

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:
20-947

PHARMACOLOGY REVIEW(S)



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

**Supervisory Pharmacologist Memorandum
ADDENDUM**

NDA NUMBER:	20-947
SERIAL NUMBER:	000 (3 rd Cycle)
DATE RECEIVED BY CENTER:	February 4, 2009
PRODUCT:	
(Proposed) Trade Name:	PENNSAID® Topical Solution
Established Name:	1.5% w/w diclofenac sodium
INDICATION:	Signs and symptoms of osteoarthritis of knee(s)
SPONSOR:	Nuvo Research, Inc.
DOCUMENTS REVIEWED:	SD-83 (September 10, 2009); Review addendum
REVIEW DIVISION:	Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)
PHARM/TOX REVIEWER:	Lawrence S. Leshin, DVM, Ph.D.
PHARM/TOX SUPERVISOR:	Adam Wasserman, Ph.D.
DIVISION DIRECTOR:	Bob Rappaport, M.D.
PROJECT MANAGER:	Jessica Benjamin

ADDENDUM

For general information please see prior Supervisory review for detailed background and the primary review from Dr. L. Steven Leshin. This addendum is focused on the narrow question of whether lymphomas observed in the 26-week rat toxicology study of the penetration enhancer dimethyl sulfoxide (DMSO) used to support its inclusion in the drug product constitutes a “signal” warranting pre-approval evaluation of carcinogenicity. Although novel excipients (by identity, route, or high level) are subject to nonclinical requirements to demonstrate safety for human use, DMSO was identified as a concern warranting significant safety information at the conclusion of the 2nd cycle and the Applicant was informed in a teleconference of 2007 held with the Division Director Dr. Bob Rappaport that if the chronic toxicology studies conducted in a rodent and non-rodent model do not reveal a “signal” for tumorigenesis, a dermal carcinogenicity study – which the Sponsor was to have started – could be completed post-approval.

As has been clearly reported in the original review of the study in Dr. Leshin’s review of the 3rd cycle submission and his addendum to this review (as well as my original Supervisory memo) the Sponsor reported findings of malignant lymphoma in 2 females; 1 at the low dose (LD; 9%) and 1 at the mid-dose (MD; 45.5%) DMSO. There were no instances of animals with lymphoma at the high-dose (HD; 90%) and no similar findings in treated males or control animals of either gender. The rarity of the finding in a 6-month study in this strain, despite the lack of dose-response or observation in both genders was considered sufficiently unusual to be considered a “signal” by both Dr. Leshin, myself, and ultimately by the Associate Director for Pharmacology/Toxicology Dr. Paul Brown who wrote a memo of concurrence.

The Applicant was made aware that we were not convinced this did not represent a signal for tumorigenesis. They responded in serial fashion with multiple submissions to address our concern (see Dr. Leshin’s table of submissions and their content in the addendum to his review). After review of these initial submissions, they were informed the nonclinical discipline had reached the final decision to recommend a Complete Response until the dermal carcinogenicity study was completed that would provide evidence in the animal model for of a lack of carcinogenic potential for DMSO.

The Applicant subsequently engaged the laboratory conducting the original 26-week study _____ as well as an expert Pathology Working Group to review and perform additional analyses on the tissue sections to support their position that the observations of lymphoma while unusual represented no more than incidental background findings of a spontaneous nature. Dr. Leshin has detailed these in his addendum but for convenience I will summarize his evaluation here:

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1) *Re-examination of tissues by —*

- Thymomas (epithelial origin) which were unreported in the original study (only control and HD groups received a full evaluation) were noted in two LD males, one benign and one malignant. Later review by the Pathology Working Group (described below) only examined control and HD groups and the animals previously identified by — with tumors. No other notable findings were revealed in any other group. The Applicant considers these tumors to represent an incidental finding due to lack of dose response and presence in only one gender. Dr. Leshin considers the observation of two additional rare tumors (though unrelated to lymphoma) to add weight to the concerns of a “signal”.

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2) *Tissue-type testing*

- Lymphomas identified in the two animals were evaluated for cell-type by immunohistochemical methods revealing one was a T-cell lymphoma while the other was unidentified by T- or B-cell-specific antibodies suggesting these lymphomas were of different cell origin and subsequent examination demonstrated a different tissue distribution between the two animals; these differences according to the Applicant further reduces the likelihood this represents a “signal”. Dr. Leshin agrees that this data suggests the lymphomas may have arisen from different cell types which would naturally have a different distribution but does not agree that a carcinogen must necessarily produce the same tumor in the same tissue in different animals.

3) *Genetic testing*

- The Applicant has stated they are conducting genetic testing on the two rats to see if they were siblings but as of this submission the results have not been provided. Dr. Leshin does not believe this likely will be a compelling argument if not combined with evidence the other non-tumor-bearing animals are not genetic siblings.

4) *Pathology Working Group (PWG) evaluation and final independent assessment*

- The Applicant formed a committee of six notable veterinary pathologists and scientists — many of whom have been employed by or served on Advisory Committees for — and are recognized experts in toxicologic pathology and authorities on chemical carcinogenesis. The PWG read coded slides blinded to treatment and identified the 4 tumors identified by — in 4 separate animals and verified the lack of pre-neoplastic or proliferative response in these tissues from all other animals in treated or control groups. The PWG concluded the malignant lymphomas as well as the epithelial thymomas were incidental spontaneous neoplasms unrelated to treatment with

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DMSO based principally on complete lack of dose response and absence of target organ toxicity and pre-neoplastic lesions and therefore do not represent a signal for potential carcinogenicity. Upon completing the report, an independent evaluation of the collected analyses was reviewed by another noted expert in toxicologic pathology _____ who concurred with this opinion. Dr. Leshin takes issue with some of the PWG peripheral arguments (i.e. genotoxic data on DMSO and metabolites) and believes it difficult to confidently conclude there is a lack of a potential carcinogenicity signal given the occurrence of 2 more tumors uncovered in the subsequent evaluation (epithelial thymomas) within this 6-month treatment period though he agrees the lack of dose-response and very high exposure levels achieved (58X – 623X human) in all treatment groups renders any finding, if true, of questionable relevance to human therapeutic use of Pennsaid. In Dr. Leshin's opinion, the observation of 4 young DMSO-treated rats in a 26 week study with tumors is improbable and that, though the signal is weak, this warrants the continued recommendation for non-approval until definitive data can be obtained from the dermal carcinogenicity study currently underway.

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I agree with Dr. Leshin that it will be of questionable usefulness to document the genetic relationship between the animals with lymphoma – which has not been provided at this time in any event – and it is difficult to confidently extrapolate carcinogenic potential from a 26-week treatment period (which is why this information does not suffice for a carcinogenicity evaluation in the first place). The lack of dose-response could look quite different at the end of 12- or 24-months of treatment. However, the narrow issue involved is whether or not the lymphomas, and now epithelial thymomas, represent a “signal” of potential carcinogenicity. The Applicant has assembled an impressive panel of experts in carcinogenesis on their own initiative, several of whom the Agency would likely have chosen to be independent experts on an Advisory Committee on this subject. The tissues from the study were read blind – unlike the original evaluation – and the panel was in complete agreement as to the findings and, combined with a number of additional arguments well described by Dr. Leshin, unanimously concluded these represented incidental and spontaneous findings and collectively was not a signal. The confirmation by another independent expert of the overall findings adds weight to what I believe already was an excellent and thorough evaluation of the study results by the Pathology Working Group. Therefore, upon consideration of these additional analyses and evaluations combined with arguments made previously by the Applicant – principally the lack of dose-response and absence of proliferative or pre-neoplastic findings, I do not agree with Dr. Leshin that the single incidence of lymphoma in a low- and mid-dose female and epithelial thymoma in 2 low-dose males represents a “signal” for potential carcinogenicity of DMSO in the 26-week toxicology study.

Carcinogenicity studies are normally required prior to approval; however, the prior agreement reached with the Applicant was that the ongoing dermal carcinogenicity study could be completed post-approval in the absence of a signal from chronic toxicology

studies. Based on the present understanding of the data, the carcinogenicity study will not be required pre-approval. Additionally, the information available suggests the risk to humans with approval of this product in the absence of a definitive animal carcinogenicity bioassay for DMSO is low due to the absence of pre-neoplastic lesions from chronic minipig and rat toxicology studies at DMSO exposures far in excess of the maximum in human as well as the lack of demonstrated genotoxicity. If the application is approved on this cycle, the dermal carcinogenicity study should be considered a Post-Marketing Requirement and will be completed within several years of initial marketing. Should the dermal study reveal tumors thought to be related to the DMSO vehicle the Agency can take appropriate action while still relatively small numbers of individuals have been exposed for a relatively brief period.

It is noted that the Applicant has added additional animals to the ongoing dermal carcinogenicity study of DMSO that provides a dose range that will explore toxicity at exposures more relevant to human use. As I am now in agreement with the Applicant that the lymphomas do not represent a "signal" of concern, the Applicant may terminate this aspect of their study without providing the data to the Agency; in addition, it is not certain what degree of confidence a "negative" finding would have provided.

Additionally, further rationale has been provided by the Applicant in regards to the risk of label leachables with handling of the drug product. Please see the CMC Review #5 of Dr. Olen Stephens (6-AUG-2009) for detail. Dr. Stephens considered their argument that the finding on stability testing that approximately _____ and over 3 years "smudged" the label which was otherwise legible was evidence that the levels of leachables under this long-duration at realistic temperatures was significantly more relevant than the extraction study done under strong conditions which removed all writing from the label. The CMC discipline considers this risk addressed when used according to the label and no follow up evaluation is considered warranted. Although the argument provided does not contain quantitative information as to compound levels which may leach from the label with repeated use, especially when not used according to the label (i.e. without hand-washing between applications), I believe this concern is speculative enough that it should not preclude approval. The Applicant can be asked to address this risk as a post-approval commitment to conduct a more representative leachable study and the data generated from that evaluation will dictate whether further regulatory action is warranted.

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Regulatory Recommendation: Approval

Additional Studies Recommended:

1. Completion of the ongoing dermal carcinogenicity study as a Post-Marketing Requirement. The Applicant may be informed that the 6-month interim evaluation is considered unnecessary and the groups may be terminated without evaluation.

2. The Applicant should, as a post-marketing commitment, conduct a leachable study of the drug container or label under conditions more representative of product use in order to evaluate levels which may be liberated over the duration of use.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI

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

/s/

ADAM M WASSERMAN
10/05/2009

PAUL C BROWN
10/06/2009

I concur with the conclusions of the supervisory pharmacologist and with the regulatory recommendation for approval.



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

ADDENDUM TO JULY 23, 2009 REVIEW OF NDA 20-947

NDA NUMBER:	20-947
SERIAL NUMBER:	000 (Complete Response to Deficiencies)
DATE RECEIVED BY CENTER:	Feb 4, 2009
PRODUCT:	PENNSAID® Topical Solution (1.5% w/w diclofenac sodium)
INTENDED CLINICAL POPULATION:	Topical treatment for relief of the signs and symptoms of osteoarthritis of the knee(s)
SPONSOR:	Nuvo Research Inc.
DOCUMENTS REVIEWED:	SD-74, June 12, 2009 SD-75, June 26, 2009 SD-76, July 13, 2009 SD-77, July 14, 2009 SD-78, July 24, 2009 SD-79, July 31, 2009 SD-80, Aug 12, 2009 SD-81, Aug 18, 2009 SD-82, Aug 21, 2009 SD-83, Sept 10, 2009
REVIEW DIVISION:	Division of Anesthesia, Analgesia and Rheumatology Products (HFD-170)
PHARM/TOX REVIEWER:	L. Steven. Leshin, DVM, PhD
PHARM/TOX SUPERVISOR:	Adam Wasserman, PhD
DIVISION DIRECTOR:	Bob A. Rappaport, MD
PROJECT MANAGER:	Jessica Benjamin

TABLE OF CONTENTS

Background	3
Submissions and Communications	4
Summary of Previous and New Findings	7
Study Findings	7
Re-examination of Tissues by —	9
Re-examination of Tissues by the Pathology Working Group	10
Interpretation and Discussion	13
Dose Relationships	13
Statistical Considerations	13
Toxicological Signal	16
Second Toxicological Study	16
Approved Products Containing DMSO	17
Unfair Treatments	18
Conclusion	20
Recommendation	21
Appendix 1: DMSO Pharmacokinetics of Human, Minipig, Rat	22
Appendix 2: Approved DMSO-containing products	23

b(4)

BACKGROUND

This Addendum to the initial Pharmtox Review is focused on the issue of lymphomas that occurred in a 6 month rat toxicology study and if a decision on approval of the NDA should be made prior at this review cycle or upon completion and submission of the carcinogenicity study which is ongoing. Additional brief discussion is provided to other issues raised by the Applicant during the extended submission period.

Regulatory

This NDA is in its third review cycle, originally submitted in 1998 but withdrawn prior to review completion (CMC inspection issues), then resubmitted in 2002 (issues with the clinical efficacy studies), 2006, and 2009. The 2006 resubmission and review of this NDA resulted in an Approvable (Complete Response) action in Dec 2006 mainly due to deficiencies related to the lack of adequate characterization for safety of the excipient, dimethylsulfoxide (DMSO). The Applicant had not been informed of nonclinical issues concerning DMSO from previous NDA reviews in 1998 and 2002. Partly due to this lack of communication and relying on available scientific data, an agreement was reached during a June 4 2007 teleconference that a decision upon resubmission of the Application (Complete Response resubmission) would be made without the final results of the 2-year rat carcinogenicity study of DMSO (the study would be continued postmarketing), if there were no toxicological "signals" in the long term studies of DMSO applied topically. The FDA statement from the June 4, 2007 meeting minutes:

As a clarification, you should initiate your dermal carcinogenicity study now. However, if the dermal tox studies do not show any signals, you can continue the dermal carcinogenicity study post-approval.

The rat dermal carcinogenicity study was recently initiated (July 2009). There were issues with the acceptance of initial SPA protocol ((IND 42,773 SD-82, Aug 31, 2007). The second SPA protocol (IND 42,773 SD-83, Dec 1, 2008) was acceptable with revised doses (ECAC notification of the Applicant Jan 14, 2009). These are described in the July 23, 2009 Pharmtox Review. A delayed decision, until the carcinogenicity study is submitted and reviewed, would mean a decision would not be forthcoming for approximately 3 years, and would not affect current treatments for patients with osteoarthritis, as this would be the second topical diclofenac for this indication. Based on

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Since June 3, 2009 (during the review cycle) when the Applicant was notified of our concerns with the findings of lymphomas (incidence of 1 in the low dose, 1 in the mid dose), in the rat 6-month DMSO topical dosing toxicological study, they have continually submitted information in an attempt to address our concerns. The PDUFA decision date for this NDA was extended 3 months to Nov 3, 2009 with the submission of SD-79, a nonclinical major amendment received Aug 3, 2009. This submission lacked all the information indicated they would submit, which was finally submitted on Sept 10

approximately half-way into the extended review period and 2 weeks after the original anticipated review completion date necessary for allowing upper management review.

The Pharmtox Review of the Complete Response was filed into DARRTS on July 23, 2009, but only superficially addressed these late submissions. This Addendum to that Review is focused on the issue of lymphomas and if a decision on the NDA should be made at this review cycle or upon completion and submission of the carcinogenicity study. This review focuses on the question "Does the occurrence of 2 rats with lymphomas in the 26 week repeat-dose dermal toxicity study constitute a plausible 'toxicological signal' requiring the Sponsor to complete the carcinogenicity study pre-approval?" The Applicant contends that the findings of lymphomas do not constitute a signal to delay a decision on the Application, but the Pharmtox Reviewer does not agree.

Submissions and Communications

Supp. Doc	Received Date	Submit Date	Comment
		06/3/2009	PharmTox Information Request for occurrence of lymphomas in rats of similar strain and study duration
74 N-000BZ	06/15/2009	06/12/2009	Nuvo Response letter (N-000-BZ), info about lymphoma and neuroblastomas, and a statistical analysis by Nuvo
		06/19/2009	Teleconference discussion of pre-decision concerns about lymphomas and label leachables, suggest Nuvo submit all supporting information for their interpretations
75 N-000BZ	06/29/2009	06/26/2009	Nuvo Response to request for additional information Summary (page 25) Also Nuvo requests that they be advised as to the final recommendations of the Associate Director of Pharmacology and Toxicology (p. 25)
	June 26, 2009 submission: • summary of the rat 26-week DMSO dermal toxicology study findings • commentary on considerations in evaluating possible "signals" and on the rationale for the a posteriori selection of the threshold for "signal" assessment • status of dermal carcinogenicity study • discussion of the lymphoma data • statistical analysis and historical control data • comments on the option to exclude DMSO from FDA's Inactive Ingredient List • CMC concern regarding safety risk of leaching of label and the following attachment: • ATTACHMENT 1—Sprague Dawley Rat Selected Neoplastic Data 26 Week Studies 09-02 Audited • ATTACHMENT 2—Sprague Dawley Rat Neoplastic Data 2 Year Studies 08-04 Audited • ATTACHMENT 3—Literature References ○ Chandra et al 1992. Spontaneous neoplasms in aged sprague-dawley rats. Arch Toxicol 66:496-505. ○ da Silva Franchi 2003. Thymic lymphomas in Wistar rats exposed to N-methyl-N-nitrosourea (MNU). Cancer Sci. 94:240-243. ○ Elmore and Peddada 2009. Points to consider on the statistical analysis of rodent cancer bioassay data when incorporating historical control data. Toxicol Pathol June 10, 2009 OnlineFirst, Vol?, 12-16. ○ Fujui, et al 2008. Assessment of the carcinogenic potential of mitemcinal (GM-611):		

			Increased incidence of malignant lymphoma in a rat carcinogenicity study. Toxicology and Applied Pharmacology 228: 1-7 ○ Giknis and Clifford 2004. Compilation of Spontaneous Neoplastic Lesions and Survival in Crl:CD (SD) Rats from Control Groups, March 2004, Charles River Laboratories ○ Greaves, M.F. 2004. Biological Models for Leukemia and Lymphoma (pp. 351-372). In Buffler, P. et al (Eds). Mechanisms of Carcinogenesis: Contributions of Molecular Epidemiology. IARC Scientific Publication No. 157, Lyon, 2004. ○ Gross et al. 1986. C-type virus particles in Spontaneous and virus-induced leukemia and malignant lymphomas in mice and rats. Cancer Research 46: 2984-2987. ○ Irons, R.D. 1988 Studies on the mechanisms of chemically induced leukemia/lymphoma. CIIT (Chemical Industry Institute of Toxicology) Activities 8(1): 1-7, and 85. ○ Kinashi et al 2000. Dimethyl sulfoxide protects against thermal and epithermal neutron-induced cell death and mutagenesis of chinese hamster ovary (CHO) cells. Int. J. radiation oncology Biol. Phys. 47: 1371-1378. ○ McFee et al 1989. Assays of three carcinogen/non-carcinogen chemical pairs for in vivo induction of chromosome aberrations, sister chromatid exchanges and micronuclei. Environmental and Molecular Mutagenesis 14:207-220. ○ Nakazawa et al 2001. Spontaneous neoplastic lesions in aged Sprague-Dawley rats. Exp. Anim. 50: 99-103. ○ Norpoth et al 1986. Investigations on metabolism, genotoxic effects and carcinogenicity of 2,2'-dichlorodiethylether. J. Cancer Res Clin Oncol. 112:125-130. ○ Norpoth et al 1988. Investigations on metabolism and carcinogenicity of 1,1,2-trichloroethane. J. Cancer Res Clin Oncol. 114:158-162. ○ Richie et al. Proviral structure and differentiation antigen phenotype of spontaneous and chemically induced AKR lymphomas. Cancer Research 45:2802-2806. ○ Silver, J. 1986. Genetic study of lymphoma induction by friend murine leukemia virus in crosses involving AKR mice. JNCI 77:793-799. ○ Smith et al. 2003. Vehicular effects of dimethyl sulfoxide (DMSO) on dermal carcinogenesis in tetradecanoyl phorbol acetate (TPA)-treated hemizygous Tg.AC transgenic mice. Society of Toxicology Abstract, Toxicological Sciences 72 (S-1, March 2003): Abstract # 422. ○ Son, W-C. 2004. Factors contributory to death of young Sprague-Dawley rats in carcinogenicity studies. Toxicology Letters 153:213-219. ○ Son, W-C. and Gopinath, C. 2004. Early occurrence of spontaneous tumors in CD-1 mice and Sprague-Dawley rats. Toxicologic Pathology 32:371-374. ○ Vogin, et al. 1970. Chronic Toxicity of DMSO in primates. Toxicology and Applied Pharmacology 16:606-612. ○ Warren et al 1990. Chemical induction of thymomas in AKR mice: interaction of chemical carcinogens and endogenous murine leukemia viruses. Comparison of N-methyl-N-nitrosourea and methyl methanesulphonate. Molecular Carcinogenesis 3:126-133. ○ Watanabe et al 1990. Radioprotective Effects of dimethyl sulfoxide in golden hamster embryo cells exposed to rays at 77 K. II. Protection from lethal, chromosomal, and DNA damage. Radiation Research 124: 73-78. • ATTACHMENT 4—Summary Report: Carcinogenicity of DMSO • ATTACHMENT 5—Statistical Assessment • ATTACHMENT 6—Summary of Products Containing DMSO • ATTACHMENT 7—Maibach H., 1989, Percutaneous transportation of drugs (pp. 11-25). In K. Brune (Ed.) Percutaneous Antirheumatic Therapy
76	07/14/2009	07/13/2009	paper submission, same as June 26 submission, but sent to Drs. Brown and Jacobson Kram
77	07/16/2009	07/14/2009	Nuvo Submission to Drs. D.K. Jacobson-Kram and P. Brown,

N-000-BZ			additional summary of information and material that was in June 2009 submissions
			07/23/2009 Teleconference discussion Nuvo with A. Wasserman and J. Benjamin PT is not recommending approval due to lymphomas in rat, final Director decision has not been made also label leachables require resolution, washing hands is insufficient (no FDA minutes in DARRTS, see SD-81)
78	07/27/2009	07/24/2009	Resubmission/After Action- Complete indicated dosing for the rat carc study has begun, and added animals for parallel treatments with an interim sacrifice April 2010, but state sacrificed after at least after 26 weeks; 25/sex/DMSO dose, dosed 1X/day with 3 DMSO doses as in carc study (different from the 6 month tox study previously conducted); also health screen of 3-4/sex at 1, 6, 12, 18, and 24 months, plus toxicokinetic animals
			07/28/2009 Teleconference discussion Nuvo with R. Rappaport and J. Benjamin Further discussion as on July 23 (no FDA minutes in DARRTS, see SD-81)
79 N-000-BS	08/03/2009	07/31/2009	Major Amendment--extend clock to Nov 4, 2009 additional information provided arising from teleconference of July 23, July 28, also indicated additional information to be forthcoming
	July 31, 2009 submission 1) assessment of 2 independent pathologists _____ 2) new pathological assessment of lymphoma different morphologies 3) new info on 2nd lymphoma 4) new statistical analysis of lymphoma occurrence 5) assessment by toxicologist _____ 6) literature about DMSO (previously submitted info) 7) new literature of DMSO and lymphomas 8) discussion of what constitute a signal 9) proposal for a Pathology Working Group Stated Forthcoming Data: a) genetic testing to determine if 2 lymphomas were in sibling rats b) tissue type testing to determine if lymphomas were different c) histopath exam of additional lymphoid tissues in rats of the 26-week study d) report of the Pathology Working Group e) request for FDA pathologist to join PWG		
80	08/13/2009	08/12/2009	Describes the pathology working group to assess lymphomas indicated was cc to Dr. Paul Brown
81 N-000-MT	08/19/2009	08/18/2009	Applicant submitted Minutes for the teleconferences of July 23 and July 28, 2009
82 N-000-BS	08/24/2009	08/21/2009	Applicant submitted additional preliminary data and, provided an update on when more data would be submitted
	Aug 21, 2009 submission 1) the 2 lymphomas are of different types, 2) another path assessment _____ 3) two new genotoxic studies supporting the lack of genotoxicity 4) notice of addition path analysis in early Sept 5) reminder of pathology working group invitation 6) reminder of comments for the label leachable issue		
83		09/10/09	Pathology working group response from additional examination of

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N-000-BB	tissues and provided references
	Sept 10, 2009 submission • Attachment I: Report of the Pathology Working Group (PWG) held on August 31, 2009 by ○ Report ○ CVs of Members of the PWG ○ McMartin, ON, Sahota, PS, Gunson, DE, Han Hsu, H, and Spaet, RH (1992). Neoplasms and related proliferative lesions in control Sprague-Dawley rats from carcinogenicity studies. Historical data and diagnostic considerations. Toxicologic Pathology, 20:212-225. ○ Mohr U, (ed.). (1993). International classification of rodent tumors: Part I: The rat. IARC Scientific Publications. 4. Hematopoietic System, Lyon, France, No. 122, p5-21. ○ Pearse, G (2006). Histopathology of the thymus. Toxicologic Pathology, 34:515-547. ○ PENNSAID® Topical Solution NDA 20-947, Nonclinical pharmacology and toxicology summary, February 4, 2009. ○ Son, W and Gopinath, C (2004). Early occurrence of spontaneous tumors in CD-1 mice and Sprague-Dawley rats. Toxicologic Pathology, 32:371-374. ○ Stefanski, SA, Elwell, MR, and Stromberg, PC (1990). Pathology of the Fischer rat reference and atlas. Chapter 22. Spleen, lymph nodes, and thymus. Boorman, GA, Eustis, SL, Elwell, MR, Montgomery, CA, and MacKenzie, WF (eds.). Academic Press, San Diego, CA. pp. 369-393. ○ Squire, RA (1989). The interpretation of equivocal or marginal animal carcinogenicity tests. Cell Biology and Toxicology, 5:371-376. ○ Ward, JM, Erexson, CR, Faucette, LJ, Foley, JF, Dijkstra, C, and Cattoretti, G (2006). Immunohistochemical markers for the rodent immune system. Toxicologic Pathology, 34:616-630. • Attachment II: Assessment by _____ • Attachment III: Pathology Interpretation for the 26-week rat — Study Report (includes newly sectioned tissue for histopathological evaluation as requested by — in preparation for the PWG) ○ Historical control Data from 26-week studies conducted at _____ ○ Son, W and Gopinath, C (2004). Early occurrence of spontaneous tumors in CD-1 mice and Sprague-Dawley rats. Toxicologic Pathology, 32:371-374. ○ Pathology Result Tables (Summary and Individual) from the 26-week rat study (data pages marked "unaudited data")

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SUMMARY OF PREVIOUS AND NEW FINDINGS

Study Findings:

Study title: **DMSO: a 26-week dermal toxicity study in Sprague-Dawley rats followed by a 12-week recovery period**

Study no.: **RD-1000-07-05 (#1447-001)**

E-location: \pharmtox\tox\rd-1000-07-05.pdf

Conducting laboratory and location: _____

Date of study initiation: Aug 1, 2007

GLP compliance: yes;

QA report: yes

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Drug: Dimethyl Sulfoxide USP, Lot USP061018, Purity unknown

Vehicle: Consisted of the excipients used in the Sponsor's DMSO-containing drug product, PENNSAID®, a 1.5% w/w diclofenac sodium Topical Solution

Key study findings:

- The NOAEL for local dermal toxicity was 9% (corresponding to a 3-time daily application of a total of 175.5 mg/day) based upon the findings of erosions/ulcers 1 (of 50 animals) in the 45.5% DMSO group and 9 (of 50 animals) in the 90% DMSO group,
- There was no NOAEL for systemic toxicity due to a death from malignant lymphoma in the low dose female group.
- There were 7 deaths (1 at 9%, 2 at 45.5%, and 4 at 90% DMSO dose groups). Two died from malignant lymphoma, 1 of a focal necrosis in the brainstem, 1 of urogenital tract obstruction and inflammation, and 3 of undetermined causes although 2 rats had generalized lymphoid tissue depletion.
- Rats (25/sex/dose) were administered 9%, 45.5% or 90% DMSO in Pennsaid Topical Solution vehicle (lacking diclofenac sodium) topically to their back, 3-times daily for 26 weeks, followed by a 12-week recovery period (5/sex/dose).

Additional Information can be obtained from study review starting at page 53 of the initial Pharmtox review for NDA 20-947 Complete Response filed in DAARTS July 23, 2009.

Lymphomas were found in 1 of 25 low-dose (9% DMSO) females found dead on day 129, and in 1 of 25 mid dose (45.5% DMSO) females that was euthanized in extremis on day 230, during the recovery phase, 49 days after the last dose, but not in the control and high dose (90% DMSO) females or in any male dose group as indicated in the table below with the associated toxicokinetic values.

Incidence of lymphomas in Study RD-1000-07-05, a 6-month repeated dosing with 3-month recovery including toxicokinetics and safety margins
(Refer to Appendix 1 for human, minipig, and rat pharmacokinetic comparisons)

	Males				Females			
Dose (%)	0	9	45.5	90	0	9	45.5	90
g/day	0	0.19	0.95	1.88	0	0.19	0.95	1.88
N	25	25	25	25	25	25	25	25
Occurrence animal ID day dead or euthanized	0	0	0	0	0	1 #1534 Day 129 4.3 months	1 #1574 Day 230 7.7 months (during recovery), 1.7 months after stop dosing	0
DMSO AUC ₀₋₂₄ (µg- h/mL)	Day 1	3191	20697	22509		3,592	22,445	32,367
Exposure Margin	Day 181	488	5131	13962		2,091	10,206	22,444
		14X	142X	387X		58X	283X	623X
Values for day 181. Exposure margin calculation based on human concentration of 36 µg-h/mL								

METABOLITES: Dimethyl sulfone (DMSO ₂), dimethyl sulfide (DMS)								
DMSO ₂ AUC ₀₋₂₄ (µg- h/mL)	Day 1	1522	3649	4511		1170	2889	4532
	Day 181	1875	6577	9421		2802	3753	3507
		0.8X	2.8X	4.0X		1.2X	1.6X	1.5X
Values for day 181. Exposure margin calculation based on human concentration of 2339 µg-h/mL								
DMS								
DMS is exhaled, and was not measured systemically or in exhaled air								

After notification of our concern with the finding of lymphomas, new information was presented that the recovery group animal with lymphoma was healthy at the end of the treatment period (week 26). The Applicant also stated, that lymphoma in rats is a rapidly fatal neoplasm, all of which further support the lack of biological plausibility that this second lymphoma could be DMSO related.

Reviewer: There is no evidence that DMSO and its metabolites were absent from cells in the recovery animal at the time lymphoma occurred. What is the data that lymphoma is rapidly fatal in rats? It is only detected upon death of the animal or morbidity and euthanasia, how long it is present prior to this timepoint is unknown. Their conclusion is premature and unsupported.

Re-examination of tissues by —

After notification of our concern with the finding of lymphomas, a post-study re-examination of tissues was conducted by the — pathologist. Tissues re-examined included the liver, spleen, thymus, bone marrow from the sternum and femur, and mandibular and mesenteric lymph nodes of all of main study and recovery animals. Thymomas were found in 2 males in the low dose (9% DMSO) group (*Reviewer: This was not reported in the initial report*). Rat 1026 had a benign epithelial thymoma and rat 1029 had a malignant epithelial thymoma. Thymomas are tumors of the epithelial cells of the thymus, not lymphoid cells.

Tissue-type testing

— conducted tissue-type testing to determine if the 2 lymphomas were of different types (SD-82 Aug 21, 2009, attachment 1). The lymphoma of rat 1534 was CD3+ indicative of a T-cell origin. The lymphoma of rat 1574 was negative for antibodies against CD3, and for B-cell markers CD20 and CD79a, indicating a non-B and non-T cell origin.

Reviewer: The source of antibodies used for identification of cell origin was not stated, but it is interesting that a recent article authored by —

— does not list antibodies to CD79a and CD 20 antigens as useful for detecting these antigens in rat tissue (Table 2, below). The pathologist report indicated that positive control tissue was positive, but it was not stated from what tissue and species it was obtained. Thus given the information provided, the conclusion of a lack of B cell origin may be premature. Despite this, the finding of a

CD3 positive tumor along with a CD3 negative tumor does support separate cellular origins for the lymphomas.

TABLE 2.—Detection of rat hematopoietic antigens in paraffin and frozen sections.

Antibody	Major cells expressing antigen	Source	Product number	Clone
CD3	All T-cells	Dako	A0152	
CD4	T-helper cells	Serotec	MCA55G	W3/25
CD5	Subset of T-lymphocytes, most thymocytes and NK cells	Serotec	MCA52G	OX-19
CD8	Subset of T-lymphocytes, most thymocytes and NK cells	Serotec	MCA48G	OX-8
CD11b*	Most macrophages, Kupffer cells, also dendritic cells, granulocytes	Serotec	MCA275GA	OX-42
CD25	Activated rat T cells	Serotec	MCA273R	OX-39
CD43	All leucocytes with the exception of B- lymphocytes	Serotec	MCA54G	W3/13
CD45RA	B-cells among thoracic duct lymphocytes with little labeling in bone marrow and none on thymocytes	Serotec	MCA340GA	OX-33
CD45RC	B-cells	Serotec	MCA53R	OX-22
CD68	Myeloid cells	Serotec	MCA341R	ED1
CD163	50% peritoneal macrophages, splenic & most other macrophages	Serotec	MCA342GA	ED2
CD169*	Macrophages	Serotec	MCA343R	ED3
IgM	B-cells	Vector	BA-2020	
MHC Class II	B-cells	Serotec	MCA46G	OX-6
Immunoglobulin human kappa light chain	Immunoglobulin-producing B cells	Dako	A0191	

*Works only with frozen sections.

_____ chairman of the Pathology Working Group had examined the tissues prior to the PWG meeting and noted morphologic differences in the appearance and distribution of the tumor cells in these two animals with lymphomas, suggesting they are of different lymphoid cell types. Based on this new finding and other reasons, he concluded the lymphomas are unlikely to be DMSO related.

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Reviewer: This may have been the incentive to do tissue typing described above. There is no reason why a drug such as DMSO, needs to produce identical neoplastic morphology among cells, particularly if the cell type or types from which they arise may be different.

Genetic testing

Genetic testing results to determine if the 2 lymphomas were in sibling rats was to be an available in August 2009, but it was not in the submissions of Aug 21 or Sept 10, 2009.

Reviewer: Whether the animals are or are not siblings has no real bearing on the determination of whether the finding is a toxicological signal or not. If they are attempting to demonstrate that they are siblings and therefore there is a genetic predisposition for lymphomas, they will also need to demonstrate that the other animals are not siblings for an effective argument.

Pathology Working Group analysis of the study data (submission Sept 10, 2009)

The Applicant arranged for an independent PWG to review, discuss, and interpret all of the findings of this study. The PWG consisted of a panel of expert toxicologic pathologists, all international authorities in chemical carcinogenesis:

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The FDA was also invited to provide a participant, but the Agency does not participate in these types of proceedings since regulatory decisions are not an industry-FDA collaborative process.

The Chairman of the PWG ——— requested ——— the study laboratory, to section and evaluate new tissues from the 9% and 45.5% DMSO groups of both sexes from the terminal necropsy and from all animals from the recovery necropsy. The examination of tissues observed by the study pathologist as well as newly processed slides of the save tissues was focused on the presence or absence of histopathologic evidence of toxicity (degeneration, necrosis, and atrophy) or the presence or absence of proliferative changes involving the thymus in male rats and lymphoid organs in female rats. *(Reviewer: Tissues from the 9 early mortalities, except for the two animals with lymphomas, were not examined).* Female tissues examined included the liver, spleen, thymus, bone marrow from the sternum and femur, and mandibular and mesenteric lymph nodes, the same tissue types re-examined by the ——— pathologist. Also examined were male thymus tissues from all control group and high dose rats and the thymus from the two low concentration (9% w/w) male rats with a diagnosis of thymoma. *(Reviewer: With the exception of the 2 low dose males with thymomas, tissues from the low and mid dose groups were not examined).*

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The lymphomas and thymoma findings of ——— were confirmed. The rat with the malignant lymphoma had neoplasm in liver, thymus, lymph nodes (mandibular and mesenteric) bone marrow (sternum and femur). Other tissues were indicated in the individual pathology table of the original study. The immunohistopathology tissues developed at ——— were also examined and the previous findings confirmed.

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"The PWG also found no evidence of toxicity affecting the lymphoid system or evidence of hyperplastic or pre-neoplastic changes in hematology data or histopathologic microscopic data. Based on these findings and panel discussions, the PWG unanimously concluded:"

"The occurrence of these neoplasms, either individually or collectively, does not indicate a signal for potential carcinogenicity of the vehicle formulation containing DMSO."

This conclusion was based on the following:

- Dimethyl Sulfoxide (DMSO) and its major metabolites, Dimethyl Sulfone (DMSO₂) and Dimethyl Sulfide (DMS), are not genotoxic when tested using a battery of genotoxic assays thus precluding any concern for a genotoxic effect that might account for an early appearance of neoplasms associated with treatment.

Reviewer: This is not accurate. Genetic toxicology studies of DMSO were negative without and with the presence of S9 liver preparations. DMSO₂ and DMS have not been

studied in genotoxic assays. It is not known if they form in the presence of induced concentrations of metabolic enzymes found in S9 preparations. DMSO is metabolized by oxidation to DMSO₂ and by reduction to DMS. Therefore it is unknown if the metabolites have been tested at appropriate doses. There is one publication, (Hakura et al 1993 Mutation Research 303:127-133) which determined dimethyl sulfide was not mutagenic, but in this same study DMSO was found to be mutagenic. This was discussed in the Pharmtox Review of the 2006 NDA submission in which a critique by the consultant Dr _____ indicated the positive results with DMSO were due to a strain not used routinely in genetic toxicology testing today.

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- An absence of a concentration related dose response with the incidence of malignant lymphoma or epithelial thymoma and a complete absence of either neoplasm in the high-concentration group.
- Malignant lymphoma was not present in male rats in any concentration group and epithelial thymoma was absent in all groups of female rats.
- Epithelial thymoma was not observed in any male rats in the mid and high concentration groups or in any of the treated groups of female rats.
- The two cases of malignant lymphoma represent different cell lineages, one being a T cell lymphoma and the second a non-B, non-T cell lymphoma based on the morphologic distribution of the neoplasm and immunohistochemical staining of the neoplasms.
- No evidence of toxicity affecting the lymphoid system was observed in the hematology data or histopathologic microscopic data.
- No evidence of hyperplastic or pre-neoplastic changes was observed in the hematology data or histopathologic microscopic data that would suggest early evidence of proliferative change affecting the lymphoid system.
- Low incidences of malignant lymphoma have been observed in the _____ historical control data and in the published literature in Sprague-Dawley rats of similar age.

In order to obtain another independent expert assessment, Nuvo asked _____ to review the totality of findings from the new _____ data and the PWG, including the new tissue evaluations. After a careful review of the entire dataset, _____ also concluded the findings in the 26-week rat study "do not constitute a potential signal of carcinogenicity".

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Reviewer: There is no disagreement with the study findings. There is a disagreement about the interpretation of the findings. Without knowing how the issue was presented to the PWG, their conclusions lead one to suggest that the lymphomas and thymomas are random spontaneous occurrences, completely unrelated to drug (DMSO) treatment. It is necessary to further extrapolate their conclusions to marketing the product, as the product could now be marketed, and the carcinogenicity study will be conducted postmarketing. Are we sufficiently confident the DMSO component will not contribute to malignancies in the product users prior to knowledge of the carcinogenicity study final results? The Reviewers position is that the occurrence of (now) 4 malignancies in a 6-month repeated dosing rat toxicological study that resulted in no NOAEL determination

is a very strong toxicological signal requiring evaluation of the final carcinogenicity study before a decision is made on approval.

INTERPRETATION AND DISCUSSION

Dose relationship

The lack of a dose-response relationship for the occurrence of lymphoma and thymomas is a reasonable explanation that these findings are unrelated to DMSO administration and may not be pertinent in terms of human therapeutic use of Pennsaid. Furthermore, the DMSO exposure was very high, even in the low dose group (58X the human equivalent dose by AUC comparison) and mid dose (223X) female groups. In fact, for carcinogenicity studies drugs producing toxicokinetic exposure of 25-fold over the human exposure is an acceptable highest dose to be administered. Alternatively, it conceivable a threshold was exceeded by the low dose, in which no dose-response relationship would be expected in this dosing regimen. Perhaps lymphomas were induced by a metabolite such as dimethyl sulfone (DMSO₂) or dimethyl sulfide (DMS), however, this is unlikely since males lacked lymphomas at 2- to 3-fold higher DMSO₂ AUC values. There is a gender difference in dose exposure, with greater DMSO exposure and lesser DMSO₂ exposure in females than males for the same amount of DMSO applied to the animal surface. Gender differences in rodent carcinogenicity findings are not unusual, and it is recommended that they not be combined for carcinogenicity analysis.

The potential role of the metabolites in carcinogenicity is unknown since there is minimal toxicological information about them, although it is known that DMS is a tissue irritant. Most of these supporting literature studies for the lack of carcinogenicity potential of DMSO involve short term in vivo or in vitro studies. While useful for mechanistic understanding, in vitro studies obviously lack all the metabolic and cellular interactions involved in an in vivo assay. *(The Reviewer has not recently re-examined the 160+ literature studies submitted in the previous review cycles).*

It is important to note that the carcinogenicity study initiated in July 2009 differs from this toxicology study, that animals are being dosed 1X/day at lower doses, and this will still provides AUC exposures exceeding the maximal human therapeutic exposure by 2-, 6-, and 67-fold, overlapping at the highest dose with the low dose toxicology study AUC in which a lymphoma occurred.

Statistical Considerations

In the Applicant's initial response of June 12, 2009 and in SD-75 (June 26, 2009, attachment 5), they presented—— historical control data, a study by Son and Gopenath 2004, and a statistical analysis. Additional literature studies have been submitted since then. The Applicant calculated the 95% confidence interval for the last 5 years of 6-month duration studies in —— historical control data as 2.46% for males and 5.23% for

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females. Since the actual incidence in the study was 0% for males and 2.6% in females they claim this is a random event and not a toxicological signal of concern.

In SD-79 (July 31, 2009, appendix B) a new statistical analyses was conducted by an independent expert statistician, _____ demonstrating the lack of statistical plausibility that the lymphoma(s) could be DMSO related. These new statistical analysis considered the new information relating to the timing of the second lymphoma.

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Based on the historical control data, published reports of lymphomas in young rats within the study age group, the absence of a dose-response the 6-month rat study, and their statistical analysis indicating the lymphoma findings were within the realm of random occurrences, the Applicant concluded that the lymphomas were not drug related and thus do not constitute a toxicological signal.

The Reviewer agrees that rat spontaneous lymphomas are rare events and occur within the same age range and strain (Sprague-Dawley) as those in 6-month rat toxicology study as indicated in the published literature. However, the Reviewer disagrees that the finding of 2 cases of lymphoma in the Nuvo study of 200 rats is not larger than the incidence of this finding that would be expected to occur by chance. The Reviewer notes that although the basis for the above confidence intervals was not described, it appeared to be derived from the incidence per number of animals in the control groups of the _____ studies. Lymphoma occurred in 1 male control group at and incidence 1 of 15 rats and in 2 females control groups both with an incidence 1 of 10 rats, resulting in an incidence of 6.67% in the male group and 10% in both females groups. It is noteworthy that the majority of the 40 control groups contained 15 to 25 animals per group which would have produced an incidence of 4-6% per group. At its simplest level, their analysis is highly biased by the size of the study group.

