relatively high concentrations (≥7.6 mM NAP; ≥ 5.9 mM SS)...these concentrations exceed the recommended maximum concentration of 10 mM because 7.6 + 5.9 = 14.5 mM".

(b) the combination was negative in the other genetic toxicology assays (i.e., Ames, in vitro mouse lymphoma tk assay, and in vivo micronucleus assay).

(c) according to current labeling, sumatriptan and naproxen were negative in carcinogenicity studies; in addition, naproxen was negative in carcinogenicity studies conducted for MT 100 (2-year rat study, naproxen alone and in combination; 26-week p53 transgenic mouse assay, naproxen in combination with metoclopramide).

I have discussed the genotoxicity data with experts in the field (David Jacobson-Kram, Ph.D. Associate Director for Pharmacology/Toxicology, OND/CDER; Martha Moore, Ph.D., Director, Division of Genetic and Reproductive Toxicology, NCTR). Based on my understanding of these discussions and relevant guidance (cf. Guideline for Industry: Specific Aspects of Regulatory

Genotoxicity Tests for Pharmaceuticals April 1996 ICH S2A; Guidance for Industry and Review Staff: Recommended Approaches to Integration of Genetic Toxicology Study Results January 2006, Pharmacology and Toxicology; OECD Guideline for the Testing of Chemicals, Guideline 473: In Vitro Mammalian Chromosome Aberration Test, February 1997), it is my opinion that the positive findings in the in vitro chromosomal aberration assays cannot be dismissed for the following reasons:

(a) positive findings in the repeat in vitro CHO assay were not associated with excessive cytotoxicity and, as noted above, naproxen alone at a concentration producing a similar degree of cytotoxicity (as measured by reduction in cell count) was negative. Also, it is not appropriate to combine the molar concentrations of each compound when considering the limit concentration of 10 mM. The purpose of the 10 mM criterion for an adequate high concentration is to ensure that a single compound is tested at a sufficiently high concentration. To my knowledge, the sponsor did not argue that the combined concentrations of sumatriptan and naproxen in this assay compromised the results based on, for example, adverse assay conditions.

(b) It is true that the other in vitro and the in vivo genetic toxicology assays were negative; however, there is no apparent basis for dismissing a reproducible positive signal in one component of the standard battery of genetic toxicology assays based solely on negative findings in other assays comprising the battery.

(c) It is acknowledged that sumatriptan was negative in carcinogenicity studies in mouse (78-week) and rat (104-week) and that naproxen was negative in a 2-year carcinogenicity study in rats (8-24 mg/kg/day) and, in combination with metoclopramide, in a 26-week p53 transgenic mouse assay (50 mg/kg). However, none of these studies tested the combination of sumatriptan and naproxen. It is my opinion that, rather than lessening the concern, it is the lack of a signal for carcinogenicity in these studies that heightens the concern regarding a possible synergistic effect of the combination. (It is of note that, due to the sensitivity of the rodent to the gastrointestinal effects of NSAIDs, naproxen could not be evaluated in any of the carcinogenicity studies at more than a fraction of clinically relevant doses or plasma exposures.)

One other factor that needs to be taken into consideration is the carcinogenicity findings for approved products for acute or prophylactic treatment of migraine. Information provided in labeling is summarized in the following table (MRHD = maximum recommended human dose for migraine treatment/prophylaxis based on mg/m^2):

DRUG	CARCINOGENICITY STUDY FINDINGS	
	MICE	RATS
AMERGE (naratriptan)	negative	thyroid follicular and benign C-cell adenomas; plasma AUCs at no-effect doses were 40 and 29 times that in humans. In nitrite-supplemented study, benign lymphocytic thymomas at all doses.
AXERT (almotriptan)	negative	negative
BLOCADREN (timolol maleate)	benign and malignant pulmonary tumors, benign uterine polyps, mammary adenocarcinomas; no effect dose is 40 times MRHD (timolol increase serum prolactin in rodents)	adrenal pheochromocytomas; no-effect dose is 80 times MRHD.
DEPAKOTE (valproic acid)	benign pulmonary adenomas at 0.4-0.8 times MRHD	subcutaneous fibrosarcomas ; no effect doses is 0.8 times MRHD
FROVA (frovatriptan)	negative in 84-wk study; 26-week study in p53 mice, subcutaneous sarcomas (attributed to transponders, not clinically relevant); at no effect dose, AUC180 times that at MRHD	pituitary adenomas at doses producing AUC 250 times AUC at MRHD
INDERAL (propranolol)	negative	negative
MAXALT (rizatriptan)	negative	negative
MIGRANAL (DHE mesylate)	no data available	no data available
RELPAX (eletriptan)	hepatocellular adenomas; AUC at no-effect dose is 7 times AUC at MRHD	testicular interstitial cell adenoma; AUC at no-effect dose is 2 times AUC at MRHD
IMITREX (sumatriptan)	negative	negative
TOPAMAX (topiramate)	urinary bladder tumors (smooth muscle tumor histomorphologically unique to mice); no-effect dose is 4 times MRHD	negative
ZOMIG (zolmitriptan)	negative	thyroid follicular cell adenoma at a dose producing AUC 3000 times AUC at MRHD

None of the drugs currently approved for acute or prophylactic treatment of migraine has a strong signal for carcinogenicity. For only three (naratriptan, valproic acid, and eletriptan) were clinically relevant tumor findings observed at doses similar to the MRHD (on a mg/m^2 basis). For naratriptan, the thymomas were observed in a special nitrite-supplemented study in rat designed to assess the potential for naratriptan to form a mutagenic (nitrosated) product in the stomach; no stomach findings were detected. Therefore, a strong signal for carcinogenicity may be an approvability issue for a product intended for acute treatment of migraine.

