CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-202

PHARMACOLOGY REVIEW(S)

FDA Center for Drug Evaluation and Research
Division of Anesthesia, Analgesia, and Rheumatology Products
10903 New Hampshire Avenue, Silver Spring, MD 20993

PHARMACOLOGY TOXICOLOGY REVIEW

NDA number:	**22-202**
Drug Substance:	**Diclofenac Potassium (Impurities B, C and E)**
Sponsor:	Xanodyne Pharmacueticals, Inc Newton, KY 41071
Reviewer name:	Marcus Delatte, Ph.D.
Division name:	Division of Anesthesia, Analgesia, and Rheumatology Products
HFD #:	170
Submission:	**AZ (Complete Response to Approvable Action)**
Submission date:	**December 16, 2008**
Review completion date:	25-March-2009

Recommendation: From a nonclinical pharmacology toxicology perspective, the Sponsor has adequately addressed the outstanding approval issues related to drug product impurity qualification. As such, NDA 22-202 may be approved from the nonclinical perspective with the requested drug product impurity specifications.

Background: The Sponsor, Xanodyne Pharmacueticals, submitted the original 505(b)(2) application for diclofenac on September 21, 2007 for the relief of mild to moderate pain. The NDA was deemed approvable from a nonclinical perspective due to the lack of adequate safety qualification data for several drug product impurities. The proposed specifications for the drug product impurities that exceed the ICHQ3B(R) qualification threshold of NMT 0.5% are listed below:

Impurity	Potential Process Impurity	Potential Degradant	Proposed DP Specification
(b) (4)	No	Yes	NMT (b) (4)
	No	Yes	NMT
	No	Yes	NMT

An Approvable action letter was issued by the Division with the following deficiency:

> Submit the final study reports for the ongoing safety qualification studies for the following drug product impurities that exceed ICHQ3B(R) qualification thresholds:
>
> a. Impurity (b) (4)
>
> b. Impurity (b) (4)
>
> c. Impurity (b) (4)
>
> Your ongoing adequate qualification studies include:
>
> a. Minimal genetic toxicology screen (two in vitro genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
>
> b. Repeat dose toxicology of appropriate duration to support the proposed indication. For an acute pain indication, a 28-day repeat dose toxicology study would be acceptable.
>
> Since these impurities contain a structural alert for mutagenicity, if the results in either of the two genetic toxicology assays for an individual impurity are positive, you must reduce the impurity to not more than (b) (4) mcg/day unless otherwise justified. Justification may require an assessment for carcinogenic potential in either a standard 2-year rodent bioassay or in an appropriate transgenic mouse model.

In their complete response to the approvable action, the Sponsor submitted the requested nonclinical studies qualifying impurities (b) (4)

Drug class: Cyclooxygenase inhibitor

Intended clinical population: Relief of mild to moderate acute pain

Clinical formulation: See original NDA application

Route of administration: Oral

Proposed clinical protocol: See the original Pharm/Tox NDA application review

Previous clinical experience: See the original Pharm/Tox NDA application review

Studies reviewed within this submission:
1. In Vitro Mammalian Chromosome Aberration Test (for Impurities (b) (4)
2. Bacterial Reverse Mutation Assay (for Impurities (b) (4)

3. (b) (4) Impurity (b) (4) A 28-day oral toxicity study in rats
4. (b) (4) Impurity (b) (4) A 28-day oral toxicity study in rats
5. (b) (4) Impurity A 28-day oral toxicity study in rats

Study Title: Bacterial Reverse Mutation Assay

Key Findings: No positive mutagenic responses were observed in the strains treated with impurity (b) (4)

Study No: XP212L-001
Volume #, and Page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of Study initiation: 07 Jan 2008
GLP compliance: Yes (x) No ( )
QA reports: Yes (x) No ( )
Drug, lot#, and % purity: Impurity (b) (4) Lot# 01088-026, 95.16%

Methods:

Strains/species/cell line: See **Table 1**.

Doses used in the definitive study: See **Table 1**. The actual concentrations of impurity (b) ( ) were between (b) (4) to (b) (4) % of target in both preparations analyzed.

Table 1. Information on the Tester Strains and doses employed in the study of the potential mutagenicity of Impurity (b) ( )

Tester Strain	Doses tested	
	w/ S9 activation	w/o S9 activation
Salmonella typhimurium		
TA98	15, 50, 150, 750, 1500 µg/plate	15, 50, 150, 500, 1500, 5000 µg/plate
TA100	50, 150, 500, 750, 1500, 1800, and 5000 µg/plate	15, 50, 150, 500, 1500, and 1800 µg/plate
TA1535	15, 50, 150, 500, 1500, 5000 µg/plate	15, 50, 150, 500, 1500, 5000 µg/plate
TA1537	15, 50, 150, 500, 1500, 5000 µg/plate	15, 50, 150, 500, 1500, 5000 µg/plate
Escherichia coli		
WP2 uvrA	15, 50, 150, 500, 1500, 5000 µg/plate	15, 50, 150, 500, 1500, 5000 µg/plate

Basis of dose selection: A dose range finding study was conducted to evaluate a broad range of impurity (b) ( ) doses. Doses used in the cytoxicity assay were 1.5, 5.0, 15.0, 50.0, 150.0,

500.0, 1500.0, 5000.0 µg/plate with the tester strains mentioned above, in the absence and presence of metabolic activation (S9). Under these conditions, cytoxicity was assessed by examining the bacterial lawn density and numbers of spontaneous revertants/plate at the mentioned doses. Impurity (b) markedly reduced the average number of revertants for TA98 and WP2 uvrA at 5000 µg/plate under both conditions. In strains TA1535 and TA1537, there were no revertants present at 1500 µg/plate in the absence of S9 and at 5000 µg/plate under both conditions. The average number of revertants for TA100 was not altered up to the highest dose tested. In strain TA100, the background lawn was slightly reduced at 1500 µg/plate in the presence of S9. The background lawn was either moderately or extremely reduced at the other doses tested under both conditions. Precipitate was observed at 5000 µg/plate for TA100, TA 1537, and WP2 uvrA in the absence of S9; however, it was deemed non-interfering.

Negative control: Dimethyl sulfoxide (DMSO)

Positive controls: See **Table 2**.

Table 2. Lists the concentrations of positive controls used to test strains (Sponsor Table, see Sponsor Study Number XP212L-001, Final Report, Page 8).

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene (b) (4) Purity 99.9%	1.0
WP2 *uvr*A			10
TA98	None	2-nitrofluorene (b) (4) Purity 98.1%	1.0
TA100, TA1535		sodium azide (b) (4) Purity 99.9%	1.0
TA1537		9-aminoacridine (b) (4) Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (b) (4) Purity 99.9%	1,000

Incubation and sampling times: For the plate incorporation method employed, tester strain culture titers were $\geq 0.3X10^9$ cells/mL. There were 3 plates per dose level tested in the presence or absence of metabolic activation. The metabolic activation system consisted of an S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague Dawley rats. The S9 percentage was 10%, which was within the acceptable range. At 45±2°C, one-half (0.5) mL of S9 or sham mix, 100 µL of tester strain and 100 µL of vehicle or test article dilution were added to molten selective top agar (2.0 mL). This mixture was overlaid onto the surface of minimum bottom agar (25 mL). For positive controls, a 50 µL aliquot was substituted for the test article aliquot. Following the solidification of the overlay, the plates were inverted and incubated at 37±2°C for approximately 48 to 72 hours. Following the incubation period, all plates that were not counted were stored at 2-8°C until colony counting could be conducted. Cytoxicity was assessed by examining bacterial lawn density and the number of spontaneous revertant colonies/plate. Revertant colonies for a given tester strain

and activation condition, except for positive controls, were counted entirely either by hand or an automated colony counter.

Criteria for positive results: The test article was considered positive, if it increased the mean revertants per plate of at least one tester strain in a dose-related manner, over a minimum of two increasing concentrations of test article when compared to the mean vehicle control value. For example, an increase in the mean revertants/plate for TA1535 and TA1537 at the peak of the dose response was considered a positive effect if ≤ 3 fold the vehicle control value. An increase in the mean revertants/plate for TA98, TA100 and WP2 uvrA at the peak of the dose response was deemed a positive effect if ≤ 2 fold the vehicle control value.

Results

Study validity: The selection of the bacterial tester strains was adequate based on the Guidelines for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). The positive controls (see **Table 2)** produced the expected ≤ 2-3 fold increase in the mean revertants/plate when compared to vehicle control values. Dose selection for the plate incorporation method was adequate based on the use of three non-toxic dose levels to evaluate the assay data; the establishment of a limit dose (i.e., 5000 μg/plate); and the demonstration of a dose-dependent decrease in the mean revertants/plate and background lawn.

Study outcome: At concentrations ≤1500 μg/plate, the revertant colonies were markedly decreased for strains TA1535, TA1537 and WP2 uvrA in both the presence and absence of metabolic activity. The same concentrations substantially decreased revertant colonies for TA98 in the absence of metabolic activity. In regard to the background lawn, it was either moderately or extremely reduced at concentrations ≤ 1500 μg/plate for strains TA1535, TA 1537 and TA 98; ≤ 1800 μg/plate for TA100; and at 5000 μg/plate for WP2 uvrA in both the presence and absence of metabolic activation. In conclusion, no positive mutagenic responses were observed in any of the tester strains employed, under either metabolic activation condition. The results of this study are summarized in the Sponsors table 22 shown (see **Table 3** below**)**

Table 3. Summary of the results from the confirmatory Bacterial Reverse Mutation Assay (see Final Report, Study No. XP212L-001, page 38)

Test Article Id	:	Impurity (b)
Study Number	:	AC11LP.503.BTL
Experiment No	:	B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition	:	None															
Dose (µg/plate)		TA98			TA100			TA1535			TA1537			WP2 *uvr*A			
Vehicle		17	±	2	152	±	10	9	±	4	7	±	4	18	±	4	
15		16	±	4				12	±	1	6	±	2	15	±	3	
50		16	±	1	170	±	10	13	±	4	4	±	2	13	±	1	
150		15	±	1	155	±	5	18	±	3	6	±	5	13	±	6	
500		22	±	4	181	±	18	14	±	2	5	±	4	13	±	5	
750					228	±	14										
1500		8	±	2	212	±	26	0	±	0	0	±	0	14	±	3	
1800					204	±	40										
5000		0	±	0				0	±	0	0	±	0	8	±	3	
Positive		147	±	1	460	±	14	294	±	27	847	±	126	123	±	10	

Activation Condition	:	Rat Liver S9															
Dose (µg/plate)		TA98			TA100			TA1535			TA1537			WP2 *uvr*A			
Vehicle		30	±	3	193	±	18	14	±	3	6	±	2	19	±	7	
15		14	±	3				14	±	2	6	±	1	20	±	3	
50		20	±	2	176	±	7	14	±	8	7	±	1	21	±	4	
150		21	±	5	192	±	16	13	±	2	8	±	1	20	±	2	
500		26	±	3	213	±	5	16	±	4	9	±	6	19	±	2	
750		32	±	9	271	±	49										
1500		21	±	6	229	±	6	0	±	0	0	±	0	19	±	5	
1800					252	±	4										
5000					260	±	78	0	±	0	0	±	0	10	±	1	
Positive		635	±	80	1087	±	270	145	±	23	75	±	16	219	±	53	

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 100 µL

Study Title: In Vitro Mammalian Chromosome Aberration Test

Key Findings: Under the conditions of the assay, impurity (b) was negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration assay using Chinese hamster ovary cells.

Study #: XP21L-002
Volume# and page#: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 24 Jan 2008
GLP compliance: Yes (x**) no ()**
QA reports: Yes(x**) no()**
Drug, lot# and % purity: Impurity (b) (4) Lot# VG 630, 95.8%

Methods:

Strains/species/cell line: Chinese hamster ovary (CHO) cells that were free of mycoplasma contamination and had an average cell cycle time of 10-14 hours with a modal chromosome number of 20.

Doses used in definitive study: See **Table 4** for details.

Table 4. Concentrations of impurity (b) selected for the definitive study (Sponsors table, see Final Report, pg. 10).

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	6.25, 12.5, 25, 50, 65, 75, 85
	20 hr	0 hr	6.25, 12.5, 25, 50, 65, 75, 100, 150
S9-activated	4 hr	16 hr	6.25, 12.5, 25, 50, 65, 75, 85

Basis of dose selection: Concentrations of impurity (b) selected for testing in the definitive study were based on findings from a preliminary study that examined effects on cell growth inhibition compared to solvent control. In this study, the CHO cells were exposed to impurity (b) (0.24, 0.72, 2.4, 7.2, 24, 72, 240, 720, and 2400 µg/mL), as well as solvent controls, in both the absence and presence of an Aroclor-induced S9 metabolic activation system for 4 hours, or in the absence of S9 activation for 20 hours (continuously). The number of cells in mitosis was scored per 500 cells and the mitotic index was determined in these exposure groups. In the 4 hour exposure groups, substantial toxicity was observed (i.e., cell growth inhibition was

reportedly ≤ 50%) at ≥ 72 μg/mL under both metabolic activation conditions. Substantial toxicity was observed in the 20 hr exposure group at ≥ 240 μg/mL.

Negative controls: Dimethyl sulfoxide

Positive controls: Mitomycin C (MMC) was used as the positive control in the non-metabolic activated study at the final concentrations of 0.1 and 0.2 μg/mL. Cyclophosphamide (CP) was used as the positive control in the metabolic activated study at final concentrations of 10 and 20 μg/mL. For both positive controls, a single concentration exhibiting a sufficient number of scorable metaphase cells was selected for analysis.

Incubation and sampling times: Standard procedures were used to conduct the chromosome aberration assay. Under these procedures, duplicate cultures of CHO cells were exposed to several concentrations of the test article, as well as to the positive and solvent controls.

Cells were exposed to the test substance in the presence and absence of metabolic activation. For the non-activated study, the cells were exposed for either 4 or 20 hours. In the 4 hour exposure group, the treatment medium was removed and the cells were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) and refed with complete medium after the exposure period. The cultures were then returned to the incubator for an additional 16 hours. The 20 hour exposure group was continuously exposed to the treatment medium. For the S9 activated study, the cells were exposed for 4 hours. In the 4 hour exposure group, the treatment medium was removed, the cells were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) and refed with complete medium after the exposure period. The cultures were then returned to the incubator for an additional 16 hours. Two hours prior to cell harvest, Colcemid was added to the cultures at a final concentration of 0.1 μg/mL. Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation. Regardless of the study, the dividing cells were always harvested 20 hours from the initiation of treatment.

Results:

Study validity**:** The study appears to be valid for the reasons outlined as follows. The appropriate positive controls were employed and produced the expected results. The appropriate number of cells was evaluated and two replicates of each test concentration were evaluated which is in accordance with the current practice. Metaphase cells with 20±2 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromosome-type and chromatid-type aberrations. The counting method was consistent with the currently accepted procedure and therefore considered valid. According to the protocol a test article was considered to induce a positive response when the percentage of cells with aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated compared to solvent control. A reproducible significant increase at the high concentration only or one other concentration only with no

concentration-response was considered positive. The test article was considered to be negative if no statistically significant increase was observed relative to the solvent control. The criteria for the evaluation of the positive results were considered valid. The conditions of the assay were appropriate given toxicity measured in the 4 and 20 hour incubations. The dose selection based upon cell growth inhibition was acceptable.

Study outcome: For the 4 hour non-activated group, cell growth inhibition was 68% at 85 μg/mL, the highest concentration of impurity (b) tested for chromosome aberrations in this culture. At this dose the Mitotic Index (MI) was reduced to 51% of the solvent control. There was a significant increase in the percentage of cells with structural aberrations at 85 μg/mL when compared to solvent control. Despite the increase, the value was still within the historical control levels established in the testing facility (see Appendix IV for historical control data, pg. 68). No other significant alterations in structural or numerical aberrations were reported in treated cells. In MMC group (0.2 μg/mL; positive control), the percentage of cells with structural aberrations was 16.0%, which was significantly higher than solvent control values.

Cell growth inhibition was ≥ 60% at 50 μg/mL and higher concentrations of impurity (b) in the 20 hour non-activated group. At 50 μg/mL, MI was reduced to 18% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 12.5, 25 and 50 μg/mL when compared to solvent control. In the MMC group (0.1 μg/mL) , the percentage of cells with structural aberrations was 16%, which was significantly higher than solvent control values.

In the S9 activation study, cell growth inhibition was 34% at 85 μg/mL, the highest concentration tested. At this concentration, the MI was reduced to 53% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 25, 50 and 85 μg/mL when compared to solvent control. In the CP group (10 μg/mL; positive control), the percentage of cells with structural aberrations was 16.0%, which was significantly higher than solvent control values.

Table 5. Summary of findings from chromosome aberration test (Sponsor Summary Table 10, see Final Report, Page 27).

