CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-239

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: July 10, 2009

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

Subject: NDA 22-239, Sumavel™ DosePro™ (sumatriptan injection), submission dates 5 May 2008 (0003/SU), 30 July 2008 (0009/BP), 15 January 2009 (0020/AZ), 13 April 2009 (0023/C)

NDA 22-239 is a 505(b)(2) application for Sumavel DosePro (sumatriptan injection) for the treatment of migraine and cluster headache. The indications, maximum daily dose (12 mg/day), and route of administration (subcutaneous) are the same as that of the Reference Listed Drug, Imitrex Injection (approved in 1992 under NDA 20-080).

In the original NDA, the sponsor (Zogenix Inc.) submitted nonclinical studies to assess the following:

- Two impurities (Impurities 1 and 3) with specification limits exceeding the qualification threshold.
- The potential toxicity of extractables and leachables related to the clinical device.

Additional studies (including a 90-day toxicity study in rat and an embryo-fetal development study in rabbit) were submitted during the review cycle to address the specification limits proposed for Impurities 1 and 3. Due to the timing of submission, these studies were not reviewed, but were considered necessary to support approval.

A Complete Response (CR) letter, issued on October 31, 2008, conveyed the following nonclinical deficiencies:

- "The positive finding in the in vitro chromosomal aberration assay in human lymphocytes…raises the concern that one or more impurities (e.g., Impurity 1 and Impurity 3) present in the stressed/spiked sumatriptan drug lot tested may have genotoxic potential. Since impurities are unlikely to confer any clinical benefit, the presence of a genotoxic impurity in the clinical drug product, unless

unavoidable, is not acceptable. Therefore, you will need to further investigate this issue prior to approval."

- "We acknowledge that you have submitted additional studies (including a 90-day oral [sic] toxicity study in rat and an embryo-fetal development study in rabbit) to address the specification limits proposed for Impurities 1 and 3. However, they were not included in the original NDA, and were not submitted in time to allow for review during this cycle. They will need to be reviewed and found adequate prior to approval."

In response to the Agency's CR letter, the sponsor provided the following:

- An in vitro chromosomal aberration assay in human lymphocytes for Impurities 1 and 3.
- A combination in vivo micronucleus and Comet assay in mouse for Impurity 3.

These studies and the 90-day and embryo-fetal development (and dose-ranging) studies were reviewed in detail by Dr. Thompson (Pharmacology/Toxicology NDA Review and Evaluation, D. Charles Thompson, R.Ph, Ph.D., 7 July 2009). Based on his review, Dr. Thompson has concluded that the sponsor has adequately qualified Impurities 1 and 3 and recommends approval.

Comments

I concur with Dr. Thompson's recommendation based on the following:

- The sponsor qualified Impurities 1 and 3 at levels of (b) (4) respectively, in a 90-day subcutaneous toxicity study in rat and a subcutaneous embryo-fetal development study in rabbit. The proposed specification limits for these impurities are (b) (4) respectively. Although there is not a margin between the levels qualified and the proposed limits based on percentage, the daily dose of Impurities 1 and 3 administered at the high doses of sumatriptan used in these studies (100 mg/kg in rat, 40 mg/kg in rabbit) provide margins of 80-100 fold compared to the daily doses at the maximum recommended clinical dose (12 mg/day) on a body surface area (mg/m^2) basis.
- No unique toxicity or exacerbation of toxicity due to the presence of Impurities 1 and 3 (spiked sumatriptan) compared to sumatriptan alone was detected in the 90-day rat study.
- As Dr. Thompson notes, sumatriptan alone was not tested in the embryo-fetal development study in rabbit. This is a deficiency and would have made the data difficult to interpret except that no adverse effects on maternal or embryo-fetal development were observed. Clearly, higher doses of sumatriptan could have been tolerated.

  The lack of embryo-fetal (and maternal) effects is notable, considering the adverse effects reported for i.v. sumatriptan (cf. labeling for Imitrex Injection) at doses less than the maximum human dose of 12/day (based on mg/m^2). The high

dose of sumatriptan (spiked with Impurities 1 and 3) tested in the sponsor's study was 65-fold higher than the maximum human dose. There are insufficient data to determine the extent to which differences in route of administration (subcutaneous versus intravenous) may account for this difference. However, since the sponsor's study appeared adequately conducted using the clinical route, it is an acceptable test of Impurities 1 and 3.

- The results of the in vitro chromosomal aberration assay in human peripheral lymphocyte were negative for Impurity 1, but equivocal for Impurity 3 in the absence of metabolic activation. At the highest concentration tested (10 μM), Impurity 3 was reproducibly positive with 21-hour treatment. However, there was evidence of excessive cytotoxicity at this concentration in one of the tests. The in vivo micronucleus/Comet assay in mouse was negative for Impurity 3. Overall, the data do not suggest a genotoxic concern for either impurity.

Recommendation

From a pharmacology/toxicology standpoint, there is no objection to approval of the NDA.

Labeling recommendations

Recommendations have been provided (Memo dated 31 October 2008).

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
7/15/2009 08:37:40 AM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY NDA REVIEW AND EVALUATION

Application Number:	22-239
Submission Number/ code/CDER Stamp Date:	0003/SU/5 May 2008
	0009/BP/30 July 2008
	0020/AZ/15 January 2009
	0023/C/13 April 2009
PDUFA Date:	15 July 2009
Product:	Sumavel™DosePro™ (sumatriptan injection)
Indication:	migraine and cluster headache
Applicant:	Zogenix, Inc.
Review Division:	Neurology Products, HFD-120
Reviewer:	D. Charles Thompson, R.Ph., Ph.D., D.A.B.T.
Supervisor/Team Leader:	Lois M. Freed, Ph.D.
Division Director:	Russell G. Katz, M.D.
Project Manager:	Lana Y. Chen, R.Ph.

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1. Executive Summary

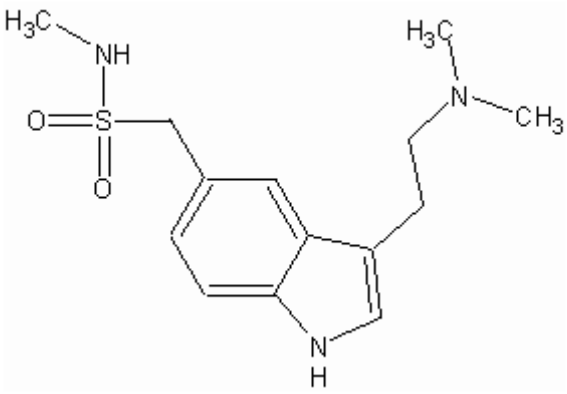
1.1. Recommendations

1.1.1. Approvability: It is concluded that the sponsor has adequately qualified the proposed drug product specification limits of (b) (4) and (b) (4) for the (b) (4) impurities, Impurity 1 and Impurity 3, in the finished sumatriptan succinate drug product. It is recommended that the NDA 22-239 be approved with said drug product specification limits.

2. Drug Information

2.1. Drug:

2.1.1. Trade name: Sumavel™ DosePro™ (sumatriptan injection) needle-free delivery system
2.1.2. Pharmacological class: selective 5-hydroxytryptamine1 ($5\text{-}HT_1$) receptor subtype agonist
2.1.3. CAS RN: 103628-48-4
2.1.4. Generic name: sumatriptan succinate
2.1.5. Code name: N/A
2.1.6. Chemical name: 3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]-N-methylmethanesulphonamide hydrogen butanedioate
2.1.7. Molecular formula/molecular weight: $C_{14}H_{21}N_3O_2S \bullet C_4H_6O_4$ / 413.5
2.1.8. Structure:



2.2. Clinical formulation:

2.2.1. Drug formulation: Sumatriptan succinate solution at a concentration of 6 mg sumatriptan base per 0.5 mL solution (same drug product formulation as RLD, Imitrex® Injection)
2.2.2. Comments on excipients: sodium chloride USP and water for injection USP
2.2.3. Comments on impurities/degradants: see comments under Regulatory Background below

2.3. Proposed clinical population and dosing regimen:

2.3.1. Target population: migraine and cluster headache patients
2.3.2. Dosing route/regimen: subcutaneous injection (needle-free)/acute treatment

3. Regulatory Background

3.1. Relevant IND/s, NDA/s, and DMF/s:

- NDA 20-080 Imitrex® (sumatriptan succinate) Injection (approved 1992)
- NDA 20-132 Imitrex® (sumatriptan succinate) Tablet (approved 1995)
- NDA 20-626 Imitrex® (sumatriptan) Nasal Spray (approved 1997)
- IND 71,275

3.2. Interactions with Agency

NDA 22-239 was originally received on 31 December 2007, followed by 16 amendments submitted between 20 March and 17 October of 2008. For a number of reasons, the application was deemed not approvable and a Complete Response (CR) letter was issued to the sponsor on 31 October 2008. In a review dated 31 October 2008, the previous reviewer (A. Powell, Ph.D.) characterized as follows the nonclinical issues that factored into the issuance of the CR letter (A. Powell, 31 October 2008):

> The sponsor has proposed drug product specification limits of (b) (4) and (b) (4) for the (b) (4) impurities, Impurity 1 and Impurity 3. These specification limits are above the threshold for qualification as defined in Guidance for Industry–Q3B(R2) Impurities in New Drug Products [July 2006, ICH Revision 2]. The sponsor was not able to demonstrate comparable levels of these impurities in the innovator drug product Imitrex® (sumatriptan succinate) Injection. Thus, to support the proposed specification limits the sponsor would need to qualify Impurities 1 and 3 in the following assays: (1) a test for gene mutation in bacteria (Ames assay) and (2) either an in vitro chromosomal aberration assay in mammalian cells or an in vitro mouse lymphoma tk assay (with colony sizing), (3) an embryo-fetal development study in a single species, and (4) a repeat-dose toxicity study of 90 days duration in a single animal species. The sponsor submitted the two in vivo studies four and seven months into the 10 months review cycle, and thus these studies have not been reviewed....

The sponsor submitted the reports of the two in vitro genetic toxicology studies with the original submission, and these were reviewed. The Ames test was negative and qualified Impurities 1 and 3 at (b) (4) and (b) (4), respectively; however, it is not unreasonable to use these data to support the proposed specification limits of (b) (4) for Impurity 1 and (b) (4) for Impurity 3. The positive in vitro chromosomal aberration assay conducted with the "stressed" and spiked formulation is problematic. According to the label for the innovator product, Imitrex® (sumatriptan succinate) Injection, sumatriptan was negative in the in vitro chromosomal aberrations assay in human peripheral lymphocytes, and the current assay did not include a nondegraded and unspiked sumatriptan control. Thus, it is reasonable to conclude that the results of the forced degrading and/or spiking of the sumatriptan succinate formulation led to the positive genotoxic response.

These nonclinical issues and Division recommendations for how to address them were conveyed to the sponsor in the 31 October CR letter in the following manner:

1. The positive finding in the in vitro chromosomal aberration assay in human lymphocytes (Study 961611) raises the concern that one or more impurities (e.g., Impurity 1 and Impurity 3) present in the stressed/spiked sumatriptan drug lot tested may have genotoxic potential. Since impurities are unlikely to confer any clinical benefit, the presence of a genotoxic impurity in the clinical drug product, unless unavoidable, is not acceptable. Therefore, you will need to further investigate this issue prior to approval.

   Since we recognize that the conditions used to produce the "stressed" sumatriptan may have resulted in the formation of impurities that would not be formed under normal storage conditions, we would recommend that you conduct a repeat in vitro chromosomal aberration assay in which Impurities 1 and 3 are tested directly. Alternatively, the study could be conducted using sumatriptan spiked with Impurities 1 and 3 at levels providing a substantial margin above the specification limits. Of course, other approaches may be acceptable. We would recommend that if you choose to test sumatriptan spiked with the impurities, you not use the clinical formulation (12 mg/mL); it artificially limits the concentrations that can be tested.

   If this repeat assay is adequately conducted and negative, no further action is necessary. If it is positive, then the genotoxic impurities would need to be identified and specification limits set to a level that would result in a total daily dose of ≤1.5 μg/day of each impurity. If more than one structurally similar impurity is identified, then the specification limits would need to be set so that the combined total daily dose would not exceed 1.5 μg/day. If such limits are not achievable, then additional genetic toxicology studies may be conducted in an attempt to further characterize the genotoxic potential (cf. Guidance for Industry and Review Staff; Recommended Approaches to Integration of Genetic Toxicology Study Results. FDA/CDER, January 2006). If the data from those studies indicated an overall lack of genotoxic potential, then no further action would need to be taken.

2. We acknowledge that you have submitted additional studies (including a 90-day oral toxicity study in rat and an embryo-fetal development study in rabbit) to address the specification limits proposed for Impurities 1 and 3. However, they were not included in the original NDA, and were

not submitted in time to allow for review during this cycle. They will need to be reviewed and found adequate prior to approval.

Thus, the present review consists of an evaluation of the in vivo toxicity data identified above that were previously submitted late in the first review cycle and not reviewed, as well as the sponsor's submitted response to the above-noted CR letter.

3.3. Studies reviewed within this submission:

- A 90-Day Toxicity Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 Administered by the Subcutaneous Route to Rats with a 14-Day Recovery Period (YQT00004)
- A Rising Dose and Multiple Dose Tolerance Study of Stressed Sumatriptan Solution Formulation Administered by the Subcutaneous Route to Rats (YQT00003)
- Subcutaneous Dosage-Range Developmental Toxicity Study of Stressed Sumatriptan Solution Formulation in Rabbits (YQT00006)
- Subcutaneous Developmental Toxicity Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 in Rabbits Including a Satellite Toxicokinetic Evaluation (YQT00007)
- Sumatriptan DosePro Degradation Products (Impurities 1 and 3) Chromosome Aberration Test (962496)
- Mouse bone marrow micronucleus test and detection of DNA damage in the liver and peripheral blood of treated mice using the Comet assay (8200929)

3.4. Studies <u>not</u> reviewed within this submission: none

3.5. Previous reviews referenced: 31 October 2008 (A. Powell, Ph.D.)

Note: Portions of this review excerpted from the sponsor's submission are identified as such.

4. Pharmacology: no data submitted

5. PK/ADME/TK: no data submitted

6. General Toxicology:

6.1. Single-dose toxicity: no data submitted

6.2. Repeat-dose toxicity:

Study title: A 90-Day Toxicity Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 Administered by the Subcutaneous Route to Rats with a 14-Day Recovery Period

Key study findings:

- Dosing formulation analysis indicated sumatriptan levels fell within ± 10% of target; levels of impurities 1 and 3 were predominantly low, but within 20% of target values.
- Two animals were found dead, one Group 4 male (#146, Day 54) and one Group 5 male (#162, Day 76); histopathology was suggestive of injection site bacterial infection and chronic peritonitis; all remaining animals survived to scheduled euthanasia.
- Convulsions were observed in one Group 4 male (#156, Day 85, duration ~30 seconds) and one Group 5 male (#159, Day 72, duration ~45 seconds).
- For most hematology/clinical chemistry parameters, values obtained in Groups 4 and 5 (i.e., same, high dose of sumatriptan with and without, respectively, spiked impurities 1 and 3) were generally affected qualitatively and quantitatively in a similar manner, suggesting minimal or no adverse impact from the added presence of the impurities.
- Within the variability of the data, organ weight values in Groups 4 and 5 were generally affected qualitatively and quantitatively in a similar manner, suggesting minimal or no adverse impact from the added presence of the impurities.
- Histopathology findings revealed no clearly discernible indication that the added presence of the spiked impurities 1 and 3 induced any additional pathology over and above that observed with sumatriptan drug substance alone. Observations at the four injection sites in almost all animals revealed varying grades of subcutaneous fibrosis, mixed cell inflammation, and hemorrhage. The majority of mid- and high-dose animals also exhibited varying grades of epidermal ulceration, fibrinosuppurative exudate, and hyperplasia, as well as dermal fibrosis at the injection sites. Most of these observations were still evident in recovery animals following the recovery period.
- The toxicokinetic study design employed was inadequate and yielded information that was minimally useful, at best, in support of overall study objectives.

Study no.: YQT00004
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: 15 November 2007
GLP compliance: Yes, with exceptions as reproduced below

- Characterization and stability analyses of the bulk test article, sumatriptan succinate, were not conducted in compliance with the GLP regulations.
- Characterization and stability analyses of the bulk test articles, Impurity 1 (lot number 07-06633-25) and Impurity 3 (lot numbers 07-066-31-27, TYG-YCH-006-2, TYG-YCH-006-1, and ACM-M0013-SD-13-28-25), were not conducted in compliance with GMP procedures.
- Due to an extremely limited amount of Sumatriptan Impurity 3, a retention sample was not collected for each lot of this test article.
- Long-term refrigerated stability at 1.5, 15 and 50 mg/mL and 24-hour room temperature stability at 1.5 mg/mL were conducted following in-life completion.

QA statement: Yes
Drug, lot #, and % purity: sumatriptan succinate (lot# 6046171, purity not defined); sumatriptan impurity 1 (lot# 07-066-33-25, purity 71% as free base); sumatriptan impurity 3 (4 different lots from 3 different suppliers: lot# 07-066-31-27, purity 91% as area% and 63% as free base; lot# TYG-YCH-006-2, purity 79% as free base; lot# TYG-YCH-006-1, purity 79% as free base; and lot# ACM-M0013-SD-13-28-25, purity 46% as free base)

The stock solutions/dosing formulations were prepared weekly and dispensed into autoclaved amber glass vials for up to 8 days of dosing, stored refrigerated, and dispensed daily for dosing. The stock solutions/dosing formulations were removed from the refrigerator for at least 30 minutes prior to dosing and stirred continuously prior to and during dosing. Dosing formulation analysis was conducted on behalf of the sponsor by (b) (4) .

Methods:

Doses: see sponsor's study design summary table reproduced below (based on results from dose range-finding study, YQT00003, reviewed below)
Species/strain: Sprague Dawley Crl:CD(SD) rats
Number/sex/group or time point (main study): see sponsor's study design summary table reproduced below
Route: subcutaneous injection at one of four injection sites, rotated daily (not indicated by sponsor, but presumed to be dorsal)
Formulation/vehicle: Sterile Water for Injection (lot# 709325F)
Dosing solution analyses/drug stability and homogeneity: dosing formulation solution concentration analysis was performed weekly over Weeks 1-10 for all study groups, for Group 4 in Week 11, and for Groups 2, 3, and 4 in Week 12; homogeneity was deemed not necessary with aqueous solutions; stability was assessed on study only for Group 2 during Week 1
Dose volume/infusion rate: once daily from Days 1-90 (for those animals scheduled for euthanasia on Days 91 and 105) or Days 1-91 (for those animals scheduled for euthanasia on Day 92); also see sponsor's study design summary table reproduced below
Satellite groups used for toxicokinetics or recovery: see sponsor's study design summary table reproduced below
Age: approximately 8 weeks at randomization
Weight: at randomization: 247-288 g, males and 181-208 g, females (main study); 255-283 g, males and 178-207 g, females (TK study)

Unique study design or methodology (if any): animals were housed individually in suspended steel cages; food (PMI Nutrition International Certified Rodent Chow® #5002) and water were available ad libitum.

Experimental Design for the Toxicity and Toxicokinetic Phases

Group No.	No. of Animals				Test Material	Sumatriptan Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration[a] (mg/mL)
	Toxicity (Recovery)		Toxicokinetics					
	Male	Female	Male	Female				
1	10 (5)	10 (5)	9	9	Sterile Water for Injection	0	2	0
2	10	10	9	9	Sumatriptan aqueous formulation solution containing Impurity 1 at (b) (4) and Impurity 3 at (b) (4)	3	2	1.5
3	10	10	9	9	Sumatriptan aqueous formulation solution containing Impurity 1 at (b) (4) and Impurity 3 at (b) (4)	30	2	15
4	10 (5)	10 (5)	9	9	Sumatriptan aqueous formulation solution containing Impurity 1 at (b) (4) and Impurity 3 at (b) (4)	100	2	50
5	10 (5)	10 (5)	--	--	Sumatriptan aqueous formulation solution (no impurities)	100	2	50

[a]The concentration for dosing was adjusted by normalization to sumatriptan free base. Impurity 1 and Impurity 3 were included at a concentration of (b) (4) of sumatriptan base, respectively.

Observation times and results:

Dosing formulation analysis/stability: analysis of dosing formulation preparations (see summary table below) indicated sumatriptan levels fell within ±10% of target, while levels of impurities 1 and 3 were predominantly low, but within 20% of target values; preparations were reported to be stable for 24 hours at room temperature and 14 days at 5°C

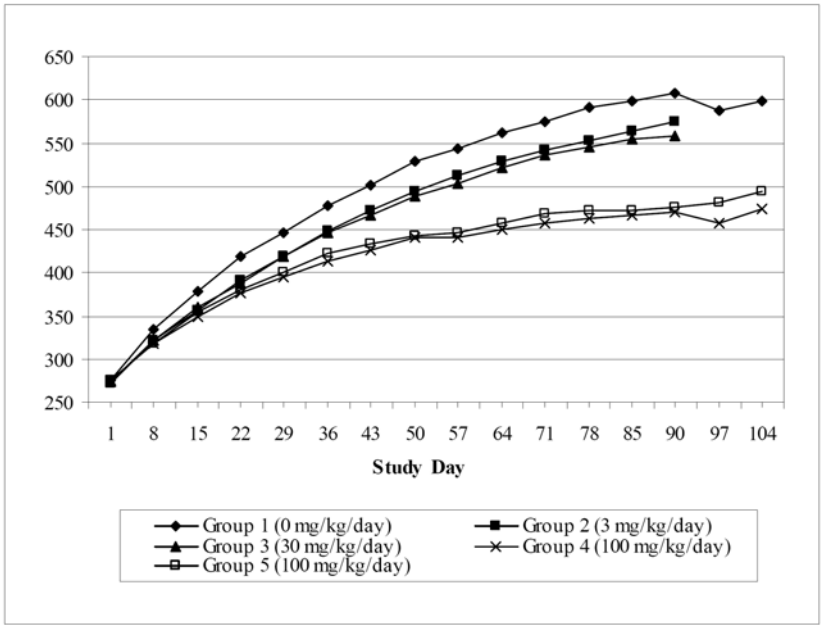
			Mean Concentration (mg/mL) (% Target)												
Group (Dose/Day)	**Analyte**	**Target (mg/mL)**	**Day 1**	**Day 14**	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**	**Week 8**	**Week 9**	**Week 10**	**Week 11**
2 (3 mg/kg)	Drug Substance	1.5	1.50 (100)	1.52 (101)	1.51 (101)	1.52 (101)	1.50 (100)	1.49 (99)	1.46 (97)	1.55 (103)	1.49 (99)	1.50 (100)	1.51 (101)	1.50 (100)	
	Impurity 1														(b) (4)
	Impurity 2														
3 (30 mg/kg)	Drug Substance	15			14.44 (96)	15.09 (101)	15.23 (102)	14.94 (100)	14.92 (99)	14.67 (98)	14.89 (99)	14.89 (99)	15.00 (100)	15.15 (101)	
	Impurity 1														(b) (4)
	Impurity 2														
4 (100 mg/kg)	Drug Substance	50			50.85 (102)	50.24 (100)	50.49 (101)	50.20 (100)	49.50 (99)	50.27 (101)	49.55 (99)	49.55 (99)	49.41 (99)	49.74 (99)	50.41 (101)
	Impurity 1														(b) (4)
	Impurity 2														
5 (100 mg/kg)	Drug Substance	50			49.18 (98)	50.01 (100)	50.52 (101)	50.19 (100)	50.27 (101)	50.44 (101)	49.79 (100)	49.76 (100)	49.64 (99)		

Mortality: Observations were performed twice daily. Two animals were found dead, one Group 4 male (#146, Day 54) and one Group 5 male (#162, Day 76); histopathology was suggestive of injection site bacterial infection and chronic peritonitis. All other animals survived to scheduled euthanasia.