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Dr. Adam Wasserman, as described in his Supervisory Review, "consulted to Dr. Stella Machado along with a request to provide an estimate of the probability of seeing two animals with lymphoma in a single study of this size assuming it is a spontaneous and random occurrence occurring at the frequency of the HCD. Please refer to the statistical consult for detail but briefly the probability was calculated to be approximately 2.7% chance of seeing 2 observations in 100 females and 5.8% in 200 animals consisting of 100 females and 100 males. A Bayesian approach yielded "credible intervals" (analogous to 95% confidence intervals) which were exceeded by the observed data and were therefore considered statistically significant. Dr. Machado and a colleague _____ felt that based on a statistical argument they cannot rule out that DMSO might have produced the lymphomas."

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This new analysis considering the timing of the second lymphoma is not relevant. The second lymphoma resulted in extensive metastasis, such that the animal was euthanized during the recovery phase 49 days after the last DMSO dose. It is not known when the lymphoma arose. It is also not known if measurable quantities are DMSO or metabolites were present at the time of death. It is known that DMSO distributes readily to all tissues. Rat (Otsuki et al., 2002; Hucker et al 1966) and monkey (Layman and Jacob,

1985), and human (Wilhite and Katz 1984) studies indicated that DMSO or its metabolites may reside in the body many days if not weeks.

The Applicant has further argued that since the lymphomas have different tissues of origin they should be considered as individual findings, further reducing the incidence to 2 types of unrelated neoplasms for lymphomas. Even with tissue typing differences, there is no reason to think that DMSO, if lymphomas were drug related, exerts its effect in the same tissues of different animals identically. Furthermore, considered as toxicological signals, there is no reason for not collectively grouping them.

Since the findings of 2 thymomas were revealed, also rare malignancies, it seems even more improbable that 4 "young" animals would spontaneously develop malignancies in a 6-month toxicology study.

Incidence of Lymphoma

	Males	Females
Nuvo study		
Incidence / number of total animals in study	0/125 (0%)	2/125 (1.6%)
Studies		
Number of studies (over previous 5 year time period)	39	40
Incidence in control groups / number of total animals	1/767 (0.13%)	2/785 (0.25%)
Description for the above Incidences Incidences Group Route Strain Study Date Number per Dose Group Incidence (%)	V Oral gavage CD[CrI:CD (SD) 5/4/06 – 12/28/06 N=15 1 (6.7%)	AA Oral gavage CD[CrI:CD (SD) 10/19/06 – 5/17/07 N=10 1 (10.0%) AG Oral gavage CD[CrI:CD (SD) 1/10/07 – 10/3/07 N=10 1 (10.0%)
Son and Gopinath 2004		
Rat data of carcinogenicity studies; 20 rat studies 1990 to 2002 at Huntingdon Life Sci, UK; Oral gavage or dietary administration; Data from deaths in control group during weeks 1-50 Lymphoma data presented below, thymomas were not mentioned in the study	1284 male (at least 50/control group) 48 deaths 17 from neoplasms 6/1284 (0.46%)	1264 (at least 50/control group) 4 deaths 34 from neoplasms 4/1264 (0.32%) Tables below

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Females: 4 within 50 weeks of the study

TABLE 4.—Tumor incidence profiles expressed by time of death in female SD rats.

Time of death (weeks on study)	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	Total up to 50 weeks	Total between 50 and 80 weeks
Total no. of deaths	0	1	2	2	1	2	2	3	10	20	43	264
Lymphoid/multicentric Lymphoma			2	1			1		1	1	4	6
Histiocytic sarcoma											2	1

In paper discussion section: Although a lymphoma (fatal) was reported in a female rat in week 11 of a carcinogenicity study, the same tumor (incidental) was recorded in a female rat during a routine health check prior to the start of a carcinogenicity study in our laboratory

Males: 6 within 50 weeks of study

TABLE 3.—Tumor incidence profiles expressed by time of death in male SD rats.

Time of death (weeks on study)	1-5	6-10	11-15	16-20	21-25	26-30	31-35	36-40	41-45	46-50	Total up to 50 weeks	Total between 50 and 80 weeks
Total no. of deaths	1	0	1	3	2	7	4	9	11	3	48	266
Lymphoid/multicentric Lymphoma				2		2		1	1		6	4
Myeloid leukemia								1			1	1
Mononuclear cell leukemia						1					1	

Son 2004

Sprague-Dawley rats in carcinogenicity study control groups (n=20/group)

total 1284 males and 1264 females,

deaths in first year due to malignant lymphoma occurred in **6 males** from weeks 11 to 50, $6/1284 = 0.46\%$, and in **5 females** from weeks 11 to 50, $5/1264 = 0.39\%$

Thymomas were not mentioned in the study

McMartin et al 1992

neoplasms in historical controls of 2 year carc studies (9 studies, 1170 Sprague-Dawley rats) conducted at Ciba-Geigy Corp, Summit NJ

Site; Neoplasm or finding	Males		Females	
	Range %	Cumulative average %	Range %	Cumulative average %
Thymus	(530)		(557)	
Thymoma, benign	0.0-1.7	0.4	0.0-2.2	0.6
Systemic	(585)		(585)	
Granulocytic leukemia	0.0-1.7	0.3	0.0-1.7	0.2
Malignant lymphoma	0.0-1.7	1.2	0.0-1.7	0.7
Sarcoma, histiocytic	0.0-5.7	2.6	0.0-5.7	1.2

Toxicological Signal

They appear to be concern about what constitutes a "signal", and that we are retrospectively applying criteria to their detriment.

A neoplastic finding, especially two occurrences in fairly young rats, is considered a toxicological signal, and now there are 4 occurrences upon re-examination of tissues. Tumor findings contribute to establishing a NOAEL value for toxicological studies in drug development. There was no NOAEL value established in females and now in males since neoplasia occurred in the low dose groups. These "signals" are indicators for follow-up studies and monitoring in human clinical trials, although for human neoplasms, they are unlikely to occur for a number years. The lymphoma and thymomas signals in this case might be considered "weak" because of their absence in the higher female doses. However, collectively these "signals" indicate that further study is warranted, and this would be a carcinogenicity study.

Second Toxicological Study

In SD-78, (July 24, 2009) the Sponsor proposed a second 6-month toxicology study "in parallel" with the carcinogenicity study. In SD-82 (Aug 21, 2009) they indicated this was started soon after the carcinogenicity study. The doses and dose frequency of this toxicology study are identical to the carcinogenicity study, and both are different from the 6-month toxicology study of this review. As discussed at the ECAC meeting in Jan 2009, carcinogenicity doses of 3, 9, and 45.5% DMSO will be applied dermally once daily (25/sex/dose) producing expected exposure margins of 2X, 6X, and 67X, respectively. These low and mid dose exposures are substantially less than those at which lymphomas were observed. This new study will include toxicokinetic animals. They also indicated that a health screen for various viruses will be obtained from 3-4/sex at 1, 6, 12, 18, and 24 months, but their animal allocation does not include these as separate animals.

The Applicant did not explain why they are doing this parallel study, but it seems likely if no neoplasms were found, they would submit this newly completed study as evidence that DMSO does not present a signal of toxicological concern. Possibly they would then resubmit the NDA prior to completion of the carcinogenicity study, shortening the approval time by approximately 2 years. The Reviewer does not see how this will negate the previous results. Perhaps the Applicant has other reasons for doing this study.

DMSO Literature

An expert opinion from a toxicologist with significant experience in the study of DMSO, _____ stated that the lymphomas were not DMSO related. The Applicant also submitted additional publications, not previously included in our NDA, providing further evidence that DMSO is not carcinogenic.

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Most of the cited information was previously submitted and not relevant. If there was an appropriately conducted carcinogenicity study, it would have been submitted already. Previously submitted published studies do not meet ICH requirements for carcinogenicity studies.

The Applicant claimed that their clinical study of the measurement of DMSO in healthy volunteers in addition to the literature demonstrating DMSO is a naturally occurring endogenous substance "casts substantial doubt on the biological plausibility that it causes lymphoma or is otherwise carcinogenic." The amount of detectable DMSO obtained in foods or produced endogenously is so miniscule compared to that following drug application that this argument is not relevant.

Approved Products Containing DMSO

The Applicant returned to an argument offered in previous years that there are other approved products containing DMSO on the market. They listed the following products RIMSO-50, Viadur, Dermagraft, Onyx Liquid Embolic System (LES), Dolobene Gel, Domoso, Synotic Otic Solution, Idoxuridine, Dolicur. As indicated in the table in Appendix 2, Synotic Otic Solution, Domoso, Dolicur are veterinary products. Dolobene Gel and Dolicur are not approved in the US. Except for RIMSO-50, for which the indication and the route and frequency of administration are different from Pennsaid, the level of DMSO exposure in the approved human products is extremely low ($\mu\text{g/day}$) and for short term use compared to g/day and continuous chronic use for Pennsaid.

Unfair Treatment

The Applicant raised the issue that DMSO is unfairly being singled out for scrutiny, as compared to cocoyl caprylocaprate of the recently approved Voltaren Gel. This was also raised in the submission of the Feb 2009 Complete Response. They stated the concern related previously by FDA regarding the penetration enhancement properties of DMSO and Pennsaid has been fully addressed in a manner consistent with requests and advice on study designs from the Pharmacology/Review team and to an extent that appears to exceed the requirements for other topical products, in general, and, in particular, for Voltaren Gel and cocoyl caprylocaprate.

They seem to be missing the point that the issue is not with effectiveness of DMSO or any component of their product, but on safety and toxicological profile. Both DMSO and cocoyl caprylocaprate are excipients in a topical diclofenac gel. DMSO (45.5% of product; human application of 73 mg/kg/day) is used as a penetration enhancer. Cocoyl caprylocaprate _____ is an emollient and it has a type IV DMF on file with the Agency. They have quite different chemistries. The Pennsaid nonclinical program has relied almost exclusively on the literature of old studies, pre GLP. From the time of the initial IND submission, the Applicant claimed the product contains DMSO as only a penetration enhancer and should not be subjected to the combination product development plan. However, this penetration enhancement was never adequately demonstrated (this submitted in vitro data did not support this claim). Since it was a 505b2 application for diclofenac, the safety of the DMSO excipient was never addressed, although it was raised in the initial nonclinical reviews. It became an issue during review of the clinical studies because DMSO had been cited in the literature as an anti-inflammatory and analgesic agent. In retrospect, absence of DMSO characterization is the factor that contributed to all of the major drug development issues

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including this one. The Reviewer acknowledges that DMSO does have an uphill climb to prove safety due to issues that arose in the 1960's concerning its safety (mainly eye-related toxicity). Since DMSO drug development was essentially halted at that point, further investigations as to the cause of those adverse events were not conducted, even though they were not observed in subsequent years. This resulted in a lingering air of caution about DMSO.

Based on separate studies (not head to head drug PK comparison, thus different assays, etc), DMSO in Pennsaid's formulation does, in fact, enhance the penetration of diclofenac better than Voltaren's formulation. The table below indicates that approximately the same diclofenac AUC is obtained in patients treated for osteoarthritis, but the amount of diclofenac applied with Pennsaid treatment was approximately one third the amount of Voltaren Gel.

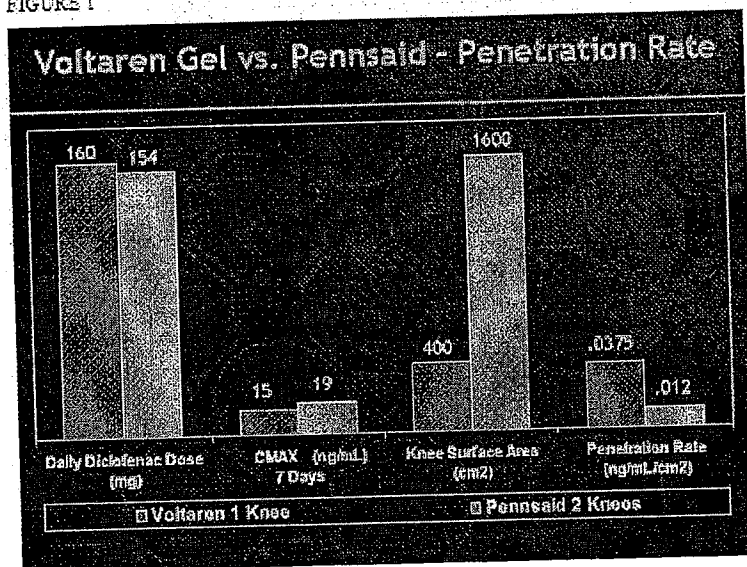
	Pennsaid	Voltaren Gel	Oral Voltaren
	1.5% w/w	1%	
Multiple doses	154 mg/day treated 2 knees for 7 days (4 x 80 drops/day); maximal dose.	480 mg/day treated 2 knees and 2 hands for 7 days (4 x 12 g/day); maximal dose	150 mg 3 x 50 mg/day
C_{max} (ng/mL)	19	54	2270
AUC_{0-t} AUC_{0-inf} AUC₀₋₂₄ (ng-h/mL)	695 745	807	3890
Pennsaid 154 mg/day resulted in approximately similar diclofenac AUC as that of 480 mg Voltaren Gel (both at max recommended dose), Also assumes that the application areas are the same, note that Pennsaid is applied "with the hand", but not "to the hand", so an exact comparison cannot be made.			

In SD-75 (June 29, 2009) an analysis of Pennsaid and Voltaren Gel were compared for penetration rate as indicted in the graph below. The analysis is not only incorrect and misleading, the "penetration rate" is not a penetration rate. The Reviewer is unclear why it is even presented, perhaps it came from a marketing/financial presentation. The question at this point of drug development is not efficacy but safety. Even if the graphical presentation was accurate it contributes nothing to product safety.

The study of Maibach (1989) was submitted to support the enhanced penetration ability with use of DMSO and includes absorption differences at different skin sites in humans, along with some comparisons with animals. The vast majority of this data is based on steroid absorption. There is some DMSO information suggesting it enhances absorption, but the Applicant is incorrect in using this information to support less absorption by the hands during application to the knees as there is no support for this hypothesis in this paper.

Voltaren Gel with cocoyl caprylocaprate versus Pennsaid with DMSO can be seen in the Figure 1 below. Note: The apparent percutaneous diclofenac flux rate for Voltaren Gel is approximately 3 times higher than Pennsaid.

FIGURE 1



Penetration Rate

Voltaren ($0.0375 \text{ ng/mL} \cdot \text{cm}^2$): calculated: 15 ng/mL divided by 400 cm^2

Pennsaid ($0.012 \text{ ng/mL} \cdot \text{cm}^2$): calculated: 19 ng/mL divided by 1600 cm^2

CONCLUSIONS

There is no disagreement with the pathological assessments of neoplasia in the rat 6-month toxicology study of DMSO. There is a disagreement with the meaning and interpretation of the finding.

The Applicant argues that the finding is atypical, random, and differences exist between the malignancies (morphology, B/T-cell lineage) that indicate to them that the lymphomas and thymomas should be considered unrelated. Therefore, there is no toxicological signal of concern and no reason to delay a decision on the NDA. The excessively high dose of DMSO is a plausible explanation for the lymphomas although it is unclear why there is no dose effects, resulting in very high DMSO AUC concentrations as well as DMSO metabolites (for which there is minimal knowledge of their toxicology). There was not a NOAEL. The Applicant provided little elaboration of the role of metabolites and the toxicokinetic data.

The Reviewer interprets the findings as a "significant toxicological signal" that warrants completion of the carcinogenicity study before an Application decision. Any

toxicological study in which malignancies are found in the drug exposure group (whether below, at, or above historical background, and especially if there is no associated NOAEL) would be treated in the realm of drug development as a signal requiring further study. This may contrast with an interpretation of environmental chemicals (for which the Pathology Working Group has extensive experience), since drugs are intended to enter the body, while environmental chemicals are studied to determine an avoidance paradigm. While it initially appears there is no dose-relationship, one of many criteria for demonstrating drug causality, the study was not a carcinogenicity study, and the appearance of 2 lymphomas in fairly young animals are rare events. New findings of 2 thymomas were also noted upon re-examination of lymphoid tissues. So there are 4 malignancies detected in the study, for which there is no obvious DMSO dose relationship and no obvious explanation for their occurrence.

The Pathology Working Group has interpreted the findings using criteria of typical of a carcinogenicity study, when in fact this is not a carcinogenicity study. The results of the 6-month DMSO study were considered by the PWG as incidental findings largely due to the absence of preneoplastic signs in related tissues of other animals, lack of a dose response and obvious mechanistic explanation. The Reviewer agrees that the evidence of a dose relationship is lacking. However, given that the study was only 6 months of dosing with 3 months recovery period, no NOAEL was determined, and there is minimal rat DMSO toxicological information for reference (the drug development program relied mostly on published non-GLP studies), these results raise concern. The few shorter (3-month) rat studies conducted to support a dermal carcinogenicity study also had some troubling toxicological findings that included non-dose related nephroblastomas in one study, and kidney-related adverse events (isosthenuria), although no lymphomas or thymoma.

While the absence of dose-related effects is troubling, the presence of 4 malignancies in a 6 month toxicology study is even more concerning. There is insufficient information to determine that they are not drug related. Furthermore, as a toxicological study, no NOAEL was obtained due to the presence of lymphomas and thymomas. The Reviewer is unaware of any drug development toxicological studies in which neoplasms were disregarded for any reason (including within a historical control distribution) in the determination of a NOAEL. The Application is at the approval stage missing a critical piece for completion of the safety profile and an important toxicological finding that necessitates clarification for completion of the safety profile. The Applicants analysis has not alleviated my concern over the carcinogenicity issue. A carcinogenicity study is designed to determine the carcinogenic potential of DMSO, which the available information is unable to adequately address. Prior to a decision for approval it would be prudent to obtain definitive data from the carcinogenicity study.

RECOMMENDATION ON APPROVABILITY:

The application should not be approved until the final report for the rat carcinogenicity study of dermally applied dimethylsulfoxide (DMSO) has been submitted and reviewed. This recommendation has not changed from the initial NDA review filed July 23, 2009.

APPENDIX 1: Pharmacokinetics of DMSO

Study #	Human		Minipig			Rat		
	2663 (2006 submission)	2656 (2006 submission)	RD-1000-07-06 (2009 submission)			RD-1000-07-05 (2009 submission)		
Dose Methodology	Single dose 2.4 mL total; 2 knees (1.2 mL each);	Multidose 9.6 mL total; (1.2 mL each application for each knee) 4X-daily for 1 week	Multidose 4.5 mL applied to 300 cm ² until week 20, then 5.6 mL to 375 cm ² area 3X-daily for week 52			Multidose 0.65 mL applied to area ranging from 3.5 to 7 cm ² (based on size of rat, not less than 10% of body surface area) 3X-daily for 26 weeks		
% DMSO	45.5%	45.5%	9%	45.5%	90%	9%	45.5%	90%
Dose DMSO Total g/day mg/cm ² /application ⁺ g/kg/day*	1.3 g 0.81 0.022	5.2 g* 0.81 0.087	1.6 1.8 0.05	8.2 9.1 0.27	16.2 18.0 0.54	0.19 M 9, F 12 M 0.32, F 0.54	0.95 M 45, F 64 M 1.58, F 2.71	1.88 M 88, F 125 M 3.13, F 5.37
Cmax (µg/mL) [#]	0.48	1.21	M ¹ 2.31 F ¹ 0.0	20.3 2.7	53.3 9.5	M ¹ 46.2 F ¹ 161	469 1049	1047 1696
Tmax (h) [#]	8.4	3.8	M 12.0 F 0.0	8.8 8.5	11.3 10.2	M 2 F 8	1 1	1 1
AUC(µg-h/mL) [#]	9.2	36.0	M 40.7 F 0.0	408 30.1	1099 189	M 488 F 2091	5131 10206	13962 22444
t _{1/2} (h)	8.4	43.1						

* Applicant indicated DMSO amounts of 0.65 mg per application and 5.2 g/day for the treatment of both knees (800 cm²/knee) at 4-times/day, but this differs from previously indicated values.

+ dose per application area, Human applied 4-times daily, Minipig applied 3-times daily, Rat applied 3-times daily; rat values from week 26 dose, gender differences exist in dosing do to size differences

based on end of study TK values, week 52 in minipig, week 26 in rat

* for minipig: based on week 52 body weights; for rat: based on week 26 body weight (constant dose applied to growing animals, so at start of the study the dose in g/body weight was larger)

¹ M - male, F - female; note values varied substantially between genders, so they were not averaged into a single value.

APPENDIX 2: Regulatory History of DMSO

DMSO was approved for interstitial cystitis as a 50% solution (NDA 17-788, RIMSO-50, approved 1978; Research Industries Corp., now marketed by Bioniche Pharma) in which the disease condition, and its method and frequency of application (bladder intravesical instillation of 50 mL, once every 2 weeks) is substantially different from the Sponsor's proposed indication and administration (topical, 4 times daily). In Nov 2002, a generic product was approved (ANDA 76-185, Bioniche Pharma).

Other drug products with DMSO (CAS # 67-68-5) as the inactive ingredient include those listed in the following table:

Drug Product	Route; Dosage Form	DMSO amount	Indication/use
Enteryx: (no longer marketed?)	IV (infusion); powder for injection suspension, lyophilized	9.6 g	Indicated for endoscopic injection into the lower esophageal sphincter for the treatment for gastroesophageal reflux disease. Enteryx is a medical device comprised of an injectable solution of ethylene vinyl alcohol copolymer dissolved in DMSO
Viadur (leuprolide acetate implant, 9 amino acid peptide analog of GnRH)	Subcutaneous; implant	104 mg within a capsule; Deliver ~ 173 µg DMSO/day	A sterile nonbiodegradable, osmotically driven miniaturized implant designed to deliver leuprolide acetate for 12 months at a controlled rate. Contains 72 mg leuprolide acetate dissolved in 104 mg DMSO. At 120 µg leuprolide/day ; therefore estimate by ratio to deliver 173 ug DMSO/day).
Dermagraft (Human Fibroblast-Derived Dermal Substitute; fibroblasts seeded onto a bioabsorbable polyglactin mesh scaffold)	Topical; dressing	— Almost all DMSO is rinsed away before application. Applied once weekly.	Demagraft is packaged with a saline-based cryoprotectant. This solution supplemented with 10% DMSO and bovine serum to facilitate term frozen storage of the product. Single-use. The product is rinsed three times before use, applied weekly.
Idoxuridine 5%	Solution	in dimethyl sulfoxide (to aid absorption)	may be painted onto the lesions of cutaneous herpes simplex and herpes zoster four times daily for 4 days.
Onyx® Liquid Embolic System Micro Therapeutics, Inc. Irvine, CA, U.S.A	Non-adhesive liquid embolic agent	The Onyx Liquid Embolic System (LESTM) consists of a 1.5 ml vial of Onyx, a 1.5 ml vial of DMSO , and three 1 ml Onyx delivery syringes.	Onyx® is a non-adhesive liquid embolic agent comprised of EVOH (ethylene vinyl alcohol) copolymer dissolved in DMSO (dimethyl sulfoxide), and suspended micronized tantalum powder to provide contrast for visualization under fluoroscopy. The Onyx Liquid Embolic System (LESTM) consists of a 1.5 ml vial of Onyx, a 1.5 ml vial of DMSO, and three 1 ml Onyx delivery syringes.

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Veterinary Products

Product	DMSO amount	Date Approved	indication
Synotic Otic Solution (fluocinolone acetone and 60% DMSO)	60% DMSO	United States	Dogs only: topical application to the ear canal
DOMSO Solution	90% DMSO	United States and Canada?	Horse: for topical prescription use
DOMSO Gel	90% DMSO	United States and Canada?	Horse: for topical prescription use
DOMOSO Solution	99% DMSO	United States and Canada?	Horse: for topical prescription use
DOMOSO Gel			
Dolicur		Canada	
Synsac Solution		United States and Canada	

Foreign Products

Product	DMSO amount	Country	Date Approved	indication
RIMSO-50	50% solution	Canada	1979	interstitial cystitis
Kemsol 70% DMSO solution	70% solution	Canada		scleroderma
Dolobene Gel 50 g tube	15%	Germany approved in another 13 countries; marketed in 10 (Austria, China, Czech Republic, Georgia, Hungary, Luxembourg, Poland, Russian Federation, Slovakia and Ukraine).	1994	topical use

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI
NDA-20947	ORIG-1	DIMETHAID RESEARCH INC	PENNSAID(DICLOFENAC SODIUM)1.5% TOP LOTI

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

/s/

LAWRENCE S LESHIN
10/02/2009

ADAM M WASSERMAN
10/02/2009

Please see my Supervisory Addendum which explains my lack of concurrence with the regulatory recommendations made by Dr. Leshin.



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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER: 20-947
SERIAL NUMBER: 000 (2nd Resubmission)
DATE RECEIVED BY CENTER: June 28, 2006
PRODUCT: PENNSAID® Topical Solution
(1.5% w/w diclofenac sodium)

INTENDED CLINICAL POPULATION: Topical treatment for relief of the signs
and symptoms of osteoarthritis of the
knee(s)

SPONSOR: Dimethaid International Inc.
DOCUMENTS REVIEWED: eCTD submission
REVIEW DIVISION: Division of Anesthesia, Analgesia and
Rheumatology Products (HFD-170)

PHARM/TOX REVIEWER: L. Steven. Leshin, DVM, PhD
PHARM/TOX SUPERVISOR: R. Daniel Mellon, PhD
DIVISION DIRECTOR: Bob A. Rappaport, M.D.
PROJECT MANAGER: Paul Balcer

Date of review submission to Division File System (DFS): December 21, 2006

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW.....	10
2.6.1 INTRODUCTION AND DRUG HISTORY.....	10
2.6.2 PHARMACOLOGY.....	16
2.6.2.1 Brief summary	16
2.6.2.2 Primary pharmacodynamics	16
2.6.2.3 Secondary pharmacodynamics	21
2.6.2.4 Safety pharmacology	22
2.6.2.5 Pharmacodynamic drug interactions.....	26
2.6.3 PHARMACOLOGY TABULATED SUMMARY.....	26
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	27
2.6.4.1 Brief summary	27
2.6.4.2 Methods of Analysis.....	28
2.6.4.3 Absorption	28
2.6.4.4 Distribution.....	32
2.6.4.5 Metabolism	37
2.6.4.6 Excretion.....	39
2.6.4.7 Pharmacokinetic drug interactions.....	40
2.6.4.8 Other Pharmacokinetic Studies.....	40
2.6.4.9 Discussion and Conclusions	40
2.6.4.10 Tables and figures to include comparative TK summary	41
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	42
2.6.6 TOXICOLOGY.....	43
2.6.6.2 Single-dose toxicity	43
2.6.6.4 Genetic toxicology.....	55
2.6.6.5 Carcinogenicity.....	74
2.6.6.6 Reproductive and developmental toxicology.....	77
2.6.6.7 Local tolerance	79
2.6.6.9 Discussion and Conclusions	81
2.6.6.10 Tables and Figures (Sponsor Tables).....	83
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	86

EXECUTIVE SUMMARY

I. RECOMMENDATION

A. RECOMMENDATION ON APPROVABILITY

From a Pharmacology and Toxicology perspective this application is not approvable. The overall opinion of this reviewer was that the product development program was not adequate for the proposed indication of chronic topical use. Therefore, certain critical issues were not addressed that impacts both the safety and efficacy of the product. These include:

- The effect of sunlight (visible and UV light spectrum) on product stability is unknown.
- Information concerning dermal carcinogenicity and photocarcinogenicity is insufficient or lacking.
- The effect of skin conditioners, moisturizers, and other topical compounds on uptake of diclofenac was not addressed.

Because the active ingredient, diclofenac, is an approved drug, and this application is a 505(b)(2), the Sponsor did not perform non-clinical studies, except 3 genetic toxicology studies. From a pharmacological and toxicological perspective, the drug product is a combination drug, consisting of both diclofenac and dimethyl sulfoxide (DMSO). Both are approved drugs, both have well documented anti-inflammatory ability, but have different mechanisms of action. The Sponsor's argued that DMSO is an excipient in their formulation, although it comprises 45.5% of the formulation. They base this claim on the use of DMSO for enabling diclofenac's penetration through the skin and its lack of efficacy in improving measures of osteoarthritis symptoms during earlier clinical testing. However, this lack of DMSO efficacy was not demonstrated until the Phase 3 study (PEN-03-112). The reason for stating DMSO as an excipient, rather than an active agent, during the initial development stages was not adequately explained. This reviewer was also unable to identify if a definitive conclusion was reached in earlier discussions with the Agency concerning the nomenclature for the DMSO component. It appears from their drug development program that it was not considered a combination drug, and the emphasis was on the efficacy and safety of topical diclofenac. However, the contribution of DMSO to safety and efficacy kept reappearing and contributed substantially to non-approval of earlier NDA submissions. With this resubmission, some previous concerns have been resolved, but others, as indicated above, still exist.

B. RECOMMENDATION FOR NONCLINICAL STUDIES

- 1. The Sponsor should determine the effects of visible and UV light spectrums on product stability and characterize the dermal and systemic toxicity of degradants if found.**

It is known from studies for the approved product, Solaraze™ (NDA 21-005; the Sponsor has not referenced this product and lacks Right of Reference to this product), that exposure of diclofenac to direct sunlight results in a number of

photodegradant products. In the presence of DMSO, it is unknown whether these will occur, due to the possible free radical scavenger activity of DMSO. However, DMSO is also very liable in sunlight, and its degradation products are not well documented. This has bearing on both efficacy and safety of the drug product.

2. **The Sponsor should characterize the carcinogenic potential of their topical product by conducting dermal carcinogenicity and photocarcinogenicity studies.** Carcinogenicity studies in which diclofenac was administered orally were conducted for Voltaren (diclofenac sodium, NDA 19-201, Novartis, referenced drug). However, dermal carcinogenicity studies have not been performed with Pennsaid. The Pennsaid product will also be exposed to sunlight and therefore photocarcinogenicity studies should be performed. If the Pennsaid product lacks absorption in UVA and UVB spectrums of wavelength, then it would not be necessary to perform the photocarcinogenicity studies, as per the May 2003 Guidance for Industry: Photosafety Testing.

Dermal carcinogenicity and photocarcinogenicity studies were conducted for the approved product, Solaraze™ (NDA 21-005; the Sponsor has not adequately referenced this product). Those studies used mice and the exposures obtained were significantly lower than the clinical exposure to the drug product due to mortalities from gastrointestinal lesions. This may have resulted from ingestion associated with their grooming behavior or absorption of the drug from the site of application.. Studies could be designed to minimize oral intake from dermal applications.

C. RECOMMENDATIONS ON LABELING

Based on previous and current reviews the following labeling changes in red italics are recommended:

Make DMSO a more prominent component:

Each 1 mL of solution contains 16.05 mg of diclofenac sodium. In addition, PENNSAID® contains the following inactive ingredients: dimethyl sulfoxide (DMSO, **45.5% w/w**), propylene glycol, alcohol _____ glycerin and purified water.

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Fertility studies have not been conducted with PENNSAID®. — diclofenac, male and female rats — administered 4 mg/kg/day did not affect fertility. In rats, maternal toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival.

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Pregnancy

Teratogenic Effects. Pregnancy Category C.

b(4)

Nursing Mothers

It is not known whether this drug is excreted in human milk. Because many drugs are excreted in human-milk and because of the potential for serious adverse reactions in nursing infants from PENNSAID®, _____

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b(4)

II. Summary of nonclinical findings

A. Brief Overview of Non-Clinical Findings

The lack of clearly delineating this product as a combination drug early in the development phase had a substantial impact on the non-clinical pharmacology and toxicology program. Both diclofenac and DMSO were approved for other routes of administration and indications. By ignoring the well known, but not fully characterized by Agency standards, toxicity of DMSO and not studying their potential interaction as a combination product, there were and still are unknowns in the toxicology of this product. Rather than empirically determining potential toxicologies that are essential to identify, the Sponsor has consistently cited published papers and drug monographs in support of the pharmacology and toxicological safety, especially for DMSO. The Sponsor considered DMSO an excipient, despite its comprising 45.5% of the drug product and its previous approval as a drug for treatment of interstitial cystitis and use in veterinary medicine as an anti-inflammatory. Collectively, the submitted published studies are inadequate in terms of meeting the Agency's toxicological study requirements for a new drug product. There were no preclinical dermal toxicology studies in which both diclofenac and DMSO have been administered together. Individually, the toxicologies and anti-inflammatory pharmacodynamic action arising from topical application of DMSO and of diclofenac are similar in many regards. However, their mechanisms of action are dissimilar and therefore, there is a potential for toxicological consequences when applied simultaneously and frequently as in the proposed indication.

Overall, reports on the toxicity of DMSO in animals indicate a lack of significant toxic manifestation in the milligram per kilogram body weight doses. The Sponsor provided published literature to support the safety of DMSO alone from a non-clinical perspective, yet the toxicological concerns regarding the use of DMSO was not resolved. There are many cases in which conflicting or inconsistent results exist in the literature. Those studies that do not support the Sponsor's assertion regarding the safety of DMSO were dismissed as having been performed with a low grade of DMSO, or with DMSO of an unknown purity, thus assumed to be contaminated. The purity of DMSO was rarely provided in the studies and it was rarely indicated that concentrations and purity were verified as would have been required if those studies were performed by the Sponsor. Therefore, this reviewer cannot adequately determine if those studies, indicative of potential safety concerns, are in fact a result of DMSO itself, or a degradant/contaminant. Conversely, it is difficult to be assured that the studies supporting the safety of DMSO lack these degradants/contaminants, or were just underdosed.

The non-clinical toxicology profile of diclofenac is typical of NSAIDs. Its main toxicity is directed toward the gastrointestinal tract, resulting in erosion of the epithelium due to its inhibitory actions on cyclooxygenase isozymes, both COX-1 and COX-2, altering prostaglandin production.

The topical route of administration for Pennsaid[®], is different from the referenced compound, Voltaren, taken orally. The toxicology of topically applied diclofenac was not studied by the Sponsor and there were few nonclinical publications describing its topical toxicology and safety. These studies were either of too short a duration or provided insufficient information to adequately describe potential safety issues for the proposed use of Pennsaid[®] (see Regulatory History section in 2.6.1. Introduction and Drug History. There was insufficient study of the distribution, duration, and toxicokinetics of DMSO applied topically. There was no information about the distribution, duration and toxicokinetics of diclofenac applied with DMSO. There was no information about a potential depot formation of DMSO and/or diclofenac within the site of application. There was no information about the effect of sunlight exposure on the drug product stability, toxicity of potentially formed products, or effectiveness of the product. Since DMSO enhanced penetration, there was no information on its ability to transfer other compounds into the body, such as environmental toxicants (insect repellants, soil contaminants), sunscreens, moisturizing lotion, cosmetics, or other topically applied compounds for which internal toxicity is not usually tested.

During drug development, two *in vitro* absorption and bioavailability studies were performed with human skin using radioactively labeled diclofenac (UCSF95SU03, UCSF96SU05; included in IND 42,773) and submitted with the initial NDA submission. Other doses of diclofenac and the concentration of its biologically active excipient, DMSO, were not studied for either efficacy safety. It was never explained why this dose and formulation were chosen.

In this NDA resubmission, the Sponsor provided 3 new genotoxicity studies. Doses were based on the drug product, Pennsaid[®], rather than the active compound, diclofenac. However, for the two *in vitro* studies testing mutagenesis (bacterial assay) and clastogenicity (human lymphocyte micronucleus assay), the maximal dose of active substance, diclofenac and the biologically active excipient, dimethyl sulfoxide (DMSO), concentrations were 72-fold and 2-fold lower, respectively, than ICH guidance recommendations. No evidence of clastogenicity was observed in an *in vivo* mouse micronucleus study with doses of Pennsaid[®] near the maximum tolerated oral dose of 12 mg/kg (approximately 178 mg/kg diclofenac and 5.86 g/kg DMSO).

However, a few published studies reported positive findings for DMSO in bacterial reverse mutation and chromosomal aberration assays. DMSO (100%) was not carcinogenic when applied to hamster oral pouch, or administered cutaneously or subcutaneously to mice for 76 to 94 weeks. It was teratogenic when administered intraperitoneally to hamsters, rats and mice at 2.5 to 12 g/kg. DMSO is classified as Pregnancy Category C. DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically.

B. Pharmacologic Activity

Diclofenac is an NSAID with anti-inflammatory, analgesic and antipyretic properties. The sodium (Voltaren®) and potassium (Cataflam®) salts of diclofenac are approved for osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, analgesia and primary dysmenorrhea in immediate-release, delayed release or extended release forms. The pharmacological effects of the diclofenac are thought to be mediated through inhibition of cyclooxygenase isozymes, both COX-1 and COX-2, altering prostaglandin production.

Because of its unique ability to readily penetrate the skin, DMSO is a suitable carrier for other substances. Pennsaid® contains 45.5% DMSO. Apparently, DMSO is being used only as a carrier and penetration enhancer in Pennsaid® because the sponsor did not claim any pharmacological efficacy for DMSO in this drug product. However, numerous pharmacological activities have been attributed to DMSO. Studies have shown that DMSO has anti-inflammatory and analgesic properties. Because of its free radical scavenging ability, DMSO can protect against damages caused by radiation and ischemia. DMSO affects the function of a number of enzymes. It has anti-microbial and anti-coagulative effects and is used for cryopreservation and as a solvent routinely.

C. Non-Clinical Safety Issues Relevant to Clinical Use

There is a lack of long term safety data from animal studies, since there were no studies of the diclofenac and DMSO combination. The literature references to separate studies with diclofenac and with DMSO did not address topical toxicology and toxicokinetics except for a few cases. These were of insufficient dose or duration to be useful in predicting potential safety issues for patients.

The product information regarding the potential for dermal carcinogenicity and photocarcinogenicity is insufficient or lacking. The effect of sunlight on product stability is unknown. The marketed product will be applied 4 times per day, so it will be carried around as part of the patient's daily routine. It will be exposed to sunlight and heat. It is not known what effect this has on the product's stability and safety.

As a first to be approved dermal NSAID in the United States, there is additional concern for widespread off-label use, especially for athletic injuries. Concerns regarding the safety of DMSO, which the Sponsors have claimed from early in development to be an excipient, but which has well documented toxicities, has been downplayed throughout the product's development and in the labelling.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 20-947
Review number: 3
Sequence /date/type of submission: 000/June 28, 2006/Resubmission 2
Information to sponsor: Yes

Sponsor and/or agent: Nuvo Research Inc.
(formerly Dimethaid International Inc.,
now a wholly-owned subsidiary of
Nuvo Research Inc.)
Los Abedules Appleby Gardens,
St. James, Barbados

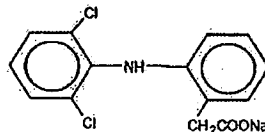
Manufacturer for drug substance: _____

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Reviewer name: L.S. Leshin
Division name: Division of Anesthesia, Analgesia and
Rheumatology Products
HFD 170
Review completion date: December 21, 2006

Drug:

Trade name: PENNSAID® Topical Solution
(1.5% w/w diclofenac sodium)
Generic name: diclofenac sodium
Chemical name: 2-[(2,6-dichlorophenyl)amino] benzeacetic acid,
monosodium salt.
CAS registry number: 15307-79-6
Molecular formula: C₁₄H₁₀Cl₂NNaO₂
Molecular weight: 318.14
Structure:



Relevant INDs/NDAs/DMFs:

NDA 19-201 (Voltaren, Novartis, diclofenac sodium for oral administration)
IND 42,773 (PENNSAID® Topical Solution)

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NDA 17-788 (RIMSO-50, Bioniche; 50% DMSO for interstitial cystitis, approved 1978)
 NDA 21-005 (Solaraze™, Bioglan Pharms Corp., 3% diclofenac gel for actinic
 keratoses)

Drug class: Non Steroidal Anti-inflammatory

Intended clinical population: Topical treatment for relief of the signs and
 symptoms of osteoarthritis of the knee(s)

Clinical formulation: The active substance is diclofenac sodium, at a
 concentration of 1.5% w/w (16.05 mg in 1 mL).
 DMSO is included as a penetration enhancer.

Composition of PENNSAID® Topical Solution (1.5% w/w diclofenac sodium)			
Ingredients	mg/g	kg/batch	%
Diclofenac sodium USP	15.0		1.5
Dimethyl Sulfoxide USP	455.0		45.5
Glycerin			
Propylene Glycol USP			
Alcohol			
Purified Water USP			
TOTAL WEIGHT OF PENNSAID® Topical Solution			100.0

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Route of administration: topical, 40 drops per knee, four times a day
 Dispense 10 drops at a time directly onto knee or
 first into hand then onto knee, spread evenly around
 front, back and sides of knee, repeat until 40 drops
 have been applied

Assuming 40 drops is approximately 1.2 ml (based on Sponsor reply Nov 8, 2006 (SN-000 BZ)
 to a FDA information request, then patients would be applying the following daily amount of
 active compounds diclofenac and DMSO:

Administered Dose of Pennsaid, Diclofenac Sodium and DMSO

Treatment	Pennsaid Dose /application	Pennsaid Dose /day	Diclofenac sodium		DMSO	
			mg/day	mg/kg/day	g/day	g/kg/day
1 knee	40 drops ≈ 1.2 mL	160 drops ≈ 4.8 mL	77 ^a	1.3 ^b	2.2 ^c	0.037 ^b
2 knees	80 drops ≈ 2.4 mL	320 drops ≈ 9.6 mL	154	2.6	4.4	0.073

^a based on 16.05 mg/mL^b based on 60 kg patient^c based on % w/w

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

Regulatory History

The following information was obtained from previous reviews and materials in DFS, and materials in the document room files for IND 42,773 and NDA 20-947.

At the time of initial IND submission, a clinical hold was recommended because of the lack of non-clinical toxicity data.

...put on hold until the sponsor submits pertinent preclinical toxicity data (or authorization to cross-reference to such data) of the two active ingredients as well as of the drug product (proposed formulation) to support the safety of the proposed clinical studies.

The sponsor provided 40 published articles in support of the product's safety in an amendment on August 17, 1993, which was also found inadequate. On September 16, 1993 the Sponsor was sent a letter detailing the deficiencies, which prevented the clinical trials from proceeding.

Clinical

- a) Cadaver skin studies are needed in order to evaluate skin penetration.
- b) Preclinical toxicology data from the two active ingredients, as well as the drug product, should be provided prior to initiation of clinical efficacy studies (see below).