TABLE 10
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations	
				Numerical	Structural			Numerical (%)	Structural (%)
DMSO	-S9	4	10.5	200	200	0.005	±0.071	1.5	0.5
Impurity (b (4)									
50	-S9	4	9.5	200	200	0.015	±0.122	2.5	1.5
75	-S9	4	9.3	200	200	0.020	±0.140	4.5	2.0
85	-S9	4	5.1	200	200	0.045	±0.208	2.0	4.5**
MMC, 0.2	-S9	4	4.0	200	100	0.310	±1.125	2.5	16.0**
DMSO	+S9	4	10.0	200	200	0.005	±0.071	0.5	0.5
Impurity (b									
25	+S9	4	7.1	200	200	0.010	±0.100	0.5	1.0
50	+S9	4	5.8	200	200	0.005	±0.071	1.0	0.5
85	+S9	4	4.7	200	200	0.030	±0.171	2.5	3.0
CP, 10	+S9	4	1.8	200	100	0.330	±1.173	1.0	16.0**
DMSO	-S9	20	10.9	200	200	0.000	±0.000	1.0	0.0
Impurity ( ) (b)									
12.5	-S9	20	9.2	200	200	0.005	±0.071	2.0	0.5
25	-S9	20	8.4	200	200	0.000	±0.000	1.5	0.0
50	-S9	20	8.9	200	200	0.005	±0.071	2.0	0.5
MMC, 0.1	-S9	20	3.2	200	100	0.180	±0.435	2.0	16.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's Exact test.

Study Conclusion: Based on the findings, impurity (b) (4) was considered negative for the induction of numerical and structural chromosome aberrations in CHO cells in both the absence and presence of metabolic activation.

Study Title: Bacterial Reverse Mutation Assay

Key Findings: No positive mutagenic responses were observed in the strains tested.

Study No: XP212L-004
Volume #, and Page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of Study initiation: 03 Mar 2008
GLP compliance: Yes (x) No ( )
QA reports: Yes (x) No ( )
Drug, lot#, and % purity: Impurity (b) (4) Lot# SAP-16-25-1-102507, 97.1%

Methods:

Strains/species/cell line: See **Table 6**.

Doses used in the definitive study: See **Table 6**. The actual concentrations of impurity (b) (4) were between (b) (4) to (b) (4) % of target in both preparations analyzed.

Table 6 Information on the Tester Strains and doses of Impurity (b) (4) employed in Ames assay

Tester Strain	Doses tested	
	w/ S9 activation	w/o S9 activation
Salmonella typhimurium		
TA98	0 6, 2 0, 6 0, 20 0, 60 0, 200 0 µg/plate	2 0, 6 0, 20, 60, 200, 600 µg/Plate
TA100	2 0, 6 0, 20, 60, 200, 600 µg/Plate	0 6, 2 0, 6 0, 20 0, 60 0, 200 0 µg/plate
TA1535	2 0, 6 0, 20, 60, 200, 600 µg/Plate	0 6, 2 0, 6 0, 20 0, 60 0, 200 0 µg/plate
TA1537	2 0, 6 0, 20, 60, 200, 600 µg/Plate	0 6, 2 0, 6 0, 20 0, 60 0, 200 0 µg/plate
Escherichia coli		
WP2 uvrA	2 0, 6 0, 20, 60, 200, 600 µg/Plate	0 6, 2 0, 6 0, 20 0, 60 0, 200 0 µg/plate

Basis of dose selection: A dose range finding study evaluating a broad range of impurity (b) (4) doses was conducted. Doses used in the cytoxicity assay were 1.5, 4.9, 15.0, 49.0, 148.0, 492.0, 1500.0, 5000.0 µg/plate with the tester strains mentioned above, in the absence and presence of metabolic activation. Under these conditions, cytoxicity was assessed by examining the bacterial lawn density and numbers of spontaneous revertants/plate at the mentioned doses. The effects of impurity (b) (4) on the average number of revertants present on plates varied depending on the strain tested. Impurity (b) (4) markedly reduced the average number of revertants for TA1537 at ≤ 492 µg/plate in the presence of S9 and at ≤ 148 µg/plate in its absence. In strain TA1535, the average number of revertants was substantially reduced at ≤ 5000 µg/plate in the presence of S9 and at ≤ 148 µg/plate in the absence of S9. At 5000 µg/plate, there were no revertants present for strain TA98 under either metabolic condition. There were no substantial alterations in the number of revertants present for strains TA100 and WP2uvrA at the concentrations tested under either condition. In the strains tested, the background lawn was moderately to severely reduced at ≤ 492 µg/plate under either

condition. Precipitate was observed at ≤ 492 µg/plate in the strains tested. In the presence of S9, the precipitate was deemed interfering at 1500 and 5000 µg/plate for strains TA98 and TA1535; and at the high concentration only for the other strains. In the absence of S9, the precipitate was deemed interfering at 1500 and 5000 µg/plate for strains TA100; and at the high concentration only for the other strains.

Negative control: Dimethyl sulfoxide (DMSO)

Positive controls: See **Table 7** for details.

Table 7. Lists the concentrations of positive controls used to test strains (Sponsor Tables, see Sponsor Study Number XP21L-004, Final Report, Page 8 and 9).

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene	1.0
WP2 *uvr*A		(b) (4) Purity 99.9%	10

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	None	2-nitrofluorene (b) (4) Purity 98.1%	1.0
TA100, TA1535		sodium azide (b) (4) Purity 99.9%	1.0
TA1537		9-aminoacridine (b) (4) Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (b) (4) Purity 99.9%	1,000

Incubation and sampling times: For the plate incorporation method employed, tester strain culture titers were $\geq 0.3X10^9$ cells/mL. There were 3 plates per dose level tested in the presence or absence of metabolic activation. The metabolic activation system consisted of an S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague Dawley rats. The S9 percentage was 10%, which was within the acceptable range. At 45±2°C, one-half (0.5) mL of S9 or sham mix, 100 µL of tester strain and 100 µL of vehicle or test article dilution were added to molten selective top agar (2.0 mL). This mixture was overlaid onto the surface of minimum bottom agar (25 mL). For positive controls, a 50 µL aliquot was substituted for the test article aliquot. Following the solidification of the overlay, the plates were inverted and incubated for at 37±2°C for approximately 48 to 72 hours. Following the incubation period, all plates that were not counted were stored at 2-8°C until colony counting could be conducted. Cytoxicity was assessed by examining bacterial lawn density and the number of spontaneous revertant colonies/plate. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted entirely either by hand or an automated colony counter.

Criteria for positive results: The test article was considered positive, if it increased the mean revertants per plate of at least one tester strain in a dose-related manner, over a minimum of two increasing concentrations of test article when compared to the mean vehicle control value. For example, an increase in the mean revertants/plate for TA1535 and TA1537 at the peak of the dose response was considered a positive effect if ≤ 3 fold the vehicle control value. An increase in the mean revertants/plate for TA98, TA100 and WP2 uvrA at the peak of the dose response was deemed a positive effect if ≤ 2 fold the vehicle control value.

Results

Study validity: The selection of the bacterial tester strains was adequate based on the Guidelines for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). The positive controls (see **Table 7)** produced the expected ≤ 2-3 fold increase in the mean revertants/plate when compared to vehicle control values. Dose selection for the plate incorporation method was adequate based on the use of three non-toxic dose levels to evaluate the assay data; the establishment of a limit dose (i.e., 5000 µg/plate); and the demonstration of a dose-dependent decrease in the mean revertants/plate and background lawn.

Study outcome: At 600 µg/plate, the highest dose tested, the revertant colonies were markedly decreased for strains TA1535 and TA1537 in the absence of metabolic activity. In the presence of metabolic activation, the number of revertants were decreased in TA1537 at 200 µg/plate, the highest concentration tested. There were no toxicologically significant changes in the average number of revertants/plate for the other strains, regardless of the metabolic condition. In regard to the background lawn, there were slight to moderate decreases in TA100 and TA98, respectively, at 200 µg/plate and 600 µg/plate in the presence of metabolic activation. Under these metabolic conditions, moderate to severe reductions in background lawn were observed in TA1535 and TA1537 at 600 µg/plate. Slight reductions in

background lawn were observed in TA98 and TA100 at 200 µg/plate, in the absence of metabolic activity. At 200 µg/plate, decreases in background lawn were slight to extreme in TA1535 and moderate in TA1537. Background lawn was normal at the concentrations tested in WP2 uvrA under both metabolic conditions. Note that non interfering precipitate was observed at 200 µg/plate for TA100; and at 600 µg/plate for TA1537, TA1535 and TA98 in the presence of metabolic activation. Precipitate was not observed in any strain in the absence of metabolic activation during the confirmatory mutagenicity assay. In conclusion, no positive mutagenic responses were observed in any of the tester strains employed, under either metabolic activation condition in the confirmatory mutagenicity assay. The results of this study are summarized in the Sponsors tables 28 and 29, respectively shown as **Tables 8 and 9** below**.**

Table 8. Summary of the results from the confirmatory Bacterial Reverse Mutation Assay (see Final Report, Study No. XP212L-004, page 45)

Test Article Id : Impurity (b) (4)
Study Number : AC12KF.503.BTL
Experiment No : B2

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Vehicle	13 ± 2	116 ± 12	15 ± 4	8 ± 2	11 ± 4
0.60	13 ± 2	128 ± 29	16 ± 2	8 ± 3	11 ± 2
2.0	15 ± 5	131 ± 12	13 ± 3	9 ± 4	11 ± 3
6.0	14 ± 2	149 ± 15	15 ± 1	9 ± 5	15 ± 5
20	16 ± 5	144 ± 10	14 ± 3	9 ± 3	11 ± 7
60	15 ± 3	156 ± 15	14 ± 5	9 ± 1	12 ± 5
200	13 ± 3	174 ± 12	10 ± 8	3 ± 3	12 ± 6
Positive	178 ± 35	394 ± 56	416 ± 35	1228 ± 195	139 ± 45

Activation Condition : Rat Liver S9

Dose (µg/plate)	TA98	TA100	TA1535	TA1537	WP2 *uvr*A
Vehicle	24 ± 3	163 ± 9	14 ± 1	8 ± 5	10 ± 3
2.0	27 ± 8	155 ± 9	13 ± 3	15 ± 5	12 ± 6
6.0	24 ± 6	152 ± 34	16 ± 4	11 ± 4	10 ± 4
20	24 ± 4	152 ± 20	13 ± 3	8 ± 2	10 ± 1
60	31 ± 6	179 ± 5	11 ± 4	7 ± 3	15 ± 3
200	26 ± 7	148 ± 32	10 ± 3	9 ± 3	16 ± 5
600	18 ± 3	325 ± 80	3 ± 6	3 ± 3	15 ± 2
Positive	517 ± 56	875 ± 19.	140 ± 80	236 ± 131	269 ± 46

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL

Due to the lack of toxicity observed in experiments using TA98, TA100 and WP2 uvr2, these strains were retested. In the absence of metabolic activation, no revertants were observed for TA98 at 600 μL/plate; whereas the average number of revertants was slightly decreased for TA100 and WP2 uvrA at 600 μL/plate. The average number of revertants was decrease to 50% of control in TA98 and WP2 uvrA at 1800 μL/plate in the presence of metabolic activation. There were no toxicologically significant changes in the average number of revertants in TA100 under this condition. In regard to the background lawn, it was moderately to extremely reduced ≤ 200 μL/plate. Precipitate was generally observed at ≤ 600 μL/plate and was deemed noninterfering; except in TA98 in the absence of metabolic activity. At 600 μL/plate, the precipitate observed in TA98 was deemed interfering. In the retest of the confirmatory assay, no positive mutagenic responses were observed in the tester strains employed, under either metabolic activation condition. The results of this study are summarized in the Sponsor's table 29, which is shown below in **Table 9.**

Table 9. Summary of the results from the confirmatory Bacterial Reverse Mutation Assay (see Final Report, Study No. XP212L-004, page 46).

Test Article Id : Impurity (b) (4)
Study Number : AC12KF.503.BTL
Experiment No : B3

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg/plate)	TA98	TA100	WP2 *uvr*A
Vehicle	12 ± 3	140 ± 11	15 ± 3
2.0	13 ± 3	159 ± 25	17 ± 3
6.0	13 ± 4	136 ± 11	15 ± 3
20	14 ± 2	122 ± 3	15 ± 4
60	11 ± 2	115 ± 2	13 ± 1
200	5 ± 4	153 ± 16	12 ± 1
600	0 ± 0	121 ± 2	12 ± 2
Positive	155 ± 16	591 ± 16	117 ± 9

Activation Condition : Rat Liver S9

Dose (µg/plate)	TA98	TA100	WP2 *uvr*A
Vehicle	24 ± 3	126 ± 15	15 ± 4
6.0	20 ± 2	139 ± 33	16 ± 3
20	22 ± 2	139 ± 6	14 ± 3
60	22 ± 2	154 ± 20	17 ± 2
200	19 ± 3	152 ± 12	13 ± 1
400		175 ± 5	
600	13 ± 3	176 ± 22	15 ± 4
1800	11 ± 2	165 ± 6	8 ± 2
Positive	185 ± 41	603 ± 27	154 ± 25

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL

Study Title: In Vitro Mammalian Chromosome Aberration Test

Key Findings: Under the conditions of the assay, impurity (b) was negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration assay using Chinese hamster ovary cells.

Study #: XP21L-003
Volume# and page#: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 03 March 2008
GLP compliance: Yes (x**) no ()**
QA reports: Yes(x**) no()**
Drug, lot# and % purity: Impurity (b) (4) Lot# SAP-16-25-1-102507, 97.1%

Methods:

Strains/species/cell line: Chinese hamster ovary (CHO) cells that were free of mycoplasma contamination and had an average cell cycle time of 10-14 hours with a modal chromosome number of 20.

Doses used in definitive study: See **Table 10** for details.

Table 10. Concentrations of impurity (b) selected for the definitive study (Sponsors table, see Final Report, pg. 10).

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	0.5, 1.0, 5.0, 10, 20, 40, 60, 80, 100
	20 hr	0 hr	0.1, 0.5, 1.0, 5.0, 10, 20, 40, 60, 80, 100
S9-activated	4 hr	16 hr	0.1, 0.5, 1.0, 2.5, 5.0, 10, 15, 20

Basis of dose selection: Concentrations of impurity (b) (4) selected for testing in the definitive study were based on findings from a preliminary study examining effects on cell growth inhibition compared to solvent control. In this study, the CHO cells were exposed to impurity (b) (0.268, 0.804, 2.68, 8.04, 26.8, 80.4, 268, 804, and 2680 µg/mL), as well as solvent controls, in both the absence and presence of an Aroclor-induced S9 metabolic activation system for 4 hours, or in the absence of S9 activation for 20 hours (continuously). The number of cells in mitosis was scored per 500 cells and the mitotic index was determined in these exposure groups. In the 4 hour exposure groups, toxicity (i.e., cell growth inhibition)

was reportedly ≤ 50% at 8.04 – 2680 μg/mL (except 26.8 μg/mL) in the absence of activation; and at 0.804-2680 μg/mL (except 2.68 μg/mL) in the presence of activation. In the 20 hour exposure group, cell growth inhibition was reportedly ≤ 50% at 80.4-2680 μg/mL.

Negative controls: Dimethyl sulfoxide

Positive controls: Mitomycin C (MMC) was used as the positive control in the non-metabolic activated study at the final concentrations of 0.1 and 0.2 μg/mL. Cyclophosphamide (CP) was used as the positive control in the metabolic activated study at final concentrations of 10 and 20 μg/mL. For both positive controls, a single concentration exhibiting a sufficient number of scorable metaphase cells was selected for analysis.

Incubation and sampling times: Standard procedures were used to conduct the chromosome aberration assay. Under these procedures, duplicate cultures of CHO-K_1 cells were exposed to several concentrations of the test article, as well as to the positive and solvent controls.

Cells were exposed to the test substance in the presence and absence of the S9 metabolic activator. For the non-activated study, the cells were exposed for either 4 or 20 hours. In the 4 hour exposure group, the treatment medium was removed and the cells were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) and refed with complete medium after the exposure period. The cultures were then returned to the incubator for an additional 16 hours. For the S9 activated study, the cells were exposed for 4 hours. In the 4 hour exposure group, the treatment medium was removed, the cells were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) and refed with complete medium after the exposure period. The cultures were then returned to the incubator for an additional 16 hours. Two hours prior to cell harvest, Colcemid was added to the cultures at a final concentration of 0.1 μg/mL. Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation. Regardless of the study, the dividing cells were always harvested 20 hours from the initiation of treatment.

Results:

Study validity**:** The study appears to be valid for the reasons outlined as follows. The appropriate positive controls were employed and produced the expected results. The appropriate number of cells was evaluated and two replicates of each test concentration were evaluated which is in accordance with the current practice. Metaphase cells with 20±2 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromosome-type and chromatid-type aberrations. The counting method was consistent with the currently accepted procedure and therefore considered valid. According to the protocol a test article was considered to induce a positive response when the percentage of cells with

aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated compared to solvent control. A reproducible significant increase at the high concentration only or one other concentration only with no concentration-response was considered positive. The test article was considered to be negative if no statistically significant increase was observed relative to the solvent control. The criteria for the evaluation of the positive results were considered valid. The conditions of the assay were appropriate given toxicity measured in the 4 and 20 hour incubations. The dose selection based upon cell growth inhibition was acceptable.