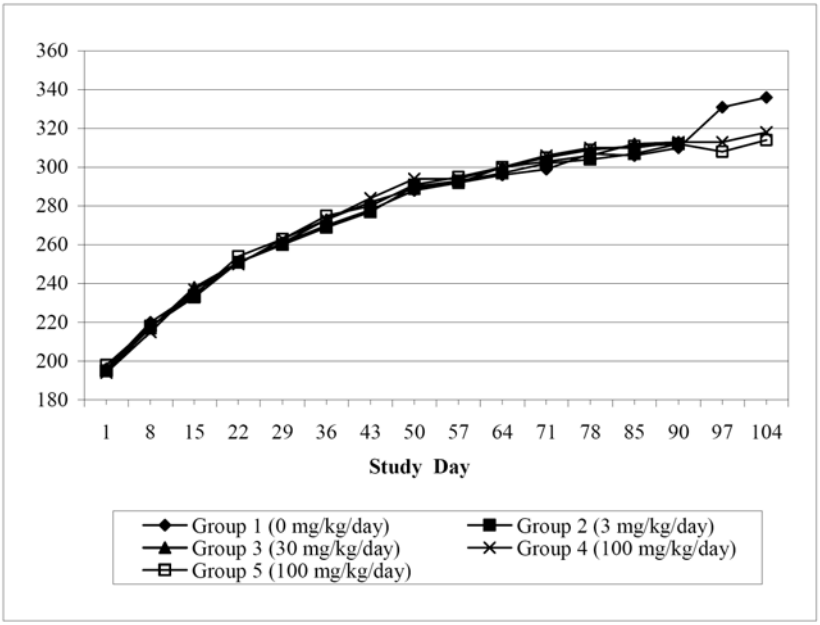
Clinical signs: Weekly detailed clinical observations and daily cage-side observations were performed. Convulsions were observed in one Group 4 male (#156, Day 85, duration ~30 seconds) and one Group 5 male (#159, Day 72, duration ~45 seconds). Other reported findings with increased incidence and/or frequency in mid- and/or high-dose groups of both sexes included vocalization, overt aggression, scabs, raised areas, and open lesions, with these last three findings continuing to be present with some resolution in recovery animals. The presence or absence of impurities 1 and 3 did not appear to be a factor in any of these findings.

Body weights: Body weights were recorded weekly (see sponsor's growth curves reproduced below). Males appeared to be more sensitive to the effects of treatment than were females.

Male Group Mean Body Weights (g)



Female Group Mean Body Weights (g)



Food consumption: Food consumption was recorded weekly. Sporadic and slight decreases in high-dose males and increases in high-dose females were considered incidental and not reflective, in particular, of the body weight loss in male animals.

Ophthalmoscopy: Performed at pre-study and during last week of treatment. No treatment-related effects reported.

ECG: not performed

Hematology: Sampling as noted in sponsor's table reproduced below; blood collected from vena cava under isoflurane anesthesia.

Samples for Clinical Pathology Evaluation

Group Nos.	Time Point	Hematology	Coagulation	Clinical Chemistry	Urinalysis
1-5[a]	Day 91/92	x	x	x	x
1, 4 and 5	Day 105	x	x	x	x

Note: "x" = scheduled sample collection.
[a]Samples were only collected from those animals scheduled for euthanasia on Days 91 and 92.

Notable effects on hematological parameters are summarized in the table on the following pages. For most parameters, values obtained in Groups 4 and 5 (i.e., same, high dose of sumatriptan with and without, respectively, spiked impurities 1 and 3) were generally affected qualitatively and quantitatively in a similar manner, suggesting minimal or no adverse impact from the added presence of the impurities. Red cell morphology was unaffected by treatment.

Clinical chemistry: Sampling as noted in sponsor's table reproduced above; blood collected from vena cava under isoflurane anesthesia. Notable effects on clinical chemistry parameters are summarized in the table on the following pages. For most parameters, values obtained in Groups 4 and 5 (i.e., same, high dose of sumatriptan

with and without, respectively, spiked impurities 1 and 3) were generally affected qualitatively and quantitatively in a similar manner, suggesting minimal or no adverse impact from the added presence of the impurities.

Selected Hematology/Coagulation Data

	Male Rats Mean ± SD (% Difference from Control)					**Female Rats** Mean ± SD (% Difference from Control)				
Parameter/ Sample Group	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Hemoglobin (g/dL)	14.3	14.8	15.0	13.1	13.0	14.0	15.1	14.6	13.5	13.0
	0.39	0.65	0.30	0.61	0.57	0.58	0.51	0.35	0.53	0.56
--main study		3.6	5.1	-8.2	-8.9		7.5	4.5	-3.6	-7.3
--recovery	14.3			13.5	13.3	14.9			13.8	14.3
	0.60			1.24	0.89	0.35			0.54	0.80
				-5.0	-6.7				-7.5	-4.0
Reticulocytes (% RBC)	2.08	2.14	2.13	4.14	4.02	2.04	1.81	1.91	3.44	3.43
	0.339	0.255	1.420	0.617	0.238	0.280	0.428	0.214	1.150	0.848
--main study		2.9	2.2	98.9	93.3		-11.6	-6.4	68.6	67.7
--recovery	2.00			4.26	3.14	2.14			1.90	2.30
	0.328			2.932	0.753	0.246			0.617	0.889
				112.7	56.8				-11.0	7.9
Platelets ($10^3/mm^3$)	1165	1094	1167	1481	1605	1197	1146	1187	1561	1599
	106.1	88.2	140.2	299.7	107.7	143.1	116.3	161.9	258.7	141.7
--main study		-6.1	0.2	27.1	37.8		-4.3	-0.8	30.4	33.5
--recovery	1147			1277	1474	1032			1441	1272
	75.8			171.0	229.2	146.6			214.0	233.6
				11.4	28.6				39.7	23.3
Leukocytes ($10^3/mm^3$)	8.19	8.58	11.08	17.11	16.16	4.97	5.53	7.26	12.14	12.25
	1.699	2.075	2.774	2.939	4.577	1.646	1.720	1.334	2.080	2.201
--main study		4.7	35.3	109.0	97.3		11.2	45.9	144.1	146.3
--recovery	9.19			12.46	13.13	4.59			8.36	7.13
	1.749			1.680	4.355	1.141			1.071	1.392
				35.6	42.9				82.3	55.4
Lymphocytes ($10^3/mm^3$)	6.37	6.21	7.62	8.66	8.01	3.76	4.24	5.62	7.44	6.83
	1.465	1.385	1.926	1.742	2.685	1.167	1.520	1.231	2.103	1.474
--main study		-2.6	19.6	35.9	25.8		12.9	49.6	98.1	81.8
--recovery	6.75			7.94	7.98	3.46			5.03	4.80
	1.964			1.459	1.628	1.240			0.776	0.711
				17.6	18.3				45.3	38.7
Monocytes ($10^3/mm^3$)	0.28	0.40	0.51	0.81	0.93	0.19	0.22	0.25	0.51	0.50
	0.086	0.110	0.175	0.252	0.253	0.076	0.102	0.094	0.119	0.111
--main study		44.6	85.1	192.7	235.3		20.5	32.4	174.6	169.2
--recovery	0.44			0.73	0.64	0.22			0.49	0.39
	0.066			0.212	0.165	0.055			0.038	0.075
				64.5	44.2				120.5	74.1
Seg. Neutrophils ($10^3/mm^3$)	1.29	1.68	2.55	6.93	6.59	0.90	0.90	1.19	3.76	4.48
	0.558	0.752	0.968	2.109	2.389	0.653	0.375	0.594	1.288	1.452
--main study		30.9	98.2	438.2	412.4		-0.6	31.9	316.4	396.3
--recovery	1.71			3.38	4.11	0.73			2.54	1.69
	0.341			1.435	2.927	0.282			1.130	0.083
				97.8	140.4				247.3	207.3
Eosinophils	0.13	0.11	0.13	0.18	0.17	0.05	0.08	0.09	0.19	0.17

	Male Rats Mean ± SD (% Difference from Control)					Female Rats Mean ± SD (% Difference from Control)				
Parameter/ Sample Group	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
(10^3/mm^3)	0.030	0.041	0.042	0.077	0.048	0.014	0.025	0.027	0.075	0.083
-main study		-14.1	2.3	44.1	32.8		52.7	58.2	238.2	207.3
--recovery	0.09			0.12	0.18	0.06			0.16	0.13
	0.019			0.031	0.094	0.018			0.051	0.027
				26.3	91.6				146.9	109.4
Basophils	0.01	0.02	0.03	0.04	0.04	0.01	0.01	0.01	0.03	0.03
(10^3/mm^3)	0.006	0.010	0.013	0.014	0.013	0.004	0.007	0.008	0.010	0.007
--main study		58.3	158.3	224.1	205.6		37.5	25.0	225.0	212.5
--recovery	0.02			0.03	0.04	0.01			0.02	0.01
	0.008			0.009	0.015	0.004			0.009	0.005
				70.0	80.0				100.0	75.0
Lg. Unstain	0.113	0.155	0.234	0.496	0.417	0.068	0.077	0.111	0.228	0.255
Cell (10^3/mm^3)	0.0445	0.0652	0.0900	0.1956	0.1111	0.0239	0.0306	0.0428	0.0857	0.0871
--main study		37.2	107.1	338.5	268.7		13.2	63.2	235.3	275.0
--recovery	0.168			0.256	0.184	0.094			0.118	0.098
	0.0263			0.0910	0.0371	0.0251			0.0311	0.0409
				52.8	9.9				25.5	4.3

Selected Clinical Chemistry Data

	Male Rats Mean ± SD (% Difference from Control)					Female Rats Mean ± SD (% Difference from Control)				
Parameter/ Sample Group	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
AST (IU/L)	75	60	69	128	119	67	79	82	100	105
--main study	12.8	10.5	14.6	44.4	18.7	10.1	50.5	24.9	22.3	19.2
		-19.9	-7.2	71.0	58.5		17.6	22.5	48.5	56.4
--recovery	59			68	96	60			217	79
	3.3			9.5	35.0	4.5			202.0	7.9
				16.6	63.8				261.8	31.4
ALT (IU/L)	26	25	25	32	31	29	45	33	32	34
--main study	3.7	4.2	3.0	5.7	4.5	7.8	59.9	13.1	7.6	5.9
		-3.3	-0.3	24.7	22.6		56.4	14.9	11.0	17.6
--recovery	26			25	26	27			105	25
	4.4			2.2	1.7	2.5			115.0	7.9
				-5.9	-2.9				296.9	-5.8
AP (IU/L)	77	63	69	87	94	39	45	55	48	75
--main study	14.0	9.2	12.9	27.9	21.0	9.8	17.7	25.7	6.9	14.2
		-18.0	-10.6	13.1	22.3		16.4	41.2	23.3	92.6
--recovery	73			72	84	36			44	38
	6.4			15.8	16.6	9.9			11.7	5.2
				-1.1	14.9				22.7	4.8
Cholesterol	33	32	39	37	39	39	44	44	44	40
(mg/dL)	8.3	8.2	10.2	4.5	7.6	9.9	12.1	9.1	5.3	6.5
--main study		-1.7	20.2	13.5	19.6		14.4	14.0	14.3	2.8
--recovery	30			37	34	43			49	39
	5.2			3.4	4.3	10.8			12.9	7.8
				23.5	11.6				14.0	-10.8

	Male Rats Mean ± SD (% Difference from Control)					**Female Rats** Mean ± SD (% Difference from Control)				
Parameter/ Sample Group	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**	**Group 1**	**Group 2**	**Group 3**	**Group 4**	**Group 5**
Triglycerides (mg/dL) --main study	43 16.6	42 14.3 -3.0	31 6.8 -28.4	31 5.5 -27.5	33 7.7 -22.7	33 9.1	34 8.5 4.2	35 10.5 7.1	30 5.2 -8.8	30 5.9 -7.9
--recovery	68 18.3			31 7.0 -54.6	29 7.4 -56.7	36 9.7			23 4.9 -35.9	26 9.7 -27.9
Albumin (g/dL) --main study	2.99 0.135	2.91 0.124 -2.6	2.86 0.125 -4.0	2.49 0.189 -16.5	2.53 0.117 -15.3	3.37 0.246	3.56 0.368 5.8	3.27 0.158 -3.0	2.99 0.255 -11.1	2.85 0.226 -15.3
--recovery	2.93 0.106			2.48 0.377 -15.5	2.46 0.389 -16.0	3.93 0.161			3.41 0.382 -13.3	3.06 0.206 -22.1
Globulin (g/dL) --main study	2.83 0.270	3.00 0.210 6.0	2.94 0.248 3.6	3.22 0.224 13.7	3.27 0.171 15.5	3.02 0.126	3.19 0.133 5.5	3.12 0.147 3.3	3.47 0.231 14.7	3.70 0.268 22.2
--recovery	2.98 0.102			3.59 0.170 20.3	3.58 0.165 19.9	3.29 0.206			3.62 0.288 10.0	3.53 0.349 7.2
Glucose (mg/dL) -main study	218 33.0	199 20.0 -8.5	159 23.6 -27.0	145 21.7 -33.3	147 21.4 -32.2	182 23.8	153 24.6 -15.7	162 23.5 -10.6	145 24.0 -20.1	128 19.5 -29.7
--recovery	195 43.6			135 32.5 -30.8	126 22.9 -35.5	186 28.1			146 13.2 -21.3	148 29.4 -20.5
Phosphorus (mg/dL) --main study	6.1 0.39	6.9 0.56 13.4	7.1 0.46 6.0	7.2 0.64 18.6	6.5 0.50 7.3	5.3 1.09	5.8 0.54 9.6	6.6 0.61 23.1	7.0 0.46 31.7	6.8 0.65 27.5
--recovery	6.5 0.22			7.2 0.46 10.7	7.2 0.36 11.7	5.8 0.49			5.7 0.39 -3.0	6.8 0.63 16.9
Potassium (mmol/L) --main study	4.60 0.173	4.77 0.199 3.7	4.69 0.286 2.0	4.96 0.259 7.7	4.71 0.253 2.4	4.03 0.279	4.38 0.337 8.7	4.25 0.191 5.4	4.43 0.371 9.9	4.43 0.286 9.8
--recovery	4.50 0.255			4.70 0.346 4.4	5.13 0.401 14.1	4.16 0.105			4.45 0.307 7.1	4.62 0.121 11.2

Urinalysis: Sampling as noted in sponsor's table reproduced above; non-fasted animals were individually housed in suspended stainless steel urine collection cages containing water bottles with ball-bearing sipper tubes and urine was collected by cage pan drainage overnight. The only notable finding was variable amounts of lysed blood cells in the urine of Group 4 and 5 animals.

Gross pathology: Animals were sacrificed via isoflurane inhalation followed by exsanguination; necropsy proceeded according to sponsor's tabular summary of terminal procedures reproduced below. Apparent treatment-related gross findings in both HD groups (both sexes) included abdominal adhesions, thickened fat, and

enlarged lymph nodes, as well as injection site thickening and discoloration. These observations were also reported in recovery animals.

Terminal Procedures

Group No.	No. of Male/Female Rats		Scheduled Euthanasia Day		Terminal Procedures			Histopathology
	Toxicity Phase	Recovery Phase	Toxicity Phase	Recovery Phase	Gross Necropsy	Tissue Collection	Organ Weights	
1	10/10	5/5	91/92	105	x	x	x	Full
2	10/10	--	91/92	--	x	x	x	Gross Lesions
3	10/10	--	91/92	--	x	x	x	Gross Lesions
4	10/10	5/5	91/92	105	x	x	x	Full
5	10/10	5/5	91/92	105	x	x	x	Full
Found Dead Animals					x	x	--	Full
Note: "x" = procedure conducted.								

Organ weights: Organ weights collected were as shown in sponsor's table reproduced below.

Organ Weights

Adrenal gland[a]	Prostate gland
Brain	Salivary gland[a]
Epididymis[a]	Seminal vesicles[a]
Heart	Spleen
Kidney[a]	Testis[a]
Liver	Thymus
Lung	Thyroid gland with parathyroid gland[a]
Ovary[a]	Uterus
Pituitary gland	
[a]Paired organ weight.	

Selected organ weight findings are summarized in the table below, showing organ weight relative to brain weight and expressed as the percent difference from control for the noted organ. Within the variability of the data, parameter values in Groups 4 and 5 were generally affected qualitatively and quantitatively in a similar manner, suggesting minimal or no adverse impact from the added presence of the impurities. Several of the observed differences from controls persisted through the recovery period.

	0 mg/kg	**3 mg/kg**	**30 mg/kg**	**100^{+} mg/kg**	**100 mg/kg**
Parameter	**Males**				
Final Body Wt (g) (mean ± SD) (% Difference from control)	588 ± 72.0	543 ± 52.5 (-7.6)	530 ± 46.0 (-9.8)	450 ± 42.0 (-23.4)	445 ± 27.7 (-24.4)
Final Brain Wt (g) (mean ± SD) (% Difference from control)	2.34 ± 0.097	2.28 ± 0.101 (-2.5)	2.27 ± 0.053 (-2.7)	2.24 ± 0.119 (-4.1)	2.18 ± 0.072 (-6.8)
--Adrenals ratio		4.7	16.1	36.3	33.8
--Pituitary ratio		-6.3	-0.1	-8.0	-6.5
--Thyroid glands ratio		3.4	-12.0	-4.6	-8.1
--Heart ratio		-3.1	2.2	-5.3	-1.8
--Liver ratio		-9.6	-14.6	-18.4	-13.9
--Spleen ratio		-2.8	3.4	42.1	29.8

	0 mg/kg	3 mg/kg	30 mg/kg	100+ mg/kg	100 mg/kg
--Thymus ratio		-16.2	-15.2	-38.9	-24.5
--Prostate ratio		14.7	11.7	-6.5	-8.0
--Testes ratio		4.0	7.1	-6.5	-1.7
	Females				
Final Body Wt (g) (mean ± SD) (% Difference from control)	284 ± 18.6	290 ± 19.0 (2.1)	293 ± 31.0 (3.2)	290 ± 27.0 (2.1)	291 ± 16.9 (2.3)
Final Brain Wt (g) (mean ± SD) (% Difference from control)	2.04 ± 0.068	2.07 ± 0.081 (1.3)	2.12 ± 0.086 (3.9)	2.06 ± 0.054 (1.0)	2.11 ± 0.092 (3.3)
--Adrenals ratio		-3.2	5.6	23.6	13.1
--Pituitary ratio		4.5	4.8	14.0	10.3
--Thyroid glands ratio		7.6	2.9	8.4	3.9
--Heart ratio		-2.8	0.8	14.0	2.7
--Liver ratio		3.1	3.0	21.0	13.5
--Spleen ratio		-3.0	7.6	45.5	44.3
--Thymus ratio		0.7	14.4	6.5	14.5
--Uterus ratio		-11.3	-3.3	-12.5	-15.2
--Ovaries ratio		-5.4	-0.6	3.8	2.3

Histopathology: All tissues and organs collected at necropsy (except animal identification; see sponsor's tabular listing reproduced below) from Group 1, 4 and 5 animals and gross lesions from all animals in all groups were examined microscopically; tissues were embedded in paraffin and stained with H&E.

Tissue Collection and Preservation

Adrenal gland (paired)	Mammary gland
Animal identification	Nerve, optic[b]
Aorta	Nerve, sciatic
Bone, femur	Ovary (paired)
Bone, sternum	Pancreas
Bone marrow, sternum	Parathyroid gland[c]
Bone marrow smear[a]	Pituitary gland
Brain (cerebrum, cerebellum, brain stem, medulla)	Prostate gland
Cervix	Salivary gland (paired)
Epididymis (paired)	Seminal vesicle (paired)
Esophagus	Skeletal muscle (thigh)
Eye (paired)[b]	Skin (mammary)
Harderian gland (paired)	Skin (untreated - hip region)
Heart	Spinal cord (cervical, thoracic, lumbar)
Injection sites(s)	Spleen
Intestine, cecum	Stomach (nonglandular and glandular)
Intestine, colon	Testis (paired)
Intestine, duodenum	Thymus
Intestine, ileum with Peyer's patch[c]	Thyroid gland (paired)
Intestine, jejunum	Tongue
Intestine, rectum	Trachea
Kidney (paired)	Urinary bladder
Liver	Uterus
Lung	Vagina
Lymph node, mandibular	Gross lesions/masses
Lymph node, mesenteric	

[a]Bone marrow smears were collected from the femur at scheduled necropsies only (for possible examination).
[b]Preserved in Davidson's fixative and then transferred to 10% neutral buffered formalin.
[c]Examined only if present in the routine section.

Adequate Battery: Yes
Peer review: No

Summarized in the table below are incidence data for selected histopathology findings from terminal sacrifice (toxicity phase) animals. In addition, there were observations at the four injection sites in almost all animals of varying grades of subcutaneous fibrosis, mixed cell inflammation, and hemorrhage. The majority of mid- and high-dose animals also exhibited varying grades of epidermal ulceration, fibrinosuppurative exudate, and hyperplasia, as well as dermal fibrosis at the injection sites. Most of these observations were still evident in recovery animals following the recovery period. There was no clearly discernible indication that the added presence of the spiked impurities 1 and 3 induced any additional pathology over and above that observed with sumatriptan drug substance alone.

