CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

20-947

CLINICAL PHARMACOLOGY AND BIOPHARMACEUTICS REVIEW(S)

CLINICAL PHARMACOLOGY REVIEW

NDA: 20-947	Submission Date: 2/4/09
Submission Type; Code:	**Resubmission** - Complete Response to December 28, 2006 Approvable Letter
Brand/Code Name:	PENNSAID® Topical Solution
Generic Name:	1.5 % w/w diclofenac sodium solution
Primary Reviewer:	David Lee, Ph.D.
Secondary Reviewer	Suresh Doddapaneni, Ph.D.
OCP Division:	2
OND Division:	Anesthesia, Analgesia, and Rheumatology Products
Sponsor:	Nuvo Research Inc. (formally Dimethaid Research Inc.)
Relevant IND(s):	42,773
Formulation; Strength(s):	1.5 % w/w
Proposed Indication:	For the treatment of the symptoms of osteoarthritis of the knee(s)
Proposed Dosage Regimen:	40 drops per knee, four times a day.

Table of Contents

1 Executive Summary

1.1 Recommendations

The Office of Clinical Pharmacology (OCP) has reviewed the Complete Response submitted on 2/4/09.

From OCP point of view, the information contained in the Complete Response is acceptable provided that a mutually acceptable agreement is reached between the Applicant and the Agency regarding the Labeling language (See 3. Detailed Labeling Recommendations section).

1.2 Phase IV Commitments

Not applicable.

1.3 Summary of CPB Findings

Nuvo Research Inc., formally known as Dimethaid Research, Inc., has submitted Complete Response to NDA 20-947 *Approvable Letter* dated 12/28/06.

Background

The following table contains a brief history of NDA 20-947 prior to this submission. The initial NDA was submitted in 12/15/97.

NDA	Action	Clin.Pharm. Information	Clin.Pharm.Reviewer
Original Submission 12/15/97	Withdrawn on 10/16/98	• One Single dose PK study (Study 106-95) • 'nested' BA study (Study 102-93-1)	Dr. D. Wang
Resubmission – Complete Response 8/1/01	Not Approval Letter 8/7/02	• One single dose PK study (resubmission of 106-95) • One single dose PK study (Study RA-CP-109-US)	Dr. D. Bashaw
Resubmission – Complete Response 6/28/06	Approvable Letter 12/28/06	• One single dose PK study (Study 2663) One multiple dose PK study (Study 2656)	Dr. D. Lee

Previously, on 6/28/06 the Applicant had submitted Complete Response to *Not Approval Letter* dated 8/7/02, and from Clinical Pharmacology perspective, the information submitted was acceptable and no further communication to the Applicant was necessary.

In this Resubmission, the Applicant conducted a relative bioavailability study (PEN-07-116), which compared Pennsaid topical solution to Solaraze Gel (NDA 21-005). The Applicant stated that they have conducted this study to address Issues 6 and 7 of Approvable Letter (Biobridge to Solaraze® Gel in order to rely on the Agency's previous finding of safety for a 2 year dermal carcinogenicity study and a 1 year photococarcinogenicity study conducted on the approved topical diclofenac product; and, data demonstrating that the same PENNSAID® photo degradants are found in the approved topical diclofenac product (Solaraze®) at a comparable or higher concentration and an assessment of the toxicological potential of the photodegradants, respectively). Additionally, the Applicant submitted DMSO/$DMSO_2$ exposure information from a clinical study, which determined the naturally occurring plasma levels of DMSO/$DMSO_2$ in healthy volunteers who are on a regular diet. This information was submitted since Pennsaid contains—— DMSO and the Applicant wanted to show that there is chronic exposure to systemic levels of DMSO/$DMSO_2$ in healthy volunteers. b(4)

Protocol PEN-07-116 was a multiple dose, an open-label, 8-week relative bioavailability study to compare the diclofenac exposure under the maximum usage. Subjects with actinic keratosis (AK) were administered with PENNSAID and Solaraze® Gel under maximum use conditions per labeling of diclofenac sodium following multiple applications of PENNSAID® Topical Solution and Solaraze® Gel (diclofenac sodium 3%). Each subject received two treatments with 3-week washout between treatments. PENNSAID® (1.5%) (40 drops (1.2 mL) per knee) was applied QID to both knees for 7 days. Solaraze® Gel (3%), 0.02 g per cm^2 (0.5 g BID for every 25 cm^2 of diseased skin), was applied BID to the confluent area of skin containing the AK lesions for 4 weeks.

The results from this study revealed that the diclofenac relative bioavailability from PENNSAID® was approximately 1/3 of Solaraze® treatment under maximum use conditions per labeling.

Parameter	PENNSAID®	Solaraze®	Ratio PENNSAID/Solaraze % (90% CI)
Uncorrected			
$C_{max(ss)}$	26.9	91.8	29.4 (21.7-39.7)
$AUC_{0-24(ss)}$	474.2	1346.2	35.2 (27.6-45.0)
Corrected for Surface Area			
$C_{max(ss)}$	0.025	0.0739	34.2 (25.0-46.9)
$AUC_{0-24(ss)}$	0.39	1.24	31.5 (24.4-40.7)

Study NRI-1000-08500 was conducted to determine the naturally occurring plasma levels of DMSO and DMSO2 from healthy subjects who are on a regular diet and not exposed to topical DMSO products. The levels of DMSO in 50 subjects were analyzed. One blood sample was taken during the study visit. The study results revealed that DMSO levels were below the limit of quantitation (55 ng/mL). The concentrations of $DMSO_2$

were quantifiable in 13 out of 50 subjects (26%). The values ranged from —— to ——— ng/mL. The geometric mean of the 13 subjects was 688.8 ng/mL and the arithmetic mean (± standard deviation) was 905.3 ± 5521.8 ng/mL. The results of this study indicated that there is chronic exposure to systemic levels of DMSO and $DMSO_2$ in healthy male and female volunteers who are on a regular diet. b(4)

Overall, the information contained in this Complete Response for diclofenac, DMSO and $DMSO_2$, are acceptable. There are no other pending Clinical Pharmacology related issues for this Complete Response submission.

2 QBR

2.1 General Attributes of the Drug – Not applicable

2.2 General Clinical Pharmacology

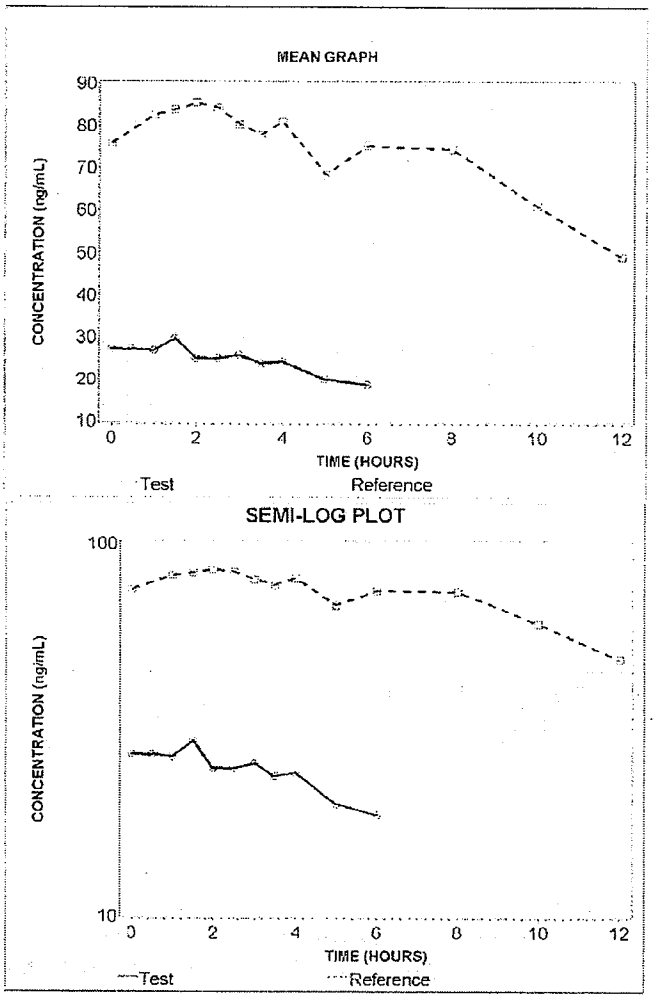
2.2.1 What is the relative bioavailability of Pennsaid compared to Solaraze Gel?

Protocol PEN-07-116 was a multiple dose, an open-label, 8-week study. The primary objective of this study was to compare the exposure under maximum use conditions per labeling of diclofenac sodium following multiple applications of PENNSAID® Topical Solution (diclofenac sodium topical solution) 1.5% w/w and Solaraze® Gel (diclofenac sodium 3%). Thirty subjects were involved in this study with a clinical diagnosis of actinic keratosis (AK) involving at least 500 cm^2 of skin on the face, head, scalp, neck, shoulders, arms and/or upper trunk, not necessarily continuous, of which at least 100 cm^2 of the total affected area was on the face, head or scalp. Subjects were required to have a total of at least 20 non-hypertrophic AK lesions on all areas combined and a total of at least 6 nonhypertrophic AK lesions on the face, head or scalp. Discontinued or withdrawn subjects who prematurely withdrew from the study were not replaced.

Each subject received two treatments with 3-week washout between treatments. PENNSAID® (1.5%) (40 drops (1.2 mL) per knee) was applied QID to both knees for 7 days. Solaraze® Gel (3%), 0.02 g per cm^2 (0.5 g BID for every 25 cm^2 of diseased skin), was applied BID to the confluent area of skin containing the AK lesions for 4 weeks.

For PENNSAID®, blood samples (6 mL each) were collected to measure diclofenac level on Days 1, 6 and 7 at 0.0 hour (pre-dose), and on Day 8 at 0.0 hour (pre-dose) and at 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0 hours. For Solaraze Gel, blood samples were collected on Days 1, 26 and 27 at 0.0 hour (pre-dose), and on Day 28 at 0.0 hour (predose) and at 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 hours following dosing.

The following mean diclofenac plasma profile was observed. (Reference: Solaraze; Test: PENNSAID)



The following parameters were derived based on the uncorrected surface area:

PK Parameter	**PENNSAID®** (N=30)	**Solaraze®** (N=27)
Cmax(ss) (ng/mL)	35.0 (28.6)	125.9 (119.3)
Cmin(ss) (ng/mL)	16.7 (10.4)	30.4 (19.4)
Cavg(ss) (ng/mL)	24.2 (16.3)	72.3 (63.5)
AUCT(ss) (ng.h/mL)	144.9 (98.0)	868.1 (762.2)
AUC0-24(ss) (ng.h/mL)	579.7 (392.2)	1736.3 (1524.4)
Tmax(ss)** (h)	1.0	2.5

* uncorrected for surface area
** median for $T_{max(ss)}$

The following parameters were derived based on the corrected surface area (per cm^2):

PK Parameter	PENNSAID® (N=30)	Solaraze® (N=27)
Cmax(ss) (ng/mL)	0.022 (0.017)	0.147 (0.131)
Cmin(ss) (ng/mL)	0.010 (0.006)	0.036 (0.021)
Cavg(ss) (ng/mL)	0.015 (0.010)	0.084 (0.069)
AUCT(ss) (ng.h/mL)	0.089 (0.058)	1.0 (0.8)
AUC0-24(ss) (ng.h/mL)	0.36 (0.23)	2.0 (1.6)

The results (90% CI of the geometric mean for ln-transformed and, both uncorrected and corrected for surface area) from this study revealed that the diclofenac relative bioavailability from PENNSAID® was approximately 1/3 of Solaraze® treatment under maximum use conditions per labeling.

Parameter	PENNSAID®	Solaraze®	Ratio, % (90% CI)
Uncorrected			
$C_{max(ss)}$	26.9	91.8	29.4 (21.7-39.7)
$AUC_{0-24(ss)}$	474.2	1346.2	35.2 (27.6-45.0)
Corrected for Surface Area			
$C_{max(ss)}$	0.025	0.0739	34.2 (25.0-46.9)
$AUC_{0-24(ss)}$	0.39	1.24	31.5 (24.4-40.7)

Summary of Safety

No serious adverse events (AEs) were reported in this study. There were 6 (20%) subjects experiencing a total of 7 AEs during treatment with PENNSAID® and 7 (25%) subjects experiencing a total of 14 AEs during treatment with Solaraze®. One application site AE occurred with PENNSAID®, and 5 application site AEs occurred with Solaraze®. The most frequent AE reported was rash at the site of application, occurring in 4 (14.3%) subjects during Solaraze® treatment. One subject withdrew from this study during the Solaraze® period because of an adverse event, an application site reaction. Two subjects withdrew voluntarily during the washout period prior to Solaraze® application (consent withdrawn; family emergency). One subject had a post-study elevation in alanine aminotransferase to 3 times the upper limit of normal and aspartate aminotransferase to 2.5 times upper limit of normal. The Applicant stated that these increases may be related to the subject's medical history (impaired renal function and diabetes), as post-study laboratory assessments indicate that these parameters remained elevated for several months following discontinuation of study drug from this study and this explanation seems reasonable.

2.3 Intrinsic Factors - Not applicable

2.4 Extrinsic Factors

2.4.1 What extrinsic factors (drugs, herbal products, diet, smoking, and alcohol use) influence exposure and/or response and what is the impact of any differences in exposure on pharmacodynamics?

Study NRI-1000-08500 was conducted to determine the naturally occurring plasma levels of DMSO and DMSO2 from healthy subjects who are on a regular diet and not exposed to topical DMSO products. The levels of DMSO in 50 subjects were analyzed. One blood sample was taken during the study visit.

The study results revealed that DMSO levels were below the limit of quantitation (55 ng/mL). The concentrations of $DMSO_2$ were quantifiable in 13 out of 50 subjects (26%). The values ranged from [redacted] ng/mL. The geometric mean of the 13 subjects was 688.8 ng/mL and the arithmetic mean (± standard deviation) was 905.3 ± 5521.8 ng/mL. b(4)

The results of this study indicated that there is chronic exposure to systemic levels of DMSO and $DMSO_2$ in healthy male and female volunteers who are on a regular diet.

2.5 General Biopharmaceutics – Not applicable

2.6 Analytical Section

2.6.1 What bioanalytical methods are used to assess concentrations?

The following table summarizes the analytical method used to measure diclofenac in plasma samples in the relative bioavailability study, PEN-07-116.

Method	Sensitivity of Method/Range
LC/MS/MS (original)	**Range:** 5.09-2546.40 ng/mL **LLOQ:** 5.09 ng/mL
LC/MS/MS (Partial Validation 1*)	**Range:** 49.72 - 49720.00 pg/mL **LLOQ:** 49.72 pg/mL
LC/MS/MS (Partial Validation 3**)	**Range:** 1.01 - 201.79 ng/mL

LLOQ = lower limit of quantitation

* The method described in SOP ANI 8972.02 was modified so as to decrease the lower and upper limits of quantitation, to change the extraction method, and to increase column length. The modified method was issued as SOP ANI 9051.00 draft-1. The revised method was partially revalidated according to SOP ANI 33.11.

** The method described in SOP ANI 9051.01 was modified to change analytical platform from API 5000 to API 4000 and to increase the calibration range from 50-50000 pg/mL to 1-200 ng/mL and was issued as SOP ANI 9391.00 draft-1. The purpose of this change was to improve the evaluation of pharmacokinetic parameters (AUC, $T_{1/2\beta}$). The revised method was partially validated according to SOP ANI 33.13.

3 Detailed Labeling Recommendations

Overall, the proposed Labeling by the Applicant is acceptable. However, the following minor modification is proposed for the Labeling. An agreement should be obtained on the proposed Labeling by the Agency. Diclofenac general information is added as well.

b(4)

21 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

__√__ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

Diagnosis and main criteria for inclusion: The study population consisted of male and female subjects diagnosed with actinic keratosis (AK) affecting at least 500 cm2 of skin on the face, head, scalp, neck, shoulders, arms, and/or upper trunk, not necessarily continuous, of which at least 100 cm2 of the total affected area was on the face, head or scalp. Subjects were required to have a total of at least 20 non-hypertrophic AK lesions on all areas combined and a total of at least 6 non-hypertrophic AK lesions on the face, head or scalp.

Test product, dose, mode of administration and batch number: 40 drops (approx. 1.2 mL) q.i.d. of PENNSAID® Topical Solution (diclofenac sodium topical solution) 1.5% w/w (Nuvo Research Inc.) to each knee; 80 drops total per application, on Days 1 to 7 and dosed once on the morning of Day 8. Lot number: HO388BE

Reference product, dose, mode of administration and batch number: 0.02 g b.i.d of Solaraze® Gel (diclofenac sodium 3%) (manufactured by Patheon Inc for Doak Dermatologics) per cm2 area of skin affected by actinic keratosis, up to a maximum of 1000 cm2 area on Days 1 to 27 and once on the morning of Day 28. Lot number: HX61 Lot number: JC04 Lot number: JC02 (used for post-study treatment)

Duration of treatment: The first dose for the first subject occurred on 27-Jun-07. The last dose for the last subject occurred on 15-Nov-07.