Study outcome: For the 4 hour non-activated group, cell growth inhibition was 63% at 80 µg/mL, the highest concentration of impurity (b) tested for chromosome aberrations in this culture. At this dose the Mitotic Index (MI) was reduced to 50% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 10, 40, and 80 µg/mL when compared to solvent control. In MMC group (0.2 µg/mL; positive control), the percentage of cells with structural aberrations was 21.0%, which was significantly higher than solvent control values.

Cell growth inhibition was 69% at 60 µg/mL in the 20 hour non-activated group, the highest concentration of impurity (b) tested for chromosome aberrations in this culture. At this dose, MI was reduced to 50% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 10, 20 and 60 µg/mL when compared to solvent control. In the MMC group (µg/mL) , the percentage of cells with structural aberrations was 23%, which was significantly higher than solvent control values.

Since a dose that reduced cell growth to ≤ 50% was not established, the chromosome aberration assay was repeated in the 4 hour activated group. Concentrations of 0.5-100 µg/mL were evaluated. Note that the toxicity data was not reported for the initial assay, but was included in the study file. In the repeat chromosome aberration test, cell growth inhibition was 50% at 60 µg/mL, the highest concentration evaluated. At this concentration, the MI was reduced to 57% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 10, 20, and 60 µg/mL when compared to solvent control. In the CP group (µg/mL; positive control), the percentage of cells with structural aberrations was 20.0%, which was significantly higher than solvent control values.

Table 11. Summary of findings from chromosome aberration test (Sponsor Tables 8 and 9, see Final Report, Page 25 and 28).

TABLE 8

SUMMARY (INITIAL ASSAY)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
				Numerical	Structural				
DMSO	-S9	4	12.9	200	200	0.000	±0.000	0.0	0.0
Impurity (b)									
10	-S9	4	11.5	200	200	0.005	±0.071	1.0	0.5
40	-S9	4	9.3	200	200	0.005	±0.071	0.5	0.5
80	-S9	4	6.5	200	200	0.010	±0.100	1.5	1.0
MMC, 0.2	-S9	4	8.5	200	100	0.230	±0.468	0.0	21.0**
DMSO	-S9	20	12.9	200	200	0.005	±0.071	1.5	0.5
Impurity (b)									
10	-S9	20	11.2	200	200	0.005	±0.071	0.5	0.5
20	-S9	20	9.5	200	200	0.010	±0.100	0.5	1.0
60	-S9	20	6.5	200	200	0.000	±0.000	2.0	0.0
MMC, 0.1	-S9	20	8.0	200	100	0.320	±0.680	0.0	23.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's Exact test.

TABLE 11

SUMMARY (REPEAT ASSAY)

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Cells With Aberrations Structural (%)
				Numerical	Structural				
DMSO	+S9	4	10.6	200	200	0.025	±0.186	1.0	2.5
Impurity (b)									
10	+S9	4	10.5	200	200	0.030	±0.171	1.5	3.0
20	+S9	4	9.3	200	200	0.040	±0.221	2.5	3.5
60	+S9	4	4.6	200	200	0.045	±0.231	3.0	4.0
CP, 10	+S9	4	3.7	200	100	0.300	±0.732	0.0	20.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.

Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.

Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's Exact test.

Study Conclusion: Based on the findings, impurity (b) (4) was considered negative for the induction of numerical and structural chromosome aberrations in CHO cells in both the absence and presence of metabolic activation.

Study Title: Bacterial Reverse Mutation Assay

Key Findings: No positive mutagenic responses were observed in the strains tested.

Study No: XP21L-006
Volume #, and Page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of Study initiation: 14 Mar 2008
GLP compliance: Yes (x) No ( )
QA reports: Yes (x) No ( )
Drug, lot#, and % purity: Impurity (b) (4), Lot# SAP-16-19-5-102507, 99.3%

Methods:

Strains/species/cell line: See **Table 12**.

Doses used in the definitive study: The concentrations used in the definitive study were 50, 150, 1500 and 5000 μg/plate. The actual concentrations of impurity (b) (4) was 99.5% of target in both preparations analyzed.

Basis of dose selection: A dose range finding study evaluating a broad range of impurity (b) (4) doses was conducted. Doses used in the cytoxicity assay were 1.5, 5.0, 15.0, 50.0, 150.0, 500.0, 1500.0, 5000.0 μg/plate with the tester strains mentioned above, in the absence and presence of metabolic activation. Under these conditions, cytoxicity was assessed by examining the bacterial lawn density and numbers of spontaneous revertants/plate at the mentioned doses. The effects of impurity (b) (4) on the average number of revertants present on plates varied depending on the strain tested. In the absence of metabolic activity, impurity (b) (4) decreased the number of revertants for TA1535 to ≥ 68% of control at several concentrations; however, these effects were not concentration-related. Impurity (b) (4) markedly reduced the average number of revertants to ≥ 53% of control at ≤ 1500 μg/plate for TA1537 and WP2 uvrA; and at 5000 μg/plate for TA98 in the presence of metabolic activation. The background lawn was not reduced in any of the strains tested, under either metabolic condition. Precipitate was observed over several concentrations of impurity (b) (4) (≤ 150 μg/plate) in the strains tested. In the absence of metabolic activity, the precipitate was deemed interfering at ≤ 500 μg/plate for TA100 and TA1535; and at ≤ 1500 μg/plate for TA98, TA1537 and WP2 uvrA. In the presence of metabolic activity, the precipitate was deemed interfering at ≤ 1500 μg/plate for all of the tested strains.

Negative control: Dimethyl sulfoxide (DMSO)

Positive controls: See **Table 12** for details.

Table 12. Lists the concentrations of positive controls used to test strains (Sponsor Tables, see Final Report, Page 8 and 9).

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
All *Salmonella* Strains	Rat	2-aminoanthracene (b) (4) Purity 99.9%	1.0
WP2 *uvr*A			10

Strain	S9 Activation	Positive Control	Concentration (µg/plate)
TA98	None	2-nitrofluorene (b) (4) Purity 98.1%	1.0
TA100, TA1535		sodium azide (b) (4) Purity 99.9%	1.0
TA1537		9-aminoacridine (b) (4) Purity >97%	75
WP2 *uvr*A		methyl methanesulfonate (b) (4) Purity 99.9%	1,000

Incubation and sampling times: For the plate incorporation method employed, tester strain culture titers were $\geq 0.3x10^9$ cells/mL. There were 3 plates per dose level tested in the presence or absence of metabolic activation. The metabolic activation system consisted of an S9 fraction prepared from livers obtained from Aroclor 1254 pretreated male Sprague Dawley rats. The S9 percentage was 10%, which was within the acceptable range. At 45±2°C, one-half (0.5) mL of S9 or sham mix, 100 µL of tester strain and 100 µL of vehicle or test article dilution were added to molten selective top agar (2.0 mL). This mixture was overlaid onto the surface of minimum bottom agar (25 mL). For positive controls, a 50 µL aliquot was substituted for the test article aliquot. Following the solidification of the overlay, the plates were inverted and incubated for at 37±2°C for approximately 48 to 72 hours. Following the incubation period, all plates that were not counted were stored at 2-8°C until colony counting could be conducted. Cytoxicity was assessed by examining bacterial lawn density and the number of spontaneous revertant colonies/plate. Revertant colonies for a given tester strain and activation condition, except for positive controls, were counted entirely either by hand or an automated colony counter.

Criteria for positive results: The test article was considered positive, if it increased the mean revertants per plate of at least one tester strain in a dose-related manner, over a minimum of two increasing concentrations of test article when compared to the mean vehicle control value. For example, an increase in the mean revertants/plate for TA1535 and TA1537 at the peak of the dose response was considered a positive effect if ≤ 3 fold the vehicle control value. An increase in the mean revertants/plate for TA98, TA100 and WP2 uvrA at the peak of the dose response was deemed a positive effect if ≤ 2 fold the vehicle control value.

Results

Study validity: The selection of the bacterial tester strains was adequate based on the Guidelines for Industry: Specific Aspects of Regulatory Genotoxicity Tests for Pharmaceuticals (ICH S2A, April 1996). The positive controls (see **Table 12)** produced the expected ≤ 2-3 fold increase in the mean revertants/plate when compared to vehicle control values. Dose selection for the plate incorporation method was adequate based on the use of three non-toxic dose levels to evaluate the assay data; the establishment of a limit dose (i.e., 5000 µg/plate); and the demonstration of a dose-dependent decrease in the mean revertants/plate and background lawn.

Study outcome: In the strains tested, the revertant colonies were not significantly altered under both metabolic conditions up to the maximum dose evaluated, 5000 µg/plate. In regard to the background lawn, it was considered normal in the strains tested. Precipitate was generally observed over several concentrations of impurity (b) (4) (≤ 500 µg/plate) in the strains tested. In the absence of metabolic activity, the precipitate was deemed interfering at ≤ 500 µg/plate for TA98, TA1535 and WP2 uvrATA100; and at ≤ 1500 µg/plate for TA100. The precipitate was deemed interfering at ≤ 500 µg/plate for TA1535 and at ≤ 1500 µg/plate for the other strains tested. In conclusion, no positive mutagenic responses were observed in any of the tester strains employed, under either metabolic activation condition in the confirmatory

mutagenicity assay. The results of this study are summarized in the Sponsors table 22, as **Table 13** below**.**

Table 13. Summary of the results from the confirmatory Bacterial Reverse Mutation Assay (see Final Report, page 39)

Test Article Id : Impurity (b) (4)
Study Number : AC12KG.503.BTL
Experiment No : B2/B3

Average Revertants Per Plate ± Standard Deviation

Activation Condition : None

Dose (µg/plate)	TA98	TA100[a]	TA1535	TA1537	WP2 *uvr*A
Vehicle	13 ± 3	178 ± 12	20 ± 3	8 ± 2	14 ± 3
50	12 ± 2	208 ± 36	20 ± 1	9 ± 2	16 ± 2
150	13 ± 2	186 ± 24	19 ± 2	5 ± 4	18 ± 5
500	17 ± 6	164 ± 11	18 ± 2	7 ± 1	15 ± 8
1500	15 ± 6	158 ± 10	21 ± 6	6 ± 2	18 ± 2
5000	12 ± 1	174 ± 26	20 ± 4	6 ± 3	23 ± 6
Positive	191 ± 151	561 ± 11	398 ± 58	1629 ± 187	211 ± 6

Activation Condition : Rat Liver S9

Dose (µg/plate)	TA98	TA100[a]	TA1535	TA1537	WP2 *uvr*A[a]
Vehicle	24 ± 7	171 ± 13	11 ± 4	6 ± 3	12 ± 3
50	28 ± 3	163 ± 19	12 ± 7	7 ± 3	15 ± 3
150	23 ± 5	148 ± 14	13 ± 9	8 ± 2	16 ± 3
500	21 ± 6	162 ± 18	10 ± 1	8 ± 4	18 ± 1
1500	19 ± 6	158 ± 6	10 ± 2	6 ± 3	10 ± 1
5000	20 ± 0	133 ± 12	17 ± 8	12 ± 2	10 ± 2
Positive	299 ± 56	525 ± 36	77 ± 18	52 ± 39	127 ± 41

Vehicle = Vehicle Control
Positive = Positive Control (50 µL plating aliquot)
Plating aliquot = 50 µL
a = data from Experiment B3

Study Title: In Vitro Mammalian Chromosome Aberration Test

Key Findings: Under the conditions of the assay, impurity (b) (4) was negative for the induction of structural and numerical chromosome aberrations in the in vitro mammalian chromosome aberration assay using Chinese hamster ovary cells.

Study #: XP21L-005
Volume# and page#: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 13 Mar 2008
GLP compliance: Yes (x**) no ()**
QA reports: Yes(x**) no()**
Drug, lot# and % purity: Impurity (b) (4) Lot# SAP-16-19-5-102507, 99.3%

Methods:

Strains/species/cell line: Chinese hamster ovary (CHO) cells that were free of mycoplasma contamination and had a average cell cycle time of 10-14 hours with a modal chromosome number of 20.

Doses used in definitive study: See **Table 14** for details.

Table 14. Concentrations of impurity (b) (4) selected for the definitive study (Sponsors table, see Final Report, pg. 10).

Treatment Condition	Treatment Time	Recovery Time	Dose levels (µg/mL)
Non-activated	4 hr	16 hr	1.56, 3.13, 6.25, 12.5, 25, 50, 75
	20 hr	0 hr	1.56, 3.13, 6.25, 12.5, 25, 50, 75, 100, 125
S9-activated	4 hr	16 hr	0.78, 1.56, 3.13, 6.25, 12.5, 25, 50

Basis of dose selection: Concentrations of impurity (b) (4) selected for testing in the definitive study were based on findings from a preliminary study to examine cell growth inhibition compared to solvent control. In this study, the CHO cells were exposed to impurity (b) (4) (0.266, 0.798, 2.66, 7.98, 26.6, 79.8, 266, 798, and 2660 µg/mL), as well as solvent controls, in both the absence and presence of an Aroclor-induced S9 metabolic activation system for 4 hours, or in the absence of S9 activation for 20 hours (continuously). The number of cells in mitosis was scored per 500 cells and the mitotic index was determined in these exposure groups. Findings in the 4 hour and 20 hour exposure groups demonstrated that cell growth inhibition was ≥ 54% at 26.6 µg/mL and higher concentrations in the absence of metabolic activity. In

the presence of metabolic activation, cell growth inhibition was reportedly ≥72% at 79.8 μg/mL or higher concentrations.

Negative controls: Dimethyl sulfoxide

Positive controls: Mitomycin C (MMC) was used as the positive control in the non-metabolic activated study at the final concentrations of 0.1 and 0.2 μg/mL. Cyclophosphamide (CP) was used as the positive control in the metabolic activated study at final concentrations of 10 and 20 μg/mL. For both positive controls, a single concentration exhibiting a sufficient number of scorable metaphase cells was selected for analysis.

Incubation and sampling times: Standard procedures were used to conduct the chromosome aberration assay. Under these procedures, duplicate cultures of CHO cells were exposed to several concentrations of the test article, as well as to the positive and solvent controls.

Cells were exposed to the test substance in the presence and absence of metabolic activation. For the non-activated study, the cells were exposed for either 4 or 20 hours. In the 4 hour exposure group, the treatment medium was removed and the cells were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) and refed with complete medium after the exposure period. The cultures were then returned to the incubator for an additional 16 hours. The 20 hour exposure group was continuously exposed to the treatment medium. For the S9 activated study, the cells were exposed for 4 hours. In the 4 hour exposure group, the treatment medium was removed, the cells were washed with calcium and magnesium-free phosphate buffered saline (CMF-PBS) and refed with complete medium after the exposure period. The cultures were then returned to the incubator for an additional 16 hours. Two hours prior to cell harvest, Colcemid was added to the cultures at a final concentration of 0.1 μg/mL. Two hours after the addition of Colcemid®, metaphase cells were harvested for both the activated and non-activated studies by centrifugation. Regardless of the study, the dividing cells were always harvested 20 hours from the initiation of treatment.

Results:

Study validity**:** The study appears to be valid for the reasons outlined as follows. The appropriate positive controls were employed and produced the expected results. The appropriate number of cells was evaluated and two replicates of each test concentration were evaluated, which is in accordance with the current practice. Metaphase cells with 20±2 centromeres were examined under oil immersion. Whenever possible, a minimum of 200 metaphase spreads (100 per duplicate treatment condition) were examined and scored for chromosome-type and chromatid-type aberrations. The counting method was consistent with the currently accepted procedure and therefore considered valid. According to the protocol a test article was considered to induce a positive response when the percentage of cells with aberrations (minus gaps) was increased in a concentration-responsive manner with one or more concentrations being statistically elevated compared to solvent control. A reproducible significant increase at the high concentration only or one other concentration only with no concentration-response was considered positive. The test article was considered to be

negative if no statistically significant increase was observed relative to the solvent control. The criteria for the evaluation of the positive results were considered valid. The conditions of the assay were appropriate given toxicity measured in the 4 and 20 hour incubations. The dose selection based upon cell growth inhibition was acceptable.

Study outcome: For the 4 hour non-activated group, cell growth inhibition was 34%, 82% and 159%, respectively, at 25 μg/mL, 50 μg/mL and 75 μg/mL. At 25 μg/mL, the Mitotic Index (MI) was reduced to 53% of the solvent control. There were no significant increases in the percentage of cells with structural aberrations up to 25 μg/mL (highest dose analysis conducted at) when compared to solvent control. In MMC group (0.2 μg/mL ; positive control), the percentage of cells with structural aberrations was 16.0%, which was significantly higher than solvent control values.

Cell growth inhibition was 57% and 74%, respectively, 12.5 μg/mL and 25 μg/mL of impurity (b) (4) At 12.5 μg/mL, the highest concentration analyzed, the MI was reduced to 43% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 3.13, 6.25 and 12.5 μg/mL when compared to solvent control. In the MMC group (0.1 μg/mL), the percentage of cells with structural aberrations was 17%, which was significantly higher than solvent control values.

In the S9 activation study, cell growth inhibition was 38%, 98% and 192%, respectively, at 75 μg/mL, 100 μg/mL and 125 μg/mL. At 75 μg/mL, the lowest of these concentrations concentration, the MI was reduced to 51% of the solvent control. There were no significant alterations in the percentage of cells with numerical or structural aberrations at 12.5, 25 and 75 μg/mL when compared to solvent control. In the CP group (10 μg/mL; positive control), the percentage of cells with structural aberrations was 16.0%, which was significantly higher than solvent control values.