Histopathology Observation	**Sex**	**0 mg/kg**	**3 mg/kg**	**30 mg/kg**	**100^{+} mg/kg**	**100 mg/kg**
Adrenal gland, within normal limits	**Males**	10/10	10/10	10/10	7/10	10/10
	Females	10/10	10/10	10/10	9/10	9/10
Bone marrow, sternum, hyperplasia, minimal	**Males**	4/10	2/10	3/10	6/10	7/10
	Females	0/10	0/10	2/10	10/10	9/10
Kidney, within normal limits	**Males**	10/10	0/1	0/0	7/10	9/10
	Females	10/10	0/1	0/0	8/10	10/10
Kidney, capsule, fibrosis, minimal	**Males**	0/10	0/1	0/0	2/10	0/10
	Females	0/10	0/1	0/0	0/10	0/10
Liver, infiltrates, mixed cell, minimal to mild	**Males**	10/10	0/0	0/1	10/10	10/10
	Females	10/10	0/0	0/0	10/10	10/10
Lymph node, mesenteric, hyperplasia, minimal	**Males**	0/10	0/0	0/0	0/10	0/10
	Females	3/10	0/0	0/0	0/10	1/10
Lymph node, mandibular, hyperplasia, minimal to marked	**Males**	10/10	0/0	0/1	9/9	10/10
	Females	10/10	10/10	10/10	10/10	10/10
Lymph node, mandibular, plasmacytosis, minimal to marked	**Males**	9/10	0/0	0/1	8/9	8/10
	Females	10/10	10/10	10/10	10/10	10/10
Spleen, hematopoiesis, minimal to moderate	**Males**	2/10	5/10	6/10	10/10	10/10
	Females	1/10	2/10	3/10	6/10	9/10
Spleen, hyperplasia, lymphoid, minimal to mild	**Males**	2/10	0/10	2/10	8/10	10/10
	Females	0/10	0/10	0/10	10/10	10/10
Spleen, capsule, fibrosis, minimal to moderate	**Males**	0/10	0/10	0/10	7/10	7/10
	Females	0/10	0/10	0/10	5/10	4/10
Spleen, capsule, inflammation, mixed cell, minimal to moderate	**Males**	0/10	0/10	0/10	5/10	5/10
	Females	0/10	0/10	0/10	0/10	0/10

Special evaluation: N/A

Toxicokinetics: Sumatriptan plasma levels were assessed on Days 1 and 90 (3 rats/sex/group) according to the schedule provided in the sponsor's tabular summary

reproduced below. No explanation is provided by the sponsor for why Group 5 was excluded from TK analyses.

TK Sample Collection Schedule

Group No.	Subgroup	No. of Males/ Females	Sample Collection Time Points (Hours Post-Dose) on Days 1 and 90					
			0 minutes[a]	10 minutes	30 minutes	60 minutes	90 minutes	24 hours
1	A	3/3	x			x		
	B	3/3		x			x	
	C	3/3			x			x
2	A	3/3	x			x		
	B	3/3		x			x	
	C	3/3			x			x
3	A	3/3	x			x		
	B	3/3		x			x	
	C	3/3			x			x
4	A	3/3	x			x		
	B	3/3		x			x	
	C	3/3			x			x

Note: "x" = scheduled sample collection.
[a]Samples collected prior to dosing on Days 1 and 90.

Reproduced below is the sponsor's summary table of estimated sumatriptan TK parameters resulting from the TK analyses conducted. Almost all plasma concentrations of sumatriptan were below the limit of detection in Group 2 animals (3 mg/kg/day). In addition, the absence of sampling by the sponsor between 1.5 and 24 hours after dosing precluded identification of a terminal elimination phase in the mid- and high-dose groups and, thus, estimation of a terminal elimination half-life.

		Males		Females	
Group (Dose)	**TK Parameter**	**Day 1**	**Day 90**	**Day 1**	**Day 90**
3 (30 mg/kg/day)	C_{max} (µg/mL)	5.29	7.02	4.88	8.28
	T_{max} (hr)	0.50	0.50	0.50	0.17
	AUC(0-t_{last}) (µg•hr/mL)	6.17	9.06	5.69	8.52
4 (100 mg/kg/day)	C_{max} (µg/mL)	14.6	15.5	11.7	16.9
	T_{max} (hr)	1.00	1.00	1.00	1.00
	AUC(0-t_{last}) (µg•hr/mL)	16.7	18.1	14.0	22.2

Study title: A Rising Dose and Multiple Dose Tolerance Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 Administered by the Subcutaneous Route to Rats

Key study findings:

- The MTD for both male and female rats in the planned 91-day repeated dose toxicity study should be considered 100 mg/kg/day, based on observations of scabbing, crusting, and thickening of the injection site at 300 mg/kg/day.

Study no.: YQT00003
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: 21 August 2007
GLP compliance: Yes, with exceptions reproduced below:

- Characterization and stability analyses of the test article were not conducted in compliance with GLP regulations.
- Test article stability analysis was not conducted at the 216 mg/mL concentration.

QA statement: Yes
Drug, lot #, and % purity: sumatriptan succinate (lot# 11315, purity not defined); sumatriptan impurity 1 (lot# ACM-MOOI3-SD-II-24-15, purity 63.8% as free base); sumatriptan impurity 3 (TYG-YCH-006-2, purity 79.0% as free base)

Methods:

Doses: see sponsor's study design summary table reproduced below
Species/strain: Sprague Dawley Crl:CD(SD) rats
Number/sex/group or time point: see sponsor's study design summary table reproduced below
Route: subcutaneous injection at one of four injection sites, rotated daily (not indicated by sponsor, but presumed to be dorsal)
Formulation/vehicle: Sterile Water for Injection (lot# 6071175)
Dosing solution analyses/drug stability and homogeneity: dosing formulation solution concentration analysis was performed as described in the sponsor's table reproduced below

Dose Formulation Samples for Analysis

Phase	Time Point	Concentration	Homogeneity[a]	Stability[b]
Rising Dose	Day 1	16.2 mg/mL	N/A	N/A
Rising Dose	Day 4	32.4 mg/mL	N/A	N/A
Rising Dose	Day 7	64.8 mg/mL	N/A	N/A
Rising Dose	Day 10	129.6 mg/mL	N/A	N/A
Rising Dose	Day 13	216.0 mg/mL	N/A	N/A
Multiple Dose	Day 1	20 and 60 mg/mL	N/A	N/A
Multiple Dose	Day 10	20 and 60 mg/mL	N/A	N/A

Note: N/A = not applicable.
[a]Homogeneity analysis was not required. Dose formulations were solutions containing water-soluble compounds.
[b]Stability was monitored under a separate protocol.

Dose volume/infusion rate: see sponsor's study design summary table reproduced below.
Satellite groups used for toxicokinetics or recovery: not performed
Age: approximately 8 weeks of age at the time of randomization (rising dose phase); approximately 11 weeks of age at the time of randomization (multiple dose phase)
Weight: 233-245 grams for the males and 171-185 grams for the females (rising dose phase); 389-435 grams for the males and 232-244 grams for the females (multiple dose phase)
Unique study design or methodology (if any): animals were housed individually in suspended steel cages; food (PMI Nutrition International Certified Rodent Chow® #5002) and water were available ad libitum.

Experimental Design for the Rising Dose Phase

Cycle No.[a]	No. of Toxicity Animals		Dose Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration[b] (mg/mL)
	Males	Females				
1	3	3	Sumatriptan aqueous formulation solution containing Impurity 1 at (b) and Impurity 3 at (b)	81	5	16.2
2				162	5	32.4
3				324	5	64.8
4				648	5	129.6
5				1296	6	216.0

[a]Animals were dosed once followed by a 3-day washout period before continuing to the next cycle.
[b]The concentration for dosing was adjusted by normalization to sumatriptan free base. Impurity 1 and Impurity 3 were included at a concentration of (b) (4) respectively, of sumatriptan base.

Experimental Design for the Multiple Dose Phase

Group No.	No. of Toxicity Animals		Dose Material	Dose Level (mg/kg/day)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)
	Males	Females				
1	3	3	Sumatriptan aqueous formulation solution containing Impurity 1 at (b) and Impurity 3 at (4)	100	5	20
2	3	3	Sumatriptan aqueous formulation solution containing Impurity 1 at (b) and Impurity 3 (4)	300	5	60

Summary Results and Conclusions: Results of the dosing formulation analyses revealed that the average results were within ± 2% of the nominal concentrations for both rising dose and multiple dose phases of the study.

In the rising dose phase of the study, two female animals were found dead on Day 14 following administration of the 1296 mg/kg dose; all remaining animals survived to scheduled euthanasia. Notable clinical signs observed in males and females after administration of 1296 mg/kg/day included tremors, decreased activity, impaired mobility, apparent hyperthermia, and partially closed eyelids. At necropsy, findings included injection site scabbing, skin scabbing and discoloration, and discoloration of the brachial or mandibular lymph nodes. Dose levels of 81, 162, 324, and 648 mg/kg in the rising dose phase were generally well tolerated in rats.

In the 10-day multiple dose phase at dose levels of 100 and 300 mg/kg/day, all animals survived to scheduled euthanasia. Clinical signs and gross necropsy findings were observed at the injection site in males and females at both dose levels; in addition, mild changes in hematology parameters were observed at the 300 mg/kg/day dose. Under the conditions of the study, a NOAEL could not be determined. It was concluded that 100 mg/kg/day should be considered the MTD for both male and female rats in the planned 91-day repeated dose toxicity study, based on observations of scabbing, crusting, and thickening of the injection site at 300 mg/kg/day.

7. Genetic toxicology

7.1 In vitro reverse mutation in bacterial cells: no data submitted

7.2 In vitro chromosomal aberrations in mammalian cells

Study title: Sumatriptan DosePro Degradation Products (Impurities 1 and 3) Chromosome Aberration Test

Key study findings:

- Criteria for a valid assay appear to have been met.
- Weakly positive evidence of clastogenicity was observed at the highest dose tested (10 mM) in the 21-hr, -S9 exposure paradigm.
- No attempt at confirming the initial negative findings in the 4-hr, +S9 exposure.

Study no.: 962496
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation:
GLP compliance: Yes, with the exceptions reproduced below from the sponsor's submission

- Stability of formulations was not determined in this study. Note that formulations were prepared on the morning of use and formulation analysis was conducted in compliance with GLP.
- The test articles were not characterized in compliance with GLP or GMP regulations. Characterization of the test articles was, however, conducted according to established SOPs, controls and approved test methodologies to ensure integrity and validity of the results generated.

QA statement: Yes
Drug, lot #, and % purity: Sumatriptan degradant, Peak 1 (lot #07-066-33-25), purity 96% via HPLC and 71% as free base; Sumatriptan degradant, Peak 3 (lot # 07-066-49-05), purity 93% via HPLC and 66% as free base

Methods

Cell line: human peripheral blood lymphocytes (healthy, non-smoking, male donors)

Concentration used in definitive study: 10 mM (3124 µg/mL)

Basis of concentration selection: regulatory guideline-prescribed limit

Negative controls: sterile water for irrigation, USP (lot #W8C07P0)

Positive controls: mitomycin C (MMC) (-S9); cyclophosphamide monohydrate (CP) (+S9)

Incubation and sampling times: 4 hours with/without exogenous metabolic activity (rat liver S9); 21 hours without S9 (see sponsor's study design summary reproduced below)

Study validity: Criteria for a valid assay appear to have been largely met; however, given the relatively weak positive response elicited by Impurity 3 at the highest dose tested in the 21-hour, -S9 treatment, confirmation of the apparent negative response in the 4-hr, +S9 treatment may be warranted.

Text Table 1 Study Design

Material	Concentration			Culture Numbers		
	Formulation	Final		4 Hours (0S9)	4 Hours (+S9)	21 Hours (0S9)
	mM†	mM	µg/mL‡			
Vehicle control	-	-	-	2	2	2
Test article 1	4.40	0.44	145	2	2	2
	6.30	0.63	205	2	2	2
	8.80	0.88	289	2	2	2
	12.5	1.25	409	2	2	2
	17.7	1.77	579	2	2	2
	25.0	2.50	819	2	2	2
	35.4	3.54	1158	2	2	2
	50.0	5.00	1637	2	2	2
	70.7	7.07	2315	2	2	2
	100	10.0	3274	2	2	2
Test article 2	4.40	0.44	138	2	2	2
	6.30	0.63	195	2	2	2
	8.80	0.88	276	2	2	2
	12.5	1.25	391	2	2	2
	17.7	1.77	552	2	2	2
	25.0	2.50	781	2	2	2
	35.4	3.54	1105	2	2	2
	50.0	5.00	1562	2	2	2
	70.7	7.07	2209	2	2	2
	100	10.0	3124	2	2	2
Mitomycin C	5.00	-	0.05	2	-	2
	10.0	-	0.10	2	-	2
	20.0	-	0.20	2	-	2
Cyclophosphamide	800	-	8.00	-	2	-
	1200	-	12.0	-	2	-
	1600	-	16.0	-	2	-

† Concentrations of the positive controls expressed in terms of µg/mL.

‡ Concentrations of test articles expressed in terms of pure anhydrous free base. Note that dose levels are separated by an exact √2 dose interval.

Results: Reproduced below is the sponsor's tabular summary of results from the current study, as well as a summary of laboratory historical control data.

Table 1 Results and Statistical Analysis

Treatment	Final Conc. (mM)	Final Conc. (µg/mL)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations b	e	B	E	other	Incidental observations † (g	G	P	C)
4 hours treatment in the absence of S9 (0S9) – Original test															
Water	-	-	7.1	100	200	1.0	2	0	0	0	0	1	1	6	0
Impurity 1	5.00	1637	7.7	108	200	3.0	6	0	0	0	0	5	0	3	0
	7.07	2315	7.6	108	200	2.5	6	0	0	0	0	5	1	1	0
	10.0	3274	7.9	112	200	1.5	3	0	1	0	0	1	0	10	0
MMC	-	0.10	6.6	93	200	9.5**	15	1	4	0	0	4	2	2	0
Impurity 3	5.00	1562	7.3	102	200	3.0	5	0	1	0	0	0	0	5	2
	7.07	2209	6.9	97	200	3.5	5	0	2	0	0	1	0	1	0
	10.0	3124	8.4	119	200	2.5	4	0	1	0	0	1	0	4	0
4 hours treatment in the presence of S9 (+S9) – Original test															
Water	-	-	6.6	100	200	3.0	6	0	0	0	0	0	0	4	1
Impurity 1	5.00	1637	6.6	99	200	2.5	5	0	0	0	0	3	1	6	0
	7.07	2315	8.3	126	200	3.0	6	0	0	0	0	7	1	6	0
	10.0	3274	7.1	107	200	4.0	7	0	1	0	0	3	0	5	0
CP	-	8.0	5.2	79	200	29.5**	71	3	13	0	0	7	7	1	1
Impurity 3	5.00	1562	5.0	75	200	3.0	5	0	1	0	0	1	0	3	0
	7.07	2209	7.9	119	200	2.5	5	0	0	0	0	3	0	5	0
	10.0	3124	8.0	121	200	4.0	8	0	0	0	0	4	0	3	0

MI, RMI	Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g	Chromatid break, exchange, gap
B, E, G	Chromosome break, exchange, gap
other	Includes pulverized chromosomes and cells with > 8 aberrations
P	Polyploidy and endoreduplication
C	Centromeric disruption
†	g, G, P and C are excluded from the calculation of % aberrant cells

Results of statistical analysis using one-tailed Fisher's exact test

*	$p \leq 0.01$ (significant)
**	$p \leq 0.001$ (highly significant)
otherwise	$p > 0.01$ (not significant)

Table 1 Results and Statistical Analysis (Cont'd)

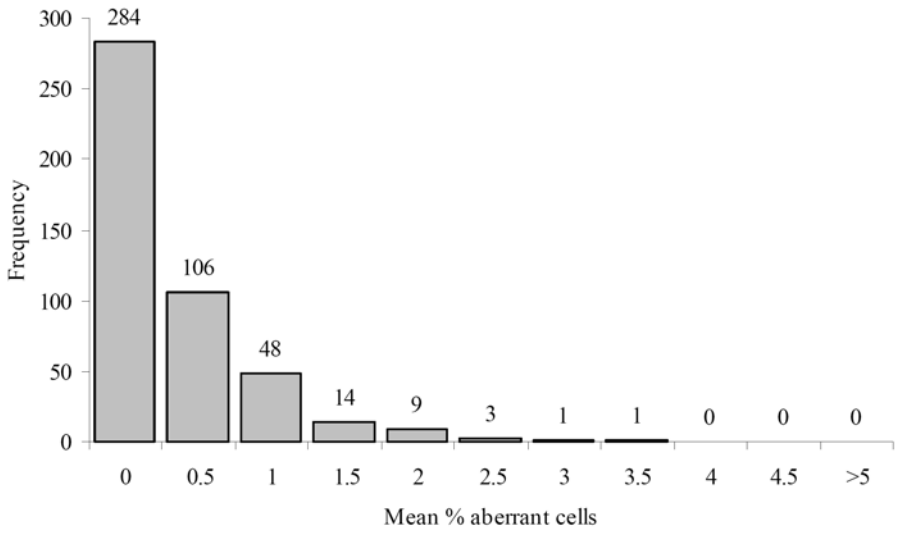
Treatment	Final Conc. (mM)	Final Conc. (µg/mL)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations b	e	B	E	other	Incidental observations † (g	G	P	C)
21 hours treatment in the absence of S9 (0S9) – Original test															
Water	-	-	3.6	100	200	1.0	0	0	2	0	0	2	1	5	0
Impurity 1	5.00	1637	3.6	102	200	4.0	8	0	1	0	0	4	0	7	0
	7.07	2315	2.7	77	200	5.5	10	0	3	0	0	4	1	1	1
	10.0	3274	2.1	60	200	3.5	6	0	1	0	0	3	1	3	0
MMC	-	0.05	3.1	88	200	12.5**	19	0	9	0	0	10	9	1	0
Impurity 3	0.88	276	5.9	167	200	4.0	6	1	1	0	0	3	1	0	1
	1.25	391	5.6	157	200	0.5	1	0	0	0	0	5	0	1	0
	5.00	1562	3.7	104	200	4.5	8	0	2	0	0	2	1	0	1
	7.07	2209	2.8	79	200	2.0	4	0	0	0	0	3	0	2	0
	10.0	3124	2.8	80	200	7.0*	12	0	2	0	0	8	2	6	0
21 hours treatment in the absence of S9 (0S9) – Supplemental test															
Water	-	-	5.9	100	200	1.5	3	0	0	0	0	3	1	4	2
Impurity 3	5.00	1562	3.5	58	200	1.5	3	0	0	0	0	7	0	1	1
	7.07	2209	2.7	46	200	4.0	9	0	1	0	0	1	0	1	0
	10.0	3124	2.9	50	181‡	[6.6]‡	12	0	1	0	0	5	0	1	1
MMC	-	0.10	4.1	69	200	15.5**	26	6	5	0	0	4	5	3	0

MI, RMI	Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g	Chromatid break, exchange, gap
B, E, G	Chromosome break, exchange, gap
other	Includes pulverized chromosomes and cells with > 8 aberrations
P	Polyploidy and endoreduplication
C	Centromeric disruption
†	g, G, P and C are excluded from the calculation of % aberrant cells
‡	200 cells not available for analysis due to a reduction in the absolute number of metaphases due to toxicity. Result in parentheses not submitted to formal statistical analysis due to toxicity and should be interpreted with caution.

Results of statistical analysis using one-tailed Fisher's exact test

*	$p \leq 0.01$ (significant)
**	$p \leq 0.001$ (highly significant)
Otherwise	$p > 0.01$ (not significant)

Figure 1 Historical Control Values



The laboratory historical mean incidence of aberrant metaphase cells for negative/vehicle control cultures for the human lymphocyte chromosome aberration test is 0.33% (SD 0.53) for 466 treatments. These QA audited results were collected from GLP compliant studies performed from 05 February 2003 to 24 January 2008.

The historical positive control values (for QA-audited and GLP compliant studies) are listed below:

Mitomycin C (4 hour 0S9):	mean 10.4%, SD 5.1, 115 treatments
Mitomycin C (21 hour 0S9):	mean 12.0%, SD 5.1, 119 treatments
Cyclophosphamide (4 hour +S9):	mean 19.6%, SD 7.5, 118 treatments

7.3 In vivo chromosomal aberrations in rodents

Study title: Mouse bone marrow micronucleus test and detection of DNA damage in the liver and peripheral blood of treated mice using the Comet assay

Key study findings:

- Criteria for a valid assay appear to have been met for both the micronucleus and the Comet assays.
- Bioanalytical assessment confirmed that systemic exposure to sumatriptan Impurity 3 did occur at the tested doses.
- There was no evidence that treatment of mice with sumatriptan Impurity 3 at up to 16 mg/kg/day induced an increase in micronuclei in the polychromatic erythrocytes of bone marrow or DNA damage in the liver and peripheral blood under the conditions of the study.

Study no.: 1000159 ((b) (4) Study Number 8200929)
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: 9 January 2009
GLP compliance: Yes
QA statement: Yes
Drug, lot #, and % purity: Sumatriptan Impurity 3 (lot #07-066-94-20), purity 86.7% (area%) and 63.1% (weight%, free base)

Methods:

Species: The test system employed was Crl:CD-1 (ICR) mice as characterized in the sponsor's summary table reproduced below.

Table 5: Animal Specification

	Range-Finder		Micronucleus/Comet Experiment[a]
Number of animals used in study	20 M	20 F	48 M
Weight range on first day of assay (g)	29-37	23-28	28-35
Approximate age on first day of dosing (weeks)	6-7		6

M Male
F Female
[a] Includes 12 satellite animals for bioanalysis

Doses used in definitive study: As defined in sponsor's summary table reproduced below

Table 2: Dose Levels - Micronucleus/Comet Experiment

Group Number	Treatment	Dose volume (mL/kg)	Dose (mg/kg/day)	No. of animals	Sample time (hours after final administration)
1	Vehicle control, WFI [a]	20	0	6M	3
2	Sumatriptan Impurity 3	20	4	6M	3
3	Sumatriptan Impurity 3	20	8 [b]	6M	3
4	Sumatriptan Impurity 3	20	16	6M	3
5	Positive control, EMS [c]	10	200	6M	3
6	Positive control, CPA [d]	10	40	6M	24

M Male
a WFI - water for injection
b Mice were treated with Sumatriptan Impurity 3 at the intended dose level stated in the table. Formulation analysis however confirmed that for day 3 (13 February 2009) the formulation as marginally outside of the specified limits (100 ± 10%). All doses from this point for the intermediate concentration have therefore been expressed as a nominal dose level administered, as confirmed by formulation analysis (Appendix 10)
c EMS - Ethyl Methanesulfonate - positive control used for the Comet assay, administered once
d CPA – Cyclophosphamide – positive control used for the mouse micronucleus assay, administered once

Basis of dose selection: Dose range-finding assay as characterized in the sponsor's summary table reproduced below. During a 2-day post-dose observation period, clinical signs of toxicity included death immediately following administration at doses of 22 mg/kg and greater. At doses of 16 mg/kg/day and lower, doses appeared to be tolerated with clinical signs confined largely to transient decreased activity. Since no substantial intersex differences in toxicity were observed, male animals only were tested in the definitive assay.