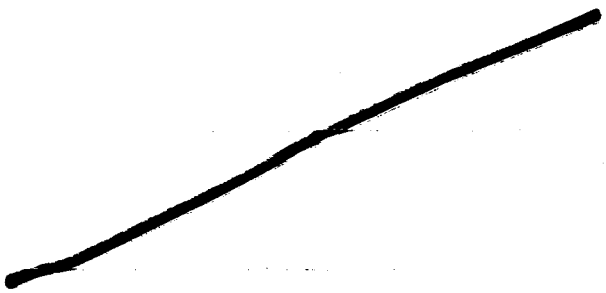
It is unlikely that conducting additional in vitro or in vivo genetic toxicology studies would provide any data that could be used to dismiss the positive response of the in vitro CHO cell assays. It is also unlikely that lifetime carcinogenicity studies or shorter-term studies in transgenic animals (e.g., p53, TgHras2) would provide meaningful data, specifically because of the sensitivity of rodents to naproxen. Drs. Jacobson-Kram and Moore have suggested the possibility of conducting a study in humans to assess the clastogenic potential of naproxen alone and in combination with sumatriptan. A number of studies have been published on evaluation of clastogenic and/or mutagenic effects in circulating lymphocytes in various populations (e.g., smokers, industrial workers, military personnel). Studies have also been conducted in patients on therapeutic doses of various medications. For example, Saxena and Ahuja (Saxena R, Ahuja YR.

Hum Genet 62(3):198-200, 1982; only abstract available) reported a significant increase in patients treated with thioridazine for 4 weeks. Ahuja et al. (Ahuja YR et al. *Arzneimittelforschung* 34(6):699-701, 1984; only abstract available) reported increases in chromosomal aberrations in patients on therapeutic doses of haloperidol. More recently, studies have been conducted to assess effects of therapeutic doses of methylphenidate on circulating lymphocytes in children (El-Zein et al. *Cancer Lett* 230(2):284-291, 2005; Walitz S et al. *Environ Health Perspect* 115:936-940, 2007). Although I have no experience with this type of investigation, it would appear to be a reasonable way to address this issue, of course, with adequate informed consent.

In addition to possibly conducting a clinical trial, the sponsor could also re-evaluate the conduct of the in vitro chromosomal aberration assays to investigate, for example, whether or not the apparent synergistic effect is an artifact of assay conditions. Another possibility would be to explore ratios other than 1:1 since the clinical formulation is not a 1:1 ratio of sumatriptan to naproxen; however, it is unclear how in vitro ratios could be compared to in vivo plasma exposures.

I recommend that the sponsor be asked to address this issue, as suggested or in some other meaningful way, prior to approval.

In case it is decided that this issue need not be addressed prior to approval, I include the following recommendations for labeling (the base document is the 24 JULY 2007 version; suggested revisions are noted as strike-out or bolded text):



3 Page(s) Withheld

_____ Trade Secret / Confidential

✓ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox- 4

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

/s/

Lois Freed
8/2/2007 06:17:22 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**21-926**
SERIAL NUMBER:	**016**
DATE RECEIVED BY CENTER:	**31 JAN 2007**
PRODUCT:	**Trexima (Sumatriptan/Naproxen)**
INTENDED CLINICAL POPULATION:	**Migraine patients**
SPONSOR:	**POZEN Inc.**
DOCUMENTS REVIEWED:	**eNDA**
REVIEW DIVISION:	**Division of Neurology Products**
PHARM/TOX REVIEWER:	**David B. Hawver, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lois Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Lana Chen**

Date of review submission to Division File System (DFS): 01 AUG 2007

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

The nonclinical package is adequate to support an approval action for NDA 21-926 TREXIMA (sumatriptan succinate/naproxen sodium) Tablets for the acute treatment of migraine.

B. Recommendation for nonclinical studies

None.

C. Recommendations on labeling

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

The data demonstrate that the combination of NAP and SS induce genotoxic effects in CHO cells in the presence and absence of S9 that are not observed at similar concentrations of either drug alone. These effects occur at moderate levels of cytotoxicity (≥ 42% reduction in cell number) and at relatively high concentrations (≥ 7.6 mM NAP; ≥ 5.9 mM SS). This reviewer believes it might be reasonable to argue that these concentrations exceed the recommended maximum concentration of 10 mM because 7.6 + 5.9 = 14.5 mM. Considering the negative findings in the mouse lymphoma assay, the bacterial reverse mutation assay, and the in vivo mouse micronucleus assay with NAP/SS, and the negative carcinogenicity findings for each of the components of the combination, this reviewer believes that including the positive genotoxicity findings in the labeling for Trexima is sufficient to address this issue. No additional nonclinical studies are needed.