The area of application of PENNSAID® for each knee consisted of the entire knee surface (front, back and sides) over an area defined as 10 cm above and below the midpoint of the patella. The estimated surface area was 700–800 cm2 per knee, about 1500 cm2 total. Each subject's actual treatment area was measured at Period 1 check-in. The areas of application of Solaraze® were the confluent area of skin containing the AK lesions and adjacent sun damaged skin. The total treated area was at least 500 cm2 and was maximized at 1000 cm2 (although there is no maximum treatment area specified in the Solaraze® FDA-approved label, the treated area was maximized at 1000 cm2 in this study to limit the exposure to topical diclofenac during Solaraze® treatment). Each subject's total affected area was measured at screening. For subjects with an affected area larger than the maximum allowed, the areas to be treated were chosen in the following order until a maximum of 1000 cm2 area was achieved: scalp/head/face; arms; shoulders; back; chest.

Criteria for evaluation:

Plasma Diclofenac: Using a validated HPLC method, plasma diclofenac sodium was analyzed from blood samples collected at the following time-points (relative to first dose of the day): Period 1 (PENNSAID®): Day 1: 0.0 hour (pre-dose) Day 6 and 7: 0.0 hour (pre-dose) Day 8: 0.0 hour (pre-dose), 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0 and 6.0 hour Period 2 (Solaraze®): Day 1: 0.0 hour (pre-dose) Day 26 and 27: 0.0 hour (pre-dose) Day 28: 0.0 hour (pre-dose), 1.0, 1.5, 2.0, 2.5, 3.0, 3.5, 4.0, 5.0, 6.0, 8.0, 10.0 and 12.0 hour.

Pharmacokinetics: The following pharmacokinetic parameters for plasma diclofenac sodium were calculated for each treatment period by standard noncompartmental methods: C ss max , Cmin ss , ss avgC , AUCτ ss , AUC ss 0–24 , T ss max . For PENNSAID® , AUCτ ss was calculated over the dosing interval of 0–6 hours, and for Solaraze® , AUCτ ss was calculated over the dosing interval of 0–12 hours. For each treatment AUCτ ss was adjusted to a 24-hour exposure parameter, AUC ss 0–24 (calculated as AUCτ ss x 2 for Solaraze® and AUCτ ss x 4 for PENNSAID®), so that a comparison between the exposure of each treatment was assessed on a daily basis.

Safety: Safety variables included adverse events, vital signs and laboratory parameters.

Statistical methods: Descriptive statistics were calculated for all pharmacokinetic parameters, with and without correction for surface area of application of study drug. Primary analysis: For the primary analysis, the Test/Reference (i.e., PENNSAID®/Solaraze®) ratio and corresponding 90% confidence interval of the geometric mean for ln-transformed C ss max and AUCss 0–24 were calculated. Secondary analysis: As a secondary analysis, the Test/Reference ratio and corresponding 90% confidence interval of the geometric mean for the ln-transformed C ss max and AUC ss 0–24 , corrected for surface area of application, were determined. Safety analysis: Adverse events that were defined as treatment-emergent were summarized for each treatment period, by the number and percentage of subjects experiencing each coded event, and by severity and relationship to the study drug. Descriptive statistics were provided for laboratory evaluations and vital sign data.

Summary and conclusions:

Summary of pharmacokinetics: Steady-state of plasma diclofenac was attained within 8 days for PENNSAID® and within 28 days for Solaraze® . The calculated Cmax(ss), Cmin(ss), Cavg(ss), AUCT(ss) and AUC0-24(ss) of diclofenac were substantially lower and Tmax(ss) was shorter following the maximum therapeutic dose of PENNSAID® compared to that observed following the application of Solaraze® as per label.

Summary Statistics of Pharmacokinetic Parameters* (mean [SD])

PK Parameter		**PENNSAID®**	**Solaraze®**
		N=30	N=27
Cmax(ss)	(ng/mL)	35.0 (28.6)	125.9 (119.3)
Cmin(ss)	(ng/mL)	16.7 (10.4)	30.4 (19.4)
Cavg(ss)	(ng/mL)	24.2 (16.3)	72.3 (63.5)
AUCT(ss)	(ng.h/mL)	144.9 (98.0)	868.1 (762.2)
AUC0-24(ss)	(ng.h/mL)	579.7 (392.2)	1736.3 (1524.4)
Tmax(ss) **	(h)	1.0	2.5

The analysis of the PENNSAID®/Solaraze® ratio (and corresponding 90% confidence interval [CI]) of the geometric mean for ln-transformed *Css* max and *AUCss* 0–24 for both uncorrected and corrected for surface area data revealed the rate and extent of bioavailabilty of diclofenac following the maximum therapeutic dose of PENNSAID® were approximately 1/3 that of Solaraze®, with upper confidence limits well below the 80–125% range.

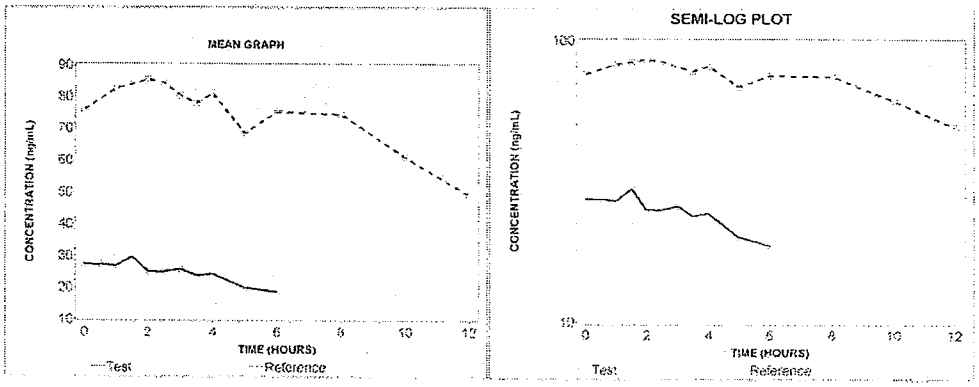
Mean Relative Bioavailability Pharmacokinetic Parameters* (ln transformed, N=27)

Parameter	**PENNSAID®**	**Solaraze®**	**Ratio, % (90% CI)**
Uncorrected			
Cmax(ss)	26.9	91.8	29.4 (21.7–39.7)
AUC0-24(ss)	474.2	1346.2	35.2 (27.6–45.0)
Corrected for Surface Area			
Cmax(ss)	0.025	0.0739	34.2 (25.0–46.9)
AUC0-24(ss)	0.39	1.24	31.5 (24.4–40.7)

Summary of safety: No serious adverse events were reported in this study. The majority of reported adverse events (AEs) were mild in severity, with none rated as severe. There were 6 (20%) subjects experiencing a total of 7 AEs during treatment with PENNSAID® and 7 (25%) subjects experiencing a total of 14 AEs during treatment with Solaraze®. One application site AE occurred with PENNSAID®, and 5 application site AEs occurred with Solaraze®. The most frequent AE reported was rash at the site of application, occurring in 4 (14.3%) subjects during Solaraze® treatment. One subject withdrew from this study during the Solaraze® period because of an adverse event, an application site reaction. Two subjects withdrew voluntarily during the washout period prior to Solaraze® application (consent withdrawn; family emergency). One subject had a post-study elevation in alanine aminotransferase to 3 times the upper limit of normal and aspartate aminotransferase to 2.5 times upper limit of normal. These increases may be related to the subject's medical history (impaired renal function and diabetes), as post-study laboratory assessments indicate that these parameters remained elevated for several months following discontinuation of study drug from this study.

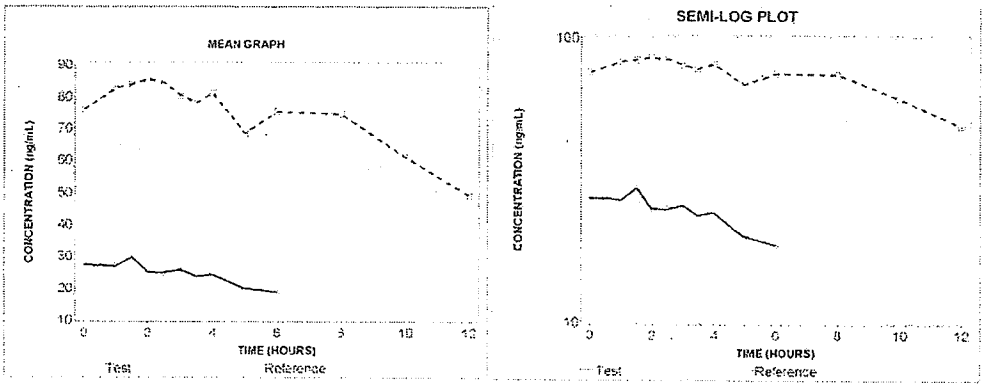
Conclusions: The results of this study confirm that the bioavailability to diclofenac following the maximum therapeutic dose of PENNSAID® is substantially less (approximately 1/3 lower) than that observed with Solaraze® treatment under maximum use conditions per labeling, even when results are corrected for different size area of application.

First time analysis profile:



This is the main profile: (Re-analysis profile: Two subjects, 001-009 and 001-010, conducted with the data reversed for the 8-hour blood samples)

Mean Graph of Diclofenac Plasma Concentration



Bioanalytical Report (Sponsor Project No. PEN-07-116)

Protocol Identification: **3589 (NRI-1000-08500)**
Study Title: **Natural Occurrence of Dimethyl Sulfoxide and Dimethyl Sulfone in Plasma of Healthy Subjects**
Sponsor: Nuvo Research Inc. 7560 Airport Road, Unit 10 Mississauga, Ontario L4T 4H4 Canada Tel: (905) 673-6980; Fax: (905) 673-0127
Study Design: This study consisted of 1 blood draw taken during 1 study visit.
Study Initiation Date: The protocol was approved by the Quebec Institutional Review Board (QC IRB) on April 8, 2008 as Version 1.0, and the study started on May 2, 2008.
Study Completion Date: May 5, 2008
Principal Investigator: Paul Y. Tam, M.D., F.R.C.P., F.A.C.P. Sub-investigators: [redacted] b(4)
Biovail Contract Research (BCR) Contact Person: Nick D'Ulisse Vice-President and General Manager Biovail Contract Research Tel.: (416) 752-3636; Fax: (416) 752-4598
Final Report Issue Date: September 12, 2008
This study was performed in compliance with Good Clinical Practice (GCP).
Investigators: Principal Investigator: Paul Y. Tam, M.D., F.R.C.P, F.A.C.P.
Publications based on this study: None
Study Visit: Blood samples were collected on May 2, 2008 and May 5, 2008
Objective: The objective of this study was to determine the naturally occurring plasma levels of dimethyl sulfoxide and dimethyl sulfone from healthy males and females.
Main Criteria for Inclusion: Non-smoking male and female subjects with a minimum age of 18 years.
Number of Subjects (planned and analyzed): Bioanalytical and statistical analyses were performed on 50 subjects who met all the inclusion and exclusion criteria.

Blood Draw Timepoints: One blood sample was taken during the study visit.

Analytical Report (Study No.: S1227) Determination of DMSO and DMSO2 in Human Plasma by LC-MS/MS (ESI(+))
Primary calibration functions were established during pre-study validation in the concentration range of ——— for DMSO and ——— for $DMSO_2$. For practical laboratory purposes a lower limit of quantification of 55.9 ng/ml (DMSO) and 377.1 ng/ml ($DMSO_2$) was used during measurement of study plasma samples with unknown concentrations. b(4)

Statistical Methods: Descriptive statistics were calculated for plasma concentrations of dimethyl sulfoxide and dimethyl sulfone.

Results:

It was not possible to quantify concentrations of dimethyl sulfoxide in any subject as each subject's level was below the limit of quantitation (55 ng/mL). The concentrations of dimethyl sulfone were quantifiable in 13 out of 50 subjects. The values ranged from ——— . The geometric mean of the 13 subjects was 688.753 ng/mL and the arithmetic mean (± standard deviation) was 905.328 ± 5521.842 ng/mL. b(4)

Discussion of supplemental data: An analysis of the detectable levels of dimethyl sulfoxide and dimethyl sulfone that included concentrations that were below the limit of quantitation showed that the arithmetic mean (± standard deviation) of dimethyl sulfoxide was 9.094 ± 11.814 ng/mL and the geometric mean was 9.611 ng/mL; range was ——— . For dimethyl sulfone, the arithmetic mean (± standard deviation) was 979.578 ± 5510.495 ng/mL and the geometric mean was 184.446 ng/mL; range from ——— . Detectable levels of dimethyl sulfoxide was found in 31 out of 50 subjects and detectable levels of dimethyl sulfone was found in 38 out of 50 subjects. Conclusion: The results of this study confirm that there is chronic exposure to systemic levels of dimethyl sulfoxide and dimethyl sulfone in healthy male and female subjects. b(4)

The results of this study show quantifiable levels of DMSO and DMSO2 in plasma of healthy male and female subjects who have not had recent exposure to DMSO containingpharmacological products. Possible sources of the plasma DMSO andDMSO2 in these subjects include naturally-occurring DMSO in food (Pearson et al.,1981; Steely, 1994), dietary supplementation with DMSO2 (known as MSM) or endogenous production of DMSO and/or DMSO2 via metabolism (Martin, 1987; Engelke et al., 2005). The data confirm that there is chronic exposure to systemic levels of DMSO and DMSO2 in healthy male and female subjects.

Final Report Issue Date: September 12, 2008

Table 14.2.1 Plasma concentrations of dimethyl sulfoxide (ng/mL) in healthy subjects

Subject	0.00
001	
002	
003	
004	
005	
006	
007	
008	
009	
010	
011	
012	
013	
014	
015	
016	
017	
018	
020	
022	
023	
024	
025	
026	
027	
028	
029	
030	
031	

Limit of quantitation = 55.9 ng/mL
BLQ = Below Limit of Quantitation ND = No Data N/AP = Not Applicable
* Mean, SD, CV and Range are calculated using a value of 0.00 ng/mL for BLQ

Study# 3589, Date: Jul 22, 2008

b(4)

Table 14.2.1 Plasma concentrations of dimethyl sulfoxide (ng/mL) in healthy subjects

Subject	0.00
032	
033	
034	
035	
036	
037	
038	
039	
040	
041	
042	
043	
044	
045	
046	
047	
048	
049	
050	
051	
052	
Mean	
SD(1)	
CV(%)	
Geometric Mean	
Range(Min)	
(Max)	
n =	

Limit of quantitation = 55.9 ng/mL
BLQ = Below Limit of Quantitation ND = No Data N/AP = Not Applicable NC = Not Calculable
* Mean, SD, CV and Range are calculated using a value of 0.00 ng/mL for BLQ

Study# 3589, Date: Jul 22, 2008

b(4)

Table 14.2.2 Plasma concentrations of dimethyl sulfone (ng/mL) in healthy subjects

Subject	0.00
001	
002	
003	
004	
005	
006	
007	
008	
009	
010	
011	
012	
013	
014	
015	
016	
017	
018	
020	
022	
023	
024	
025	
026	
027	
028	
029	
030	
031	
032	

Limit of quantitation = 377.1 ng/mL
BLQ = Below Limit of Quantitation ND = No Data N/AP = Not Applicable
* Mean, SD, CV and Range are calculated using a value of 0.00 ng/mL for BLQ

Study# 3589, Date: Jul 22, 2008

Table 14.2.2 Plasma concentrations of dimethyl sulfone (ng/mL) in healthy subjects

Subject	0.00
033	
034	
035	
036	
037	
038	
039	
040	
041	
042	
043	
044	
045	
046	
047	
048	
049	
050	
051	
052	
Mean	
SD(#)	
CV(%)	
Geometric Mean	
Range(Min)	
(Max)	
n =	

Limit of quantitation = 377.1 ng/mL
BLQ = Below Limit of Quantitation ND = No Data N/AP = Not Applicable
* Mean, SD, CV and Range are calculated using a value of 0.00 ng/mL for BLQ
** Geometric Mean is calculated with non-BLQ values (n=13)

Study# 3589, Date: Jul 22, 2008

b(4)

b(4)

Analytical Report (Study No.: S1227): Determination of DMSO and DMSO2 in Human Plasma by LC-MS/MS (ESI(+))

Primary calibration functions were established during pre-study validation in the concentration range of ———————— for DMSO and ———————— for $DMSO_2$. For practical laboratory purposes a lower limit of quantification of 55.9 ng/ml (DMSO) and 377.1 ng/ml ($DMSO_2$) was used during measurement of study plasma samples with unknown concentrations. The results for the mean day-to-day precision of DMSO and DMSO2 are contained in the following tables 1 - 2.

b(4)

DMSO quality control samples (QC)	mean day-to-day precision
[ng/ml]	[%]
4,501.0	1.01
700.4	1.38
90.1	2.62

$DMSO_2$ quality control samples (QC)	mean day-to-day precision
[ng/ml]	[%]
30,009.8	1.95
4,669.5	0.55
600.5	3.38

The analytical accuracy was assessed by comparing arithmetic QC means for deviations between nominal and measured values. From the mean relative deviations, which are depicted in the following tables 3 - 4, an acceptable level of between-day accuracy was deduced for the present investigation.