Table 1: Dose Levels - Range-Finder Experiment

Group Number	Treatment	Dose volume (mL/kg)	Dose (mg/kg/day)	No. of animals
1	Sumatriptan Impurity 3	20	1000	3M, 2F
2	Sumatriptan Impurity 3	20	700	1M, 1F
3	Sumatriptan Impurity 3	20	500	1M, 1F
4	Sumatriptan Impurity 3	20	250	1M, 1F
5	Sumatriptan Impurity 3	20	125	1M, 1F
6	Sumatriptan Impurity 3	20	31	2M, 2F
7	Sumatriptan Impurity 3	20	8	2M, 3F
8	Sumatriptan Impurity 3	20	11	3M, 3F
9	Sumatriptan Impurity 3	20	16	3M, 3F
10	Sumatriptan Impurity 3	20	22	3M, 3F

M Male
F Female

Negative controls: Water for injection

Positive controls: Ethyl methanesulfonate (EMS, used for the Comet assay) and cyclophosphamide (CPA, used for the micronucleus assay) as summarized in the sponsor's table reproduced below

Table 3: Positive Controls

Treatment	Dose volume (mL/kg)	Concentration of solution (mg/mL)	Dose of administered (mg/kg)
EMS	10	20.0	200
CPA	10	4.0	40

Incubation and sampling times: All treatments were administered via short intravenous infusion (~2 mL/minute) at time points of approximately 0, 24 and 45 hours, and animals were sampled 3 hours after the final administration (48 hours), as summarized in the sponsor's table reproduced below.

Figure 1: Test Article Administration and Endpoints

Time point (hrs)	0	24	45	48
Dose	X[a]	X[a,b]	X[b]	-
Sampling	-	-	-	X

[a] Dose affecting micronucleus endpoints
[b] Dose affecting Comet Assay endpoints

In addition, satellite animals (3/dose group) were included and sampled as shown in the sponsor's summary table reproduced below for purposes of bioanalytical confirmation of systemic exposure to sumatriptan Impurity 3

Table 6: Bioanalysis

Group	Number of animals	Dose level (mg/kg/day)	Dose volume (mL/kg)	Sample time (minutes after final administration) 5
Vehicle	3M	0	20	√
Sumatriptan Impurity 3	3M	4	20	√
Sumatriptan Impurity 3	3M	8[a]	20	√
Sumatriptan Impurity 3	3M[a]	16	20	√

M Male
√ Blood taken via cardiac puncture
a Treatment dose expressed as nominal dose administered
b Due to an unexpected death in the main study animals treated at 16 mg/kg/day on Day 1, 1 animal from this group was transferred to the main study. Consequently only 2 animals at this dose were available for bioanalysis sampling

Study validity: The sponsor's criteria for a valid micronucleus assay were stated as follows:

1. The incidence and distribution of MN PCE in vehicle control groups were consistent with the laboratory's historical vehicle control data, and
2. At least five animals out of each group were available for analysis, and
3. The positive control chemical (CPA) induced a statistically significant increase in the frequency of MN PCE.

In addition, the sponsor asserted the following findings in the micronucleus assay would be interpreted as evidence indicative of clastogenicity:

1. A statistically significant increase in the frequency of MN PCE occurred at one or more dose levels
2. The incidence and distribution of MN PCE in individual animals at such a point exceeded the laboratory's historical vehicle control data
3. A dose-response trend in the proportion of MN PCE was observed (where more than two dose levels were analyzed).

The sponsor's criteria for a valid Comet assay were stated as follows:

1. At least four animals per group were available for analysis
2. The vehicle control treatments demonstrated low levels of cytotoxicity (i.e., group mean of less than 30% clouds or 30% diffused cells) indicating correct preparation of the cell suspensions
3. The positive control treatments demonstrated a clear increase in Comet parameters over the vehicle control.

The sponsor asserted the following findings in the Comet assay would be interpreted as evidence indicative of DNA damage induction by the test article:

1. A dose related change in tail moment or tail intensity in any tissue, or
2. A marked change in tail moment or tail intensity in any tissue between the vehicle and at least a single dose group.

Results: Reproduced below are the sponsor's tables summarizing the results from the micronucleus assay and the Comet assays in liver and peripheral blood, followed by the results of the bioanalytical assessments.

Summary of micronucleus data

Table 15: Sumatriptan Impurity 3: Summary and Statistical Analysis of Micronucleus Data

Treatment (mg/kg/day)	Cell Total	% PCE	MN PCE	% MN PCE	SD	Heterogeneity		Contingency	
						X^2	Significance	X^2C	Significance
Vehicle	12000	48.85	11	0.09	0.07	4.82	NS		
4	12000	49.90	10	0.08	0.09	10.41	NS	0.00	NS
8[a]	12000	48.89	7	0.06	0.07	9.29	NS	0.50	NS
16	12000	52.12	2	0.02	0.03	4.00	NS	4.93	NS
CPA, 40[b]	12000	47.00	133	1.11	0.77			102.29	$p \leq 0.001$

Linear trend: NS
NS Not significant
MN Micronucleated
a Treatment dose expressed as nominal dose administered
b Administered as a single dose
SD Standard deviation
PCE Polychromatic erythrocytes
CPA Cyclophosphamide

Table 11: Sumatriptan Impurity 3: Summary of Comet data, Liver

Treatment (mg/kg/day)	Tail Moment ± SD	Tail Intensity ± SD
Vehicle	0.17 ± 0.04	1.04 ± 0.17
4	0.22 ± 0.10	1.37 ± 0.42
8[a]	0.20 ± 0.06	1.29 ± 0.26
16	0.14 ± 0.03	0.90 ± 0.16
Positive control, EMS, 200[a]	1.51 ± 0.56 p≤0.001 (S+T)	6.14 ± 1.80 p≤0.001 (S+)

SD Standard deviation
EMS Ethyl methanesulfonate
S+ two-sample t-test, upper tail (vehicle vs EMS)
T log-transformed
a Treatment dose expressed as nominal dose administered
b Administered as a single dose

Table 12: Sumatriptan Impurity 3: Summary of Comet data, Peripheral blood

Treatment (mg/kg/day)	Tail Moment ± SD	Tail Intensity ± SD
Vehicle	0.08 ± 0.04	0.61 ± 0.34
4	0.09 ± 0.03	0.66 ± 0.19
8[a]	0.15 ± 0.17	0.84 ± 0.65
16	0.07 ± 0.01	0.49 ± 0.07
Positive control, EMS, 200[b]	1.41 ± 0.49 p≤0.001 (S+T)	6.46 ± 2.19 p≤0.001 (S+)

SD Standard deviation
EMS Ethyl methanesulfonate
S+ two-sample t-test, upper tail (vehicle vs EMS)
T log-transformed
a Treatment dose expressed as nominal dose administered
b Administered as a single dose

Table 4 Individual plasma concentration results for Sumatriptan Impurity 3

Group	Dose (mg/kg/day)	Animal number	Concentration (ng/mL)	Mean (ng/mL)
1 (vehicle)	0	179	BLQ	BLQ
		180	BLQ	
		181	BLQ	
2	4	182	3746	4378
		183	2983	
		184	6404	
3	8	185	8389	8831
		186	10851	
		187	7254	
4	16	188	19234	19506
		189	NS	
		190	19778	

BLQ Denotes below limit of quantification (100 ng/mL).
NS Denotes no sample received as the animal was transferred to the Micronucleus / Comet study and was therefore unavailable for sampling.

7.4 Other genetic toxicity studies: no data submitted

8. Carcinogenicity: no data submitted

9. Reproductive and developmental toxicology:

9.1 Fertility and early embryonic development: no data submitted

9.2 Embryonic Fetal development

Study title: Subcutaneous Developmental Toxicity Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 in Rabbits Including a Satellite Toxicokinetic Evaluation

Key study findings:

- The study design employed by the sponsor precludes assessment of developmental toxicity unique to Impurity 1 and/or Impurity 3 versus that due to sumatriptan drug substance alone.
- Maximal mean maternal body weight decrement versus controls was ~5% in HD rabbits; however, mean maternal body weight gain from GD 7 to GD 20 was decreased 50% in HD rabbits versus that in controls.
- No apparent treatment-related adverse effects on any of the developmental parameters evaluated were observed under the conditions of the study.

Study no.: YQT00007 (PD001-0704)
Study report location: EDR
Conducting laboratory and location: (b) (4)
Date of study initiation: 16 November 2007
GLP compliance: Yes, with the exceptions noted by the sponsor and reproduced below:

- The characterization of Impurity I and Impurity 3 test articles were conducted non-GLP.
- The characterization of sumatriptan succinate test article was conducted GMP.
- Bulk stability of the impurities was not conducted.
- A specific homogeneity report bracketing the range of concentrations used on study was not available; however, stability for 14 days was tested at concentrations in a range from 1.5 mg/mL to 130 mg/mL sumatriptan. No change in concentrations were observed, thus indicating that the sumatriptan solutions were not only stable but also homogeneous, based on the average of three preparations used to evaluate stability, at those concentration ranges.
- A quality assurance statement was not available from (b) (4) regarding concentration quality assurance inspection findings, and the audit findings were not submitted to the study director for review; however the concentration analysis was conducted under FDA GLPs.
- These exceptions did not affect the quality or integrity of the study because concentrations analyses at the beginning and end of the dosing period did not demonstrate any degradation of the formulated test article (sumatriptan and impurities).

QA statement: Yes
Drug, lot #, and % purity: Sumatriptan succinate, lot 11316 (supplier lot SU004D05), purity 100.5% via HPLC (anhydrous); sumatriptan impurity 1, lot 07-066-33-25, purity 71% as free base; sumatriptan impurity 3, lot 07-066-31-27, purity 63% as free base

Methods:

Doses: see sponsor's study design summary reproduced below, which was based on results of dose range-finding study (YQT00006) reviewed below; Impurities 1 and 3 were spiked into test article dosing solutions at levels of (b) (4) respectively, of sumatriptan base

Species/strain: New Zealand White rabbits [Hra:(NZW)SPF], time-mated females (day of mating, GD-0)

Number/sex/group or time point: see sponsor's study design summary reproduced below

Route: subcutaneous injection at one of four rotating dorsal injection sites

Formulation/vehicle: 0.9% NaCl injection, USP (lot J7K438)

Dosing solution analyses/drug stability and homogeneity: The sponsor provides the sampling schedule reproduced below for apparent analysis of dosing formulation concentration analysis; however, the sponsor's manner of reporting the data apparently resulting from analyses of these samples is less than transparent. What would appear to be the relevant data are appended to the end of a report entitled, "Stability of Sumatriptan Solution for Pre-Clinical Studies" (Appendix 4, "Analytical Report"). The data are reported on what are deemed 'Revised Certificates of Analysis' for study number YQT00004, which is the 90-day rat study reviewed above that was conducted at a different test facility. However, elsewhere on the same 'Revised CoA' it also states that the data pertain to "Lot. No.: YQT00007", which is the appropriate study and the appropriate target concentration is listed. The data thus reported would appear to confirm that dosing formulation concentrations were on the low side of target, but generally fell within 20% of the target levels for Impurities 1 and 3. The sponsor also provides data (summary table reproduced below) supporting their assertion that "sumatriptan solution was stable for 15 days both at 5°C and 25°C".

Sampling			
Concentration[a]			
Sample Size: 1 mL			
Date Sampled	Date Shipped	Recipient	Shipping Conditions
19 NOV 2007[b] 29 NOV 2007[c]	19 NOV 2007 29 NOV 2007	(b) (4)	PL on cold packs

a. Quadruplicate samples (middle) were retained from the first and last preparations. Two samples of each set were sent for analysis. The remaining samples were retained at the Testing Facility and will be disposed of after issuance of the final report; disposition will be documented in the raw data.
b. First day of preparation.
c. Last day of preparation.
d (b) (4)
R - Refrigerated (2°C to 8°C)
PL - Protected from light

Table 1: Sumatriptan Concentrations for the Accompanying Stability Study for the Pre-clinical Studies

Temperature (°C)	Target conc. (mg/mL)	Initial (mg/mL	8 Days (mg/mL)	15 Days (mg/mL)
N/A	8.1	8.0	-	-
	60	59	-	-
	130	130	-	-
5	8.1	-	8.0	8.0
	60	-	59	60
	130	-	130	131
25	8.1	-	8.0	8.1
	60	-	59	60
	130	-	130	129

Dose volume/infusion rate: once daily dosing on GD 7-19, as per sponsor's study design summary reproduced below

Satellite groups used for toxicokinetics or recovery: see sponsor's study design summary reproduced below

Age: approximately 5 months at test facility receipt

Weight: 2.7-3.7 kg at test facility receipt

Unique study design or methodology (if any): Rabbits were individually housed in stainless steel cages with water available ad libitum; approximately 150 g of Certified Rabbit Chow® #5322 was available to each rabbit each day until the first day of dosage, at which time approximately 180 to 185 g of the same certified feed was offered to each rabbit each day.

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbit Numbers	
					Main Study	TK Study
I	0 (Vehicle)	0	1	20	4401 - 4420	N/A
II	10	10	1	20 + 3[b]	4421 - 4440	4481 - 4483
III	20	20	1	20 + 3[b]	4441 - 4460	4484 - 4486
IV	40	40	1	20 + 3[b]	4461 - 4480	4487 - 4489

a. The test article was considered 100% active/pure for the purpose of dosage calculations.
b. Assigned to toxicokinetic sample collection.
N/A – Not applicable.

Results:

Mortality (dams): Observations were performed at least twice daily; one Group 2 rabbit (doe 4430) was sacrificed on GD 13 due abnormal clinical signs (left hindlimb warm, swollen, and compromised use; vocalization)

Clinical signs (dams): Observations performed at least daily; soft/liquid feces was the only observation that appeared may have been related to treatment

Body weight (dams): Maternal body weights measured daily on study; results (kg, mean ± SD) are summarized in the table and sponsor's figure reproduced below. Mean maternal body weight in HD rabbits was decreased 4% versus controls on GD 19. Mean

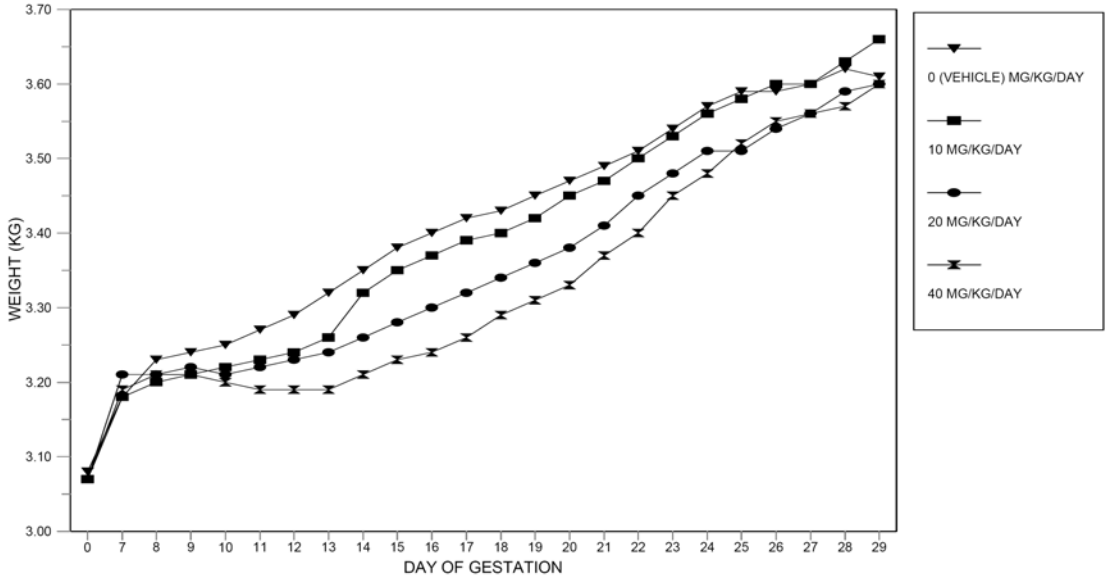
maternal body weight gain from GD 7 to GD 20 was decreased 50% in the HD group versus that in the controls, whereas this effect was almost completely reversed from GD 20 to GD 29 (i.e., weight gain was increased in the HD group by almost 50% versus controls).

Sample Time (GD)	Vehicle (20 pregnant)	10 mg/kg/day (19 pregnant)	20 mg/kg/day (19 pregnant)	40 mg/kg/day (18 pregnant)
Day 0	3.08 ± 0.20	3.07 ± 0.22	3.07 ± 0.21	3.07 ± 0.18
Day 7	3.18 ± 0.16	3.18 ± 0.25	3.21 ± 0.23	3.19 ± 0.15
Day 19	3.45 ± 0.18	3.42 ± 0.28	3.36 ± 0.24	3.31 ± 0.18
Day 29	3.61 ± 0.17	3.66 ± 0.28	3.60 ± 0.27	3.60 ± 0.20

PROTOCOL YQT00007: SUBCUTANEOUS DEVELOPMENTAL TOXICITY STUDY OF SUMATRIPTAN SOLUTION WITH SUMATRIPTAN IMPURITIES 1 AND 3 IN RABBITS INCLUDING A SATELLITE TOXICOKINETIC EVALUATION (SPONSOR'S REFERENCE NUMBER: PD001-0704)

MATERNAL BODY WEIGHTS

Figure 1



Food consumption (dams): Feed consumption was measured daily and supplementation provided to any animal consuming <50% of daily feed ration. Selected data (g/day, mean ± SD) are summarized in the table below.

Sample Time (GD)	Vehicle (20 pregnant)	10 mg/kg/day (19 pregnant)	20 mg/kg/day (19 pregnant)	40 mg/kg/day (18 pregnant)
Day 7-20	165.0 ± 18.8	163.6 ± 21.5	148.8 ± 22.6	141.0 ± 24.0
Day 20-29	127.8 ± 18.4	138.0 ± 22.3	138.8 ± 19.5	143.2 ± 22.0

Terminal and necroscopic evaluations-section data (implantation sites, pre- and post-implantation loss, etc.) and offspring (malformations, variations, etc.): Caesarean-sectioning and gross necropsy of thoracic, abdominal, and pelvic cavities on GD 29 for main study animals; uterine contents were assessed, with uteri of apparently non-pregnant rabbits visualized between glass plates to confirm absence of implantation sites; all fetuses were examined for both visceral and skeletal (Alizarin red S staining) abnormalities. The sponsor's summary figure and data tables are reproduced on the following pages. No apparent treatment-related adverse effects on any of the developmental parameters evaluated were observed under the conditions of the study.
Toxicokinetics: Blood sampling occurred on GD 7-8 and 19-20 at approximately 0.5, 2, 4, 8, and 24 hours post dosing via medial auricular artery. TK animals were sacrificed on GD 20 following last blood sampling and examined for pregnancy status, including any implantation sites, and then discarded without further evaluation. All rabbits assigned for use in toxicokinetic sample collection survived to scheduled sacrifice. All TK study rabbits (3/dose group) were pregnant, except for one doe in each of the 10 and 40 mg/kg/day groups. TK parameter estimates are summarized in the sponsor's table reproduced below; data for the two non-pregnant rabbits are excluded from these summary values.

Table 1: Dose Proportionality of Sumatriptan C_{max} and $AUC_{(0-\infty)}$ in New Zealand White Rabbit Plasma Relative to Ascending Dose Level of Sumatriptan Delivered by Subcutaneous Administration

Females

Occassions	Group No.	Dose Level (mg/kg/day)	Fold Increase	C_{max} (µg/mL)	Fold Increase	$AUC_{(0-\infty)}$ (µg•h/mL)	Fold Increase
Day 7 of Gestation	II	10	1.00	2.42	1.00	6.83	1.00
	III	20	2.00	5.59	2.31	15.0	2.20
	IV	40	2.00	10.7	1.92	31.0	2.06
Overall:			5.00		5.23		5.26
Dose Proportionality Factor:					**1.05**		**1.05**
Day 19 of Gestation	II	10	1.00	2.35	1.00	5.74	1.00
	III	20	2.00	7.87	3.35	19.2	3.35
	IV	40	2.00	19.0	2.41	40.6	2.11
Overall:			5.00		6.76		6.46
Dose Proportionality Factor:					**1.35**		**1.29**

TABLE 8 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	20	40
RABBITS TESTED	N	20	20	20	20
PREGNANT	N(%)	20(100.0)	19(95.0)	19(95.0)	18(90.0)
UNSCHEDULED SACRIFICE	N(%)	0(0.0)	1(5.3)	0(0.0)	0(0.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	20	18	19	18
CORPORA LUTEA	MEAN±S.D.	9.0 ± 1.8	9.3 ± 1.6	7.7 ± 1.6	9.1 ± 2.0
IMPLANTATIONS	MEAN±S.D.	9.0 ± 1.8	9.2 ± 1.7	7.5 ± 1.7	9.2 ± 2.0
LITTER SIZES	MEAN±S.D.	8.6 ± 1.4	8.9 ± 1.7	7.2 ± 1.5*	8.8 ± 1.8
LIVE FETUSES	N	172	161	137	159
	MEAN±S.D.	8.6 ± 1.5	8.9 ± 1.7	7.2 ± 1.5*	8.8 ± 1.8
DEAD FETUSES	N	1	0	0	0
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
RESORPTIONS	MEAN±S.D.	0.4 ± 0.7	0.3 ± 0.5	0.3 ± 0.6	0.3 ± 0.6
EARLY RESORPTIONS	N	6	5	5	4
	MEAN±S.D.	0.3 ± 0.7	0.3 ± 0.5	0.3 ± 0.6	0.2 ± 0.4
LATE RESORPTIONS	N	1	0	1	2
	MEAN±S.D.	0.0 ± 0.2	0.0 ± 0.0	0.0 ± 0.2	0.1 ± 0.3
DOES WITH ANY RESORPTIONS	N(%)	5(25.0)	5(27.8)	5(26.3)	5(27.8)
DOES WITH ALL CONCEPTUSES DEAD OR RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	20(100.0)	18(100.0)	19(100.0)	18(100.0)
PLACENTAE APPEARED NORMAL	N(%)	20(100.0)	18(100.0)	19(100.0)	18(100.0)

a. Dosage occurred on days 7 through 19 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

TABLE 9 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	20	40
LITTERS WITH ONE OR MORE LIVE FETUSES	N	20	18	19	18
IMPLANTATIONS	MEAN±S.D.	9.0 ± 1.8	9.2 ± 1.7	7.5 ± 1.7	9.2 ± 2.0
LIVE FETUSES	N	172	161	137	159
	MEAN±S.D.	8.6 ± 1.5	8.9 ± 1.7	7.2 ± 1.5*	8.8 ± 1.8
LIVE MALE FETUSES	N	91	83	66	78
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	51.5 ± 20.9	51.1 ± 17.6	47.6 ± 17.8	49.0 ± 12.2
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	42.28 ± 3.63	40.54 ± 4.41	43.21 ± 3.57	41.17 ± 4.03
MALE FETUSES	MEAN±S.D.	42.88 ± 4.44	41.94 ± 5.35	43.10 ± 4.32	41.10 ± 3.90
FEMALE FETUSES	MEAN±S.D.	41.75 ± 3.90	39.54 ± 4.42	43.05 ± 3.55	41.26 ± 4.41
% DEAD OR RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	3.9 ± 6.4	3.2 ± 5.6	3.6 ± 6.5	3.2 ± 5.6

a. Dosage occurred on days 7 through 19 of gestation.
* Significantly different from the vehicle control group value ($p \leq 0.05$).