B. Pharmacologic activity

No new pharmacologic activity studies were conducted.

C. Nonclinical safety issues relevant to clinical use

Positive genotoxicity findings should be included in the labeling.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

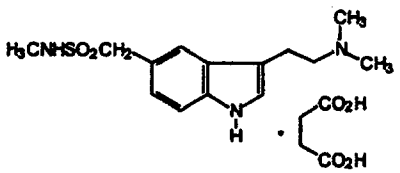
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-926
Review number: 2
Sequence number/date/type of submission:
016/31 JAN 2007/505 (b)(2) Original Application
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: POZEN Inc., Chapel Hill, NC
Manufacturer for drug substance:
Sumatriptan Succinate (SS): Glaxo Wellcome Manufacturing Pte Limited, Singapore
Naproxen Sodium (NAP): [redacted]

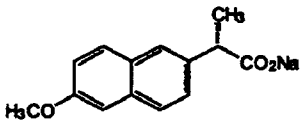
Reviewer name: David B. Hawver, Ph.D.
Division name: Division of Neurology Products
HFD #: 120
Review completion date: 30 JUL 2007

Drug:

Trade name: Trexima™ (formerly, Trexima)
Generic name: sumatriptan succinate/naproxen sodium
Code name: MT400
Chemical name:
SS: 3-[2-(dimethylamino)ethyl]-N-methyl-indole-5-methanesulfonamide succinate (1:1)
NAP: (S)-6-methoxy-(alpha)-methyl-2-naphthaleneacetic acid, sodium salt
CAS registry number: 103628-48-4 (sumatriptan succinate)
26159-34-2 (naproxen sodium)
Molecular formula/molecular weight:
sumatriptan succinate: $C_{14}H_{21}N_3O_2S \bullet C_4H_6O_4$ MW 413.5
naproxen sodium: $C_{14}H_{13}NaO_3$ MW 252.25
Structure:



sumatriptan succinate



naproxen sodium

Relevant INDs/NDAs/DMFs:
IND 68,435 MT 400 for migraine, POZEN's current IND for sumatriptan/naproxen combined in one [redacted] tablet; submitted 18 DEC 2003
IND 60,669 MT 400 for migraine, POZEN's initial IND for sumatriptan/naproxen using marketed products in combination; submitted 26 JUL 2000
NDA 20-132 IMITREX® Tablets, sumatriptan succinate for migraine; Glaxo Inc.; approved 01 JUN 1995
NDA 17-581 NAPROSYN® Tablets, naproxen for rheumatoid arthritis, now also for acute pain, ankylosing spondylitis, tendonitis, bursitis, and acute gout; Roche (originally Syntex, Inc.); approved 11 MAR 1976
NDA 18-164 ANAPROX® Tablets, naproxen sodium for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and juvenile arthritis; Roche/Syntex; approved 04 SEP 1980

[redacted]

Drug class:
Sumatriptan succinate is a selective 5-$HT1_D$ receptor agonist.
Naproxen sodium is a nonsteroidal anti-inflammatory drug (NSAID).

Intended clinical population:
The proposed indication for Trexima Tablets is for the treatment of acute migraine headache with or without aura in adults.

Clinical formulation:
Each Trexima Tablet contains 119 mg sumatriptan succinate (equivalent to 85 mg sumatriptan) and 500 mg naproxen sodium. Inactive ingredients (which are all GRAS for use in oral pharmaceuticals) include: [redacted] (microcrystalline cellulose), croscarmellose sodium, dibasic calcium phosphate, magnesium stearate, microcrystalline cellulose, [redacted] sodium bicarbonate and talc; the aqueous film coat contains sodium carboxymethyl-cellulose, maltodextrin, dextrose monohydrate, titanium dioxide, lecithin and FD&C Blue No. 2.

Route of administration: Oral tablet

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

Data reliance:
Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-926 are owned by POZEN Inc. or are data for which POZEN Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 21-926 that POZEN Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that POZEN Inc. does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 21-926.

Studies reviewed within this submission:

- 1:1 combination of Naproxen sodium and Sumatriptan succinate: In vitro Chromosome Aberration Assay with Cultured Chinese Hamster Ovary (CHO) Cells
- 1:1 combination of Naproxen sodium and Sumatriptan succinate: In Vitro Mutation Assay with L5178Y Mouse Lymphoma Cells at the TK Locus

Studies not reviewed within this submission: None.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

No Pharmacology studies were included in this submission.

2.6.2.2 Primary pharmacodynamics

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

2.6.2.5 Pharmacodynamic drug interactions

2.6.3 PHARMACOLOGY TABULATED SUMMARY

2.6.3.2 Primary Pharmacodynamics

2.6.3.3 Secondary Pharmacodynamics

2.6.3.4. Safety Pharmacology

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

No Pharmacokinetics/Toxicokinetics studies were included in this submission.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY