## 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:

- 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

**1:1 combination of Naproxen sodium and Sumatriptan succinate: In vitro Chromosome Aberration Assay with Cultured Chinese Hamster Ovary (CHO) Cells**

**Study no.:** [redacted] Study 2990/7; GSK Document WD2006/03218/00

**Volume # , and page #:** eNDA 21-926 #016, Module 4, Section 4.2.3.3.1.1, pages 1-76
**Conducting laboratory and location:** [redacted] North Yorkshire, UK
**Date of study initiation:** 04 AUG 2006
**GLP compliance:** Yes, UK 1999/2004, signed 31 OCT 2006 by Study Director
**QA reports:** yes, statement signed 31 OCT 2006 by the QA Representative
**Drug, lot #, and % purity:** Naproxen Sodium (NAP) Lot #NPXNAM-631 and #NPXNAM-635, Purity 100%; NAP doses were calculated as the free acid, using a correction factor of 1.1. Sumatriptan Succinate (SS) Lot #K058945 and #K026882, Purity 98.7% and 99.2%, respectively. SS doses were calculated as the free base, using a conversion factor of 1.4.

**Methods**

Strains/species/cell line:
CHO cells, originally supplied by [redacted] were maintained at [redacted] in tissue culture flasks containing [redacted] 5A medium with 10% (v/v) heat inactivated fetal calf serum, and 100 ug/mL gentamycin. The doubling time of this cell line is ~13 hrs, and its modal chromosome number is 21.

Doses used in definitive studies:
-S9: NAP alone at 1920 and 2500 ug/mL
SS alone at 1920 ug/mL
SS/NAP together at 1710/1710, 1815/1815, 1850/1850, and 1920/1920 ug/mL
+S9: NAP alone at 1780 and 2500 ug/mL
SS alone at 1780 ug/mL
SS/NAP together at 1640/1640, 1710/1710, 1745/1745, and 1780/1780 ug/mL

Basis of dose selection:
The primary measure of cytotoxicity in this study was mitotic index (MI), the percentage of cells in mitosis, based on scoring of at least 1000 cells per culture. Slides showing > 61% reduction in MI were not scored for chromosomal aberrations. The concentrations selected for analysis of chromosomal aberrations was agreed with the sponsor before scoring. Cytotoxicity was also measured based on cell counts (compared to mean vehicle control) and population doublings (PD = [log ($N/X_0$)]/log 2, where N = mean final cell count/culture at harvest, and $X_0$ = starting count at beginning of treatment).

Negative controls:
Purified water was used as the solvent control.

Positive controls:
4-Nitroquinolone 1-oxide (NQO, 0.25 and 0.30 ug/mL final concentration, from stock in DMSO, [redacted], was used as the positive controls in the absence of metabolic activation.

Cyclophosphamide (CPA, 6.25 and 12.5 ug/mL final concentration, from stock in DMSO, [redacted], was used as the positive control in the presence of metabolic activation.

Incubation and sampling times:
Duplicate cultures were prepared and treated with test article or positive control ± S9 metabolic activation (rat liver, [redacted], 2% final concentration) for 3 hrs as described above (vehicle controls were tested in quadruplicate cultures). Cells were harvested 17 hrs after the beginning of treatment, and 1.5 hrs after the addition of colchicine (1 ug/mL final concentration) to arrest dividing cells in metaphase. A cell count was determined from an aliquot of each cell suspension prior to centrifugation and resuspension of the cells [redacted], followed by centrifugation and resuspension several times to clean the cells. Slides were prepared after several drops of 45% (v/v) aqueous acetic acid were added to enhance chromosome spreading. Dried slides were **stained for 5 minutes in filtered 4% (v/v) Giemsia stain in Gurr's buffer (pH 6.8), rinsed,** dried, and mounted in DPX under coverslips.

The top four or five concentrations without excessive toxicity were scored for chromosome aberrations (100 metaphases from each of the duplicate flasks, providing 200 per concentration level, and 400 from the four vehicle-treated cultures). Only cells with 19-23 chromosomes were considered acceptable for analysis of chromosomal aberrations. The frequency of hyperdiploid, polyploid and endoreduplicated cells was also scored for each culture.

A 20-hr treatment in the absence of S9 was also performed, but was not analyzed for chromosomal aberrations because the 3-hr incubations without S9 were clearly positive.