TABLE 10 (PAGE 1): FETAL ALTERATIONS - CAESAREAN-DELIVERED LIVE FETUSES (DAY 29 OF GESTATION) - SUMMARY

DOSAGE GROUP		I	II	III	IV
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	10	20	40
LITTERS EVALUATED	N	20	18	19	18
FETUSES EVALUATED	N	173	161	137	159
LIVE	N	172	161	137	159
DEAD	N	1b	0	0	0
LITTERS WITH FETUSES WITH ANY ALTERATION OBSERVED	N(%)	11(55.0)	12(66.7)	9(47.4)	11(61.1)
FETUSES WITH ANY ALTERATION OBSERVED	N(%)	21(12.2)	17(10.6)	15(10.9)	18(11.3)
% FETUSES WITH ANY ALTERATION/LITTER	MEAN±S.D.	12.0 ± 12.9	11.1 ± 11.7	10.6 ± 13.8	12.7 ± 15.8

a. Dosage occurred on days 7 through 19 of gestation.
b. Values for the dead fetus were excluded from summarization and statistical analyses. Observations for this conceptus are cited on Table 22.

Study title: Subcutaneous Dosage-Range Developmental Toxicity Study of Stressed Sumatriptan Solution Formulation in Rabbits

Key study findings:

- The study design employed by the sponsor precludes assessment of developmental toxicity unique to Impurity 1 and/or Impurity 3 versus that due to sumatriptan drug substance alone.
- Mean maternal body weights of treated does were within 5% of controls throughout the study. However, mean maternal body weight gain from GD 7 to GD 20 was decreased 67% in the HD group versus that in controls and mean maternal relative feed consumption (g/kg/day) was decreased in that same group over that same time period by 18%.
- No apparent treatment-related adverse effects on any of the developmental parameters evaluated were observed under the conditions of the study.
- Based on reduction of maternal body weight gain and feed consumption at 40 mg/kg/day, selection of this dose as the top dose for the definitive study (YQT00007) by the sponsor would appear to have been reasonable.

Study no.: YQT00006
Study report location: EDR
Conducting laboratory and location: (b) (4)

Date of study initiation: 20 August 2007
GLP compliance: Yes, with the following exceptions noted by the sponsor and reproduced below

> The characterization of Impurity 1 and Impurity 3 test articles, were conducted non-GLP
>
> Bulk stability of the impurities was not conducted
>
> Prepared formulation stability at (b) (4) is not known; however, stability at (b) (4) was provided and found to be stable
>
> A specific homogeneity report bracketing the range of concentrations used on study was not available; however, stability for 14 days was tested at concentrations in a range from 1.5 mg/mL to 130 mg/mL sumatriptan. No change in concentrations were observed, thus indicating that the sumatriptan solutions were not only stable but also homogeneous, based on the average of three preparations used to evaluate stability, at those concentration ranges.
>
> A quality assurance statement was not available from (b) (4) regarding concentration quality assurance inspection findings, and the audit findings were not submitted to the study director for review.
>
> These exceptions did not affect the quality or integrity of the study because concentrations analyses at the beginning and end of the dosing period did not demonstrate any degradation of the stressed test article (sumatriptan and impurities).

QA statement: Yes

Drug, lot #, and % purity: Sumatriptan succinate, lot 11315 (supplier lot SU003B05), purity not defined; sumatriptan impurity 1, lot ACM-M0013-SD-11-24-15, purity 63.8% as free base via NMR; sumatriptan impurity 3, lot TYG-YCH-006-1, purity 79.0% as free base via NMR

Methods:

Doses: see sponsor's study design summary reproduced below (study protocol states that dosing formulations were to be spiked with Impurities 1 and 3 at (b) (4) respectively, of sumatriptan base)

Species/strain: New Zealand White rabbits [Hra:(NZW)SPF], time-mated females (day of mating, GD-0)

Number/sex/group or time point: see sponsor's study design summary reproduced below

Route: subcutaneous injection at one of four rotating dorsal injection sites

Formulation/vehicle: 0.9% NaCl injection, USP (lot J7D603/J7H606)

Dosing solution analyses/drug stability and homogeneity: reported analytical data appear to confirm that dosing formulation concentrations generally fell within 20% of the target levels for Impurities 1 (b) (4) and 3 (b) (4))

Dose volume/infusion rate: once daily dosing on GD 7-19, as per sponsor's study design summary reproduced below

Satellite groups used for toxicokinetics or recovery: not performed

Age: approximately 6 months at test facility receipt

Weight: 2.6-3.5 kg at test facility receipt

Unique study design or methodology (if any): Rabbits were individually housed in stainless steel cages with water available ad libitum; approximately 150 g of Certified Rabbit Chow® #5322 was available to each rabbit each day until the first day of dosage, at which time approximately 180 to 185 g of the same certified feed was offered to each rabbit each day.

Dosage Group	Dosage[a] (mg/kg/day)	Concentration (mg/mL)	Dosage Volume (mL/kg)	Number of Rabbits	Assigned Rabbits Numbers
I	0 (Vehicle)	0	1	5	2176 - 2180
II	1	1	1	5	2181 - 2185
III	10	10	1	5	2186 - 2190
IV	20	20	1	5	2191 - 2195
V	40	40	1	5	2196 - 2200

a. The test article was considered 100% active/pure for the purpose of dosage calculations.

Summary Results and Conclusions: Presumed pregnant New Zealand White [Hra:(NZW)SPF] (5/dose group) were subcutaneously administered sumatriptan drug substance spiked with Impurities 1 and 3 at (b) (4), respectively, once daily on GD 7 through GD 19. Dosing levels were 0 (0.9% NaCl), 1, 10, 20 and 40 mg/kg/day of sumatriptan base. All surviving rabbits were sacrificed on GD 29, Caesarean-sectioned and a gross necropsy of the thoracic, abdominal and pelvic cavities was performed. Fetuses were weighed and examined for gross external alterations and sex. Summary data are presented in the sponsor's figure and tables reproduced below.

PROTOCOL YQT00006: SUBCUTANEOUS DOSAGE-RANGE DEVELOPMENTAL TOXICITY STUDY OF STRESSED SUMATRIPTAN SOLUTION FORMULATION IN RABBITS (SPONSOR'S REFERENCE NUMBER: PD001-0703)

MATERNAL BODY WEIGHTS

Figure 1

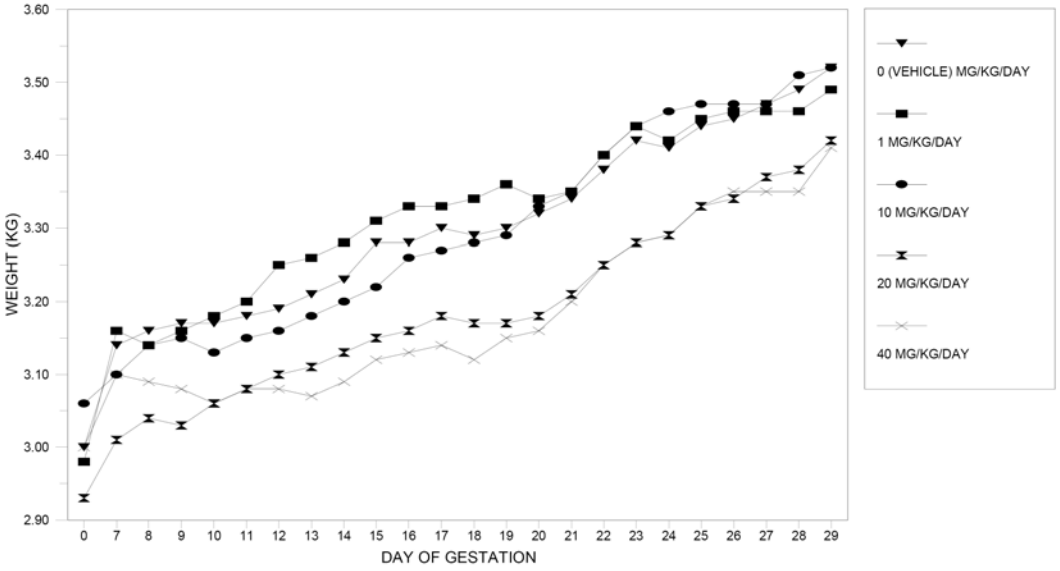


TABLE 6 (PAGE 1): CAESAREAN-SECTIONING OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	1	10	20	40
RABBITS TESTED	N	5	5	5	5	5
PREGNANT	N(%)	5(100.0)	5(100.0)	3(60.0)	5(100.0)	5(100.0)
RABBITS PREGNANT AND CAESAREAN-SECTIONED ON DAY 29 OF GESTATION	N	5	5	3	5	5
CORPORA LUTEA	MEAN±S.D.	10.2 ± 1.3	8.2 ± 0.8	9.7 ± 1.5	7.8 ± 2.2	9.0 ± 2.1
IMPLANTATIONS	MEAN±S.D.	10.2 ± 1.3	8.2 ± 0.8	9.7 ± 1.5	7.8 ± 2.2	9.0 ± 2.1
LITTER SIZES	MEAN±S.D.	10.0 ± 1.2	7.8 ± 0.8	9.3 ± 2.1	7.8 ± 2.2	8.6 ± 1.5
LIVE FETUSES	N	50	39	28	39	43
	MEAN±S.D.	10.0 ± 1.2	7.8 ± 0.8	9.3 ± 2.1	7.8 ± 2.2	8.6 ± 1.5
DEAD FETUSES	N	0	0	0	0	0
RESORPTIONS	MEAN±S.D.	0.2 ± 0.4	0.4 ± 0.5	0.3 ± 0.6	0.0 ± 0.0	0.4 ± 0.9
EARLY RESORPTIONS	N	1	1	1	0	2
	MEAN±S.D.	0.2 ± 0.4	0.2 ± 0.4	0.3 ± 0.6	0.0 ± 0.0	0.4 ± 0.9
LATE RESORPTIONS	N	0	1	0	0	0
	MEAN±S.D.	0.0 ± 0.0	0.2 ± 0.4	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
DOES WITH ANY RESORPTIONS	N(%)	1(20.0)	2(40.0)	1(33.3)	0(0.0)	1(20.0)
DOES WITH ALL CONCEPTUSES RESORBED	N(%)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
DOES WITH VIABLE FETUSES	N(%)	5(100.0)	5(100.0)	3(100.0)	5(100.0)	5(100.0)
PLACENTAE APPEARED NORMAL	N(%)	5(100.0)	5(100.0)	3(100.0)	5(100.0)	5(100.0)

a. Dosage occurred on days 7 through 19 of gestation.

TABLE 7 (PAGE 1): LITTER OBSERVATIONS (CAESAREAN-DELIVERED FETUSES) AND GROSS EXTERNAL OBSERVATIONS - SUMMARY

DOSAGE GROUP		I	II	III	IV	V
DOSAGE (MG/KG/DAY)a		0 (VEHICLE)	1	10	20	40
LITTERS WITH ONE OR MORE LIVE FETUSES	N	5	5	3	5	5
IMPLANTATIONS	MEAN±S.D.	10.2 ± 1.3	8.2 ± 0.8	9.7 ± 1.5	7.8 ± 2.2	9.0 ± 2.1
LIVE FETUSES	N	50	39	28	39	43
	MEAN±S.D.	10.0 ± 1.2	7.8 ± 0.8	9.3 ± 2.1	7.8 ± 2.2	8.6 ± 1.5
LIVE MALE FETUSES	N	26	20	17	20	17
% LIVE MALE FETUSES/LITTER	MEAN±S.D.	52.3 ± 22.9	50.4 ± 17.1	62.0 ± 8.6	50.8 ± 15.7	39.3 ± 28.1
LIVE FETAL BODY WEIGHTS (GRAMS)/LITTER	MEAN±S.D.	38.47 ± 4.25	39.49 ± 2.33	36.32 ± 1.68	39.69 ± 6.95	39.90 ± 4.29
MALE FETUSES	MEAN±S.D.	39.92 ± 4.70	41.52 ± 3.71	36.94 ± 1.50	40.54 ± 7.56	41.58 ± 2.98 [4]b
FEMALE FETUSES	MEAN±S.D.	39.09 ± 5.54	38.59 ± 4.12	35.57 ± 2.13	38.51 ± 6.91	39.01 ± 5.56
% RESORBED CONCEPTUSES/LITTER	MEAN±S.D.	1.8 ± 4.1	4.7 ± 6.5	4.2 ± 7.2	0.0 ± 0.0	3.3 ± 7.5

NO FETAL ALTERATIONS WERE IDENTIFIED AT GROSS EXTERNAL EXAMINATION

[ ] = NUMBER OF VALUES AVERAGED
a. Dosage occurred on days 7 through 19 of gestation.
b. Litter 2197 had no male fetuses.

All does survived to scheduled sacrifice. No adverse clinical signs were reported for any treatment group. Mean maternal body weights of treated does were within 5% of controls throughout the study. However, mean maternal body weight gain from GD 7 to GD 20 was decreased 67% in the HD group versus that in controls and mean maternal relative feed consumption (g/kg/day) was decreased in that same group over that same time period by 18%. Under the conditions of the study, no effects of treatment were apparent on any parameter evaluated at Caesarean-sectioning—litter averages for corpora lutea, implantations, litter sizes, live and dead fetuses, early and late resorptions, fetal body weights, percent resorbed conceptuses, and percent live male fetuses did not differ meaningfully across the dose groups. There were no dead fetuses and no does resorbed all conceptuses. No gross fetal alterations were reported for any fetus.

9.3 Prenatal and postnatal development: no data submitted

10. Special toxicology studies: no data submitted

11. Overall integrated summary and safety evaluation:

The data reviewed in the present submissions are intended to qualify sponsor-proposed drug product specification limits of (b) (4) for the (b) (4) impurities, Impurity 1 and Impurity 3, respectively, which exceed the threshold for qualification as defined in Guidance for Industry–Q3B(R2) Impurities in New Drug Products [July 2006, ICH Revision 2]. The submitted data consist of a 90-day repeated subcutaneous injection toxicity study in rats with dose range-finder and a subcutaneous developmental toxicity study in rabbits with dose range-finder, both of which tested sumatriptan drug substance spiked with Impurities 1 and 3 at (b) (4) respectively, plus an in vitro chromosome aberration assay with Impurities 1 and 3 and a combination mouse in vivo chromosome aberration (micronucleus) assay/Comet assay. All of the data originally required of the sponsor have now been submitted and reviewed.

In the 90-day rat study, dosing formulation analyses confirmed sumatriptan levels were within ± 10% of target, while levels of impurities 1 and 3 were predominantly low, but within 20% of target values. Two animals were found dead, apparently from injection site bacterial infection and chronic peritonitis; all remaining animals survived to scheduled euthanasia. Convulsions were observed in one Group 4 male and one Group 5 male, in each case on one day only. Hematology, clinical chemistry, organ weight, and histopathology findings revealed no clearly discernible indication that the added presence of the spiked Impurities 1 and 3 induced any additional pathology over and above that observed with sumatriptan drug substance alone. Observations at the four injection sites in almost all animals revealed varying grades of subcutaneous fibrosis, mixed cell inflammation, and hemorrhage. The majority of mid- and high-dose animals also exhibited varying grades of epidermal ulceration, fibrinosuppurative exudate, and hyperplasia, as well as dermal fibrosis at the injection sites. Most of these observations were still evident in recovery animals following the recovery period.

The rabbit developmental toxicity study design employed by the sponsor precluded an assessment of toxicity unique to Impurity 1 and/or Impurity 3 in that no dose group exposed to

sumatriptan drug substance alone was included. At sumatriptan doses of up to 40 mg/kg/day with Impurities 1 and 3 spiked at (b) (4) respectively, maximal mean maternal body weight decrement versus controls was ~5%; however, mean maternal body weight gain from GD 7 to GD 20 was decreased 50% in the HD rabbits versus that in controls. There were no apparent treatment-related adverse effects observed on any of the developmental parameters evaluated under the conditions of the study.

In the repeated in vitro chromosome aberration assay conducted in human peripheral blood lymphocytes with the purified Impurities 1 and 3 individually, all criteria for a valid assay appear to have been met. Weakly positive evidence of clastogenicity was observed with Impurity 3 in the 21-hr, -S9 exposure paradigm only at the highest dose tested (10 mM); Impurity 1 was negative under the same conditions. Both 4-hr exposures (+/- S9) gave no evidence of any increase in aberrant cells for either of the impurities.

Though not requested by the Division, the sponsor also submitted an in vivo chromosome aberration assay in the form of a combination micronucleus/Comet assay with Impurity 3 only, conducted in mice via the intravenous route of administration. All criteria for a valid assay as such appear to have been met. In addition, results from bioanalytical assessments confirmed that systemic exposure to sumatriptan Impurity 3 did occur at the tested doses. There was no evidence that treatment of mice with sumatriptan Impurity 3 at up to (b) (4) induced an increase in micronuclei in the polychromatic erythrocytes of bone marrow or DNA damage in the liver and peripheral blood under the conditions of the study. Based on the reported findings from this in vivo micronucleus/Comet assay, it is concluded that no further confirmation and/or follow-up of the in vitro chromosome aberration assay findings is required from the sponsor.

Thus, based on the data described above, it is concluded that the sponsor has adequately qualified the proposed drug product specification limits of (b) (4) for the (b) (4) impurities, Impurity 1 and Impurity 3, in the finished sumatriptan succinate drug product. It is recommended that the NDA 22-239 be approved with said drug product specification limits.

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/s/

Donald C Thompson
7/10/2009 10:26:51 AM
PHARMACOLOGIST

Lois Freed
7/10/2009 12:37:03 PM
PHARMACOLOGIST

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: October 31, 2008

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

Subject: NDA 22-239, Sumavel™ DosePro™ (sumatriptan injection), submission date December 28, 2007

NDA 22-239 is a 505(b)(2) application for Sumavel DosePro (sumatriptan injection) for the treatment of migraine and cluster headache. The indications, maximum daily dose (12 mg/day), and route of administration (subcutaneous) are the same as that of the Reference Listed Drug, Imitrex Injection (approved in 1992 under NDA 20-080).

In the original NDA, the sponsor submitted nonclinical studies to assess the following:

- Two impurities with specification limits exceeding the qualification threshold.
- The potential toxicity of extractables and leachables related to the clinical device.

These data were reviewed in detail by Andrea M. Powell, Ph.D.
(Pharmacology/Toxicology Review and Evaluation, NDA 22-239, 31-Oct-2008)

- The following discussion is based on the information in Dr. Powell's review.

Dr. Powell concluded that the maximum amount of individual leachables in the drug product would be below a level of concern (<1.5 µg/day), and that no toxicity testing of any of these would be necessary. Therefore, the only nonclinical issue is the specification limits for two impurities, Impurities 1 and 3, in the drug product. The sponsor's analysis of Imitrex injection confirmed that the amounts of Impurities 1 and 3 in the approved product (i.e., (b) (4), respectively) were at or below the qualification threshold and below the specification limits proposed by the sponsor (b) (4) respectively).

The only nonclinical studies submitted in the original NDA to address this impurity issue were an in vitro Ames assay and an in vitro chromosomal aberration assay in human lymphocytes using a "stressed" drug substance lot spiked with additional amounts of Impurities 1 and 3. (Additional nonclinical studies to qualify these impurities [including a

90-day subcutaneous toxicity study in rat, and a subcutaneous embryo-fetal development study in rabbit] were submitted four to seven months after NDA filing, and were not reviewed.) Based on review of the genetic toxicology study reports, Dr. Powell concluded that the in vitro Ames assay was adequate and negative, and that the in vitro chromosomal aberration assay was inadequate, but positive.

While Dr. Powell considered the in vitro Ames assay sufficient to qualify Impurities 1 and 3, Dr. Powell did note that the amount of each impurity (b) (4), respectively) was lower than the specification limit for each; however, the difference was considered too small to require a repeat study. That conclusion is reasonable, and consistent with current guidelines (cf. *Guidance for Industry Q3B(R2) Impurities in New Drug Products July 2006 ICH Revision 2).*

The in vitro chromosomal aberration assay in human lymphocytes was positive at the highest concentration tested in the absence of metabolic activation. The sponsor considered the assay negative, based on the fact that the only statistically significant increase in chromosomal aberrations occurred at the highest concentration tested and only under a single assay condition (21-hour exposure, absence of metabolic activation). However, as Dr. Powell notes, the highest concentration tested under the other assay conditions (i.e., 4-hour exposure, presence and absence of metabolic activation) was limited by the sponsor's use of the clinical formulation (concentration of 12 mg/mL) and not by cytotoxicity; therefore, higher concentrations could and should have been tested. In addition, a significant positive response detected only at the highest concentration tested cannot be dismissed since it is consistent with a potentially genotoxic impurity present at only a small fraction of the total drug substance. Even known genotoxic compounds (e.g., those used as positive controls) present as impurities may not produce a positive response, if the highest level tested is limited by the cytotoxicity of the drug substance.

Based on the labeling for Imitrex Injection, sumatriptan was negative in a standard battery of genetic toxicology studies. Therefore, it is likely that Impurity 1 and/or Impurity 3, or some other impurity(ies) formed under the "stressed" conditions is responsible for the positive signal.

Since impurities are unlikely to confer any clinical benefit, the presence of a genotoxic impurity would result in unacceptable risk. As Dr. Powell suggests, the sponsor can address this issue by lower the specification limits for each of the impurities to a level that would result in a total daily dose of ≤1.5 μg/day or, if that is not possible, then additional nonclinical studies will need to be conducted to further investigate the genotoxic potential of these impurities. The nature of those studies has been discussed in detail by Dr. Powell.