Table 15. Summary of findings from chromosome aberration test (Sponsor Summary Table 10, see Final Report, Page 28).

TABLE 10
SUMMARY

Treatment µg/mL	S9 Activation	Treatment Time	Mean Mitotic Index	Cells Scored		Aberrations Per Cell (Mean +/- SD)		Cells With Aberrations Numerical (%)	Structural (%)
				Numerical	Structural				
DMSO	-S9	4	11.8	200	200	0.055	±0.229	2.5	5.5
Impurity (b) (4)									
3.13	-S9	4	9.6	200	200	0.060	±0.258	2.5	5.5
6.25	-S9	4	7.5	200	200	0.065	±0.285	2.0	5.5
25	-S9	4	5.5	200	200	0.155	±1.018	3.5	6.5
MMC, 0.2	-S9	4	7.6	200	100	0.280	±1.111	0.0	16.0**
DMSO	+S9	4	10.4	200	200	0.025	±0.157	3.5	2.5
Impurity (b) (4)									
12.5	+S9	4	8.8	200	200	0.025	±0.157	6.0	2.5
25	+S9	4	6.7	200	200	0.020	±0.140	5.0	2.0
75	+S9	4	5.1	200	200	0.055	±0.229	6.5	5.5
CP, 10	+S9	4	3.3	200	100	0.340	±1.157	3.0	16.0**
DMSO	-S9	20	10.4	200	200	0.020	±0.140	2.0	2.0
Impurity (b) (4)									
3.13	-S9	20	9.5	200	200	0.010	±0.100	2.5	1.0
6.25	-S9	20	7.1	200	200	0.035	±0.210	3.5	3.0
12.5	-S9	20	5.9	200	200	0.020	±0.140	3.0	2.0
MMC, 0.1	-S9	20	6.8	200	100	0.180	±0.411	2.0	17.0**

Treatment: Cells from all treatment conditions were harvested 20 hours after the initiation of the treatments.
Aberrations per Cell: Severely damaged cells were counted as 10 aberrations.
Percent Aberrant Cells: *, p≤0.05; **, p≤0.01; using Fisher's Exact test.

Study Conclusion: Based on the findings, impurity (b) (4) was considered negative for the induction of numerical and structural chromosome aberrations in CHO cells in both the absence and presence of metabolic activation.

Repeat-dose toxicity

Study title: (b) (4) Impurity (b) (4) A 28-day oral toxicity study in rats.

Key study findings: Exposure to impurity (b) (4) was increased in a dose-related manner in treated rats. Biologically significant alterations were observed across various endpoints evaluated in animals from the 25/15 mg/kg group. In both genders, body weight and food consumption were decreased in treated animals. Alterations were observed in organ weights, as well as in hemolytic, macroscopic and microscopic endpoints in both genders. In females, alterations in the clinical signs exhibited and clinical chemistry endpoints measured were reported. Urine volume was increased in males. Based on these findings the NOAEL established was 5 mg/kg. There is a 69-fold safety margin for this impurity at the requested drug product specification of NMT (b) (4) % at the maximum daily diclofenac dose of 100 mg/day.

Study no.: 1516-006
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 16 May 2008
GLP compliance: Yes (x**) no ()**
QA reports: Yes(x**) no()**
Drug, lot, and % purity: Impurity (b) (4) Lot# QCLVG 653, 97.49%
Methods

Table 16. General information on the dosing schedule for impurity (b) (4) in CD rats that are approximately 6 weeks old (Sponsor table, see Sponsor Study No. 1516-006, Final Report, pg. 16).

Group Assignments			
Group Number	Dose Level (mg/kg)	Number of Animals	
		Male	Female
	Main Study		
1	0	10	10
2	0.25	10	10
3	5	10	10
4	25/15[a]	10	10
	Toxicokinetic Assessment		
5	0	4	4
6	0.25	8	8
7	5	8	8
8	25/15[a]	8	8

[a]Dose levels were lowered from 25 to 15 mg/kg for females on Day 6 and 7 and for males on beginning on Day 8.

Satellite groups used for toxicokinetics: Yes
Age: Six weeks (males/females)
Weight: Males: 260-265 grams; Females: 198-196 grams (at Day-1)

Observations and times:

Toxicokinetics: Blood samples (0.5 mL) were collected via the orbital sinus after anesthesia was administered. In treated animals, samples were collected 1, 2, 4 and 24 hours prior to dosing; as well as 24 hours following dosing on treatment Days 1 and 28. Samples in control animals were collected 1 hour postdose on Days 1 and 28. None of the animals were fasted prior to the collection of blood.

Mortality: Animals were observed for mortality, morbidity, injury, and the availability of water and food twice daily during the study.

Clinical signs**:** Clinical signs were evaluated during the acclimation period and weekly during treatment in animals. Animals were reportedly observed for alterations in the skin, fur, eyes, ears, nose, thorax, oral cavity, external genitalia, abdomen, feet and limbs. Effects on the respiratory and circulatory systems, autonomic nervous system, and central nervous system were evaluated.

Body weights**:** Body weights were measured in each animal upon their arrival at the testing facility, as well as weekly during treatment.

Food consumption**:** Food consumption for animals in the main study was measured weekly.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted in all rats prior to the start of the study, as well as at the scheduled necropsy in surviving animals from the main study.

Clinical Chemistry: Blood samples (4 mL) were collected via the abdominal vena cava on Day 8 in females from the 25/15 mg/kg group and prior to the terminal necropsy in the other main study animals. The other main study animals were fasted overnight prior to the collection of the blood sample. Note that the Sponsor attempted to collect blood samples from animals euthanized in extremis. In these animals, clotted or hemolyzed blood samples were not redrawn. These animals were euthanized after the samples were collected.

Macroscopic pathology: Macroscopic examinations were conducted in main study animals that were either found dead, euthanized in extremis, or euthanized at the scheduled necropsy. All animals were examined for external abnormalities such as masses. The thoracic, abdominal, and cranial cavities were examined for abnormalities.

Organ weights: Organ weights were measured for all surviving animals at the scheduled necropsy. Note that paired organs were measured together.

Microscopic pathology: Microscopic examinations were conducted in animals euthanized in extremis or found dead, and all rats from the main study in the 0 and 25/15 mg/kg group. In the 0.25 and 5 mg/kg group (main study animals), the jejunum, ileum, duodenum, cecum, Peyer's patch, rectum, glandular stomach, nongladular stomach, pancreas, liver, thymus, spleen, mandibular and mesenteric lymph nodes, adrenals, and bone with bone marrow (femur and sternum) were determined to be potential target organs and examined in treated animals.

Table 17. Lists the tissues collected for microscopic evaluation in rats treated with impurity (b) (4)

	Dose (mg/kg; PO)			
	0	0.25	5	25/15
Adrenals	X	X	X	X
Aorta	X	-	-	X
Femur bone with marrow	X	X	X	X
Brain[1]	X	-	-	X
Cecum	X	X	-	X
Cervix	X	-	-	X
Colon	X	-	-	X
Duodenum	X	X		X
Epididymis	X	-	-	X
Esophagus	X	-	-	X
Eye	X	-	-	X
Fallopian tube	-	-	-	-
Gall bladder	-	-	-	-
Gross lesions	X	-	-	X

	Dose (mg/kg; PO)			
	0	0.25	5	25/15
Harderian gland	-	-	-	-
Heart	X	-	-	X
Ileum	X	X	X	X
Injection site	-	-	-	-
Jejunum	X	X	X	X
Kidneys	X	-	-	X
Lacrimal gland, exorbital	X	-	-	X
Larynx	X	-	-	X
Liver	X	X	X	X
Lungs with bronchi	X	-	-	X
Joint, tibiofemoral	X	-	-	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	-	-	X
Nasal cavity	-	-	-	-
Optic nerves	X	-	-	X
Ovaries	X	-	-	X
Pancreas	X	X	X	X
Parathyroid	X	-	-	X
Peripheral nerve	-	-	-	-
Peyer's patch	X	X	X	X
Pharynx	-	-	-	-
Pituitary	X	-	-	X
Prostate	X	-	-	X
Rectum	X	X	X	X
Salivary gland	X	-	-	X
Sciatic nerve	X	-	-	X
Seminal vesicles	X	-	-	X
Skeletal muscle	X	-	-	X
Skin	X	-	-	X
Spinal cord	X	-	-	X
Spleen	X	X	X	X
Sternum with bone marrow	X	X	X	X
Stomach	X	X	X	X
Testes	X	-	-	X
Thymus	X	X	X	X
Thyroid	X	-	-	X

	Dose (mg/kg; PO)			
	0	0.25	5	25/15
Tongue	X	-	-	X
Trachea	X	-	-	X
Urinary bladder	X	-	-	X
Uterer	X	-	-	X
Uterus (both horns)	X	-	-	X
Vagina	X	-	-	X
Zymbal gland	-	-	-	-

[1] Brain tissues collected from areas that include the cerebrum, midbrain, cerebellum, and medulla/pons.

Adequate Battery: yes (x), no ( )

Peer review: yes (x), no ( )

Results

Toxicokinetics. The toxicokinetic profile for impurity (b)(4) was partially completed at the 5, 15 and 25 mg/kg treatment doses. Note that the decreased survival rate at the high dose and the lack of drug detection at various doses served to limit those data collected. Toxicokinetic endpoints were reported at Day 1 (5 and 25 mg/kg) for both genders and at Day 28 (5 and 15 mg/kg) in males only. Based on the limited data reported (see **Table 18**), the averaged C_{max} and AUC_{last} values increased in a dose-related manner and these values were not dose proportional. For example, both genders were exposed to a greater proportion of impurity (b)(4) at 25 mg/kg when compared to 5 mg/kg on Day 1. In addition, males were exposed to a greater proportion of impurity (b)(4) at 15 mg/kg when compared to 5 mg/kg. At the 5 mg/kg dose, drug accumulation was demonstrated based on the 2-fold increase in the averaged AUC_{last} values on Day 28 when compared to Day 1. In other findings reported in males, total body clearance for the 25 mg/kg treatment dose was 810 L/hr/kg at Day 1. At Day 28, total body clearance was 1281 L/hr/kg for 5 mg/kg and 343.9 L/hr/kg for 15 mg/kg. Despite these findings that clearance at Day 28 was lower for the highest dose when compared to the lowest dose mentioned, there was no conclusive evidence of drug accumulation at the 15 mg/kg treatment dose. In females, there were no data on total body clearance or conclusive evidence of drug accumulation. Together, these data demonstrated that exposure to impurity (b)(4) increased in a dose-related manner in rats and that there were no apparent gender-related differences in its toxicokinetic profile.

Table 18. The values for the C_{max}, AUC_{last} and half-life ($t_{1/2}$) for impurity (b)(4) in treated rats are presented.

	C_{max}				AUC_{last}				$t_{1/2}$			
Dose	Day 1		Day 28		Day 1		Day 28		Day 1		Day 28	
mg/kg	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
0.25	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC	NC
5	1.24	1.03	3.01	NC	1.6	1.44	3.31	NC	NC	NC	NC	NC
15			33.27				41.58				1.69	
25	14.43	9.35			25.87	17.43			3.46	NC		

In males, impurity (b) was reportedly rapidly metabolized to diclofenac when measured on Day 28. This yielded systemic exposures to dicolfenac rather than impurity (b)(4) At Day 1, the C_{max} and AUC_{last} values increased in a dose-related manner across dose groups. Similar findings were reported at Day 28; however, exposure was not determined for females from the 25/15 mg/kg group. Due to the early termination of females in the 25/15 mg/kg group and the lack of quantifiable plasma levels of impurity (b)(4) at the lower doses tested, this finding could not be determined in female rats.

Table 19. The values for the C_{max}, T_{max}, AUC_{last} and half-life ($t_{1/2}$) for impurity (b)(4) in treated rats are presented (Sponsor Table, Study # 1516-006, pg. 28).

Mean Diclofenac Toxicokinetic Parameters in Rats Given Impurity (b) (4) Orally for 28 Days					
Sex	Dose (mg/kg)	C_{max} (ng/mL)	T_{max} (hr)	t½ (hr)	$AUC_{(0-\tau)}$ (ng*hr /mL)
		Day 1			
Male	0.25	25.50	1.0	3.78	112.58
	5	390.67	1.0	5.49	2544.33
	25/15	3993.33	1.0	5.12	17721.67
Female	0.25	37.50	1.0	11.75	241.97
	5	915.00	1.0	5.01	4811.78
	25/15	4336.67	1.0	2.72	32144.07
		Day 28			
Male	0.25	40.23	1.0	2.30	89.40
	5	505.33	1.0	2.75	1998.00
	25/15	2963.33	1.0	2.05	8216.05
Female	0.25	47.40	1.0	6.24	266.48
	5	921.33	1.0	2.98	4597.98
	25/15	ND	ND	ND	ND

ND = No data; $AUC_{(0-\tau)}$ = Area under the plasma concentration-time curve over the dosing interval.

Clinical Signs. Although the clinical signs observed in treated male rats were incidental or sporadic in the study, those observed in treated female rats were biologically significant when animals from the 25/15 mg/kg group were compared to control at week 1. For example, females in the 25/15 mg/kg group reportedly exhibited clinical signs such as postured hunch, discolored hair, and piloerection in ≥ 50% of the animals.

Body weights. Statistically significant decreases in body weights were observed in animals from the 25/15 mg/kg group at week 1 for females and at each week for males. The average body weight of females was decreased by 9% when compared to control at week 1. In subsequent weeks, no body weights were reported, as none of the females from this group survived. In males, body weights reportedly decreased by ≥ 9% when compared to control at weeks 1-4. The greatest decreases were demonstrated at weeks 2 and 3, during which the averaged body weights were, respectively, 84% and 82% of control. At week 4, the averaged body weight measured was 91% of control, slightly higher than that observed over weeks 2 and 3. Together, these findings demonstrated that the averaged body weights reported were decreased in a biologically significant manner (i.e., > 10% decrease) in males only at weeks 2 and 3 of treatment.

Food consumption. Food consumption was decreased in the 25/15 mg/kg group in a statistically significant manner at week 1 in females and at weeks 1-3 in males. At week 1,

food consumption in females was 49% of control, a substantial decrease that may partially explain the 100% mortality rate in females during subsequent weeks. The averaged amount of food consumed by males was decreased by ≥ 19% when compared to control at weeks 1-3. At week four, the averaged amount was decreased by only 6% when compared to control, suggesting a recovery in food consumption. These alterations in food consumption are consistent with changes in the animal body weights reported.

Hematology. Toxicologically significant alterations were observed in various hematology parameters evaluated in animals from the 25/15 mg/kg groups (see **Table 20**). For example, the averaged values for absolute reticulocytes and eosinophils, respectively, were 209% and 191% of control. In females, significant alterations were produced across numerous endpoints. These alterations were apparent in endpoints such as platelets, absolute reticulocytes, neutrophils, monocytes and basophils, as the averaged values reported were ≥ 205% of control. Substantial reductions were apparent in endpoints such as erythrocytes, hemoglobin, lymphocytes, and eosinophils, as the averaged values reported were ≤ 71% of control.

Table 20. Lists the hematology values, represented as a percent of control, for endpoints altered in a toxicologically significant manner in animals treated with impurity (b) (4) (0.25, 5, and 25/15 mg/kg).

	Males			**Females**		
	Doses (mg/kg)			Doses (mg/kg)		
Endpoint	**0.25**	**5**	**25/15**	**0.25**	**5**	**25/15**
Absolute Reticulocytes	96%	85%	209%	114%	117%	429%
Eosinophils	97%	96%	191%	77%	88%	23%
Erythrocytes				100%	99%	67%
Hemoglobin				102%	100%	71%
Platelets				96%	103%	205%
Neutrophils				93%	110%	305%
Lymphocytes				91%	102%	62%
Monocytes				86%	82%	326%
Basophils				101%	94%	229%

Coagulation. The coagulation endpoints reported were not altered in a biologically significant manner.

Clinical Chemistry. A variety of clinical chemistry endpoints evaluated in female animals were altered in a biologically significant manner at the highest treatment dose; whereas those in male animals were altered in an incidental or sporadic manner. In females, endpoints that included phosphorus, alkaline phosphatase, GGT, AST, ALT, urea nitrogen and triglyceride were markedly increased by ≥ 38% when compared to control (see **Table 21**). In particular,

GGT and alkaline phosphatase values in the 25/15 mg/kg group were > 9-fold higher than the averaged value for the control group.

Table 21. Lists clinical chemistry endpoints that were altered in female rats treated with impurity (b) (4). The values are represented as a percentage of control.

	Females		
	Doses (mg/kg)		
Endpoint	**0.25**	**5**	**25/15**
Phosporus	97%	101%	138%
Alkaline Phosphatase	94%	94%	959%
GGT	100%	100%	1755%
AST	101%	98%	326%
ALT	101%	95%	165%
Urea Nitrogen	105%	99%	177%
Albumin	102%	99%	65%
Triglyceride	79%	89%	162%
Cholesterol	102%	108%	74%

Urinalysis. The urinalysis endpoint urine volume was increased to 176% of control, in surviving male rats from the 25/15 mg/kg group; whereas the endpoints specific gravity and pH were not altered in a biologically significant manner in either species. No data are reported for female rats from the 25/15 mg/kg group, as no animals survived throughout the study.

Macroscopic Observations. In the main study animals, there were 12 deaths (5/10 males and 7/10 females; unscheduled) from the 25/15 mg/kg group. Biologically significant macroscopic findings were observed across genders in the abdominal cavity of animals from the 25/15 mg/kg group only. These adhesions were observed in animals that either died or were euthanized on study (DOS), as well as those that had scheduled necropsies (SNC). In females, adhesions were observed in 5/7 DOS animals and 2/3 SNC animals. These adhesions were reportedly moderate to severe in the DOS animals; and either mild or severe in the two SNC animals. Macroscopic findings in male DOS rats included mild to moderate adhesions in the abdominal cavity, fluid in the abdominal and/or thoracic cavities and body fat depletion.

Table 22. List of macroscopic findings in male and female rats treated with impurity (b) (4)

	Abdominal Cavity (adhesion)							
	Vehicle		0.25 mg/kg		5 mg/kg		25/15 mg/kg	
Gender	DOS	SNC	DOS	SNC	DOS	SNC	DOS	SNC
Males	0/0	0/10	0/0	0/10	0/0	0/10	5/5	0/5
Females	0/0	0/10	0/0	0/10	0/0	0/10	5/7	2/3

Organ weights. Weights for a variety of organs were altered in animals from the 25/15 mg/kg group (see **Table 23)**. These alterations are apparent in the averaged values based on organ weight/brain weight ratios. For example, in males the average value for the right mandibular/sublingual salivary gland and the spleen were, respectively, 68% and 133% of control. In females, the averaged value for the pituitary, thymus and thyroid/parathyroid were decreased to ≤ 75% of control. The average values for organs that include the spleen, adrenal and liver were increased to ≥ 131% of control.

Table 23. Lists organ weights for male/female rats treated with impurity (b) (4) Values are represented as a percentage of control.

	Males			Females		
	Doses (mg/kg)			Doses (mg/kg)		
Organ	**0.25**	**5**	**25/15**	**0.25**	**5**	**25/15**
Salivary gland, mand/sub, rt	97%	83%	68%			
Spleen	94%	90%	133%	102%	98%	135%
Adrenal				114%	112%	143%
Liver				101%	101%	131%
Pituitary				104%	99%	74%
Thymus				98%	108%	55%
Thyroid/Parathyroid				79%	80%	75%

Microscopic observations. Biologically significant microscopic findings were observed in animals from the 25/15 mg/kg treatment group. The findings included hypercellularity, chronic-active inflammation, edema, increased extramedullary hematopoiesis, necrosis, generalized lymphoid depletion and generalized lymphoid necrosis. These findings were observed in ≥ 50% of the animals from the subgroups in which these findings were observed (see **Table 24)**. Note that the severity of these findings varied from minimal to severe in the subgroups listed.

Across genders, there was overlap in the microscopic findings observed. For example, hypercellularity in the bone marrow of the sternum and generalized lymphoid depletion in the spleen and thymus gland were reported. Chronic active inflammation in the liver; mesenteric lymph node; pancreas; duodenum and jejunum of the small intestine; as well as the glandular and nonglandular stomach were observed in treated animals. In the spleen and duodenum, respectively, increased extramedullary hematopoiesis and necrosis were reported. Note that findings from males were observed in the DOS subgroup only, whereas those in females were

observed in the DOS and/ or SNC subgroups (see **Table 24**). For instance, chronic inflammation in the abdominal cavity, mesenteric lymph node, pancreas, and duodenum and jejunum of the small intestine; increased extramedullary hematopoiesis in the spleen; and generalized lymphoid depletion in the spleen and thymus gland reportedly occurred in females from the DOS and SNC subgroups. These results provide evidence that microscopic findings overlapped across genders; but did not overlap in terms of the subgroups in which these findings were observed.

Findings observed in males only demonstrated the presence of chronic active inflammation in the rectum of the large intestine and seminal vesicles. In the pancreas and jejunum of the small intestine, respectively, edema and necrosis were reported in treated males. Observations of hypercellularity in the bone marrow of the femur and generalized lymphoid necrosis in the thymus gland were also reported. Note that all of the microscopic findings reported for males were observed in ≥ 60% of the animals from the DOS subgroup.

In females, the findings reported were observed in ≥ 57% of the animals from either the DOS and/or SNC subgroup(s). Findings of chronic active inflammation in the adrenal glands, colon of the large intestine and ileum of the small intestine were observed in the DOS subgroup only; whereas those in the abdominal cavity and spleen were observed in both subgroups. In the DOS subgroup, generalized lymphoid necrosis was reported in the spleen of treated females.

Table 24. Lists microscopic findings in rats treated with 25/15 mg/kg of impurity (b) (4)

			Group 25/15 mg/kg	
Gender	**Microscopic Finding**	**Endpoint**	**DOS**	**SNC**
Male	Hypercellularity	Bone marrow, femur	5/5	1/5
		Bone marrow, sternum	5/5	1/5
	Chronic-active inflammation	Large intestine, rectum	3/5	0/5
		Liver	3/5	0/5
		Mesenteric Lymph Node	4/5	0/5
		Pancreas	4/5	1/5
		Seminal Vesicles	3/5	0/5
		Small intestine, duodenum	3/5	1/5
		Small intestine, jejunum	3/5	0/5
		Stomach gladular	5/5	0/5
		Stomach, nongladular	3/5	2/5
	Edema	Pancreas	3/5	2/5
	↑ Extramedullary hematopoiesis	Spleen	5/5	1/5
	Necrosis	Small intestine, duodenum	3/5	0/5
		Small intestine, jejunum	3/5	0/5
	Generalized lymphoid necrosis	Thymus gland	4/5	0/5
	Generalized lymphoid depletion	Spleen	4/5	0/5
		Thymus gland	5/5	0/5
Female	Hypercellularity	Bone marrow, sternum	4/7	1/3
	Chronic-active inflammation	Adrenal glands	4/7	0/3
		Abdominal cavity	6/6	2/2
		Large intestine, colon	4/7	1/3
		Liver	6/7	1/3
		Mesenteric Lymph node	7/7	2/3
		Pancreas	6/7	2/3
		Small intestine, duodenum	7/7	2/3
		Small intestine, duodenum	4/7	0/3
		Small intestine, jejunum	7/7	2/3
		Spleen	4/7	2/3
		Stomach, gladular	5/7	1/3
		Stomach, nongladular	5/7	1/3
	↑ Extramedullary hematopoiesis	Spleen	5/7	2/3
	Necrosis	Small intestine, duodenum	4/7	0/3
	Generalized lymphoid necrosis	Spleen	4/7	1/3
	Generalized lymphoid depletion	Spleen	7/7	2/3
		Thymus gland	7/7	2/3
		Thymus gland	7/7	2/3

Study title: (b) (4) Impurity (b) (4) A 28-day oral toxicity study in rats.

Key study findings: Impurity (b) (4) exposure increased in a dose-related manner in rats, which exhibited gender-related differences with regard to its toxicokinetic profile. Biologically significant alterations were observed across various endpoints evaluated in animals from the 250 mg/kg group. In females, alterations were observed in various hematology and clinical chemistry endpoints. Urine volume was increased in males. Based on these findings the NOAEL established was 25 mg/kg. There is a 243x safety margin for this impurity at the requested drug product specification of NMT (b) (4)% at the maximum daily diclofenac dose of 100 mg/day.

Study no.: 1516-007
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 16 May 2008
GLP compliance: Yes (x**) no ()**
QA reports: Yes(x**) no()**
Drug, lot, and % purity: Impurity (b) (4) Lot# SAP-18-27-041808, 97.1%
Methods

Table 25. General information on the dosing schedule for impurity (b) (4) in CD rats that are approximately 6 weeks old (Sponsor table, see Sponsor Study No. 1516-007, Final Report, pg. 14).

Group Assignments			
Group Number	Dose Level (mg/kg)	Number of Animals	
		Male	Female
Main Study			
1	0	10	10
2	0.5	10	10
3	25	10	10
4	250	10	10
Toxicokinetics			
5	0	4	4
6	0.5	8	8
7	25	8	8
8	250	8	8

Satellite groups used for toxicokinetics: Yes
Age: Six weeks (males/females)
Weight: Males: 236-279 grams; Females: 177-212 grams (at Day-1)

Observations and times:

Toxicokinetics: Blood samples (0.5 mL) were collected via the orbital sinus after anesthesia was administered. In treated animals, samples were collected 1, 2, 4 and 24 hours prior to dosing; as well as 24 hours following dosing on treatment Days 1 and 28. Samples in control animals were collected 1 hour postdose on Days 1 and 28. None of the animals were fasted prior to the collection of blood.

Mortality: Animals were observed for mortality, morbidity, injury, and the availability of water and food twice daily during the study.

Clinical signs**:** Clinical signs were evaluated during the acclimation period and weekly during treatment in animals. Animals were reportedly observed for alterations in the skin, fur, eyes, ears, nose, thorax, oral cavity, external genitalia, abdomen, feet and limbs. Effects on the respiratory and circulatory systems, autonomic nervous system, and central nervous system were evaluated.

Body weights**:** Body weights were measured for each animal upon their arrival at the testing facility, as well as weekly during the study.

Food consumption**:** Food consumption for animals in the main study was measured weekly.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted in all rats prior to the start of the study, as well as the scheduled necropsy in surviving animals from the main study animals.

Clinical Chemistry: Blood samples (4 mL) were collected via the abdominal vena cava from all animals in the main study. Animals were fasted overnight prior to the collection of the blood sample.

Macroscopic pathology: Macroscopic examinations were conducted in main study animals that were euthanized at the scheduled necropsy. All animals were examined for external abnormalities such as masses. The thoracic, abdominal, and cranial cavities were examined for abnormalities.

Organ weights: Organ weights were measured for all surviving animals at the scheduled necropsy. Note that paired organs were measured together.

Microscopic pathology: Microscopic examinations were conducted in all rats from the main study in the 0 and 250 mg/kg group. In the 0.5 and 25 mg/kg group (main study animals), the epididymides was determined as a potential target organ and examined in treated animals.

Table 26. Lists the tissues collected for microscopic evaluation in rats treated with impurity (b) (4)

	Dose (mg/kg; PO)			
	0	0.5	25	250
Adrenals	X	-	-	X
Aorta	X	-	-	X
Femur bone with marrow	X	-	-	X
Brain[1]	X	-	-	X
Cecum	X	-	-	X
Cervix	X	-	-	X
Colon	X	-	-	X
Duodenum	X	-	-	X
Epididymis	X	X	X	X
Esophagus	X	-	-	X
Eye	X	-	-	X
Fallopian tube	-	-	-	-
Gall bladder	-	-	-	-
Gross lesions	X	-	-	X
Harderian gland	-	-	-	-
Heart	X	-	-	X
Ileum	X	-	-	X
Injection site	-	-	-	-
Jejunum	X	-	-	X
Kidneys	X	-	-	X
Lacrimal gland, exorbital	X	-	-	X
Larynx	X	-	-	X
Liver	X	-	-	X
Lungs with bronchi	X	-	-	X
Joint, tibiofemoral	X	-	-	X
Lymph nodes mandibular	X	-	-	X
Lymph nodes, mesenteric	X	-	-	X
Mammary Gland	X	-	-	X
Nasal cavity	-	-	-	-
Optic nerves	X	-	-	X
Ovaries	X	-	-	X
Pancreas	X	-	-	X
Parathyroid	X	-	-	X

	Dose (mg/kg; PO)			
	0	0.5	25	250
Peripheral nerve	-	-	-	-
Peyer's patch	X	-	-	X
Pharynx	-	-	-	-
Pituitary	X	-	-	X
Prostate	X	-	-	X
Rectum	-	-	-	-
Salivary gland	X	-	-	X
Sciatic nerve	X	-	-	X
Seminal vesicles	X	-	-	X
Skeletal muscle	X	-	-	X
Skin	X	-	-	X
Spinal cord	X	-	-	X
Spleen	X	-	-	X
Sternum with bone marrow	X	-	-	X
Stomach	X	-	-	X
Testes	X	-	-	X
Thymus	X	-	-	X
Thyroid	X	-	-	X
Tongue	X	-	-	X
Trachea	X	-	-	X
Urinary bladder	X	-	-	X
Uterer	X	-	-	X
Uterus (both horns)	X	-	-	X
Vagina	X	-	-	X
Zymbal gland	-	-	-	-

[1] Brain tissues collected from areas that include the cerebrum, midbrain, cerebellum, and medulla/pons.

Adequate Battery: yes (x), no ( )
Peer review: yes (x), no ( )

Results

Toxicokinetics. A toxicokinetic profile was determined for the 25 and 250 mg/kg doses of impurity (b)(4). Toxicokinetic endpoints were reported at Days 1 and 28 for both genders. In regard to the averaged C_{max} and AUC_{last} values, these endpoints were increased in a dose-related manner; however, these values generally were not dose proportional (see **Table 27**). For example, both genders were exposed to a substantially greater proportion of impurity (b)(4) at 25 mg/kg compared to 250 mg/kg on treatment Day 28. At Day 1, females were exposed to a slightly higher proportion of impurity (b)(4) at 250 mg/kg compared to 25 mg/kg. Note that gender-related differences were observed in the averaged C_{max} and AUC_{last} values for the

doses tested, which suggests that females were exposed to greater levels of impurity (b)(4) than males. These values were generally ≥ 2-fold higher in females when compared to males across the doses tested on Days 1 and 28. At 25 mg/kg, drug accumulation was observed in both genders based on the > 2-fold increase in the averaged AUC_{last} values reported at Day 28 compared to Day 1. This may be supported by the slightly lower values for the averaged total body clearance of impurity (b)(4) in animals on Day 28 when compared to Day 1 (see **Table 27**). For example, total body clearance at Day 28 in males and females, respectively, was ≥ 85% and ≥ 74% of Day 1 at the doses tested. Note that clearance was > 1.5-fold higher across doses in males compared to those in females on the treatment days mentioned. The half-life reported for impurity (b)(4) was 4.71-6.96 hr in males and 4.5-6.91 hr in females across Days 1 and 28. Together, these data demonstrated that exposure to impurity (b)(4) increased in a dose-related manner in rats and that there were gender-related differences in its toxicokinetic profile.

Table 27. The Cmax and AUC values for the doses studied are presented .

	C_{max}				AUC_{last}			
Dose	Day 1		Day 28		Day 1		Day 28	
mg/kg	Male	Female	Male	Female	Male	Female	Male	Female
0.5	NC	NC	NC	NC	NC	NC	NC	NC
25	4.1	8.1	5.2	14.7	20.0	31.8	45.4	82.2
250	30.0	83.9	37.7	106.2	215.7	461.9	252.4	599.0

Table 28. Lists results from the expression of data from Day 28/Day 1 for C_{max} and AUC_{last} values in rats treated with impurity (b)(4) during toxicokinetic studies.

	Drug Accumulation (Day 28/Day 1)			
Dose	C_{max}		AUC_{last}	
mg/kg	Male	Female	Male	Female
25	1.3	1.8	2.3	2.6
250	1.3	1.3	1.2	1.3

Clinical Signs. There were no biologically significant clinical findings in treated animals.

Body weights. There were no biologically significant alterations of food consumption in treated animals.

Hematology. Biologically significant increases for hematology endpoints that included neutrophils and monocytes were observed in females only at the 250 mg/kg treatment dose. At this dose, neutrophil and monocyte levels, respectively, were increased to 199% and 142% of control. All other alterations in hematology endpoints were incidental and/or sporadic in both genders.

Coagulation. There were no biologically significant alterations in the coagulation endpoints evaluated in treated rats.

Clinical chemistry. In female rats treated with 250 mg/kg, ALT and sorbitol dehydrogenase levels were decreased to 66% and 62% of control. These alterations were not biologically significant, based on the direction of the effect. All other alterations in clinical chemistry values were deemed either incidental or sporadic in treated animals.

Urinalysis. There were no biologically significant alterations in the urinalysis endpoints at the doses evaluated in both genders, except for urine volume at 250 mg/kg in males. Urine volume was increased by 66% when compared to control.

Macroscopic observations. There were no biologically significant macroscopic findings observed in treated animals. In a single male rat from the 250 mg/kg group, a mild yellow epididymal discoloration was reported at terminal necropsy.

Organ weights. There were no biologically significant alterations in the organ weights of treated animals.

Microscopic observations. There were no biologically significant microscopic findings observed in the treated animals. In 2/10 males from the 250 mg/kg group, epididymal spermatic granulomas were reported. This finding was not test-article related.

Study title: (b) (4) Impurity (b) (4) A 28-day oral toxicity study in rats.