**Results**

Study validity
Criteria for a valid assay were met for the 1:1 combination:

- **The highest concentrations analyzed (1920/1920 ug/mL –S9, 61% MI; 1780/1780** ug/mL +S9, 57% MI) both showed greater than the minimum 50% requirement for mitotic inhibition.
- At least 80% of the intended total cells per treatment (200 for test article and positive **controls; 400 for vehicle controls) were scored, except at the highest dose –S9** (1920/1920), where only 151 of the intended 200 cells were analyzed. This was not important since that concentration was clearly positive.
- The percentage of cells with aberrations in the solvent controls were within or close to laboratory historical control ranges.
- Positive control cultures showed clear, unequivocal positive responses as expected.

Study outcome:
Criteria for a positive response (chromosomal aberration (CA) frequency (excluding gaps) falling outside the historical vehicle control range, and statistically increased over vehicle controls) were met for the combination at ≥ 1815/1815 ug/mL without S9, and at ≥ 1745/1745 ug/mL with S9. No increase in the frequency of chromosomal aberrations was observed with NAP alone at up to 2500 ug/mL ± S9 or with SS alone at 1920 ug/mL (-S9) or 1780 (+S9). Both cytotoxicity (as measured by % Reduction in Cell Count) and frequency of chromosomal aberrations increased with increasing concentrations of NAP/SS. The frequency of numerical aberrations was also significantly increased at ≥ 1815/1815 ug/mL (-S9), primarily due to increased endoreduplication, but not in a clearly dose-related manner. Similarly, numerical aberrations were significantly increased at 1745/1745 ug/mL (+S9), but not at 1780/1780 ug/mL.

| 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.

**Sponsor's Conclusions:**

The sponsor concluded that the 1:1 combination of Naproxen Sodium and Sumatriptan Succinate was clastogenic when incubated with CHO cells for 3 hours in the presence and absence of metabolic activation. However, the sponsor also noted that no induction of chromosomal aberrations was observed in cultures with cytotoxicity ≤ 54% as **measured by inhibition of population doublings (PD, "a more reliable and robust measure of cytotoxicity,"** *page 29*), and that this assay would be considered negative if > 50% inhibition of PD were the cytotoxicity target used in dose selection instead of mitotic inhibition. The sponsor believes that this indicates that the chromosomal aberrations observed at higher concentrations of NAP/SS were caused by a non-genotoxic mechanism dependent on cytotoxicity, as described in Greenwood et al (*Environmental and Molecular Mutagenesis* 43:36-44, 2004).

**Reviewer's Conclusions:**

FDA has not yet adopted the cytotoxicity target of > 50% PD inhibition for the CHO chromosomal aberrations assay. Our current guidance states:

> **"The desired level of toxicity for in vitro cytogenetic tests using cell lines should be greater than** 50% reduction in cell number or culture confluency. For lymphocyte cultures, an inhibition of **mitotic index by greater than 50% is considered sufficient."**
> *(Guideline for Industry, Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals, ICH S2A, April, 1996, page 3)*

Therefore, in the present study, the most appropriate measure of cytotoxicity is reduction in cell number. The combination of Naproxen Sodium and Sumatriptan Succinate should be considered positive for clastogenicity in CHO cells, since statistically significant dose-related increases in the frequency of cells with structural aberrations were observed without S9 at NAP/SS 1815/1815 ug/mL and 1850/1850 ug/mL associated with reductions in cell number of 50% and 56%, respectively; and with S9 at NAP/SS 1745/1745 ug/mL and 1780/1780 ug/mL associated with reductions in cell number of 42% and 52%, respectively.

The data also confirm suggestions from previous studies that the combination of NAP and SS produces a synergistic effect on both toxicity (a cytotoxic and/or cytostatic effect as measured by the reduction in cell number) and clastogenicity. Without S9, 1920 ug/mL NAP + 1920 ug/mL SS reduced cell number by 68%, more than the sum of NAP and SS alone at the same dose (30% and 24%, respectively; sum = 54%). Similarly, with S9, the combination at 1780/1780 ug/mL reduced cell number by 52%, more than the sum of NAP and SS alone (31% + 0% = 31%). The frequency of cells with chromosomal aberrations was increased 20-40-fold with the highest dose of the combination, but not at all with the same doses of NAP and SS alone.

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