I concur with Dr. Powell's recommendation regarding the sponsor's need to further address the presence of potentially genotoxic impurities in the Sumavel DosePro drug product, and recommend the following wording for the action letter:

1. The positive finding in the in vitro chromosomal aberration assay in human lymphocytes (Study 961611) raises the concern that one or more impurities (e.g., Impurity 1 and Impurity 3) present in the stressed/spiked sumatriptan drug lot tested may have genotoxic potential. Since impurities are unlikely to confer any clinical benefit, the presence of a genotoxic impurity in the clinical drug product, unless unavoidable, is not acceptable. Therefore, you will need to further investigate this issue prior to approval.

   Since we recognize that the conditions used to produce the "stressed" sumatriptan may have resulted in the formation of impurities that would not be formed under normal storage conditions, we would recommend that you conduct a repeat in vitro chromosomal aberration assay in which Impurities 1 and 3 are tested directly. Alternatively, the study could be conducted using sumatriptan spiked with Impurities 1 and 3 at levels providing a substantial margin above the specification limits. Of course, other approaches may be acceptable. We would recommend that if you choose to test sumatriptan spiked with the impurities, you not use the clinical formulation (12 mg/mL); it artificially limits the concentrations that can be tested.

   If this repeat assay is adequately conducted and negative, no further action is necessary. If it is positive, then the genotoxic impurities would need to be identified and specification limits set to a level that would result in a total daily dose of ≤1.5 μg/day of each impurity. If more than one structurally similar impurity is identified, then the specification limits would need to be set so that the combined total daily dose would not exceed 1.5 μg/day. If such limits are not achievable, then additional genetic toxicology studies may be conducted in an attempt to further characterize the genotoxic potential (cf. Guidance for Industry and Review Staff; Recommended Approaches to Integration of Genetic Toxicology Study Results. FDA/CDER, January 2006). If the data from those studies indicated an overall lack of genotoxic potential, then no further action would need to be taken.

2. We acknowledge that you have submitted additional studies (including a 90-day oral toxicity study in rat and an embryo-fetal development study in rabbit) to address the specification limits proposed for Impurities 1 and 3. However, they were not included in the original NDA, and were not submitted in time to allow for review during this cycle. They will need to be reviewed and found adequate prior to approval.

Labeling recommendations

The basis for recommendations for each section is provided as bullets following each section.

(b) (4)

3 pages of draft labeling withheld immediately after this page as B4 (TS/CCI)

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
10/31/2008 05:41:11 PM
PHARMACOLOGIST

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	**22-239**
SUBMISSION TYPE:	**Original**
DATE RECEIVED BY CENTER:	**28-Dec-07**
PRODUCT:	**Sumavel™DosePro™ (sumatriptan injection)**
INTENDED CLINICAL POPULATION:	**migraine and cluster headache**
SPONSOR:	**Zogenix, Inc.**
DOCUMENTS REVIEWED:	**Submissions:** • **Original submission (28-Dec-07)** • **Response to reviewer request for information Amendment A-0015 (26-Sept-08)**
REVIEW DIVISION:	**Division of Neurology Products (HFD-120)**
PHARM/TOX REVIEWER:	**Andrea M. Powell, Ph.D.**
PHARM/TOX SUPERVISOR:	**Lois M. Freed, Ph.D.**
DIVISION DIRECTOR:	**Russell Katz, M.D.**
PROJECT MANAGER:	**Lana Chen, R.Ph.**

TABLE OF CONTENTS

Note: Portions of this review excerpted from the sponsor's submission are identified as such.

I. INTRODUCTION AND DRUG HISTORY

NDA number: 22-239

Sequence number/date/type of submission:

- Original submission, #0000, dated 28-Dec-07
- Response to reviewer request for information, Amendment A-0015, dated 26-Sept-08

Sponsor: Zogenix, Inc.

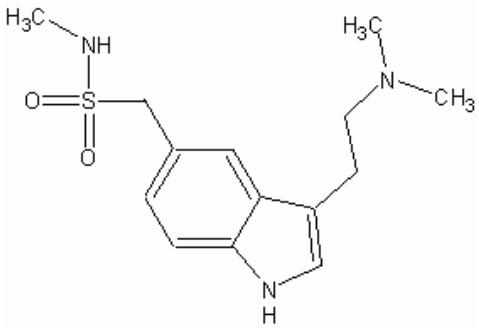
Manufacturer for drug substance: (b) (4)

Drug:

Trade name: Sumavel™ DosePro™ (sumatriptan injection) needle-free delivery system
Generic name: sumatriptan succinate
Code name: none listed
Chemical name:

- 3-[2-(dimethylamino)ethyl]-1H-indol-5-yl]-N-methylmethanesulphonamide hydrogen butanedioate

CAS registry number: 103628-48-4
Molecular formula: $C_{18}H_{27}N_3O_6S$ / Molecular weight: 413.40
Structure:



Relevant IND: IND 71,275

Relevant NDAs:

- NDA 20-080 Imitrex® (sumatriptan succinate) Injection (approved 1992)
- NDA 20-132 Imitrex® (sumatriptan succinate) Tablet (approved 1995)
- NDA 20-626 Imitrex® (sumatriptan) Nasal Spray (approved 1997)

Drug class: selective 5-hydroxytryptamine$_1$ (5-HT$_1$) receptor subtype agonist

Indication: acute treatment of migraine and cluster headache

Clinical formulation: Sumatriptan succinate solution at a concentration of 6 mg sumatriptan base per 0.5 mL solution. The excipients are sodium chloride USP and water for injection USP. This is the same drug product formulation as in the reference product Imitrex® (sumatriptan) Injection.

Route of administration: subcutaneous injection (needle-free)

II TOXICOLOGY

Genetic Toxicology

Stressed Sumatriptan Succinate Formulation Solution Bacterial Mutation Test (Study 961610)

Location within submission: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 21-June-07 (experimental phase: 22-June-07 – 18-July-07)
GLP compliance: yes with the following exceptions: (1) "concentration and stability of the test article formulations were not determined in the study", (2) "the test article was prepared and characterized using validated methodologies as per GMP requirements."
QA reports: yes
Drug, lot #, and % purity: stressed (method unspecified) sumatriptan succinate (lot # PRM-ZPH-C0024.00), supplied by (b) (4) as a 12 mg/mL aqueous solution of sumatriptan, Impurity 1 (≈ (b) (4) and Impurity 3 (b) (4) "The solution is rendered isotonic by the inclusion of 7 mg/mL sodium chloride." Sumatriptan dosing formulations were prepared fresh on the day of use under gold light.

The sponsor used the GMP compliant (non-GLP) stability data from Study # 961611, the in vitro chromosomal aberration assay in human peripheral lymphocytes, to support the stability of the test formulation used in this study. Samples of the test formulation were assayed prior to and after the conduct of the study at (b) (4) under GMP conditions. The results of the evaluation demonstrated that Impurity 1 was present a (b) (4) (b) (4) and Impurity 3 was present at (b) (4) prior to and post use, respectively.

Methods

Strains/species/cell line:
- *S. typhimurium* TA1535, TA1537, TA98, TA100
- *E. coli* WP2 *trp uvrA*

Doses used in definitive study: The following concentrations of stressed sumatriptan were tested in the plate incorporation and pre-incubation assays: 1.58, 5.0, 15.8, 50, 158, 500, 1581, and 5000 µg/plate, with the five highest concentrations evaluated for all strains and conditions. The highest concentration tested, 5000 µg/plate, is the standard upper limit for a nontoxic, readily soluble test article. The sponsor noted that there was no evidence of bacterial lawn thinning or precipitates at any concentration tested.
Vehicle controls: 0.9% NaCl
Positive controls:
- In the presence of metabolic activation: 2-aminoanthracene (in DMSO), benzo[a]pyrene (in water).
- In the absence of metabolic activation: sodium azide (in water), 9-aminoacridine (in water), 2-nitrofluorene (in DMSO), 4-nitroquinoline N-oxide (in DMSO).

Metabolic activation system: commercially prepared liver S9 from phenobarbital/5,6-benzoflavone treated male SD rats.
Incubation and sampling times:
- Plate incorporation method: 48-72 hrs incubation
- Preincubation method: 48-72 hrs

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.):

- Each test article or vehicle was run in triplicate
- At least five concentrations were evaluated per experiment
- An automated colony counter was used
- "The absence of colonies on sterility check plates confirmed absence of microbial contamination (results not shown). However, there was slight microbial contamination present (5 colonies) on the sterility check plate for the vehicle during the plate incorporation assay. It was considered that this was an isolated incident since all the individual plate counts for the test article and vehicle control appeared normal and were within the laboratory historical negative control range… , ***i.e.*** there was no effect on the results obtained and the results are considered valid."
- The sponsor's assessment of mutagenic activity was based on the following criteria:
    - *"Positive*: If treatment with the test article produced a dose-related increase in revertant colony numbers to at least twice the concurrent vehicle control levels with any bacterial strain (1.5× for strain TA100) either in the presence or absence of S9 mix. Provided mean value(s) lay outside the historical control range, this was considered to be indicative of mutagenic activity."
    - *"Negative*: If treatment with the test article did not produce a dose-related increase of at least 1.5 (strain TA100) or 2 (other strains) times the concurrent vehicle controls, it was considered to show no evidence of mutagenic activity in this test system."
    - *"Equivocal*: If the results obtained failed to satisfy the criteria for a clear positive or negative response, the results were considered equivocal. It is acceptable to conclude an equivocal response if no clear conclusion can be made. Note that the reproducibility of any apparent effect is taken into account in making any clear conclusion."

Study outcome:

- According to the report, the results of the first test were not reported due to the presence of contamination.
- The sponsor reported slight microbial contamination (5 colonies) of the vehicle control in the plate incorporation assay; however, the sponsor stated that there was no negative impact on the validity of the assay since all vehicle controls (and test article concentrations) fell within the historical control range. Review of the data indicates that this is reasonable. The plate incorporation assay in the presence and absence of metabolic activation was adequate and negative.
- The preincubation assay in the presence and absence of metabolic activation was adequate and negative.

Stressed Sumatriptan Succinate Formulation Solution Chromosome Aberration Test (Study 961611)

Location within submission: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 21-June-07 (experimental phase: 28-June-07 – 11-July-07)
GLP compliance: yes with the following exceptions: (1) "concentration and stability of the test article formulations were not determined in the study", (2) "the test article was prepared and characterized using validated methodologies as per GMP requirements."
QA reports: yes
Drug, lot #, and % purity: stressed (method unspecified) sumatriptan succinate (lot # PRM-ZPH-C0024.00), supplied by (b) (4) as a 12 mg/mL aqueous solution of sumatriptan and impurity 1 (b) (4) and impurity 3 (b) (4) "The solution is rendered isotonic by the inclusion of 7 mg/mL sodium chloride." Sumatriptan dosing formulations were prepared fresh on the day of use. According to the report, samples of the test formulation were assayed prior to and after the conduct of the study at (b) (4) under GMP conditions. The results of the evaluation demonstrated that Impurity 1 was present at (b) (4) and Impurity 3 was present at (b) (4) prior to and post use, respectively.

Methods

Strains/species/cell line: human peripheral blood lymphocytes from a single healthy, non-smoking male.
Basis of dose selection:

- "maximum practical concentration based on the formulation supplied and the compatibility of high volumes of vehicle with the test system"

Vehicle control: 0.9% NaCl
Positive controls: Mitomycin C (in the absence of metabolic activation) and Cyclophosphamide (in the presence of metabolic activation)
Metabolic activation system: commercially prepared liver S9 from phenobarbital/5,6-benzoflavone treated male SD rats.
Incubation and sampling times, and concentrations evaluated:

- 4 hrs exposure in the presence and absence of metabolic activation, with total incubation time of 21 hours.
- 21 hrs exposure in the absence of metabolic activation, with a total incubation time of 21 hrs.
- Test article concentrations: 5, 10, 20, 40, 80, 160, 320, 640, 1280, 2400 µg/ml
- Positive control concentrations: MMC: 0.05, 0.10 and 0.20 µg/ml, and CP: 8, 12, 16 µg/ml.

Results

Study validity:

- For each experiment, test article concentration formulation and control cultures were run in duplicate. At least two slides were prepared for each culture.
- Mitotic index was evaluated on 500 cells per culture.
- Slides were evaluated blinded to treatment and 200 metaphases were examined per experimental point (100 from each culture).
- Sponsor's criteria for interpreting data:
    - "A positive response is normally indicated by a statistically significant (dose-related, if applicable) increase in the incidence of aberrant cells for the treatment group compared

with the concurrent control group ($p \leq 0.01$); individual and/or group mean values should exceed the laboratory historical control range (99% limit). A negative result is indicated where group mean incidences of aberrant metaphase cells for the group treated with the test article are not significantly greater than incidences for the concurrent control group ($p > 0.01$) and where these values fall within or close to the historical control range. An equivocal response is obtained when the results do not meet the criteria specified for a positive or negative response."

Study outcome:

- The short term exposure assays in the presence and absence of metabolic activation are negative, but inadequate. The highest concentration (HC) tested was the maximum achievable concentration; however, this technical limitation was based on the sponsor's decision to use the clinical formulation (12 mg/ml) as the base formulation rather than a more concentrated formulation. The HC (2400 µg/ml or 8.1 mM) did not produce adequate cytotoxicity, and was less than the limit-dose set by the OECD (5000 µg/ml or 10 mM, whichever is lower) for freely soluble, noncytotoxic compounds. For sumatriptan, a 10 mM solution would be equivalent to 2954 µg/ml).
- The longer incubation assay (in the absence of metabolic activation) is positive only at the highest concentration tested (although the responses at the next two lower concentrations doses are higher than the negative control). The positive response is statistically significant and outside of the sponsor's historical control data.
- The sponsor's interpretation is that the response most likely the result of a non-specific toxicity. The sponsor's conclusion is provided below:
    - "A statistically significant increase in chromosome damage was observed at the highest assessable dose level (2400 µg/mL) of Stressed Sumatriptan Succinate Formulation Solution after 21 hours of exposure in the absence of S9. No increases were apparent at any other experimental point; in particular**,** the Stressed Sumatriptan Formulation did not cause any significant increase in chromosome damage at the next lower dosage of 1280 µg/mL where a 48% reduction in mitotic index was obtained (compared with 60% reduction at the highest dose). The isolated increase could be indicative of genotoxicity but, since chromosome damage was apparent at only the highest dose after prolonged exposure, it is considered most likely that it is related to non-specific toxic effects as described by Galloway (2000)†."

      † Galloway SM (2000) Cytotoxicity and Chromosome Aberrations *In Vitro*: Experience in Industry and the Case for an Upper Limit on Toxicity in the Aberration Assay. Environmental and Molecular Mutagenesis 35(3) 191-201.

- A copy of the sponsor's summary table follows.

Sponsor's historical control data:

- Negative/vehicle mean 0.30%, SD 0.48, 415 treatments
- MMC (4 hrs) mean 10.2%, SD 4.9, 99 treatments
- MMC (21 hrs) mean 11.9%, SD 4.9, 103 treatments
- CP (4 hrs) mean 19.5%, SD 7.6, 103 treatments

Table 1 Results and Statistical Analysis

Treatment	Conc. (μg/mL)	MI	RMI (%)	Number cells examined	% Aberrant	Number of aberrations					Incidental observations †			
						b	e	B	E	other	(g	G	P	C)
4 hours treatment in the absence of S9 (0S9)														
0.9% Saline	-	10.4	100	200	0.5	0	0	1	0	0	0	0	0	0
Sumatriptan	640	7.8	75	200	0.5	1	0	0	0	0	0	0	0	2
	1280	8.8	85	200	0.0	0	0	0	0	0	1	0	0	1
	2400	8.5	82	200	0.0	0	0	0	0	0	0	0	0	1
MMC	0.10	7.3	70	200	9.5**	13	1	8	0	0	4	1	0	0
4 hours treatment in the presence of S9 (+S9)														
0.9% Saline	-	7.2	100	200	0.5	1	0	0	0	0	1	0	0	2
Sumatriptan	640	8.9	124	200	0.5	1	0	0	0	0	0	0	0	0
	1280	6.9	96	200	0.0	0	0	0	0	0	0	0	0	0
	2400	7.7	107	200	0.0	0	0	0	0	0	0	0	0	0
CP	16.0	3.8	53	200	27.0**	62	5	8	0	3	14	2	0	1
21 hours treatment in the absence of S9 (0S9)														
0.9% Saline	-	9.8	100	200	0.5	0	0	1	0	0	0	0	0	0
Sumatriptan	320	9.3	94	200	0.0	0	0	0	0	0	1	0	0	0
	640	6.5	66	200	2.0	4	0	1	0	0	2	0	0	1
	1280	5.1	52	200	2.0	4	0	0	0	0	0	0	0	0
	2400‡	3.9	40	200	7.5**	24	1	3	0	0	3	0	0	1
MMC	0.20	5.7	58	200	21.5**	41	3	32	0	0	8	4	0	1

MI, RMI Mitotic Index, Relative Mitotic Index (vehicle = 100%)
b, e, g Chromatid break, exchange, gap
B, E, G Chromosome break, exchange, gap
other Includes pulverized chromosomes and cells with > 8 aberrations
P Polyploidy and endoreduplication
C Centromeric disruption
† g, G, P and C are excluded from the calculation of % aberrant cells
‡ Limit of toxicity
Results of statistical analysis using one-tailed Fisher's exact test
* $p \leq 0.01$ (significant)
** $p \leq 0.001$ (highly significant)
otherwise $p > 0.01$ (not significant)

Special Toxicology

Primary Dermal Irritation/Corrosion Study with Intraject® Capsule Sub-Assembly Extracts in Rabbits (Study 7880-103)

Location of report within submission: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 14-June-07
GLP compliance: yes, except "dosing extracts were not analyzed for stability, homogeneity, or concentration.
QA reports: yes

Purpose: "… to assess the relative level of primary skin irritation/corrosion of a test article extracts on rabbits with intact or abraded skin under semioccluded conditions."

Test article: Extracts of Intraject® Capsule Sub-assembly (manufacturing date: 21-Feb-06, batch #: 026014). The extraction technique was described by the sponsor as follows:

(b) (4)

Controls: 0.9% saline and cottonseed oil heated to approximately 70°C for 24 hrs and used within 24 hrs of heating.

Animals: male Hra:(NZW)SPF rabbits from (b) (4) weighing 2443-2628 grams and approximately 16 weeks of age at initiation of treatment. The hair was clipped from the back and flank areas on the day prior to dermal application of the test agents. Twelve application sites were defined on each animal (six per side) and on the day of application six of these sites (three per side) were abraded with a clipper blade ("deep enough to penetrate the stratum corneum, but not deep enough to cause bleeding"). Thus, on each animal there were six abraded and six intact application sites, each approximately 6 cm^2. Two abraded- and two intact sites served as untreated controls.

Test article application: Controls (saline and cottonseed oil) and extracts (saline and cottonseed oil vehicles) were each applied to an intact and abraded site, at a dose volume of 0.5 ml. The application areas were covered with gauze that was taped in place and then wrapped in plastic wrap and tape, which the sponsor describes as "semiocclusive dressing". After approximately four hours of exposure, the dressings were removed and the area washed with mild soap and water.

Dermal irritation: The assessment was conducted using the Draize technique at the following approximate time points after the removal of the dressing: 30-60 min, 24, 48 and 70-74 hours. Animals were sacrificed after the last observation without necropsy or tissue sampling.

Results: All three animals survived until sacrifice, and all gained weight during the study (95-218 grams). No animal experienced edema at any treatment site or time point in the study. The most severe finding in the study was "very slight/barely perceptible erythema". Based on the average primary dermal irritation score and primary dermal irritation index, the saline extract on abraded skin was more irritating than the relevant abraded skin controls, and the cottonseed oil extract on intact skin was more irritating than the relevant intact skin controls under the conditions of the assay. The sponsor, however, concluded that "the dermal observations were similar between the untreated sites, the blanks and the extracts."

Incidence of Primary Dermal Irritation

		erythema and eschar formation				edema			
time of evaluation (post exposure)		30-60 min	24 hrs	48 hrs	70-74 hrs	30-60 min	24 hrs	48 hrs	70-74 hrs
untreated	intact	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	abraded	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
saline control	intact	0/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3
	abraded	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
saline extract	intact	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	abraded	1/3	2/3	1/3	0/3	0/3	0/3	0/3	0/3
untreated	intact	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	abraded	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
cottonseed oil control	intact	0/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3
	abraded	1/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3
cottonseed oil extract	intact	1/3	1/3	1/3	0/3	0/3	0/3	0/3	0/3
	abraded	1/3	0/3	0/3	0/3	0/3	0/3	0/3	0/3

each finding had a score of 1 ("very slight erythema [barely perceptible]")

Summary of Dermal Irritation Scores

		average primary dermal irritation score				primary dermal irritation index
time of evaluation (post exposure)		30-60 min	24 hrs	48 hrs	70-74 hrs	
untreated	intact	0.0	0.0	0.0	0.0	0.0 – non irritating
	abraded	0.3	0.0	0.0	0.0	0.1 – slightly irritating
saline control	intact	0.0	0.3	0.3	0.0	0.2 – slightly irritating
	abraded	0.0	0.0	0.0	0.0	0.0 – non-irritating
saline extract	intact	0.0	0.0	0.0	0.0	0.0 – non-irritating
	abraded	0.3	0.7	0.3	0.0	0.4 – slightly irritating
untreated	intact	0.0	0.0	0.0	0.0	0.0 – non-irritating
	abraded	0.0	0.0	0.0	0.0	0.0 – non-irritating
cottonseed oil control	intact	0.0	0.0	0.0	0.0	0.0 – non-irritating
	abraded	0.3	0.3	0.3	0.0	0.3 – slightly irritating
cottonseed oil extract	intact	0.3	0.3	0.3	0.0	0.3 – slightly irritating
	abraded	0.3	0.0	0.0	0.0	0.1 – slightly irritating

- average primary dermal irritation score per interval = the total dermal irritation score (erythema and edema) for all the animals divided by the number of animals (3) for each observation period.
- primary dermal irritation index = sum of the individual index scores, divided by the number of animals (3).
- the sponsor uses a 4 point system for classification of irritancy potential based on the primary dermal irritation index: 0 = non-irritating, 0.1-2.0 = slightly irritating, 2.1-5.0 = moderately irritating, 5.1-8.0 = severely irritating.

Dermal Sensitization Study of Intraject® Capsule Sub-Assembly Extracts in Guinea Pigs – Maximization Test (Study 7880-104)

Location of report within submission: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 27-June-07
GLP compliance: yes, except "dosing extracts were not analyzed for stability, homogeneity, or concentration."
QA reports: yes

Purpose: "to assess the contact sensitization potential of Intraject® Capsule Sub-Assembly Extracts when administered by intradermal injection and topical application in guinea pigs."