DMSO quality control samples (QC)	mean between-day accuracy
[ng/ml]	[%]
4,501.0	-3.62
700.4	-1.14
90.1	-3.89

$DMSO_2$ quality control samples (QC)	mean between-day accuracy
[ng/ml]	[%]
30,009.8	1.14
4,669.5	-2.70
600.5	-3.75

Precision and Accuracy of Determination of Calibration Standards

DMSO concentration	precision CV	mean deviation
[ng/ml]	[%]	[%]
5,592.0	1.08	-0.48
2,236.8	0.40	2.02
1,118.4	2.90	-0.21
559.2	2.66	-1.90
279.6	3.31	-1.53
139.8	3.51	-2.83
55.9	6.50	4.93

$DMSO_2$ concentration	precision CV	mean deviation
[ng/ml]	[%]	[%]
37,706.7	1.40	0.19
15,082.7	0.15	-0.30
7,541.3	1.65	-0.26
3,770.7	2.86	-0.29
1,885.3	7.44	-0.41
942.7	3.77	1.61
377.1	7.66	-0.55

4.3 Consult Review (including Pharmacometric Reviews) - Not applicable.

4.4 Cover Sheet and OCPB Filing/Review Form

Office of Clinical Pharmacology and Biopharmaceutics New Drug Application Filing and Review Form			
General Information About the Submission			
	Information		Information
NDA Number	20-947	Brand Name	PENNSAID
OCPB Division (I, II, III)	II	Generic Name	Diclofenac sodium 1.5 % w/w
Medical Division	HFD-170	Drug Class	-
OCPB Reviewer	David Lee	Indication(s)	osteoarthritis
OCPB Team Leader	Suresh Doddapaneni	Dosage Form	solution
		Dosing Regimen	40 drops per knee, four times a day
Date of Submission	2/4/09	Route of Administration	Topical
Estimated Due Date of OCPB Review	-	Sponsor	Nuvo Research Inc. (formally Dimethaid Research Inc.)
Medical Division Due Date		Priority Classification	Complete Response
PDUFA Due Date	8/4/09		
Clin. Pharm. and Biopharm. Information			

	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X	2	2	
I. Clinical Pharmacology				
Mass balance:				
Isozyme characterization:				
Blood/plasma ratio:	x			
Plasma protein binding:	x			
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:				
multiple dose:	x	1	1	
Patients-	x	1	1	
single dose:				
multiple dose:				
Dose proportionality -				
fasting / non-fasting single dose:				
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:				
In-vivo effects of primary drug:				
In-vitro:				
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				Deferral
geriatrics:				
renal impairment:				
hepatic impairment:				
PD:				
Phase 1:				
Phase 2/3:				
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -				
Data rich:				
Data sparse:				
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:				
alternate formulation as reference:				

Bioequivalence studies -				
traditional design; single / multi dose:				
replicate design; single / multi dose:				
Food-drug interaction studies:				
Dissolution:				
(IVIVC):				
Bio-wavier request based on BCS				
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:				
Chronopharmacokinetics				
Pediatric development plan				
Literature References				

Filability and QBR comments		
	"X" if yes	Comments
Application filable ?	X	Reasons if the application is not filable (or an attachment if applicable) For example, is clinical formulation the same as the to-be-marketed one?

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

/s/

David Lee
6/25/2009 01:51:15 PM
BIOPHARMACEUTICS

Suresh Doddapaneni
7/1/2009 05:54:46 AM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY REVIEW

NDA: 20-947	Submission Date: 6/28/06
Submission Type; Code:	**Resubmission**-Complete Response to August 7, 2002 NA Letter
Brand/Code Name:	PENNSAID® Topical Solution
Generic Name:	1.5 % w/w diclofenac sodium solution
Primary Reviewer:	David Lee, Ph.D.
Secondary Reviewer	Suresh Doddapaneni, Ph.D.
OCP Division:	2
OND Division:	Anesthesia, Analgesia, and Rheumatology Products
Sponsor:	DIMETHAID INTERNATIONAL INC.
Relevant IND(s):	42,773
Formulation; Strength(s):	1.5 % w/w
Proposed Indication:	For the treatment of the symptoms of osteoarthritis of the knee(s)
Proposed Dosage Regimen:	40 drops per knee, four times a day.

Table of Contents

1 Executive Summary

1.1 Recommendations

The Office of Clinical Pharmacology / Division of Clinical Pharmacology II (OCP/DCP-II) has reviewed the Complete Response submitted on 6/28/06.

From OCP point of view, the information contained in the Complete Response is acceptable provided that a mutually acceptable agreement is reached between the Applicant and the Agency regarding the Labeling language (See Labeling Review section).

1.2 Phase IV Commitments

Not applicable.

1.3 Summary of CPB Findings

In this submission, Dimethaid International Inc. has submitted Complete Response to NDA 20-947 Not Approval Letter dated 8/07/02. The resubmission includes new data from 3 phase III clinical trials and 2 PK studies to respond fully to the deficiencies. The Applicant stated that every technical section has been updated, integrating the new data (clinical studies and literature references) with the previously submitted summaries. The Applicant stated that the pivotal safety and efficacy trial, PEN-03-112, provides conclusive evidence that PENNSAID applied topically to a knee is efficacious for that particular knee.

The initial NDA was submitted in 12/15/97. The NDA contained one single-dose PK study (106-95), and the 'nested' BA study (102-93-1). The dosage in the single-dose study was 1 mL to one knee only. The Applicant withdrew the NDA 10/27/98 due to deficiencies resulting from pre-approval inspection of the facility. Subsequent to the withdrawal of the NDA (see *FDA correspondence dated July 20, 2000*), the Division informed the folllowing to the Applicant with regards to the Pharmacokinetics and Bioavailability section of the NDA:

> "These sections were found to be acceptable from the Clinical Pharmacology and Biopharmaceutics perspective at the time of the NDA 20-947 review process (8/17/98) prior to its withdrawal, provided that the sponsor adequately addressed labeling comments. These labeling comments were not conveyed to the sponsor during the time that NDA 20-947 was active, however, they are provided to you at this time to assist in your plans to submit a new NDA."

The NDA was resubmitted on 8/7/01 as a stand alone submission. In support of the human PK data, it contained the previously reviewed single-dose PK study 106-95 (n=4) and a new nested bioavailability study, RA-CP-109-US (PK). Subsequently, a non-approvable letter was issued on 8//7/02, and it was communicated to the Applicant that

since DMSO may be an active component of Pennsaid® and that Pennsaid® may represent a combination drug product, the PK of DMSO following topical administration would need to be evaluated prior to approval. The following Clinical Pharmacology /Biopharmaceutics requirement was noted:

> "Based on both the amount of diclofenac contained in this solution and the proposed conditions of use, no additional systemic pharmacokinetic information is needed relative to the diclofenac component of this product. However, you have not provided an adequate evaluation of the pharmacokinetics of DMSO following topical administration. As part of the clinical program for this product, you should undertake a study whereby both single dose and multiple dose pharmacokinetic sampling is undertaken for DMSO and its major metabolites with both an adequate number of samples and subjects. This study must be conducted prior to approval."

In this Resubmission, as requested by the Division, the Applicant conducted both a single dose PK study (Study No. 2663) and a multiple dose PK study (Study No. 2656) in early 2003. The dose of Pennsaid® Topical Solution (1.5% w/w diclofenac sodium) in the new studies was to 2 knees, as opposed to the single-dose PK study No.106-95, in which the dose was to one knee only. The protocols were submitted prior to commencing the studies and were found to be acceptable (*see FDA correspondence dated January 24, 2003*) for the assessment of the bioavailability of diclofenac and DMSO (and its metabolites). The new PK studies incorporate the dosing of Pennsaid® under maximal conditions per the proposed labeling, Applicant is citing Voltaren® as the reference listed product for this 505(b)(2) application.

Study 2663 was an open-label, single-dose PK study. A total of 80 drops of Pennsaid® containing 43 mg of diclofenac sodium were applied on both knees (40 drops per knee), the same dosing of Pennsaid® per the proposed labeling. Diclofenac Cmax of 8.05 ng/mL was reached in a mean of about 11 hrs (median value of 10 hours) and diclofenac remained measurable up to 72 hrs post dose in 18 subjects. Mean t1/2 of diclofenac was 37 hrs (13 subjects). Diclofenac AUC_{0-inf} was 196.3 ng.hr/mL. The apparent clearance was about 244.7 L/hr. Maximum DMSO Cmax of 0.5 µg/mL was reached in a mean of about 8.5 hours (median value of 8.0 hours). Mean DMSO t1/2 was 8.4 hrs (9 subjects). DMSO AUC_{0-inf} was 9.1 µg.hr/mL. The apparent clearance was approximately 163.5 L/hr. Calculation of the pharmacokinetic parameters of $DMSO_2$ was not possible since the majority of the concentrations were below the lower level of quantitation (LLOQ). During this study, 11 subjects experienced a total of 26 adverse events. All adverse events either resolved spontaneously or with non-medical treatment. All the subjects were able to complete the study period. Seven subjects did not experience any adverse event.

Study 2656 was an open-label, multiple dose pharmacokinetic study involving a total of 80 drops of Pennsaid® (40 drops per knee) applied on both knees over 7 days with the final dose on the morning of Day 8. The daily diclofenac dose in Pennsaid® applied was 172 mg (21.5 mg per knee, four times a day). On Days 6, 7 and 8, mean trough plasma concentrations of diclofenac were 23.9 ng/mL, 20.0 ng/mL and 17.4 ng/mL, respectively.

Diclofenac reached near steady state on Day 6. After the last dose of Pennsaid® on Day 8, mean plasma diclofenac C_{max} was 19.4 ng/mL and mean T_{max} was 4.0 hrs. The mean diclofenac $AUC_{0\text{-}inf}$ was 745.2 ng.hr/mL. The apparent terminal half-life ($t_{1/2}$) was 79.0 hrs. The daily DMSO dose in Pennsaid® applied was 5.2 g (0.65 mg per knee, four times a day). On Days 6, 7 and 8, trough plasma concentrations of DMSO were 1.3, 1.0 and 1.1 µg/mL, respectively. DMSO reached plasma concentration levels at or near steady state on Day 6. Following the last dose of Pennsaid® on Day 8, mean DMSO C_{max} was 1.2 µg/mL and mean T_{max} value was 3.8 hrs. The mean DMSO $AUC_{0\text{-}inf}$ was 36.0 µg.hr/mL. The mean apparent terminal half-life ($t_{½}$) was 43 hrs. For $DMSO_2$, plasma trough concentrations on Days 6, 7 and 8 were 15.7 µg/mL, 16.7 µg/mL and 16.5 µg/mL, respectively. These values were not statistically different between each other, when tested by ANOVA model, indicating that $DMSO_2$ reached plasma concentration levels at or near steady state on Day 6. Following the last dose of Pennsaid® on Day 8, $DMSO_2$ C_{max} value was 18.0 µg/mL and mean T_{max} value was 9.4 hrs. The mean $DMSO_2$ $AUC_{0\text{-}t}$ was 1525.3 µg.hr/mL and the mean $AUC_{0\text{-}inf}$ was 2339.2 µg.hr/mL. The mean apparent terminal halflife ($t_{½}$) was 61 hrs. During this study, 11 subjects experienced a total of 34 adverse events. Adverse events, probably related to the study medication (bad breath, dry skin, itching of the knee, burning sensation of the knee), were mild in severity at onset and they resolved spontaneously. In addition, for this study, 10 patients were randomly selected for platelet aggregation analysis. Based on the results presented for this analysis, the average change from the addition of the aggregation agents ADP, collagen, epinephrine and arachidonic acid were 101.3%, 99.8%, 109.8% and 99.0%, respectively. These results indicated that there was no significant difference between baseline platelet aggregation results and the final platelet aggregation results obtained for the randomly selected subjects who were administered Pennsaid® Topical solution.

Based on the single-dose Pennsaid® Study 2663, which provides maximally 8.05 ng/mL of diclofenac sodium (40 drops of Pennsaid® to two knees), when compared to a single oral dose of Voltaren® (50 mg tablet; C_{max} was 1500–1600 ng/mL) (see *USP:DI 2000*), the maximum blood levels of diclofenac sodium in Pennsaid® were more than 187–200 times lower than oral administration of diclofenac sodium.

Comparison of the times to reach maximum concentrations after administration of the drug products revealed that it took significantly longer for diclofenac sodium to reach C_{max} after topical application (11 hrs) than oral administration (1–4 hrs). Moreover, comparing the area under the serum concentration time curve for diclofenac sodium between topically applied Pennsaid® and orally administered Voltaren®, the $AUC_{0\text{-}inf}$ for Pennsaid® (containing 43 mg diclofenac sodium) was 196.9 ng.hr/mL and the $AUC_{0\text{-}inf}$ for enteric-coated Voltaren® (50 mg) was reported to be 6.3 µg.hr/mL (El-Sayed *et al.*, 1988).

Overall, the information contained in this Complete Response for diclofenac, DMSO and $DMSO_2$, are acceptable. There are no other issues for this Complete Response.

2 QBR

2.1 General Attributes of the Drug – Not applicable

2.2 General Clinical Pharmacology

2.2.1 What are the single dose and multiple dose PK parameters of diclofenac and DMSO?

Study 2663 was an open-label, single-dose pharmacokinetic study, a total of 80 drops of Pennsaid® containing 43 mg of diclofenac sodium were applied on both knees (40 drops per knee), per the proposed dosing. Serial blood samples were obtained up to 240 hours post-dose. The primary objective of this study was to determine the PK of diclofenac sodium, dimethyl sulfoxide (DMSO) and its major metabolite, dimethyl sulfone ($DMSO_2$). Validated bioanalytical assay methods were employed for the analysis of diclofenac sodium (see *FB-2002-13.pdf*), DMSO (see *FB-2003-24.pdf*) and $DMSO_2$ (see *FB-2003-08.pdf*) in plasma samples. Bioanalytical tests were carried out by ——— ——— Pharmacokinetic and statistical analyses were performed on data obtained from 18 normal, healthy, non-smoking male and female subjects. b(4)

A maximum diclofenac sodium concentration in plasma of 8.05 ng/mL was reached in a mean of about 11 hrs (median value of 10 hours) and diclofenac remained measurable up to 72 hrs post dose in 18 subjects. Four subjects still had measurable diclofenac concentrations 240 hrs post-dose. Mean elimination half-life of diclofenac sodium was 37 hrs (13 subjects) and the elimination rate constant was approximately 0.024 hr^{-1}. AUC_{0-t} for diclofenac sodium was about 177.5 ng.hr/mL and AUC_{0-inf} was 196.3 ng.hr/mL. The apparent clearance was about 244.7 L/hr.

Maximum DMSO concentration in plasma of 0.5 µg/mL was reached in a mean of about 8.5 hours (median value of 8.0 hours) and DMSO remained measurable up to 24 hrs post dose in 18 subjects. Mean elimination half-life of DMSO was 8.4 hrs (9 subjects) and the elimination rate constant was approximately 0.114 hr^{-1}. The AUC_{0-t} for DMSO was, on average, 8.7 µg.hr/mL hrs and AUC_{0-inf} was 9.1 µg.hr/mL. The apparent clearance was approximately 163.5 L/hr.

Calculation of the pharmacokinetic parameters of $DMSO_2$ was not possible since the majority of the concentrations were below the lower level of quantitation (LLOQ). Since only plasma was collected for pharmacokinetic measurements, neither urinary excretion nor renal clearance is available for diclofenac sodium and DMSO.

During this study, 11 subjects experienced a total of 26 adverse events. All adverse events either resolved spontaneously or with non-medical treatment. All the subjects were able to complete the study period. Seven subjects did not experience any adverse event.

Study 2656 was an open-label, multiple dose pharmacokinetic study involving a total of 80 drops of Pennsaid® (40 drops per knee) applied on both knees over 7 days with the final dose on the morning of Day 8. This is the same dosing of Pennsaid® under maximal conditions per the proposed labeling. Serial blood samples were collected up to 360 hours following the last dose on Day 8. Additionally, pre-dose samples were collected on Day 6, 7 and 8. The primary objective of this study was to determine the pharmacokinetics of diclofenac sodium, DMSO and its major metabolite, $DMSO_2$. Validated bioanalytical assay methods were employed for the analysis of diclofenac sodium (see *FB-2002-13.pdf*), DMSO (see *FB-2003-24.pdf*) and $DMSO_2$ (see *FB-2003-08.pdf*) in plasma samples. Bioanalytical tests were carried out by ———— b(4)
—— Twenty (20) subjects received Pennsaid® and 19 subjects completed the study. Pharmacokinetic and statistical analyses were performed on data obtained from the 19 subjects who completed the study.

The daily diclofenac dose in Pennsaid® applied was 172 mg (21.5 mg per knee, four times a day). On Days 6, 7 and 8, mean trough plasma concentrations of diclofenac were 23.9 ng/mL, 20.0 ng/mL and 17.4 ng/mL, respectively. After the last dose of Pennsaid® on Day 8, mean plasma C_{max} value of diclofenac was 19.4 ng/mL and mean T_{max} was 4.0 hrs. The mean AUC_{0-t} was 695.4 ng.hr/mL and mean AUC_{0-inf} was 745.2 ng.hr/mL. The apparent terminal half-life ($t_{1/2}$) was 79.0 hrs.