Preclinical

- a) If further development of this product is planned, it will be necessary to separate out the pharmacodynamic activity contributed by the drug diclofenac and DMSO, which has anti-inflammatory properties and therefore cannot be considered an inactive excipient as you propose. In addition, the following are required:
 - b) A PK study of _____ in animals should be performed to determine the amount of diclofenac absorbed from _____. Other PK parameters should also be determined, such as elimination half-life and systemic distribution. The metabolic profile for diclofenac should be determined for this route of administration.
 - c) A drug interaction study with DMSO should be conducted.
 - d) A toxicity study will be needed to determine if diclofenac toxicity is increased by this route of exposure. The length of this study will depend on the proposed duration of human use. For chronic use in humans, a 90-Day study will be required.

b(4)

In addition, the following concerns were to be addressed as soon as possible:

- e) It was recommended that skin metabolism information be obtained from cell culture techniques.
- f) A rationale should be submitted to indicate there is no change in the systemic carcinogenicity or teratogenicity potential, or the potential to produce skin cancer, or mediate photocarcinogenicity by the intended route of administration. Animal PK data and metabolic profile can be the starting basis for the rationale.
- g) It should be determined if serum protein binding of diclofenac is altered by expected human serum concentrations of DMSO.

At a meeting with Dimethaid held on 6/6/95, the following information was exchanged:

- 1) FDA agreed the metabolic profile from cadaver skin could substitute for a cell culture profile to obtain skin metabolism data.
- 2) The potential for skin sensitization, skin phototoxicity, and photocontact allergy will be evaluated by Dimethaid in clinical studies.
- 3) Human transdermal bioavailability could be substituted for the recommended animal study.
- 4) Safety data from humans evaluated in Canada could replace a 90-day animal study.

During a meeting June 6, 1995 between the FDA and Dimethaid Research Inc. it was agreed that instead of the customary 90-day rat toxicity study, the safety data generated on humans in Canada through the EDR program, and the phase III open-labeled study #105-95, would be sufficient.

Subsequently, in a letter dated June 7, 1995, the sponsor was allowed to proceed with the clinical trials.

NDA 20-947 was originally submitted on Dec 15, 1997. The Sponsor provided literature references for diclofenac sodium and DMSO to support the nonclinical pharmacology and toxicology section of the NDA. The Applicant withdrew the NDA in Oct 27, 1998 because the contract manufacturing facility was not prepared for the pre-approval inspection due to ongoing renovations that were beyond the control of the Sponsor. The Pharmacology and Toxicology review was not completed at the time of withdrawal and there is no record of feedback concerning non-clinical findings being sent to the Sponsor after they withdrew the NDA.

The NDA was resubmitted August 7, 2001. At that time, the human safety data was found to be inadequate. A non-approvable (NA) letter was issued August 7, 2002, which stated the following about the human safety data.

Safety:

Inadequate data was provided to demonstrate long-term safety for this drug product.

In addition, demonstration of safety in situations of co-administered therapies, which is likely to occur once a topical agent is approved, is needed. No safety information on the use of Pennsaid with daily oral anti-inflammatory and analgesic therapies was provided.

There are no clinical laboratory data and no data on co-administration with other drugs (except rescue acetaminophen) in either Study 109 or 109-US.

There are substantial differences in reporting of some adverse events (AEs) between Studies 109 and 109-US and other NDA studies. For example, arthralgia is listed to occur between 1.9-3.7% for the Pennsaid groups in Study 109 and 109-US, but 34.5% in Study 107-96. Similarly, arthralgia was noted in 1.9-4.6% of patients treated with DMSO in Study 109 and 109-US, but in 40% of DMSO treated patients in Study 107-96. It is unclear why this difference exists. Since DMSO is an important component of Pennsaid, all AEs in these two groups in Study 109 and 109-US are considered to occur with Pennsaid. Therefore, there is no clear understanding of the adverse event profile of Pennsaid versus a "non-diclofenac" 45.5% DMSO-containing control. Under ICH guidelines the Sponsor is required to submit safety data from the sample size of 300-600 patients treated for 6 months and from 100 patients treated for 12 months. You have submitted data from two open-label, long-term studies, EDR and 105-95.

There are no AEs reported for EDR. The rates of AEs in Study 105-95 are very low compared to controlled studies even for skin reactions that are very common with the use of this drug. This is suggestive of very significant underreporting and, therefore, cannot be viewed as adequate safety data for long-term use of the drug.

Because of the above deficiencies, the safety of Pennsaid has not been adequately demonstrated in this NDA.

The nonclinical pharmacology and toxicology summary was the same as the 1997 submission. The Division requested on Sept 12, 2001, that the Applicant provide an update on the reproductive toxicity, genotoxicity, dermal toxicity and carcinogenicity of DMSO (i.e. any recent study reports or information, besides those that have been submitted with previous filing of the NDA). An updated summary was submitted October 5, 2001, and on May 8, 2002, additional literature references for genotoxicity, mutagenicity and carcinogenicity of DMSO were submitted. In the non-approvable (NA) letter of August 7, 2002 for this second submission, no specific nonclinical issues were communicated to the Sponsor. The pharmacology/toxicology reviewer and supervisor concurred that the application was approvable from a nonclinical perspective.

NDA 20-947 was resubmitted June 28, 2006, with comments from the Sponsor that it was their understanding, based on the non-approvable letter, that there were no outstanding issues regarding the non-clinical pharmacology and toxicology section of NDA 020-947 submitted in August 2001. As agreed to by the Division (February 13, 2006 FDA Advice Letter), new literature references to the NDA were provided. The Sponsor also included 3 genotoxicology studies conducted with the "to be marketed" formulation of Pennsaid® Topical Solution. The new nonclinical literature references covered toxicological and pharmacokinetic studies of DMSO, which the Sponsor considers to be an excipient.

For (b)(2) applications:

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 20-947 are owned by Nuvo Research Inc. or are data for which Nuvo Research Inc. has obtained a written right of reference. Any information or data necessary for approval of NDA 20-947 that Nuvo Research Inc. does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Nuvo Research Inc. does not own (or from FDA reviews or summaries of a previously

approved application) is for descriptive purposes only and is not relied upon for approval of 20-947.

Studies reviewed within this submission:

Study Location (e-submission)	Study Title
Genotoxicology	
phamtox\tox\17623.pdf	Mutagenicity study of PENNSAID® in the <i>Salmonella typhimurium</i> reverse mutation assay (<i>in vitro</i>)
pharmtox\tox\17625.pdf	<i>In vitro</i> assessment of the clastogenic activity of PENNSAID® in cultured human peripheral lymphocytes
pharmtox\tox\17626.pdf	Micronucleus test of PENNSAID® in bone marrow cells of the NMRI mouse by oral administration

The following sections were not included in this submission.

II. Pharmacology Studies
a. Pharmacodynamics
b. Safety Pharmacology
c. Other Pharmacology Studies
III. Pharmacokinetics
a. Absorption
b. Distribution
c. Metabolism
d. Excretion
e. Other
IV. Toxicology
a. Single dose toxicity studies
b. Repeat dose toxicity studies
d. Carcinogenicity studies
e. Reproduction toxicity studies
f. Supplemental toxicity studies

Information concerning these topics was obtained from previous reviews and the references to published articles submitted in the previous and current submission, (see List of References in Appendix 1).

Studies not reviewed within this submission:

Published articles listed in Appendix 1 were submitted in the previous and current submissions of this NDA. These articles were not formally reviewed in template format.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary

The active ingredient of Pennsaid® is diclofenac, a well characterized nonsteroidal anti-inflammatory drug. It is a phenylacetic acid derivative with anti-inflammatory, analgesic and antipyretic properties. The sodium (Volteren®) and potassium (Cataflam®) salts of diclofenac are approved for osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, analgesia and primary dysmenorrhea in immediate-release, delayed release or extended release forms. The pharmacological effects of the diclofenac mediated through inhibition of both the COX-1 and COX-2 isoforms of the cyclooxygenase enzyme, preventing synthesis of prostaglandins involved in therapeutic and adverse effects of nonsteroidal anti-inflammatory drugs.

Pennsaid® contains 45% dimethyl sulfoxide (DMSO), used as a penetration enhancer for diclofenac in Pennsaid®. DMSO is a polar, aprotic hydroscopic solvent. The Sponsor considered DMSO as an excipient in Pennsaid®, and claims that it lacks pharmacological efficacy in their clinical trial. An abundance of studies in animals indicates that DMSO itself has anti-inflammatory properties; although it's often associated analgesic properties are not well established. Studies have demonstrated numerous biological effects of DMSO, which include protection against damage caused by radiation and ischemia due to its free radical scavenging ability, and effects on enzyme function, anti-microbial activity, and anti-coagulative effects. DMSO is used routinely as a solvent for hydrophobic compounds and for cryopreservation. In addition, DMSO is found naturally in the environment and in a large number of fruits, vegetables, grains and beverages.

2.6.2.2 Primary pharmacodynamics

MECHANISM OF ACTION: DICLOFENAC

Early studies of diclofenac effects (Menassé et al., 1978), demonstrated *in vitro* that diclofenac inhibited prostaglandin synthesis, specifically prostaglandin E synthetase with an IC₅₀ of 1.6 µM.

Comparison of Prostaglandin Synthetase Inhibition between NSAIDs
(Microsomal Fractions of Bovine Seminal Vesicles (*in vitro*) (Sponsor's Table)

PREPARATION	Inhibition of Prostaglandin Synthetase (IC ₅₀ µM)
Diclofenac Sodium	1.6
Acetylsalicylic Acid	3,300
Indomethacin	5.6
Naproxen	5.7

[Menassé et al. 1978]

Potential effects on the synthesis of other prostaglandins, or other related inflammatory pathways, were not provided by the Sponsor.

MECHANISM OF ACTION: DIMETHYL SULFOXIDE (DMSO)

DMSO is used as a skin penetrant and carrier of diclofenac sodium in the Pennsaid® formulation. DMSO enhances the percutaneous absorption of substances of molecular weight less than 3,000 daltons. Within the skin, the absorbed substances tend to accumulate, creating a dermal reservoir. DMSO has a permeability constant of 300 $\mu\text{cm}/\text{min}$ and a cumulative penetration rate of 8 $\mu\text{g}/\text{cm}^2/\text{min}$ (Allenby et al., 1969).

The Sponsor provided a summary of the current hypothesis of DMSO actions along with the evidence upon which this is based. Kligman A.M. (1965) carried out some of the early work on the topical application of DMSO and noted that "it is only with rather concentrated solutions (above 70%) that penetration enhancement becomes really striking". However, it is clear that at lower concentrations of DMSO penetration enhancement occurs, but the rate of transport is reduced. Topical applications of DMSO induce changes in cell size and in the extracellular matrix of the outer cell layers of skin that comprises the barrier for percutaneous penetration. In the basal epidermal layer, cells became enlarged and separate from the underlying granular cells producing a subcorneal cleft. In the intermediate layer, cells enlarge to a lesser degree. In the superficial layer, fibrils clump together or totally disappear. The plasma membrane of the cells involved remains intact (Montes 1967).

Sandborn et al. (1975) on the basis of electron micrographic studies, proposed that DMSO enters cells through a "pore" system in the cell membrane; and, further, that passage within a cell takes place via a microcirculatory tubule system, such that processes are directed and are not a matter of random distribution in the cytoplasm. This could facilitate the uptake and transcellular transport of substances that would normally be prevented by the cell membrane. DMSO may open potential pores in the cell membrane and carry dissolved substances with it through the membrane. This theory would explain the deep penetration and lack of "sink" effect. Guy and Maibach (1983) concluded "that the vascular supply of the skin is not always a perfect "sink" and that deeper penetration may be a far more ubiquitous phenomenon than had ever been thought possible". Such deeper penetration would deliver more active compound locally to underlying tissue than would other more commonly employed routes of administration.

Alsop (1984) noted that the penetration process is reversible and occurs without affecting the integrity of most membranes. The mechanisms are not understood but the polar nature of DMSO, its ability to hydrogen bond, and its relatively small and compact structure all could contribute to its unique property of skin penetration.

Kurihara-Bergstrom (1986) concluded from physicochemical studies using extracts of stratum corneum, that barrier impairment of the stratum corneum is due in part to elution of DMSO-soluble components from the horny tissue layer. Delamination of horny layer proteins enhances diffusion. Khan and Kellaway (1989) used differential scanning calorimetry to examine the effect of DMSO on stratum corneum and also concluded that DMSO-enhanced penetration resulted from an interrelationship between delipidation and protein denaturation of stratum corneum. This effect only occurred at DMSO concentrations above 60%. Ghosh and Banga (1993) in a review on transdermal drug delivery consider there to be two potential routes through the skin: between the cells (intercellular route) or through the protein-filled cells of the stratum

corneum and across the lipid-rich regions (transcellular route). Within the skin the absorbed substances tend to accumulate, creating a dermal reservoir.

DRUG ACTIVITY RELATED TO PROPOSED INDICATION: DICLOFENAC

The Sponsor provided references and summarized the early studies demonstrating diclofenac anti-inflammatory and analgesic properties. Studies by Menassé et al., (1978), demonstrated an *in vivo* analgesic potency in a mouse and rat paw pain models (see Sponsor's table below).

Analgesic Potency of Diclofenac and Other Anti-inflammatory Agents (Mouse and Rat)

Preparation	Analgesic Potency		
	Phenyl- <i>p</i> -benzo-quinone test, Mouse (ED ₅₀ * mg/kg p.o.)	Acetic Acid Test, Rat (ED ₅₀ mg/kg p.o.)	Atacrynic Acid Test, Rat (ED ₅₀ mg/kg p.o.)
Diclofenac Sodium	4.3	2.5	1.4
Acetylsalicylic Acid	165	65	88
Flufenamic Acid	>250	125	Not Tested
Ibuprofen	44	10	Not Tested
Indomethacin	2.7	2	2.5
Naproxen	22	Not Tested	Not Tested
Ocyphenylbutazone	220	60	93
Phenylbutazone	95	35	62
Sudoxican	10	Not Tested	Not Tested

*ED₅₀=median effective dose

[Menassé et al. 1978]

In rats, edemas produced by carrageen and kaolin were inhibited, and adjuvant arthritis was reduced by smaller oral doses of diclofenac sodium compared to other NSAIDs as indicated in the Sponsor's table below.

Effect of Diclofenac on Edema

PREPARATION	Inhibition of Oedema Induced By Carrageen (ED ₅₀ mg/kg/p.o.)	Inhibition of Oedema Induced By Kaolin (ED ₅₀ mg/kg/p.o.)	Reduction of Adjuvant Arthritis (ED ₅₀ mg/kg/p.o.)
Diclofenac Sodium			
Acetylsalicylic Acid			
Indomethacin			
Naproxen			

b(4)

Topical diclofenac in pain and inflammation

Topical emulsion of diclofenac diethylammonium salt increased the pain threshold, ie. analgesia, in rats similar to other compounds with analgesic properties indicated in the Sponsor's table below from a study of Hiramatsu et al. (1990).

Antinociceptive Effect in the Rat, Randall and Selitto Test

Treatment	Pain Threshold (g)		% Increase Over Controls
	Mean ^a	Median ^b	
Control	131.9 ± 29.8	128 ± 52	-
1.16% diclofenac diethylammonium salt, lipids, isopropyl alcohol, propylene glycol	206.9 ± 66.8	182 ± 107	57
1% indomethacin, macrogol 300, sodium thiosulfate, benzyl alcohol, gelatin	211.0 ± 97.7	180 ± 109	60
5% etofenamat, aromatics, macrogel 400	200.7 ± 71.1	171 ± 119	52
1% adrenal extract (corresponding to 0.02% prednisolone), 0.2% mucopolysaccharide-sulphuric acid ester, 2% salicylic acid	187.1 ± 46.0	189 ± 83	42

^a Mean ± S.D. (n=9-10, control n = 15)^b Median ± interquartile range

Topical application of diclofenac sodium also exhibited anti-inflammatory qualities. Kyuki et al. (1983) examined the anti-inflammatory effect of sodium diclofenac ointments on topical application in various acute, subacute and chronic inflammatory animal models of inflammation (Sponsor's table below). Formulations were prepared in three bases: lipophilic (ointment), emulsion (cream), and carboxyvinyl polymer (gel). On the basis of screening in four of the models, it was concluded that the cream had the most significant anti-inflammatory activity and was used for future studies. Cream formulations containing 0.5, 0.75, 1.0, and 1.5% sodium diclofenac were then compared in the carrageenan edema, UV erythema and croton oil edema tests. They found that the 1% cream was the optimum formulation. The anti-inflammatory effect of the 1% diclofenac cream was compared with topical 1% indomethacin gel, 5% bufexamac cream and Mobilat ointment (a mixture of adrenal extract, heparin-like substances and salicylic acid). The diclofenac cream produced a 17% inhibition of vascular permeability, a 33% inhibition of carrageenan induced skin edema, and a 34% reduction in croton oil induced edema. The cream also produced a significant reduction in carrageenan induced swelling in the rat paw and UV erythema tests. In adjuvant arthritis, diclofenac cream reduced the swelling in the treated paw and slightly in the untreated paw. A 1% diclofenac sodium cream also inhibited the pain due to pressure stimulation of an inflamed rat paw. The analgesic effect occurred shortly after application of the cream.

Effects of Topical Application of Preparations on Various Models of Acute Inflammation (Mice)
(Sponsor's Table)

Test Ointments	Vascular Permeability		Carrageenan Skin Edema		Croton Oil Ear Edema	
	Bluing Area (mm ²) Mean ± S.E.	Inhibitory %	Swelling % Mean ± S.E.	Inhibitory %	Swelling % Mean ± S.E.	Inhibitory %
Control	126.0 ± 2.3	-	83.9 ± 4.2	-	125 ± 8.6	-
DF Cream	104.0 ± 3.9	17.4	55.9 ± 4.2	33.4	82.1 ± 9.0	34.5
BM Cream	103.6 ± 3.4	17.8	62.2 ± 5.5	25.9	90.0 ± 7.6	28.0
ML Ointment	100.8 ± 4.9	20.0	62.3 ± 4.9	25.7	81.8 ± 7.2	34.7
IM Gel	91.7 ± 3.1	27.2	59.0 ± 3.8	29.7	99.0 ± 5.0	21.0

(Kyuki et al. 1983)

DF Cream = 1% Diclofenac sodium

BM Cream = Anderm, 5% bufexamac cream

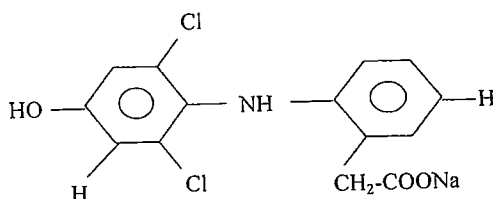
ML Ointment = Mobilat, 2% salicylic acid, 0.2% mucopolysaccharide poly-sulphuric acid ester and 1% suprarenal extract

IM Gel = Inteban, 1% indomethacin

Effect Metabolites of Diclofenac

Studies by Menasse et al. (1978) indicate that oral administration of diclofenac metabolites were relatively ineffective analgesics and antipyretics, with ED₅₀ >100 mg/kg compared to 0.26 mg/kg for diclofenac sodium. The most active metabolite, Metabolite I (see diagram below), was about 15 times less potent than diclofenac in the acetic acid test. In the yeast-induced fever test, Metabolite I was about 60 times less active than diclofenac.

Metabolite I



DRUG ACTIVITY RELATED TO PROPOSED INDICATION: DMSO

The enhanced penetration of diclofenac by DMSO was studied using human skin tissue *in vitro* by Hartway et al., (1996) and Hewitt et al., (1997). These studies are described in the Section 2.6.4.3 Absorption in Section 2.6.4. Pharmacokinetics/Toxicokinetics.

There were no studies that examined the diclofenac absorption into or through skin in the presence of DMSO, the basis for the proposed formulation.

The anti-inflammatory effect of DMSO was reported by Gorog and Kovacs (1968) in a rat study in which carrageenan-induced paw edema was improved by both oral and topical administration. It was also reported that mycobacterium-induced arthritis was inhibited to a greater extent by

dermal administration of DMSO rather than by oral administration. Similar inhibitory results were reported from topical administration of DMSO to rats with arthritis induced with 6-sulfanilamidazole.

2.6.2.3 Secondary pharmacodynamics

DICLOFENAC

There were no secondary pharmacodynamic studies referenced for diclofenac. The Sponsor mentioned some aspects adverse events that are commonly caused by nonsteroidal anti inflammatory drugs and related some of these to altered prostaglandin production, but did not provide information on prostaglandin types or diclofenac interactions with cyclooxygenase isozymes. Thus, they did not distinguish between toxicological and pharmacological effects.

Inhibition of wound healing

In an early study of topical NSAID creams Kyuki et al., (1983) found that while diclofenac cream had analgesic and anti-inflammatory properties, it inhibited the proliferation of granulation tissue similar to indomethacin and bufenamic preparations, resulting in a significant inhibitory effect on wound healing.

DIMETHYL SULFOXIDE (DMSO)

The Sponsor provided little information about the secondary actions of DMSO. (See Section 2.6.3. Tabulated Summary). Additional information was obtained through a drug indexing information service (Micromedex Inc.).

DMSO may act as a local anesthetic (Percy and Carson, 1981). DMSO lowers the conduction velocity of isolated nerves in animals, and provided pain relief equal to morphine when given topically, SQ, IP, and IV, but not orally (Jimenez and Wilkens, 1982). The anti-inflammatory actions of DMSO may be due to its ability to scavenge free radicals, stabilize lysosomal membranes (Jimenez and Wilkens, 1982), and produce vasodilation. DMSO is a free radical hydroxide trapper (Brayton, 1986). DMSO has an effect on collagen fibers. The mechanism is unknown but may have to do with dissolving collagen and leaving elastic fibers intact. In animals, DMSO temporarily reduced autoantibody levels. Its use may have a temporary immunosuppressive effect. DMSO enhanced cholinergic contractions in isolated guinea pig central airways induced by transmural electrical field stimulation (Schreiber and Slapke, 1990). The authors speculate that enhancement of neurotransmission might be a possible cause of DMSO-induced neurotoxicity. DMSO may induce histamine release from mast cells (Brayton, 1986).

2.6.2.4 Safety pharmacology

There was no safety pharmacology information provided by the Sponsor. Diclofenac is an approved drug for oral, topical, and ophthalmic uses. Neither the safety pharmacology of diclofenac, DMSO, nor the combination of diclofenac with DMSO was studied by the Sponsor. DMSO was approved for interstitial cystitis (NDA 17-788, RIMSO-50, Research Industries Corp., now owned by Bioniche Pharma) in which the disease condition, and its method and frequency of application (bladder intravesical instillation, once every 2 weeks) is substantially different from the Sponsor's proposed indication and administration (topical, 4 times daily).

Neurological effects

No studies were submitted or referenced.

Cardiovascular effects

No studies were submitted or referenced.

Pulmonary effects

No studies were submitted or referenced.

Renal effects

DMSO

Bennett et al. (1983) intravenously administered equal volumes (1g/kg body weight) of 40% DMSO or normal saline into the lingual vein under ethyl ether anesthesia over a period of 15 minutes. After 3 days of daily injections, the rats were sacrificed, their serum creatinine was measured and their kidneys were microscopically examined for damage. They found that there was no significant difference between the serum creatinine levels of the test and control groups and that there was no visual evidence of histologic damage.

They also induced renal failure in rats by intravenous injection of differing amounts of potassium dichromate. Half of these rats were given daily injections of 1 g/kg DMSO intraperitoneally as a 67% solution. Controls were injected intravenously with normal saline and then half of these rats were given daily injections of 1 g/kg DMSO intraperitoneally as a 67% solution. After 1, 3 and 5 days rats were sacrificed and their serum creatinines were determined. They found no evidence of added nephrotoxicity in rats with dichromate induced renal failure treated with DMSO versus those treated only potassium dichromate.

Gastrointestinal Effects

Diclofenac

Ménasse et al. (1978) measured the gastrointestinal tolerability of different anti-inflammatory drugs by injecting ^{51}Cr -labelled erythrocytes intravenously into rats and then orally administering various doses of drugs. The amount of radioactivity in their feces, before and for three days after a single oral dose of each anti-inflammatory drug, was used as an indicator of the amount of gastro-intestinal bleeding. A dose of 17 mg/kg diclofenac sodium caused a blood loss of 150 μL in 72 hours. When considered in relation to its pharmacodynamic efficacy, diclofenac had a therapeutic/toxic ratio 8.1:1, higher than other anti-inflammatory preparations studied (Sponsor's table below). None of the 4 metabolites of diclofenac had pronounced deleterious effect on the gastrointestinal mucosa of rats.

Gastrointestinal Tolerability of Diclofenac Compared with other NSAIDs

Preparation	Gastro-Intestinal Bleeding (GIB) (mg/kg p.o.)	Tolerability Index (GIB/ED ₅₀)
Diclofenac Sodium	17	8.1
Acetylsalicylic Acid	240	0.3
Flufenamic Acid	110	3.0
Ibuprofen	180	1.1
Indomethacin	5	1.0
Naproxen	48	2.4
Oxyphenylbutazone	>300	-
Phenylbutazone	113	2.3
Sudoxicam	15	0.5

p.o.: oral gavage administration

(Ménasse et al., 1978)

Acute toxicity values and the dose needed to induce gastro-intestinal bleeding in the rat for diclofenac and its metabolites are presented in the Sponsor's Table below.

Comparative Acute Toxicity Data (Rat) (Ménasse et al. 1978)

Preparation	Acute Toxicity		
	Sex	LD ₅₀ (mg/kg p.o.)	95% Confidence Limits
Diclofenac Sodium	M	240	195 - 294
	F	226	186 - 274
Acetylsalicylic Acid	F	2170	1870 - 2510
Flufenamic Acid	M + F	595	-
Ibuprofen	-	1600	-
Indomethacin	-	12	-
Naproxen	-	543	-
Oxyphenylbutazone	M	655	534 - 805
	F	631	518 - 769
Phenylbutazone	M	608	538 - 687
	F	660	581 - 764
Sudoxicam	-	157	-

The most potent diclofenac metabolite was at least 5-fold less effective in producing gastrointestinal bleeding than diclofenac, but the LD₅₀ concentration was similar, as indicated in the Sponsor's table below.

Metabolite Effects on Gastrointestinal Bleeding

Preparation	Gastro-intestinal bleeding (mg/kg p.o.)	Acute Toxicity	
		Sex	LD ₅₀ (mg/kg p.o.)
Diclofenac sodium	17	M	240
		F	226
Metabolite I	> 100	M	240
		F	190
Metabolite II	~ 300	M	1,430
		F	1,580
Metabolite III	no effect at 300	M/F	> 1,000
Metabolite IV	300	M/F	774

Other: Inhibition of Human Platelet AggregationDiclofenac

Jobin and Gagnon (1971) found that diclofenac sodium is a very potent inhibitor of collagen-induced aggregation and is partial inhibitor of secondary aggregation by ADP. Diclofenac sodium (referred to as GP45840 in their study) partially inhibited collagen-induced aggregation of human platelets, and was about 400 times more potent than oxyphenbutazone. Diclofenac also partially inhibited ADP-induced secondary aggregation. The inhibition never reached 100% for either platelet aggregation pathway at the doses tested.

TABLE 1. The inhibition of collagen-induced platelet aggregation by GP 44296, GP 45840, and by some pyrazole compounds

Compounds	No. of experiments	Minimal concentration consistently giving $\geq 65\%$ inhibition (nmoles/l)
GP 44296	3	0.045
GP 45840	8	0.002
Phenylbutazone	3	0.64
Oxyphenbutazone	9	0.92
Sulfinspyrazone	3	1.34

TABLE 2. The inhibition of ADP-induced platelet aggregation by GP 44296, GP 45840, and by some pyrazole compounds

Compounds	No. of experiments	Concentration (mmoles/l)	Inhibition of first phase of aggregation (%)	Inhibition of second phase of aggregation (%)
GP 44296	3	0.36	59 (57-61)*	92 (85-96)
	4	0.18	36 (32-41)	85 (79-92)
GP 45840	4	0.002	—	61 (45-83)
Phenylbutazone	5	1.28	—	66 (57-80)
Oxyphenbutazone	9	1.84	—	68 (48-76)
Sulfinpyrazone	4	1.34	—	53 (33-76)

NOTE: Final ADP concentration, 0.5-2.5 μ mole/l.

*Mean and extreme values observed.

2.6.2.5 Pharmacodynamic drug interactions

There was no information of drug interaction provided by the Sponsor in the nonclinical pharmacology sections

2.6.3 PHARMACOLOGY TABULATED SUMMARY

Summary Table of Pharmacology Studies (Sponsor's Table)

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Pharmacology - Primary Action Studies					
Menassé <i>et al.</i>	1978	Diclofenac sodium	Mice, rats	Subplantar, intraperitoneal, intramuscular	Diclofenac sodium inhibited prostaglandin biosynthesis <i>in vitro</i> and <i>in vivo</i>; high degree of anti-inflammatory, analgesic, and antipyretic activity
Hiramatsu <i>et al.</i>	1990	Diclofenac diethylammonium salt	Rats	Subcutaneous	Pain thresholds were significantly elevated after treatment
Kyuki <i>et al.</i>	1983	Diclofenac sodium cream	Mice, rats, guinea pigs	Topical	Diclofenac sodium cream had an anti-inflammatory effect that was superior or comparable to similar existing anti-inflammatory ointments
Allenby <i>et al.</i>	1969	DMSO	Human skin samples	N/A	DMSO did not increase the skin clearance rate but permeability of the skin barrier layer increased; causing ultrastructural modifications to stratum corneum
Pharmacology - Effects Related to Possible Adverse Reactions					
O'Brien	1986	Diclofenac	40,000 clinical trial subjects and 85,000 patients from clinical reports	N/A	- No unusual adverse reactions with diclofenac - Appeared to be a safe, effective, and well-tolerated NSAID, especially in the elderly, who have the highest risk for adverse reactions
Vogin <i>et al.</i>	1970	Daily dose of 1, 3, or 9 ml/kg body weight of 90% (v/v) aqueous DMSO	Rhesus monkeys	Oral, dermal	Local effects observed were scaling and flaking of the skin at the site of drug application in dermally treated animals

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

DICLOFENAC

Topical Application

The percutaneous absorption of diclofenac sodium 1% (w/w) in a cream formulation (Voltaren[®] cream)¹ was investigated for effects on pain and analgesia in mice by Kyuki et al. (1983). The pharmacokinetics of the diclofenac sodium 1% (w/w) cream formulation was characterized in the guinea pig and rabbit by Riess et al. (1986). In the guinea pig, percutaneous absorption of diclofenac sodium amounted to 3–6% of the dose when the cream formulation was applied in doses of 40, 100, or 320 mg to 10 cm² of skin surface (location not identified) under an occlusive dressing and left in place for 6 hours. Plasma concentrations reached a plateau at 1.5 hours after application and remained at this level until removal of the residual cream (assumed to be 6 hours after application). During daily administration the steady state levels in muscle tissue in the immediate vicinity of the application site were about 3 times higher than a tissue sample taken from a distant muscle (locations not identified). When applied topically to the knee joints of rabbits, diclofenac penetrated into the patellar ligament, the fatty tissues and the synovial fluid (see Sponsor's Table below).

Table 37: Distribution of Diclofenac Sodium in Animals

Species	Dose	Route of Administration	Results
Mouse	10 mg/kg of ¹⁴ C-labelled diclofenac sodium	Intravenous	The highest concentrations of radioactively labeled diclofenac sodium were in the blood
Guinea pig	Oral: 1 mg/kg ¹⁴ C-labelled diclofenac diethylammonium Topical: 1.16% ¹⁴ C-labelled diclofenac diethylammonium applied twice daily for 6 days in a quantity approx. 400 mg/kg	Oral, topical	- Oral: 1 hour after a single dose, the concentrations of radiotracer in variously located muscles are identical - Topical: 16 hours after final application, the ¹⁴C-activity in the muscle beneath the site of application was roughly 3 times higher than in a tissue sample taken from a remotely situated muscle
Rabbit	One knee joint was treated with 1 mg ¹⁴ C-labelled diclofenac sodium in 100 mg cream	Topical	- The ¹⁴C-activity 5 hours after application was much higher in the patellar ligament of the treated knee than in the fatty tissue and in the synovial fluid - In the untreated contralateral knee the ¹⁴C-activity in the synovial fluid corresponded to that in the blood

(Riess et al., 1978, 1986)

¹ Voltaren Cream has not been approved in the United States.

Oral Administration

The Sponsor cited information from the Voltaren label. Diclofenac is rapidly and almost completely absorbed and is distributed to blood, liver and kidneys when given orally under fasting condition. It is highly protein-bound (approximately 99.5%). The plasma concentration increases linearly, reaching maximum in about 2.5 hours. There is no accumulation at therapeutic doses. About 90% of the administered dose is eliminated within 72 hrs. Urinary excretion accounts for 40-60% of the dose, mainly as hydroxyl conjugates of diclofenac.

DIMETHYL SULFOXIDE (DMSO)

Topical Application

Following topical application, dimethyl sulfoxide is absorbed and distributed in the tissues and body fluids. Dimethyl sulfoxide is metabolized by oxidation to dimethyl sulfone or by reduction in dimethyl sulfide. No residual accumulation of dimethyl sulfoxide occurs, however dimethyl sulfone can persist in serum for longer than two weeks after a single bladder intravesical instillation of RIMSO-50 (50% DMSO). Dimethyl sulfoxide and dimethyl sulfone are excreted in the urine and feces. Dimethyl sulfide is eliminated through the breath and skin and is responsible for the characteristic odor reported from patients using DMSO.

2.6.4.2 Methods of Analysis

There was no information concerning methods of analysis. The methods for each study were described within the study.

2.6.4.3 Absorption

Pennsaid® Topical Solution

There were no studies performed with Pennsaid Topical Solution applied to animals. Two *in vitro* studies characterized the effect of Pennsaid Topical Solution on ¹⁴C-diclofenac absorption in human cadaver skin tissue.

Hartway et al. 1996; Study UCSF95SU03
NDA, Vol. 1.17, p. 19)

This was comparison study of the absorption of diclofenac sodium through human cadaver skin with or without DMSO in the carrier solution.

Methods: Human male or female viable thigh skin, 500 microns thick from four different sources, was taken from 32, 38, and 53 year old male and female cadavers. Skin (1 cm²) was mounted in glass cells of the flow-through design containing Eagles MEM in one reservoir. ¹⁴C-Diclofenac sodium was added to Pennsaid Topical Lotion, Pennsaid Placebo, and Pennsaid Control Lotion (Lotion was the term used, instead of Solution, during early drug development;

composition was identical). A 1-5 $\mu\text{Ci}/10 \mu\text{L}/\text{cm}^2$ dose was administered to the skin preparations. Cell contact time was 6 and 24 hours. The receptor fluid was assayed by ^{14}C -TLC at 6 time points over 24 hours. The treatments were:

- A) 1000 ppm ^{14}C - _____ Lotion (1.5% diclofenac sodium, 45% DMSO, 11% EtOH, 11% propylene glycol, and water.
- B) Replicate of A.
- C) Base lotion without DMSO (1.5% 1000 ppm ^{14}C -diclofenac sodium, 11% glycerin, 11% EtOH, 11% propylene glycol, and water.
- D) Replicate of C.
- E) ^{14}C -Diclofenac in 45% DMSO, 11% glycerin, 11% EtOH, 11% propylene glycol and water.
- F) Radiolabeled chemical to be used as positive control for skin metabolism and viability.

b(4)

Results: The results are presented in the Sponsor's Table, modified by the reviewer, below. The percentage of ^{14}C -diclofenac that was absorbed through or deeply into the skin after 24 hours was higher for the Pennsaid® formulation (5.12%) than for the formulation without DMSO (3.63%). For Pennsaid®, the amounts of diclofenac transferred through the skin continued to increase at 16 hours, whereas for the formulation lacking DMSO, there was a peak at 12 hours and then a decrease in the amount of diclofenac transferred through the skin. Extraction and TLC analysis of the organic materials from the percutaneously absorbed diclofenac indicated that >90% of the diclofenac sodium was absorbed as intact drug. The known metabolites were also found. In addition, there were unidentified radioactive spots on the TLC plates that were thought to be conjugated, water-soluble metabolites.

In Vitro Percutaneous Absorption of ^{14}C -Diclofenac Na in Human Skin
(Percent of Dose Absorbed, Mean $\pm$ SD; Reviewer modified table)

	Base lotion With 45.5% DMSO		Base lotion Without DMSO		Base lotion With 45.5% DMSO
	A	B	C	D	E
Receptor Fluid	1.34 $\pm$ 1.10	0.44 $\pm$ 0.06	1.02 $\pm$ 0.72	1.12 $\pm$ 0.62	0.72 $\pm$ 0.34
Tape Strips	4.41 $\pm$ 2.47		7.02 $\pm$ 3.63		
Skin Digest	3.78 $\pm$ 2.60		2.61 $\pm$ 0.64		
Receptor Fluid+ Skin Digest	5.12		3.63		
Total Absorption	9.53		10.65		
Surface Wash	87.4 $\pm$ 7.2	90.6 $\pm$ 5.1	84.7 $\pm$ 4.2	87.2 $\pm$ 3.8	95.1 $\pm$ 10.8
Total	95.7 $\pm$ 4.5	91.1 $\pm$ 5.2	95.3 $\pm$ 1.8	87.4 $\pm$ 3.9	95.8 $\pm$ 11.1

Percent absorption was measured as accumulation in the receptor fluid (1.34 $\pm$ 1.10). The total absorbed in formulation A was 9.53% and 10.65% in formulation C over 24 hr. Receptor fluid diclofenac content increased up to 12 hr, decreased between 12 and 17 hr, then increased up to 24 hr. These data indicate the Pennsaid® formulas A and C, with or without DMSO, are both absorbed through viable human skin.

Reviewer's Comment: It was unclear from the table where the data for the sentence "The percentage of ^{14}C -diclofenac that was absorbed through or deeply into the skin after 24 hours was higher for the

Pennsaid® formulation (5.12%) than for the formulation without DMSO (3.63%)” arose, so the reviewer add that row to the Table.

Hewitt et al., 1997; Study UCSF96SU05
NDA, Vol. 1.17, p. 91.

This *in vitro* study demonstrated that multiple topical applications of diclofenac sodium to viable human skin increased the amount of diclofenac absorbed compared to a single application, and the absorption was also greater from Pennsaid® Solution than from an aqueous solution. Diclofenac in the Pennsaid® formulation resulted in approximately 35% absorption through or deeply into the cadaver skin at 48 hours after a single dose.

Reviewer Comments: Interestingly, with multiple doses, while greater amounts of diclofenac were absorbed compared to a single dose, the percentage absorbed was less, perhaps indicating a saturation phenonema. The Sponsor did not address this issue. They did not mention if it was further pursued in an *in vivo* animal model or clinically through dose-response testing coupled with pharmacokinetic analysis.

Methods: Human leg skin was obtained from five different cadavers. Skin thickness was 500 microns. ¹⁴C-Diclofenac sodium (6 mg) of 99% radio-chemical purity and 9.76 mCi/mmol was added to 400 µL of Pennsaid® lotion, giving a 1.5% formulation. The 9 dose formulations, A through H, are indicated below in the reviewer modified Sponsor’s table. Treatment I was a viability control monitoring glucose metabolism to lactate.

Results: Total absorption included recovery from stratum corneum, recovery from skin, and recovery from receptor fluid. These data indicate that a greater amount of drug was absorbed/penetrated the cadaver skin with multiple applications. On the other hand, the percent of drug absorbed/penetrated from the total amount applied was less in the single dose. It is of interest that diclofenac sodium was also absorbed from an aqueous ethanol solution (C, D, G, H), but less than that from the Pennsaid® Lotion.

The mean amount of drug absorbed in the stratum corneum was approximately 11.6% (2.0%-24.9% range) from a single application of the 30 µg lotion, 11.8% (3.5-21% range) from a single application of 75 µg lotion, 10.3% (4.7-18.5% range) from multiple applications of the 240 µg lotion, and 6.0% (2.4-13.1% range) from multiple applications of the 600 µg lotion.

Diclofenac Absorption Through/Into Human Cadaver Leg Skin
(Percent of Dose Absorbed, Mean±SD; Reviewer modified table)

	Single Dose				Multiple (8) Doses			
	1.5% (1000 ppm) ¹⁴ C-Diclofenac sodium in PENNSAID Lotion Single dose		1.5% (1000 ppm) ¹⁴ C-Diclofenac sodium in HPLC grade H ₂ O (+10% EtOH) Single dose		1.5% (1000 ppm) ¹⁴ C- Diclofenac sodium in PENNSAID Lotion. Multiple doses, at times 0, 4, 8, 12, 24, 28, 32, and 36 hr.		1.5% (1000 ppm) ¹⁴ C- Diclofenac sodium in HPLC grade water (+10% ethanol). Multiple doses, at times 0, 4, 8, 12, 24, 28, 32, and 36 hr	
μL	2	5	2	5	16 (2 μL x 8)	40 (5 μL x 8)	16 (2 μL x 8)	40 (5 μL x 8)
	A	B	C	D	E	F	G	H
μg/skin	30	75	30	75	240	600	240	600
Total Diclofenac Absorption (μg) Mean (sd)	10.18 (6.70)	26.26 (17.50)	8.33 (1.51)	12.25 (5.66)	40.14 (17.58)	85.61 (41.36)	9.44 (2.87)	35.68 (18.97)
% (sd)	33.92 (22.32)	35.01 (23.34)	27.76 (5.03)	16.34 (7.54)	16.72 (7.32)	14.27 (6.89)	3.94 (1.20)	5.94 (3.16)
receptor fluid	11.39 (11.62)	13.05 (16.57)	7.63 (7.85)	4.03 (3.25)	2.04 (1.40)	1.93 (1.88)	0.32 (0.29)	0.97 (0.68)
total skin residue less stratum corneum	10.97 (7.83)	10.14 (7.36)	14.29 (2.79)	5.01 (4.02)	4.37 (2.56)	6.34 (3.99)	1.08 (0.61)	1.41 (0.91)
stratum corneum (=10 tape strips)	11.56 (9.29)	11.82 (6.47)	5.84 (2.83)	7.03 (3.20)	10.32 (5.01)	6.00 (5.08)	2.54 (1.79)	3.56 (3.11)
total absorption (SC+Skin+RF)	33.92 (22.32)	35.01 (23.34)	27.76 (5.03)	16.34 (7.54)	16.72 (7.32)	14.27 (6.89)	3.94 (1.20)	5.94 (3.16)
total recovery of radioactivity	96.37 (6.67)	92.83 (18.58)	107.24 (22.71)	110.57 (15.12)	100.74 (7.09)	90.55 (3.01)	82.04 (9.66)	76.78 (8.22)

DMSO

Ursin et al. (1995) determined the DMSO permeation rate in living human skin samples *in vitro*, and compared these values with the ³H-water permeation rate to normalize values for comparison with other solvents and to determine skin integrity (from the magnitude of the water permeation value and DNA synthesis after solvent exposure). Skin samples were obtained from females during plastic surgery of the breast and the epidermis was separated from the dermis. Permeability measurements were determined using a 2 chamber Franz Diffusion Cell over which the skin was stretched to provide a exposed area of 0.64 cm² and thickness of 300 to 600 μm.

DMSO had a permeability rate of 176 g/m²/h and the rate for [³H] water was 14.8 g/m²/h. The volume results can be expressed in units of cm/h (those usually associated with permeability constant) by multiplying the values in cm³/m²h by 10⁻⁴ m²/cm² to give cm³/cm²h which reduces to cm/h.