Test article: Extracts of Intraject® Capsule Sub-assembly (manufacturing date: 21-Feb-06, batch #: 026014).
The extraction technique was described by the sponsor as follows:

(b) (4)

Controls: 0.9% saline and cottonseed oil heated to approximately 70°C for 24 hrs and used within 24 hrs of heating.

Animals: male Crl:(HA)BR guinea pigs weighing 314-362 grams and approximately 5 weeks of age at the initiation of treatment. On Day 1 a 4 cm x 6 cm area of hair was clipped from the back of the animals. Animals were assigned to groups as depicted in the following table:

study group	designation	test substance	# animals
1	test group 1	saline extract	10
2	test group 2	cottonseed oil extract	10
3	irritation control group 1	saline	5
4	irritation control group 2	cottonseed oil	5

Dosing: The sponsor describes the dosing regimen as follows:

- "On Day 1 of study, each group received intradermal injections of saline extract, cottonseed oil extract (Test Groups 1 and 2, respectively), or saline or cottonseed oil (Irritation Control Groups 1 and 2, respectively). On Day 7, all animals were pretreated with 10% sodium lauryl sulfate in petrolatum and then on Day 8, the induction sites were wiped clean and the test article extracts (saline extract or cottonseed oil extract for Test Groups 1 and 2) or saline or cottonseed oil (irritation control groups) were applied topically and covered for 48 hours. On Day 22, two weeks after the topical applications, all of the animals (test and

irritation groups) received a challenge dose (treated with the saline or cottonseed oil extract and saline or cottonseed oil alone) and covered for 24 hours."

With regard to the intradermal injections on Day 1, there were three types of injections per animal in the clipped area: anterior – Freund's complete adjuvant (FCA) emulsion only, medial-test article only, posterior-test article in FCA emulsion.

With regard to dermal application on Day 8, the appropriate test substance was applied to the previous injection areas as a saturated 2 cm x 4 cm piece of filter paper affixed to the skin with tape. The dressing was removed after 48 hrs and the skin washed and dried.

With regard to the Day 22 challenge, the appropriate test article extract (~ 0.4 mL) was applied to shaved skin via an adhesive patch on the right side of the animal and was held in place for 24 hrs. The left side was treated similarly with the saline or cottonseed oil control. These patches were sealed with tape and dental dam to achieve complete occlusion. After 24 hrs the dressings and patches were removed and the area washed with soap and water. Evaluation was conducted 24 and 48 hours after patch removal.

Results:

Mortality: all survived until scheduled sacrifice.

Clinical observations: animals were observed once daily for general cage-side observations.

designation	test substance	findings
test group 1	saline extract	1/10 – thin dosing day 14
test group 2	cottonseed oil extract	1/10- thin with few feces dosing day 14
irritation control group 1	saline	1/5 - thin & 1/5 thin with few feces dosing day 14
irritation control group 2	cottonseed oil	3/5 – thin on dosing day 14 (2) and 18 (1)

Body weights: recorded prior to initiation of treatment on Day 1 and then at termination.

designation	test substance	body weight (g) Day 1	body weight (g) Day 25	change in BW Day 1-25 (g)
test group 1	saline extract	333 ± 12.6	390 ± 30.3	57 ± 29.6
test group 2	cottonseed oil extract	339 ± 12.2	401 ± 33.5	62 ± 32.9
irritation control group 1	saline	341 ± 18.1	396 ± 27.9	56 ± 18.4
irritation control group 2	cottonseed oil	337 ± 17.7	374 ± 16.8	37 ± 28.3

Observation of induction site: The induction site was observed for changes in general condition prior to induction dose and then on Days 3 and 11.
Evaluation of challenge reaction: The sponsor describes this as follows:

- "On Day 24, the challenge sites were clipped with an electric clipper before the first reading. The challenge sites were scored approximately 24 hours after removal of the patches. The sites were scored again at approximately 48 hours (Day 25) mainly to detect weak, slowly developing reactions. Redness constituted the minimum criterion of allergic reaction. Strongly sensitized animals displayed a vivid redness, associated with indurated swelling. The reactions were scored according to the 4-point scale…"

Summary Incidence (by number of test sites) of Effect at Induction Sites on Days 3 and 11						
			saline		cottonseed oil	
day	finding	injection site	extract	control	extract	control
Day 3	erythema	test substance alone	0/20	6/10	2/20	0/10
		FCA alone	20/20	10/10	20/20	10/10
		test substance + FCA	20/20	10/10	20/20	10/10
	edema	test substance alone	0/20	0/10	9/20	8/10
		FCA alone	20/20	10/10	20/20	10/10
		test substance + FCA	19/20	10/10	20/20	10/10
Day 11	erythema	test substance alone	0/20	0/10	0/20	0/10
		FCA alone	2/20	0/10	0/20	0/10
		test substance + FCA	0/20	0/10	0/20	0/10
	edema	test substance alone	9/20	3/10	6/20	0/10
		FCA alone	17/20	10/10	17/20	10/10
		test substance + FCA	10/20	7/10	17/20	10/10
	sore/scab	test substance alone	3/20	0/10	0/20	0/10
		FCA alone	18/20	10/10	20/20	10/10
		test substance + FCA	17/20	9/10	20/20	10/10
	crust/hard skin – no scab	test substance alone	14/20	10/10	19/20	7/10
		FCA alone	0/20	0/10	0/20	0/10
		test substance + FCA	1/20	1/10	0/20	0/10
	fissure	test substance alone	1/20	0/10	0/20	0/10
		FCA alone	0/20	0/10	0/20	0/10
		test substance + FCA	0/20	0/10	0/20	0/10
	denuded	test substance alone	0/20	0/10	0/20	0/10
		FCA alone	0/20	0/10	0/20	0/10
		test substance + FCA	0/20	0/10	0/20	0/10

- On each animal there were two injection sites each for the following test formulations: test article alone (extract or vehicle), FCA alone, test article + FCA.

Dermal Reactions to Challenge Application					
		dermal response @ 24 hr		dermal response @ 48 hr	
		extract	control	extract	control
saline	sensitization test group	30% (3/10)	0% (0/10)	10% (1/10)	0% (0/10)
	irritation control group	60% (3/5)	20% (1/5)	0% (0/5)	0% (0/5)
cotton seed oil	sensitization test group	10% (1/10)	0% (0/10)	0% (0/10)	0% (0/10)
	irritation control group	0% (0/5)	20% (1/5)	0% (0/5)	0% (0/5)

- All positive responses were category 1- scattered mild redness
- Incidence is the parameter of interest, not severity
- Note that in the non-concurrent positive control assay no animal in irritation group had a dermal reaction at any time point and there were no dermal reactions to the vehicle controls. Positive control animals had dermal responses at the following rates: 50% (4/8) at the low dose and 100% (8/8) at the high dose at 24 hrs, and 38% (3/8) at the low dose and 100% (8/8) at the high dose at 48 hrs. All dermal reactions in the positive control groups were category 1 (scattered mild redness).

Sponsor's rating criteria:

Maximization Ratings	
Sensitization Rate (%)*	Classification
0	Not a Sensitizer
>0 – 8	Weak Sensitizer
9 – 28	Mild Sensitizer
29 – 64	Moderate Sensitizer
65 – 80	Strong Sensitizer
81 – 100	Extreme Sensitizer

* Percentage of animals exhibiting a dermal reaction at challenge.

During the induction portion of the experiment the test article extracts produced a greater incidence of dermal findings when compared to the appropriate control as listed below:

- On Day 3, erythema in the cottonseed oil extract group.
- On Day 11, edema in the saline and cottonseed oil extract groups, sore/scab in the saline extract group, crust/hard skin in the cottonseed oil extract, and a single incidence of fissuring in the saline extract group.

With regard to the challenge application, the parameter of importance is incidence and not severity of response. The saline extract clearly produces a dermal response (scattered mild redness) at 24 hrs post challenge; however, there was no clear evidence of hypersensitivity. The dermal response persisted to the 48 hr post challenge time point in one animal from the sensitization group; however, there were no responses at the saline control sites and no responses in the irritation group. According to the evaluation system provided, this would categorize the saline extract as a mild sensitizer. The cottonseed oil extract produced a lower incidence of dermal response than the saline extract; however, at neither post challenge time point (24 or 48 hrs) was there evidence of hypersensitivity.

Based on the results of the challenge application, the sponsor concluded that the saline and cottonseed oil extracts were not sensitizers, under the conditions of the assay; however, the basis for the conclusion is not clear.

Biological Reactivity (Intracutaneous Injection) Study with Intraject® Capsule Sub-Assembly Extracts in Rabbits (Study 7880-105)

Location of report within submission: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 14-June-07
GLP compliance: yes, except "dosing extracts were not analyzed for stability, homogeneity, or concentration.
QA reports: yes

Purpose: "The purpose of this study was to evaluate biological responses to extracts of the test material following single intracutaneous injections into rabbits according to the method described in USP <88> Biological Reactivity, In Vivo (Intracutaneous Injection Test, USP, 2002)."

Test article: Intraject® Capsule sub assembly (manufacturing date: 21-Feb-06, batch #: 026014).
The extraction technique was described by the sponsor as follows:

- (b) (4)

Controls: 0.9% saline and cottonseed oil heated to approximately 70°C for 24 hrs and used within 24 hrs of heating.

Animals: two male Hra:(NZW)SPF rabbits from (b) (4). At the initiation of treatment, the animals were 15-16 weeks old and weighed 2347-2471 grams. On the day prior to dosing the fur on either side of the spinal column was clipped. After the last evaluation, the animals were sacrificed and discarded without necropsy.

Dosing: The sponsor describes the dosing regimen as follows:

- "Each prepared extract or blank was administered by intracutaneous injection (0.2 mL per site) … Group 1 animals received 5 injections each of Sodium Chloride for Injection Test Article Extract and Cottonseed Oil Test Article Extract on their right sides and 5 injections each of the corresponding blanks on their left sides. Care was taken to have adequate space between each injection site. The injection sites were identified with a marking of indelible ink for evaluation during the study. Fading location marks were remarked as often as needed to ensure identification."
- Deviation: "The extracts were vigorously shaken for 3 minutes by the study director, but this information was not documented on the extraction preparation sheets. The storage conditions for the saline blank and saline extracts were not documented in the data."

Clinical observations: mortality/morbidity checks were conducted twice daily; general cage-side observations were conducted daily for each animal. Both animals survived until scheduled necropsy. According to the sponsor, both animals were noted as normal during observations (no data were submitted).

Body weights: body weights were obtained on the day of dosing and at termination. Both animals gained weight during the study period (75 & 124 grams).

Dermal/Local Irritation: Injection site evaluations were conducted prior to dosing and at approximately 24, 48, and 72 hours post injection, using the Draize scoring technique.

Summary of Dermal Irritation following Intracutaneous Injection			
	24 hrs	48 hrs	72 hrs
saline control	erythema in 1/2 (3/5 sites)	no findings	no findings
saline extract	erythema in 1/2 (5/5 sites) edema in 2/2 (5/5 sites each)	erythema in 1/2 (5/5 sites) edema in 2/2 (5/5 sites each)	erythema in 1/2 (5/5 sites) edema in 2/2 (3/5 & 5/5 sites)
cotton seed oil control	no findings	no findings	no findings
cotton seed oil extract	erythema in 2/2 (1/5 & 5/5 sites) edema in 2/2 (5/5 & 5/5)	erythema in 1/2 (5/5 sites) edema in 2/2 (5/5 sites each)	erythema in 1/2 (5/5 sites) edema in 2/2 (3/5 & 5/5 sites)
• All findings were severity category one, very slight (barely perceptible).			

Intracutaneous injection of both the saline- and cottonseed oil extracts of the capsule subassembly produced persistent edema and erythema; with severity of very slight/barely perceptible.

The sponsor did not discuss the implications, if any, of these findings and concluded:

- "… since the mean irritation scores between the test article extract (0.72 for the sodium chloride for injection extract and 0.73 for the cottonseed oil extract) and the corresponding blank (0.05 for the sodium chloride for injection blank and 0.0 for the cottonseed oil blank) were less than 1.0 then the requirements of the test were met."

Biological Reactivity (Systemic Injection Test) of Intraject® Capsule Sub-Assembly Extracts in Albino Mice (Study 7880-106)

Location of report within submission: electronic
Conducting laboratory and location: [redacted] (b) (4)
Date of study initiation: 14-June-07
GLP compliance: yes, except "dosing extracts were not analyzed for stability, homogeneity, or concentration."
QA reports: yes

Purpose: "The purpose of this study was to evaluate biological responses to extracts of the test material following single intraperitoneal and intravenous injections into albino mice according to the method described in USP <88> Biological Reactivity, In Vivo (Systemic Injection Test, USP 25, 2002)."

Test article: Intraject® Capsule sub assembly (manufacturing date: 21-Feb-06, batch #: 026014).
The extraction technique was described by the sponsor as follows:

- [redacted] (b) (4)

Controls: 0.9% saline and cottonseed oil heated to approximately 70°C for 24 hrs and used within 24 hrs of heating.

Animals: male Crl:CD-1® (ICR) mice from [redacted] (b) (4). At initiation of treatment animals weighed 18.2 – 25.6 grams and were approximately 5 weeks old. Animals were sacrificed and discarded after the last observation period without necropsy.

Dosing: single injection as follows:

group	treatment	route	dose	# animals
1	saline extract	intravenous	50 mL/kg	5
2	saline control	intravenous	50 mL/kg	5
3	cottonseed oil extract	intraperitoneal	50 mL/kg	5 *
4	cottonseed oil control	intraperitoneal	50 mL/kg	5

* - one animal received 0.7 mL instead of the intended dose of 1.3 mL, due to insufficient dosing solution.

Clinical observations: mortality/morbidity checks were conducted twice daily; general cage-side observations were conducted daily for each animal. Detailed observations were conducted immediately post dose, and approximately 4, 24, 48 and 72 hrs post dose. One cottonseed oil control animal did not have an immediate post dose observation. All animals were listed as normal at each observation time point and all survived until scheduled necropsy.

Body weights: weights were obtained prior to the initiation of experiment and at termination. All animals gained weight over the course of the study (Days 1-4).

group	treatment	body weight (g) Day 1	body weight (g) Day 4	body weight change (g)
1	saline extract	22.9 ± 2.74	25.6 ± 2.94	2.6 ± 0.78
2	saline control	23.4 ± 1.37	26.6 ± 0.75	3.2 ± 0.78
3	cottonseed oil extract	22.8 ± 2.76	25.3 ± 2.99	2.5 ± 0.89
4	cottonseed oil control	23.1 ± 1.86	26.6 ± 2.02	3.5 ± 0.72

The criteria for toxicity in this study were treatment-related mortality/morbidity, clinical observations and changes in normal body weight gain over the 72 hr post dose observation period. There was no effect of treatment on any of these parameters.

MEM Elution Assay (Study 7880-107)

Location of report within submission: electronic
Conducting laboratory and location: (b) (4)
Date of study initiation: 05-July-07
GLP compliance: yes, except (1) stability, purity and characterization of the extract was not provided, and (2) test formulations were not analyzed for stability, homogeneity, or concentration verification.
QA reports: yes

Purpose: "to evaluate the cytotoxicity of an MEM Culture Medium extract of Intraject Capsule Sub-Assembly to L-929 mammalian cells *in vitro*."

Test article: Intraject® Capsule sub-assembly, unfilled, 0.5 ml, stoppered (batch #: 026014) extracted in MEM medium as described by the sponsor:

- (b) (4) A blank (b) (4) control carried through the same procedures used to extract the test articles was also provided. The extracts of the Intraject Capsule Sub-Assemblies and the blank control were transparent, light red liquids.

  (b) (4) . No evidence of contamination was observed. However, the extraction for sterility testing was not properly documented and results from the first MEM Elution assay were used to confirm that there was no contamination."

Positive control: 10% solution of ethanol in MEM medium supplemented with 5% fetal bovine serum and gentamycin.
Negative control: extract from USP Negative Control Plastic Reference Standard extracted in MEM for approximately 24 hours at 35-38 °C.
Blank control: MEM medium supplemented with 5% fetal bovine serum and gentamycin carried through the same procedures as used in extraction of test material.
Cell line: mouse fibroblast L-929 cells (C3H/An Mouse Fibroblast cells, ATCC CCL-1; NCTC clone 929 (L-929)), from (b) (4)

Methodology: near confluent monolayers of fibroblast cells were incubated in the presence of the test extract, or controls (positive, negative and blank) at 35-38°C for a minimum of 48 hours. The first evaluation for signs of cytotoxicity was conducted after approximately the first 24 hrs of incubation. The cultures were examined again for cytotoxicity after a minimum of 48 hours, then fixed, stained and reexamined. According to the protocol, if there was a difference noted in the evaluation between the live and fixed cells, the results of the fixed cells was used. All cultures were run in triplicate. The assay was repeated due to a problem in the initial assay with the positive response at 24 hrs.

Results: All replicates of the test article extracts, the negative and blank controls were evaluated as non-reactive, grade "0" (cell cultures noted with "discrete intracytoplasmic granules; no cell lysis") at 24 and 48 hours in both assays. In the first assay at the 24 hr time point only, the positive control did not provide an acceptable response; at 48 hrs in the first assay, as well as both time points in the second assay, the positive response was adequate.

III. SUMMARY AND EVALUATION

This is a review of Zogenix Incorporated's 505(b)(2) NDA for Sumavel™ DosePro™ (sumatriptan injection) needle-free delivery system for subcutaneous use, with the intended clinical indications of the acute treatment of migraine attacks with and without aura, and the acute treatment of cluster headache episodes. (During development [under IND 71,275], the product was known as Intraject® Sumatriptan.) This is a drug/device combination that employs a novel, sterile, prefilled, single-use, needle-free device to deliver sumatriptan succinate to the subcutaneous tissue at the same dose, concentration and volume as for the marketed innovator product, Imitrex® (sumatriptan succinate) Injection for subcutaneous use. Furthermore, the sumatriptan solution is identical in composition to the marketed Imitrex® injection (the reference listed drug) with regard to the concentrations of sumatriptan salt and the excipients. It is the use of the novel needle-free device that distinguishes this application from a generic drug application.

No nonclinical studies were submitted to address the local toxicity and local distribution of sumatriptan administered with this needle-free device, and none were requested or required. According to the Clinical Pharmacology and Biopharmaceutics review team, Sumavel™ DosePro™ is bioequivalent to the reference product, Imitrex® (sumatriptan succinate) Injection. Therefore, from a Pharmacology / Toxicology standpoint, the only issues that would need to be addressed are impurities and leachables.

Impurities: In the cover letter to the original NDA submission (28-Dec-07) the sponsor states the following:

> No additional nonclinical studies were required for this 505(b)(2) NDA submission, as agreed upon during the 28 June Pre-IND and 11 June 2007 pre-NDA meetings. However, Zogenix has initiated nonclinical studies to support the qualification of two (b) (4) Impurity 1 and Impurity 3, in order to extend the expiry of the product. Both of these impurities are found in the reference products sumatriptan injection (NDA 20-080) and sumatriptan nasal spray (NDA 20-626). Nonclinical studies, in addition to *in vitro* gentoxicity/mutagenicity testing, are being conducted to qualify Impurity 1 and Impurity 3 at (b) (4) respectively. FDA agreed that these additional nonclinical studies were not required for NDA filing, but a repeat-dose 90 day toxicity study and an embryo-fetal development study would be required to support an extended expiration dating of the product (11 June 2007 pre-NDA meeting minutes).

During the preIND meeting for this product the Division did agree that additional nonclinical safety or toxicology studies would not be needed to support approval of the sumatriptan with the needle-less subcutaneous injection device (at that time the sponsor was Aradigm). At that point in the development program, impurities were not specifically discussed. Furthermore, at the time of the 30-day safety review date for the original IND (submitted 29-Sept-06, sponsor now Zogenix), no drug product impurities were above the level of qualification, except under accelerated conditions. Thus, again, qualification of impurities was not an issue.

A preNDA meeting was held on 11-June-07. Reproduced below is the discussion of the only nonclinical question from the preNDA package and meeting (reproduced from the official minutes dated 15-Sept-07).

6.1 Nonclinical Question

Question 1: Except for mutagenicity and genotoxicity studies for impurities 1 and 3, there will be no additional nonclinical data in Module 4 of the eCTD. Does the Agency agree with our understanding?

FDA response: No. In addition to the in vitro genotoxicity studies, you would need to test impurities 1 and 3 in a repeat dose toxicity study of 14-90 days duration in one species and an embryo-fetal development study in one species. These studies should be conducted using a clinically relevant route of administration.

Alternatively, you can reduce your drug product specifications for these impurities to levels that are below the ICH qualification threshold.

Meeting discussion: The sponsor asked if impurities 1 and 3 would need to be qualified if the levels of these impurities in the Intraject® formulation were comparable to those in marketed formulations. The division stated some concern regarding comparisons to the nasal spray, but indicated that the sponsor should submit the relevant data for review.

The sponsor noted that 12-month stability data are available; however, the data will be updated to extend stability to >12 months. Sponsor plans to submit an NDA in September of 2007.

[Note added: to support an NDA for a chronic use drug product, a bridging repeat-dose toxicity study to qualify an impurity should be of 90 days duration.]

Therefore, the sponsor's statement that the Division agreed that no additional nonclinical studies would be required to support the approval of the 505(b)(2) application is not accurate. Furthermore, the sponsor's statement that "FDA agreed that these additional nonclinical studies were not required for NDA filing, but a repeat-dose 90 day toxicity study and an embryo-fetal development study would be required to support an extended expiration dating of the product …" is not accurate, based on (1) divisional policy that the final study reports for all nonclinical studies that would be required to support marketing of an NDA be submitted at the time of the original NDA filing, and (2) the lack of a statement of agreement to an exception to divisional policy in the official meeting minutes.

In the original NDA submission (28-Dec-07), the sponsor had reported only 18 month stability data for Sumavel™ DosePro™. According to these data, impurity levels were considered to be reasonably within the threshold limits for qualification, and similar to levels that the sponsor had documented for the innovator product. The sponsor proposed drug product specification limits of (b) (4) for Impurities 1 and 3, respectively; however, they stated that interim specification limits of (b) (4) would be used for these impurities until submission of the drug product stability updates and submission of the final reports for additional nonclinical data in May 2008.

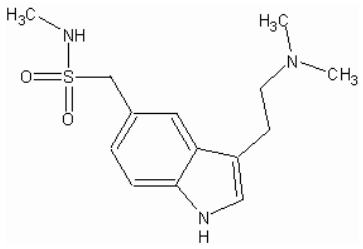
During the course of the NDA review cycle, the sponsor submitted drug product stability data for up to 24 months that demonstrates that the amounts of Impurities 1 and 3 increase over time. With regard to Impurities 1 and 3, the proposed drug product specification limits, that would support a 24-month drug product expiration date, exceed the threshold for qualification as defined in Guidance for Industry– Q3B(R2) Impurities in New Drug Products [July 2006, ICH Revision 2]. The general qualification thresholds for degradation products from that guidance document are listed in the table below.