The daily DMSO dose in Pennsaid® applied was 5.2 g (0.65 mg per knee, four times a day). On Days 6, 7 and 8, trough plasma concentrations of DMSO were 1.3 µg/mL, 1.0 µg/mL and 1.1 µg/mL, respectively. These values were not statistically different when tested by ANOVA model, indicating that DMSO reached plasma concentration levels at or near steady state on Day 6. Following the last dose of Pennsaid® on Day 8, mean C_{max} value of DMSO was 1.2 µg/mL and mean T_{max} value was 3.8 hrs. The mean AUC_{0-t} was 31.9 µg.hr/mL. The mean AUC_{0-inf} was 36.0 µg.hr/mL. The mean apparent terminal half-life ($t_{½}$) was 43 hrs.

For $DMSO_2$, plasma trough concentrations on Days 6, 7 and 8 were 15.7 µg/mL, 16.7 µg/mL and 16.5 µg/mL, respectively. These values were not statistically different between each other, when tested by ANOVA model, indicating that $DMSO_2$ reached plasma concentration levels at or near steady state on Day 6. Following the last dose of Pennsaid® on Day 8, C_{max} values of $DMSO_2$ were 18.0 µg/mL and mean T_{max} value was 9.4 hrs. The mean AUC_{0-t} was 1525.3 µg.hr/mL. The mean AUC_{0-inf} was 2339.2 µg.hr/mL. The mean apparent terminal halflife ($t_{½}$) was 61 hrs.

During this study, 11 subjects experienced a total of 34 adverse events. Adverse events, probably related to the study medication (bad breath, dry skin, itching of the knee, burning sensation of the knee), were mild in severity at onset and they resolved spontaneously. One patient (subject no. 01) had an episode each of headache, pneumonia and fever, for which he was treated with concomitant medications. The events resolved. Another patient (subject no.13) had an adverse event recorded as paleness, which resolved with non-medical treatment. In addition, for this study, 10 patients were randomly selected for platelet aggregation analysis. Based on the results presented for this analysis, the average change from the addition of the aggregation agents ADP,

collagen, epinephrine and arachidonic acid were 101.3%, 99.8%, 109.8% and 99.0%, respectively. These results indicated that there was no significant difference between baseline platelet aggregation results and the final platelet aggregation results obtained for the randomly selected subjects who were administered Pennsaid® Topical solution.

2.3 Intrinsic Factors - Not applicable

2.4 Extrinsic Factors - Not applicable

2.5 General Biopharmaceutics

2.5.1 What is the in vivo relationship of the proposed to-be-marketed formulation to the pivotal clinical trial formulation in terms of comparative exposure?

All batches of solution (Pennsaid®, placebo, vehicle-control) used in the pivotal clinical trials PEN-03-112, RA-CP-109-US, RA-CP-109, (excluding study 107-96), the long-term safety trial PEN-03-112E, the active-controlled clinical study RA-CP-110 and the new single-dose (2663) and multiple-dose (2656) pharmacokinetic studies were manufactured by Dimethaid Manufacturing Inc. under Good Manufacturing Practices.

The supplier of the active ingredient, diclofenac sodium, and all inactive ingredients, dimethyl sulfoxide, alcohol, propylene glycol and glycerin, have remained the same.

In general, the manufacturing process has remained the same, with only a change in the manufacturing facility site [redacted] Dimethaid Manufacturing Inc. (Pennsaid® manufacturing site since June 1998, for Lots used in PK studies RA-CP-109US, 2656, and 2663). Similar equipment was used to manufacture Pennsaid® at each of the sites identified. The Applicant stated that the change in manufacturing facility had no impact on the quality of the finished drug product.

b(4)

Drug Formulation Development Summary of Pivotal Clinical Trials

Study Number	Lot Number	Dosage Form and Strength	Batch Size	Formulation / Significant Manufacturing Change (if any) / Reason for Change	Effect of Change
2663	**Pennsaid®: B0063AG**	Lotion: 1.5% (w/w) diclofenac sodium in 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water	481.5 kg	Manufacturer: Dimethaid Manufacturing Inc., Varennes, Quebec, Canada	Not applicable
2656	**Pennsaid®: B0065AJ**	Lotion: 1.5% (w/w) diclofenac sodium in 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water	481.5 kg	Manufacturer: Dimethaid Manufacturing Inc., Varennes, Quebec, Canada	Not applicable
RA-CP-109-US11	**Pennsaid®: A0004A12; Control–DMSO: A0002A**	Dosage form: lotion Pennsaid®: 1.5% (w/w) diclofenac sodium in 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin ——— propylene glycol and ——— purified water Control–DMSO: 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water	Pennsaid®: 481.5 kg Control–DMSO: 481.5 kg	Manufacturer: Dimethaid Manufacturing Inc., Varennes, Quebec, Canada Reason for Change: Facility is a wholly-owned subsidiary of Nuvo Research Inc. (formerly Dimethaid Research Inc.) Formulation: No Change	The change in manufacturing facility does not affect the safety or efficacy of Pennsaid® . The suppliers for the drug substance and the inactive ingredients remained the same. The release and stability specifications remained unchanged.
Protocol 106-95 (H-832-11989-01)	4063	Lotion: 1.5% (w/w) diclofenac sodium in 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water	350 kg	Manufacturer: ——— ——— ——— (same lot as used in Protocol 102-93-1)	Not applicable

b(4)

b(4)

b(4)

b(4)

Protocol 102-93-1	**Pennsaid®: 40052/4063 ; Placebo: 40054; Control: 40053**	Dosage form: lotion Pennsaid®: 1.5% (w/w) diclofenac sodium in 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water Placebo: ——— (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water Control: 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water	Pennsaid® lot#4063: ———	Manufacturer: ——— ——— ——— ———Reason for change: ——— plant more conveniently located. Formulation: No change	The manufacturing procedures were not changed and ——— ——— complied with current GMP requirements, therefore, the change in the manufacturing facility had no impact on the quality of the finished product. Additionally, the suppliers for the drug substance and the inactive ingredients remained the same
			Pennsaid® lot#40052: ——— Placebo:—— Control: ———	Manufacturer: ——— ——— ———	

b(4)

b(4)

Drug Formulation Development Summary of Other Clinical Trials

Study Name and Number	Lot Number	Dosage Form and Strength	Batch Size
Cumulative Skin Irritation 100-8913	Pennsaid®: 40052 Placebo (1): 40053 Placebo (2): 40054 Control: 116701 (extemporaneously compounded by a licensed pharmacist)	Dosage form: lotion Pennsaid®: 1.5%(w/w) diclofenac sodium in 45.5%(w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water Placebo (1): 45.5%(w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water **Placebo (2): 4.55%(w/w) dimethyl** sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water **Control: ——— diclofenac** sodium, 45.5%(w/w) dimethyl sulfoxide, ——— ethanol, ——— glycerin, ——— propylene glycol and ——— purified water	Pennsaid®: Placebo (1):——— Placebo (2):——— Control: not recorded
Skin Sensitization 101-89-213	Pennsaid®: 4063 Placebo-L: 40054	Pennsaid®: same as above Placebo-L: same as placebo (2)	Pennsaid®: ——— Placebo (1):———

b(4)

b(4)

	Placebo-H: 40053	Placebo-H: same as placebo (1)	Placebo (2)
Skin Photoirritation 103-93-213	Pennsaid®: 4063 Placebo-L: 40054 Placebo-H: 40053	Pennsaid®: same as above Placebo-L: same as placebo (2) Placebo-H: same as placebo (1)	Pennsaid®: —— Placebo (1) —— Placebo (2) ——
Skin Photosensitization 104-93-313	Pennsaid®: 4063 Placebo-L: 40054 Placebo-H: 40053	Pennsaid®: same as above Placebo-L: same as placebo (2) Placebo-H: same as placebo (1)	Pennsaid®: —— Placebo (1) —— Placebo (2) ——
Emergency Drug Release Program13	4038 & 4063	Pennsaid®: 1.5%(w/w) diclofenac sodium in 45.5%(w/w) dimethyl sulfoxide, —— ethanol, —— glycerin, —— propylene glycol and —— purified water	——
Study Name and Number	**Lot Number**	**Dosage Form and Strength**	**Batch Size**
Open Investigational New Drug Study 105-9513	4063 & 68152	Pennsaid®: 1.5%(w/w) diclofenac sodium in 45.5%(w/w) dimethyl sulfoxide, —— ethanol, —— glycerin, —— propylene glycol and —— purified water	——
Protocol 107-9613	Pennsaid®: 967191 Control: 967192/ 977132 Placebo: 967193/ 977131	Pennsaid®: as above Control: same as placebo (1) Placebo: same as placebo (2)	Pennsaid®: —— Control: —— Placebo: ——
Protocol 108-97	Pennsaid®: 1014 Control–DMSO: 7 Control–Diclofenac: 6 Placebo: 8	Pennsaid®: as above Control–DMSO: same as placebo (1) Control–Diclofenac: 1.5% diclofenac sodium, —— DMSO, —— ethanol, —— purified water Placebo: —— DMSO, —— ethanol, —— purified water	Pennsaid®: —— Control–DMSO: —— Control–Diclofenac: —— Placebo: ——
Protocol RA-CP-109	Pennsaid®: PR-09514 Control: PR-094	Pennsaid®: as above Control: same as placebo (1)	Pennsaid®: —— Control: ——
Protocol RA-CP-11015	**Pennsaid®: A0030/ A0031/ B0065; Placebo: B0051/ B0062/ C0098**	**Pennsaid®: as above Placebo: —— —— dimethyl sulfoxide, —— ethanol, and —— purified water**	Pennsaid®: —— Placebo: ——
Protocol PEN-03-11215	**Pennsaid®: D0148/ D0149/ F0267/ F0278; Placebo: D0146/**	**Pennsaid®: as above Placebo: —— —— dimethyl sulfoxide, —— ethanol, —— glycerin, —— propylene**	Pennsaid®: —— Placebo: —— (D0146 & D0152)/ —— (F0269)/ —— (F0286)

b(4)

	D0152/ F0269/ F0286; Vehicle-control: D0147/ D0153/ F0268/ F0284	glycol, and——— purified water Vehicle-control: 45.5% (w/w) dimethyl sulfoxide, ——— ethanol, ——— ——— glycerin, ——— propylene glycol and ——— purified water	Vehicle-control: ——— (D0147 & D0153). ——— (F0268)/ ——— (F0284)
Protocol PEN-03-112E15	D0149/ D0150/ E0181/ E0234/ E0244/ F0261/ F0267/ F0278/ F0290/ F0298	Pennsaid®: as above	———

b(4)

b(4)

2.6 Analytical Section

2.6.1 What bioanalytical methods are used to assess concentrations?

The following table summarizes the analytical methods used in Pennsaid Solution:

Study Number	Submission Date	Type of Biological Fluid	Drug Substance	Method	Sensitivity of Method/Range	Specificity
2663	IND 42,773 submitted: Dec. 24, 2002	Plasma	Diclofenac sodium	LC/MS/MS	**Limit of quantitation: 0.05 ng/mL Range: 0.05–5.0 ng/mL Linearity: r2 ≥ 0.990**	Method specific for diclofenac sodium
			Dimethyl sulfoxide	LC/MS/MS	**Limit of quantitation: 0.05 ng/mL Range: 0.05–5 µg/mL Linearity: r2 ≥ 0.988**	Method specific for dimethyl sulfoxide
			Dimethyl sulfone	LC/MS/MS	**Limit of quantitation: 2.0 ng/mL Range: 2.0–100 µg/mL Linearity: r2 ≥ 0.996**	Method specific for dimethyl sulfone
2656	IND 42,773 submitted: Dec. 24, 2002	Plasma	Diclofenac sodium	LC/MS/MS	**Limit of quantitation: 0.05 ng/mL Range: 0.05–5.0 ng/mL Linearity: r2 ≥ 0.990**	Method specific for diclofenac sodium
			Dimethyl sulfoxide	LC/MS/MS	**Limit of quantitation: 0.05 µg/mL Range: 0.05–5 µg/mL Linearity: r2 ≥ 0.988**	Method specific for dimethyl sulfoxide

			Dimethyl sulfone	LC/MS/MS	**Limit of quantitation: 2.0 ng/mL Range: 2.0–100 µg/mL Linearity: r2 ≥ 0.996**	Method specific for dimethyl sulfone
RA-CP-109-US	IND 42,773 submitted: Nov. 2000	Plasma	Diclofenac sodium	LC/MS/MS	**Limit of quantitation: 0.05 ng/mL**	Precise and accurate method for diclofenac in human plasma
			Dimethyl sulfoxide	LC/MS/MS	**Limit of quantitation: 50 ng/mL**	Method specific for dimethyl sulfoxide
H-832-11989-01 (Protocol 106-95)	IND submitted: May 30, 95	Plasma and urine	Diclofenac sodium	Radioactivity	**Lower limit of detection: less than 0.15 ng/mL Linear concentration Range: 0.15 –150 µg/mL**	Method specific for diclofenac sample Response corrected for background response
			Dimethyl sulfoxide	GC/MS (SIM)	**Lower limit of detection: 0.1 µg/mL Minimum S/N Ratio of 9:1 Range: 0.1–10 µg/mL Linearity: r2 ≥ 0.995**	Method specific for dimethyl sulfoxide Sample response corrected for blank response
Protocol 102-93-1	IND submitted: Sept. 93	Plasma and synovial fluid	Diclofenac sodium	GC/MS (MS-MS)	**Lower limit of detection: 1 ng/mL Minimum S/N ratio of 2:1 Range: 1–200 ng/mL Linearity: r2 ≥ 0.97 Lower limit of quantitation: 10ng/mL Range: 5–200 ng/mL Linearity: r2 ≥ 0.99**	Method specific for diclofenac sodium Sample response corrected for blank response
Protocol 102-93-1	IND submitted: Sept. 93	Plasma	Dimethyl sulfoxide	GC	**Lower limit of detection: 1 µg/mL S/N ratio of 2:1 Range: 1.0–25.0 µg / mL Linearity: r2 ≥**	Method specific for DMSO Sample response corrected for blank response

					0.98	

3 Detailed Labeling Recommendations

Overall, the proposed Labeling by the Applicant is acceptable. However, the following minor modification is proposed for the Labeling. An agreement should be obtained on the proposed Labeling by the Agency.

b(4)

1 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

__√__ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

4 Appendices

4.1 Proposed Package Insert (Original and Annotated)

Proposed Text of Labeling for PENNSAID®
PENNSAID® TOPICAL SOLUTION
(1.5% w/w diclofenac sodium)

b(4)

14 Page(s) Withheld

_____ § 552(b)(4) Trade Secret / Confidential

√ § 552(b)(4) Draft Labeling

_____ § 552(b)(5) Deliberative Process

[redacted]

b(4)

[redacted]

4.2 Individual Study Review

Protocol No.: 2663

A Single-Dose Pharmacokinetic Evaluation of Pennsaid® Topical Solution (1.5% w/w Diclofenac Sodium Topical Solution) In Normal Healthy Non-Smoking Male and Female Subjects

Investigators:
Principal Investigator: Paul Y. Tam, M.D., F.R.C.P., F.A.C.P.

[redacted]

Study Center:

[redacted]

b(4)

Objectives:
The primary objective of this study was to determine the pharmacokinetics of diclofenac sodium, dimethyl sulfoxide and its major metabolite, dimethyl sulfone, after a single application of Dimethaid's novel formulation, Pennsaid® Topical Solution to both knees of each subject.

Investigational Product, lot number and mode of administration:

PENNSAID TOPICAL SOLUTION, Lot 0: B0063AG, was administered topically as follows:

Each subject was instructed to apply the study solution to clean knees, total 40 drops per knee: 10 drops each to the front of the knee, to each side and to the back. To avoid spillage, the subject was instructed to apply and spread 5 drops at a time, directly onto his/her hand, and then onto the site. The application left the area visibly wet for several minutes and was applied without massaging. Study solution was applied to both knees; the order of application did not matter. After each dosing, subjects waited for the application sites to dry prior to dressing. The subject washed his/her hands after each complete application. The subject showered at least once a day.

Dosing time was determined when the first drop was applied to the first knee.

The drug was provided to each subject in one multiple-dose, 30 mL container.

Eighteen subjects (9 males, 9 females) with a mean age of 33 years (range = 22 to 46 years) were enrolled in this study. The subjects' mean height was 174 cm (range = 163 to 188 cm) and the mean weight was 75 kg (range = 54 to 90 kg). The subjects consisted of 14 Caucasians, 1 Asian and 3 Blacks.

All of the subjects in this study were healthy, non-smoking males and females.

Subject No.	Race	Gender	Age (years)	Height (cm)	Weight (kg)	BMI (kg/(m)^2)	Frame
01	Caucasian	Male	30	173	83	27.73	Large
02	Black	Female	27	175	77	25.14	Large
03	Caucasian	Male	25	185	71	20.75	Medium
04	Caucasian	Female	31	178	79	24.93	Large
05	Caucasian	Male	29	188	90	25.46	Medium
06	Asian	Female	27	170	67	23.18	Medium
07	Caucasian	Male	40	180	87	26.85	Medium
08	Caucasian	Female	28	165	62	22.77	Medium
09	Caucasian	Male	29	165	68	24.98	Medium
10	Caucasian	Female	45	163	77	28.98	Large
11	Caucasian	Male	34	178	81	25.56	Medium
12	Black	Female	32	175	76	24.82	Medium
13	Caucasian	Male	34	183	75	22.40	Medium
14	Caucasian	Female	41	168	67	23.74	Medium
15	Caucasian	Male	22	178	82	25.88	Medium
16	Black	Female	22	173	65	21.72	Medium
17	Caucasian	Male	45	180	85	26.23	Medium
18	Caucasian	Female	46	150	54	24.0	Medium

Mean (All subjects)	33	174	75	24.73
S.D. (±)	8	9	10	2.10

Analytical Procedure:
Validated bioanalytical assay methods were employed for the analysis of diclofenac sodium, dimethyl sulfoxide and its metabolite, dimethyl sulfone, in plasma samples.