2.6.4.4 Distribution

DICLOFENAC

A summary of the studies of the distribution of diclofenac is presented in the Sponsor's table below.

Table 37: Distribution of Diclofenac Sodium in Animals

Species	Dose	Route of Administration	Results
Mouse	10 mg/kg of ^{14}C -labelled diclofenac sodium	Intravenous	The highest concentrations of radioactively labeled diclofenac sodium were in the blood
Guinea pig	Oral: 1 mg/kg ^{14}C -labelled diclofenac diethylammonium Topical: 1.16% ^{14}C -labelled diclofenac diethylammonium applied twice daily for 6 days in a quantity approx. 400 mg/kg	Oral, topical	- Oral: 1 hour after a single dose, the concentrations of radiotracer in variously located muscles are identical - Topical: 16 hours after final application, the ^{14}C-activity in the muscle beneath the site of application was roughly 3 times higher than in a tissue sample taken from a remotely situated muscle
Rabbit	One knee joint was treated with 1 mg ^{14}C -labelled diclofenac sodium in 100 mg cream	Topical	- The ^{14}C-activity 5 hours after application was much higher in the patellar ligament of the treated knee than in the fatty tissue and in the synovial fluid - In the untreated contralateral knee the ^{14}C-activity in the synovial fluid corresponded to that in the blood

(Riess et al., 1978, 1986)

PROTEIN BINDING OF DICLOFENAC

Riess et al. (1978) using equilibrium dialysis, found that *in-vitro* protein binding capacity of diclofenac sodium, when diclofenac was administered in therapeutic doses, 99.7% of the concentration attained in the serum was bound to protein. Diclofenac did not interfere with the protein binding of other drugs such as tolbutamide, salicylates, warfarin or prednisolone. However, the protein binding of diclofenac was impaired by high doses of anionic drugs such as salicylic acid.

DMSO

Studies of the distribution and pharmacokinetics/toxicokinetics of DMSO are summarized in the Reviewer's table below.

ADME Studies with DMSO in Animals

Reference	Species	Doses	Findings
Kaye et al. (1983)	mice Swiss Webster	Intravenous 1.5 mg/g of 15%	$T_{1/2}$ = 1.5 minutes C_{max} = 1 minute

		DMSO	AUC (0-480 minutes) = 313 mg.min/mL peak plasma DMSO: 3.12 ± 0.48 mg/mL body clearance of DMSO: 4.79 mL/min/kg DMSO was rapidly and extensively distributed in all tissues and was not concentrated in any particular tissue By 8 hours, there was little DMSO found in plasma and tissue
McDermot et al. (1967)	rat	Dermal undiluted DMSO, to 25 cm ² area on the clipped ventral surface	More than 90% of the applied DMSO dose was absorbed with evaporative losses of less than 1%. DMSO was found in the selected tissues: brain, muscle, kidney, lung, liver, heart and blood Peak concentrations were reached at 1.5 to 2 hours highest concentration kidney lowest concentration lung. Elimination: Approximately 85% of the applied dose was excreted unchanged in the urine in 24 hours
Kolb et al. (1967)	rats,	Dermal	half-life of DMSO in blood was found to be 10 hrs distributed in all organs studied decreased within 2 to 24 hrs after application 50% of the dose was eliminated in the urine after 8 hrs 75% of the dose was eliminated after 24 hrs Elimination: 50% of the dose was eliminated in the urine in 6 hrs and was eliminated after 24 hrs elimination pattern for was similar for all 3 administration methods
	rabbits	Dermal	higher DMSO concentration found in the cornea than in other parts of the eye or in blood plasma. Maximal concentration is reached after 4 hrs and by 24 hrs the levels are lower than the concentration plasma. Elimination: 70% of the applied dose was excreted in the urine on Day 1, 80% of the applied dose was excreted on Day 2 90% of the applied dose was excreted by Day 10
	beagle dogs	Dermal, single application the skin was excised Days 1, 4, 7 and 14	80% penetrates the skin barrier after 4 hrs 90% of DMSO applied penetrates after 24 hrs linear decline of radioactivity in the dermis over two weeks On day 14, 0.037% of the applied dose was in the dermis

			Elimination: studied following single and repeated cutaneous application, oral and intravenous application. One-half of the dose was eliminated via the kidneys following intravenous application with an elimination half-life of 1.5 days. The elimination half-life was also 1.5 days following oral administration. However, for cutaneous administration, between 12 and 25% of the dose were eliminated in the urine during the first 24 hrs and 37 to 48% during the 7 day test period.
Hucker et al. (1965)	rat	Intraperitoneal Oral Dermal ³⁵S-DMSO 500 mg/kg,	Intraperitoneal plasma radioactivity was the highest after 0.5 hrs following with a T_{1/2} of 5 to 6 hrs. Dermal application: plasma level concentration of radioactivity was constant for 6 hours Elimination: IP and Oral: At 22 hours, radioactivity in urine 60-85% Dermal At 22 hours, radioactivity in urine 36-50% of administered dose
Hucker et al. (1966)	Rat male Sprague-Dawley Rabbit	³⁵S-DMSO 0.55 g/kg, orally IP, dermally	Rat: High concentrations of radioactivity in plasma 0.5 hr after dosing by all routes. Tmax was reached in 0.5-1 hr for oral and intraperitoneal routes and 2 hrs for the dermal route of administration. The plasma concentration levels then declined with an average half-life of approximately 6 hrs At the end of 24 hr the plasma levels had declined to 5-10% of the peak concentration. Accumulation of radioactivity was appreciable in all tissues after 0.5 hr following oral administration. A similar trend in radioactivity was observed in dermal administration, though levels were lower than observed in oral administration. Elimination Within 24 hrs, approximately 67% of the dose was excreted in urine and 4 to 10% of the dose was excreted in the feces. An average of 6% of the dose was found in respired air. Approximately 10-15% of the administered dose of DMSO was metabolized to DMSO₂.

Hucker et al. (1966)	Guinea pig	Intraperitoneal 0.55 g/kg ³⁵ S-DMSO	Elimination 52% of the dose was excreted in urine within 24 hr and 4% in the feces additional 16% of the dose was excreted within 24 to 48 hr
Hucker et al. (1966)	Rabbits, New Zealand White	Dermal 0.55 g/kg ³⁵ S-DMSO	Concentrations of radioactivity were appreciable after 0.5 hr in all tissues except the lens and increased sharply after 4 hr. Levels in the lungs, heart, plasma, bile, aqueous humor, vitreous humor and cornea were higher than those in other tissues. Elimination: 30% of the administered dose was excreted in the urine after 24 hrs. This excretion rate is much lower than the excretion rate in rats, suggesting that there is accumulation of DMSO and DMSO ₂ in rabbits.
Layman and Jacob (1985)	Rhesus monkeys (Macaca mulatta)	daily oral doses of 3 g DMSO/kg for 14 days gastric intubation of 3 grams DMSO per kg body weight as a 50% solution in water for 14 days (total of 210g of DMSO)	average peak serum concentration of 2.3 mg/mL at 4 h which declined relatively rapidly to about 0.95 mg/mL after 24 hours half-life of 16 hours elimination rate constant was $K_e = 0.693/16$ or about 4% per hour with continued daily oral administration, the average peak serum DMSO concentration increased slightly from 0.95 to 1.1 mg/mL on Day 2 and then reached a steady state concentration of 0.9 mg/mL after 4 days (not different) after oral DMSO administration was terminated, serum DMSO declined rapidly and was not detected after 72 hours DMSO ₂ became detectable in serum after 2 hours and increased slowly to 0.18 mg/mL at 24 hours DMSO ₂ attained a steady state concentration of 0.34 mg/mL after 4 days of treatment. When oral DMSO was stopped after 14 days, the mean DMSO ₂ serum concentration declined slowly over the next 96 hours and trace amounts were detected at 120 hours half-life was calculated to be 38 hours and elimination rate constant was 0.018 or 2% per hour
Wong et al. (1971)	miniature pigs, male	Dermal 15 g of 80% DMSO gel applied to the shaved back of each pig area of approx. 250 cm ²	absorbed within 4 hours principal metabolite was dimethyl sulfone (DMSO ₂). DMSO was excreted predominantly unchanged in the urine Elimination: excretion of DMSO diminished rapidly and was not detected after 3 days DMSO ₂ excretion was still pronounced 5 days after drug application was stopped. Approximately 1.6% of the dose was excreted in the feces and a substantial amount of

Tiews et al. (1975)	cows and calves	topical and parenteral application ¹⁴ C DMSO	dimethyl sulfide (DMS) was detected in the expired air. Elimination lung and kidney appeared to be the principal excretory organs in bovine Excretion via feces is negligible, and excretion via milk after intramammary application is 2.03% of the applied dose Excretion via urine reaches peak values within 6 to 12 hours, then, decreases sharply, disappearing within 12 to 28 hr after application. ¹⁴C excretion in exhaled air follows an identical pattern Concomitant with the decline in DMSO, there was an increase in DMSO₂ concentration in the urine, reaching peak values after 30–40 hrs in calves and 6–12 hrs in cows. The DMSO₂ concentration returned to normal values within 7 days in calves and 3–5 days in cows
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ADME of DMSO in Humans

Reference	Doses	Findings
from review of Willhite and Kazt (1984)	Dermal 1 g/kg DMSO	peak serum concentration within 4 h, with a $t_{1/2}$ = 20 h dimethyl sulfone concentration peaked between 72-96 h postadministration with $t_{1/2}$ = 72 h Elimination At 120 h, 50% of the total dose was excreted in urine as DMSO and 9.6% as dimethyl sulfone. Within 480 h, 22% of the total dose was excreted as dimethyl sulfone. Cumulative urinary excretion of DMSO and dimethyl sulfone was linear and exponential, respectively, following 0.5 g/kg DMSO p.o. per day for 14 days
	Dermal 2 g of 90% DMSO	Eight hours after application, 20% remained at the application site. The maximum concentration plateau in the blood occurred 4-6 h postapplication with no change in the plateau for the next 1.5-3 days. Elimination: Within 24 h, 10-15% of the total dose appeared in the urine. After 7 days, 40% was excreted in the urine. At 6 h and 24 h, 1% and 1.5-3.0% of the total dose, respectively, had been expired. Dimethyl sulfide expiration ceased after 24 h.
	Dermal 80% DMSO for 30 min per day for 8 days	25-40% of the dose was absorbed per day After 9 days, DMSO was not detectable in the plasma, while dimethyl sulfone was detectable until day 15; dimethyl sulfone $t_{1/2}$ = 2.5-3.0 days
	Dermal 1 g/kg [³⁵ S]-DMSO	maximum serum level between 4-8 h with $t_{1/2}$ = 11-14 h. The dimethyl sulfone peak serum concentration occurred between 36-72 h with $t_{1/2}$ = 60 to 70 h. Elimination: At 48 h, 13.0% of the total dose was eliminated in the urine as DMSO, while 3.1 % of the dose was eliminated as dimethyl

		sulfone; 17% of the dose was excreted as dimethyl sulfone by 456 h. There was negligible excretion in the feces.
	Intravenous 2.0 g of 50% DMSO	Elimination: elimination of 80% of the total dose in 7 days; $t_{1/2}$ = 4 days. Negligible amounts were excreted in feces. Dimethyl sulfide expiration accounted for 3% of the total dose.

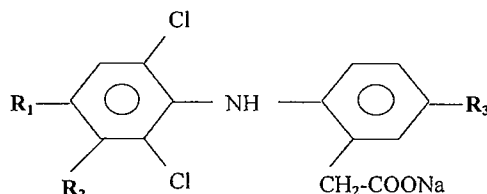
2.6.4.5 Metabolism

DICLOFENAC SODIUM

In a human study, Riess et al. (1978) found that mainly hydroxylated metabolites of diclofenac were formed, which are excreted in the urine in the form of glucuronides accounting for about 60% of the dose administered. Oral doses of 25 mg and 50 mg of diclofenac sodium given as enteric-coated tablets, lead to mean maximum plasma concentrations of approximately 0.5 pg/mL and 1.0 pg/mL, respectively, after about 2 hours. Four hours after administration of these doses, the levels still detectable in the plasma were approximately 10% of the maximum concentrations.

Diclofenac Metabolites

Diclofenac Molecule with Metabolite Relevant Binding Sights



Metabolite	R ₁	R ₂	R ₃
I	OH	H	H
II	H	H	OH
III	OH	H	OH
IV	H	OH	H

Menassé et al. (1978)

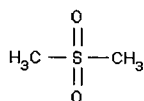
Biotransformation of diclofenac sodium is species-specific. The various biotransformation products of diclofenac sodium identified in the urine and bile of rat, dog, Rhesus monkey and baboon are shown in the Sponsor's table below. The percentages for the individual metabolites are in respect of the total ^{14}C radioactivity excreted in the urine or bile of each species. Diclofenac metabolism in the dog differed from the other species. The extent of its biliary excretion varied from species to species. In the rat and dog, the drug was subject to enterohepatic circulation according to Riess et al 1978.

Metabolites of diclofenac excreted by percent in the urine or bile

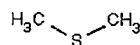
Type of Metabolite	Rat		Dog		Monkey		Baboon	
	Urine	Bile	Urine	Bile	Urine	Bile	Urine	Bile
Conjugates of Diclofenac	<5%	30-40%	5-10%	80-90%	10-20%	0%	10-20%	0%
Diclofenac Taurine Conjugate	0%	0%	20-30%	0%	0%	0%	0%	0%
Conjugates of the 4'-hydroxy Derivative of Diclofenac	20-30%	10-20%	<5%	<5%	10-20%	0%	20-30%	0%
Conjugates of the 5-hydroxy Derivative of Diclofenac	5-10%	5-10%	<5%	<5%	20-30%	0%	10-20%	0%
Conjugates of the 3'-hydroxy Derivative of Diclofenac	0%	0%	0%	0%	5-10%	0%	5-10%	0%
Conjugates of the 4',5-dihydroxy Derivative of Diclofenac	<5%	<5%	0%	0%	5-10%	0%	5-10%	0%

*Riess et al. (1978)***DMSO**

DMSO is metabolized to dimethyl sulfone (DMSO₂) and dimethyl sulfide (DMS)



Dimethyl Sulfone



Dimethyl Sulfide

Both DMSO₂ and DMS occur naturally in a variety of food products, and therefore in people, they are endogenously present in the plasma (DMSO₂ at levels ranging from 0–25 µmol/L) (Engelke et al. 2005). A very minor amount (1%) of DMS is formed from methanethiol, a catabolite of the amino acid methionine, which may contribute to endogenous levels of DMS, DMSO, and DMSO₂ (Engelke et al. 2005). Additionally, DMSO₂ has been detected in the cerebral spinal fluid and the brain, as it can cross the blood brain barrier; however, only in individuals taking dietary supplements with DMSO₂ (Rose et al., 2000; Lin et al., 2001; Engelke et al., 2005). Normal urinary excretion of DMSO₂ ranges from 4 to 11 mg/day (Williams et al., 1966a).

The metabolism of radiolabelled DMSO₂ was investigated in 5-week-old male Wistar rats administered 0.47 g DMSO₂/kg body weight/day via oral gavage for 7 days (Otsuki et al., 2002). Radioactivity was evenly distributed throughout the tissues, which according to the authors indicated permeation of the whole system. Approximately 3.5–10% of the radioactivity remained incorporated in the tissues after 7 days, with higher levels retained in the blood, spleen, and skin. The authors suggested that this indicated DMSO₂ was metabolized to other sulfur-containing metabolites, but this was not determined. After 7 days of dosing, 60–79% of the

radioactivity was excreted in the urine and 5–18% was excreted in the feces, with average daily urinary and fecal radioactive excretion of 70 and 10%, respectively (Otsuki et al., 2002).

Radiolabelled DMSO₂ was subcutaneously injected into female New Zealand rabbits at a dose of 1.82×10^6 cpm daily for 4 days, after which urine was collected for 20 days (Williams et al., 1966b). The authors reported 76% of the administered radioactivity was collected as DMSO₂ in the urine over this period. No DMS was detected in the breath of rabbits.

In the same experiment, female New Zealand rabbits were subcutaneously injected with non-radioactive DMS (1 mL DMS/day; exact dose could not be determined due to volatility of DMS), daily for 4 days. Approximately 8.6% and 9.8% of the administered dose of DMS was reported to be excreted as DMSO and DMSO₂, respectively, over a period of 6 days. DMS also was eliminated via the expired air, indicated by the strong characteristic garlic-like odour on the animals' breath during the experiment.

Layman and Jacob (1985) evaluated the absorption, metabolism and excretion of DMSO₂ in Rhesus monkeys (n=3) after an administration dose of 3 g DMSO/kg body weight/day via oral gavage for 14 days. Levels of DMSO₂ were subsequently measured in serum, urine and feces. DMSO₂ was detected in the blood within 2 hours of dosing and reached a steady-state concentration after 4 days. The half-life for DMSO₂ was reported to be 38 hours and 120 hours after dosing was terminated, no DMSO₂ was detected in the blood. Approximately 60 and 16% of the administered DMSO dose was excreted in the urine as un-metabolised DMSO and DMSO₂, respectively, with no detection of either compound in the feces. Although DMS was not measured, the authors reported the smell of DMS on the breath of monkeys.

2.6.4.6 Excretion

DICLOFENAC

In the rat and dog, excretion is predominantly biliary, whereas in the rhesus monkey, 80% of the dose was excreted through the kidneys as indicated in the Sponsor's table below.

Table 39: Excretion of Diclofenac Sodium in Animals

Species	Dose	Route of Administration	Results
Rat	5 mg/kg of ¹⁴ C-labelled diclofenac sodium	Oral, intravenous	• Excretion is predominantly biliary
Dog	5 mg/kg of ¹⁴ C-labelled diclofenac sodium	Oral, intravenous	• Excretion is predominantly biliary
Rhesus monkey	5 mg/kg of ¹⁴ C-labelled diclofenac sodium	Oral, intravenous	• Excretion is through the kidneys for 80% of the dose

(Riess et al., 1978)

DMSO

Following oral administration of DMSO, some DMSO may be excreted unchanged in the urine; however, approximately 15% is oxidized to DMSO₂ in the liver and up to 5% is converted to DMS (Tsuruta et al., 2001). In addition, DMSO₂ may be excreted unchanged or as sulfur-containing metabolites in the urine (Otsuki et al., 2002), whereas DMS is released into the expired air via the lungs (Tsuruta et al., 2001).

2.6.4.7 Pharmacokinetic drug interactions

There were no nonclinical studies describing the pharmacokinetic effects of drug interactions on either diclofenac or DMSO.

2.6.4.8 Other Pharmacokinetic Studies

There were no other pharmacokinetic studies.

2.6.4.9 Discussion and Conclusions

Most of the studies were conducted prior to the development of the current guidelines for drug approval and, as with the toxicology studies, lack information about the purity of the DMSO preparations used. These studies also did not examine the distribution of DMSO or diclofenac in as many organs and tissues as expected in current studies. There was no information about the potential depot effect produced by DMSO at the site of topical application and its impact on diclofenac pharmacokinetics, especially potential lingering effects upon discontinuation of dosing. Based on the short-term *in vitro* human cadaver skin studies, a saturation phenomena may develop at the site of application. Further investigations of this possibility in animal models or clinically were not investigated. It is not known if the amount of DMSO and/or diclofenac are far in excess of that needed for absorption, or insufficient. There were no studies submitted to indicate that this combination of DMSO and diclofenac are the most appropriate.

2.6.4.10 Tables and figures to include comparative TK summary

Comparison of ADME data for diclofenac sodium in species used in oral toxicology studies.
(Riess, W. et al. 1978, 1986; Sponsor's Table)

Characteristics	Species			
	Rat	Dog	Monkey	Human
Absorption	100% (oral)	100% (oral)	100% (oral)	100% (oral)
Serum half-life	n/a	n/a	n/a	1 hour (oral) (Voltaren®) 70.3±32.3 hours (PENNSAID®)
Tissue Accumulation	(Oral) Kidneys, Liver	(Oral) Kidneys, Liver	(Oral) Kidneys, Liver	(Topical) Synovial Fluid and Tissue Surrounding Joint
Major Metabolic Product	30-40% Conjugates of Diclofenac (Bile). 20-30% Conjugates of 4'-Hydroxy-Diclofenac (Urine).	80-90% Conjugates of Diclofenac (Bile). 20% Diclofenac Taurine Conjugate (Urine).	20-30% Conjugates of 5-Hydroxy-Diclofenac (Urine). 10-20% Conjugates of Diclofenac (Urine). 10-20% Conjugates of 4'-Hydroxy-Diclofenac (Urine).	20-30%(Urine), 10-20% (Bile) Conjugates of 4'-Hydroxy-Diclofenac.
Protein Binding	n/a	n/a	n/a	≥99% to serum albumin
Excretion (%/hours), major route.	Mostly Biliary (n/a)	Mostly Biliary (n/a)	80% Renally	90% in 96 hours, mostly renal

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Pharmacokinetic Studies with Diclofenac and DMSO (Modified from Sponsor's Table)

Authors / Investigators	Treatment	Animal Species	Administration Route	Results
ADDMU Studies				
Reiss W., et al., 1978	Diclofenac Sodium	Human, Rat, Dog, Monkey, Baboon	Oral	-other than the kidney or liver, the highest concentration of radioactive-labelled diclofenac was recorded in the blood, this characteristic does not differ from other NSAID drugs of the lipophilic acid type -oral dose is totally absorbed in man, but the total amount excreted within the first 96 hours accounts for 90% of the administered dose -within its therapeutic concentration range diclofenac becomes bound to the proteins in human serum to the extent of 99.7%, but it is not expected that diclofenac itself will interfere with the protein binding of other drugs rather it may be displaced more easily itself
Reiss W., et al., 1986	Diclofenac Sodium	Guinea-pig, Rabbit, Man	Topical	-in man daily topical application yielded steady state plasma levels of diclofenac -overall, transdermal release of diclofenac is superior to oral administration in cases where well-defined areas near the body surface need to be treated, thus high concentrations of the drug in the rest of the body can be avoided
Hartway T., et al., 1996	Diclofenac Sodium	Human Skin Samples	N/A	-single dose <i>in vitro</i> percutaneous absorption showed that PENNSAID® delivers diclofenac at 9.53% dose for a 24 hour dosing period -diclofenac showed continual and ever increasing absorption through the full 24 hour dosing period with the full PENNSAID® formulation, whereas the same formulation without DMSO showed decreased diclofenac delivery after 12 hours dosing - diclofenac is the major constituent delivered through the human skin
Hewitt P.G., et al., 1997	Diclofenac Sodium	Human Skin Samples	N/A	-multi-dose <i>in vitro</i> percutaneous absorption showed that PENNSAID® statistically increased the penetration rate of diclofenac into and through the skin, as compared to an aqueous solution of diclofenac - PENNSAID® exhibited enhanced absorption through human skin when applied a multiple number of times and this enhanced absorption was maintained over 48 hours

2.6.6 TOXICOLOGY

2.6.6.2 Single-dose toxicity

PENNSAID® TOPICAL SOLUTION.

No single dose toxicity studies were performed by the Sponsor with Pennsaid® Topical Solution.

DICLOFENAC SODIUM

The major toxicity observed in the animal studies was gastrointestinal ulceration. Gastro-intestinal tolerability of rats towards known gastrointestinal irritants was tested by Mennasé et al. (1978) as described in Section 2.6.2.4 of Safety Pharmacology. Of the analgesic compounds studied, diclofenac was found to be one of the least damaging. The most potent metabolite of diclofenac was at least 5-fold less effective effective in producing gastrointestinal bleeding than diclofenac, but the LD₅₀ concentration was similar.

DMSO

The acute and chronic toxicity of DMSO was summarized by Willhite and Kazt (1984).

Intravenous: A single intravenous injection of 2.5 to 10 g/kg DMSO in mice or rats resulted in decreased motor activity and myasthenia. Death was preceded by tremors, myasthenia, dyspnea and convulsions. Single intravenous injection of 200 mg/kg DMSO into anesthetized cats produced apnea, transient hypotension and hemolysis with hemoglobinuria. Single doses of more than 400 mg/kg were fatal. Injection of 5000-10,000 mg/kg i.v. in lightly anesthetized dogs caused a dose dependent, transient tachycardia, tachypnea and hypertension.

Intraperitoneal: Parenteral administration of DMSO was associated with tail catatony and hypothermia in mice. Profuse lacrimal secretions and diuresis with some hemoglobinuria were observed in rats. Injection of rats with 1.2-4.8 mL/kg, DMSO ,i.p., produced a dose-related increase in periportal fatty infiltration. The maximum effect occurred 12 h after administration and regressed after 24 h.

Oral: Oral intubation of 10-40 g/kg DMSO in mice resulted in immediate decreased motor activity, ataxia, myasthenia and bradypnea. Rats also showed polyuria and polydipsia. The administration of 14.5 g/kg DMSO to rats caused hyperemia and eye inflammation.

Inhalation: Inhalation of 1600, 2000 or 2900 mg/m³ for 4, 24 or 40 h respectively, by rats, resulted in elevated serum urea nitrogen levels in the 2900 mg/m³ group. Garlic breath and microscopic pulmonary edema were seen in all groups.

Topical: Topical application of DMSO resulted in localized skin dryness in rhesus monkeys. This effect was also evident in the clinical trials on Pennsaid.

A number of older studies identified the DMSO LD₅₀ for various species and routes of administration. These are listed in the following Sponsor's tables. The acute LD₅₀ of DMSO is in the g/kg of body weight range, regardless of route or animal species.

Acute Toxicity of DMSO For the Mouse and Rat (Caujolle *et al.*, 1967)

Route of Administration		Intravenous injection	Intraperitoneal injection	Subcutaneous injection	Oral route
MDNF*	mouse	5.0	14.0	12.2	≥ 14.0
	rat	≥ 6.0	11.1	10.0	≥ 15.0
LD ₅₀ 24 hrs	mouse	11.0	20.1	16.0	NE*
	rat	NE*	13.7	13.7	NE
MDAF**	mouse	14.8	> 22.1	> 22.5	14.0
	rat	> 6.0	15.0	> 15.0	> 15.0
Time of crisis	mouse	5 days	3 days	2 days	NE
	rat	NE	3 days	2 days	NE
Sublethal dose	mouse	5.0	14.0	12.2	≥ 14.0
	rat	≥ 6.0	10.0	10.0	≥ 15.0
LD ₅₀ limit	mouse	9.2	18.0	15.3	NE*
	rat	NE	13.1	13.5	NE

Results expressed in g/kg.

*MDNF=Maximum dose never fatal

**MDAF=Minimum dose always fatal

NE=Evaluation unable to be made because of too great a volume administered to animals

Acute Lethal Toxicity (LD₅₀) of DMSO (g/kg) (Willson *et al.*, 1965; and modified by reviewer)

Route of Administration	Intravenous injection	Intraperitoneal injection	Subcutaneous injection	Oral route	Topical	Dermal
Mouse	5.75	5.75		16.5-24.6	21.4, 50	
	2.66			7.78		
Rat	8	5.36		17.4-28.3	28.3, 40	
	2.12			3.46		
Guinea Pig	2.54			22.2		
Rabbit				3.88		
Dog				10		11
Monkey				4		11

Smith *et al.*, 1967Brown *et al.*, 1967

CIBA-GEIGY Canada Ltd, 1989

Dimethyl Sulfone (DMSO₂) and Dimethyl Sulfide (DMS)Several acute toxicity studies with DMSO₂ and DMS are presented in the Sponsor's table below.

Table 13: Acute Toxicity Studies on DMSO₂ and DMS

Route of Administration	Test Species	LD ₅₀ Value (mg/kg body weight)	Reference
DIMETHYL SULFOXIDE (DMSO)			
Oral	Rat	>5,000	NTIS, 1991
		>2,000	Horváth <i>et al.</i> , 2002
Topical	Rabbit	>5,000	NTIS, 1991
DIMETHYL SULFIDE (DMS)			
Oral	Rat	3,300	Koptyaev, 1967
		535	Marhold, 1972
	Mouse	3,700	Koptyaev, 1967
Topical	Rabbit	>5,000	Levenstein, 1975
Intraperitoneal	Mouse	8,000	Ashwood-Smith, 1961

The LD₁₀₀ values for DMS of 5,400 and 6,300 mg/kg body weight were reported in rats and mice, respectively (Koptyaev, 1967). These lethal doses of DMS were reported to kill “the majority of the animals” within 24 hours, with only a few animals living as long as 5 days, and acute poisoning characterized by motor incoordination, lateral posture, followed by deep narcosis and death (Koptyaev, 1967).

Acute Topical Application of DMSO

Smith et al. (1967) applied 60% or 100% DMSO dermally to the shaved backs of dogs and monkeys at doses of 3,300 to 33,000 mg/kg/week for 6 months. The skin became transiently reddened and warm from application, particularly with undiluted DMSO. Within 3 weeks erythema, desquamation and focal skin lesions occurred which lasted for the duration of treatment. No changes in behaviour, body weight, hematological parameters, blood chemistry or urinalyses were observed. No differences in organ weights were seen at necropsy and the only DMSO related effect seen upon microscopic examination was desquamation of the upper layers of the skin. The deeper layers were not involved.

2.6.6.3 Repeat-dose toxicity

PENNSAID® TOPICAL SOLUTION.

During a meeting in 1995 between the United States Food and Drug Administration (FDA) and Dimethaid Research Inc. it was agreed that instead of the customary 90-day rat toxicity study, the safety data generated on humans in Canada through the Emergency Drug Release (EDR) program and an Open-Labeled study #105-96, would be sufficient. (See the Regulatory History in section 2.6.1 Introduction and Drug History, and the Clinical Review).

DICLOFENAC SODIUM

The following toxicological data from the Voltaren (Diclofenac Sodium Tablets and Ophthalmic Solution) New Drug Submissions was obtained by the Sponsor through a Freedom of Information request.

Multidose Toxicity Study of Orally Administered Diclofenac (mg/kg)

(CIBA-GEIGY Canada Ltd., 1989; Sponsor's Table modified by reviewer)

Species	Period	Doses (mg/kg/day)	No signs of Intoxication	Reversible signs of toxicity, mainly G.I. tract	Minimum Lethal Dose
Rat	3 months	0.25, 1.0, 2.0	2	-	6
	6 months		1	2	4
	98 weeks		0.25	-	1
Dog	3 months	0.5	-	0.5	2
Rhesus Monkey	6 months	5-15	-	5-15	75
Baboon	12 months	0, 5, 10, 30	-	5	10

A summary of the diclofenac toxicological studies provided by the Sponsor are presented in the Reviewer's table below.

Diclofenac Toxicological Studies of NDA 19-201

Species	Dose	Findings
Rats Male and female	0.25, 1.0, 2.0 mg/kg/day; Oral daily dosing for 59 weeks (high-dose groups) to 98 weeks (low- and intermediate-dose groups).	High dose-related mortality rates resulted in termination of high-dose administration after 59 weeks Mortality: high mortality rate was caused by severe dose-dependent ulceration of the gastrointestinal tract with perforated ulcers leading to peritonitis and subsequent sequelae Body weight gain and feed consumption: similar between groups Hematology: neutrophilic leucocytosis and anemia occurred in the high- and intermediate-dose groups, particularly females at weeks 52 and 98, respectively Females: had enlarged adrenals and depressed glucose, increased alkaline phosphatase Histopathology: mucosal ulceration of the small intestine, lymphagiectasis, lymphoid hypoplasia, and plasma cell hypoplasia of the mesenteric lymph node, foci of hepatocytic hyperplasia, adrenal cortical atrophy and prostatitis No increase in tumor incidence was observed in the drug-treated groups as compared to the control group
Baboons (Papio spp.),	0, 5, 15 (reduced to 10 on day 254) and 50	All dose levels: ulceration of the gastrointestinal tract. Ulceration was confined to the colon in the low-dose group but was present in the stomach and small intestine also in the other

	(reduced to 30 on day 38) mg/kg/day Oral daily dosing in gelatin capsules for up to 52 weeks	two groups In the recovery groups (control, low, and intermediate), no intestinal lesions were present Body weights: less than controls; constipation, with occasional episodes of diarrhea, was a marked feature Food consumption-and body-weight gains: were within normal limits Hematology: Anemia and an increased ESR were observed in the high-dose group. Recovery group: similar to controls Chemistry: in all treated groups, there was a dose-related fall in serum albumin levels Recovery group: similar to controls
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DMSO

Repeated Dose Studies of DMSO were reviewed in the previous NDA submission. A brief summary is provided here.

Dermal Application

Undiluted DMSO applied topically to mice twice a week for 30 weeks failed to produce dermal injury. Treatment of the Achilles tendon of mice between 0-21 days by rinsing the skin with 100 μ L of 70% DMSO decreased the mean separation force of the tendons 20.2%. After 3 weeks, the mean separation force underwent a net increase. After 4 weeks, the mean separation force decreased 9.4%.

Dermal application of 10 mL DMSO to the backs of rabbits for 15-20 days resulted in dry, cracked, scaly skin. At 1 month post-induction of the Dumonde Glynn model of arthritis in rabbits, the animals were treated with 80% DMSO at 1 g/kg topically to the knees, with one 0.5 mL injection of 80% DMSO at 1 g/kg to the knees, or with nothing for 5 days per week. After 3 months, knees treated with topical DMSO showed radiographic erosion, macroscopic cartilage loss, synovial hyperplasia, inflammatory infiltrate and increased joint circumference when compared to the control knees. Following 90 days of 50% DMSO at 8 mL/kg or 100% DMSO at 4 mL/kg to rabbits, lenticular changes were observed."

Daily dermal or oral administration of 10 g/kg DMSO to rabbits caused lines of discontinuity in the lens at the end of the second week, haziness in the central nuclear region with continued administration, and by 5 weeks the lens became dense and myopic. After 10 weeks of continued administration, the lens cortex returned to normal and the nucleus was clear, but the lens was harder due to an increase in insoluble protein. Dermal application of 50% or 90% DMSO at the rate of 1.5 mL per kg per day of neat DMSO for 6 months to the normal or abraded skin of rabbits caused similar ocular disturbances.

Pigs required daily dermal application of 2.7 mL/kg DMSO to show ocular toxicity. Daily dermal application to pigs of 4.5 mL/kg 90% DMSO produced alterations of the lens.

In dogs, 0.4 g/kg DMSO i.v., daily, for 33 days and 1 mL/kg DMSO applied dermally to shaved backs 5 days per week for 15 months failed to induce toxicity. Dogs injected daily with 0.3-2.4 g/kg DMSO i.v. 6 days per week for 4 weeks showed perivascular inflammation and intravascular thrombi at the injection site. The dogs developed garlic breath, anemia, reticulocytosis, erythroid hyperplasia in the bone marrow, hemoglobinuria and hematuria. In the liver, spleen, kidney and at the injection sites, an Fe-containing pigment collected. Cloudy swelling and granularity of the hepatic parenchyma were noted. Following 1.2 g per kg per day, biliary thrombi were seen, while at higher dosages, bilirubinuria was present. Serum transaminase values were slightly elevated. At 2.4 g/kg, both males died during the first injection, while the two females survived for another 3 weeks. Dermal application of 3.3-33 g per kg per week of 60-100% DMSO for 6 months to the shaved backs of dogs caused skin desquamation.

Three dogs were given daily doses of approximately 2.5, 5.0 or 10 g/kg p.o. 50% DMSO initially; then 25% DMSO after a few days. After 14 days, one dog died (average daily dose, 5.3 g/kg). Elevated hemoglobin, hematocrit, SGOT and SGPT were reported. Treatment continued with the second and third dogs receiving 5 g/kg for 35 days and 3.8 g/kg/day for 48 days, respectively. At the end of the treatment, faint anterior and posterior cortical bands in the lens of the eyes were present. Additional treatment of both dogs with 10 g/kg/day for 13 days produced myopia and lenticular changes. Dogs given daily oral doses of 5 g/kg DMSO 5 days per week for 9 weeks and 2.5 g/kg DMSO 5 days per week for 18 weeks exhibited myopia in the central region of the lens and hyperopia in the cortical region.

Subcutaneous Administration

Chronic exposure of mice to DMSO by subcutaneous injection resulted in slightly irritated skin and mucosa, with pathological changes in the kidney and liver. Repeated doses of 2.5 g/kg, i.p. in mice caused tubulonephritis with diffuse necrosis of the intraperitoneal organs in the area of injection.

In mice and rats, chronic subcutaneous administration of DMSO produced muscle necrosis and inflammation with edema at the injection site. Mice developed liver adhesions, but rats failed to show consistent pathologic changes in any organ.

Intraperitoneal Administration

Rats injected i.p. with 0.5-8.0 g/kg DMSO 6 days per week for 4 weeks showed fibrinopurulent peritoneal exudate and peritoneal adhesions at the injection site. After the injections, all mice except those receiving the lowest dosage were restless, irritable and weak. The rate of growth was decreased in the 4-8 g/kg mice. The 8 g/kg mice showed reticulocytosis, anemia and increased mortality. Rats given 2 or 4 g/kg DMSO, i.p., daily, for 28 days showed decreased weight gain and increased mortality. In vitro incubation and histological examination of rat

kidney slices failed to show nephrotoxicity. Rats injected with DMSO daily at 7.5 mL/kg i.p. for 4 days showed fatty degeneration with congestion of the liver sinusoids, rounding of the liver margin, and fibrinous strands within the peritoneum.

Oral Administration

Oral intubation of 50% DMSO into rats at 5 g/kg for 45 days caused a slight loss in body weight, liver cell necrosis and inflammation with irritation of portal spaces. Daily intubation of 18 mL/kg 50% DMSO for 5 days per week for 18 months caused minimal changes in rat body weight, hematological values and the eye.

Diclofenac sodium was given orally to male and female rats in doses of 0.25, 1.0 and 2.0 mg/kg/day from 59 weeks (high-dose groups) to 98 weeks (low and intermediate dose groups). High dose-related mortality rates resulted in termination of high dose administration after 59 weeks; the high mortality rate was caused by severe dose dependent ulceration of the gastrointestinal tract, with perforated ulcers leading to peritonitis and sequelae. Body weight gains and feed consumption of the treated groups were close to the controls. Hematologic patterns showing neutrophilic leucocytosis and anemia were seen in the high and intermediate dose groups, particularly females at weeks 52 and 98, respectively. Female animals tended to develop enlarged adrenals and eventually experienced depressed glucose and elevated alkaline phosphatase levels. Histology studies carried out on the tissues of the control, low and intermediate dose groups showed drug related changes including mucosal ulceration of the small intestine, lymphangiectasis, lymphoid hypoplasia, and plasma cell hypoplasia of the mesenteric lymph nodes, foci of hepatocytic hyperplasia, adrenal cortical atrophy and prostatitis. No increase in tumor incidence was observed in the drug-treated groups as compared to the control group.

Oral administration of 50% DMSO to dogs 5 days per week for 2 years at 2-18 mL per day was associated with weight loss, hematological changes and diuresis. At the highest dose, myopia occurred after 5-10 weeks with transitory equatorial lens opacities by the 5th month. Similar changes occurred more slowly with lower doses. Early termination of DMSO administration led to a decrease in the myopia, but did not prevent opalescence. Decreased concentrations of soluble protein (α -crystallin), glutathione and water, with an increase in the amount of insoluble protein in the lens, were reported.

Diclofenac sodium was administered orally in gelatin capsules once daily to baboons (*Papio* spp.) at dose levels of 0, 5, 15 (reduced to 10 on day 254) and 50 (reduced to 30 on day 38) mg/kg/day for up to 52 weeks. At all dose levels studied, diclofenac caused ulceration of the gastrointestinal tract. Ulceration was confined to the colon in the low dose group but was present in the stomach and small intestine also in the other two groups. Body weights were below controls. Constipation, with occasional episodes of diarrhea, was a marked feature. In all treated groups, there was a dose related fall in serum albumin levels. Anemia and an increased ESR were observed in the high-dose group. In the recovery groups (control, low, and intermediate), no intestinal lesions were present. Food consumption and body-weight gains were within normal limits. Hematology parameters were comparable to controls and serum albumin levels returned towards normal values.

In monkeys, 0.4-4.0 g/kg DMSO i.v. or p.o. per day for 5 days or 4.0 g/kg DMSO, i.v., per day, 5 days per week for 9.5 weeks, showed a dose related hemodilution and marked fluctuations in serum glutamic pyruvic transaminase, blood glucose, and platelet and leukocyte counts. No gross abnormalities were evident. Weekly cutaneous application of 33 g/kg 60-100% DMSO for 6 months to the shaved backs of monkeys resulted in skin desquamation. Monkeys orally administered 9 mL/kg DMSO daily for 14 weeks manifested lens changes, while monkeys receiving 5 g/kg/day for 100 days failed to show ocular toxicity. Daily i.v. doses of 3 g/kg 40% DMSO for 9 days caused diuresis, transient hemolysis, and central respiratory stimulation.

In humans, chronic dermal application of DMSO resulted in itching, whealing, erythema, eosinophilia, garlic breath, fatigue, headache, nausea, dizziness, burning or aching eyes, exfoliation, and pigmentation at the site of application. Volar skin surfaces of the knee and elbow, and skin of the face, neck and axillae were more sensitive to the local irritation caused by DMSO than other portions of the body. Topical application of 80% DMSO daily for 12 weeks failed to cause ocular toxicity in humans. Instillation of 7.5-60% DMSO in tetrahydrozoline for 1-15 months in patients with intraocular inflammatory diseases and ocular disorders failed to induce ocular toxicity. A review of papers concerned with topical application of DMSO revealed an absence of ocular toxicity in humans. Daily injection of 1 g/kg i.v. 10-40% DMSO resulted in dose-dependent hemolysis with hemoglobinuria and no sign of nephrotoxicity. Administration of DMSO i.v. has been reported to cause cerebral infarction and hepatic toxicity, but others have not considered DMSO the etiological agent.

Inhalation

Repeated inhalation of 200 mg/m³ DMSO, 7 h per day, 5 days per week for 6 weeks was not toxic in rats. Garlic breath was evident. Injury did not occur when DMSO was applied daily to the clipped backs of guinea pigs for 28 days.

Ocular Toxicity

The ocular toxicosis of DMSO was summarized in a number of review articles. Administration of DMSO causes change in relucency of the cortical fibres of the lens relative to relucency of the nucleus producing a refracting surface more convex than the lens surface, creating a myopic condition. Young dogs receiving 5g/kg/day orally for 9 weeks showed a decrease in the normal relucency of the lens cortex. Similar lenticular changes were seen by the 90th day of topical administration of 4g/kg/day to young rabbits, and twice daily topical application of 45g/kg to young swine. Changes in refractive index nucleus (myopia), transitory appearance of dense white opalescence in the nuclear region of the lens and changes in vitreous humour observed as linear cracks behind the lens have been reported in dogs following 5-10 weeks of administering with DMSO orally, or topically at 9 mg/kg as a 50% solution.