Key study findings: Impurity (b) (4) exposure increased in a dose-related manner in rats, which exhibited gender-related differences with regard to its toxicokinetic profile. Biologically significant alterations were observed across various endpoints evaluated in animals from the 250/75 mg/kg group. In both genders, food consumption and organ weights, as well as hematology, clinical chemistry, and macroscopic and microscopic endpoints were reportedly altered. Based on these findings the NOAEL established was 25 mg/kg. There is a 243x safety margin for this impurity at the requested drug product specification of NMT (b) (4)% at the maximum daily diclofenac dose of 100 mg/day.

Study no.: 1516-008
Volume #, and page #: Electronic submission
Conducting laboratory and location: (b) (4)

Date of study initiation: 15 May 2008
GLP compliance: Yes (x**) no ()**
QA reports: Yes(x**) no()**
Drug, lot, and % purity: Impurity (b) (4) Lot# EVK-2-55-7-041708, 99.8%
Methods

Table 29. General information on the dosing schedule for impurity (b) (4) in CD rats that are approximately 6 weeks old (Sponsor table, see Sponsor Study No. 1516-008, Final Report, pg. 15).

Group Assignments			
Group Number	Dose Level (mg/kg/day)	Number of Animals	
		Male	Female
	Main Study		
1	0	10	10
2	0.5	10	10
3	25	10	10
4M	250	10	10
4F[a]	250/75		
	Toxicokinetic Assessment		
5	0	4	4
6	0.5	8	8
7	25	8	8
8M	250	8	8
8F[a]	250/75	8	8

[a]The dose level was lowered from 250 to 75 mg/kg/day beginning on Day 6.

Satellite groups used for toxicokinetics: Yes
Age: Six weeks (males/females)
Weight: Males: 227-288 grams; Females: 171-224 grams (at Day-1)

Observations and times:

Toxicokinetics: Blood samples (0.5 mL) were collected via the orbital sinus after anesthesia was administered. In treated animals, samples were collected 1, 2, 4 and 24 hours prior to dosing; as well as 24 hours following dosing on treatment Days 1 and 28. Samples in control animals were collected 1 hour postdose on Days 1 and 28. None of the animals were fasted prior to the collection of blood.

Mortality: Animals were observed for mortality, morbidity, injury, and the availability of water and food twice daily during the study.

Clinical signs**:** Clinical signs were evaluated during the acclimation period and weekly during treatment. Animals were reportedly observed for alterations in the skin, fur, eyes, ears, nose, thorax, oral cavity, external genitial, abdomen, feet and limbs. Effects on the respiratory and circulatory systems, autonomic nervous system, and central nervous system were evaluated.

Body weights**:** Body weights were measured for each animal upon their arrival at the testing facility, as well as weekly during the study.

Food consumption**:** Food consumption for animals in the main study was measured weekly.

Ophthalmoscopy: Ophthalmoscopic examinations were conducted in all rats prior to the start of the study, as well as at the scheduled necropsy in surviving animals from the main study.

Clinical Chemistry: Blood samples (3.5-4 mL) were collected via the abdominal vena cava on Day 9 in females from the 250/75 mg/kg group and at the terminal necropsy for all other animals from the main study. Animals were fasted overnight prior to the collection of the blood sample.

Macroscopic pathology: Macroscopic examinations were conducted in all main study animals that were either found dead, euthanized in extremis, or euthanized at the scheduled necropsy. All animals were examined for external abnormalities such as palpable masses. The thoracic, abdominal, and cranial cavities were examined for abnormalities.

Organ weights: Organ weights were measured for all surviving animals at the scheduled necropsy. Note that paired organs were measured together.

Microscopic pathology: Microscopic examinations were conducted in all rats from the main study in the 0 and 250 mg/kg group. In the 0.5 and 25 mg/kg group (main study animals), the liver, glandular stomach, spleen, pancreas, cecum, Peyer's patch, thymus, and the mesenteric

and mandibular lymph nodes were determined as a potential target organ and examined in treated animals.

Table 30. Lists the tissues collected for microscopic evaluation in rats treated with impurity (b) (4)

	Dose (mg/kg; PO)			
	0	0.5	25	250/75
Adrenals	X	-	-	X
Aorta	X	-	-	X
Femur bone with marrow	X	-	-	X
Brain[1]	X	-	-	X
Cecum	X	X	X	X
Cervix	X	-	-	X
Colon	X	-	-	X
Duodenum	X	-	-	X
Epididymis	X	-	-	X
Esophagus	X	-	-	X
Eye	X	-	-	X
Fallopian tube	-	-	-	-
Gall bladder	-	-	-	-
Gross lesions	X	-	-	X
Harderian gland	-	-	-	-
Heart	X	-	-	X
Ileum	X	-	-	X
Injection site	-	-	-	-
Jejunum	X	-	-	X
Kidneys	X	-	-	X
Lacrimal gland, exorbital	X	-	-	X
Larynx	X	-	-	X
Liver	X	X	X	X
Lungs with bronchi	X	-	-	X
Joint, tibiofemoral	X	-	-	X
Lymph nodes mandibular	X	X	X	X
Lymph nodes, mesenteric	X	X	X	X
Mammary Gland	X	-	-	X
Nasal cavity	-	-	-	-
Optic nerves	X	-	-	X
Ovaries	X	-	-	X

	Dose (mg/kg; PO)			
	0	0.5	25	250/75
Pancreas	X	X	X	X
Parathyroid	X	-	-	X
Peripheral nerve	-	-	-	-
Peyer's patch	X	X	X	X
Pharynx	-	-	-	-
Pituitary	X	-	-	X
Prostate	X	-	-	X
Rectum	-	-	-	-
Salivary gland	X	-	-	X
Sciatic nerve	X	-	-	X
Seminal vesicles	X	-	-	X
Skeletal muscle	X	-	-	X
Skin	X	-	-	X
Spinal cord	X	-	-	X
Spleen	X	X	X	X
Sternum with bone marrow	X	-	-	X
Stomach	X	X	X	X
Testes	X	-	-	X
Thymus	X	X	X	X
Thyroid	X	-	-	X
Tongue	X	-	-	X
Trachea	X	-	-	X
Urinary bladder	X	-	-	X
Uterer	X	-	-	X
Uterus (both horns)	X	-	-	X
Vagina	X	-	-	X
Zymbal gland	-	-	-	-

[1] Brain tissues collected from areas that include the cerebrum, midbrain, cerebellum, and medulla/pons.

Adequate Battery: yes (x), no ( )

Peer review: yes (x), no ( )

Results

Toxicokinetics. A toxicokinetic profile was determined for the 25 and 250 mg/kg doses of impurity (b)(4). Toxicokinetic endpoints were reported at Day 1 in both genders and Day 28 for males only. In regard to the averaged C_{max} and AUC_{last} values, these endpoints were increased in a dose-related manner; however, the values determined were generally not dose-proportional. For example, male rats were exposed to a greater proportion of impurity (b)(4) at 25 mg/kg when compared to the 250 mg/kg treatment dose for both Days 1 and 28. Separately, females were exposed to a greater proportion of impurity (b)(4) at 250 mg/kg when compared to the 25 mg/kg treatment dose for Day 1. At 25 mg/kg, there was a slight accumulation of drug in both genders based on the 1.5-fold increase in the averaged AUC_{last} values on Day 28 compared to Day 1. In males, this finding may be supported by the demonstration that the total clearance of drug was slightly higher at Day 1 (322.65 L/hr/kg) compared to Day 28 (215.05 L/hr/kg). At Day 28, total body clearance was demonstrated to increase in a dose-related manner, as demonstrated by the > 4-fold higher value at 250 mg/kg compared to 25 mg/kg (see **Table 31**). Findings in females were only reported for the 25 mg/kg dose (Day 28), at which the averaged total body clearance was 528.88 L/hr/kg. This value was 2.5 fold higher in females when compared to males at the same treatment dose. In males, the averaged half-life of impurity (b)(4) was 3.81 hours at Day 1. These values were not reported at other time points and/or in female rats. Together, these data demonstrated that impurity (b)(4) exposure increased in a dose-related manner in rats and that there were gender-related differences in its toxicokinetic profile.

Table 31. The C_{max} and AUC_{last} values for the doses studied are presented.

	C_{max}				AUC_{last}			
Dose	Day 1		Day 28		Day 1		Day 28	
mg/kg	Male	Female	Male	Female	Male	Female	Male	Female
0.5	NC	NC	NC	NC	NC	NC	NC	NC
25	12.89	4.28	19.67	7.33	76.2	25.25	116.25	38.62
250	33.3	82.03	34.2	NC	306.67	455.43	257.81	NC

Clinical Signs. All clinical signs observed were either sporadic or incidental.

Body Weights. There were no biologically significant alterations in the reported body weights.

Food Consumption. Alterations in food consumption were observed in both genders. Food consumption in females treated with 250 mg/kg was reduced to 83% of control in week 1. Note that this dose was lethal in 70% of the rats at week 2, and in all surviving animals at week 3. There were no biologically significant alterations in food consumption by male rats, despite reports of the lethal effects of the 250 mg/kg dose in a rat during weeks 3 and 4.

Hematology. There were substantial increases in a variety of hematology endpoints evaluated in both genders at the 250 mg/kg dose (see **Table 32**). In both genders, the averaged amount of absolute reticulocytes and neutrophils was increased. The averaged number of absolute reticulocytes was 174% and 514% of control, respectively, in males and females. In regard to neutrophils, this endpoint was increased to 194% and 363% of control, respectively, in male and female rats. The averaged values for these endpoints were substantially higher in females when compared to males. In females, the averaged amount of platelets and monocytes, was increased to 212% and 367% of control. The averaged amount of eosinophils in males was increased to 132% of control.

Table 32. Hematology values are expressed as a percentage of control in rats administered impurity (b) (4) in a 28-day repeat dose toxicity study.

	Males			**Females**		
	Doses (mg/kg)			Doses (mg/kg)		
Endpoint	**0.5**	**25**	**250**	**0.5**	**25**	**250**
Eosinophils	127%	97%	132%			
Platelets	100%	101%	111%	103%	94%	212%
Absolute Reticulocytes	117%	112%	174%	110%	90%	514%
Neutrophils	99%	94%	194%	165%	168%	363%
Monocytes				168%	134%	367%
Leukocytes				144%	116%	115%

Coagulation. The coagulation endpoints evaluated were not markedly altered, except for APTT in female rats at 250 mg/kg. At this dose, APTT in females was 70% of control.

Clinical Chemistry. There were marked increases in a variety of clinical chemistry endpoints evaluated. In both genders, triglycerides and GGT levels were increased at 250 mg/kg. Triglycerides were increased in males and females, respectively, to 132% and 251% of control. In regard to GGT levels in males and females, respectively, the averaged amounts were increased to 150% and 330% of control. Note that these alterations were substantially higher in treated females when compared to males. In males, total bilirubin was ≥ 150% at the doses tested. These effects were not considered biologically significant at the 0.5 and 25 mg/kg doses, given the lack of microscopic findings in the liver. In females, ALT, urea nitrogen, and alkaline phosphatase levels were markedly increased compared to control. At the 250 mg/kg dose, urea nitrogen and alkaline phosphatase were ≥ 150% of control. ALT levels were 128%, 166% and 162%, respectively, at the 0.5, 25 and 250 mg/kg doses. The alterations at the 0.5 and 25 mg/kg doses were not deemed biologically significant, based on the variability of ALT levels across individual subjects and the lack of microscopic findings at these doses.

Urinalysis. There were no biologically significant alterations in the urinalysis endpoints evaluated in treated rats.

Macroscopic Observations. There were six unscheduled deaths reported in animals (2/10 males and 4/10 females) from the 250/75 mg/kg group. In this group, adhesions were observed in the abdominal cavity of male and female rats. Adhesion was observed in 2/2 males and 2/4 females from the DOS subgroup. All other macroscopic observations were deemed either sporadic or incidental.

Organ Weights. Organ weights in treated rats were comparable to controls, except for the liver and spleen in female animals at 250 mg/kg. At this dose, the liver and spleen weights expressed as a percentage of body weight were increased to 156% and 187% of control, respectively. Biologically significant microscopic observations were observed in these organs for both genders at the 250 mg/kg dose (see **Tables 34 and 35)**.

Table 33. Organ weight/Body weight values are expressed as a percentage of control in rats administered impurity (b)(4) (0.5, 25, and 250 mg/kg; oral) in a 28-day repeat dose toxicity study.

	Male			**Female**		
	Doses (mg/kg)			Doses (mg/kg)		
Organ	**0.5**	**25**	**250**	**0.5**	**25**	**250**
Liver	102%	103%	114%	96%	105%	156%
Spleen	100%	104%	119%	106%	95%	187%

Microscopic Observations. Biologically significant microscopic observations were reported in the 250/75 mg/kg group. These observations were generally reported in rats from the DOS subgroup. The only exception was in females from the SNC subgroup, which were observed to have had increased extramedullary hematopoiesis in the spleen of 5/6 animals. In the DOS subgroup, microscopic observations were reported in various organs and body cavities (see **Table 34 and 35)** of ≥ 50% of the animals from either gender. In both genders, microscopic findings were observed in the abdominal cavity, large intestine, liver, lymph node, pancreas, small intestine, spleen, stomach, thymus gland, and urinary bladder. The severity of these and the other microscopic findings varied across male and female animals. In males, necrosis was minimal in the spleen and was either minimal or mild in the mesenteric lymph node. Moderate lymphoid necrosis was generalized in the thymus gland. Acute inflammation was observed in the cecum of the large intestine and it was deemed mild. Subacute inflammation was observed in the prostate gland and it was deemed minimal. Peritoneal inflammation ranged from minimal to mild across treated animals. For example, minimal peritoneal inflammation was observed in the kidneys; colon of the large intestine; mesenteric lymph node; ileum and jejunum of the small intestine; and the nongladular areas of the stomach. Mild peritoneal inflammation was observed in the abdominal cavity; epididymus; cecum of the large intestine; pancreas; duodenum of the small intestine; spleen; and urinary bladder. In the seminal vesicles and the glandular areas of the stomach, peritoneal inflammation ranged

from minimal to mild in severity. Bacterial colonies observed in the cecum of the large intestine and liver and were deemed moderate and mild, respectively. Generalized lymphoid depletion was deemed as mild or moderate in the spleen, and minimal in the thymus gland. Cardiomyopathy was reported and deemed minimal. Thrombus in the lung and cecum of the large intestine, respectively, was deemed and minimal and mild.

Table 34. Microscopic findings observed in male rats treated with impurity (b) (4) (250/75 mg/kg group) are shown.

	Impurity (b) (4)		Group	
Gender	**Microscopic Finding**	**Endpoint**	**250/75 mg/kg**	
Male			**DOS**	**SNC**
	Necrosis	Lymph node, mesenteric	2/2	2/8
		Spleen	2/2	0/8
	Generalized lymphoid necrosis	Thymus gland	2/2	0/8
	Degeneration/necrosis	Abdominal cavity	2/2	1/8
		Large interstine, cecum	1/2	0/8
		Pancreas	1/2	1/8
	Acute inflammation	Large intestine, cecum	1/2	0/8
	Subacute inflammation	Prostate gland	2/2	0/8
	Peritoneal inflammation	Abdominal cavity	2/2	1/8
		Epididymides	2/2	1/8
		Kidneys	1/2	0/8
		Large intestine, cecum	1/2	0/8
		Large interstine, colon	1/2	0/8
		Lymph node, mesenteric	2/2	0/8
		Pancreas	2/2	1/8
		Seminal vesicles	2/2	1/8
		Small intestine, duodenum	1/2	0/8
		Small intestine, ileum	1/2	1/8
		Small intestine, jejunum	1/2	0/8
		Spleen	1/2	0/8
		Stomach gladular	2/2	0/8
		Stomach nonglandular	2/2	0/8
		Testees	2/2	0/8
		Urinary bladder	1/2	0/8
	Bacterial Colonies	Large interstine, cecum	1/2	0/8
		Liver	1/2	1/8
	Generalized Lymphoid Depletion	Spleen	2/2	0/8
		Thymus gland	2/2	0/8
	Cardiomyopathy	Heart	1/2	2/8
	Thrombus	Large intestine, cecum	1/2	0/8
		Lung	1/2	0/8

In female rats from the DOS subgroup the location and severity of the microscopic findings listed in **Table 35** varied. For example, necrosis was observed in the spleen (mild), mandibular (minimal) and mesenteric (minimal or mild) lymph node. Lymphoid necrosis was generalized in the thymus gland and deemed mild. Minimal necrosis and degeneration were observed in the liver. Female animals exhibited peritoneal inflammation that varied in severity across a variety of organs and the abdominal cavity. Minimal peritoneal inflammation was observed in the colon of the large intestine, ovaries, and uterus. Minimal or mild peritoneal inflammation was observed in the rectum of the large intestine; mesenteric lymph node; duodenum, ileum and jejunum of the small intestine; spleen; glandular and nonglandular areas of the stomach; and the urinary bladder of treated females. Either minimal, mild or moderate peritoneal inflammation was observed in the abdominal cavity, cecum of the large intestine, liver, and pancreas of treated females. In treated females, the severity of bacterial colonies was deemed minimal in the mesenteric lymph node; and either mild or moderate in the abdominal cavity, small intestine, and spleen. The severity of the bacterial colonies ranged from minimum to mild in the cecum of the large intestine and minimal to moderate in the liver. In the liver and spleen there were reports of increased extramedullary hematopoiesis in female animals. The severity of this finding was minimal in the liver, whereas it was deemed as either mild or moderate in the spleen of treated females. Treated animals exhibited generalized lymphoid depletion that ranged from minimal to mild.