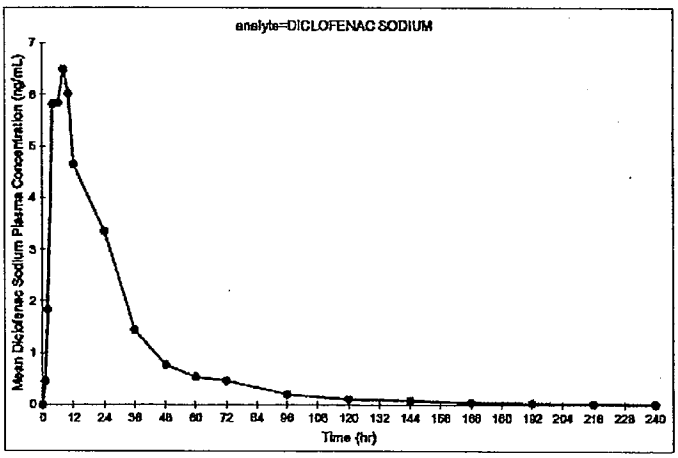
Criteria for evaluation:
Pharmacokinetics:
The following pharmacokinetic parameters for diclofenac sodium and dimethyl sulfoxide were calculated by standard non-compartmental methods: C_{max}, AUC_{0-t}, AUC_{0-inf}, T_{max}, K_{el}, $t_{½}$, and CL/F. Originally, parameters for detection of diclofenac sodium, dimethyl sulfoxide and dimethyl sulfone were specified in the protocol. Following laboratory analysis it was determined that, for dimethyl sulfone, the majority of the results were below the limit of quantitation for the analytical method used.
Safety:
All adverse events were tabulated by subject number.

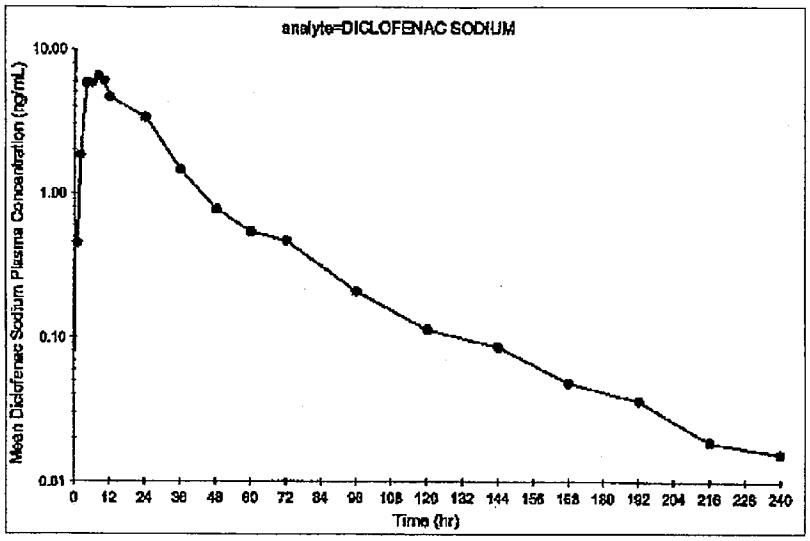
Statistical methods:
Descriptive statistics (mean, standard deviation, range and coefficient of variation [CV]) were performed for all PK parameters. The pharmacokinetic results obtained from this single-dose study may be combined with data generated from other multi-dose studies for Pennsaid® Topical Solution.

Results:

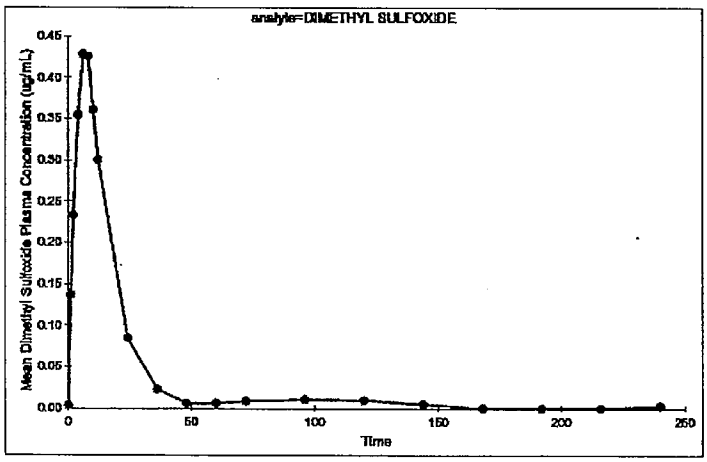
1. Figures:

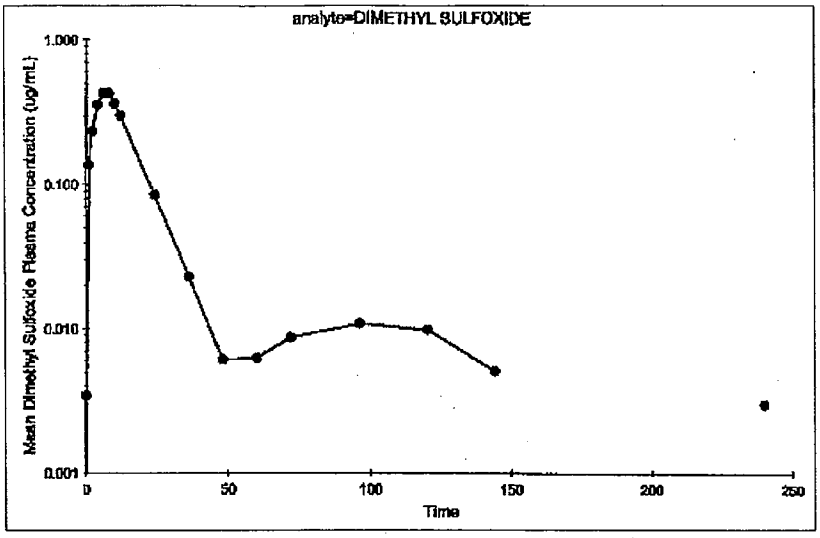
Mean (SD) Plasma Concentration-Time Profiles for Diclofenac Sodium (ng/mL /mL)





Mean (SD) Plasma Concentration-Time Profiles for Dimethyl Sulfoxide (ug/mL)





2. PK parameters

SUMMARY OF SINGLE-DOSE PHARMACOKINETIC RESULTS: Diclofenac Sodium (for two knee application)

Pharmacokinetic Parameters	*PENNSAID® TOPICAL SOLUTION* n = 18 Mean ± SD
AUC0-t (ng.hr/mL)	177.506 ± 72.624
AUC0-inf (ng.hr/mL)†	196.265 ± 68.471
Cmax (ng/mL)	8.051 ± 5.935
Tmax (hr)	11.010 ± 6.438
t½ (hr)†	36.716 ± 20.818
Kel (hr-1)†	0.0237 ± 0.0098
CL/F (L/hr)†	244.664 ± 84.723
†n = 13	

SUMMARY OF SINGLE-DOSE PHARMACOKINETIC RESULTS: Dimethyl Sulfoxide (for two knee application)

Pharmacokinetic Parameter	*PENNSAID® TOPICAL SOLUTION* n = 18 Mean ± SD
AUC0-t (g.hr/mL)	8.719 ± 4.611
AUC0-inf (g.hr/mL)†	9.174 ± 3.751
Cmax (g/mL)	0.475 ±0.335
Tmax (hr)	8.451 ± 2.708
t½ (hr)†	8.422 ± 7.307
Kel (hr-1)†	0.1136 ± 0.0470
CL/F (L/hr)†	163.492 ± 64.139
†n = 9	

CONCLUSION:
After a single administration of PENNSAID.. TOPICAL SOLUTION a maximum diclofenac concentration in plasma of 8.05 ng/mL was reached in about 10 hours, and diclofenac remained measurable up to 72 hours post-dose in 18 subjects. Four subjects still had measurable diclofenac concentrations 240 hours post-dose. Mean elimination half-life was 37h (13 subjects). Maximum DMSO concentration in plasma of 0.48 ..g/mL was reached in about 8 hours, and DMSO remained measurable up to 24 hours post-dose in 18 subjects. Mean elimination half-life was 8.42h (9 subjects).

Protocol No.: 2656

MULTI-DOSE PHARMACOKINETIC EVALUATION OF ———— TOPICAL SOLUTION (1.5% WNV DICLOFENAC SODIUM TOPICAL SOLUTION) IN NORMAL HEALTHY NON-SMOKING MALE AND FEMALE SUBJECTS

b(4)

Objectives:

The primary objective of this study was to determine the pharmacokinetics of diclofenac sodiuin, dimethyl sulfoxide and its major metabolite, dimethyl sulfone, after multiple applications of Dimethaid's novel formulation of Pennsaid Topical Solution to both knees of each subject.

Analytical Procedure:

Validated bioanalytical assay methods were employed for the analysis of diclofenac sodium, dimethyl sulfoxide and its metabolite, dimethyl sulfone, in plasma samples.

Main criteria for inclusion:

Normal, healthy, non-smoking male and female subjects within an age range of 18 to 55 years. All the subjects in this study were healthy, non-smoking males and females.

Subject No.	Race	Gender	Age (years)	Height (cm)	Weight (kg)	BMI (kg/m^2)	Frame
01	Asian	Male	42	180	79	24.38	Medium
02	Caucasian	Female	42	168	74	26.22	Medium
03	Asian	Male	37	165	72	26.45	Medium
04	Caucasian	Female	25	173	67	22.39	Large
05	Asian	Male	39	180	80	24.69	Medium
06	Caucasian	Female	43	173	64	21.38	Medium
07	Caucasian	Male	35	180	74	22.84	Medium
*08	Caucasian	Female	42	173	61	20.38	Medium
09	Caucasian	Male	24	180	81	25.00	Medium
10	Caucasian	Female	37	160	69	26.95	Medium
11	Caucasian	Male	26	175	73	23.84	Medium
12	Black	Female	33	157	48	19.47	Medium
13	Caucasian	Male	31	168	60	21.26	Small
14	Caucasian	Female	38	157	63	25.56	Medium
15	Caucasian	Male	42	178	80	25.25	Medium
16	Caucasian	Female	28	157	52	21.10	Medium
17	Black	Male	24	175	73	23.84	Medium
18	Caucasian	Female	18	165	56	20.57	Large
19	Caucasian	Male	31	185	87	25.42	Medium
20	Caucasian	Female	22	168	65	23.03	Medium

Mean (All subjects)	33	171	69	23.50
S.D. (±)	8	9	10	2.24
Mean (Completing subjects*)	33	171	69	23.67
S.D. (±)	8	9	10	2.17

* Subject #08 did not complete the study.

Investigational Product, lot number and mode of administration:

PENNSAID TOPICAL SOLUTION, Lot #: B0065AJ, was administered topically as follows:

Each subject was instructed to apply the study solution to a clean knee, total 40 drops per knee: 10 drops each to the front of the knee, to each side and to the back. To avoid spillage, the subject was instructed to apply and spread 5 drops at a time, directly onto his/her hand, and then onto the site. The application left the area visibly wet for several minutes and was applied without massaging. Study solution was applied to both knees; the order of application did not matter. After each dosing, subjects waited for the application site to dry prior to dressing. The subject washed his/her hands after each complete application to both knees. The subject showered at least once a day.

The subject washed his/her hands after complete application to both knees. The subject continued regular applications of the study solution to both knees four times daily (7:00 am, 12:00 pm, 5:00 pm and 10:00 pm) on Day 1 through Day 7 and on the morning of Day 8 (7:00 am).

Days 1 and 6:

The subject applied the first dose of study solution to both knees in the clinic under supervision. The subject applied the second, third and fourth doses of the study solution at home.

Days 2 to 5:

The subject applied all four doses of the study solution at home.

Day 7:

The subject applied the first dose of the study solution in the clinic and then exited. The second and third doses were applied at home. The fourth dose was applied in the clinic.

Day 8:

The subject applied the last dose of the study solution to both knees in the clinic following an overnight fast of at least ten hours.

The drug was provided to the subjects in two multi le-dose 60 mL containers.

Sample Collection:

Blood samples (10 mL each) were drawn on Days 1, 6, 7 and 8 at 0.0 hour (pre-dose) and at 1.0, 2.0, 4.0, 6.0, 8.0, 12.0, 24.0, 36.0, 48.0, 60.0, 72.0, 96.0, 120.0, 168.0, 216.0, 264.0, 312.0 and 360 hours (post 0.0 hour) following application of the last dose of drug, on Day 8.

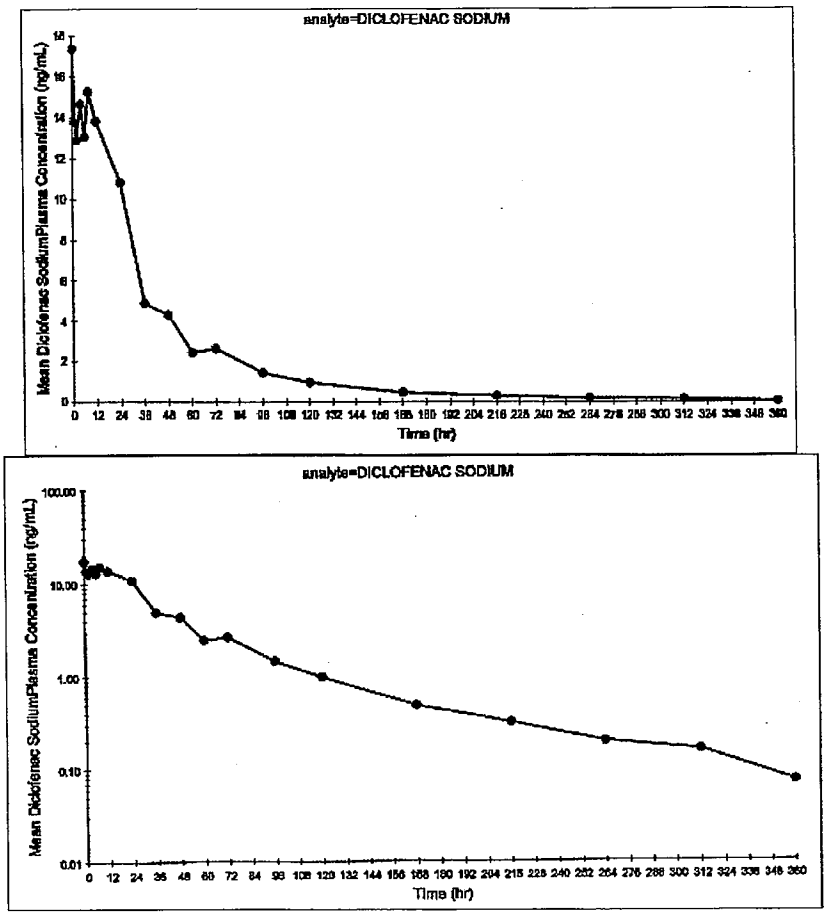
Criteria for evaluation:
Pharmacokinetics:
The following pharmacokinetic parameters for diclofenac sodium, dimethyl sulfoxide and dimethyl sulfone were calculated by standard non-compartmental methods:
AUC_{0-t}, AUC_{0-inf}, C_{max}, T_{max}, K_{el}, and $t_{½}$ when data permits.
Safety:
All adverse events were tabulated by subject number.

Statistical methods:
Descriptive statistics (mean, standard deviation and CV) were performed for all PK parameters.