The pathophysiology of these ocular effects is unknown. It was reported that the lens does not accumulate DMSO. Radiolabelled DMSO, applied cutaneously to the rabbit resulted in radioactivity being detected in all tissues except the lens within 30 minutes. After 4 hours DMSO was detected in high concentrations in the cornea, aqueous humour and vitreous body, but not in the lens. The lens changes are not caused by hydration or dehydration, and DMSO does not

appear to bind to lens protein. Concentrations of urea, glutathione, uric acid and amino acids were decreased in an affected lens while the albuminoid concentration was increased. In studies of DMSO as ophthalmic drops, no lenticular changes were noted that could be attributed to the drug. It was summarized that no lenticular changes have been seen in humans with DMSO by any route of administration.

A summary of the ocular toxicity studies in animals is presented in the Reviewer's table below.

Ocular Toxicity of DMSO in Animals

Reference/Notes	Species	Dose	Findings
Brown et al., 1963			No detrimental effects
Smith et al., 1967	Rabbits, applied into the conjunctival sac	Single dose	
		diluted (25 and 60% in saline)	dose-related edema and erythema of the orbital tissues within two hours
		undiluted (100%) DMSO	lacrimation and drooping of the lower eyelid
authors speculated that if the hypothesis that there are no species-related differences in response to DMSO is correct, that prolonged treatment in man could have adverse effects on the eye doses required to produce ocular changes in the dog are far in excess of those that have been used clinically for musculoskeletal and inflammatory conditions in humans	dog	Multiple daily oral dosing of	Normal hematology and blood chemistry
		3.8 g/kg for 48 days (n=1) then 10 g/kg/day 25% solution in distilled water for an additional 13 days	faint anterior and posterior cortical bands in the lens of each eye Lens changes were seen on slit-lamp examination carried out pre and post treatment And after 25% solution Slit-lamp examination revealed myopic changes and further lenticular changes (cortical banding and nuclear hazing)
		5 g/kg for 35 days (n=1) then 10 g/kg/day 25% solution in distilled water for an additional 13 days	No ocular changes Lens changes were seen on slit-lamp examination carried out pre and post treatment And after 25% solution Slit-lamp examination revealed myopic changes and further lenticular changes (cortical banding and nuclear hazing) 8 months after treatment, lesions are still present
Jacob and Wood (1967) Review article	dogs, rabbits and pigs	Large doses, particularly by oral route	causes change in the refractive index with progressive myopia in the lens nucleus and an increase in hyperopia in the lens cortex chemical analysis reduction of the usual soluble protein, urea, glutathione, uric and amino acid concentrations in the lens of affected eyes no lens changes were observed in the following species at the doses noted

	pigs	27 weeks of topical DMSO at 2.7- 4.5 g/kg doses	lenticular changes reversible effect: 2 months after cessation of treatment, lens alterations regressed																																				
Human cited in Jacob and Wood (1967)		84 patients on DMSO therapy (average dose and duration = 18.5mL of 90% DMSO for 2.5 months)	examined ophthalmoscopically. No toxicity to the eye was observed																																				
Vogin et al (1970)	young rhesus monkeys	chronic oral (gastric intubation) and dermal daily doses of 1, 3 or 9 mL/kg of 90% (v/v) DMSO solution administered in two divided doses 7 days a week for 18 months (74-87 weeks) topical applications covered the entire abdominal area	rhesus monkey could tolerate DMSO administered daily for about 18 months at doses up to 9 mL/kg dermally or 3 mL/kg orally without frank evidence of intolerance no lenticular changes of reluctance and progressive myopia even in high doses or prolonged treatment (9mL/kg orally for 39 or 53 weeks, 3mL/kg orally for 82 weeks, or 3mL/kg dermally for 83 weeks) Deaths: Dermal: 9 mL/kg/day, n=1 accidental Oral: 9 mL/kg/day, n=5 as result of oral administration (technical errors??) Body weight, blood pressure, heart rate, respiratory rate, body temperature, water consumption, neurologic reflexes and ophthalmologic examinations, Electrocardiograms, blood chemistries and hematological examinations, Gross and histopathology was completed terminally on 39 tissues and/or organs all topically treated animals exhibited scaling and flaking of the skin in the area of application, several had erythema, not dose related no other adverse behavioral or physical signs were seen that could be attributed to topical DMSO																																				
<table border="1"> <thead> <tr> <th>Animals/group</th><th>Route</th><th>Route</th><th>Dose</th></tr> </thead> <tbody> <tr> <td>3 (2 female, 1 male)</td><td>oral</td><td></td><td>control</td></tr> <tr> <td>3 (2 female, 1 male)</td><td></td><td>dermal</td><td>control</td></tr> <tr> <td>4 (2 female, 2 male)</td><td>oral</td><td></td><td>1mL/kg/day</td></tr> <tr> <td>4 (2 female, 2 male)</td><td></td><td>dermal</td><td>1mL/kg/day</td></tr> <tr> <td>4 (2 female, 2 male)</td><td>oral</td><td></td><td>3mL/kg/day</td></tr> <tr> <td>4 (2 female, 2 male)</td><td></td><td>dermal</td><td>3mL/kg/day</td></tr> <tr> <td>6 (3 female, 3 male)</td><td>oral</td><td></td><td>9mL/kg/day</td></tr> <tr> <td>6 (3 female, 3 male))</td><td></td><td>dermal</td><td>9mL/kg/day</td></tr> </tbody> </table>				Animals/group	Route	Route	Dose	3 (2 female, 1 male)	oral		control	3 (2 female, 1 male)		dermal	control	4 (2 female, 2 male)	oral		1mL/kg/day	4 (2 female, 2 male)		dermal	1mL/kg/day	4 (2 female, 2 male)	oral		3mL/kg/day	4 (2 female, 2 male)		dermal	3mL/kg/day	6 (3 female, 3 male)	oral		9mL/kg/day	6 (3 female, 3 male))		dermal	9mL/kg/day
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4 (2 female, 2 male)	oral		3mL/kg/day																																				
4 (2 female, 2 male)		dermal	3mL/kg/day																																				
6 (3 female, 3 male)	oral		9mL/kg/day																																				
6 (3 female, 3 male))		dermal	9mL/kg/day																																				
Wood et al. (1973)	young Rabbit, lenses cultured in vitro	0, 0.1, 0.3, and 0.5 moles DMSO	Lens changes were noted after one day with 0.5 molar DMSO (stated that months of drug therapy would be necessary to produce similar effects in vivo in rabbits).																																				

Rubin and Barnett (1975)	young dogs	oral DMSO to dogs 5 gm DMSO/kg after 9 weeks	Oral and dermal treatment caused lens changes characterized by a decrease in normal relucency of the lens cortex At lower dose levels the change was observed later								
	young pigs	dermal 4.5 ml of 90% DMSO/kg twice daily	Similar changes by 90 days of treatment								
	rabbits	dermal 8 ml 50% DMSO/kg/day and 4 ml 100% DMSO/kg/day and higher	changes were seen after 90 days of dermal application								
Oe la Torre et al. 1981	rhesus monkeys six male Rhesus monkeys, weighing 3-6 kg.:	an iv dose of 3 g/kg/day once daily for 9 consecutive days observed for 4 months after dosing	No effect on urinalysis, blood count, blood clotting times, serum electrolytes, SGOT, LDH and serum bilirubin suggesting that there was no damage to the liver, kidneys or eyes Daily fundusoscopic examinations revealed no abnormalities in any animal double-blind slit lamp examinations carried out the day before and again at 10 and 120 days after drug administration reflected no changes in refraction or translucency of the lens in the control or test animals No other ocular abnormalities were noted in any animal before, during or 18 weeks post-drug								
<table><tr><td># animals</td><td>Dose</td></tr><tr><td>4</td><td>3 g/kg DMSO in a 40% solution with saline</td></tr><tr><td>1</td><td>2 g/kg DMSO 40% with saline</td></tr><tr><td>1</td><td>3 mL/kg physiological saline (control)</td></tr></table>		# animals	Dose	4	3 g/kg DMSO in a 40% solution with saline	1	2 g/kg DMSO 40% with saline	1	3 mL/kg physiological saline (control)		
# animals	Dose										
4	3 g/kg DMSO in a 40% solution with saline										
1	2 g/kg DMSO 40% with saline										
1	3 mL/kg physiological saline (control)										
	dog	at or below 1 g/kg	Liver damage and hemorrhagic gastroentropathy occurred								
Rubin, (1983)	Mice	oral: 2,500 mg/kg/day for 6 weeks	hepatotoxicity and nephrotoxicity some degeneration of liver and an indication of nephritis								
Review	rats	1,000 to 5,000 mg/kg/day for 6 weeks	degenerative liver changes and signs of nephritis.								

Termination of clinical testing with DMSO by the FDA occurred on November 11, 1965, on the basis of toxicological findings of changes in the lens of the eyes of lower animals, first seen in dogs in a Merck-supported study. Follow-up studies revealed that sufficiently high doses of DMSO for a prolonged time can alter the nucleus of the lens of the eye. No lens changes were noted in Rhesus monkeys at 5 g/kg/day for 100 days. The testing ban was partly rescinded by the FDA at a later date and further relaxed on completion of a controlled study in man. A very

extensive toxicology study of DMSO was conducted with 80% DMSO gel at 1 gm/kg per day, by daily dermal application. This was estimated to be 3 to 30 times the usual dose in humans. Jacob and Wood (1967) noted that the average human topical dose was 0.1-0.2 g/kg/day. Additionally, human subjects had been ophthalmologically examined after DMSO therapy lasting as long as 2.5 years, with no ocular toxicity.

2.6.6.4 Genetic toxicology

PENNSAID® TOPICAL SOLUTION

The Sponsor performed three genotoxicology studies with the “to be marketed” formulation. The results indicated a lack of mutagenicity or clastogenicity as indicated in the reviewer’s table below. Reviews of these studies are presented later. Because these studies used this formulation, rather than the biologically active ingredients, individually or together, the maximum concentrations of diclofenac and DMSO used in the *in vitro* studies were approximately 70-fold and 2-fold, respectively, less than recommended maximal concentrations in the ICH and FDA Guidances for Industry. The concentrations used in the *in vivo* mouse micronucleus test were within the range expected to demonstrate toxicity.

Pennsaid® Genetic Toxicology Studies

Report, Location	Species/Strain	Doses			Key Findings
		Pennsaid® µg/plate	Diclofenac µg/plate	DMSO µg/plate	
Mutagenic: Bacteria Reversion Assay	<i>Salmonella typhimurium</i>	100	1.39	45.6	Not mutagenic with or without metabolic activation
		316	4.3	144	
		1000	13.9	456	
		3160	43.9	1441	
		5000	69.5	2280	
Clastogenic Human lymphocytes, <i>in vitro</i>	human lymphocytes	Pennsaid® µg/mL	Diclofenac µg/mL	DMSO µg/mL	Not clastogenic (no chromosomal or chromatid damage with or without metabolic activation)
		312.5	4.34	142.5	
		625	8.68	285	
		1250	17.36	570	
		2500	34.72	1140	
Clastogenic Mouse micronucleus, <i>in vivo</i>	NMRI mouse oral administration	Pennsaid® mL/kg	Diclofenac mg/kg	DMSO mg/kg	Not clastogenic up to maximum tolerated dose of 12 mg/kg, oral dose, examined at 24 and 48 hours
		3	45	1465	
		6	89	2930	
		12	178	5860	
		20	297	9768	

DICLOFENAC SODIUM

For the approval of Voltaren (NDA 19-201) genetic toxicology studies demonstrated a lack of mutagenic and clastogenic activity in a variety of test systems that included Ames, yeast (*Saccharomyces cerevisiae* MP-1) and *in vitro* mouse lymphoma L5178Y cell mutation tests, dominant lethal studies and germinal epithelial chromosomal studies in male mice, and nucleus anomaly and chromosomal aberration studies in Chinese hamster bone marrow cells.

DMSO

Soon after the first resubmission of this NDA, the Division requested additional genotoxicity and carcinogenicity information. The Sponsor submitted a safety update on the genotoxicity and carcinogenicity of DMSO to the NDA (SU, May 9, 2002). Included in this submission was a review and critique of the submitted published studies by _____. His curriculum vita was provided as evidence of his expertise. His conclusion was that the genotoxic potential of DMSO was exceedingly low:

b(4)

“the preponderance of the evidence shows that DMSO does not produce gene mutation in the standard bacterial tester strains or mammalian cell lines, induce chromosome aberration or micronuclei in mammalian cells *in vitro* or *in vivo*, or sister chromatid exchange in mammalian cells *in vitro*. The weight of evidence from *in vivo* bone marrow studies in rats and mice also support the conclusion that DMSO would not produce genetic damage at non-toxic doses. In fact, there are a number of reports that DMSO protects against chromosomal damage induced by radiation and chemicals.”

In support of this conclusion he noted the common practice to use DMSO as a standard solvent for hydrophobic compounds undergoing testing in the Ames mutagenicity assays (George and Kuttan, 1997; Mortelmans and Zeiger, 2000). DMSO had no mutagenic activity at either low or high doses. The few *in vitro* and *in vivo* studies that did see mutagenic effects with DMSO had from design or methodological faults and required further validation, discussed below.

Zeiger et al (1992) conducted the U.S. National Toxicology Program that examined the potential for DMSO genotoxicity. DMSO (99% pure) was tested as a coded chemical in two independent laboratories at concentrations up to 10,000 µg/plate, without and with S9 liver metabolic enzyme preparations. No toxicity or mutagenicity was seen at any of the concentrations tested, and both laboratories reported similar results.

In vitro studies

A few studies provided evidence for DMSO genotoxicity.

Hakura et al. (1993) tested DMSO (Dötait-Spectrosol grade, not otherwise described) using the preincubation procedure at dose levels up to 1 mL/plate. Mutagenicity occurred in *Salmonella* strains TA1537 and TA2637 at DMSO concentrations between 0.8 and 0.9 mL/plate without S9, and between 0.85 and 0.95 mL/plate with rat liver S9. Mutagenicity occurred in *E. coli* strain WP2uvrA, only with S9, at DMSO concentrations between 0.9 and 1.0 mL/plate. The concentrations of DMSO producing the increased numbers of mutant colonies also had low survival rates, 41% to 0.1% for the *Salmonella* strains, and 61% to 31% for the *E. coli* strains. There was no indication if this poor survival of the cells affected their response to DMSO.

_____ provided the following commentary:

b(4)

These positive results (which occurred at toxic doses) contrast with the results obtained from Dr. Ames' laboratory (McCan et al. 1975) and their recommendation that DMSO be used as a solvent vehicle for test chemicals because of its lack of effect (Maron et al., 1981; Zeiger et al., 1992).

Based on knowledge of the responses of the various Ames tester strains, it is unexpected to see a response only in strain TA1537 and its modified version TA2637. Strain TA2637 is not used routinely and not recommended by Ames, the ICH, or the OECD. Chemicals that are mutagenic at the DNA target sequence in strain TA1537 tend also to be mutagenic in strain TA97, but DMSO was not mutagenic in this strain (McCan et al., 1975; Zeiger et al., 1992). These compounds also contain a planar, intercalating aromatic ring structure, which DMSO lacks. Additionally, given the widespread use of DMSO as the solvent control, and the widespread use of strain TA1537, one would expect to see more reports of mutagenicity than actually occur. In addition, the positive response reported by Hara et al. (1993) in *E. coli* WP2uvrA, does not conform to what is known about the genetics and responsiveness of this strain. While *Salmonella* T1537 responds only to a very specific, molecular intercalation effect, and chemicals that are positive only in this strain produce only frameshift mutations, the *E. coli* strain identifies chemicals that produce base-pair substitution mutations, not chemicals that only produce frameshift mutations. In recognition of this pattern of responses, the ICH (1996) and an international expert working group (Gatehouse et al., 1994) recommended that *E. coli* WP2uvrA, which measures mutations at AT sites within the DNA be interchangeable with *Salmonella* strain TA102 and TA104, which also measure base-pair substitution mutations at AT sites within the DNA.

In vivo studies

A large number of in vivo rodent studies used DMSO as a solvent control, rather than as the test chemical. A few studies used DMSO itself as the test chemical.

Kapp and Eventoff (1980) administered male rats from 1 to 100% DMSO, intraperitoneal, at 5 mL/kg daily, for 5 days. Bone marrow cells were examined 24 hours after the last injection. There was no significant difference in the number of chromosome breaks compared to controls, but the numbers of chromatid breaks were elevated. The incidences of chromosome exchanges, rings and dicentric chromosomes were significantly increased following DMSO exposure. After 100% DMSO, there was a significant increase in the number of damaged cells. Overall, the incidence of aberrant cells increased from 10% at the 1% dose level to 68% at the 100% DMSO dose level. Tate and Krek (1980) also positive chromosome aberration response

Other studies using similar protocols found no effects

Anwar et al. (1989)

Loveday et al. (1990)

Amcher and Zelljadt (1984) noted, without presenting the data that DMSO was not mutagenic in mouse lymphoma cells.

Caspar et al. (1988) summarized the results from numerous studies in mouse lymphoma

cells in two laboratories under the auspices of the NTP, and showed that DMSO did not induce an increase in mutations.

As mentioned in ———critique, DMSO is available in various grades of purity. The DMSO used by Kapp and Eventoff (1980) was the lowest available purity, whereas, Anwar et al. (1989) used reagent grade, which has a higher purity and lower (or none) levels of measurable contaminants, and did not find chromosome damage. In the majority of *in vivo* studies, DMSO was used as the vehicle control rather than as the test chemical. However, there are sufficient data from these vehicle control studies to support a determination that DMSO does not produce chromosome damage in rodent bone marrow cells, measured as micronuclei or chromosome aberrations. The weight of evidence, therefore, supports the conclusion that DMSO does not induce gene mutations or chromosome aberrations in mammalian cells.

b(4)

In summary, the preponderance of the evidence indicates that DMSO does not produce gene mutations in the standard bacteria tester strain or mammalian cell lines, induce chromosome aberrations or micronuclei in mammalian cells *in vivo* or *in vitro*, or sister chromatid exchanges (SCE) in mammalian cells *in vitro*. The weight of the evidence from *in vivo* bone marrow studies in rats and mice also support a conclusion that DMSO would not produce genetic damage at nontoxic doses. In fact, there are a number of reports that DMSO protects against chromosome damage induced by radiations and chemicals.

Reviews of New Genetic Toxicology Studies

Study title: Mutagenicity study of PENNSAID® in the *Salmonella typhimurium* reverse mutation assay (in vitro)

Key study findings: Pennsaid® was not mutagenic in the *Salmonella typhimurium* reverse mutation assay at concentrations up to 5000 µg/plate (equivalent up to — µg/plate diclofenac and — µg/plate DMSO). There was no increase in revertant colonies of the *Salmonella typhimurium* strains TA98, TA100, TA102, TA1535 and TA1537, during incubations without or with metabolic activation, using either the plate incorporation or preincubation methodology.

b(4)

Reviewer's Comment: These concentrations of the active drug substance and biologically active excipient are below the 5000 µg/plate ICH recommendations.

Study no.: 17623

E-submission: phamtox\tox\17623.pdf

Conducting laboratory and location: _____

b(4)

Date of study initiation: Jan 9, 2004

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Pennsaid®, Batch D0168AE — w/w diclofenac sodium, — w/w DMSO, — ethanol)

b(4)

Vehicle: water for injection

Methods

Strains/species/cell line: *Salmonella typhimurium* strains

Strain Designation	Histidine Gene Locus Affected	Additional Mutations			Type of Mutation Detected
		Repair	LPS	R-factor	
TA 98	<u>his</u> D 3052	<u>uvr</u> B ⁻	<u>rfa</u> ⁻	pKM 101	Frameshift
TA 100	<u>his</u> G 46	<u>uvr</u> B ⁻	<u>rfa</u> ⁻	pKM 101	Base-pair substitution
TA 102	<u>his</u> G 428	<u>wild-type</u>	<u>rfa</u> ⁻	pKM 101	Base-pair substitution
TA 1535	<u>his</u> G 46	<u>uvr</u> B ⁻	<u>rfa</u> ⁻	-	Base-pair substitution
TA 1537	<u>his</u> C 3076	<u>uvr</u> B ⁻	<u>rfa</u> ⁻	-	Frameshift

rfa: partial loss of lipopolysaccharide (LPS) barrier that causes increased permeability to macromolecules

uvr B: loss of DNA excision repair system

R-factor (pKM 101): plasmid, thought to cause an increased error-prone DNA repair

Doses used in definitive study: 100, 316, 1000, 3160 and 5000 µg/plate

The Sponsor stated that doses used were 100, 316, 1000, 3160 and 5000 µg/plate. However, the final product Pennsaid[®], Batch D0168AE density _____ was used to calculate the weight prepare each test concentration. The actual concentrations of diclofenac and dimethyl sulfoxide are indicated in the following table, based on the Pennsaid[®] composition of _____ w/w diclofenac sodium and _____ w/w DMSO.

Active Component Concentrations		
Pennsaid [®] µg/plate	Diclofenac µg/plate	DMSO µg/plate
100		
316		
1000		
3160		
5000		

Reviewer's Comment: The maximum concentration of the active drug, diclofenac, was 72-fold lower than the recommended ICH guidance of 5000 µg/plate. The maximum concentration of the biologically active excipient, DMSO, was approximately 2.2-fold lower than this recommended dose. There was no indication of cytotoxicity that may explain why these low concentrations were used.

Basis of dose selection:

A preliminary cytotoxicity test was carried out to determine the non-toxic dose range, using strain TA100. Doses (up to 5000 µg/plate or 100 µl/plate) were plated (Vogel-Bonner minimal media plates) with strain TA100, in triplicates, both without and with S9 activation.

Negative controls: water for injection

Positive controls:

a) without metabolic activation	
sodium azide ¹ in H ₂ O ² (10 µg/plate)	TA 1535, TA 100
2-nitro-fluorene ¹ in DMSO ³ (10 µg/plate)	TA 98
9-amino-acridine ¹ in ethanol, abs. ⁴ (100 µg/plate)	TA 1537
methyl methane sulfonate (MMS) ¹ in DMSO ³ (1300 µg/plate)	TA 102
b) with metabolic activation	
2-anthraccene amide ¹ in DMSO ³ (2 µg/plate)	TA 98, TA 102, TA 1537
cyclophosphamide ¹ in aqua ad iniectionis ² (1500 µg/plate)	TA 100, TA 1535

Incubation and sampling times:

Cultures were prepared using standard techniques, either plate incorporation or preincubation methods. Triplicate plates were used for each concentration. Two independent experiments were conducted with and without metabolic activation for each culture preparation method. Plates were incubated for 48 hours, and then background lawn and colonies were evaluated. Sterility was confirmed by incubations without bacteria.

S9 metabolic activator was obtained as the post-mitochondrial fraction of liver from 20 to 30 rats treated with Aroclor 1254. The protein content of the S9 fraction was 29.92 mg/mL S9. The cytochrome P-450 content was 0.44 nmol/mg. It was stored in liquid nitrogen and prepared fresh and filter sterilized on the experimental day.

Results

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

The genotypes were regularly confirmed.

A response was positive if the following criteria were met:

- the number of revertants is significantly increased ($p \leq 0.05$; U-test according to MANN and WHITNEY, see reference 3.) compared with the solvent control to at least 2-fold for TA 98, TA 100 and TA 102 and 3-fold for TA 1535 and TA 1537, in both independent experiments, and
- a significant concentration (log value)-related effect ($p \leq 0.05$; Spearman's rank correlation coefficient, see reference 3.) is observed, and
- the histidine independence of the revertants has been confirmed by streaking random samples onto histidine-free agar plates, and
- positive results are reproducible

Cytotoxicity was defined as a scarce background lawn (50% reduction compared with the solvent control).

Study outcome:

In the preliminary test, there were no signs of cytotoxicity up to 5000 µg/plate Pennsaid with or without metabolic activation. No mutagenic effect (no increase in revertant colony numbers as compared with control counts) was observed for Pennsaid® tested up to 5000 µg/plate in any of the five test strains (plate incorporation method, data summarized below; or preincubation method, similar data, not presented in this review) without and with metabolic activation. Positive controls showed the expected increase in revertant colonies.

Reviewer Comments: The reviewer concurs with the Sponsor's conclusions. However, the results did not substantially contribute to determination of the genotoxic safety of the active components since their concentrations were far below ICH guideline recommendations. Usually

strains from a second species of bacteria, *Escherichia coli*, are also used in bacteria mutagenic studies, but these were not incorporated into the Sponsor studies. Revertants of *Escherichia coli* WP2 uvrA occur by base substitution at A-T sites, which is similar to changes that occur with *S.typhimurium* strain TA 102, used in the above study.

TABLE 2
Mutagenicity study of
Pennsaid®
in the *Salmonella typhimurium* reverse mutation assay (in vitro)

Item (µg/plate)	Plate incorporation test without metabolic activation Number of reverted colonies				
	TA 98	TA 100	TA 102	TA 1535	TA 1537
mean values ± SD					
Pennsaid®					
5000	M	24.7	154.0	305.3	13.3
	SD	4.0	4.0	1.5	2.1
3160	M	27.0	154.3	264.0	11.7
	SD	8.9	16.1	17.3	0.6
1000	M	34.3	155.3	264.0	14.0
	SD	9.7	19.6	17.1	2.0
316	M	35.0	164.0	280.0	13.0
	SD	5.6	19.7	6.1	1.0
100	M	25.0	173.3	271.0	13.3
	SD	3.0	5.8	18.7	1.2
Negative control 100 µL/plate					
	M	36.3	165.0	258.3	14.3
	SD	11.5	12.5	14.0	1.2
Untreated control					
	M	39.3	144.0	257.0	12.7
	SD	4.9	9.8	5.3	2.1
Positive control item					
	2-Nitro- fluorene	Sodium azide	Methyl- methane sulfonate	Sodium azide	Amino- acridine
Concentration µg/plate					
	10	10	1300	10	100
	M	435.7	802.0	534.7	691.7
	SD	5.7	19.1	21.7	4.0

TABLE 2
Mutagenicity study of
Pennsaid®
in the *Salmonella typhimurium* reverse mutation assay (in vitro)

Plate incorporation test with metabolic activation Number of reverted colonies						
Item (µg/plate)		TA 98	TA 100	TA 102	TA 1535	TA 1537
mean values ± SD						
Pennsaid®						
5000	M	30.3	184.3	277.7	16.0	4.7
	SD	3.8	15.7	31.2	1.0	1.5
3160	M	29.0	166.7	266.3	12.7	5.0
	SD	2.0	20.7	12.5	2.1	1.0
1000	M	32.7	170.0	261.3	12.0	5.0
	SD	0.6	18.7	5.0	1.0	1.0
316	M	38.0	174.7	262.0	13.3	5.0
	SD	11.3	19.6	6.1	1.2	1.7
100	M	31.3	159.7	275.3	13.0	4.0
	SD	3.1	5.7	9.1	1.0	1.0
Negative control 100 µL/plate						
	M	40.3	181.3	251.3	15.7	8.0
	SD	10.2	18.0	3.2	0.6	1.0
Untreated control						
	M	48.7	180.3	262.0	19.0	7.7
	SD	11.7	12.7	24.6	1.0	2.5
Positive control item		2-Anthra- cene amide	Cyclophos- phamide	2-Anthra- cene amide	Cyclophos- phamide	2-Anthra- cene amide
Concentration µg/plate		2	1500	2	1500	2
	M	436.0	790.7	962.7	530.3	689.7
	SD	3.6	6.4	130.1	3.5	2.5

Study title: In vitro assessment of the clastogenic activity of PENNSAID® in cultured human peripheral lymphocytes

Key study findings: Pennsaid® was not clastogenic during *in vitro* incubations with human lymphocytes at concentrations up to 5000 µg/mL (equivalent up to — µg/mL diclofenac and — µg/mL DMSO). There was no increase in chromosomal or chromatid damage during incubations in the absence or presence of metabolic activation.

b(4)

Reviewer's Comment: These concentrations of the active drug substance and biologically active excipient are below the 5000 µg/plate ICH recommendations.

Study no.: 17625

E-submission: phamtox\tox\17625.pdf

Conducting laboratory and location: _____

b(4)

Date of study initiation: Feb 9, 2004

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Pennsaid®, Batch D0168AE — w/w diclofenac sodium, — w/w DMSO,
— ethanol).

b(4)

Vehicle: water for injection

Methods

Strains/species/cell line: human lymphocytes

Human peripheral blood was obtained by venipuncture from healthy donors known to be without any medication and collected in heparinised vessels.

Reviewer's Comment: It is not clear whether one person contributed cells for all the tests or if cells from different individuals were each used in different tests.

Doses used in definitive study: 312.5, 625, 1250, 2500, 5000 µg/mL Pennsaid®

Pennsaid® was dissolved and diluted to the appropriate concentrations in sterile water prior to the treatment of the cells. The density — was used to calculate the weight of test item required to prepare each test concentration.

b(4)

Reviewer's Comment: As in the previous study, the final product Pennsaid, Batch D0168AE was used. The actual concentrations of diclofenac and dimethyl sulfoxide are indicated in the following table, based on the Pennsaid composition of — w/w diclofenac sodium and — w/w DMSO.

b(4)

Active Component Concentrations

Pennsaid® µg/mL	Diclofenac µg/mL	DMSO µg/mL
312.5		
625		
1250		
2500		
5000		

b(4)

Basis of dose selection:

The concentrations for the main study were chosen following a preliminary toxicity test. A preliminary toxicity study was carried out employing Pennsaid® at concentrations of 10, 25, 100, 250, 1000, 2500 and 5000 µg/mL medium in an experiment with metabolic activation (in the presence of S9 mix, 4-h exposure) and without metabolic activation (24-h exposure). Lymphocyte cultures were established for 48 hours, then treatments were added and cultures were incubated for an additional 24 hours at 37°C before harvesting cells for analysis. One slide per culture was prepared. One thousand lymphocytes per culture were examined at a magnification of x400; the mitotic index was calculated as the percentage of lymphocytes examined which were in metaphase.

In this preliminary experiment, no signs of cytotoxicity were observed up to the highest tested concentration of 5000 µg Pennsaid®/mL medium in the experiments without and with metabolic activation (24-h and 4-h exposure). Therefore 5000 µg Pennsaid/mL without and with metabolic activation was used in the main study.

Negative controls: sterile water

Positive controls: Mitomycin C was used in the absence of metabolic activation
Cyclophosphamide in the presence of metabolic activation

Incubation and sampling times:

All cultures were run in duplicate using blood from a different donor. Two independent experiments with and without metabolic activation were carried out. The preparation of the Aroclor 1254-induced rat S9 fraction was prepared on site and stored in liquid nitrogen until used. The S9 mix was freshly prepared on the day of the test containing 10% S9.

After 48 hours of culture in complete medium the tubes were centrifuged, and the cell pellet resuspended to 4.5 mL (for S9 mix addition) or 5.0 mL with treatment medium. S9 mix (0.5 mL) was added to the appropriate cultures. Treatments were added and the tubes were incubated for 4 hours in a shaking water bath at 37°C. Cells were then washed and incubated again for 20 hours without S9. In a second set of the experiment a continuous treatment of 24 hours without metabolic activation was carried out and the 4-hour treatment with metabolic activation was repeated.

Experiment 1:

Hours	Absence of S9 4-h exposure / 24-h sampling	Presence of S9 4-h exposure / 24-h sampling
0	commence treatment	commence treatment
+ 4	remove treatment medium, wash and add recovery medium	remove treatment medium, wash and add recovery medium
+ 22	add colcemid	add colcemid
+ 24	harvest, prepare slides	harvest, prepare slides

Experiment 2:

Hours	Absence of S9 24-h exposure / 24-h sampling	Hours	Presence of S9 4-h exposure / 24-h sampling
0	commence treatment	0	commence treatment
	24-hour continuous treatment	+ 4	remove treatment medium, wash and add recovery medium
+ 22	add colcemid	+ 22	add colcemid
+ 24	harvest, prepare slides	+ 24	harvest, prepare slides

Two hours before termination, cell division was arrested by the addition of colcemid. After incubation an additional 2 hours, the cells were harvested, fixed in freshly prepared methanol: glacial acetic acid fixative (4:1, v/v), spread on slides, stained with Giemsa and examined. The slides were examined under low power (x 100 objective) and those areas judged to be of sufficient technical quality were located and examined under high power (x 1000, oil immersion objective).

Results

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

The highest concentration employed in the main study should suppress the mitotic index by 50 - 80%. The lowest dose should be in the region of the solvent (negative control). Four concentrations of the test item in addition to the solvent negative control and positive control were investigated both in the absence and in the presence of metabolic activation. For each treatment and culture 100 metaphases per plate were examined, if possible. Observed aberrations were noted and scored according to Savage (1975):

Gap	Achromatic region in chromatid(s) not greater than the width of a chromatid; scored as single-stranded or double-stranded.
Break	Achromatic region in chromatid(s) greater than the width of a chromatid or a discontinuity with displacement; scored as chromatid or chromosomal.
Fragment	Any free displaced portion of chromatid material.
Exchange	Aberration arising from an exchange between two or more chromosomes which results in the products re-uniting to form a dicentric or polycentric structure. These may be chromosome or chromatid interchanges. In studies of this type, where full karyotyping is not undertaken and chromosome banding has not been performed, only asymmetrical or chromatid exchanges will normally be recognised.
Other types of Aberrations	E.g. isochromatid/isochromatid exchanges such as dicentric chromosomes, centric rings, pulverised metaphases or polyploidy.

Metaphases were assigned to one of the following 5 categories:

1	normal metaphases
2	metaphases with 1 - 2 aberrations
3	metaphases with multiple aberrations
4	pulverised metaphases: extreme ($\geq 50\%$) fragmentation of chromosomal material
5	polyploidy

In addition, the total number of gaps was recorded in 100 metaphases. Metaphases which differed from the normal diploid complement (46) were excluded from evaluation. However, variations of the normal chromosome number were noted (polyploidy).

To examine the toxicity of the test item 1000 cells were scored and the mitotic index was calculated as the percentage of cells in metaphase. The assessment was carried out by a comparison of the number of chromosome aberrations of the samples with those of the solvent control, using the exact test of Fisher ($p < 0.05$).

Chromatid gaps are not generally considered examples of true chromosomal aberrations. Therefore only the total numbers of cells with aberrations exclusive of gap damage were analyzed. However, the numbers of cells with aberrations including gap damage were also tabulated.

Not all treatments were evaluated according to the Sponsor because it was thought that they would provide no further information. Those not evaluated were:

- 312.5 μg Pennsaid[®]/mL (in the experiments without metabolic activation, 4-h and 24-h exposure)
- 0.1 μg mitomycin C/mL (in the experiments without metabolic activation,

- 4-h and 24-h exposure)
- 312.5 µg Pennsaid®/mL (in the experiments with metabolic activation, 4-h exposure)
- 10 µg Cyclophosphamide/mL (in the first and second experiment with metabolic activation, 4-h exposure)

A positive clastogenic response was indicated if:

- the number of chromosomal aberrations is significantly (at $p < 0.05$) increased compared with the solvent control
- the increase observed is concentration-dependent
- both duplicate cultures lead to similar results
- the increase should not occur in the severely cytotoxic range (mitotic index < 0.25), as it is known that high cytotoxicity causes artifacts in the form of aberrations in *in vitro* chromosomal aberration tests.

Study outcome:

In the main study, there were no signs of cytotoxicity without and with metabolic activation (24-h and 4-h exposure).

For tests without metabolic activation (4- and 24-hour exposure), the mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Pennsaid® at concentrations from 625 to 5000 µg/mL medium (4-h and 24-h exposure) in the absence of metabolic activation ranged from 0.5% to 3.5%. The results obtained are considered to be within the normal range of the negative reference item where a mean incidence of chromosomal aberrations (excluding gaps) of 1.5% was observed after a 4-hour and 24-hour exposure.

For tests with metabolic activation (4-hour exposure) The mean incidence of chromosomal aberrations (excluding gaps) of the cells treated with Pennsaid® at concentrations from 625 to 5000 µg/mL medium in the presence of metabolic activation ranged from 0.5% to 3.0%. The results obtained are considered to be within the normal range of the negative reference item where a mean incidence of chromosomal aberrations (excluding gaps) of 3.0 or 0.5 was observed in the first and second experiment, respectively.

The incidence of chromosomal aberrations (excluding gaps) of the negative reference items without and with metabolic activation ranged from 0.0% to 5.0% for the last 30 experiments (most recent background data, not audited by the QAU-department).

Reviewer's Comments: The reviewer concurs with the Sponsor's conclusions, although the absence of counting the low dose treatments indicates the analysis was not done blindly which can bias the results. As above, the results did not substantially contribute to determination of the genotoxic safety of the active components since their concentrations were far below ICH guideline recommendations.

A Summary of the results is presented table 2, below.

Table 2 Chromosome analysis in human peripheral lymphocytes in vitro
SUMMARY

1st experiment 4-h exposure				2nd experiment 24-h exposure			
Treatment (μ g/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps	Treatment (μ g/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps
without metabolic activation							
<i>aqua ad infectabilia</i>							
0	1.00	200	1.5	0	1.00	200	1.5
Pennsaid®							
625	1.32	200	1.0	625	1.06	200	1.5
1250	1.22	200	1.0	1250	1.19	200	0.5
2500	1.10	200	2.0	2500	0.93	200	1.5
5000	0.96	200	3.5	5000	1.01	200	0.5
Mitomycin C							
0.2	1.00	200	13.5 *	0.2	0.59	200	18.5 *

= mitotic index: number of metaphases/1000 cells; negative control = 1.00

* = significantly different from negative control ($p \leq 0.05$)

1st experiment 4-h exposure				2nd experiment			
Treatment (μ g/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps	Treatment (μ g/mL medium)	Mitotic index#	Number of metaphases scored	% of cells with aberrations excluding gaps
with metabolic activation							
<i>aqua ad infectabilia</i>							
0	1.00	200	3.0	0	1.00	200	0.5
Pennsaid®							
625	1.36	200	1.0	625	1.03	200	1.0
1250	1.10	200	1.0	1250	0.98	200	0.5
2500	0.73	200	2.0	2500	0.85	200	1.5
5000	0.78	200	3.0	5000	1.02	200	1.5
Cyclophosphamide							
20	0.62	200	23.0 *	20	0.95	200	11.0 *

= mitotic index: number of metaphases/1000 cells; negative control = 1.00

* = significantly different from negative control ($p \leq 0.05$)

Study title: Micronucleus test of PENNSAID® in bone marrow cells of the NMRI mouse by oral administration

Key study findings: In the mouse *in vivo* micronucleus assay, Pennsaid® was not clastogenic in bone marrow cells (polychromatic erythrocytes) when administered up to the maximum tolerated dose of 12 mL/kg b.w., p.o.

Study no.: 17626

E-submission: pharmtox\tox\17626.pdf

Conducting laboratory and location: _____

b(4)

Date of study initiation: Feb 9, 2004

GLP compliance: yes

QA reports: yes

Drug, lot #, and % purity:

Pennsaid, Batch D0168AE — w/w diclofenac sodium, — w/w DMSO,
— ethanol)

b(4)

Vehicle: 0.8% aqueous hydroxypropylmethylcellulose gel (Methocel)

Methods

Strains/species/cell line: Crl:NMRI mice, 5 males and 5 females per sampling interval and group, about 6 week of age, 18-23 g.

Doses used in definitive study: 3, 6 and 12 mL Pennsaid®/kg b.w. The maximum feasible administration volume did not exceed 2 mL/100 g body weight.

Active Component Concentrations

Pennsaid mL/kg	Diclofenac mg/kg	DMSO mg/kg
3		
6		
12		
20		

b(4)

Basis of dose selection:

The dose levels were selected based on a preliminary oral acute toxicity study of Pennsaid® in NMRI mice given doses of 2, 4, 8, 12 and 20 mL/kg b.w. and followed for 48 hours. No signs of systemic toxicity were noted after single oral administration of Pennsaid® up to 8 mL/kg b.w. The animal treated with 12 mL/kg b.w. died on day 4, the animal treated with 20 mL/kg b.w. died on day 2.

The highest dose was defined as the dose producing signs of toxicity such that higher dose levels, based on the same dosing regimen, would be expected to produce lethality. The highest dose may also be defined as the dose that produces some indication of toxicity of the bone

marrow (e.g. reduction of the proportion of immature erythrocytes among total erythrocytes in the bone marrow).

Negative controls: 0.8% aqueous hydroxypropylmethylcellulose gel (Methocel)

Positive controls: cyclophosphamide (27 mg/kg b.w., i.p.)

Incubation and sampling times:

In the main study, doses of 3, 6 and 12 mL Pennsaid®/kg b.w. were administered orally and animals were sampled at 24 hours. Another group was sampled at 48 hours after administration of 12 mL Pennsaid®/kg b.w. Positive and negative control groups were also included in the 24 hour groups, but only a negative control included in the 48 hour groups.

Group	Dose Pennsaid® (mL/kg b.w., p.o.)	Sampling time (h)	Number and sex of animals	Animal no.
1 negative reference/vehicle	20 mL 0.8% aqueous hydroxypropylmethylcellulose gel	24	5 m 5 f	1 - 5 6 - 10
2 low dose	3	24	5 m 5 f	11 - 15 16 - 20
3 intermediate dose	6	24	5 m 5 f	21 - 25 26 - 30
4 high dose	12	24	5 m 5 f	31 - 35 36 - 40
5 positive reference	27 mg cyclophosphamide/ kg b.w., i.p.	24	5 m 5 f	41 - 45 46 - 50
6 negative reference/vehicle	20 mL 0.8% aqueous hydroxypropylmethylcellulose gel	48	5 m 5 f	51 - 55 56 - 60
7 high dose	12	48	5 m 5 f	61 - 65 66 - 70

Immediately after sacrifice, bone marrow smears were prepared from the femur, dried, fixed in methanol, and stained with hematoxylin-eosin.

Results

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

Two thousand (2000) erythrocytes were evaluated per animal for the incidence of micronuclei. To assess bone marrow toxicity, the ratio of polychromatic (PCE) to normochromatic erythrocytes (NCE) was determined for each animal after counting a total of 1000 erythrocytes. The assessment was carried out by a comparison of the samples with the positive and the vehicle references, using a chi-square test corrected for continuity. The PCE/NCE ratios and frequencies of micronucleated PCE in vehicle reference animals were compared with historical control ranges to determine whether or not the assay was acceptable. For each group, inter-individual variation in the numbers of micronucleated PCE was estimated by means of a heterogeneity chi test.

The numbers of micronucleated PCE in each treated group (males and females, separately and combined) were then compared with the numbers in vehicle reference groups by using a 2 x 2 contingency table to determine chi (Colquhoun, 1971). Probability values of $p < 0.05$ were accepted as significant.

The micronucleus test was considered valid if:

- the heterogeneity chi² test provided evidence of acceptable variability between animals within a group
- the incidence of micronucleated PCE in the vehicle reference groups fell within or close to the historical vehicle control range as given in Appendix 6
- at least 7 animals {males and females together} out of each group at each kill were available for analysis
- the positive reference chemical (CPA) induced clear and statistically significant increases in the frequencies of micronucleated PCE.

The micronucleus test was considered positive in this assay if:

- a statistically significant increase in the frequency of micronucleated PCE occurred for at least one dose at one kill time
- the frequency of micronucleated PCE at such a point exceeded the historical control range iii)
- corroborating evidence was obtained, for example, increased but statistically insignificant frequencies or micronucleated PCE at other doses or kill times, or dose response profiles.