Table 35. Microscopic findings observed in female rats treated with impurity (b) (4) 250/75 mg/kg group) are shown.

	Impurity (b) (4)		Group	
Gender	**Microscopic Finding**	**Endpoint**	**250/75 mg/kg**	
Female	Necrosis	Lymph node, mandibular	3/4	1/6
		Lymph node, mesenteric	3/4	0/6
		Spleen	2/4	1/6
	Generalized lymphoid necrosis	Thymus gland	3/4	1/6
	Degeneration/necrosis	Liver	2/4	1/6
	Peritoneal inflammation	Abdominal cavity	2/4	1/6
		Large intestine, cecum	3/4	1/6
		Large interstine, colon	3/4	1/6
		Large intestine, rectum	4/4	0/6
		Liver	4/4	1/6
		Lymph node, mesenteric	3/4	1/6
		Ovaries	3/4	0/6
		Pancreas	4/4	2/6
		Small intestine, duodenum	3/4	1/6
		Small intestine, ileum	4/4	1/6
		Small intestine, jejunum	3/4	1/6
		Spleen	3/4	2/6
		Stomach gladular	4/4	2/6
		Stomach nonglandular	3/4	0/6
		Urinary bladder	4/4	1/6
		Uterus w/ cervix	4/4	1/6
	Bacterial Colonies	Abdominal cavity	2/4	0/6
		Large interstine, cecum	3/4	1/6
		Liver	3/4	1/6
		Lymph node, mesenteric	2/4	0/6
		Small intestine, ileum	3/4	0/6
		Spleen (bacterial colonies)	2/4	0/6
	↑ extramedullary hematopoiesis	Liver	3/4	2/6
		Spleen	4/4	5/6
	Generalized Lymphoid Depletion	Spleen	4/4	1/6

OVERALL CONCLUSIONS AND RECCOMENDATIONS

Conclusions: The Sponsor has conducted the required minimal genetic toxicology screen for Impurities (b) (4) The results indicated that none of these impurities demonstrate evidence of genotoxicity under the conditions of the assays. The Sponsor has also conducted 28-day repeat dose toxicity studies in the rat for each impurity. Based on the safety margins calculated below (see **Table 36)**, there is a ≥ 69-fold safety margins for the levels of each impurity tested. Doses for these impurities were determined from the proposed specifications for each impurity and the maximum daily dose for this drug product. The safety margins presented were established by determining human equivalent doses (HED) for an average 60 kg person and converting them into body surface area. The margins calculated should be adequate, given the maximum dose of diclofenac proposed (25 mg QID or 100 mg/day).

Table 36. Safety margins established using NOAEL doses in preclinical studies in rats and doses proposed for clinical studies.

	Maximum Diclofenac dose is 100 mg/day		(b) (4)		(b) (4)	
	Impurity (b) (4)		**Impurity** (b) (4)		**Impurity** (b) (4)	
	Human	Animal (NOAEL)	Human	Animal (NOAEL)	Human	Animal (NOAEL)
Amount	0 7 mg/day		1 mg/day		1 mg/day	
Dose	0 011667 mg/kg/day	5 mg/kg/day	0 01667 mg/kg/day	25 mg/kg/day	0 01667 mg/kg/day	25 mg/kg/day
HED	0 431667 mg/m^2	30 mg/m^2	0 61667 mg/m^2	150 mg/m^2	0 61667 mg/m^2	150 mg/m^2
Safety Margin = # 69 X			**Safety Margin = 243 X**		**Safety Margin = 243 X**	

Recommendations: From a nonclinical pharmacology toxicology perspective, the Sponsor has adequately addressed the outstanding approval issues related to drug product impurity qualification. As such, NDA 22-202 may be approved from the nonclinical perspective with the requested drug product impurity specifications.

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/s/

Marcus S Delatte
5/1/2009 04:15:19 PM
PHARMACOLOGIST

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I concur with Dr. Delatte's recommendation that NDA 22-202 may be approved from a nonclinical pharmacology toxicology perspective.

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:	**22-202**
SERIAL NUMBER:	**000**
DATE RECEIVED BY CENTER:	**09/21/2007**
PRODUCT:	**Zipsor™ (diclofenac potassium) Soft Gelatin Capsule**
INTENDED CLINICAL POPULATION:	**Proposed indication is for the relief of mild to moderate pain.**
SPONSOR:	**Xanodyne Pharms Inc.**
DOCUMENTS REVIEWED:	**Module 2 and 4 of eCTD**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**R. Daniel Mellon, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Tanya Clayton**

Date of review submission to Division File System (DFS): June 23, 2008

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical pharmacology toxicology perspective, NDA 22-202 is approvable, if the Sponsor provides adequate safety qualification for the drug product impurities that exceed ICHQ3B(R) qualification thresholds and contain a structural alert for mutagenicity.

B. Recommendation for nonclinical studies

Submit the final study reports for the ongoing safety qualification studies for the following drug product impurities that exceed ICHQ3B(R) qualification thresholds:

1. Impurity (b) (4)
2. Impurity (b) (4)
3. Impurity (b) (4)

As previously communicated to the Division, your ongoing adequate qualification studies include:

- Minimal genetic toxicology screen (two *in vitro* genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
- Repeat dose toxicology of appropriate duration to support the proposed indication. For an acute pain indication, a 28-day repeat dose toxicology study would be acceptable.

Since these impurities contain a structural alert for mutagenicity, if the results in either of the two genetic toxicology assays for an individual impurity are positive, you must reduce the impurity to not more than (b) (4) mcg/day unless otherwise justified. Justification may require an assessment for carcinogenic potential in either a standard 2-year rodent bioassay or in an appropriate transgenic mouse model.

C. Recommendations on labeling

Sponsor's Proposed Labeling	Recommended Labeling	Rationale/Comment
8 USE IN SPECIFIC	**8 USE IN SPECIFIC**	

3 pp. withheld following this page as draft labeling (b)(4) CCI/TS

No new nonclinical studies for diclofenac were submitted with this NDA. The application relies upon the previous findings of safety for the referenced drug application, Cataflam.

The Sponsor also submitted copies of several published reports on the reproductive and developmental effects of diclofenac and proposed to include language in the label. These studies appear to contribute to the understanding of the potential developmental effects of diclofenac and should be included in the label.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

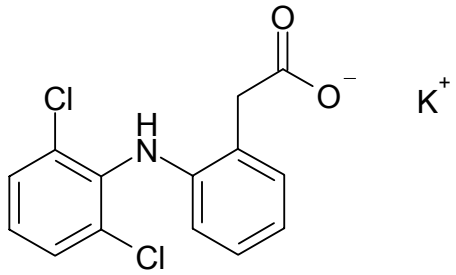
NDA number: 22-202
Review number: 1
Sequence number/date/type of submission: 21-Sept-2007 / Original NDA
Information to Sponsor: Yes (X) No ( )
Sponsor and/or agent: Xanodyne Pharmaceuticals, Inc.
Newport, KY

Manufacturer for drug substance: (b) (4)

Reviewer name: R. Daniel Mellon, Ph.D.
Division name: Division of Anesthesia, Analgesia and Rheumatology Products (DAARP)
HFD #: 170
Review completion date: June 21, 2008

Drug:

Trade name: Zipsor™
Generic name: Diclofenac potassium soft gelatin capsules
Code name: DPSGC
Chemical name: 2-((2,6-dichlorophenyl)amino)-benzeneacetic acid, monopotassium salt
CAS registry number: 15307-81-0
Molecular formula/molecular weight: $C_{14}H_{10}Cl_2KNO_2$ / 334.24 g/mol
Structure:



Relevant INDs/NDAs/DMFs:

NDA#	Drug Name	Div	Strength (route)	Marketing Status	AP Date	Indication	Company
20-142	Cataflam	DAARP	50 mg	AP	24-Nov-1993	• For treatment of primary dysmenorrhea • For relief of mild to moderate pain • For relief of the signs and	Novartis

						symptoms of osteoarthritis • For relief of the signs and symptoms of rheumatoid arthritis	

IND#	Product	Status	Division	Indication	Status	Sponsor
(b) (4)						
63,308	Diclofenac potassium soft gelatin capsules	Active	DAARP	Treatment of acute pain in adults	17-Sept-2001	Xanodyne Pharmacal, Inc.

DMF#	Subject of DMF	Holder	Submit Date
(b) (4)	(b) (4)	(b) (4)	(b) (4)

Drug class: NSAID

Intended clinical population: The drug product is proposed for the treatment of mild to moderate pain.

Clinical formulation: DPSGC is a liquid formulation of diclofenac potassium encapsulated in soft gelatin capsules. The formulation of the drug product is presented in the Sponsor's table below. There are no pharmacology toxicology concerns regarding the excipients in the drug product formulation.

TABLE 3.2.P.1.2.1:
DRUG PRODUCT QUANTITATIVE COMPOSITION

COMPONENT	REFERENCE TO QUALITY STANDARD	FUNCTION	mg per Capsule	% w/w
FILL SOLUTION				
DICLOFENAC POTASSIUM	In-house	Active	25.0[1]	(b) (4)
Polyethylene Glycol 400	NF			
Glycerin (b) (4)	USP			
Sorbitol Solution (b) (4)	USP			
Povidone (b) (4)	USP			
Polysorbate 80	NF			
Hydrochloric Acid. (b)	In-house			
(b) (4)	NF	(b) (4)	N/A	N/A
(b) (4)				
Gelatin (b) (4) (b) (4)	NF.	(b) (4)		See Footnote[2]
Sorbitol (b) (4)	In-house			
Glycerin (b)	USP			
(b) (4)	USP			
(b) (4)				
Isopropyl Alcohol	USP			
Mineral Oil	USP			
(b) (4)	In-house			
		Total		**400.00**

[1] The actual amount of diclofenac potassium used is corrected for its purity.

(b) (4)

Route of administration: Oral

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

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of 22-202 are owned by Xanodyne or are data for which Xanodyne has obtained a written right of reference. Any information or data necessary for approval of 22-202 that Xanodyne does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Xanodyne does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of 22-202.

This is a 505(b)(2) NDA and the Sponsor is referencing the Agency's previous findings of safety and efficacy of Novartis's Cataflam (NDA 20-142) for the nonclinical data.

Studies reviewed within this submission:

Study Title	Document #	Submission (Date)
Other Toxicity		
(b) (4) A Single Ascending Oral Administration Toxicity Study in Rats	Study AAIRES000334	21-Sept-2007

Studies not reviewed within this submission:

All studies submitted were reviewed for this NDA application.

2.6.2 PHARMACOLOGY

No new pharmacology studies were completed or required to support of this NDA.

2.6.2.1 Brief summary

The FDA approved labeling for Cataflam (2006) provides the following information:

> Cataflam® (diclofenac potassium immediate-release tablets) is a nonsteroidal anti-inflammatory drug (NSAID) that exhibits anti-inflammatory, analgesic, and antipyretic activities in animal models. The mechanism of action of Cataflam, like that of other NSAIDs, is not completely understood but may be related to prostaglandin synthetase inhibition.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: As stated in the proposed drug product labeling:

> The mechanism of action of Zipsor, like that of other NSAIDs, is not completely understood but may involve inhibition of the cyclooxygenase (COX-1 and COX-2) pathways. Diclofenac's mechanism may also be related to prostaglandin synthetase inhibition. The analgesic mechanism of action needs further elucidation.

Drug activity related to proposed indication: Diclofenac has anti-inflammatory, anti-nociception, and anti-pyretic effects.

2.6.2.3 Secondary pharmacodynamics

No new studies were completed or required to support of this NDA.

2.6.2.4 Safety pharmacology

No new safety pharmacology studies were completed or required to support of this NDA. The reader is referred to the FDA approved drug labeling or warnings and precautions related to the cardiovascular, gastrointestinal, renal, and hypersensitivity risks related to diclofenac.

2.6.2.5 Pharmacodynamic drug interactions

No new nonclinical drug interaction studies were completed or required to support of this NDA. The reader is referred to the FDA approved drug labeling for further information.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the Sponsor]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

NA

2.6.4.1 Brief summary

NA

2.6.4.2 Methods of Analysis

[see under individual study reviews]

2.6.4.3 Absorption

No new absorption studies were completed or required to support of this NDA. As the proposed formulation has a different pharmacokinetic profile from the referenced drug product, the labeling will be updated with the new human data. The reader is referred to the clinical pharmacology and biopharmaceutics review for the absorption data specific to this drug product.

2.6.4.4 Distribution

No new distribution studies were completed or required to support of this NDA. The FDA approved labeling for Cataflam (2006) provides the following information:

> The apparent volume of distribution (V/F) of diclofenac potassium is 1.3 L/kg.
>
> Diclofenac is more than 99% bound to human serum proteins, primarily to albumin. Serum protein binding is constant over the concentration range (0.15-105 μg/mL) achieved with recommended doses.
>
> Diclofenac diffuses into and out of the synovial fluid. Diffusion into the joint occurs when plasma levels are higher than those in the synovial fluid, after which the process reverses and synovial fluid levels are higher than plasma levels. It is not known whether diffusion into the joint plays a role in the effectiveness of diclofenac.

2.6.4.5 Metabolism

No new metabolism studies were completed or required to support of this NDA. The FDA approved labeling for Cataflam (2006) provides the following information:

> Five diclofenac metabolites have been identified in human plasma and urine. The metabolites include 4'-hydroxy-, 5-hydroxy-, 3'-hydroxy-, 4',5-dihydroxy- and 3'-hydroxy-4'-methoxy diclofenac. In patients with renal dysfunction, peak concentrations of metabolites 4'-hydroxy-and 5-hydroxy-diclofenac were approximately 50% and 4% of the parent compound after single oral dosing compared to 27% and 1% in normal healthy subjects. However, diclofenac metabolites undergo further glucuronidation and sulfation followed by biliary excretion.
>
> One diclofenac metabolite 4'-hydroxy-diclofenac has very weak pharmacologic activity.

2.6.4.6 Excretion

No new excretion studies were completed or required to support of this NDA. The FDA approved labeling for Cataflam (2006) provides the following information:

> Diclofenac is eliminated through metabolism and subsequent urinary and biliary excretion of the glucuronide and the sulfate conjugates of the metabolites. Little or no free unchanged diclofenac is excreted in the urine. Approximately 65% of the dose is excreted in the urine and approximately 35% in the bile as conjugates of unchanged

diclofenac plus metabolites. Because renal elimination is not a significant pathway of elimination for unchanged diclofenac, dosing adjustment in patients with mild to moderate renal dysfunction is not necessary. The terminal half-life of unchanged diclofenac is approximately (b) (4).

2.6.4.7 Pharmacokinetic drug interactions

No new nonclinical drug interaction studies were completed or required to support of this NDA. The reader is referred to the FDA approved drug labeling for further information.

2.6.4.8 Other Pharmacokinetic Studies

NA

2.6.4.9 Discussion and Conclusions

NA

2.6.4.10 Tables and figures to include comparative TK summary

NA

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the Sponsor]

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: No new toxicology studies on diclofenac were submitted. The Sponsor submitted an ascending dose escalation study for impurity (b) (4) however this study is not adequate to qualify the safety of the proposed impurity specification.

Genetic toxicology: Diclofenac was negative in the standard battery of genetic toxicology studies. There are three drug product impurities that have not yet been adequately characterized for genotoxic potential. Like diclofenac, these compounds also contain structural alerts for genotoxicity and should be adequately characterized for safety.

Carcinogenicity: Carcinogenicity assessment for diclofenac sodium is provided by reference to Cataflam. Diclofenac was negative in both rat and mouse bioassays.

Reproductive toxicology: Reproductive and developmental toxicity is provided by reference to Cataflam as well as a review of the literature from 1996 to 2007. Several new publications were submitted and reviewed. The studies provide additional information regarding the potential developmental effects of diclofenac and should be included in the labeling.

2.6.6.2 Single-dose toxicity

No new single-dose toxicity studies with diclofenac were completed or required to support of this NDA.

2.6.6.3 Repeat-dose toxicity

No new repeat-dose toxicity studies with diclofenac were completed or required to support of this NDA. The following study was conducted to the potential toxicity of drug substance Impurity (b) (4)

Study title: (b) (4) **A Single Ascending Oral Administration Toxicity Study in Rats**

Key study findings: The acute toxicity of (b) (4) was evaluated in rats via an ascending dose escalation design (6.7, 50, 109, and 175 mg/kg via oral gavage).

- Treatment related mortality occurred following dosing with 109 mg/kg.
- There was clear reduced body weight gain in animals treated with 50 mg/kg and higher; however, as there was no control group, the lowest dose may also have produced changes in body weight.
- Clinical signs of soft stool were noted at 50 mg/kg, which progressed to dark red tarry stools with evidence of GI perforations in some animals following treatment with 109 and 175 mg/kg.
- The study can not be used to determine a clear NOAEL for the drug treatments as it is not clear if the findings were due to the last dose administered or the cumulative doses received. However, a crude NOAEL of 6.7 mg/kg and a LOAEL of 50 mg/kg could be estimated.
- The study can not be used to justify the safety of impurity levels in the drug product since it is not a controlled study and is not of adequate duration to support the proposed indication.