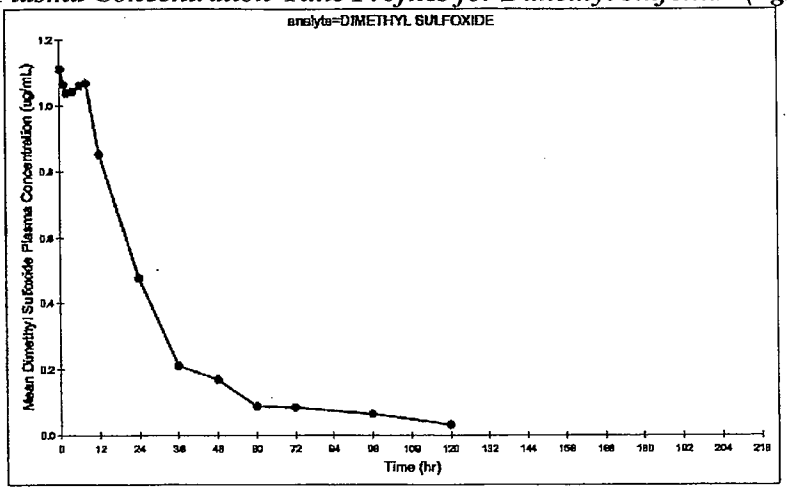
Results:

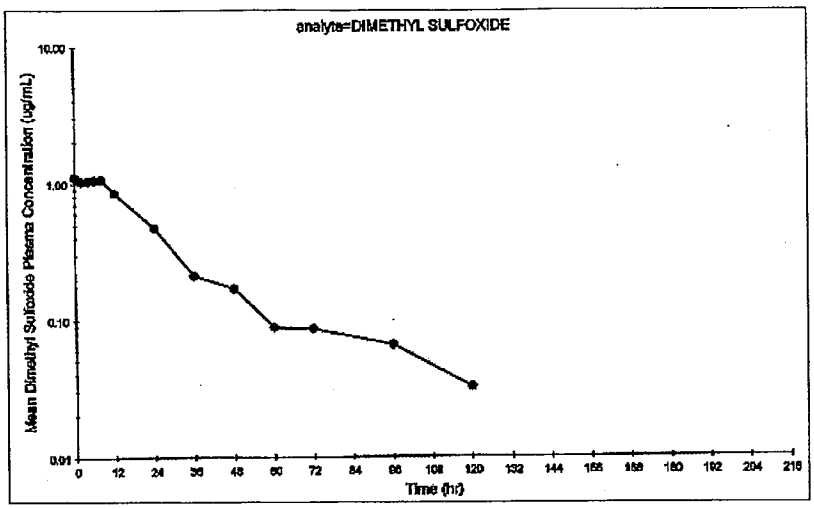
1. Figures:

Mean (SD) Plasma Concentration-Time Profiles for Diclofenac

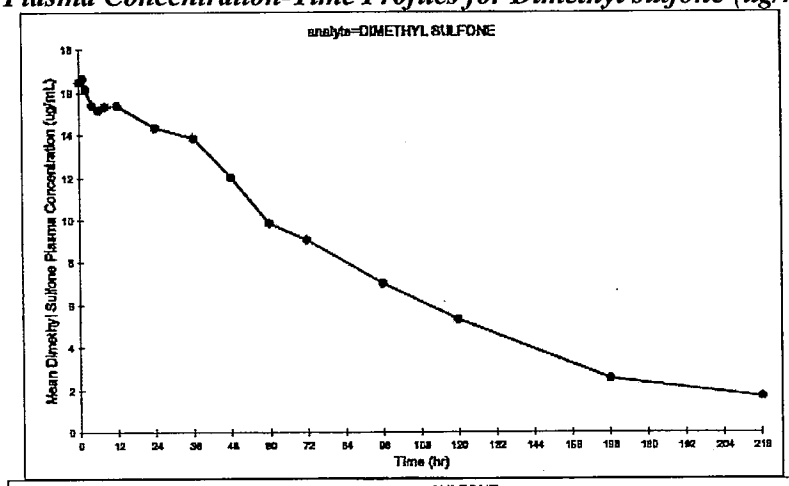


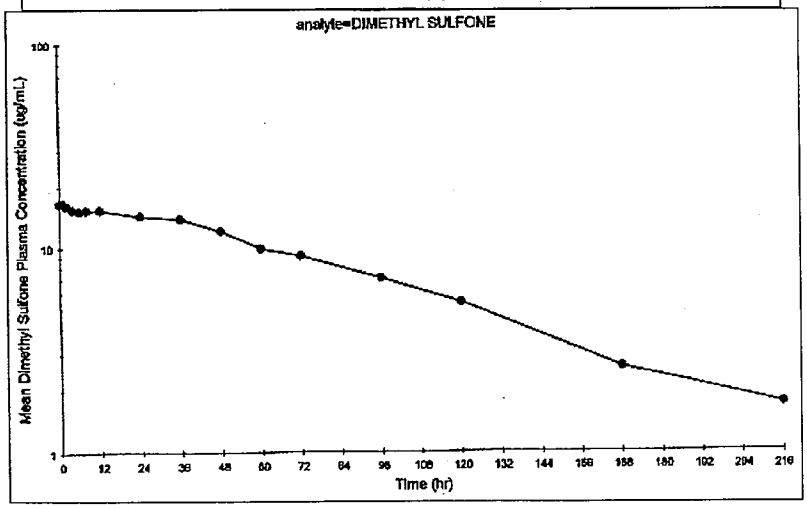
Mean (SD) Plasma Concentration-Time Profiles for Dimethyl sulfoxide (ug/mL)





Mean (SD) Plasma Concentration-Time Profiles for Dimethyl sulfone (ug/mL)





2. Tables

SUMMARY OF MULTIPLE-DOSE PHARMACOKINETIC RESULTS: DICLOFENAC SODIUM (for two-knee application)

			PENNSAID® TOPICAL SOLUTION
Pharmacokinetic Parameters			n = 19
			Mean ± SD
AUC0-t (ng.hr/mL)			695.398 ± 348.866
AUC 0-inf (ng.hr/mL)†			745.192 ± 374.740
Cmax		(ng/mL)	19.415 ± 9.326
Tmax		(hr)	4.005 ± 6.541
t1/2 (hr)†			78.972 ± 38.133
Kel (hr –1)†			0.0105 ± 0.0040
† n = 15			

SUMMARY OF MULTIPLE-DOSE PHARMACOKINETIC RESULTS: DIMETHYL SULFOXIDE (for two-knee application)

		PENNSAID® TOPICAL SOLUTION
Pharmacokinetic Parameters		n = 19
		Mean ± SD
AUC 0-t (mcg.hr/mL)		31.887 ± 15.520
AUC 0-inf (mcg.hr/mL)†		35.979 ± 15.419
Cmax	(mcg/mL)	1.206 ± 0.575
Tmax	(hr)	3.842 ± 3.468
t1/2 (hr)†		43.123 ± 22.968
Kel (hr –1)†		0.0213 ± 0.0124
†n = 13		

SUMMARY OF MULTIPLE-DOSE PHARMACOKINETIC RESULTS: DIMETHYL SULFONE (for two-knee application)

		PENNSAID® TOPICAL SOLUTION
Pharmacokinetic Parameters		n = 19
		Mean ± SD
AUC 0-t (mcg.hr/mL)		1525.292 ± 1065.245
AUC 0-inf (mcg.hr/mL)†		2339.190 ± 1276.555
Cmax	(mcg/mL)	18.033 ± 10.587
Tmax	(hr)	9.397 ± 13.341
t1/2 (hr)†		61.287 ± 18.376
Kel (hr –1)†		0.0123 ± 0.0039
†n = 11		

Discussion:

Diclofenac Sodium

Based on 19 completing subjects, the pharmacokinetics of diclofenac topical solution were assessed on Day 8, after 7 days of 4 daily administrations (40 drops four times a day). The diclofenac dose was 43 mg QID = 172 mg/day. On Days 6, 7 and 8, mean ±

SD pre-dose concentrations were 23.872 ± 19.192 ng/mL, 20.036 ± 14.963 ng/mL and 17.377 ± 7.174 ng/mL respectively. These values were not statistically different (p=0.0004), indicating achievement of steady state. On Day 8, C_{max} values (mean ± SD) were 19.41 ± 9.33 ng/mL and mean T_{max} values were 4.005 h. The AUC_{0-t} mean ± SD values were 695.398 ± 348.866 ng.h/mL. The mean (± SD) apparent terminal $t_{½}$ was 78.97 ± 38.13 h, mean ± SD AUC_{0-inf} was 745.192 ± 374.740 ng.h/mL.

Dimethyl Sulfoxide

Based on 19 completing subjects, the pharmacokinetics of DMSO after administration of diclofenac topical solution were assessed on Day 8, after 7 days of 4 daily administrations (40 drops four times a day). The DMSO dose was 1.3g QID = 5.2 g/day. On Days 6, 7 and 8, mean ± SD pre-dose concentrations were 1.292 ± 0.930 mcg/mL, 1.033 ± 0.667 mcg/mL and 1.111 ± 0.570 mcg/mL respectively. These values were not statistically different (p <0.0001), indicating achievement of steady state. On Day 8, C_{max} values (mean ± SD) were 1.206 ± 0.575 mcg/mL and mean T_{max} values were 3.842 h. The AUC_{0-t} mean ± SD values were 31.887 ± 15.520 mcg.h/mL. The mean (± SD) apparent terminal $t_{½}$ was 43.123 ± 22.968 h, mean ± SD AUC_{0-inf} was 35.979 ± 6.219 mcg.h/mL.

Dimethyl Sulfone

Based on 19 completing subjects, the pharmacokinetics of $DMSO_2$ after administration of diclofenac topical solution were assessed on Day 8, after 7 days of 4 daily administrations (40 drops four times a day). On Days 6, 7 and 8, mean ± SD pre-dose concentrations were 15.651 ± 1.652 mcg/mL, 16.684 ± 11.843 mcg/mL and 16.485 ± 10.389 mcg/mL respectively. These values were not statistically different (p <0.0001), indicating achievement of steady state.

On Day 8, C_{max} values (mean ± SD) were 18.033 ± 10.587 mcg/mL and mean T_{max} values were 9.397 h. The AUC_{0-t} mean ± SD values were 1525.292 ± 1065.245 mcg.h/mL. The mean (± SD) apparent terminal $t_{½}$ was 61.287 ± 18.376 h, mean ± SD AUC_{0-inf} was 2339.190 ± 1276.555 mcg.h/mL.

Platelet aggregation

For this study, ten subjects were randomly selected for platelet aggregation analysis (see Memos-to-File dated 12-18-2002 and 01-07-2003). Based on the results presented for this analysis, the average change from the addition of the aggregation agents ADP, collagen, epinephrine and arachidonic acid were 101.31%, 99.81%, 109.85% and 99.04%, respectively. These results indicate that there was no significant difference between baseline platelet aggregation results and the final platelet aggregation results obtained for the randomly selected subjects who were administered PENNSAID® TOPICAL SOLUTION. Therefore, the topical administration of PENNSAID® TOPICAL SOLUTION does not have a significant effect on platelet aggregation.

Subject Number	Visit	Date	% Aggregation			
			ADP	Collagen	Epinephrine	Arachidonic Acid
020	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		105.71	112.86	101.43	101.35
004	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		128.07	110.96	143.14	101.41
014	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		96.20	101.43	96.25	113.16
007	Baseline	14/01/2003				
	Final	21/01/20033				
	% Change		93.33	89.61	100.00	103.95
006	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		84.00	91.25	178.05	126.58
001	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		111.11	116.67	92.31	125.00
010	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		87.50	88.51	91.57	88.89
002	Baseline	14/01/2003				
	Final	21/01/20033				
	% Change		115.94	108.96	111.43	108.11
011	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		117.91	108.22	118.18	106.49
015	Baseline	14/01/2003				
	Final	21/01/2003				
	% Change		73.33	69.62	66.20	15.49
Summary						
Average % Change			101.31	99.81	109.85	99.04

b(4)

CONCLUSION:

The objective of this study was to describe the pharmacokinetics of diclofenac, DMSO and its metabolite $DMSO_2$ after multiple doses, using a topical solution. The treatment (40 drops, applied on the knee four times a day) was carried on for 7 days, and the pharmacokinetic profile was characterized on Day 8 after the 29th administration.

After PENNSAID® TOPICAL SOLUTION administration for 7 days, steady state was achieved on Day 6 (after 20 doses) for the 3 products analyzed. On Day 8, diclofenac remained measurable up to 360 hours post-dose in 11 subjects. The inter-subject variability was around 45-50% for C_{max} and AUCs. On Day 8, DMSO and $DMSO_2$ remained measurable up to 120 hours (10 subjects) and 216 hours (9 subjects) post-dose respectively. The inter-subject variability was around 45-50% for C_{max} and AUCs.

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

/s/

David Lee
11/13/2006 02:31:09 PM
BIOPHARMACEUTICS

Suresh Doddapaneni
11/13/2006 07:14:38 PM
BIOPHARMACEUTICS

Clinical Pharmacology/Biopharmaceutics Review

Diclofenac Sodium Lotion 1.5%
NDA 20-947 (resubmission)
PENNSAID™
Reviewer: E.D. Bashaw, Pharm.D.
WW

Dimethaid Research, Inc.
144 Steelcase Road W.
Markham, Ontario L3R 3JD
Submission Date:
Aug. 1, 2001

Review of a Re-Submission

Background

This NDA was originally submitted in 1998 and found to be non-approvable for clinical reasons. The drug product itself is a topical lotion containing 1.5% diclofenac as an active ingredient. While not classified as an active ingredient the formulation also contains —— dimethyl sulfoxide (DMSO), a solvent that has been rumored to, but never conclusively shown to possess anti-inflammatory properties of its own. This NDA was originally reviewed in 1998 by Dr. Dan Wang of the Division of Pharmaceutical Evaluation-III. At that time the NDA was found to be non-approvable on the basis of a lack of demonstration of clinical efficacy.

b(4)

In this re-submission the sponsor has re-submitted all of the original NDA data from the 1998 submission and the results of a new in vivo clinical study (RA-CP-109-US, see Attachment 1) in which pk samples were obtained. As in the previous trials no detectable plasma concentrations were seen in this study (0/46 samples). Also contained in this resubmission were the results of an in vitro skin permeation study that attempts to demonstrate the existence of a sub-stratum corneum depot for diclofenac (see Attachment 2). This data was submitted to address an issue noted by Dr. Wang regarding the low apparent topical bioavailability of diclofenac from their radiolabeled trial.

Conclusion

The results of the new in vivo clinical study with pk sampling are consistent with that seen previously. However, the poor choice of plasma sampling times (baseline, and study exit) precludes us from making definitive statements regarding the potential bioavailability of either diclofenac or DMSO. As for the in vitro data, while suggestive it still does not provide an answer to the question regarding the extent of depot effect and its impact on bioavailability estimates for diclofenac and DMSO.

Recommendation

Based on both the amount of diclofenac contained in this solution and the proposed conditions of use, no additional systemic pharmacokinetic information is needed relative to the diclofenac component of this product. However, the sponsor has yet to provide an adequate evaluation of the pharmacokinetics of DMSO following topical administration. As part of the clinical program for this product the sponsor should undertake a study

whereby both single dose and multiple dose pharmacokinetic sampling is undertaken for DMSO and its major metabolites with both an adequate number of samples and subjects. This study should be conducted prior to approval.

In order to address the issue of local (sub-stratum corneum) depot formation, the sponsor is encouraged to evaluate the uptake of DMSO and diclofenac into the different layers of skin using microdialysis.

E. Dennis Bashaw, Pharm.D.
Team Leader, Pharmacokinetics
HFD-880

Secondary Review: Arzu Selen, Ph.D., Deputy Director, DPE-III_____

Attachment 1

Study # RA-CP-109-US: A Double-Blinded, Vehicle-Controlled, Two-Way Parallel Clinical Trial To Confirm The Safety And Efficacy Of PENNSAID® In The Treatment Of The Osteoarthritic Knee.

Description of Pharmacokinetic Segment of Study:
Pharmacokinetic study of PENNSAID® Topical Solution (1.5% diclofenac sodium) -Determination of the concentrations of diclofenac sodium in plasma and DMSO in whole blood samples of patients treated with PENNSAID® and the control carrier lotion without diclofenac.

Investigators:

b(4)

Center for Analysis:

b(4)

Studied Period (years): 0.23 yrs
December 2000 (date of first enrolment); May 2001 (date of last analysis)

Objectives:
To determine the relative extent of transdermal absorption of diclofenac sodium and dimethyl sulfoxide (DMSO) into the blood following repeated dose, longer term topical application of PENNSAID® Topical Solution (1.5% diclofenac sodium).

Study Design:
Double blind, parallel study.

Number of Subjects:
46 total, 23 treated with PENNSAID®, 23 treated with Control-DMSO lotion.

Diagnosis and Criteria for Inclusion:
Patients with osteoarthritis of the knee(s). All were arbitrarily chosen for testing and gave blood samples.

Test Product, **Dose and Mode of Administration, Batch Number:** Topical administration: 40 drops of PENNSAID™ (about I mL) were applied by the patient, 10 drops at a time, to an area 10 cm proximal, 10 cm distal, 5 cm medial and 5 cm lateral to the center of the knee cap and distributed evenly without massage, 4 times daily.

PENNSAID® Topical Lotion [Batch A0004A]	Percentage
diclofenac sodium	1.5 %
dimethyl sulfoxide	45.5%
alcohol ———	
glycerin	
propylene glycol	
purified water	

b(4)

Duration of Treatment: 84 days (12 weeks).

Study Procedure and Endpoints:
Baseline blood samples were taken from the first 46 patients to be randomized, prior to treatment. Final blood samples were taken at the final visit. Diclofenac sodium level was measured in plasma via HPLC. DMSO level was measured in whole blood via LC MS/MS.

Statistical Methods:

The dates of the first and last application and number of days of application of PENNSAID® or Control-DMSO lotion were monitored. From this data conclusions were drawn regarding the levels of diclofenac sodium and DMSO after repeated topical application of PENNSAID® or Control-DMSO lotion.

Results and Conclusions:

Serum diclofenac sodium None of the patients in the PENNSAID® group had quantifiable concentrations of plasma diclofenac sodium (LOQ = 50ng/mL). Two patients in the Control-DMSO group had quantifiable plasma levels of diclofenac sodium. One patient (19004) was found to have been taking Voltaten® (diclofenac sodium) for 9 days prior to final visit and blood sample (as recorded on the CRF as a prohibited medication), accounting for the unexpected level of diclofenac in this patient's blood. The second patient (06007) was taking Vioxx® prior to being randomized on January 4, 2001 and resumed taking it on January 6, 3 days prior to final visit and blood sample on January 9, 2001. It is understood that metabolites of Vioxx® may interfere with the measurement of diclofenac sodium via the HPLC method used in this study. Thus, what appears to be diclofenac sodium in this patient's blood may actually be metabolites of Vioxx®.