Study outcome:

No signs of systemic toxicity were noted after administration of Pennsaid® up to the maximum tolerated dose level of 12 mL/kg b.w.

The maximum tolerated dose of 12 mL Pennsaid®/kg b.w., p.o., did not result in an increase in the incidence of micronucleated polychromatic erythrocytes (PCE). The incidences of PCEs for the combined male and female animals receiving 12 mL/kg were 1.9 and 1.7 micronuclei per 1000 PCEs for the samples collected 24 and 48 hours, respectively. The negative control values were 1.1 and 1.6, respectively. Cyclophosphamide resulted in a significant increase of 9.2 micronuclei per 1000 PCEs. The ratio of polychromatic to normochromatic erythrocytes (NCE) was not affected by treatment.

Table 1, below, presents the summarized data. There was no effect of Pennsaid on either the PCE/NCE ratios or the numbers of micronucleated PCE. The numbers of micronucleated PCE were also similar to those seen in historical controls

Table 1

SUMMARISED DATA OF THE MUTAGENICITY STUDY

Test item mL/kg b.w., p.o.	Sampling time (h)	Number of polychromatic erythrocytes scored per group*	Ratio PCE/NCE#			Micronucleated polychromatic erythrocytes mean frequency per 1000 PCE			Significances
			males	females	mean*	males	females	mean*	
Pennsaid®									
0	24	20000	0.47	0.43	0.45	1.5	0.7	1.1	-
3	24	20000	0.47	0.38	0.42	1.7	2.3	2.0	n.s.
6	24	20000	0.57	0.44	0.50	2.2	1.3	1.8	n.s.
12	24	20000	0.50	0.40	0.45	1.3	2.4	1.9	n.s.
0	48	20000	0.60	0.60	0.60	1.9	1.3	1.6	-
12	48	20000	0.71	0.47	0.59	1.6	1.8	1.7	n.s.
Cyclophosphamide									
27 mg/kg b.w., i.p.	24	20000	0.42	0.56	0.49	10.4	7.9	9.2	s.
s.	significant at $p \leq 0.05$		PCE	polychromatic erythrocytes					
n.s.	not significant at $p \leq 0.05$		NCE	normochromatic erythrocytes					
*	males and females combined		#	per 1000 counted cells					
n	male		f	female					

Reviewer's Comment: The maximal tolerated doses for mice, based on DMSO concentrations in Pennsaid®, fall within the range of studies described in the published literature.

2.6.6.5 Carcinogenicity

PENNSAID® TOPICAL SOLUTION

No carcinogenicity studies were performed with Pennsaid® Topical Solution. It is recommended that photocarcinogenicity study be performed since this is a topical product and there are no studies of Pennsaid's® photosensitization or photocarcinogenicity potential.

DICLOFENAC SODIUM

One carcinogenic study in rat was submitted to the original NDA 19-201 in 1983. A second species study was suggested as a Phase IV commitment in Pharmacology Toxicology review of stamped Sept 20, 1985, because a new guidance ICH-S1A, finalized in March 1996. A second animal species study, in mice, was submitted in March 1989.

The Sponsor cited the carcinogenicity information of the Voltaren label to support the safety of Pennsaid®.

A 2-year carcinogenicity study in rats given dietary diclofenac sodium up to 2 mg/kg/day (or 12 mg/m²/day, approximately the human dose) did not significantly increase the incidence of tumors. There was a slight increase in benign mammary fibroadenomas in mid-dose-treated (0.5 mg/kg/day or 3 mg/m²/day) female rats (high-dose females had excessive mortality), but the increase was not significant. There was a positive dose-related trend with respect to adrenal medullary hyperplasia, mammary fibroadenomas and subcutaneous tissue fibromas in females, as well as with C-cell adenomas of the thyroid in males. The differences in the incidence between the various groups, including control, were small, and were considered to reflect the variation in the spontaneous occurrence of these incidental lesions, common in old laboratory rats.

In a 2-year mouse study, only controls and animals at the two lower daily doses of 0.1 and 0.3 mg/kg (0.9 mg/m²/day) showed survival sufficient for assessment of carcinogenic potential. The two higher daily doses of 1 (≈3 mg/m²/day) and 2 mg/kg resulted in a shortening of lifespan, particularly in males, as a consequence of ulceration and/or perforation of the small intestine and therefore prevented evaluation. The known susceptibility of rodents to non-steroidal anti-inflammatory drugs, resulting in high mortality at dose levels close to the therapeutic dose, is considered to be a rodent-specific effect. Diclofenac sodium at doses up to 0.3 mg/kg/day (0.9 mg/m²/day) in males and 1 mg/kg/day (3 mg/m²/day) in females did not reveal any oncogenic potential.

DMSO

A large number of carcinogenicity studies have been performed with DMSO, however most of these studies were of insufficient duration and sometimes dose to satisfy current drug approval in the United States.

In 1991, RTECS (1991) stated that DMSO was an equivocal tumorigenic agent by their criteria based on a review of the literature.

On May 9, 2002, the Sponsor provided a safety update on the genotoxicity and carcinogenicity of DMSO. This update is a review of literature and evaluation of the reports in literature by

_____ whose curriculum vitae was provided as evidence of his expertise. _____

_____ concluded that

“although none of these studies have been performed according to the currently accepted 2-year rat and mouse carcinogenicity study, the combined weight of the animal studies, in conjunction with the report of DMSO’s lack of mutagenicity, and the finding that it does not induce pre-neoplastic transformation in vitro, is sufficient to support the presumption that DMSO would not be carcinogenic in rodents. Overall, the mutagenicity, cell transformation, and carcinogenicity data are consistent, and support a determination that DMSO should be classified as not mutagenic or carcinogenic.”

b(4)

Related studies cited by the Sponsor to support the lack of carcinogenic potential of Pennsaid® include the following:

Vogin et al (1970), treated normal, uninitiated Rhesus monkeys with pharmaceutical grade DMSO (1, 3 or 9 mL/kg), either topically or orally, 7 days/week for 74-87 weeks (up to 18 months). Gross and histopathology of the entire body and all organs at the end of the experiment showed no carcinogenic or preneoplastic effects of DMSO at the sites of administration or at other sites. These results indicate that long term topical application or oral consumption of large doses of DMSO does not pose an increased risk for cancer.

Using DMSO as a control in models of chemical carcinogenesis of hamster oral pouch mucosa, Elzay (1967) observed no tumors in the oral pouches of Syrian hamsters treated with DMSO (100%) topically three times per week for 11 weeks and observed for 12 more weeks. Similarly, Shklar et al (1969) observed no tumors in the oral pouch of Syrian hamsters treated topically with DMSO (100%) for 12 weeks. In both models, tumors were observed only in groups treated with a chemical carcinogen and not in groups treated with DMSO alone suggesting that DMSO does not act as a carcinogen.

Van Duuren et al (1967) used DMSO topically as a control to test the skin carcinogenicity of various compounds. They found that DMSO, applied topically at full strength (100%), 3 times per week for over 94 weeks to Swiss albino mice, showed no visible signs of skin toxicity and, no tumorigenic effects. In a similar model, testing another battery of carcinogenic compounds, the same study group (Van Duuren et al, 1971) also used DMSO as a control and found that DMSO (100%), injected subcutaneously into Swiss albino mice weekly for 76 weeks showed no

tumorigenic effects, while tumors were observed in a saline treated control group. Likewise, Kanisawa and Suzuki, observed no liver or kidney carcinogenic effects of DMSO (0.1ml/20g body weight) given by gastric intubation for 50 weeks.

In a study to test the potentiation of antineoplastic compounds in normal and leukaemic F344 rats, Warren et al (1975), provided DMSO (1%, 2%) in the drinking water for over 43 weeks. They observed no carcinogenic potential of DMSO in the normal rats, nor any cancer promotive effects in leukaemic rats. In addition, they found that the therapeutic effect of an antineoplastic compound was increased in tumor-bearing rats fed DMSO. In a similar model, Thuning et al (1983) have shown that DMSO (2%), given in the drinking water to F344 rats for over one year, was not toxic or carcinogenic. In the same study, DMSO (0.25 to 32%), fed in the drinking water to F344 rats bearing inguinal tumors had no tumor protective or enhancing effects by itself, but did enhance the antineoplastic potency of chemopreventive drugs dissolved in it. DMSO's enhancement of chemotherapeutic drug activity has also more recently been shown in a rat bladder cancer model using 50% DMSO (Yaman et al, 1999). These results suggest that DMSO is not carcinogenic, has no promotive effect on existing cancers and may enhance the effect of chemotherapeutic therapy.

Effect on cellular growth kinetics

The Sponsor also submitted a number of studies that support DMSO ability to protect against cancer and as a chemo-protective agent. The studies indicated that DMSO that induces cellular differentiation *in vitro* in a number of different human and animal cancer cell lines associated in some studies with a reduction in cell proliferation.

Neoplastic transformation of mammalian cells *in vitro* has been proposed as an indicator of the carcinogenic potential of substances. DMSO has been tested in a number of such test systems. At concentrations of 0.0625 - 2% in the culture medium, DMSO did not induce neoplastic transformation in Syrian hamster embryo (SHE) cells using a clonal assay (reported by Heidelberger et al., 1983). DMSO also did not induce neoplastic transformation in the pH 6.7 SHE cell transformation assay at doses up to 5000 µg/ml in either a 24-hr or 7-day exposure regimen (LeBoeuf et al. 1996).

The lack of response in the *in vivo* neoplastic transformation studies in SHE cells using the currently recommended protocols support the mutagenicity results and carcinogenicity results, and suggests that DMSO will not be carcinogenic in rodents in the standard regulatory protocol. Although no 2-year rodent studies have been performed using the currently recommended protocols, there are a large number of rodent carcinogenicity studies, and one chronic monkey study, that provide persuasive evidence for the noncarcinogenicity of DMSO. The Sponsor concluded that overall, the mutagenicity, cell transformation, and carcinogenicity data are consistent, and support a determination that DMSO should be classified as not mutagenic or carcinogenic.

2.6.6.6 Reproductive and developmental toxicology

PENNSAID® TOPICAL SOLUTION

There were no reproduction and developmental toxicological studies with Pennsaid® Topical Solution.

DICLOFENAC SODIUM

The Sponsor submitted information from the reproductive toxicology studies in support of Voltaren (NDA 19-201), which was made available through a Freedom of Information request to the FDA.

The effects of diclofenac sodium on fertility and reproduction were studied in a Segment I study in rats and rabbits. Doses of 2 and 4 mg/kg/day were given orally to male and female rats with no noticeable effect on fertility (CIBA-GEIGY Canada Ltd., 1989). Dosing was carried out during pre-mating and mating periods. At the higher dose (more than 4 mg/kg/day), prolonged gestation and dystocia were observed. Pregnant rabbits treated with oral doses of 5 or 10 mg diclofenac sodium/animal/day during the gestation period showed a dose-dependent increase in resorption rates, reduced fetus weights, and abnormal skeletons (CIBA-GEIGY Canada Ltd., 1989).

Teratogenic effects of diclofenac sodium were studied in a Segment II study in mice, rats and rabbits. Oral doses of diclofenac sodium in mice and rats at 2, 3, 10 and 20 mg/kg/day showed no teratogenic effects on fetuses. At doses higher than 4 mg/kg/day, prolonged gestation, dystocia and embryotoxicity were observed. However, at doses approaching 20 mg/kg/day, pronounced gastrointestinal effects were observed. Definite embryotoxicity was observed at the highest dose in rabbits (80 mg/m2/day) although there was no evidence to suggest teratogenicity (CIBA-GEIGY Canada Ltd., 1989). In addition, diclofenac sodium was found to impede mouse palatal fusion in vivo and in vitro due to prostaglandin synthetase inhibition (Montenegro and Palomino, 1990). Diclofenac sodium (200 µL) was injected intramuscularly into pregnant mice on Day 13.5 of gestation. Diclofenac sodium produced low frequencies of cleft palate and fusion of the palatine shelves was inhibited. Study results are presented in Tables 24 and 25.

DMSO

The Sponsor provided support for the reproductive safety of DMSO by submitting published studies. These studies were mostly concerned with the teratogenic effects of DMSO, since the published literature contains information stating the teratogenic effects of DMSO as the only reproductive toxicologic concern. The review by Willhite and Katz (1984) summarized this information.

Oral dosing of mice with 5-12 g/kg 50% DMSO on days 6-12 of gestation failed to cause malformations in the fetuses. However, i.p. administration caused malformed limbs (4%), anencephalia (2%) and celosomia (1%) in surviving fetuses. The resorption rate was not affected. Daily oral administration of 5 g/kg DMSO to both male and female rats for 4

days prior to mating and to the females throughout gestation failed to interfere with either embryonic development or fertility. An i.p. injection of 1.0 mL/kg 40% DMSO in saline in rats on day 15 of gestation was associated with 44% reduction in the weight of the ovaries and a 34% reduction in the weight of the genital tract of the female offspring at 6 months post-partum. A 9% reduction in body weight and a 3% reduction in the weight of the testes occurred in the male offspring, but there was no effect on the combined weights of the seminal vesicles and the coagulating gland at 6 months post partum.

An i.p. injection of 40% DMSO in saline at 1.0 mL/kg on day 13 of gestation in rats was not teratogenic. Offspring of rats given 8-10 g/kg of a 50% solution of DMSO on days 6-12 of gestation exhibited malformations of the abdominal wall, central nervous system, limbs, jaw and tail. The largest dose killed 4 of 14 dams.

Injection of rats with 10.25 g/kg DMSO s.c. on days 8, 9 and/or 10 of gestation failed to induce gross or skeletal anomalies in the offspring, but the number of resorptions was significantly increased. Injection of 5.5 g/kg i.p. on day 8 of gestation in the hamster induced dilated embryonic intercellular spaces, cell shrinkage and enlarged peripheral vascular channels in the cephalic and axial nonsegmental mesoderm. Administration of DMSO to pregnant hamsters at 2.5 or 5.5 g/kg i.v. or at 5.5 or 8.25 g/kg i.p. on day 8 of gestation resulted in fetal exencephaly, rib fusion, microphthalmia, limb abnormalities and cleft lip.

Administration of 5 g/kg of 50% DMSO p.o. or 4 g/kg of 50% DMSO s.c. on days 6-14 of gestation failed to alter significantly the fetal rabbit body weights or the abortion or malformation rates.

These studies consistently showed that the teratogenic effect of DMSO in mammals is species specific and varied between studies. In one study in the hamster (Ferm, 1966b), levels of 5.5 – 8.2 g/kg DMSO administered i.p. caused malformations, whereas oral administration of levels below 2.5 g/kg were not teratogenic. However, in a second study (Robens, 1969), an oral dose of 5.5 g/kg DMSO was not teratogenic. In comparison, a dose as high as 12 g/kg/day DMSO administered i.p. in the mice caused only a slight increase in malformations (Caujolle et al., 1967) and a dose of 5 g/kg orally in the rat was also not teratogenic (Räegnier and Richard, 1998). Additionally, in the rabbit, an oral dose of 5 g/kg DMSO and a dose of 4g/kg DMSO, s.c., were both not teratogenic (Caujolle et al., 1967).

Therefore, although the exposure levels of DMSO i.p. in the hamster that were teratogenic (93 to 140 times higher than the maximal topical exposure of DMSO in Pennsaid®), an oral dose of 5.5 g/kg in the hamster (93 times higher than with Pennsaid®) was not teratogenic. Furthermore, in the mice, rat and rabbit, DMSO exposure levels 68 to 203 times higher than the maximal topical exposure of DMSO in Pennsaid® were not teratogenic.

Reviewer's Comment:

The Sponsor presented evidence that DMSO is capable of producing terata, but only at very high doses given either orally or by injection. They argue that this route of administration which allowed for much greater bio-availability of DMSO, and hence more potential for teratogenic

effects, than topical applications. Thus, the topical application of DMSO should be safe. In addition, the Sponsor suggests that the teratogenic effects of DMSO are close to the lethal dose of DMSO in studies of various vertebrate classes that include amphibians, birds, and mammals. The Sponsor did not provide the lethal doses, but the RTEC (2001) data provided lethality for intraperitoneal doses of DMSO in mice and rats (table below). The teratogenic effects noted in studies described above were near or within the lethal dose range, as the Sponsor suggested.

Teratogenic and Lethal Doses of DMSO

Species	DMSO Dose producing Teratogenic Responses	DMSO Lethal Dose*
mice	IP, 5-12 g/kg of 50% DMSO.	LD ₅₀ = 2.5 g/kg, IP,
rat	IP, 5-12 g/kg of 50% DMSO.	LD ₅₀ = 8.2 g/kg, IP,
hamster	IP, 5-12 g/kg of 50% DMSO.	unknown
rabbit	topical dose of 5 g/kg for the 2 days, followed by 2.5 g/kg for 8 days	unknown

* from RTEC, 2001; Registry of Toxic Effects of Chemical Substances. National Institute for Occupational Safety and Health. Cincinnati, OH (Internet Version). Edition expires 2001; provided by Thompson MICROMEDEX, Greenwood Village, CO.

2.6.6.7 Local tolerance

There were no local tolerance studies performed by the Sponsor. The Sponsor did not provide a summary of published relevant nonclinical information. The Sponsor did provide, in an earlier submission, dermal safety studies conducted in healthy human subjects and osteoarthritis patients. There were a number of methodological concerns with these studies, including lack of compliance with Good Clinical Practice regulations. These studies were never repeated. See Appendix 3 and the Medical Review for additional information.

Numerous publications indicated that both diclofenac and DMSO, individually, have irritant properties when applied to the skin. There were no animal studies characterizing the effect of combined application. One cannot predict if it at the doses applied, the effect would be synergistic. Also it is not known if the effect attenuates with repeated application.

A toxicological study of diclofenac sodium administered to rabbit eyes was conducted for approval of the referenced drug, Voltaren (NDA 19-201). In that study, 0.1 ml of 0.3% or 0.5% diclofenac sodium in buffered aqueous solution was introduced into the conjunctival sac of the left eye of rabbits once a day for 5 consecutive days. This resulted in minimal transient conjunctival reaction. No lesions were observed.

Smith et al., 1967 found that a single dose of DMSO (25 or 60% solution, diluted in saline, (amount applied unknown) into the conjunctival sac of rabbit eyes, resulted in dose-related edema and erythema of orbital tissues within 2 hours. Application of undiluted DMSO (100%) resulted in lacrimation and drooping of the lower eyelid.

2.6.6.8 Special toxicology studies

DMSO

Ursin et al. (1995) determined the DMSO permeation rate in living human skin samples *in vitro*, and compared these values with the ^3H -water permeation rate to normalize values for comparison with other solvents and to determine skin integrity (from the magnitude of the water permeation value and DNA synthesis after solvent exposure). Skin samples were obtained from females during plastic surgery of the breast and the epidermis was separated from the dermis. Permeability measurements were determined using a 2 chamber Franz Diffusion Cell over which the skin was stretched to provide a exposed area of 0.64 cm^2 and thickness of 300 to 600 μm .

DMSO had a permeability rate of $176\text{ g/m}^2/\text{h}$ and the rate for $[\text{}^3\text{H}]$ water was $14.8\text{ g/m}^2/\text{h}$. The volume results can be expressed in units of cm^3/h (those usually associated with permeability constant) by multiplying the values in $\text{cm}^3/\text{m}^2/\text{h}$ by $10^{-4}\text{ m}^2/\text{cm}^2$ to give $\text{cm}^3/\text{cm}^2/\text{h}$ which reduces to cm/h . Based on viability and DNA synthesis data (incorporation of 5-bromo-2'-deoxyuridine), DMSO was one of the least toxic of the compounds studied (Table below).

Viability Data: Positive Cells Remaining After Two Hours of Solvent Exposure

Solvent	# Fields (N)	Average # Positive Cells per Field
No Exposure	10	31.0
Methyl ethyl ketone	2	30.0
Sulfolane	3	29.0
DMSO	5	22.4
Propylene carbonate	3	22.3
Ethanol	3	18.3
Butyl acetate	3	17.7
Octyl acetate	3	15.7
Gamma-butyrolactone	2	13.0
Toluene	3	6.3
N-methyl-2-pyrrolidone	3	3.0
Dimethyl acetamide	4	0
Dimethyl formamide	4	0
Methylene chloride	4	0

Ursin et al. (1995)

2.6.6.9 Discussion and Conclusions

Overall, reports on the toxicity of DMSO in animals indicate a lack of significant toxic manifestation in the milligram per kilogram body weight doses. The Sponsor provided published literature to support the safety of DMSO alone from a non-clinical perspective, yet the toxicological concerns regarding the use of DMSO was not resolved. There are many cases in which conflicting or inconsistent results exist in the literature. Those studies that do not support the Sponsor's assertion regarding the safety of DMSO were dismissed as having been performed with a low grade of DMSO, or with DMSO of an unknown purity, thus assumed to be contaminated. The purity of DMSO was rarely provided in the studies and it was rarely indicated that concentrations and purity were verified as would have been required if those studies were performed by the Sponsor. Therefore, this reviewer cannot adequately determine if those studies, indicative of potential safety concerns, are in fact a result of DMSO itself, or a degradant/contaminant. Conversely, it is difficult to be assured that the studies supporting the safety of DMSO lack these degradants/contaminants, or were just underdosed.

The non-clinical toxicology profile of diclofenac is typical of NSAIDs. Its main toxicity is directed toward the gastrointestinal tract, resulting in erosion of the epithelium due to its inhibitory actions on cyclooxygenase isozymes, both COX-1 and COX-2, altering prostaglandin production.

The topical route of administration for Pennsaid, is different from the referenced compound, Voltaren, taken orally. The toxicology of topically applied diclofenac was not studied by the Sponsor and there were few nonclinical publications describing its topical toxicology and safety. These studies were either of too short a duration or provided insufficient information to adequately describe potential safety issues for the proposed use of Pennsaid (see Regulatory History section in 2.6.1. Introduction and Drug History. There was insufficient study of the distribution, duration, and toxicokinetics of DMSO applied topically. There was no information about the distribution, duration and toxicokinetics of diclofenac applied with DMSO. There was no information about the supposed depot formation of DMSO and/or diclofenac within the site of application. There was no information about the effect of sunlight exposure on the drug product stability, toxicity of potentially formed products, or effectiveness of the product. Since DMSO enhanced penetration, there was no information on its ability to transfer other compounds into the body, such as environmental toxicants (insect repellants, soil contaminants), sunscreens, moisturizing lotion, cosmetics, or other topically applied compounds for which internal toxicity is not usually tested.

During drug development, two *in vitro* absorption and bioavailability studies were performed with human skin using radioactively labeled diclofenac (UCSF95SU03, UCSF96SU05; included in IND 42,773) and submitted with the initial NDA submission. Other doses of diclofenac and the concentration of its biologically active excipient, DMSO, were not studied for either efficacy safety. It was never explained why this dose and formulation were chosen.

In this NDA resubmission, the Sponsor provided 3 new genotoxicity studies. Doses were based on the drug product, Pennsaid, rather than the active compound, diclofenac. However, for the

two *in vitro* studies testing mutagenesis (bacterial assay) and clastogenicity (human lymphocyte micronucleus assay), the maximal dose of active substance, diclofenac and the biologically active excipient, dimethyl sulfoxide (DMSO), concentrations were 72-fold and 2-fold lower, respectively, than ICH guidance recommendations. No evidence of clastogenicity was observed in an *in vivo* mouse micronucleus study with doses of Pennsaid near the maximum tolerated oral dose of 12 mg/kg (approximately ——— diclofenac and ——— DMSO).

b(4)

However, a few published studies reported positive findings for DMSO in bacterial reverse mutation and chromosomal aberration assays. DMSO (100%) was not carcinogenic when applied to hamster oral pouch, or administered cutaneously or subcutaneously to mice for 76 to 94 weeks. It was teratogenic when administered intraperitoneally to hamsters, rats and mice at 2.5 to 12 g/kg. DMSO is classified as Pregnancy Category C. DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically.

2.6.6.10 Tables and Figures (Sponsor Tables)

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Pharmacology—Primary Action Studies					
Menasse <i>et al.</i>	1978	Diclofenac sodium	Mice, rats	Subplantar, intraperitoneal, intramuscular	Diclofenac sodium inhibited prostaglandin biosynthesis <i>in vitro</i> and <i>in vivo</i>, high degree of anti-inflammatory, analgesic, and antipyretic activity
Hiramatsu <i>et al.</i>	1990	Diclofenac diethylammonium salt	Rats	Subcutaneous	Pain thresholds were significantly elevated after treatment
Kyuki <i>et al.</i>	1983	Diclofenac sodium cream	Mice, rats, guinea pigs	Topical	Diclofenac sodium cream had an anti-inflammatory effect that was superior or comparable to similar existing anti-inflammatory ointments
Allenby <i>et al.</i>	1969	DMSO	Human skin samples	N/A	DMSO did not increase the skin clearance rate but permeability of the skin barrier layer increased, causing ultrastructural modifications to stratum corneum
Pharmacology—Effects Related to Possible Adverse Reactions					
O'Brien	1986	Diclofenac	40,000 clinical trial subjects and 85,000 patients from clinical reports	N/A	- No unusual adverse reactions with diclofenac - Appeared to be a safe, effective, and well-tolerated NSAID, especially in the elderly, who have the highest risk for adverse reactions
Vogin <i>et al.</i>	1970	Daily dose of 1, 3, or 9 ml/kg body weight of 90% (v/v) aqueous DMSO	Rhesus monkeys	Oral, dermal	Local effects observed were scaling and flaking of the skin at the site of drug application in dermally treated animals

Table 1: Summary of Nonclinical Studies

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Acute Toxicity Studies					
CIBA-GEIGY Canada Ltd.	1989	Diclofenac sodium	Mice, rats, guinea pigs, rabbits	Oral, intravenous	LD₅₀ values were well within the 95% confidence limits
Menassé <i>et al.</i>	1978	Diclofenac	Mice, rats	Oral	Gastrointestinal tolerability was better than other highly effective nonsteroidal anti-inflammatory drugs (NSAIDS)
Willson <i>et al.</i>	1965	2.5, 5.0, and 10 g/kg doses of undiluted DMSO intravenously; 10, 20, and 40 g/kg doses of undiluted DMSO orally	Mice, rats	Oral, intravenous	- Lethal doses by either route caused death within the first 24 hours - Generalized depression and decreased motor activity were the most common signs of toxicity
Caujolle <i>et al.</i>	1967	DMSO	Mice, rats	Oral, intravenous, intraperitoneal, subcutaneous	- Rapid speed of diffusion through the tissues - No characteristic lesions through examination of tissues of dying mice or rats - DMSO (50%) appeared to cause immediate but slight toxic effect - Most significant symptoms observed were Straub's reaction and hypothermia in the mouse and profuse lacrimal secretions in the rat when doses nearing sublethal level during parenteral injection
Fishman <i>et al.</i> New literature reference p. 74	1969	Rats: DMSO concentrations of approx. 2900 mg/m ³ for 24 hrs, 2000 mg/m ³ for 40 hrs, 1600 mg/m ³ for 4 hrs, 200 mg/m ³ for 7 hrs, Mice: 2.5-4.5 g/kg DMSO	Mice, rats	Oral, intravenous, inhalation	- The oral LD₅₀ of DMSO in rats was found to be 14.5 g/kg with 95% confidence limits of 13.4 to 15.7 g/kg - The intravenous LD₅₀ in Swiss mice was 3.1 g/kg with 95% confidence limits of 2.7 to 3.5 g/kg - The LD₅₀ after acute inhalation in rats was >1600 mg/m³

Table 1: Summary of Nonclinical Studies

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Rosenkrantz <i>et al.</i> New literature reference p. 17	1963	Mice: 2.01–7.98 g/kg undiluted DMSO (iv) and 10.0–31.7 g/kg undiluted DMSO (sc); Rats: 2.53–7.98 g/kg undiluted DMSO (iv) and 10.0–31.7 g/kg undiluted DMSO (sc)	Mice, rats	Intravenous (iv), subcutaneous (sc)	- The LD₅₀ of DMSO by the intravenous route was 3.8 g/kg in mice and 5.25 g/kg in rats - The LD₅₀ of DMSO by the subcutaneous route was 20.5 g/kg in mice and rats
Rosenkrantz <i>et al.</i> New literature reference p. 18	1963	Rabbits: 0.1 or 1.0 mL undiluted DMSO (im); Guinea pigs: 0.1 or 1.0 mL undiluted DMSO (sc)	Rabbits, guinea pigs	Intramuscular (im), subcutaneous (sc)	Gross and micropathologic changes after a single intramuscular or subcutaneous injection of undiluted DMSO were relatively mild
Bennett <i>et al.</i> New literature reference p. 44	1983	DMSO 40% DMSO, 3 daily injections	Rat	Intravenous	No change from baseline in the blood urea nitrogen, serum creatinine, or creatinine clearance observed after 3 days of DMSO infusions
Horváth <i>et al.</i> New literature reference p. 1459	2002	Acute: Single dose of 2.0 g/kg DMSO ₂ Subchronic: 1.5 g/kg DMSO ₂ for 90 days	Rat	Oral	A single gavage dose of 2 g/kg was well-tolerated by both male and female rats; resulted in no adverse events or mortality; thus LD₅₀ was >2 g/kg
NTIS New literature reference p. 6 of 13	1991	DMSO ₂	Rat, rabbit	Oral, topical	The LD₅₀ was found to be > 5000 mg/kg
Koptyaev New literature reference p. 317	1967	Single administration of dose ranging from 900 to 7200 mg/kg DMS	Rat, mouse	Oral gavage	The LD₅₀ was 3700 mg/kg for mouse and 3300 mg/kg for rat

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Marhold New literature reference p. 2	1972	DMS	Rat	Oral	The LD₅₀ was reported to be 535 mg/kg
Ashwood-Smith New literature reference p. 43	1961	2250, 4500, 8100 mg/kg DMSO; 5000 mg/kg DMS	Female R Mice (a cross between PCT female and C3HX 101 male)	Intraperitoneal	- An increase in dose of DMSO above 4500 mg/kg did not result in greater x-ray protection - The LD₅₀ was 8000 mg/kg after an intraperitoneal dose of 5000 mg/kg
Levenstein New literature reference p. 365	1975	DMS	Rabbit	Topical	The LD₅₀ was found to be > 5000 mg/kg
Sub-chronic/Chronic/Carcinogenicity and Related Toxicity Studies					
CIBA-GEIGY Canada Ltd.	1989	Diclofenac sodium	Rats, mice, dogs, rhesus monkeys, baboons	Oral	- No increase in tumour incidence was observed in the diclofenac-treated groups as compared to the control group - No intestinal lesions were present - In a 2-year study in mice, diclofenac sodium was not carcinogenic
Kanisawa and Suzuki	1978	0.1 mL DMSO/20g body weight; 50 weeks	Male ddY mice	Gastric intubation	- No metastasis was detected in mice given DMSO - Co-administration of DMSO with ochratoxin A did not affect levels or patterns of ochratoxin tumours
Van Duuren <i>et al.</i>	1971	0.05 mL 100% DMSO; 3 times per week for 76 weeks	Female ICR/Ha Swiss mice	Subcutaneous	DMSO showed no signs of local tumors
Iverson <i>et al.</i>	1981	5 administrations; 0.2 mL solution of MCA in DMSO, at one week intervals for 75 weeks	Male and female hr/hr Oslo strain mice	Topical	50% DMSO has a moderate, but significant inhibitory effect on MCA-induced skin carcinogenesis

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Jacoby and Weiss	1986	Single-stage study: benzo(a)pyrene in 40 μ L of DMSO, twice each week for 20 weeks	Inbred C3H male mice induced by DMBA and benzo(a)pyr- ene, and by the promoter, phorbol-12- myristate- 13-acetate (PMA)	Topical	Higher numbers of tumours were induced when benzo(a)pyrene was applied in DMSO than in acetone
Jacoby and Weiss	1986	Two-stage study: DMBA was applied in toluene and PMA was applied in 40 μ L of DMSO, biweekly for 15 weeks	Outbred CD-1 male mice	Topical	- DMSO inhibited DMBA- induced tumours - When DMSO was applied before PMA at a site remote from PMA and DMBA administration, the number of tumours doubled
Slaga and Fischer	1983	1 μ g TPA in 0.2 mL acetone or DMSO	Sencar mice	Topical	The tumour promoting activity of promoter phorbol acetate was reduced > 99% when administered in DMSO, rather than in acetone
Stenback and Garcia	1975	Administration of 0.02 mL DMSO to mice biweekly for 50 weeks	Female Swiss mice	Topical	No deaths or tumours were induced by DMSO treatment
Van Duuren <i>et</i> <i>al.</i> New literature reference p. 523	1966	0.1 mL DMSO applied three times weekly to the backs of mice for 52 weeks	Female ICR/Ha Swiss mice	Topical	No tumours were induced

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Van Duuren <i>et al.</i>	1967	100% DMSO applied 3 times per week for > 94 weeks	Female ICR/Ha Swiss mice	Topical	- DMSO produced no sign of skin toxicity or tumorigenicity - DMSO had no tumour modulating activity when applied with the carcinogen dimethylbenzanthracene
Hozumi New literature reference p. 162	1969	Experiment I: 0.25 mL DMSO (0.25%) painted on the shaved dorsal skin 3 times a week for 2 weeks, followed by once a week for 18 weeks; Experiment II: 0.25 mL DMSO (0.25%) painted on the shaved dorsal skin 3 times a week for 18 weeks	Female ICR Swiss mice	Topical	- Experiment I: one mouse developed a papilloma on the 127th day - Experiment II: no tumour was observed
Bingham and Falk New literature reference p. 174	1970	50 mg DMSO applied to the skin 3 times per week for the lifetime of the animal or until a tumour appeared	Male C3H mice	Topical	No tumour was developed
Stoll <i>et al.</i>	2001	12.5 µg/mL TPA in 99.8% anhydrous DMSO applied 3 days/week for up to 26 weeks	Male & female FVB/N Tg.AC transgenic mice	Topical	- Use of DMSO as the solvent reduced the number of papillomas per affected animals (by 50 to 66% in male and female Tg.AC mice) to TPA - Solvents comprised of mixtures of DMSO and acetone also slowed the onset and reduced the magnitude of response of affected Tg.AC mice to TPA; more pronounced in females than in males

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Smith <i>et al.</i> New literature reference p. 96	2003	Incremental doses of TPA in DMSO and DMSO only (vehicle control), applied 3 times a week for 12 weeks	Male and female TPA-treated hemizygous Tg.AC transgenic mice	Topical	At low doses of TPA (< 10 µg per dose) the vehicular effects of DMSO resulted in decreased incidences and multiplicities, and increased latency periods for the appearance of papillomas
McCabe <i>et al.</i>	1986	5% DMSO	Sprague Dawley rats	Oral	Strong chemoprotective effects of DMSO on benign and malignant tumours with no evidence of DMSO toxicity
Habs <i>et al.</i>	1978	Single dose, 2 mL 10% DMSO (purity >99.7%)	Male And female BD- VI rats	Oral gavage	A single gavage dose of 2 mL of 10% DMSO did not induce stomach, or other tumours in the rat
Noel <i>et al.</i>	1975	50% DMSO	Rat	Oral gavage	- Mortalities were few and could not be related to DMSO treatment - No changes in the retina or vitreous
Fletcher and Dennis	1967	50 ppm DMSO; 18 months	Sprague Dawley rat initiated with DMBA	Drinking water	- DMSO showed no pro-carcinogenic activity - DMSO+DMBA-treated animals had fewer tumours than animals receiving DMBA without DMSO
O'Dwyer <i>et al.</i>	1988	5% DMSO; up to 9 months	Male Sprague Dawley rat; initiated with DMH	Drinking water	- DMSO decreased the number of poorly differentiated tumours versus saline control animals - DMSO did not alter the time to tumour, or tumour incidence
Warren <i>et al.</i>	1975	1% and 2% DMSO ingested daily for > 300 days	Fischer 344 rats	Drinking water	- No gross evidence of toxicity in DMSO-treated healthy animals - Therapeutic effect of antineoplastic compounds was increased in DMSO-treated tumour-bearing rats

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Thuning <i>et al.</i>	1983	2% DMSO ingested for > a year	Fischer 344 rats	Drinking water	• Ingestion of 2% DMSO for >1yr was well-tolerated • DMSO did not significantly increase the oral toxicity of a series of antitumour drugs
Malejka- Giganti <i>et al.</i>	1999	Single dose of 50 μ L DMSO	30-day old and 50-day old female Sprague Dawley rats	Intramammary injection	• Animals were followed for up to 82 weeks after injection • There was a low-level induction of tumours in the mammary glands of DMSO-injected animals
Willson <i>et al.</i>	1965	DMSO	Rats (ip), dogs (iv)	Intravenous (iv), Intraperitoneal (ip)	• The body weight of rats receiving 8 g/kg/day (highest dose) intraperitoneally was markedly affected • Varying degrees of restlessness and irritability occurred following all intraperitoneal injections of DMSO, followed by a period of general depression and weakness at all but the lowest dosage level (0.5 g/kg/day) • No deaths occurred during or following intravenous injections at doses of 1.2 g/kg or less • Many of the dogs showed signs of discomfort during injection at all dose levels, most noticeable when undiluted DMSO was used; signs of discomfort were minimal when diluted DMSO was used

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Rosenkrantz <i>et al.</i> New literature reference p. 18	1963	Rats: 0-5.0 g/kg/day DMSO for 30 days and 10 g/kg/day DMSO for 15 days (SC); 2.5 g/kg/day DMSO for 15 days (IV); Dogs: 0.4 g/kg/day DMSO for 33 days (IV)	Rats (sc and iv), Dogs (iv)	Subcutaneous (sc), Intravenous (iv)	Rats: - Systemic toxicity in rats with subcutaneous injection of 10 g/kg/day DMSO or with intravenous injection of 2.5 g/kg/day DMSO for 2 weeks was not possible due to local necrotic changes that prevented the continuation of treatment for longer periods - At lower subcutaneous dose levels, despite some local necrotic changes, there was no systemically toxic effects; histopathologic changes were relatively minor Dogs: - There were no significant hematologic or biochemical changes - Gross changes observed at autopsy and histopathologic changes were minor and incidental
Norpoth <i>et al.</i> New literature reference p. 127	1986	0.25 mL DMSO applied once/week for 2 years	Male and female Sprague- Dawley rats	Subcutaneous	- There was no significant difference in the tumours identified between the DMSO-treated and untreated control animals - There was no statistical difference between the median survival time of the untreated and DMSO-treated male and female rats
Norpoth <i>et al.</i> New literature reference p. 160	1988	0.25 mL DMSO applied once/week for 2 years	Male and female Sprague- Dawley rats	Subcutaneous	Histological studies on both malignant and benign tissue changes were negative (only organs and tissues with macroscopic disorders were examined)

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Sander <i>et al.</i>	1974	0.5 mL DMSO	SIV-50 rats	Subcutaneous	• There was no induction of local or systemic tumours
Fishman <i>et al.</i> New literature reference p. 74	1969	200 mg/m ³ DMSO	Male Sprague Dawley rats	Inhalation	• A NOEL was established at 2000 mg/m ³
Stenback New literature reference p. 63	1970	0.02 mL DMSO	Sprague Dawley rats	Topical	• No neoplastic or preneoplastic changes were detected in rats treated with DMSO alone
Purchase and Van der Watt New literature reference p. 280	1973	0.1 mL DMSO applied biweekly for 70 weeks	Male Wistar rats	Topical	• No tumour was induced in the DMSO-treated and untreated control animals
Quarles <i>et al.</i> New literature reference p. 4528	1979	0.2 ml/100 g body weight DMSO was administered on Day 10 of gestation; fetal cell cultures prepared on Day 13 of gestation	Hamster	Intraperitoneal	• There was no indication of transformation induced by DMSO in a host-mediated assay
Elzay	1967	100% DMSO applied 3 times per week for 11 weeks; observed for 12 more weeks	Syrian golden hamster	Topical (cheek pouch)	• DMSO alone did not affect animal mortality and did not induce tumours
Dachi <i>et al.</i> New literature reference p. 1184	1967	DMSO applied 3 times a week for 52 weeks	Male golden Syrian hamsters	Topical (cheek pouch)	• None of the animals which received DMSO alone developed tumours
Shklar <i>et al.</i>	1969	100% DMSO applied 3 times per week for 12 weeks	Syrian hamsters	Topical (buccal pouch)	• DMSO produced no alterations in the pouches of animals at either 8 or 12 weeks • In several animals at 12 weeks, there were slight degenerative changes in the connective tissues directly beneath the epithelium

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Siegel and Shklar New literature reference p. 772	1969	70% DMSO applied 3 times per week for 10 weeks	Syrian golden hamsters	Topical (buccal pouch)	- Gross observation: animals treated with DMSO presented a normal appearance after 10 weeks - Microscopic observations: the left buccal pouches of animals treated with DMSO alone were normal
Suri <i>et al</i> New literature reference p. 383	1971	100% DMSO applied 3 times a week for 21 weeks	Syrian golden hamsters	Topical (buccal pouch)	Application of DMSO did not result in the development of leukoplakia or tumours
Vogin <i>et al</i> Resubmitted p. 606	1970	Oral: 1, 3, 9 mL/kg Topical: 1, 3, 9 mL/kg 90% (v/v) aqueous DMSO applied daily for up to 87 weeks (equivalent to 0.099, 2.97 and 8.91 g/kg/day)	Rhesus monkey	Oral, topical	- Animals given the highest oral dose of DMSO could not tolerate it and died between weeks 15 and 53 of the study period (group of 6 animals, 1 died accidentally, and 5 died due to DMSO causing emphysema and atelectasis. - No toxicological or pathological changes attributable to oral administration of 1 or 3 mL/kg or topical application of 1, 3, or 9 mL/kg of DMSO were found - Pathologic changes with 9 mL/kg DMSO given orally were emphysema and atelectasis, which may have been secondary to regurgitation and/or tracheal inspiration (how about the path changes with the highest oral dose that led to deaths... what caused the deaths?)

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
de la Torre <i>et al.</i> New literature reference p. 49	1981	3 g/kg and 2 g/kg DMSO in a 40% solution with saline, given daily for 9 consecutive days	Rhesus monkeys	Intravenous	After monitoring the animals for 4 months post-treatment, there was no significant or long-lasting changes recorded in any of the parameters studied (including blood chemistry, hematology, urine, ocular, neurological, and cardiovascular systems)
McCann <i>et al.</i>	1975	DMSO	Bacterial species	N/A	DMSO was found to be non-carcinogenic when subjected to the <i>Salmonella</i>/microsome test
Heidelberger <i>et al.</i>	1983	0.0625 – 2% DMSO	Syrian hamster embryo (SHE) cells	Cell culture	At concentrations of 0.0625 – 2% in the culture medium, DMSO did not induce neoplastic transformation in SHE cells using a clonal assay
LeBoeuf <i>et al.</i>	1996	DMSO (99.5% purity)	Syrian hamster embryo (SHE) cells	Cell culture	DMSO did not induce neoplastic transformation in the SHE cell transformation assay at DMSO doses up to 5000 µg/mL in both 24-hr and 7-day exposure regimens
Horváth <i>et al.</i> New literature reference p. 1459	2002	DMSO ₂	Rat	Oral gavage	- No reported deaths, clinical signs of toxicity, changes in body weight, or food consumption - No significant effects on biochemical or haematological parameters were reported, and there were no changes in organs weights - No gross lesions were identified following necropsy.