Drug Product Qualification Thresholds	
Maximum Daily Dose of Drug Substance	Thresholds for Degradation Products *
< 10 mg	1.0% or 50 µg TDI, whichever is lower
10 mg - 100 mg	0.5% or 200 µg TDI, whichever is lower
>100 mg - 2 g	0.2% or 3 mg TDI, whichever is lower
> 2 g	0.15%
* expressed as percentage of the drug substance or as daily intake of the degradant	

The proposed maximum daily dose for sumatriptan in the Sumavel™ DosePro™ is two injections (6 mg/0.5 ml each). Thus, the maximum recommended daily dose is 12 mg and the qualification threshold associated with this dose is 60 µg (representing 0.5% of the drug substance). The sponsor has proposed specification limits of (b) (4) for Impurity 1 (b) (4) and (b) (4) for Impurity 3 (b) (4) The specification limits for both impurities are above the threshold for qualification.

The structures for sumatriptan and these impurities follow.

Sumatriptan



$C_{14}H_{21}N_3O_2S$
Mol. Wt. (succinate salt): 413.5
Mol. Wt. (free-base): 295.4

(b) (4)

If the sponsor had demonstrated that Impurities 1 and 3 were present in the innovator product at levels greater than, or equal to, those in the proposed specifications limits (b) (4) for Impurities 1 and 3, respectively), no additional nonclinical studies would have been required. In an attempt to address this issue, the sponsor compared the amount of Impurities 1 and 3 in the three stability lots of Sumavel™ DosePro™ to a single lot of Imitrex® (sumatriptan succinate) Injection (see table below for details). The

sponsor was not able to support the proposed specifications for Impurities 1 and 3 through comparison to Imitrex® (sumatriptan succinate) Injection.

Comparison of the Amount of Impurities #1 and #3 in Imitrex® Injection and Sumavel™ DosePro™					
Impurity	Imitrex® Injection lot 183832	Sumavel™ DosePro™			Sumavel™ DosePro™ specification limits
		lot PD05139	lot PD05140	lot PD05141	
	≥ 18 mo *	18 mo → 24 mo	18 mo → 24 mo	18 mo → 24 mo	
#1					(b) (4)
#3					

- Sumavel™ DosePro™ stored for 18 and 24 months at 25°C / 60% RH
- Imitrex® Injection was tested at only one time point ("after storage at 25°C / 60% RH for 18 months and within a month of product expiry")

The sponsor also tried to qualify the amounts of Impurities 1 and 3 in Sumavel™ DosePro™ through comparison to Imitrex® Nasal Spray. The data are summarized in the following two tables. The first based on the amount of the impurities in the formulations, and the second based on the amount delivered (although by different routes) when the maximum recommended single dose was administered. Even if the differences in the route of administration could be ignored, neither the amount nor the dose of Impurities 1 and 3 in the Imitrex® Nasal Spray would qualify the amount or dose that would be permitted with the proposed specification limits for Sumavel™ DosePro™.

Comparison of the Amount of Impurities #1 and #3 in Imitrex® Nasal Spray and Sumavel™ DosePro™					
Impurity	Imitrex® Nasal Spray lot B491	Sumavel™ DosePro™			Sumavel™ DosePro™ specification limits
		lot PD05139	lot PD05140	lot PD05141	
	initial → 3mo → 6 mo	18 mo → 24 mo	18 mo → 24 mo	18 mo → 24 mo	
#1					(b) (4)
#3					

- Sumavel™ DosePro™ stored for 18 and 24 months at 25°C / 60% RH
- Imitrex® Nasal Spray was stored at 40°C / 75% RH for the 3 and 6 months time points.
- Imitrex® Nasal Spray tested at three time points "Assuming a 24 month expiry, the initial time point represents approximately 8 months from the date of drug product manufacture, the 3-month accelerated time points represent approximately 20 months from the date of drug product manufacture and the 6-month accelerated time point represents approximately 32 months from the date of drug manufacture."

Comparison of the Dose of Impurities #1 and #3 (µg/dose) in Imitrex® Nasal Spray and Sumavel™ DosePro™					
Impurity	Imitrex® Nasal Spray lot B491	Sumavel™ DosePro™			Sumavel™ DosePro™ specification limits
		lot PD05139	lot PD05140	lot PD05141	
	initial → 3mo → 6 mo	24 mo	24 mo	24 mo	
#1					(b) (4)
#3					

- Based on data summarized in the table above for the maximum recommended single dose of Imitrex® Nasal Spray (20 mg), and Sumavel™ DosePro™ (6 mg).

Therefore, to support the sponsor's proposed drug product specification limits for Impurities 1 and 3 (b) (4) the sponsor needs to qualify the impurities with an appropriate battery of nonclinical tests using either the isolated impurities or the drug substance containing levels of the

impurities that are at least equal to the proposed specification limits. The battery of nonclinical tests include: (1) a test for gene mutation in bacteria (Ames assay), (2) either an in vitro chromosomal aberration assay in mammalian cells or an in vitro mouse lymphoma tk assay (with colony sizing), (3) a 90-day repeat dose toxicity study in one species, and (4) and embryo-fetal development study in one species. Alternatively, the sponsor would need to reduce the specifications for Impurities 1 and 3 to a level that does not exceed the threshold for qualification (i.e., ≤ 0.5%).

The following two studies, conducted to support the qualification of drug product Impurities 1 and 3, were submitted in the original NDA and were reviewed:

1. Study # 961610, an in vitro bacterial mutation assay (Ames test) using a "stressed" sumatriptan succinate formulation.

2. Study # 961611, an in vitro chromosome aberration assay in human peripheral lymphocytes using a "stressed" sumatriptan succinate formulation.

The additional nonclinical studies listed below, conducted to support the proposed drug product specifications for Impurities 1and 3, were submitted 4-7 months into the 10-month original review cycle, and have not been reviewed. Studies necessary for drug approval need to be submitted at the time of NDA filing. These studies are necessary, and will need to be reviewed prior to approval.

1. Study # YQT00003, A Rising Dose and Multiple Dose Tolerance Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 Administered by Subcutaneous Route to Rats. This study was submitted on 02-May-08.

2. Study # YQT00004, A 90-Day Toxicity Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 Administered by the Subcutaneous Route to Rats with a 14-Day Recovery Period. This study was submitted first as an audited draft on 02-May-08, and then as a final report on 29-July-08.

3. Study # YQT00006, Subcutaneous Dosage-Range Developmental Toxicity Study of Stressed Sumatriptan Formulation in Rabbits. This study was submitted on 02-May-08.

4. Study # YQT00007, Subcutaneous Developmental Toxicity Study of Sumatriptan Solution with Sumatriptan Impurities 1 and 3 in Rabbits Including a Satellite Toxicokinetic Evaluation. This study was submitted on 02-May-08.

Genetic Toxicology Studies: In order to qualify Impurities 1 and 3 to the proposed drug product specifications of (b) (4), respectively, the sponsor conducted an Ames test and an in vitro chromosomal aberration assay in human peripheral lymphocytes with a "stressed" sumatriptan formulation. The production process for the stressed formulation was not described in the study reports. In response to the Division's 25-Sept-08 request for further information, the sponsor provided a description of the process (26-Sept-08, Amendment A-0015).

The drug formulation used in these two genetic toxicology assays was produced in a two step process. First glass containers of bulk solution of the drug product (Intraject® sumatriptan solution) were stored at 50°C for 35 days. Since this "stressing" process did not produce adequate levels of Impurities 1 and 3 (b) (4) the stressed solution was also spiked with synthesized Impurities 1 and 3. The following table compares the amount of impurities found in the different formulations tested.

Comparison of the Amount of Impurities in the Formulation used for Genetic Toxicology Studies, Imitrex® Injection and Sumavel™ DosePro™

Impurity	Sumavel™ DosePro™ genetox formulation		Sumavel™ DosePro™ stability lots ^	Sumavel™ DosePro™ specification limits	Imitrex® Injection lot 183832
	"stressed"	"stressed" and spiked	24 mo		≥ 18 mo *
1	(b) (4)				
3					
related compound C					
2b					
related compound A					
5					
6					

- Sumavel™ DosePro™ stored for 18 and 24 months at 25°C / 60% RH
- * Imitrex® Injection was tested at only one time point ("after storage at 25°C / 60% RH for 18 months and within a month of product expiry")
- ^ = stability lots PD05139, PD05140, PD051541
- Stressing procedure: 35 days at 50°C

The Ames test (Study 961610) was negative; however, it qualified Impurities 1 and 3 to levels lower than intended due to insufficient spiking of the test formulation (see table below). The differences between the levels of Impurities 1 and 3 actually tested and the intended levels are small and do not warrant a repeat assay.

		Impurity 1	Impurity 3
amount in stressed & spiked sample	%	(b) (4)	
	corresponding daily dose		
proposed specification limit	%		
	corresponding daily dose		
qualification limit	%		
	corresponding daily dose		

In the 21 hour exposure component of the in vitro chromosomal aberration assay in human peripheral lymphocytes (Study 961611), there was a statistically significant increase in the percentage of aberrant cells at the highest concentration tested (which was associated with a 60% reduction in mitotic index). Although the sponsor states that all test article responses were within historical control range, this contention is not supported by the data. This positive response was clearly outside the laboratory's historical control.

The sponsor concluded that the positive response was most likely due to non-specific toxicity, rather than direct genetic toxicity based on (1) lack of a significant effect at the next lower concentration which was associated with a 48% reduction in mitotic index, (2) the lack of a positive response in other components of the assay (i.e., the short exposures in the presence or absence of metabolic activation). Thus, the sponsor considers the results of this assay negative.

It is true that the positive response occurred only at a concentration that is associated with excessive cytotoxicity, and was not evident at the next lower concentration of the formulation tested. These

mitigating factors would be reasonable considerations for impurities assayed directly; however, they do not negate the positive response when considering the genotoxic potential of impurities assessed only as part of a forcibly degraded and spiked sumatriptan formulation.

Also, the negative results obtained in the short term exposure components of the assay (in the presence and absence of metabolic activation) cannot be used to negate the positive response because these components were inadequate. The highest concentration tested was the maximum achievable concentration; however, this technical limitation was based on the sponsor's decision to use the clinical formulation (12 mg/ml) as the base formulation rather than a more concentrated formulation. This concentration (2400 µg/ml or 8.1 mM) did not produce adequate cytotoxicity, and was less than the limit-dose set by the OECD (5000 µg/ml or 10 mM, whichever is lower) for freely soluble, noncytotoxic compounds.

Furthermore, the label for Imitrex® Injection states that sumatriptan was negative in the in vitro chromosome aberration assay in human peripheral lymphocytes. Therefore, it would appear that the results of the "stressing" and/or spiking of the sumatriptan succinate formulation led to the positive response. Therefore, the results of this assay should be considered positive and the sponsor will need to further address this issue.

The qualification of impurities by testing a formulation that has been subjected to forced degradation, while acceptable (if adequate levels of the impurities are achieved), is less than ideal. Forced degradation is not a selective process and can potentially produce impurities that would not be formed under the normal conditions of storage and handling. A positive genotoxic response due to an impurity that would not be present under the normal conditions of use and storage would have little clinical relevance.

An additional disadvantage of this technique is that the maximum concentrations of the impurities evaluated may be limited by the properties of the base solution (in this case sumatriptan), and not by the properties of the impurities (such as solubility, or cytotoxicity). This shortcoming also applies to the testing of spiked formulations. An adequately conducted assay using a spiked and/or force degraded formulation could potentially falsely characterize an impurity as nongenotoxic, because the maximum concentration of the "genotoxic" impurity that could be tested as part of the whole formulation was inadequate. Also, a positive response would necessitate additional assays to determine which of the impurities is genotoxic.

Testing synthesized Impurities 1 and 3 directly would address the concerns in the most reliable and straightforward manner.

The sponsor should conduct an additional in vitro chromosomal aberration assay in human peripheral lymphocytes. It is strongly recommended that the sponsor conduct the assay using synthesized Impurities 1 and 3 directly. From a regulatory standpoint it is acceptable to qualify impurities using a sumatriptan formulation containing levels of impurities that are at least equal to the proposed specification limits (achieved through forced degradation and/or spiking); however, these testing methods are less than ideal. The technical limitations of these methodologies could potentially lead to both false positive and false negative results, and if a positive response is detected, additional studies will be necessary to determine which of the impurities is genotoxic. Also, if the impurities are tested as part of a sumatriptan formulation, each of the impurities should be present at levels much higher than the proposed specification limits to ensure adequate testing, an unspiked/nondegraded sumatriptan control should be incorporated into the assay, and the clinical formulation (12 mg/mL) should not be used as the base solution, since this restricts the concentrations that can be tested due to technical limitations.

If the subsequent testing demonstrates that neither Impurity 1 nor Impurity 3 is genotoxic, no further action would be necessary. The course of regulatory action is more complicated if either of these impurities is demonstrated to be genotoxic. Based on current policy, the drug product specification limit for a genotoxic impurity would need to be reduced to a level that would result in a total daily intake (TDI) not to exceed 1.5 µg/day. The proposed specification limits for Sumavel™ DosePro™ would permit a TDI of up to (b) (4) for Impurities 1 and 3, exceeding the TDI limit by (b) (4), respectively. Furthermore, the sponsor has demonstrated that that Impurities 1 and 3 are present in Imitrex® Injection at levels that would correspond to total daily intakes of (b) (4), exceeding the TDI limit by (b) (4), respectively (based on a single lot tested after 18 months of controlled storage and within a month of its labeled expiry date). The sponsor also demonstrated that Impurities 1 and 3 were present in Imitrex® Nasal Spray at levels that would correspond to total daily doses of (b) (4), exceeding the TDI limit by (b) (4), respectively (based on a single lot tested at a time point estimated to represent approximately 32 months post manufacture). The sponsor did not submit data generated with Imitrex® Tablet; therefore, it is not known whether these (b) (4) impurities are present in the tablet formulation.

Therefore, if either Impurity 1 or 3 is demonstrated to be genotoxic, the resulting regulatory actions potentially would have ramifications for all other sumatriptan formulations.

Leachables: The sponsor identified potential leachables from the parts of the device that have contact with the drug product, i.e., the capsule sub-assembly (piston, interface seal, stopper, sleeve, and capsule), and submitted a report (8800-1353-RPO) entitled, "Intraject Sumatriptan Extractables and Leachables Overview". This report was not reviewed since, after 24 months storage (at 25°C / 60RH), no leachables were present at levels above 1 µg/mL (confirmed by the CMC reviewer, David Claffey, PhD).

Leachables are specifically not covered under Guidance for Industry–Q3B(R2) Impurities in New Drug Products [July 2006, ICH Revision 2], and therefore, the thresholds for qualification of impurities as defined by this document would not apply. However, for Sumavel™ DosePro™ the threshold for qualification would be (b) (4) based on the maximum recommended daily dose of 12 mg), which exceeds the amount of individual leachables detected in the drug product (not more than 1 µg/mL) after 24 months of storage in the capsule sub-assembly. Furthermore, based on current Agency standards, impurities that have been demonstrated to be clearly genotoxic are permitted in a drug product at levels that result in a total daily intake not to exceed 1.5 µg/day. Based on a comparison to these standards, it is not necessary to characterize the toxicity of the individual leachables.

Special Toxicity Studies: Five nonclinical studies were conducted to assess the toxicity of extracts of the device capsule sub-assembly (the components that are in contact with the drug product). Three different extraction paradigms were used to produce the leachables.

The first extraction paradigm was tested in one in vitro assay system, i.e., the MEM elution assay (Study 7880-107). The extract, prepared by heating the Intraject® capsule sub-assemblies filled with MEM cell culture medium (35-38°C for ≈ 24 hrs), was considered to be non-reactive when evaluated for the ability to induce cytotoxicity in mouse fibroblast L-929 cell cultures when compared to an appropriate control.

The second and third extraction paradigms were based on a saline extraction and a cottonseed oil extraction. Each was tested in four in vivo assay systems. For each of these assays, extracts were prepared by heating Intraject® capsule sub-assemblies filled with either saline or cottonseed oil to ≈70°C for 24 hours. The results obtained with the extracts were compared to blank saline or cottonseed oil controls.

1. In the primary dermal irritation/corrosion study in rabbits (Study 7880-103; 4 hr semi-occluded exposure) the saline extract was more irritating to abraded skin and the cottonseed oil extract was

more irritating to intact skin than the relevant controls. The irritation was manifested only as very slight/barely perceptible erythema. While the effects of the extracts were very slight, the sponsor's conclusion that the dermal effects were similar between the untreated sites, the blanks and the extracts does not appear to be supported by the data.

2. In the dermal sensitization study in guinea pigs (Study 7880-104) using the maximization test method, the saline and cottonseed oil extracts produced greater incidences of dermal findings during the induction portion of the assay when compared to the appropriate controls (erythema in the cottonseed oil extract group on Day 3, edema in the saline and cottonseed oil extract groups, sore/scab in the saline extract group, crust/hard skin in the cottonseed oil extract group, and a single incidence of fissuring in the saline extract group on Day 11). In the challenge component of the assay, the saline extract produced a greater incidence of dermal response (scattered mild redness) at 24 hrs post challenge; however, there was no clear evidence of hypersensitivity. In one animal from the sensitization group, the dermal response persisted to the 48 hr post challenge. According to the evaluation system provided, this would categorize the saline extract as a mild sensitizer. The cottonseed oil extract produced a lower incidence of dermal response than the saline extract; however, at neither post challenge time point (24 or 48 hrs) was there evidence of hypersensitivity. The basis for the sponsor's conclusion that the saline extract was not a sensitizer is not clear.

3. In the biological reactivity intracutaneous injection study in rabbits (Study 7880-105), both saline and cotton seed oil extracts produced persistent but very slight/barely perceptible edema and erythema.

4. In the biological reactivity systemic injection test in albino mice (Study 7880-106), neither saline extracts nor cottonseed oil extracts had an effect on mortality, morbidity, clinical signs or body weight (the only parameters assessed) when administered as single intravenous or intraperitoneal injections.

Based on these studies, it is clear that the saline and cottonseed oil extracts contain some leachable component or components that are slightly irritating to the skin when administered as intracutaneous or intradermal injections or applied topically to the skin, and the irritation can be persistent. Local irritation data derived on leachables or extractables obtained under such extreme conditions (24 hrs at 70°C) are not relevant to the labeled clinical storage and handling of Sumavel™ DosePro™ (store at room temperature (20-25°C) with excursions permitted between 15 and 30°C).

IV. RECOMMENDATIONS

The current submission is not approvable from a Pharmacology/Toxicology standpoint. The sponsor has proposed drug product specification limits of (b) (4) for the (b) (4) impurities, Impurity 1 and Impurity 3. These specification limits are above the threshold for qualification as defined in Guidance for Industry–Q3B(R2) Impurities in New Drug Products [July 2006, ICH Revision 2]. The sponsor was not able to demonstrate comparable levels of these impurities in the innovator drug product Imitrex® (sumatriptan succinate) Injection. Thus, to support the proposed specification limits the sponsor would need to qualify Impurities 1 and 3 in the following assays: (1) a test for gene mutation in bacteria (Ames assay) and (2) either an in vitro chromosomal aberration assay in mammalian cells or an in vitro mouse lymphoma tk assay (with colony sizing), (3) an embryo-fetal development study in a single species, and (4) a repeat-dose toxicity study of 90 days duration in a single animal species. The sponsor submitted the two in vivo studies four and seven months into the 10 months review cycle, and thus these studies have not been reviewed. The sponsor should be advised that the adequacy of the two in vivo toxicity studies conducted to qualify Impurities 1 and 3 (Study # YQT00004, the 90-day repeat dose toxicity study in rat, and Study # YQT00007, the embryo-fetal development study in rabbits) has not been determined, since the study reports were submitted too late for consideration in this review cycle.

The sponsor submitted the reports of the two in vitro genetic toxicology studies with the original submission, and these were reviewed. The Ames test was negative and qualified Impurities 1 and 3 at (b) (4) respectively; however, it is not unreasonable to use these data to support the proposed specification limits of (b) (4) for Impurity 1 and (b) (4) for Impurity 3. The positive in vitro chromosomal aberration assay conducted with the "stressed" and spiked formulation is problematic. According to the label for the innovator product, Imitrex® (sumatriptan succinate) Injection, sumatriptan was negative in the in vitro chromosomal aberrations assay in human peripheral lymphocytes, and the current assay did not include a nondegraded and unspiked sumatriptan control. Thus, it is reasonable to conclude that the results of the forced degrading and/or spiking of the sumatriptan succinate formulation led to the positive genotoxic response.

To resolve this issue, the sponsor should conduct an additional in vitro chromosomal aberration assay in human peripheral lymphocytes. It is strongly recommended that the sponsor conduct the assay using synthesized Impurities 1 and 3 directly; however, the less ideal option of testing the drug substance in combination with the impurities is acceptable. If the impurities are tested as part of a sumatriptan formulation, the impurities should be present at levels much higher than the proposed specification limits to ensure adequate testing, an unspiked/nondegraded sumatriptan control should be incorporated into the assay, and the clinical formulation (12 mg/mL) should not be used as the base solution, since this restricts the concentrations that can be tested due to technical limitations.

It is recommended that the sponsor resolve this issue prior to approval of the NDA. In addition, the 90-day repeat dose toxicity study in rat (Study YQT00004) and the embryo-fetal development study in rabbit (Study YQT00007) that were submitted during the review cycle need to be reviewed prior to NDA approval. The embryo-fetal development study may provide valuable data for the labeling of this product. The current label for Imitrex® Injection does not include the results of an embryo-fetal development study in rabbit by the subcutaneous route.

V. LABELING RECOMMENDATIONS

The base document used for the labeling recommendations is the Draft Labeling Text (1.14.1.3) submitted in Amendment A0002, the most recent version submitted to the NDA.

From a Pharmacology/Toxicology standpoint, the recommended labeling for Sumavel™ DosePro™ is notably different than the sponsor's proposed labeling which was based on minor modifications to the labeling for the reference compound, Imitrex® (sumatriptan succinate) Injection. The more significant recommendations proposed here were based on two issues: (1) removal of dose comparisons (on a mg/m^2 basis) across routes of administration (as in the Imitrex® labeling), and (2) comparing animal exposure to the maximum recommended human daily dose, rather than the maximum recommended human single dose, to make the labeling consistent with the other members of this pharmacologic class.

HIGHLIGHTS OF PRESCRIBING INFORMATION

(b) (4)

4 pages of draft labeling withheld immediately after this page as B4 (TS/CCI)

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

/s/

Andrea Powell
10/31/2008 05:45:05 PM
PHARMACOLOGIST

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Please see memo for comments.