Whole Blood DMSO
One patient (06-033) had a detectable level of DMSO at baseline, before application of the first dose of study lotion. The literature suggests that some foods contains substantial amounts of DMSO, particularly tea, coffee, tomatoes, etc. (see Section 5: Nonclinical Pharmacology and Toxicology Studies, Volume 7A, Page 190). Final level values ranged substantially and for the most part relate to the interval between last application and blood sample. One does note that the occasional patient had a low level detectable 1-2 following the final application. The highest levels occurred where the interval between last application and blood sample was just a few hours. The highest recorded value of ——————— would appear to be a 'peak' value and is hardly a significant level of exposure.

b(4)

These values do contrast with the values noted in study #106-96, a single-dose pharmacokinetic study where the peak values in two patients were less than 50 ngm/mL, suggesting that there may be minor accumulation of DMSO, but to insignificant levels of exposure.

Pennsaid® Study RA-CP-109-US Individual Patient Listing: Plasma Diclofenac Sodium Concentration: Baseline Final

Patient Number	Treatment	BASELINE (ng/ml)	Final (ng/ml)
02002	pennsaid	not detected	blq
05001	pennsaid	not detected	not detected
05002	control-dmso	not detected	not detected
06002	pennsaid	not detected	blq
06003	control-dmso	not detected	not detected
06004	pennsaid	blq	blq
06006	control-dmso	not detected	blq
06007	control-dmso	blq	———
06008	pennsaid	not detected	blq
06011	control-dmso	blq	not detected
06012	pennsaid	not detected	blq
06014	control-dmso	blq	blq
06015	pennsaid	not detected	blq
06016	control-dmso	not detected	not detected
06018	control-dmso	blq	blq
06019	pennsaid	blq	blq
06020	pennsaid	blq	blq
06023	control-dmso	blq	blq
06024	pennsaid	blq	blq
06025	control-dmso	not detected	not detected
06028	pennsaid	not detected	blq
06029	control-dmso	not detected	not detected
06031	pennsaid	not detected	not detected
06032	control-dmso	blq	not detected
06033	control-dmso	blq	blq
06036	pennsaid	blq	blq
06037	pennsaid	not detected	blq
08003	pennsaid	not detected	blq
09001	control-dmso	blq	blq
15001	control-dmso	not detected	not detected
15002	pennsaid	blq	blq
15003	pennsaid	blq	blq
15005	control-dmso	not detected	not detected
15007	pennsaid	blq	not detected
15009	control-dmso	blq	not detected
15012	control-dmso	not detected	not detected
15014	pennsaid	not detected	not detected
19002	pennsaid	not detected	blq
19003	control-dmso	not detected	not detected
19004	control-dmso	not detected	———
19005	pennsaid	not detected	not detected
19006	control-dmso	not detected	not detected
19007	pennsaid	blq	blq
19008	pennsaid	blq	not detected
19009	control-dmso	not detected	blq
19011	control-dmso	not detected	blq

b(4)

b(4)

*Protocol violators, see text.
N/A: Not Available. Not Detected: (4-10 ng/ml)- BLQ: Below limit of quantification 50ng/ml)

HPLC Assay Method		DICLOFE8.M			
Method name: The HPLC bioanalytical method for diclofenac in human plasma with UV detection					
Method code: ANA010517		Approval date: 01-05-18			
Column(s)/ temperature (if other than ambient):					
Mobile phase (specify gradient program if applicable):					
Detector /wavelength (if applicable):					
Flow rate:					
Injection volume:					
Reference solution conc. (absolute/percent): Preparation:					
Sample solution conc. (absolute/percent): Preparation:		1 mL of sample 1 mL of internal standard 3 mL of acetone			
Typical retention time:					
System suitability solution:		Same as Reference solution			
System suitability (tests and limits):		Peak ratio RSD ≤ 3 Retention Time RSD ≤ 3 Peak Symmetry ≤ 3 Resolution ≤ 2			
Method of quantification:		Method of integration is DICLOFE9.M we integrate manually the peaks that were not well integrated. We used internal standard for the quantification.			
Other information:		None			
HPLC Assay Method Validation					
Specificity					
Linearity	Number of concentrations Range (absolute/percent) Slope Y-Intercept Correlation coefficient (r^2)	9 50.0-5000.0 ng/mL 0.0001378074 0 0.9999			
Accuracy	Diclofenac (ng/mL) Number of replicates Accuracy	50 18 92.9%	150 18 96.6%	2000 15 93.9%	4000 18 94.2%
Precision - Repeatability	Conc.(s) (absolute/percent) Number of replicates %CV	50 18 -5.7%	150 18 -3.4%	2000 15 -6.1%	4000 18 -5.8%
Precision - Intermediate Precision (days/analysts/equipment)	Parameter (s) altered: Result (avg/rsd):	HPLC system/column			
Precision - Reproducibility (inter-laboratory if applicable)	Testing sites: Sample: Result (avg/rsd):	Not applicable			
Robustness	Stability of analytical solutions: Other variables/effect:	21 Days			
Copies of typical chromatograms may be found in Volume/Page:		Project Binder			
Company(s) responsible for method validation:					
Other information:		None			

b(4)

b(4)

Attachment 2

Table #21: Percentage Dose Absorbed Through and Into Human Skin *In Vitro* After Topical Application of Diclofenac Sodium in PENNSAID® or an Aqueous Solution

Formulation		Mean	SD
Single Dose			
2 µL PENNSAID® lotion (30µg/skin)	Receptor Fluid	11.39	11.62
	Total Skin Residue less the Stratum Corneum	10.97	7.83
	Stratum Corneum (=10 tape strips)	11.56	9.29
	Total Absorption (SC+Skin+RF)	33.92	22.32
	Total Recovery of Radioactivity	96.37	6.67
5 µL PENNSAID® lotion (75µg/skin)	Receptor Fluid	13.05	16.57
	Total Skin Residue less the Stratum Corneum	10.14	7.36
	Stratum Corneum (=10 tape strips)	11.82	6.47
	Total Absorption (SC+Skin+RF)	35.01	23.34
	Total Recovery of Radioactivity	92.83	18.58
2 µL Aqueous Diclofenac Sodium (30µg/skin)	Receptor Fluid	7.63	7.85
	Total Skin Residue less the Stratum Corneum	14.29	2.79
	Stratum Corneum (=10 tape strips)	5.84	2.83
	Total Absorption (SC+Skin+RF)	27.76	5.03
	Total Recovery of Radioactivity	107.24	22.71
2 µL Aqueous Diclofenac Sodium (75µg/skin)	Receptor Fluid	4.03	3.25
	Total Skin Residue less the Stratum Corneum	5.01	4.02
	Stratum Corneum (=10 tape strips)	7.03	3.20
	Total Absorption (SC+Skin+RF)	16.34	7.54
	Total Recovery of Radioactivity	110.57	15.12
Multi-Dose			
2 µL PENNSAID® lotion X 8 (240µg/skin)	Receptor Fluid	2.04	1.40
	Total Skin Residue less the Stratum Corneum	4.37	2.56
	Stratum Corneum (=10 tape strips)	10.32	5.01
	Total Absorption (SC+Skin+RF)	16.72	7.32
	Total Recovery of Radioactivity	100.74	7.09
5 µL PENNSAID® lotion X 8 (600µg/skin)	Receptor Fluid	1.93	1.88
	Total Skin Residue less the Stratum Corneum	6.34	3.99
	Stratum Corneum (=10 tape strips)	6.00	5.08
	Total Absorption (SC+Skin+RF)	14.27	6.89
	Total Recovery of Radioactivity	90.55	3.01
2 µL Aqueous Diclofenac Sodium X 8 (240µg/skin)	Receptor Fluid	0.32	0.29
	Total Skin Residue less the Stratum Corneum	1.08	0.61
	Stratum Corneum (=10 tape strips)	2.54	1.79
	Total Absorption (SC+Skin+RF)	3.94	1.20
	Total Recovery of Radioactivity	82.04	9.66
2 µL Aqueous Diclofenac Sodium X 8 (600µg/skin)	Receptor Fluid	0.97	0.68
	Total Skin Residue less the Stratum Corneum	1.41	0.91
	Stratum Corneum (=10 tape strips)	3.56	3.11
	Total Absorption (SC+Skin+RF)	5.94	3.16
	Total Recovery of Radioactivity	76.78	8.22

[Hewitt *et al.* 1997]

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

/s/

Dennis Bashaw
8/1/02 04:48:00 PM
BIOPHARMACEUTICS

Arzu Selen
8/1/02 05:05:33 PM
BIOPHARMACEUTICS

CLINICAL PHARMACOLOGY / BIOPHARMACEUTICS REVIEW

NDA 20-947	**SUBMISSION DATE:** 1/8/98, 3/31/98, 4/7/98, 4/29/98
PRODUCT: Pennsaid™ (diclofenac sodium) 1.5% topical lotion	
SPONSOR: Dimethaid Research Inc. 144 Steelcase Road W. Markham, Ontario L3R 3J9	**REVIEWER:** Dan Wang, Ph.D.
	TYPE OF SUBMISSION: Original

NDA 20-947 REVIEW

BACKGROUND

The applicant submitted an NDA for Pennsaid™, a topical lotion containing the NSAID diclofenac sodium in a patented carrier, for the localized treatment of osteoarthritic pain. Pennsaid™ was designed for transdermal delivery of diclofenac sodium to the site of pain without incurring significant systemic distribution and the attendant risk of adverse effects associated with oral NSAID therapy. Pennsaid™ contains dimethyl sulfoxide (DMSO) which enhances the penetration of materials through the skin and into underlying tissues by changing the structure of the stratum corneum and opening pores in the cell membrane. This product was developed by Dr. Ed Sandborn first in 1975 and then through trial and error the formulation was finalized. Though it has not been approved anywhere, by the early 1990's over 8000 prescriptions had been written by numerous physicians in Canada.

In this submission, two studies were included in Human Pharmacokinetic and Bioavailability section. Study #106-95/H832-11989-01 studied the pharmacokinetic behavior of diclofenac sodium and dimethyl sulfoxide (DMSO), including the rate and extent of transdermal absorption, the time of peak urinary excretion and the total urinary recovery with ^{14}C-diclofenac sodium. Study #102-93-1 was a phase III clinical efficacy trial. Blood from 10 patients was collected for diclofenac and DMSO analysis. This study was intended to roughly gauge whether there was any significant long term plasma accumulation of diclofenac sodium or DMSO, as compared to the results noted in Study #106-95/H832-11989-01. However, this study was not performed in compliance with Good Clinical Practices regulation, thus can not be used to support this NDA.

The sponsor has also provided some literature articles which studied the mechanism of action of topical NSAIDs, gender effect, age effect and effect of renal impairment on disposition of diclofenac sodium. These literature articles were also reviewed by the reviewer.

On April 29, 1998, the sponsor informed the Agency that the manufacturer for PENNSAID™ has been changed from ——————— to ———————. It is understood by the reviewer from the chemist that the manufacture site is the only factor involved in this change. b(4)

RECOMMENDATIONS

1. The applicant has adequately studied the pharmacokinetics of diclofenac sodium after topical application of PENNSAID™. See "**CONCLUSIONS**" section of the review for the results of the studies submitted and those from literature.

2. Since the manufacture site is the only change involved for the to be marketed formulation and PENNSAID™ is a ———— liquid, no additional *in vivo* pharmacokinetic study or *in vitro* release study are needed for the to be marketed formulation from clinical pharmacology and biopharmaceutics point of view. b(4)

3. The applicant should be informed of **COMMENTS #1** and **LABELING COMMENTS.**

TABLE OF CONTENTS Page No.

FORMULATION

PENNSAID™ Topical Lotion is a clear, colorless, ———— liquid. PENNSAID™ has the following quantitative composition indicating it is a ——— solution of diclofenac in DMSO: b(4)

INGREDIENT	CONCENTRATION (mg per g)
Diclofenac Sodium USP	15.0
Dimethyl Sulfoxide USP	455.0
Glycerin USP	b(4)
Propylene Glycol USP	
Ethanol USP	
Purified Water USP	
Total	*1000.0*

SUMMARY OF BIOAVAILABILITY STUDIES

1. STUDY #106-95/H832-11989-01

TITLE: Diclofenac Transdermal Bioavailability in Man

OBJECTIVES: This study was designed to determine the pharmacokinetic behavior of diclofenac sodium and dimethyl sulfoxide (DMSO) when applied topically in PENNSAID™. Parameters determined included the rate and extent of transdermal absorption, the time of peak urinary excretion and the total urinary recovery.

FORMULATION: Pennsaid topical lotion (lot #4063), a colorless liquid, was supplied by the sponsor. ^{14}C-diclofenac sodium ——————————, was supplied by ——————————— Radioactive PENNSAID™ was produced by adding ^{14}C-diclofenac sodium to the standard product to yield a specific activity of 50.36 μCi/g. b(4)

STUDY DESIGN: This is a single dose, non-blinded, single site, non-vehicle controlled study. Six healthy, non-smoking subjects, five men and one woman (4 white, 1 African-American, 1 native American), age ranging from 34-76 yrs (mean 48.7) were included in the study. The first two subjects were studied as a 'pilot' to determine the optimal times for blood and urine sampling. Diclofenac sodium pharmacokinetics were studied in all six subjects. DMSO pharmacokinetics were studied only in the first two 'pilot' subjects.

A single 1.0 ml dose of [^{14}C]-diclofenac PENNSAID™ was applied to a 200 cm^2 area (20 cm x 10 cm) of the left knee of each subject. After the application, the dosed area was kept clear of clothing for the first 10 hours of dosing, then clothing was allowed to touch the knee. The subjects were requested not to wash the dosed area for 24 hours. Twenty-four hours after dosing, the dosed site was washed using qauze pads, IVORY liquid soap and water. Individual qauze pads were placed in a borosilicate glass vial containing 10 ml of methanol to extract ^{14}C content.

Ten ml blood samples were collected at 15, 30, 45 minutes, 1, 1.5, 2, 3, 4, 5, 6, 8, 12, 24, 48, and

72 hours after dosing for 'pilot group'. Urine samples were collected at time 0, all individual voided urine within the first 24 hours, and all daily voided urine for 5 days. For the main group (the remaining four subjects), blood samples were collected at 1, 2, 4, 6, 8, 10, 24, 48, 72, 96 and 120 hours after dosing. Urine samples were collected prior to dose application (0), 0-2, 2-4, 4-6, 6-8, 8-10, 10-24, 24-48, 48-72, 72-96, 96-120 hours after application.

ASSAY: All radioactivity measurements were conducted using a Model 1500 Liquid Scintillation Counter (Packard Instruments). The counter was audited for accuracy using sealed samples of quenched and unquenched standards according to the instrument manual. Background control and test samples were counted in duplicate where possible for 3 or 5 minutes. The lower limit of detection was less than 0.15 ng/ml. Linear concentration range is 0.15 ng/ml - 150 μg/ml. The method is specific for diclofenac sample response corrected for background response. The lower limit of detection was 0.1 μg/mL for DMSO.

DATA ANALYSIS: Based on control and test sample counts, the percent of radioactivity recovered from the applied dose was determined. The equivalent of concentration of [^{14}C]-diclofenac was calculated from these values based on the specific activity and purity of [^{14}C]-diclofenac, plus non-labeled diclofenac in Pennsaid topical lotion. Individual plasma total radioactivity, expressed as ng equivalents of 14C-diclofenac, were utilized to construct total radioactivity-time profiles and for calculation of noncompartmental pharmacokinetic parameters.

Noncompartment PK parameters from plasma levels of each subject versus time data were determined by using the program PCNONLIN. These parameters included Cmax, Tmax, AUC(0-last), and $t_{1/2}$.

RESULTS:

The 'pilot' group was studied first and the result of the study was used to adjust the study design for the main group. Blood and urine were analyzed for the 'pilot' group and it was discovered that the radioactive purity of the diclofenac had apparently deteriorated to well below 90%. The diclofenac sodium was then re-purified to a radioactive purity of 99.0% and was then used in a new batch of PENNSAID™ for the remaining 4 'main study' subjects. Only the results from 'main group' were used to calculate pharmacokinetic parameters (AUC, Cmax and percent of dose recovered from the urine).

The results from the 'pilot' group showed that the radioactivity recovered from skin after 24 hour exposure period was 18.82 ± 0.63% of the administered dose. The peak urinary excretion was reached at 48-72 hour period. The total urinary radioactivity recovery was 3.50% and 2.46% of administered dose for subjects 1 and 2 respectively. The plasma levels for subjects 1 and 2 are shown in the figure below along with those for subjects of 'main group'.

Diclofinac plasma concentration profile

b(4)

Because of observed long absorption phase (tmax of 24 and 48 hours for subjects 1 and 2, respectively) and long half-life, the sampling period for the subjects in 'main group' was extended from 72 to 120 hours.