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Morton and Siegel New literature reference p. 227	1986	DMSO ₂ (3% in drinking water)	MRL/Ipr, C3H/Ipr, or male BXSB mice	Oral (drinking water)	No clinical signs of toxicity
Wood <i>et al.</i> New literature reference p. 530	1971b	DMSO ₂	Rabbit	Oral (drinking water)	No significant changes observed in organ weights or following histological examination of the kidney, lung, liver, spleen, heart, adrenal
Butterworth <i>et al.</i> New literature reference p. 15	1975	0 (control), 2.5, 25 or 250 mg DMS/kg/day for 14 weeks	15 male and 15 female Rats	Oral gavage	- No deaths, clinical signs of toxicity, changes in food consumption, or body weights - No changes in haematological parameters were reported - Transient changes in organ weights were reported, however were not supported following histological examination (which included brain, pituitary, thyroid, heart, liver, stomach, small intestine, caecum, spleen, kidneys, adrenals and gonads, salivary gland, trachea, oesophagus, colon, rectum, lymph nodes, lung, aorta, pancreas, urinary bladder, uterus, and skeletal muscle) - Histological findings were not significantly different in severity or frequency between treatment and control animals

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Koptyaev New literature reference p. 317	1967	Medium term toxicity experiments using 300 and 600 mg/kg DMS administered daily for 70 days	32 Albino Rats	Oral gavage	- Lung liver and adrenal weights were reportedly increased (dose group not specified) - No significant changes in blood counts, blood cholinesterase and catalase activity, haemoglobin, vascular permeability, or oxygen consumption were reported
Koptyaev New literature reference p. 317	1967	Long-term toxicity experiment using 0.0015, 0.015, 0.6, 15 mg/kg DMS for 7.5 months	25 Albino Rats 18 Rabbits	Oral gavage	- DMS possesses a medium degree of toxicity without marked cumulative properties - No functional disturbances were elicited in the course of long term toxicological experiment on animals using DMS in doses of 0.6 and 0.015 mg/kg
Wood <i>et al.</i> New literature reference p. 530	1971b	DMS	Rabbit	Oral (drinking water)	- Increased lung weights and pulmonary congestion (with some blood spots) were reported in the lung, and pyelonephritis was reported in the kidney following histological examination - No significant changes observed in organ weights or following histological examination of the liver, spleen, heart, adrenal
Koptyaev New literature reference p. 317	1967	Long-term toxicity experiment using 0.0015, 0.015, 0.6, 15 mg/kg DMS for 7.5 months	18 Rabbits 25 Albino Rats	Oral gavage	- Some decrease in the rate of weight gain amongst rabbits treated with 15 mg/kg, and decreased blood cholinesterase and catalase activity - No significant changes between the test animals and control in blood counts, haemoglobin, vascular permeability, or oxygen consumption

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Special Toxicity Studies					
Jobin and Gagnon New literature reference p. 480	1971	Diclofenac Sodium	N/A	N/A	• Diclofenac sodium was a potent inhibitor of collagen-induced aggregation and it partially inhibited secondary aggregation by ADP
Ursini <i>et al.</i> New literature reference p. 651	1995	DMSO	Living Human Skin Samples	N/A	• Based on the ability of skin cells to undergo DNA synthesis, one of the solvents judged to be the least toxic was DMSO
Brown <i>et al.</i> New literature reference p. 691	1963	Undiluted DMSO dropped into conjunctival sac of the right eye	Rabbits	Ocular irrigation	• No ill effects were noted in any of the treated eyes
Conquet <i>et al.</i> New literature reference p. 132	1977	1/10 ml undiluted DMSO in both eyes	Male and Female Albino Rabbits	Ocular irritation	• Undiluted DMSO had no effect on corneal thickness 2 hrs, 3, 7 and 11 days following instillation • Based on the Draize procedure, 24 hrs after instillation, the treated eyes were not significantly different than the control
Brobyn	1975	Humans: 80% DMSO gel at 1g/kg/day for 14 days and 90 days Animals: 5 g/kg/day for 3 months	Humans, rabbits, dogs and swine	Not specified	• Three months application of 5 g/kg DMSO in these animals resulted in a change in the refractive index of the lens • No significant changes in humans for the 14 day study; no changes suggestive of an abnormal refractive index characteristic of changes observed in animals • 1.0 g/kg doses of DMSO daily for as long as 90 days produced no significant toxicity in humans

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Rubin and Barnett New literature reference p. 333	1967	0, 2.5, 5, 10, 20, and 40 gm/kg/day DMSO, five days a week	Beagle dogs	Gastric intubation	- Changes in the lens were seen in 4 dogs during the 9th week - Histologic examination failed to reveal any anatomic changes
	1967 p. 339	DMSO	Rabbits, dogs (Pembroke hire Corgis) and swine	Oral, dermal	- Dogs: no changes observed until 68th day of administration; bilateral lens changes which became more apparent as dosing continued - Rabbits: bilateral changes in lens - Swine: bilateral lens changes; less pronounced than seen in dogs and rabbits
Kleiberger New literature reference p. 381	1967	Daily doses of 9 ml/kg DMSO in 50% aqueous solution for the first 3 days then 5 ml/kg/day in 25% aqueous solution	Beagle Dogs	Stomach probe	- After 3 days, there was a marked reduction of hyperopia - One week later, refraction of all eyes had changed in the direction of myopia
Barnett and Noel New literature reference p. 1115	1967	9 ml/kg/day and 3ml/kg/day DMSO in 50% aqueous solution 5 days a week for 6 months	Monkeys	Oral	Monkeys that received 9 g/kg/day DMSO developed lenticular changes within 14 weeks
Brobyn	1975	11 g/kg/day dermally and 5 g/kg/day orally DMSO	Monkeys	Oral, dermal	No lens changes resulted
de la Torre et al. New literature reference p. 54	1981	3 g/kg and 2 g/kg DMSO in a 40% solution with saline, given daily for 9 consecutive days	Rhesus monkeys	Intravenous	- Daily fundusoscopic examination during the testing period did not reveal any abnormalities in any of the monkeys tested - No changes in refraction or translucency of the lens and no other abnormalities were noted in any animal before, during, or 18 weeks after DMSO administration

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Rubin and Mattis New literature reference p. 83	1966	DMSO	Monkeys	Not specified	* No lens changes resulted
Rengstorff et al. New literature reference p. 308	1972	DMSO pure (12/treatment group) DMSO technical grade (1:1; Solvent:saline 4/group, and 5% Solvent in saline 12/group) DMSO given 3 times a week for 3 weeks	Guinea pigs	Cutaneous (topical application)/ subcutaneous	* Topical application: With Pure DMSO: 11 animals no effect in lens and 1 animal developed transient isolated vacuolated areas in periphery of lens that disappeared completely after month 3. 0.5% technical grade: 6 animals showed no effect in lens and 3 animals extensive vacuolated areas at six months. • Subcutaneous application in 1:1: Pure DMSO: 2 animals showed no effect and 2 animals transient vacuolae that dissapeared in 3 or 4 month of observation. 4 animals injected with technical grade DMSO showed no effect in lens • Subcutaneous (5% solvent saline) Pure DMSO: 9 animals no effect on lens, 2 animal transient extensive vacuolae (3 to 6 months) Technical grade DMSO: 8 showed animals no effect of DMSO in the lens, 4 transient vacuolated areas and 2 animals extensive vacuolated areas in pheriphery of lens.

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Edds and Kirkham New literature reference p. 1305	1967	120 ml DMSO twice daily and 4 times daily	Horses	Dermal	Dermal administration of about 0.6 g/kg/day DMSO for about 2 months caused a slight change in static refraction towards myopia.
Wood <i>et al.</i> New literature reference p. 352	1967	Direct application: 10, 15, 30, 100% DMSO applied three times/day for 6 months; Oral: 5% DMSO; Topical: 100% DMSO	Rabbits	Direct application/ Oral/ Topical	- Direct application: no difference between treated and control eyes of the same animal; temporary lacrimation in the eye receiving 100% DMSO; no evidence of change in the iris, cornea, lens, retina, conjunctiva and lids - Oral: presence of well-defined lines of discontinuity in the lens within 1-2 weeks following daily DMSO ingestion; after 2-3 weeks of DMSO ingestion, 1-3 concentric reflex bands developed in the lens; after 4-5 weeks, lens nucleus appeared to be more dense - Topical: appearance of a zone of discontinuity which was more cortically oriented
Wood <i>et al.</i> New literature reference p. 520	1971a	1 or 5 g/kg/day DMSO for 30 days	Rabbits	Intraperitoneal/ Dermal	Rabbits treated with 1 g/kg/day DMSO failed to demonstrate lenticular biomicroscopic changes; rabbits treated with 5 g/kg/day DMSO developed lens changes
Wood <i>et al.</i> New literature reference p. 530	1971b	10 g/kg/day DMSO for 13 weeks	Rabbits	Oral	DMSO-treated animals developed progression towards decreasing hyperopia

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Wood <i>et al.</i> New literature reference p. 530	1971b	5 g/kg/day DMSO ₂ for 13 weeks	Rabbits	Oral	No significant effects were reported in any of the rabbits administered with DMSO₂ when compared to control rabbits administered with drinking water alone
Wood <i>et al.</i> New literature reference p. 530	1971b	2 g/kg/day DMS for 13 weeks	Rabbits	Oral	No lenticular changes were reported following examination of the eyes of DMS-treated rabbits
Reproduction Studies					
CIBA-GEIGY Canada Ltd.	1989	Rats: 2 and 4 mg/kg/day Diclofenac Sodium Mice and Rats: 2, 3, 10, and 20 mg/kg/day Diclofenac Sodium Rabbits: 5 or 10 mg/animal/day Diclofenac Sodium	Rat, mice, rabbit	Oral	- Doses of diclofenac sodium up to 4mg/kg/day during pre-mating, mating, gestation and lactation in rats showed no noticeable effect on fertility, but at higher doses prolonged gestation, dystocia and embryotoxicity were also observed - Doses up to 20mg/kg/day showed no teratogenic effect in mice and rat fetuses; at higher doses, pronounced gastrointestinal effects were observed - Pregnant rabbits treated with 5 or 10 mg/kg/day diclofenac sodium throughout gestation showed dose-dependent increase in resorption rate, reduced fetus weight and abnormal skeletons
Montenegro and Palomino	1990	Diclofenac sodium	Mice of AKR strain	Intramuscular (<i>in vivo</i>) and cell culture (<i>in vitro</i>)	<i>In vivo</i>, diclofenac sodium produced low frequencies of cleft palate <i>In vitro</i>, fusion of the palatine shelves was inhibited by diclofenac sodium

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Caujolle <i>et al.</i>	1967	DMSO	Chick, mouse, rat, rabbit	Oral/ intraperitoneal/ subcutaneous/ cell culture	- Mammalian species showed infrequent malformations, which occurred when high doses were repeatedly given - DMSO produced varying abnormalities in chick embryos depending on dose and development exposure stage
Ferm	1966a	Single dose of 0.5 ml of 100 % DMSO	Golden Hamster	Intravenous, intraperitoneal	No embryocidal or teratogenic effect was noted until levels of 2g/kg were reached. At higher levels, malformations including rib fusions, microphthalmia, limb abnormalities, cleft lip and especially exencephaly were found
Robens	1969	5 ml/kg and 10 ml/kg DMSO	Hamster	Oral	Doses of DMSO above 10mL/kg, demonstrated specific malformations including exencephaly, cranial pimple, short or curved tail, shortened mandible, fused ribs, cleft palate and spina bifida
Overman New literature reference p. 42A	1979	DMSO	Hamster	Topical	Treatment with DMSO resulted in delayed palate closure in embryos
Schmitt New literature reference p. 95	1988	20 second external application of 0.04%, 0.4%, 4.0% DMSO	Mice	Cutaneous	There was a dose-related increase in abnormal embryos; the litter size decreased significantly in the DMSO-treated mice compared to the sterile distilled water-treated controls

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Gray and Kavlock New literature reference p. 414	1984	DMSO	Female mice (CD-1)	Subcutaneous	DMSO treatment did not lead to significantly different responses from the control animals in the Chernoff-Kavlock assay
Juma and Staples	1967	90% aqueous solution of DMSO at 10.25 g/kg/day	Sprague- Dawley female rats	Subcutaneous	- DMSO did not significantly affect maternal survival, weight gain, or fetal weight - There was a significant decrease in live fetuses per litter and a significant increase in the number of resorptions in the animals receiving 3-day administration of DMSO - No skeletal alterations were seen in any dosages
Wickramaratne New literature reference p. 80	1987	10 ml/kg DMSO	Rats	Oral gavage	DMSO did not affect litter size and was not teratogenic
Raegnier and Richard New literature reference p. 256	1998	0, 200, 1000 or 5000 mg/kg/d DMSO	Rats	Oral	- No abnormal clinical signs, unscheduled deaths, abortions or macroscopic findings were observed - No effects were observed on pre- and post-implantation losses, the number of live fetuses or sex ratio - No external malformations, soft tissue malformation, skeletal variations or malformations or other anomalies were noted - None of the dose levels tested was teratogenic

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Stula and Krauss New literature reference p. 52	1977	5.0 ml DMSO	Rats	Topical	DMSO caused slight maternal mortality and had no effect on embryo mortality or teratogenicity
Courtney <i>et al.</i> New literature reference p. 865	1970	DMSO	Mice, rats	Oral/ subcutaneous injection	There was no increase in the incidence and type of anomalies observed in the animals treated with DMSO alone compared with non-treated group
Little <i>et al.</i> New literature reference p. 11	1995	Injection volume of 0.1 ml DMSO	Mice	Subcutaneous	Compared to an untreated control group, DMSO-treated group did not show any effect on litter size, fetal birth weights, sex ratio, or anogenital distances in males and females.
Dix <i>et al.</i> New literature reference p. 58	1977	8 ml DMSO/kg/day	Mice	Oral intubation	- There were no significant differences between the DMSO-treated and untreated control groups with respect to fetal survival, although DMSO fetuses were significantly lighter - Fetuses from the DMSO-treated group had a higher incidence of delayed ossification and skeletal abnormalities - There was no significant increase in extra ribs for the DMSO-control group as compared to the untreated control group
Kavlock <i>et al.</i> New literature reference p. 343	1979	0.1 ml DMSO/day (approximately 3.3 g/kg/day)	Mice	Oral intubation	DMSO did not affect any parameter in maternal or fetal health when compared to sham-intubated controls

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Iyer <i>et al.</i> New literature reference p. 372	1993	3.2 g/kg DMSO	Mice	Oral gavage	- The number of live fetuses, dead fetuses, resorptions and the sex ratio in the live fetuses were similar in the DMSO-treated and untreated groups - Maternal body weights were significantly lowered in the DMSO-treated group compared to the untreated control - No significant differences in major malformations were seen between the DMSO-treated and untreated groups
Gill <i>et al.</i> New literature reference p. 2143	1981	0.7 ml DMSO	Hamster	Intraperitoneal	DMSO alone produced a congenital malformation rate of 13.8% compared to no observed congenital malformations when sorbitol or carboxymethylcellulose was injected alone
Nomura <i>et al.</i> New literature reference p. 573	1984	2.5 µl/g body weight of DMSO	Mice	Intraperitoneal	DMSO-alone treated mice did not differ from untreated mice in regards to the percentage of live fetuses or fetal malformations
Sharma <i>et al.</i> New literature reference p. 228	1985	2.5, 5.0, 10.0, 20.0 mg/kg DMSO	Mice	Intraperitoneal	No DMSO effects on sister chromatid exchange (SCE) induction in femoral bone marrow of dams or livers of fetuses were observed
Pérez-Pastén <i>et al.</i> New literature reference p. 167	2006	0.1%, 0.2% and 0.5% (v/v) DMSO	CF1 mice	Cell culture	Co-treatment of mouse embryos (grown in sodium arsenite) with DMSO partially reduced neural tube defects and facial dysmophology

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Dresser <i>et al.</i>	1992	0.25%, 0.50%, 0.75%, 1.00%, 1.25%, 1.5% and 1.75% v/v DMSO	Xenopus	Cell culture	There was no observable effect limit of mortality equal to 1.75% DMSO and a teratogenic index equal to 1.20% DMSO
Mutagenicity Studies					
Kadotani <i>et al.</i>	1984	Diclofenac sodium	Bacterial Species	N/A	No evidence of mutagenicity in the Ames Reversion Test using <i>S. typhimurium</i> and <i>E. coli</i> strains
McCann <i>et al.</i>	1975	DMSO	Bacterial Species	N/A	No evidence of mutagenicity using the standard Ames <i>Salmonella</i>/microsome assay
Maron <i>et al.</i>	1981	500 µl DMSO/plate	Bacterial Species	N/A	No evidence of mutagenicity
Zeiger <i>et al.</i>	1992	DMSO	Bacterial Species	N/A	No evidence of mutagenicity
Hakura <i>et al.</i>	1993	DMSO conc. Of 14%, 25%, 29%, 33%, 37%, or 40%	Bacterial Species	N/A	DMSO is mutagenic at cytotoxic concentrations in strains TA1537 and TA 2637
Loveday <i>et al.</i>	1990	DMSO	Chinese hamster ovary (CHO) cells	Cell culture	DMSO did not induce chromosome aberrations under any test conditions
Latt <i>et al.</i>	1981	DMSO	CHO cells	Cell culture	DMSO did not induce sister-chromatid exchange in CHO cells when tested with or without metabolic activation
Armstrong <i>et al.</i>	2000	Cell co-treated 20 hrs with 0.25 or 1% DMSO	CHO cells	Cell culture	DMSO had no effects on colony forming efficiency, aberrant cell induction, DNA strand break or DNA adduct production
Tates and Kriek	1981	1 or 10% DMSO	CHO cells	Cell culture	DMSO (19%) produced chromosomal aberrations and sister chromatic exchanges when tested with metabolic activation

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Amacher <i>et al.</i> New literature reference p. 459	1980	DMSO	L5178Y mouse lymphoma cells	Cell culture	DMSO was not found to be mutagenic at the TK locus of L5178Y cells
Amacher and Zelljadt	1984	0.5% DMSO for 5 hours	Chinese hamster ovary (CHO) cells	Cell culture	DMSO did not induce mutations at the HGPRT locus of CHO cells
Casparly <i>et al.</i>	1988	DMSO	L5178Y mouse lymphoma cells	Cell culture	Addition of DMSO to the cell cultures at 1:0% with or without S9 did not lead to change in mutation frequency
Watanabe <i>et al.</i>	1990	0.128-1.28 M DMSO	Syrian golden hamster embryo cells	Cell culture	Treatment with DMSO did not induce chromosome aberrations, but did reduce radiation-induced breaks
Kinashi <i>et al.</i>	2000	10% DMSO for 15 mins	CHO cells	Cell culture	- DMSO (10%) was not mutagenic - DMSO was protective against radiation induced cell killing and DNA mutations
Yee <i>et al.</i>	1972	10% and 40% DMSO	Yeast cells	Cell culture	- Exposure to increasing concentrations of DMSO resulted in decreased cell viability, but not cell lysis or protein leakage - Inclusion of DMSO in growth medium resulted in the conversion of yeast cultures to respiratory deficient petites
Hoffmann <i>et al.</i>	1993	0.125M, 0.25M, 0.5M, 1M, 2M DMSO	Human cells	Cell culture	Treatment of human lymphocytes in culture with 0.25M and 1.0M DMSO did not affect the proliferation index or micronucleus (MN) frequency

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Laffon <i>et al.</i>	2001	50 µl of 1% DMSO	Human cells	Cell culture	• Treatment of human lymphocytes in culture with 0.13M DMSO did not induce DNA strand breaks or cause any changes in the cell proliferation rate
Kocan <i>et al.</i>	1982	0.5% DMSO	Rainbow trout gonadal cells	Cell culture	• Treatment of rainbow trout gonadal cells with DMSO for 48 hours did not produce any chromosome aberrations
Mollett	1976	1% DMSO	<i>Drosophila melanogaster</i>	Provided in feed	• DMSO did not induce any genetic alterations in larvae at the concentration tested
Traut	1983	2% DMSO	<i>Drosophila melanogaster</i>	Provided in feed	• No evidence of the ability of DMSO to induce numerical chromosome aberrations in oocytes
Vogel and Nivard	1993	1% DMSO	<i>Drosophila melanogaster</i>	Provided in feed	• No evidence of mutagenicity
Lee <i>et al.</i>	1983	DMSO	<i>Drosophila melanogaster</i>	Provided in feed	• DMSO causes no sex-linked recessive lethal gene mutations, aneuploidy or whole sex-chromosome loss in any germ cell stage or exposure level tested
Valencia <i>et al.</i>	1984	DMSO	<i>Drosophila melanogaster</i>	Provided in feed	• DMSO is non-clastogenic
Kapp and Eventoff	1980	5 ml/kg of 1%, 10%, 50%, and 100% DMSO	Rats	Intraperitoneal	• Resultant percent of aberrant cells per animal was found to increase from 10% aberrant cells at 1% DMSO to 68.7% aberrant cells at 100% DMSO
Anwar <i>et al.</i>	1989	1.25, 3.75, or 12.5% DMSO at a volume of 0.01 ml/gm b.w.	Mice	Oral gavage	• No significant chromosome breakage or micronuclei at any level of DMSO given

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
MacGregor <i>et al.</i>	1983	DMSO	Mice	Intraperitoneal	No micronucleus cells were induced in bone marrow polychromatic or normochromatic erythrocytes
McFee	1989	0.1 ml of DMSO	Mice	Intraperitoneal	Animals that received one intraperitoneal injection of 0.1 mL of 100% DMSO showed equivalent levels of chromosome aberrations and sister chromatid exchanges as animals receiving corn oil or saline
McFee <i>et al.</i>	1989	0.1 ml of DMSO	Mice	Intraperitoneal	The carcinogen, o-toluidine HCl produced micronucleus in mice when administered in saline, but no MN was produced when the same dose of carcinogen was administered in DMSO
Bali <i>et al.</i>	1990	DMSO	Mice	Intraperitoneal	DMSO did not induce any increase in micronuclei in bone marrow
Hrelia <i>et al.</i>	1990	5 g/kg DMSO	Mice	Intraperitoneal	Administration with DMSO reduced the metronidazole-induced gene conversion in yeast recovered from the liver, lungs and kidneys
MacGregor <i>et al.</i>	1983	2.5 ml of DMSO	Rabbits	Intraperitoneal	There was no induction of SCE
Hakura <i>et al.</i> New literature reference p. 127	1993	DMS/DMSO ₂	Bacterial Species	N/A	DMSO₂ and DMS were both negative in the Ames assay at a concentration of 100 µM/plate in <i>Salmonella typhimurium</i>
Nakamura <i>et al.</i> New literature reference p. 12	1990	0.2%, 0.4%, 0.6% DMS	Bacterial Species	N/A	DMS was (if sufficient data and study is credible, it is better to give your assessment in assertive terms) non-genotoxic at non-toxic doses of 0.2, 0.4 and 0.6%

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Dooley <i>et al.</i> New literature reference p. 39	1987	DMS	Mouse lymphoma cells	Cell culture	DMS was non-mutagenic in mammalian cells in the absence of metabolic activation
Leuschner Study No. 17623 New nonclinical study report	2004	Pennsaid®	Bacterial Species	N/A	No mutagenic effect was observed for Pennsaid® tested up to 5000 µg/plate in any of the five test strains in two independent experiments with and without metabolic activation
Leuschner Study No. 17625 New nonclinical study report	2005	Pennsaid®	Human lymphocytes	Cell cultures	Pennsaid® tested up to concentrations of 5000 µg/mL medium in the presence and absence of metabolic activation employing one exposure time (with S9) and two exposure times (without S9) revealed no indication of mutagenic properties with respect to chromosomal or chromatid damage
Leuschner Study No. 17626 New nonclinical study report	2005	Pennsaid®	Mouse	Oral	Pennsaid® tested up to the maximum tolerated dose of 12 mL/kg b.w. showed no mutagenic properties in the mouse bone marrow micronucleus study at sampling times of 24 hours and 48 hours
ADME Studies					
Riess <i>et al.</i>	1978	Diclofenac Sodium	Rat, dog, monkey, baboon	Oral	In addition to the kidney or liver, the highest concentration of radioactive-labelled diclofenac was recorded in the blood

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Riess <i>et al.</i>	1986	Guinea pigs: 320, 100, or 40 mg of 1% (w/w) diclofenac sodium cream applied to 10 cm ² of skin under an occlusive dressing and left in place for 6 h Rabbits: 1 mg diclofenac sodium in 100 mg cream	Rabbit, guinea pig	Topical	- Rabbit: diclofenac sodium penetrated into patellar ligament, the fatty tissues and the synovial fluid - Guinea pig: during daily administration, steady state levels in muscle tissue in the vicinity of application site were about 3 times higher than a tissue sample taken from a distant muscle
Hartway <i>et al.</i> UCSF study #95SU03	1996	Diclofenac Sodium in 3 doses: 1) 1.5% 1000 ppm diclofenac sodium and 45.5% DMSO in Pennsaid® base 2) 1.5% 1000 ppm diclofenac sodium in Pennsaid® base 3) 2.38% 1587 ppm diclofenac sodium and 45.5% DMSO in Pennsaid® base	Human Skin Samples	N/A	- Single dose <i>in vitro</i> percutaneous absorption showed that Pennsaid® delivers diclofenac at 9.53% dose for a 24 hour dosing period - Diclofenac showed continual absorption through the 24 hour dosing period with Pennsaid®, whereas the same formulation without DMSO showed decreased diclofenac delivery after 12 hours - Diclofenac is the major constituent delivered through the human skin
Hewitt <i>et al.</i> UCSF study #96SU05	1997	2 µl and 5 µl of 1.5% (1000ppm) Diclofenac Sodium	Human Skin Samples	N/A	- Multi-dose <i>in vitro</i> percutaneous absorption showed that Pennsaid® statistically increased the penetration rate of diclofenac into and through the skin, as compared to an aqueous solution of diclofenac - Pennsaid® exhibited enhanced absorption through human skin when applied multiple times and this enhanced absorption was maintained over 48 hours

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Kaye <i>et al.</i> New literature reference p. 1223	1983	15% DMSO at a dosage of 1.5 mg/g	Mice	Intravenous	- The peak plasma DMSO concentration was 3.12 ± 0.48 mg/mL; calculated body clearance of DMSO was 4.79 mL/min/kg - DMSO was rapidly and extensively distributed in all tissues; at the end of 8 hrs, there was little DMSO found in plasma and tissue
Hucker <i>et al.</i> New literature reference p. 256	1965	500 mg/kg of DMSO	Rats	Oral, intraperitoneal, topical	- Plasma radioactivity was the highest after 0.5 hrs following intraperitoneal administration with a half-life of 5-6 hrs. - Radioactivity in the urine after 22 hrs represented 60-85% of intraperitoneal and oral doses and 36-50% of the dermal dose
McDermot <i>et al.</i> New literature reference p. 475	1967	About 200 mg of undiluted DMSO	Rats	Topical	- Peak concentrations in all tissues studied were reached in 1.5-2 hrs; highest concentration was found in the kidneys and the lowest concentration of DMSO was found in the lungs - About 85% of the applied dose was excreted unchanged in the urine within 24 hrs
Kolb <i>et al.</i> New literature reference p. 35	1967	50% or 90% DMSO Rats: 1 g/kg 90% DMSO i.v or 0.1g 90% DMSO cutaneously Rabbits: 1 g/kg of 90% DMSO orally and cutaneously Dogs: 2 g of 90% DMSO i.v or 2 g of 50% DMSO orally or 1 g of 90% DMSO cutaneously	Rats; rabbits, dogs	Intraperitoneal, subcutaneous, intravenous	- Following cutaneous application, the half-life of DMSO in blood was 10 hrs in rats; DMSO was distributed in all organs studied and decreased within 2-24 hrs after application - DMSO was absorbed and distributed in all organs in the rabbits; following cutaneous application, a higher DMSO concentration is found in the cornea; on Day 1, 70% of the applied dose was excreted in the urine

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
					In beagle dogs, 80% of the applied dose penetrates the skin barrier after 4 hrs and 90% of the applied dose penetrates the skin after 24 hrs; there was a linear decline of radioactivity in the dermis over 2 weeks and on Day 14, only 0.037% of the applied dose could be found on the dermis
Hucker <i>et al.</i> New literature reference: p. 176	1966	0.55 g/kg of DMSO	Rats, rabbits, guinea pigs	Oral, intraperitoneal, topical	- High concentrations of radioactivity were detected in plasma 0.5 hr after dosing by all routes T_{max} was reached in 0.5-1 hr for oral and intraperitoneal routes and 2 hrs for the dermal route of administration - Approximately 10-15% of the administered dose of DMSO was metabolized to $DMSO_2$
Layman and Jacob New literature reference: p. 2431	1985	3 g DMSO/kg B.W. per day for 14 days	Rhesus monkeys	Oral	- An average serum concentration of 2.3 mg/mL was observed after 4 hrs, which declined to about 0.95 mg/mL after 24 hrs - After oral DMSO administration was terminated, serum DMSO declined rapidly and was not detected after 72 hrs; $DMSO_2$ became detectable in serum after 2 hrs and increased slowly to 0.18 mg/mL at 24 hrs

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Wong <i>et al.</i> New literature reference p. 34	1971	15g of DMSO 80% gel applied t.i.d.	Miniature pigs	Topical	- DMSO was totally absorbed within 4 hrs - The principal metabolite was dimethyl sulfone (DMSO₂) - Approximately 1.6% of the dose was excreted in the feces and a substantial amount of dimethyl sulfide (DMS) was detected in the expired air
Hewes <i>et al.</i> New literature reference p. 139	1975	5.0 ml, 10.0 ml, or 8.5 ml of 90% DMSO; 15.0 ml of 60% DMSO	Cows and calves	Topical and parenteral	- The lung and kidney appeared to be the principal excretory organs - Excretion via urine reached peak values within 6-12 hrs, then decreased sharply within 12-28 hrs after application - Concomitant with the decline in DMSO, there was an increase in DMSO₂ concentration in the urine
Otsuki <i>et al.</i> New literature reference p. 313	2002	5.0×10^{-3} mol day ⁻¹ kg ⁻¹ body weight of DMSO ₂ once per day for 7 days	Rat	Oral gavage	- Radioactivity was evenly distributed throughout the tissues - Approximately 3.5-10% of the radioactivity remained incorporated in the tissues after 7 days, with higher levels retained in the blood, spleen, and skin - After 7 days of dosing, 60 to 79% of the radioactivity was excreted in the urine and 5 to 18% was excreted in the feces, with average daily urinary and fecal radioactive excretion of 70 and 10%, respectively

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Williams <i>et al.</i> New literature reference p. 87	1966b	1.82×10^6 cpm DMSO ₂ for 4 days	Rabbit	Subcutaneous injection	76% of the administered radioactivity was collected as DMSO₂ in the urine over 20 days - No DMS was detected in the breath of rabbits
Layman and Jacob New literature reference p. 2431	1985	3 g DMSO/kg B.W. per day for 14 days	Rhesus monkey	Oral gavage	- DMSO₂ was detected in the blood within 2 hours of dosing and reached a steady-state concentration after 4 days - The half-life for DMSO₂ was reported to be 38 hours and 120 hours after dosing was terminated, no DMSO₂ was detected in the blood - Although DMS was not measured, the authors reported the smell of DMS on the breath of monkeys
Williams <i>et al.</i> New literature reference p. 87	1966b	DMS was diluted with an equal volume of sesame oil and 2 ml of the mixture was injected on each of 4 consecutive days.	Rabbit	Subcutaneous injection	- Approximately 8.6 and 9.8% of the administered dose of DMS was excreted as DMSO and DMSO₂, respectively, over a period of 6 days - DMS also was eliminated via the expired air, indicated by the strong characteristic garlic-like odour on the animals' breath during the experiment

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

Because the active ingredient, diclofenac, is an approved drug, and this application is a 505(b)(2), the Sponsor did not perform non-clinical studies, except 3 genetic toxicology studies. From a pharmacological and toxicological perspective, the drug product is a combination drug, consisting of both diclofenac and dimethyl sulfoxide (DMSO). Both are approved drugs, both have well documented anti-inflammatory ability, but have different mechanisms of action. The Sponsor's argued that DMSO is an excipient in their formulation, although it comprises 45.5% of the formulation. They base this claim on the use of DMSO for enabling diclofenac's penetration through the skin and its lack of efficacy in improving measures of osteoarthritis

Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Marhold New literature	1972	DMS	Rat	Oral	The LD₅₀ was reported to be 535 mg/kg
Authors/ Investigators/ Reference	Date	Treatment	Species/ Tissues	Administration Route	Results
Jacoby and Weiss	1986	Single-stage study: benzo(a)pyrene in 40 µL of DMSO, twice each week for 20 weeks	Inbred C3H male mice induced by DMBA and benzo(α)pyr- ene, and by the promoter, phorbol-12- myristate- 13-acetate (PMA)	Topical	Higher numbers of tumours were induced when benzo(a)pyrene was applied in DMSO than in acetone
Jacoby and Weiss	1986	Two-stage study: DMBA was applied in toluene and PMA was applied in 40 µL of DMSO, biweekly for 15 weeks	Outbred CD-1 male mice	Topical	- DMSO inhibited DMBA- induced tumours - When DMSO was applied before PMA at a site remote from PMA and DMBA administration, the number of tumours doubled
Slaga and Fischer	1983	1 µg TPA in 0.2 mL acetone or DMSO	Sencar mice	Topical	The tumour promoting activity of promoter phorbol acetate was reduced > 99% when administered in DMSO, rather than in acetone
Stenback and Garcia	1975	Administration of 0.02 mL DMSO to mice biweekly for 50 weeks	Female Swiss mice	Topical	No deaths or tumours were induced by DMSO treatment
Van Duuren <i>et al.</i> New literature reference p. 523	1966	0.1 mL DMSO applied three times weekly to the backs of mice for 52 weeks	Female ICR/Ha Swiss mice	Topical	No tumours were induced

symptoms during earlier clinical testing. However, this lack of DMSO efficacy was not demonstrated until the Phase 3 study (PEN-03-112). The reason for stating DMSO as an excipient, rather than an active agent, during the initial development stages was not adequately explained. This reviewer was also unable to identify if a definitive conclusion was reached in earlier discussions with the Agency concerning the nomenclature for the DMSO component. It appears from their drug development program that it was not considered a combination drug, and the emphasis was on the efficacy and safety of topical diclofenac. However, the contribution of DMSO to safety and efficacy kept reappearing and contributed substantially to non-approval of earlier NDA submissions. With this resubmission, some previous concerns have been resolved, but others, as indicated above, still exist.

The lack of clearly delineating this product as a combination drug early in the development phase had a substantial impact on the non-clinical pharmacology and toxicology program. Both diclofenac and DMSO were approved for other routes of administration and indications. By ignoring the well known, but not fully characterized by Agency standards, toxicity of DMSO and not studying their potential interaction as a combination product, there were and still are unknowns in the toxicology of this product. Rather than empirically determining potential toxicity that are essential to identify, the Sponsor has consistently cited published papers and drug monographs in support of the pharmacology and toxicological safety, especially for DMSO. The Sponsor considered DMSO an excipient, despite its comprising 45.5% of the drug product and its previous approval as a drug for treatment of interstitial cystitis and use in veterinary medicine as an anti-inflammatory. Collectively, the submitted published studies are inadequate in terms of meeting the Agency's toxicological study requirements for a new drug product. There were no preclinical dermal toxicology studies in which both diclofenac and DMSO have been administered together. Individually, the toxicity and anti-inflammatory pharmacodynamic action arising from topical application of DMSO and of diclofenac are similar in many regards. However, their mechanisms of action are dissimilar and therefore, there is a potential for toxicological consequences when applied simultaneously and frequently as in the proposed indication.

Overall, reports on the toxicity of DMSO in animals indicate a lack of significant toxic manifestation in the milligram per kilogram body weight doses. The Sponsor provided published literature to support the safety of DMSO alone from a non-clinical perspective, yet the toxicological concerns regarding the use of DMSO was not resolved. There are many cases in which conflicting or inconsistent results exist in the literature. Those studies that do not support the Sponsor's assertion regarding the safety of DMSO were dismissed as having been performed with a low grade of DMSO, or with DMSO of an unknown purity, thus assumed to be contaminated. The purity of DMSO was rarely provided in the studies and it was rarely indicated that concentrations and purity were verified as would have been required if those studies were performed by the Sponsor. Therefore, this reviewer cannot adequately determine if those studies, indicative of potential safety concerns, are in fact a result of DMSO itself, or a degradant/contaminant. Conversely, it is difficult to be assured

that the studies supporting the safety of DMSO lack these degradants/contaminants, or were just underdosed.

The non-clinical toxicology profile of diclofenac is typical of NSAIDs. Its main toxicity is directed toward the gastrointestinal tract, resulting in erosion of the epithelium due to its inhibitory actions on cyclooxygenase isozymes, both COX-1 and COX-2, altering prostaglandin production.

The topical route of administration for Pennsaid, is different from the referenced compound, Voltaren, taken orally. The toxicology of topically applied diclofenac was not studied by the Sponsor and there were few nonclinical publications describing its topical toxicology and safety. These studies were either of too short a duration or provided insufficient information to adequately describe potential safety issues for the proposed use of Pennsaid (see Regulatory History section in 2.6.1. Introduction and Drug History. There was insufficient study of the distribution, duration, and toxicokinetics of DMSO applied topically. There was no information about the distribution, duration and toxicokinetics of diclofenac applied with DMSO. There was no information about the supposed depot formation of DMSO and/or diclofenac within the site of application. There was no information about the effect of sunlight exposure on the drug product stability, toxicity of potentially formed products, or effectiveness of the product. Since DMSO enhanced penetration, there was no information on its ability to transfer other compounds into the body, such as environmental toxicants (insect repellants, soil contaminants), sunscreens, moisturizing lotion, cosmetics, or other topically applied compounds for which internal toxicity is not usually tested.

During drug development, two *in vitro* absorption and bioavailability studies were performed with human skin using radioactively labeled diclofenac (UCSF95SU03, UCSF96SU05; included in IND 42,773) and submitted with the initial NDA submission. Other doses of diclofenac and the concentration of its biologically active excipient, DMSO, were not studied for either efficacy safety. It was never explained why this dose and formulation were chosen.

In this NDA resubmission, the Sponsor provided 3 new genotoxicity studies. Doses were based on the drug product, Pennsaid, rather than the active compound, diclofenac. However, for the two *in vitro* studies testing mutagenesis (bacterial assay) and clastogenicity (human lymphocyte micronucleus assay), the maximal dose of active substance, diclofenac and the biologically active excipient, dimethyl sulfoxide (DMSO), concentrations were 72-fold and 2-fold lower, respectively, than ICH guidance recommendations. No evidence of clastogenicity was observed in an *in vivo* mouse micronucleus study with doses of Pennsaid near the maximum tolerated oral dose of 12 mg/kg (approximately 178 mg/kg diclofenac and 5.86 g/kg DMSO).

However, a few published studies reported positive findings for DMSO in bacterial reverse mutation and chromosomal aberration assays. DMSO (100%) was not

carcinogenic when applied to hamster oral pouch, or administered cutaneously or subcutaneously to mice for 76 to 94 weeks. It was teratogenic when administered intraperitoneally to hamsters, rats and mice at 2.5 to 12 g/kg. DMSO is classified as Pregnancy Category C. DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically.

Unresolved toxicology issues (if any):

There is a lack of long term safety data from animal studies, since there were no studies of the diclofenac and DMSO combination. The literature references to separate studies with diclofenac and with DMSO did not address topical toxicology and toxicokinetics except for a few cases. These were of insufficient dose or duration to be useful in predicting potential safety issues for patients.

The product information regarding the potential for dermal carcinogenicity and photocarcinogenicity is insufficient or lacking. The effect of sunlight on product stability is unknown. The marketed product will be applied 4 times per day, so it will be carried around as part of the patient's daily routine. It will be exposed to sunlight and heat. It is not known what effect this has on the product's stability and safety.

The Sponsor should determine the effects of visible and UV light spectrums on product stability and characterize the dermal and systemic toxicity of degradants if found. It is known from studies for the approved product, Solaraze™ (NDA 21-005), that exposure of diclofenac to direct sunlight results in a number of photodegradant products. In the presence of DMSO, it is unknown whether these will occur, due to the possible free radical scavenger activity of DMSO. However, DMSO is also very liable in sunlight, and its degradation products are not well documented. This has bearing on both efficacy and safety of the drug product.

The Sponsor should characterize the carcinogenic potential of their topical product by conducting dermal carcinogenicity and photocarcinogenicity studies. Carcinogenicity studies in which diclofenac was administered orally were conducted for Voltaren (diclofenac sodium, NDA 19-201, Novartis, referenced drug). However, dermal carcinogenicity studies have not been performed with Pennsaid. The Pennsaid product will also be exposed to sunlight and therefore photocarcinogenicity studies should be performed. If the Pennsaid product lacks absorption in UVA and UVB spectrums of wavelength, then it would not be necessary to perform the photocarcinogenicity studies, as per the May 2003 Guidance for Industry: Photosafety Testing.

As a first to be approved dermal NSAID in the United States, there is additional concern for widespread off-label use, especially for athletic injuries. Concerns regarding the safety of DMSO, which the Sponsors have claimed from early in development to be an excipient, but which has well documented toxicities, has been downplayed throughout the product's development and in the labeling.

Recommendations:

From a Pharmacology and Toxicology perspective this application is not approvable. The overall impression of this reviewer was that the product development program was not oriented toward the proposed indication of chronic topical use. Therefore, certain critical issues were not addressed that impacts both the safety and efficacy of the product. These include:

- The effect of sunlight (visible and UV light spectrum) on product stability is unknown.
- Information concerning dermal carcinogenicity and photocarcinogenicity is insufficient or lacking.
- The effect of skin conditioners, moisturizers, and other topical compounds on uptake of diclofenac was not addressed.

Suggested labeling:

Based on previous and current reviews the following labeling changes in red italics are recommended:

b(4)

2 Page(s) Withheld

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

 ✓ § 552(b)(4) Draft Labeling

 § 552(b)(5) Deliberative Process

Reviewer: L.S. Leshin

NDA 20-947

Reviewer Signature

L.S. Leshin, D.V.M., Ph.D

Supervisor Signature

D. Mellon, Ph.D.

Concurrence Yes ___ No ___

Appendix 1 SUPPORTING DOCUMENTATION

The following publications were submitted to IND or NDA 20,947. Information derived from some of the articles was used in this review, but they were not formally reviewed.