Study no.: Sponsor study number AAIRES000334
Volume #, and page #: Module 4
Conducting laboratory and location: (b) (4)

Date of study initiation: 12-Mar-2003
GLP compliance: Yes
QA report: yes (X) no ( )
Drug, lot #, and % purity: (b) (4)
QCL-AAI-DCI1031003, 89.8% purity.

Methods

Doses: 6.7, 50, 109, and 175 mg/kg (actual doses based on purity of compound).

Species/strain: Sprague Dawley (Crl:CD®(SD)IGSBR) rat

Number/sex/group or time point (main study):

Route, formulation, volume, and infusion rate: Oral gavage,

Satellite groups used for toxicokinetics or recovery: None.

Age: 63-76 days of age at study initiation.

Weight: males 330-365 g; females 255-277 g

Sampling times:

Unique study design or methodology (if any): This is a single ascending single dose study. A total of 11 rats (5 males and 6 females) were tested. Each dose was separated by 8 days. Rats were housed individually.

Observations and times: (these parameters can be captured separately here or described in connection with each endpoint under the results section.

Mortality: Animals were observed at least twice a day for mortality and morbidity.
Clinical signs: Animals were observed predose and at 0.5, 1, 4, 8, 12 and 24 hours after dose administration and at least twice daily during the interval between dosing.
Body weights: Body weights were measured pretest, before each new treatment and twice during the interval between treatments.
Food consumption: Not recorded.
Ophthalmoscopy: Not recorded.
EKG: Not recorded.
Hematology: Not completed.
Clinical chemistry: Not completed.
Urinalysis: Not completed.
Gross pathology: Animals were euthanized by carbon dioxide asphyxiation. Tissues in the list below plus any macroscopic lesions were collected in saved in 10% neutral buffered formalin.
Organ weights (specify organs weighed if not in histopath table): Organ weights were not obtained.
Histopathology: Adequate Battery: yes ( ), no (X)— the study did not include actual analysis of the histopathology, rather the tissues were saved but not evaluated.
Peer review: yes ( ), no (X)

Results

Mortality: One female was euthanized following treatment with the 6.7 mg/kg dose after being found in the moribund condition; this animal had a perforated esophagus which

was attributed to the dosing procedure and is not treatment related. There were no deaths in the 50 mg/kg dose group. One female died following treatment with 109 mg/kg. One male and one female were found dead following treatment with the 175 mg/kg dose. Two additional females were euthanized following treatment with 175 mg/kg dose due to moribund condition. The Sponsor attributes the deaths in the 109 and 175 mg/kg treatment groups to be treatment-related. The overall incidence of deaths were higher in the females compared to males.

Clinical signs: Clinical signs following treatment with 50, 109 and 175 mg/kg doses included soft feces which progressed to liquid feces to dark black/red and tarry stools after the higher doses. Consistent with these findings, the animals demonstrated an unkempt appearance, dehydration, and weight loss.

Body weights: Average weight gain over a 7 day period following dosing of 6.7, 50, and 109 mg/kg was 21, 9 and 8 grams, respectively. There was an average of 40 gram weight loss over the three day period following the dose of 175 mg/kg.

Gross pathology: Macroscopic observations included findings in the GI tract (stomach, duodenum, jejunum, cecum, colon, and rectum, which included dark red/brown/black gelatinous fecal matter, a thick black tarry substance, and multiple red foci in the mucosa following the 109 and 175 mg/kg doses. Perforation of the GI tract occurred in several animals, which appears to have resulted in multiple adhesions of various abdominal organs following the 109 and 175 mg/kg doses.

Histopathology: Adequate Battery: yes ( ), no (X)—the study did not include actual analysis of the histopathology, rather the tissues were saved but not evaluated.
Peer review: yes ( ), no (X)

Histopathology inventory (optional)

Study	
Species	Rat
Adrenals	x
Aorta	x
Bone Marrow smear	x
Bone (femur)	x
Brain	x
Cecum	x
Cervix	
Colon	x
Duodenum	x
Epididymis	x
Esophagus	x
Eye	x
Fallopian tube	
Gall bladder	
Gross lesions	
Harderian gland	

Heart	x
Ileum	x
Injection site	
Jejunum	x
Kidneys	x
Lachrymal gland	x
Larynx	
Liver	x
Lungs	x
Lymph nodes, cervical	
Lymph nodes mandibular	x
Lymph nodes, mesenteric	x
Mammary Gland	x
Nasal cavity	
Optic nerves	
Ovaries	x
Pancreas	x
Parathyroid	x
Peripheral nerve	
Pharynx	
Pituitary	x
Prostate	x
Rectum	x
Salivary gland	x
Sciatic nerve	x
Seminal vesicles	
Skeletal muscle	x
Skin	x
Spinal cord	x
Spleen	x
Sternum	x
Stomach	x
Testes	x
Thymus	x
Thyroid	x
Tongue	x
Trachea	x
Urinary bladder	x
Uterus	x
Vagina	
Zymbal gland	

X, histopathology performed
*, organ weight obtained

2.6.6.4 Genetic toxicology

No new genetic toxicology studies were submitted in support of this NDA. The Sponsor is relying upon the Agency's previous findings for Cataflam. The FDA approved

labeling for Cataflam (2000) contained the following information, which should be included in the proposed labeling for Zipsor:

> Diclofenac sodium did not show mutagenic activity in in vitro point mutation assays in mammalian (mouse lymphoma) and microbial (yeast, Ames) test systems and was nonmutagenic in several mammalian in vitro and in vivo tests, including dominant lethal and male germinal epithelial chromosomal aberration studies in Chinese hamsters.

2.6.6.5 Carcinogenicity

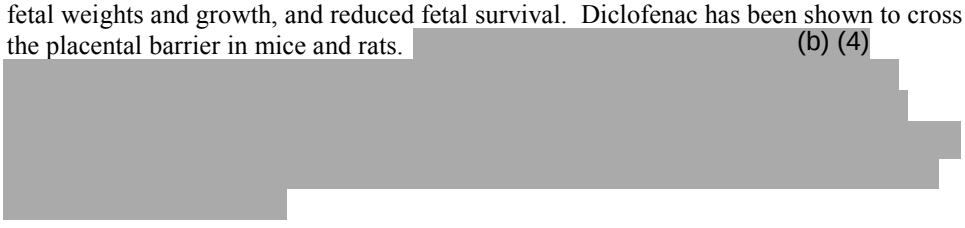
No new carcinogenicity studies were submitted in support of this NDA. The Sponsor is relying upon the Agency's previous findings for Cataflam. The FDA approved labeling for Cataflam (2000) contained the following information, which should be included in the proposed labeling for Zipsor, updated to include appropriate exposure ratios:

> Long-term carcinogenicity studies in rats given diclofenac sodium up to 2 mg/kg/day (or 12 mg/m^2/day, approximately the human dose) have revealed no significant increase in tumor incidence. There was a slight increase in benign mammary fibroadenomas in mid-dose-treated (0.5 mg/kg/day or 3 mg/m^2/day) female rats (high-dose females had excessive mortality), but the increase was not significant for this common rat tumor. A 2-year carcinogenicity study conducted in mice employing diclofenac sodium at doses up to 0.3 mg/kg/day (0.9 mg/m^2/day) in males and 1 mg/kg/day (3 mg/m^2/day) in females did not reveal any oncogenic potential.

2.6.6.6 Reproductive and developmental toxicology

No new reproductive and developmental toxicology studies were submitted in support of this NDA. The Sponsor is relying upon the Agency's previous findings for Cataflam. The FDA approved labeling for Cataflam (2000) contained the following information, which should be included in the proposed labeling for Zipsor:

> Diclofenac sodium administered to male and female rats at 4 mg/kg/day (24 mg/m^2/day) did not affect fertility.
>
> **Pregnancy, Teratogenic Effects, Pregnancy Category B**
> Reproductive studies have been performed in mice given diclofenac sodium (up to 20 mg/kg/day or 60 mg/m^2/day) and in rats and rabbits given diclofenac sodium (up to 10 mg/kg/day or 60 mg/m^2/day for rats, and 80 mg/m^2/day for rabbits), and have revealed no evidence of teratogenicity despite the induction of maternal toxicity and fetal toxicity. In rats, maternally toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival. Diclofenac has been shown to cross the placental barrier in mice and rats. (b) (4)



Sui et al. reported results from thirty patients in Hong Kong undergoing legal surgical termination of pregnancy between 8 and 12 weeks gestation that were given two doses of

diclofenac (50 mg diclofenac in the form of Cataflam) before the procedure (Siu et al., 2000). According to this publication, diclofenac was detectable in all fetal tissue samples at a concentration similar to that found in maternal venous samples. Maternal serum concentrations ranged from 24.6 to 842.4 ng/mL (mean 183.9 ng/mL) and fetal tissue drug concentration ranged from 17.4 to 665.7 ng/g (mean 279 ng/g). The mean maternal/fetal diclofenac ratio (maternal serum concentration divided by fetal tissue drug concentration) was 0.95. This study demonstrates that diclofenac crosses the human placenta and can reach fetal tissue.

The Sponsor conducted a review of the literature since the 1996 approval of Cataflam to determine if there were new reproductive and developmental toxicity data for diclofenac. Several studies were described and submitted from the published literature.

Chan and colleagues examined the effect of diclofenac on the developing rat embryo using a whole rat embryo culture model (Chan et al., 2001). Embryos were exposed to diclofenac concentrations of 0, 1.5, 2.5, 5, 7.5, and 15 mcg/mL during the critical period of organogenesis. Specifically embryos were explanted at gestational day 9.5 and cultured in culture medium containing Sprague-Dawley rat serum and Dulbecco's modified eagle medium (DMEM), penicillin G and streptomycin sulfate at final concentrations of 60 and 100 mcg/mL, and diclofenac sodium solution at the desired final concentration. Embryos were maintained in culture for 48 hours then examined for morphology. Overall embryonic growth as measured by yolk sac diameter, crown-rump length and number of somites were not affected by diclofenac concentrations up to 15 mcg/mL, which is about 14-times the plasma concentration of diclofenac after a single oral dose of Zipsor (Cmax 1.087 ± 0.419 mcg/mL; data from proposed package insert). Rat embryos exposed to concentrations of diclofenac ≥ 7.5 mcg/mL had lower morphological scores for development of caudal neural tube, flexion, and hindlimb bud. Concentrations of 5 mcg/mL and below did not produce evidence of teratogenicity. Follow-up studies by this same group provided evidence that the embryonic toxicity may be due to free oxygen radical damage (Chan et al., 2002).

Gökçimen et al. administered either physiological serum or diclofenac sodium (1 mg/kg, IP) daily to pregnant rats from gestation day 5 to 20. Offspring were allowed to be born and tissue from the liver, kidney, and testes were obtained 4 weeks after birth. The authors report that diclofenac treatment significantly prolonged the gestation period compared to control treatment. The liver of offspring born from diclofenac treated dams showed enlargement of the periportal area, sinusoidal dilation, bile duct proliferation, pyknosis in the nucleus, and vacuolar degeneration in parenchymal cells of the liver. There were no significant effects noted in the kidneys or testes (Gokcimen et al., 2001).

The potential effects of diclofenac on the developing nervous system have been reported in the literature. Kudo et al. report that treatment of mouse neural stem cells in vitro with diclofenac (≥10 μM) for 2 days induced the death of the NSC in a concentration dependent manner. Similar concentrations of naproxen, indomethacin, aspirin or ibuprofen did not result in cell death. Diclofenac concentrations of 10 μM also inhibited proliferation and differentiation into neurons (Kudo et al., 2003).

Gökçimen et al. administered either physiological serum or diclofenac sodium (1 mg/kg, IP) daily to pregnant rats from gestation day 5 to 20. Offspring were allowed to be born and brain tissue was obtained at either 4 or 20 weeks after birth. There was a significant cell loss in total neuron number of hippocampal pyramidal cells in 20 week old animals but not in 4 week old animals. In contrast, the authors report a significant decrease in dentate gyrus granule cells in 4-week old pups born from animals treated with diclofenac sodium compared to control. At 20-weeks, there was an increased number of dentate gyrus granule cells compared to controls (Gokcimen et al., 2007). Using the same drug treatment protocol, this group also report that diclofenac sodium treatment during gestation also resulted in a significant reduction in the total number of cerebellar Purkinje cells in offspring compared to control (Ragbetli et al., 2007).

Collectively, the Sponsor has proposed to include the following information in the drug product label based on the above literature references:

> Literature studies have shown that diclofenac has been shown to exert direct teratogenic effects on rat embryos at doses of 7.5 and 15 µg/mL, and diclofenac exposure to pregnant rats can lead to prolonged gestation as well as liver toxicity and neuronal loss in offspring.

Following review of the literature references provided, inclusion of the information is acceptable. The information from the rat embryo studies should be clearly noted as being from in vitro exposure. The dose of 1 mg/kg via IP in the rat, although via a different route of administration, is still below the daily human dose of 100 mg/kg when compared on a body surface area basis.

2.6.6.7 Local tolerance

No new studies were conducted in support of this NDA submission.

2.6.6.8 Special toxicology studies

No new studies were conducted in support of this NDA submission.

2.6.6.9 Discussion and Conclusions

The Sponsor has referenced the Agency's previous findings of safety for Cataflam® (diclofenac potassium) in support of this NDA. From the nonclinical pharmacology toxicology perspective, this is acceptable as the exposure to diclofenac potassium in Zipsor™ is comparable to that from the referenced drug. However, the Sponsor's drug product contains several impurities that exceed the ICH Q3B(R) qualification threshold of NMT 0.5% for drug products with a maximum daily dose of 10 mg to 100 mg.

Specifically, the Sponsor has proposed the following drug product (DP) specifications:

Impurity	Potential Process Impurity	Potential Degradant	Proposed DP Specification
(b) (4)	Yes	Yes	NMT (b) (4)
	No	Yes	NMT
	No	Yes	NMT
	No	Yes	NMT

The structures of the above impurities are presented below:

(b) (4)

As noted in the table above, impurities (b) (4) all exceed the ICH qualification thresholds and also contain a structural alert for mutagenicity (b) (4)). The Sponsor is currently completing a minimal genetic toxicology screen for impurities (b) (4) as well as a 28-day repeat dose toxicology study with a drug

product formulation containing elevated levels of these three compounds. These studies will be required to support the proposed drug product specifications.

2.6.6.10 Tables and Figures

None.

2.6.7 TOXICOLOGY TABULATED SUMMARY

None.

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: The Sponsor has referenced the Agency's previous findings of safety of Cataflam to support the nonclinical pharmacology toxicology portions of this NDA. The submitted and referenced information is adequate to allow approval of the NDA application from the nonclinical perspective with the exception of the lack of information on the impurities in the drug product that exceed ICH Q3B qualification thresholds. The Division communicated this deficiency to the Sponsor in the 74-day letter and the Sponsor has already initiated studies to characterize the potential genetic toxicity and general toxicity of the impurities to support the proposed specifications. According to the Sponsor's communication dated February 28, 2008, the genetic toxicology studies are expected to be completed according to the following schedule:

Impurity		Start	Complete
(b) (4)	Point mutation assay	January 11, 2008	March 8, 2008
	Chromosome aberration assay	January 29, 2008	April 11, 2008
	Point mutation assay	March 6, 2008	May 9, 2008
	Chromosome aberration assay	March 20,2008	May 29, 2008
	Point mutation assay	March 18, 2008	May 12, 2008
	Chromosome aberration assay	April 1, 2008	June 12, 2008

Likewise, the Sponsor indicated that they have begun efforts to conduct a 28-day repeat dose toxicology study in rats to support qualification of impurities (b) (4) however, the study report will not be available until late September 2008.

Unresolved toxicology issues (if any): Submit the final study reports for the ongoing safety qualification studies for the following drug product impurities that exceed ICHQ3B(R) qualification thresholds:

4. Impurity (b) (4)
5. Impurity (b) (4)
6. Impurity (b) (4)

As previously communicated to the Division, your ongoing adequate qualification studies include:

- Minimal genetic toxicology screen (two *in vitro* genetic toxicology studies, e.g., one point mutation assay and one chromosome aberration assay) with the isolated impurity, tested up to the limit dose for the assay.
- Repeat dose toxicology of appropriate duration to support the proposed indication. For an acute pain indication, a 28-day repeat dose toxicology study would be acceptable.

Since these impurities contain a structural alert for mutagenicity, if the results in either of the two genetic toxicology assays for an individual impurity are positive, you must reduce the impurity to not more than (b) (4) mcg/day unless otherwise justified. Justification may require an assessment for carcinogenic potential in either a standard 2-year rodent bioassay or in an appropriate transgenic mouse model.

Recommendations: From the nonclinical pharmacology toxicology perspective, NDA 22-202 is approvable, if the Sponsor provides adequate safety qualification for the drug product impurities that exceed ICHQ3B(R) qualification thresholds and contain a structural alert for mutagenicity.

Suggested labeling: See executive summary.

APPENDIX/ATTACHMENTS

Reference List

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