The pharmacokinetic parameters determined from plasma concentration-time profile for the 'main group' are summarized in Table 26 (APPENDIX). The mean $AUC_{0\text{-}120hr}$ (SD), Cmax (SD), and Tmax (SD) values are 717.64 (123.98) ng eq-hr/ml, 11.80 (4.23) ng eq/ml, and 30 (12) hrs, respectively. The sponsor also calculated the terminal half-life using PCNONLIN. The mean half-life determined by the sponsor was about 70 hours (including both 'pilot' and 'main' groups). Given the mean tmax of 30 hours, the observed terminal phase were less than one half-life for the 'pilot group' and a little more than one half-life for the 'main group', assuming the disposition follows one compartment model. Therefore, the estimates for terminal phase half-life may not be reliable. The reviewer double checked the $t_{1/2}$ calculation using WinNonlin. Except for subject 3, none of the $t_{1/2}$ values reported by the sponsor can be obtained using WinNonlin using any reasonable or non-reasonable combination of terminal phase data points. For subject 3, the $t_{1/2}$ of 117.4 was obtained by using only the last two points (t = 96 and 120hr). This is considered not appropriate by the reviewer after examining the data. The reviewer re-calculated the $t_{1/2}$ using WinNonlin. The results are summarized below. It was initially thought by the reviewer that the $t_{1/2}$ obtained from the 'pilot group' should not be included in the calculation of mean value because of the long $t_{1/2}$ and relatively short sample collection period. However, since the $t_{1/2}$ values obtained from the two subjects of the 'pilot group' were very similar to those of the 'main group', they were also included when calculating the mean value for $t_{1/2}$.

The $t_{1/2}$ values calculated by the sponsor and reviewer:

	subject 1	subject 2	subject 3	subject 4	subject 5	subject 6	mean (SD)
Sponsor	31.01	37.23	117.40	73.13	74.73	88.40	70.32 (32.28)
Reviewer	66.79	40.67	41.39	31.43	91.51	48.13	53.32 (22.13)

The reviewer believed that the $t_{1/2}$ calculated by the reviewer should be used as an estimate for $t_{1/2}$ of PENNSAID™.

The following table summarized the percentage of radioactivity recovered from skin wash and

urine of the 'main group' based on total radioactivity.

	Subject 3	Subject 4	Subject 5	Subject 6	Mean (SD)
Skin wash	20.93	15.49	36.18	31.61	26.05 (9.51)
Urine	3.70	5.29	1.61	5.52	4.03 (1.81)

The earliest urinary radioactivity appeared at 0-2 hour collective period after topical dosing. The peak urinary excretion was reached at 24-48 hour period for all subjects in the 'main group'. The value of total urinary radioactivity recovery was 4.03% of administered dose by 120 hours after dosing. The percentage of dose absorbed is calculated by the following equation:

Percent Dose Absorbed = (^{14}C urinary excretion $_{topical}$)/(^{14}C urinary excretion $_{iv}$)*100

The IV dose is assumed to be 100% bioavailability due to injection, and the above equation accounts for ^{14}C excretion by other body routes (feces, CO_2) or retained in the body. Based on literature report, 61% of diclofenac sodium administered to man is excreted in urine. Given this, the percent dose absorbed after topical PENNSAID™ administration is (4.03/61)*100 = 6.61%. Skin surface wash of the knee after 24 hour dosing recovered 26.05% dose. Thus, dose accountability is 32.66% dose. The sponsor indicated that since the topical application is non-occlusive and without protection for 14 hours after dosing, it was assumed that the amount of remainder of the dose was lost to clothing and bedding. This assumption is considered reasonable by the reviewer.

No DMSO was found in the plasma of two patients in 'pilot group', taken 15 minutes and 24 hours after application of PENNSAID™, with a lower detection limit of 0.1 μg/mL.

2. STUDY #102-93-1

TITLE: A double-blind, placebo controlled, three-way parallel study of the safety and efficacy of PENNSAID™ treatment of osteoarthritic knee

The sponsor indicated in the submission that this study was not performed in compliance with Good Clinical Practices regulation. Thus, this study can not be used to support this NDA. The following is a brief summary of the study.

This was a phase III clinical efficacy trial. Patients had applied 1 ml of randomly assigned study lotion 4 times a day for 42 days. Blood samples were taken at the first visit and at the exit visit from volunteer subjects at each site. This was intended to roughly gauge whether there was any significant long-term plasma accumulation of diclofenac sodium or DMSO, as compared to the results noted in Study #106-95/H832-11989-01. The mean time between last dose of lotion and blood sampling was 11.2 hours. Synovial fluid samples were obtained at the exit visit from 2 volunteer patients at 13 hours and 6.9 hours following the last application of PENNSAID™.

After unblinding, blood from 10 patients and synovial fluid from 2 patients determined to have used the active PENNSAID™ was analyzed for diclofenac sodium by GC/MS with a lower limit of quantification of 10 ng/ml. The blood samples were also analyzed for DMSO by GC with a lower limit of quantification of 1.0 μg/ml.

It was concluded that after 42 days of treatment with approximately 1.0 ml of PENNSAID™, four times a day that, 1) the plasma concentrations of diclofenac sodium in 9 patients measured from 1-25 hours to 37 hours (mean=12.9) hours and from one patients measured at 20 days after the last application of PENNSAID™ were less than 10 ng/ml (under the limit of quantification); 2) the synovial fluid concentrations of diclofenac sodium from 2 patients taken 6.9 hours and 13 hours after the last application of PENNSAID™ were less than 10 ng/ml; 3) the blood concentrations of dimethyl sulfoxide in 5 patients, measured from 2.75 hours to 37 hours (mean=14.2 hours) and from one patient measured at 20 days after the last application of PENNSAID™, were less than 1.0 μg/ml (under the limit of quantification).

LITERATURE REPORTS

1. Percutaneous Absorption

Percutaneous absorption of diclofenac has been previously reported for various diclofenac topical formulations. In the article "*Percutaneous Absorption of Diclofenac*", 1986, W. Riess et al. reported that after applying 1.16% Voltaren Emulgel (5mg/cm^2) to non-occluded skin and left in place for 12 hours, the percutaneous absorption in man amounted to 6% of the dose applied. The pattern of the diclofenac metabolites in human urine was identical after topical and oral administration. In man daily topical application of the 1% Voltaren cream (10 mg/cm^2) is doses of 2.5 g t.i.d. yielded steady state plasma levels of diclofenac of 20 - 40 nmol/l (6.36 - 12.7 ng/ml, MW=318.13). After the Voltaren Emulgel had been applied to the hands of 8 arthritic patients for 3 day in a dosage of 2.5 g q.i.d., diclofenac was found to be present in concentrations of ≥ 6.36 ng/ml in the plasma, of ≥ 117.7 ng/ml in the synovial fluid, and of ≥ 130.4 ng/g in the synovial tissue. The authors claimed that the topical application of diclofenac over an inflamed joint resulted in synovial fluid drug concentrations which exceeded plasma concentrations suggesting a direct penetration of the drug into the joint. However, a different result was obtained from another study.

In article "*Diclofenac concentrations in synovial fluid and plasma after cutaneous application in inflammatory and degenerative joint disease*"(J. Radermacher et a., Br. J. Clin. Pharmac (1991), 31), 10 patients with bilateral knee joint effusions were treated topically with a gel containing 1% diclofenac (80 mg t.i.d.). They were randomized to receive diclofenac gel to one knee and a placebo gel preparation to the other knee. It was observed that plasma concentrations of total drug were higher than synovial fluid concentrations in both knees (40.6±4.7 ng/ml vs. 25.5±3.6 (diclofenac gel) and 21.6±2 ng/ml (placebo gel), respectively). The free drug concentrations were essentially the same (113.3±22.5 pg/ml vs. 115.5±16.3 and 98.8±11.6 pg/ml). It was

indicated by the author that the small difference in diclofenac concentrations between the two knees indicated that any direct transport of across the skin accounted for only a small fraction of drug reaching the joint; at least 85% of the dose absorbed must penetrate via the systemic circulation. The small difference might have been due to direct contamination despite the precautions taken. Efficacy end points have also been evaluated in this study. There was no difference in the placebo treated and diclofenac gel-treated knees throughout the study with regard to maximal knee flexion. However, both placebo and diclofenac gel treated knees showed significant improvement of mobility. Joint circumference decreased significantly during the period of investigation in both knees, but there was no significant difference between knees. The authors concluded that transcutaneous application of diclofenac gel to knee joints gave rise to plasma and synovial fluid diclofenac concentrations which were lower than those observed after oral or parenteral administration of the drug. Local accumulation of diclofenac by direct transport or diffusion into the knee joint seemed unlikely.

2. Gender Difference

"*Percutaneous absorption of diclofenac in healthy volunteers after single and repeated topical application of Diclofenac Emulgel*", A. Sioufi et al., Biopharmaceutics & Drug Disposition, Vol 15, 1994. It has been reported in this article that comparison between the results obtained with 5 men and 5 women revealed no distinct differences related to gender with regard to the drug disposition.

3. Patients with impaired renal function

"Pharmacokinetics of diclofenac sodium (Voltaren) and metabolites in patients with impaired renal function", H. Stierlin et al, Scand J Rheumatology, Suppl. 22, 1978. The results of this study show that diclofenac sodium is extensively metabolized. The plasma concentration of unchanged diclofenac is not increased when renal function is reduced. The plasma concentration of total diclofenac metabolites, on the other hand, tend to be higher in patients with impaired renal function than in normal subjects. The authors indicated that these metabolites were largely present in conjugated form. Conjugation usually reduces pharmacological activity to a considerable extent, so that no accumulation of pharmacologically active substances is to be expected. Patients with renal insufficiency may therefore be given the same doses of diclofenac sodium as patients with normal renal function.

4. Age effect

"Pharmacokinetic studies on diclofenac sodium in young and old volunteers", J.V. Willis et al, Scand J Rheumatology, Suppl. 22, 1978. A single 50 mg enteric-coated tablet of diclofenac sodium (Valtaren) was administered to two groups, each consisting of eight female subjects. The subjects in one group were young adults all aged less than 22 years, while those in the other group were all above 62 years. The results show that the plasma concentration-time profiles, relative bioavailability, and urinary excretion pattern of the drug in the two groups were similar.

Thus, in the absence of interacting factors, such as drugs and disease, the ability to absorb, metabolize, and excrete diclofenac sodium does not appear to be influenced by age.

CONCLUSIONS

1. Low plasma levels of total radioactivity were obtained after application of PENNSAID™ to the knee. The mean $AUC_{0\text{-}120hr}$ (SD), Cmax (SD), and Tmax (SD) values are 717.64 (123.98) ng-hr/ml, 11.80 (4.23) ng/ml, and 30 (12) hrs, respectively.

2. The half-lives reported by the sponsor were found not to be the best estimates from the available data. The reviewer recalculated the half-lives using WinNonlin. The mean half-life was 53.32 ± 22.13 hours. However, due to the long half-life and relatively short period of sample collection, the half-life estimated from this study may not be reliable. It should also be noted that this half-life represent the elimination half-life of the total radioactivity (including both diclofenac and its metabolites).

3. After 24 hour application of PENNSAID™ to the knee, the percentage of the applied dose of diclofenac sodium that was systemically absorbed was 6.0 ± 1.5% based on urinary recovery of total radioactivity up to 120 hours. The actual percentage absorbed should be more than 6.0% but less than 8% since PENNSAID™ has a long half-life of about 53 hours.

4. No dimethyl sulfoxide was found in the plasma of two patients, taken 15 minutes and 24 hours after application of PENNSAID™, using a GC/MS method with a lower limit of detection of 0.1 μg/ml.

5. The mechanism of how diclofenac sodium is absorbed into systemic circulation has not been studied by the sponsor. Percutaneous absorption of topical diclofenac has been reported for Valtaren Emulgel. In one study, much higher diclofenac levels were observed in synovial fluid and tissue than in plasma suggesting a direct penetration of the drug into the joint. However, in another study, similar diclofenac levels were obtained from plasma and synovial fluid. The diclofenac levels in synovial fluid were also similar for those taken from the knee treated with Valtaren Emulgel and the knee treated with placebo. This suggests a penetration via the systemic circulation.

6. It has been reported in the literature that dose adjustment for gender, age and impaired renal function is not necessary.

COMMENTS (need to be sent to the sponsor)

1. The sponsor indicated in the submission that the half-lives of diclofenac sodium following application of PENNSAID™ were calculated using PCNONLIN. The reviewer examined this calculation using WinNonlin and the same result could not be obtained by using any

reasonable combination of terminal phase data. The mean half-life obtained by the reviewer was 53.32 ± 22.13 hours. It should be noted that the half-life estimated from this study may not be reliable due to the long half-life and relatively short period of sample collection.

LABELING COMMENTS

1. In **Pharmacokinetics** section of the labeling, the pharmacokinetic parameters are summarized in Table 1. These parameters were obtained from STUDY #106-95 using all 6 subjects studied. However, in the study report for STUDY #106-95, the sponsor indicated that for the 'pilot' group (n=2) the radioactive purity of the diclofenac had apparently deteriorated to well below 90%. The results from these two subjects were only to be used to determine the optimal times for blood and urine sampling for the 'main' group. The diclofenac sodium was re-purified to a radioactive purity of 99.0% and was then used in a new batch of PENNSAID™ for the remaining 4 'main' group subjects. The sampling time of 72 hours used for the two 'pilot' subjects were found to be too short to define PK parameters considering the long half-life of diclofenac following topical administration. The sampling time were extended to 120 hours for the 'main' group. Only the results from the 'main' group were used to calculated pharmacokinetic parameters. The reviewer believes that the PK parameters summarized in Table 1 should be consistent with those in the study report for STUDY #106-95. In addition, the half-life of diclofenac following topical application of PENNSAID™ 1.5% should be 53.32 ± 22.13 hours. The following table should be included in the labeling:

TABLE 1: PENNSAID™ Pharmacokinetic Parameters determined from total radioactivity

PK Parameters	Normal Healthy Adults* [n=4] (Age: Mean 55 years Range: 45-76)
Systemic Bioavailability (%)	6.61 ± 2.96
Plasma Cmax ** (ng eq/ml)	11.80 ± 4.23
Plasma tmax** (hr)	30 ± 12
Total Urinary Recovery** (%)	4.03 ± 1.81

*1 Caucasian female, 2 Caucasian males and 1 Native American male
** measured as total radioactivity

2. In **Pharmacokinetics** section of the labeling, under ***Metabolism***, it is stated that ——— ——— (as oral administration). The sponsor should provide reference for this statement. b(4)

8/17/98
Dan Wang, Ph.D.
Division of Pharmaceutical Evaluation III

FT initialed by E. Dennis Bashaw, Pharm.D. EB 8/17/98

cc:
NDA 20-947(Original)
HFD-550(lutwak)
HFD-880(Division file)
HFD-880(Bashaw)
HFD-880(Wang)
HFD-344(Viswanathan)
CDR: Attn: Barbara Murphy

DIV

FEB 5 1998

CLINICAL PHARMACOLOGY / BIOPHARMACEUTICS REVIEW

NDA 20-947	**SUBMISSION DATE:** 1/8/98
PRODUCT: Pennsaid™ (diclofenac sodium) 1.5% topical lotion	
SPONSOR: Dimethaid Research Inc. 144 Steelcase Road W. Markham, Ontario L3R 3J9	**REVIEWER:** Dan Wang, Ph.D.
	TYPE OF SUBMISSION: Original

45-Day Fileability Review

BACKGROUND

The applicant submitted a NDA for Pennsaid™, a topical lotion containing the NSAID diclofenac sodium in a patented carrier, for the localized treatment of osteoarthritic pain. Pennsaid™ was designed for transdermal delivery of diclofenac sodium to the site of pain without incurring significant systemic distribution and the attendant risk of adverse effects associated with oral NSAID therapy. Pennsaid™ contains dimethyl sulfoxide (DMSO) which enhances the penetration of materials through the skin and into underlying tissues by changing the structure of the stratum corneum and opening pores in the cell membrane. This product was developed by Dr. Ed Sandborn first in 1975 and then through trial and error the formulation was finalized. Though it has not been approved, by the early 1990's over 8000 prescriptions had been written by numerous physicians in Canada.

In this submission, two studies were included in Human Pharmacokinetic and Bioavailability section. Study #106-95/H832-11989-01 studied the pharmacokinetic behavior of diclofenac sodium and dimethyl sulfoxide (DMSO), including the rate and extent of transdermal absorption, the time of peak urinary excretion and the total urinary recovery with ^{14}C-diclofenac sodium. Six patients participated the study. The results showed average Cmax was 9.7 ng/mL and occurred around 32 hours after the application of Pennsaid. Diclofenac transdermal bioavailability was found to be 6%. Study #102-93-1 was a phase III clinical efficacy trial. Blood from 10 patients was collected for diclofenac and DMSO analysis. The lower limit of quantification was 10 ng/mL for diclofenac and 1.0 µg/mL for DMSO. The mean time between last dose and lotion and blood sampling was 11.2 hours. The results showed that both diclofenac sodium and DMSO levels were below the limit of quantification. Synovial samples from 2 patients were taken 13 hours and 6 hours 55 minutes following the last dose and analyzed for diclofenac sodium. These samples were found to contain less than 10 ng/mL. Please also see attached for study summary table.

RECOMMENDATION

1. NDA 20-947 is fileable to the Division of Drug Evaluation III, OCPB.

2. The applicant should provide the full report for Study #102-93-1 including assay