PHARMACOLOGY

Author (Year Published)/ Title/Citation	Year Submitted/Location
Diclofenac	
Kadar D. (1989) Anti-inflammatory analgesics In: Kalant H, Roschlau WHE. Principles of medical pharmacology. 5th Ed. Toronto: BC Decker Inc; 1989. pp. 325-326	2001 NDA, SN-000, Vol. 1.6, p. 46-49 1997 NDA, SN-000, Vol. 16, p. 12
Menassé R, Hedwall PR, Krartz J, Pericin C, Riesterer L, Sallmann A, Ziel R, Jaques R. (1978) Pharmacological properties of diclofenac sodium and its metabolites Scand. J. Rheumatol. 22 Suppl: 5-16	IND 42773, SN-007, Vol. 1, p 171, 173-177. 2001 NDA, SN-000, Vol. 1.6, p. 50-62 1997 NDA, SN-000, Vol. 16, p. 14
Hiramatsu Y, Akita S, Salamin PA, Maier R. (1990) Assessment of topical non-steroidal anti-inflammatory drugs in animal models Arzneim.-Forsch./Drug Res 40(II)10: 1117-1124.	2001 NDA, SN-000, Vol. 1.6, p. 63-71 1997 NDA, SN-000, Vol. 16, p. 26 IND 42773
Kyuki K, Shibuya T, Tsurumi K, Fujimura H. (1983) Anti-inflammatory effects of diclofenac sodium ointment (cream) in topical application Jap. J. Pharmacol. 33: 121-132.	2001 NDA, SN-000, Vol. 1.6, p. 72-84 1997 NDA, SN-000, Vol. 16, p. 34 IND 42773, SN-000, Vol. 1, p. 246 IND 42773, SN-007, Vol. 1, p 172, 173
O'Brien WM. (1986) Adverse reaction to non-steroidal anti-inflammatory drug diclofenac sodium with other non-steroidal anti-inflammatory drugs Am. J. Med. 80 Suppl 4B: 70-80	2001 NDA, SN-000, Vol. 1.6, p. 90 1997 NDA, SN-000, Vol. 16, p. 48
Szczeklik A, Gryglewski RJ, Czerniawska-Mysik G, Pieton R. (1977) Asthmatic attacks induced in aspirin-sensitive patients by diclofenac and naproxen Br. Med. J. 23: 231-232	2001 NDA, SN-000, Vol. 1.6, p. 102-104 1997 NDA, SN-000, Vol. 16, p. 59
Dimethyl Sulfoxide	
Kligman, AM. (1965) Topical pharmacology and toxicology of dimethyl sulfoxide, parts 1 and 2 JAMA 193: 796-804, 193: 923-928	IND 42773, SN-000, Vol. 1, p. 21 IND 42773, SN-007, Vol. 1, p. 183, 205 2001 NDA, SN-000, Vol. 1.6, p. 105-121 1997 NDA, SN-000, Vol. 16, p. 61
Allenby AC, Creasey NH, Edginton JAG, Fletcher JA, and Schock C. (1969) Mechanism of action of accelerants on skin penetration Br. J. Derm. 4: 47-55	2001 NDA, Vol. 1.6, p. 121-130 1997 NDA, SN-000, Vol. 16, p. 76
Vogin, E.E., Carson, S., Cannon, G. (1970) Chronic toxicity of DMSO in primates. Toxicol & Applied Pharmacol 16:606-612.	IND 42773, SN-000, Vol. 1, p 21. IND 42773, SN-007, Vol. 1, p 192, 193 2001 NDA, SN-000, Vol. 1.6, p. 131-138 1997 NDA, SN-000, Vol. 16, p. 85

PHARMACOKINETICS/TOXICOKINETICS

Author (Year Published)/ Title/Citation	Year Submitted/Location
Diclofenac	
Reiss W, Stierlin H, Degen P, Faigle JW, Gerardin A, Moppert J, et al. (1978) Pharmacokinetics and metabolism of anti-inflammatory agent Voltaren Scand. J. Rheumatol. 22: 17-29.	2001 NDA, SN-000, Vol. 1.6, p. 141-154 1997: SN-000, Vol. 16, p. 382 IND 42773, SN-007, Vol. 1, p. 178-9, 180, 183
Riess W, Schmid K, Botta L, Kobayashi K, Moppert J, Schneider W, et al. (1986) Percutaneous absorption of diclofenac Arzneimittelforschung 1986; 36: 1092-1096. Pharma Res Dev 3-7.	2001 NDA, SN-000, Vol. 1.6, pp. 155-160 1997: SN-000, Vol. 16, p. 395 IND 42773, SN-007, Vol. 1, p. 180
Chan KKH, Kunjbala HV, Brandt KD. (1987) <i>In vitro</i> protein binding of diclofenac sodium in plasma and synovial fluid J. Pharm. Sci. 76: 105-108	2001: NDA, Vol. 1.6, p. 161-165
Hartway T, Poblete N, Wester RC, Maibach HI. (1996) Report Number UCSF95SU03 <i>In vitro</i> percutaneous absorption of [¹⁴ C]-diclofenac sodium in human skin Final Report. San Francisco (CA): Surge Laboratory, Department of Dermatology, University of California; 1996 Dec, Sponsored by Dimethaid Research Inc.	2001 NDA, SN-000, Vol. 6, p. 166-288 1997 NDA, SN-000, Vol. 17, p. 1
Hewitt PG, Wester RC, Maibach HI. (1997) Report UCSF96SU05 Bioavailability of [¹⁴ C] – Diclofenac in vitro in human skin. Preliminary Report. San Francisco (CA): Surge Laboratory, Department of Dermatology, University of California; 1997 Oct, Sponsored by Dimethaid Research Inc	2001 NDA, SN 000, Vol. 6, p. 257 1997 NDA, SN-000, Vol. 17, p. 91
Dimethyl Sulfoxide (DMSO)	
Dugard P, Embery G. (1969) The influence of dimethylsulphoxide on the percutaneous migration of potassium butyl [35S]sulphate, potassium methyl [35S]sulphate and sodium [35S]sulphate. Br J Dermatol 81(Suppl. 4):69- 74	2006, pharmtox\pubs\dugard1969brjdermatol.pdf
Engelke UFH, Tangerman A, Willemsen MAAP, Moskau D, Loss S, Mudd SH, Wevers RA. (2005) Dimethyl sulfone in human cerebrospinal fluid and blood plasma confirmed by one-dimensional 1H and two-dimensional 1H-13C NMR. NMR Biomed 18(5): 331- 336.	2006, pharmtox\pubs\engelke2005nmrbiomed.pdf
Hucker HB, Ahmad PM, Miller EA. (1965) Physiological disposition and metabolism of dimethyl sulfoxide (DMSO). Fed Proc Fed Am Soc Exp Biol 24: 256 (Abstract).	2006, pharmtox\pubs\hucker1965fedproc.pdf
Hucker HB, Ahmad PM, Miller EA. (1966) Absorption, distribution and metabolism of dimethyl sulfoxide in the rat, rabbit and guinea pig. J Pharmacol Exp Ther 154: 176-184.	2006, pharmtox\pubs\hucker1966jpharmacolexpther.pdf

Jobin F, Gagnon FT. (1971) Inhibition of human platelet aggregation a dibenzazepine compound (GP 44296) and by N-(2,6-dichlorophenyl)-aminophenylacetic acid (GP 45840). Can J Physio Pharm 49: 479-481.	2006, pharmtox\pubs\jobin1971canjphysiolpharmacol.pdf
Kaye TS, Egorin MJ, Riggs CE Jr., Olman EA, Chou FT, Saleman M. (1983) The plasma pharmacokinetics and tissue distribution of dimethyl sulfoxide in mice. Life Sci 33(13): 1223-1230.	2006, pharmtox\pubs\kaye1983lifesci.pdf
Kolb KH, Jaenicke G, Kramer M, Schulze PE. (1967) Absorption, distribution and elimination of labeled dimethyl sulfoxide in man and animals. Ann N Y Acad Sci 141: 85-95.	2006, pharmtox\pubs\kolb1967annnyacadsci.pdf
Koptyaev VG. (1967) Experimental determination of maximum permissible concentration of dimethyl sulfide in water bodies. Gig Sanit 32(1-3): 315- 320.	2006, pharmtox\pubs\koptyaev1967gigsanit.pdf
Layman DL, Jacob SW. (1985) The absorption, metabolism and excretion of dimethyl sulfoxide by Rhesus monkeys. Life Sci 37(25): 2431-2437.	2006, pharmtox\pubs\layman1985lifesci.pdf
Lin A, Nguy CH, Shic F, Ross BD. (2001) Accumulation of methylsulfonylmethane in the human brain: Identification by multinuclear magnetic resonance spectroscopy. Toxicol Lett 123(2&3): 169-177.	2006, pharmtox\pubs\lin2001toxicollett.pdf
McDermot HL, Finkbeiner AJ, Zanette B. (1966) The fate of dimethyl sulfoxide applied to the skin of the rat. Can J Physiol Pharmacol 45: 475-478.	2006, pharmtox\pubs\mcdermot1966canjphysiolpharmacol.pdf
Otsuki S, Qian W, Ishihara A, Kabe T. (2002) Elucidation of dimethylsulfone metabolism in rat using a 35S radioisotope tracer method. Nutr Res 22(3): 313-322.	2006, pharmtox\pubs\otsuki2002nutrres.pdf
Rose SE, Chalk JB, Galloway GJ, Doddrell DM. (2000) Detection of dimethyl sulfone in the human brain by in vivo proton magnetic resonance spectroscopy. Magn Reson Imaging 18(1): 95- 98.	2006, pharmtox\pubs\rose2000magnresonimag.pdf
Sulzberger MB, Cortese, Jr. TA, Fishman L, Wiley HS, Peyre Kovich PS. (1967) Some effects of DMSO on human skin in vivo. Ann NY Acad Sci 141: 437-450.	2006, pharmtox\pubs\sulzberger1967annnyacadsci.pdf
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Williams KIH, Burstein SH, Layne DS. (1966b) Metabolism of dimethyl sulfide, dimethyl sulfoxide, and dimethyl sulfone in the rabbit. Arch Biochem Biophys 1966b; 117(1): 84-87.	2006, pharmtox\pubs\williams1966archbiochembiophysb.pdf

TOXICOLOGY: SINGLE DOSE STUDIES

Author (Year Published)/ Title/Citation	Year Submitted/Location
Diclofenac	
CIBA-GEIGY Canada Limited (1989) VOLTAREN (diclofenac sodium) Product Monograph (Date of Revision Nov. 7, 1989)	IND 42773, SN-007, Vol. 1, p. 187-8, 196-7, 199-200, 208 2001 NDA, SN-000, Vol 1.6, p. 291 1997 NDA, SN-000, Vol. 16, p. 163
Medical Economics Data Production Company (1994) Voltaren Physician's Desk Reference. 48th ed. Montvale NJ: The Company; p.996-998.	SN-000, Vol 7, p. 74 1997 NDA, SN-000, Vol. 16, p. 195
Menassé R, Hedwall PR, Krartz J, Pericin C, Riesterer L, Sallmann A, Ziel R, Jaques R. (1978) Pharmacological properties of diclofenac sodium and its metabolites. Scand J Rheumatology 22 Suppl: 5-16.	IND 42773, SN-007, Vol. 1, p. 171, 173-177 2001 NDA, SN-000, Vol 1.6, p. 50 1997 NDA, SN-000, Vol. 16, p. 125
Dimethy Sulfoxide	
Willson JE, Brown DE, Timmens EK. (1965) A toxicologic study of dimethyl sulfoxide. Toxicology and Applied Pharmacology 7: 104-112.	2001 NDA, SN-000, Vol 6, p. 325 1997 NDA, SN-000, Vol. 16, p. 137
Caujolle FME, Caujolle DH, Cros SB, Calvet M-MJ. (1967) Limits of toxic and teratogenic tolerance of dimethyl sulfoxide. Ann NY Acad Sci 141:110-125.	2001 NDA, SN-000, Vol 1.6, p. 335 1997 NDA, SN-000, Vol. 16, p. 146

General Toxicology	
Diclofenac	
CIBA-GEIGY (1989) Voltaren (diclofenac sodium) – product monograph Ciba-Geigy Canada (Date of Revision Nov. 7, 1989).	2001 NDA, Vol. 1.6, pp. 291-323
(1978) Pharmacological properties of diclofenac sodium and its metabolites Scand. J. Rheumatol. 22: 5-16	2001 NDA, Vol. 1.6, pp. 50-62
DMSO	
Willson JE, Brown DE, Timmens EK. (1965) A toxicologic study of dimethyl sulfoxide Tox. Appl. Pharmacol. 7: 104-112	2001 NDA, Vol. 1.6, pp. 325-334
(1967) Limits of toxic and teratogenic tolerance of dimethyl sulfoxide Ann. N.Y. Acad. Sci. 141: 110-125	2001 NDA, Vol. 1.6, pp. 335-351
Physician's Desk Reference (1994) Voltaren Physician's Desk Reference 48 th Ed., Medical Economics Data Production Company, Montvale NJ: The Company; 1994	2001 NDA, Vol. 1.7, pp. 74-78
Vogin EE, Carson S, Cannon G, Linegar CR, Rubin LF. (1970) Chronic toxicity of DMSO in primates Tox. Appl. Pharmacol. 16: 606-612	2001 NDA, Vol. 1.6, , p. 131-138 1997 NDA, SN-000, Vol. 16, p. 209

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DMSO General Toxicology	
Ashwood-Smith MJ. (1961). The radioprotective action of dimethyl sulphoxide and various other sulphoxides. Int J Radiat Biol 3: 41-48.	2006: pharmtox\pubs\ashwoodsmith1961intjradbiol.pdf
Barnett KC, Noel PR. (1967). Dimethyl sulfoxide and lens changes in primates. Nature 1967; 214: 115-116.	2006, pharmtox\pubs\barnett1967nature.pdf
Bennett W, Bristol T, Weaver W, Muther R. (1983). Lack of nephrotoxicity of dimethyl sulfoxide in man and laboratory animals. Ann NY Acad Sci 1983; 411:43-47.	2006, pharmtox\pubs\bennett1983annnyacadsci.pdf
Brayton CF. (1986) Dimethyl sulfoxide (DMSO): A review. Cornell Vet. 76: 61-90.	2006, pharmtox\pubs\brayton1986cornellvet.pdf
Brobyn, RD. (1975) The human toxicity of dimethyl sulfoxide. Ann NY Acad Sci 243: 497-506.	IND 42773, SN-000, Vol. 1, p. 23 IND 42773, SN-007, Vol. 1, p. 207 2001: NDA, SN-000, Vol. 7, p. 80 1997 NDA, SN-000, Vol. 16, p. 199
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Fishman EG, Jenkins LJ Jr., Coon RA, Jones RA. (1969) Effects of acute and repeated inhalation of dimethyl sulfoxide in rats. Toxicol Appl Pharmacol 15: 74-82.	2006, pharmtox\pubs\fishman1969toxicolapplpharmacol.pdf
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Garcia C. (1983). Ocular toxicology of dimethyl sulfoxide and effects on retinitis pigmentosa. Ann NY Acad Sci 1983;411:48-51.	2006, pharmtox\pubs\garcia1983annnyacadsci.pdf
Horváth K, Noker PE, Somfai- Relle S, Glavits R, Financsek I, Schauss AG (2002) Toxicity of methylsulfonylmethane in rats. Food Chem Toxicol 40(10):1459-1462.	2006, pharmtox\pubs\horvath2002foodchemtox.pdf
Jacob S, Appleton J. MSM. (2003) The Definitive Guide. A comprehensive review of the science and therapeutics of methylsulfonylmethane. 2003:107-121.	2006, pharmtox\pubs\jacob2003msm.pdf
Kleberger KE. (1967). An ophthalmological evaluation of DMSO. Ann N Y Acad Sci 1967; 141: 381.	2006, pharmtox\pubs\kleberger1967annny acadsci.pdf
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Morton JJ, Siegel BV. (1986) Effects of oral dimethyl sulfoxide and dimethyl sulfone on murine autoimmune lymphoproliferative disease. Proc Soc Exp Biol Med 183(2):227-230.	2006, pharmtox\pubs\morton1986procsocexpbiolmed.pdf
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this page is the manifestation of the electronic signature.**

/s/

Lawrence Leshin
12/21/2006 04:32:04 PM
PHARMACOLOGIST

R. Daniel Mellon
12/21/2006 04:53:02 PM
PHARMACOLOGIST
I concur with Dr. Leshin's recommendation that NDA 20947
is not approvable from the pharmacology toxicology perspective.



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

**PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION
SUPERVISOR'S SECONDARY REVIEW**

NDA NUMBER: 20-947
SERIAL NUMBER: 000 (Second Resubmission)
DATE RECEIVED BY CENTER: June 28, 2006
PRODUCT: PENNSAID® Topical Solution
(1.5% w/w diclofenac sodium)

INTENDED CLINICAL POPULATION: Topical treatment for relief of the signs
and symptoms of osteoarthritis of the
knee(s)

SPONSOR: Dimethaid International Inc.
REVIEW DIVISION: Division of Anesthesia, Analgesia, and
Rheumatology Products (HFD-170)

PHARM/TOX REVIEWER: Lawrence S. Leshin, DVM, Ph.D.
PHARM/TOX SUPERVISOR: R. Daniel Mellon, Ph.D.
DIVISION DIRECTOR: Bob A. Rappaport, M.D.
PROJECT MANAGER: Paul Balcer

Date of review submission to Division File System (DFS): December 21, 2006

Executive Summary

I. Recommendations

A. Recommendation on approvability

In my opinion, from the nonclinical pharmacology toxicology perspective, NDA 20-947 should not be approved. The following deficiencies exist for this application:

- 1) The Sponsor has not provided data to demonstrate the safety of the drug product in terms of the potential for the DMSO in the drug product to increase the absorption of other potentially toxic substances (for example, DEET or sunscreen, household chemicals, residual dry-cleaning chemicals, etc).
- 2) The Sponsor has not provided an adequate toxicological evaluation of the drug product formulation containing DMSO. DMSO has not previously been approved for chronic topical use on intact skin and therefore is considered a novel excipient and should be adequately qualified via repeat-dose toxicology studies with pharmaceutical grade DMSO. Although the literature references provided address some of the safety concerns, they do not address the potential combined toxicity due to absorption of the DMSO, diclofenac, glycerin, propylene glycol and ethanol from the drug product application.
- 3) The Sponsor has not provided adequate identification and characterization of the potential extractable and leachable components from the container closure system. I concur with the recommendations for additional identification and safety qualification data that have been made by the Chemistry, Manufacturing and Controls (CMC) Review Team of Drs. Sue Ching Lin, Ali Al Hakim, and Ravi Harapanhalli.
- 4) The Sponsor has not provided adequate safety qualification for an identified drug product impurity described as _____ that contains a structural alert for mutagenicity that has been identified by the CMC review team. I concur with their recommendations for a minimal genetic toxicology screen.
- 5) The Sponsor has not provided adequate characterization for the potential carcinogenic effects of this drug product. The oral carcinogenicity studies completed for the referenced drug (Voltaren) do not provide adequate characterization of the carcinogenic potential of this topical drug product formulation. The Sponsor should conduct dermal carcinogenicity studies with the drug product formulation or provide adequate scientific rationale for why such information is not needed.

b(4)

- 6) The Sponsor has not provided adequate information regarding the photostability of the drug product in order to provide an assessment of the potential toxicity associated with photodegradation products, if present.

B. Recommendation for nonclinical studies

- 1) Provide data to support the safe use of the drug product via characterization of the potential risk associated with the use of DMSO via the topical route of administration. Specifically address the potential for the following:
 - a. Will the use of topical DMSO and the resulting increase in the permeability of the skin result in the inadvertent absorption of toxicants from the environment?
 - b. Does application of DMSO to the knee via the hands lead to alterations in the permeability of the skin on the palms as well as the knee?
 - c. Given the rapid distribution of DMSO in the body, does application of DMSO to one site in the body (such as the knee) lead to changes in the skin permeability at other sites indirectly (such as the back)?
 - d. What is the duration of the skin changes and enhanced permeability of the skin following single exposure and repeated exposure? Your proposed drug product labeling recommends that patients wait 20 minutes prior to applying other products. What is the basis for the 20 minutes?
 - e. Will exposure to DMSO via the topical route lead to an increase in the potential systemic exposure to viral or bacterial agents?
- 2) Conduct chronic repeat-dose dermal toxicology studies with the final drug product formulation (pharmaceutical grade DMSO) in two species including full histopathology and assessments of the systemic exposure to the inactive ingredients in the drug product.
- 3) Identify the extractables and leachables from the drug product container closure system and provide a toxicological risk assessment for the potential exposures to these substances. This risk assessment should include characterization of the potential toxicity following exposure to any extracted compounds of the container closure system, including the external dyes used for labeling. Data from published literature may be submitted in lieu of animal studies, if available.
- 4) Provide a minimal genetic toxicology screen (one in vitro mutagenicity assay and one in vitro assay for chromosomal damage) for the drug substance impurity

described as _____ or reduce the levels of this impurity to not more than 1.5 ug/day total daily intake.

b(4)

- 5) Assess the potential carcinogenic effect of the drug product formulation via dermal carcinogenicity studies in two species or provide adequate scientific rationale for why such information is not needed.
- 6) Assess the photostability of the drug product and provide a toxicological risk assessment for the potential exposure to any photodegradants noted.

C. Recommendation for Labeling:

NOTE: The referenced drug product for this NDA is Voltaren. The current approved drug product labeling for Voltaren does not contain all of the nonclinical information listed in the original drug product label. The nonclinical information appears to have been inadvertently omitted from the label sent to the sponsor during a recent labeling update. However, the data in the original approved drug product label still represents the Agency's findings for this NDA and therefore should be included in this drug product.

The drug product label proposed by the sponsor does not contain reproductive toxicology data for DMSO. Since the DMSO in this drug product is significantly high and the use of this excipient is novel, and DMSO has been shown to be teratogenic in the literature, information should be included in the label.

II. Summary of nonclinical findings

A. Regulatory Summary (Pharmacology Toxicology):

The Sponsor opened IND 42,773 on 10-Jun-1993. The initial IND submission was placed on partial hold. On September 16, 1993, the Division forwarded a list of both preclinical and clinical deficiencies to the sponsor. As noted in the PharmTox Review of the initial NDA submission, the following comments were relayed to the sponsor following receipt of the initial IND:

1. Clinical
 - a) Cadaver skin studies are needed in order to evaluate skin penetration.
 - b) Preclinical toxicology data from the two active ingredients, as well as the drug product, should be provided prior to initiation of clinical efficacy studies (see below).
2. Preclinical
 - a) If further development of this product is planned, it will be necessary to separate out the pharmacodynamic activity contributed by the drug diclofenac and DMSO, which has anti-inflammatory properties and therefore cannot be

considered an inactive excipient as you propose. In addition, the following are required:

- b) A PK study of _____ in animals should be performed to determine the amount of diclofenac absorbed from _____. Other PK parameters should also be determined, such as elimination half-life and systemic distribution. The metabolic profile for diclofenac should be determined for this route of administration.
- c) A drug interaction study with DMSO should be conducted.
- d) A toxicity study will be needed to determine if diclofenac toxicity is increased by this route of exposure. The length of this study will depend on the proposed duration of human use. For chronic use in humans, a 90-Day study will be required.

b(4)

In addition, the following concerns were to be addressed as soon as possible:

- e) It was recommended that skin metabolism information be obtained from cell culture techniques.
- f) A rationale should be submitted to indicate there is no change in the systemic carcinogenicity or teratogenicity potential, or the potential to produce skin cancer, or mediate photocarcinogenicity by the intended route of administration. Animal PK data and metabolic profile can be the starting basis for the rationale.
- g) It should be determined if serum protein binding of diclofenac is altered by expected human serum concentrations of DMSO.

At our meeting with Dimethaid held on 6/6/95, the following information was exchanged:

1. FDA agreed the metabolic profile from cadaver skin could substitute for a cell culture profile to obtain skin metabolism data.
2. The potential for skin sensitization, skin phototoxicity, and photocontact allergy will be evaluated by Dimethaid in clinical studies.
3. Human transdermal bioavailability could be substituted for the recommended animal study.
4. Safety data from humans evaluated in Canada could replace a 90-day animal study.

The Agency removed the partial hold on June 7, 1995. An advice letter was forwarded to the sponsor on June 20, 1995, to which several responses from the sponsor were obtained.

NDA 20-947 was initially received by the Agency 16-Dec-1997. The initial pharmacology toxicology review was completed by Dr. Almon W. Coulter on July 16, 1998. From his review, it is clear that the nonclinical sections of the NDA were comprised entirely of literature references. However, it should be noted that Dr. Coulter does reference the drug product label and company monograph for Voltaren, as well as the drug product monograph for RIMSO-50. Dr. Coulter's recommendations noted that "There are no objections to the

approval of this drug, based on the pharmacology/toxicology data submitted to this NDA.” He recommended that information on the carcinogenicity, mutagenicity and reproductive toxicity of DMSO be included in the label.

The initial NDA application was withdrawn in on October 27, 1998 prior to an action being taken. According to the sponsor, the submission was withdrawn due to a manufacturing facility not being ready for inspection. The NDA was then resubmitted August 27, 2001. On September 12, 2001, the review Division requested the following additional information:

- Please refer to the minutes from the meeting held on June 5, 2000. You have indicated that pre-clinical skin sensitivity studies were performed with Pennsaid. Please provide the study reports from these studies.
- In addition, please provide an update on the reproductive toxicity, genotoxicity, dermal toxicity and carcinogenicity of DMSO, i.e. any recent study reports or information, besides those that have been submitted with previous filing of the NDA.

Dr. Hamid Amouzadeh was the primary reviewer for the 2001 resubmission; Dr. Robert Osterberg was the supervisory pharmacologist at the time. Dr. Amouzadeh’s review indicates that there were no nonclinical studies submitted, rather, the Sponsor’s submission contained a number of published papers and drug monographs. Drs. Amouzadeh and Osterberg recommended that the NDA application be considered approvable without specific nonclinical studies needed. The review clearly notes that information regarding the potential ocular toxicity of DMSO should be included in the label, and specifically references the RIMSO-50 drug product label.

A “Not Approvable” letter issued on August 8, 2002 included the following items that contain safety concerns that could be addressed in part via nonclinical studies (see letter for complete list):

Inadequate data was provided to demonstrate long-term safety of this drug product.

In addition, demonstration of safety in situations of co-administered therapies, which is likely to occur once a topical agent is approved, is needed.

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

Additional requests:

Under 21 CFR 314.50(d)(5)(vi)(b), we request that you update your NDA by submitting all safety information you now have regarding your new drug. The safety update should include data from all non-clinical and clinical studies of the drug under consideration regardless of indication, dosage form, or dose level.

1. Review of the literature and labels for DMSO, along with efficacy results for Study 102-93-1 and adverse events (i.e. paresthesia at site) in Study 107-96 suggest DMSO

is an active component of Pennsaid. Therefore, Pennsaid may represent a combination product which will need to be studied as such in future trials.

The latest resubmission of this NDA was received in June of 2006. This submission was filed as a 505(b)(2) submission and lists the reference drug as Voltaren Tablets (NDA 19-201). The 2006 submission contains three new genetic toxicology studies that were completed with the actual drug product as well as additional publications from the literature on both diclofenac as well as DMSO. The submission also contains summaries of toxicological data which were submitted in the RIMSO-50 (DMSO) and Voltaren (Diclofenac Ophthalmic Solution) New Drug Submissions that the sponsor stated were made available through Freedom of Information. Patent certification was not provided for these products. Dr. Lawrence Leshin completed the primary review of this submission.

B. Brief overview of nonclinical findings

No toxicology studies were completed by the sponsor for diclofenac either via the systemic or dermal route of administration. The sponsor is relying upon the Agency's previous findings of safety for the approved oral diclofenac sodium drug product Voltaren.

The Sponsor provided a multitude of published reports regarding the potential toxicity of DMSO. As noted in the reviews of Drs. Coulter, Amouzadeh, and Leshin, the existing published nonclinical toxicology data on DMSO suggests that the purified substance primarily has demonstrated ocular toxicity that has not been reported in the published literature in humans. The challenge regarding the interpretation of the studies in the published literature is the lack of specific information regarding the purity of the DMSO used in the studies and control over the conditions of its handling. As a result, although supportive of the application, these studies do not provide definitive data for this drug product.

The Sponsor submitted three genetic toxicology studies for the PENNSAID drug product in support of the current submission. These genetic toxicology studies were not specifically requested by the Agency in the not approvable letter. Dr. Leshin's review of these studies concludes that the drug product was negative under the conditions tested in the standard battery of genetic toxicity studies.

C. Pharmacologic activity

Diclofenac is a member of the NSAID class of drugs and demonstrates anti-inflammatory, analgesic, and antipyretic activities. Although DMSO has been approved as an active agent by CDER and the CVM, the sponsor maintains that this ingredient is not active for this indication and therefore has described it as an inactive ingredient. It is included as a penetration enhancer.

The drug product formulation is a topical solution to be applied directly to the knee. The formulation and maximum recommended daily dosage is summarized in the table below:

D. Brief History of DMSO as a Drug

DMSO was first synthesized in 1866 as a byproduct of paper manufacturing. In 1963, Stanley Jacob, M.D. reported that DMSO could penetrate skin and produce local analgesia, decrease pain and promote healing of injured tissue. DMSO first became the subject of clinical investigation, with the approval of the FDA, in 1964.

There have been a total of 7 NDAs filed with the Agency for DMSO as an active ingredient. These applications are listed in the table below:

NDAs for DMSO as an Active Ingredient Filed with the Agency:

b(4)

The first NDA submitted to the Agency for the use of DMSO as an active agent was received on _____ from _____ was for _____
for the proposed indication of _____

b(4)

The NDA was reviewed and considered "incomplete" by the Agency. According to a letter dated December 9, 1965, the NDA was incomplete for the following reasons:

It fails to include adequate animal studies from which an evaluation of the safety of the drug preparation can be made.

It fails to include adequate clinical data to sufficiently demonstrate whether the drug preparation is safe and effective in use under the submitted labeling.

On November 10, 1965, a conference was held between the Food and Drug Administration and Industry representatives from Wyeth, Squibb, Geigy, Merck Sharp & Dohme. The conference was apparently held to discuss concerns regarding ocular toxicity in animal

models reported by Merck. According to Agency records, the FDA concluded that "all human studies should stop except those involving the use of the drug in possible life-threatening situations, until the animal findings are cleared up."

A Federal Register notice was published on September 10, 1968 (FR 12776) that outlined the FDA's position as follows:

(a) (1) Chronic-toxicity studies with dimethylsulfoxide (DMSO) in animals, including dogs, rabbits, and swine, reported by a consulting laboratory in England and by a number of laboratories in the United States show that the administration of dimethylsulfoxide (DMSO) causes changes in the refractive index of the lens of the eyes of such animals. On the basis of these reports, clinical testing of dimethylsulfoxide (DMSO) preparations was discontinued for a time and later returned under restricted conditions.

(2) An adequate, controlled human toxicity study (Phase I) involving short-term cutaneous application of 1 gram of dimethylsulfoxide (DMSO) per kilogram of body weight daily for 14 consecutive days has recently been completed. Data obtained, not previously available, show that when dimethylsulfoxide (DMSO) was applied topically to the skin of healthy volunteers, it did not produce adverse effects upon the eyes of the subjects. Mild, apparently reversible, changes were seen suggesting that the drug may have some effects upon the liver and upon the hematopoietic system in some subjects.

(b) A comprehensive evaluation of all available data on dimethylsulfoxide (DMSO) preparations justifies further clinical investigation of the drug in treating certain serious conditions. Although reports concerning the use of dimethylsulfoxide (DMSO) in relatively benign conditions are equivocal regarding its efficacy, short-term clinical use has been established as reasonably safe by adequate Phase I studies. Under appropriate protocols, further short-term clinical investigations in the treatment of such benign conditions can be justified.

In 1972, an ad hoc Committee on Dimethyl sulfoxide was established by the National Academy of Sciences-National Research Council, at the request of FDA. According to the overview of the final report, the Committee "was asked to review the scientific information on the toxicity and clinical effectiveness of DMSO and to determine whether that information supported the restrictions imposed by the Food and Drug Administration on the use of the drug." The Committee arrived at the following conclusions:

1. The position of the Food and Drug Administration with respect to the clinical use of DMSO in man, as published in the Federal Register of September 10, 1968, is, in general, supported by the information reviewed. The minor reservation concerns the published criteria for acceptance of individual applications for clinical investigation which, in the view of the Committee, are overly restrictive.

2. DMSO produces side effects, particularly in the skin, in most persons treated and there have been sporadic cases in which DMSO has, with reasonable confidence, been linked to acute generalized urticaria in man. There is also evidence that in some species of laboratory animals DMSO in doses somewhat higher than those contemplated for man produces a unique alteration of the lens. The nature of these side effects, the import of the animal data, and the incidence of adverse reactions alone would not warrant withholding the drug in clinical circumstances in which it gave promise of saving life or in which it would clearly be more effective than currently available treatment in arresting a disease process, reducing disability, or relieving pain.

3. The nature of the evidence of effectiveness of DMSO is not such as to warrant the release of the drug for prescription in general medical practice at this time. There is suggestive evidence that DMSO may be effective in the treatment of acute traumatic injury and nontraumatic painful shoulder and in relieving the pain of rheumatoid arthritis. Some investigators have claimed that DMSO may be superior to currently available forms of treatment in cutaneous scleroderma and in interstitial cystitis, but the evidence does not allow a conclusion to be drawn in that respect. As a vehicle, DMSO may enhance the effect of other therapeutic agents such as fungicides and antiviral agents.

4. In view of the toxicity and lack of demonstrated efficacy in prior studies, the use of the drug should be restricted to investigational circumstances until it can be clearly demonstrated in some clinical condition that its therapeutic effect warrants the attendant side effects.

5. More reliable data are needed on the toxicity of DMSO and on its mechanism of action.

DMSO was approved as a drug substance by the United States Food and Drug Administration on 04/04/1978 for the symptomatic relief of patients with interstitial cystitis (NDA 17-788, RIMSO-50, Bioniche Pharma). RIMSO-50 is a 50% aqueous solution of DMSO (each mL contains 0.54 gm DMSO). According to the dosage and administration section of the approved package insert, 50 mL of RIMSO-50 should be instilled directly into the bladder by catheter or aseptic syringe and allowed to remain for 15 minutes. The medication is expelled by spontaneous voiding. The treatment should be repeated every two weeks until maximum symptomatic relief is obtained (total DMSO dose of 27 gm every two weeks; however, a portion of this dose is voided). Thereafter, time intervals between therapeutic applications may be increased appropriately. A generic version of this drug has also been approved by the FDA on November 29, 2002 (ANDA 76-185, Bioniche (Canada)). There are no unexpired patents on either product. As noted in the above table, all other NDAs were either not approved or were withdrawn by the Sponsor during the review process.

The sponsor is not claiming that DMSO is an active agent in the PENNSAID drug product formulation. Rather, they describe DMSO as an inactive ingredient. The FDA has approved three NDA applications that contain DMSO as an inactive ingredient. Two of these NDAs are

for the treatment of serious infections and are not considered chronic indications. The third NDA is for the treatment of advanced prostate cancer via a subcutaneous implant that contains a relatively small amount of DMSO. It is clear from the table below, that the use of DMSO in the PENNSAID drug product is significantly greater in terms of total dose and duration. Although a topical drug product containing DMSO has been approved, this drug product is not considered a chronic indication and is used to treat and prevent infections of wounds in second and third degree burns. The presence of DMSO in this drug product does not increase the potential risk associated with absorption of environmental agents, as these individuals already have compromised dermal integrity and the sites of application are likely being protected from all environmental exposures.

NDA applications where DMSO is listed as an Inactive Ingredient:

Viadur = Leuprolide acetate (Alza)	150	21-088 (3-Mar- 2000)	Subcutaneous; Implant	Palliative treatment of advanced prostate cancer	104 mg inactive (released over 12 months = 0.28 mg/day)
PENNSAID = Diclofenac (Dimethaid)	170	20-947 Pending Received 16-Dec- 1997	Topical lotion	Osteoarthritis	45.5% DMSO

A search of the Agency's databases indicates that a large number of INDs have been submitted to the agency for the use of DMSO as an active ingredient. The table below is sorted by Status date (ascending order). The data indicate that a total of 10 commercial INDs were terminated between November 19 and November 25, 1965. The termination of these INDs appears to have been driven primarily by the concerns for ocular toxicity based on data obtained in animals. Likewise, _____ was determined to be incomplete (current designation of Not Approvable) on December 9, 1965.

DMSO Applications from CDMS							
APPL TYPE	APPL NO	SPONSOR/APPLICANT	DRUG NAME	RECEIVED DATE	ORF CODE	APPL STATUS	STATUS DATE

3 Page(s) Withheld

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

 § 552(b)(4) Draft Labeling

 § 552(b)(5) Deliberative Process

b(4)

E. Nonclinical safety issues relevant to clinical use:

1) **Inadequate characterization of a novel excipient.** The primary concern regarding the safety of this drug product resides in the formulation. This product contains 45.5% DMSO. According to the FDA's Guidance for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients, the use of DMSO fulfills the criteria of a new excipient. Specifically, the guidance states:

In this guidance, the phrase *new excipients* means any inactive ingredients that are intentionally added to therapeutic and diagnostic products, but that: (1) we believe are not intended to exert therapeutic effects at the intended dosage, although they may act to improve product delivery (e.g., enhance absorption or control release of the drug substance); and (2) are not fully qualified by existing safety data with respect to the currently proposed level of exposure, duration of exposure, or route of administration.

The FDA has never approved any human drug products containing DMSO for use on intact skin.

There has been a long history of concerns regarding the use of DMSO via the topical route of administration, either as an active agent or as an inactive ingredient. The amount of DMSO in this drug product and the proposed directions for use will result in the daily exposure of over 4 grams of DMSO per day. The sponsor has only provided literature references to support the safety of the DMSO in this drug product. The purity of the DMSO tested in the published studies is frequently not reported and therefore the do not provide definitive data regarding the safe use of this excipient. This application would serve as the basis for the Agency's determination of the safety for the topical use of DMSO (45.5% and below) for a chronic indication. The setting of such a precedent should be based on well-controlled GLP studies with characterized pharmaceutical grade DMSO. Nonclinical studies provide the opportunity to obtain histological evaluations of tissues that can not be obtained from clinical data.

DMSO has been reported to be teratogenic in the published literature. The sponsor has concluded that the teratogenic effects appear to occur at exposures that should not be obtained following the use of the PENNSAID product. Given the large number of published nonclinical studies, which employed a variety of routes of administration and different sources of DMSO, it is not clear how best to label the product. If the sponsor wishes to rely upon the published literature, a clear statement that DMSO has been shown to be teratogenic in various animal models should be made. In the absence of clear PK data from a GLP reproductive toxicology studies with the drug product, extrapolation of exposure margins from published should be avoided.

2) **Inadequate characterization of the absorption of other drug excipients.** The drug product formulation contains significant levels of DMSO. Aside from the safety concerns with the use of this excipient via the topical route of administration, the DMSO will also result in significant absorption of all other excipients as well as any agent that it solubilizes. Therefore, the use of glycerin and propylene glycol in topical drug products alone does not provide adequate support for their safety in this drug product. The systemic absorption of these ingredients will also be enhanced by the DMSO. Based on a worst case scenario, this drug product may result in systemic absorption of _____ of glycerin and propylene glycol per day. The sponsor should provide an assessment of the systemic absorption of the glycerin and propylene glycol from the drug product formulation and justify the safety of such exposures.

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Composition of PENNSAID Topical Solution (1.5% diclofenac sodium) 40 drops per knee, four times per day (average of 1.2 mL/40 drops)				
Ingredient	%	mg/ application/knee	mg/application/ 2 knees	TDI (4 times per day)
Diclofenac sodium USP	1.5	19.3 mg*	38.6 mg	154.4 mg
Dimethyl sulfoxide USP	45.5	602.78 mg	1205.5 mg	4822.27 mg
Glycerin				
USP				
Propylene glycol				
USP				
Alcohol,				
USP				
Purified water				
USP				
Total	100.00			
* as per the sponsor: 1 mL solution x 1.5% diclofenac sodium= 15 mg/mL x 1.07 (specific gravity) = 16.05 mg/mL; 1.2 mL (40 drops)= 19.3 mg diclofenac sodium DMSO: 1 mL solution x 45.5% DMSO= 455 mg/mL x 1.104 (specific gravity) = 502.32 mg/mL; 1.2 mL(40 drops) = 602.78 mg DMSO Glycerin Propylene glycol: Alcohol				

b(4)

b(4)

3) **DMSO as a carrier for other potentially toxic substances.** The proposed drug product contains an excipient, DMSO, which, when applied via the topical route of administration raises concern regarding the potential for this excipient to lead to absorption of compounds from environmental exposures that can not be adequately controlled for without further information regarding the duration and extent of vulnerability. Based on the known properties of this compound, particular concern exists regarding the potential for the product to lead to an increase in the exposure to genotoxic agents in the environment, compounds that may be extracted from the container closure system including any dyes used for printing on the outside of the container, and other compounds such as impurities that could produce delayed toxicities that would not be noted in the existing clinical trials or preclinical toxicology studies. The extent and clinical implications of such exposures for any given study participant would be virtually impossible to study in a clinical trial and may not appear until years after acute or cumulative exposures.

The concern that DMSO may carry other dissolved chemicals into the body is illustrated in hazard management guidelines regarding exposure to DMSO. For example, HAZARDTEXT®, an online Hazard Management guideline, provides the following warning: "Warning: Dimethyl sulfoxide may act as a vehicle for other solvents. When dimethyl sulfoxide is a constituent in a mixture, the mixture as a whole must be evaluated (Mansdorf, 1995). MEDITEXT®, an online medical management database notes that under the heading Acute Exposure: With Therapeutic Use: "DMSO readily penetrates the skin and may carry other dissolved chemicals into the body." The MEDITEXT document further describes actions that should be taken following dermal exposure with the comment that "DMSO carries other substances through the skin. Take careful history as to what other chemicals might be involved, and observe for toxic effects from these chemical." Specific statements regarding the general carrier properties of DMSO are also made in MEDITEXT as follows "CARRIER PROPERTIES – It is especially important to remember that a DMSO exposure may result in symptoms not due to DMSO. This may be due to drug interactions or other substances being carried into the body. DMSO may carry water soluble drugs through the skin and provide a dermal reservoir for sustained effect" and "DMSO enhances the percutaneous absorption of substances or molecular weight less than 3,000. It also decreases movement of water soluble substances once in the skin, thus creating a dermal reservoir."

The concern regarding the potential carrier effect of DMSO was also raised by the medical reviewer for _____ as noted in the Addendum to Summary dated December 20, 1966 as follows:

b(4)

If DMSO truly acts as a penetrant carrier, and there is some doubt about this action of DMSO particularly depending upon the size and type of molecule being carried, herein lies on the DMSO's most potentially serious drawbacks to its use in clinical medicine; this danger results from the fact that toxic absorption may result inadvertently or result from the use of DMSO with a preparation that is not meant for internal use (this could happen frequently in the field of dermatology where frequent use of noxious compounds are applied topically and are not meant for absorption, but could potentially be absorbed with concurrent use of DMSO.

4) **Inadequate data regarding the dermal carcinogenicity of the drug product.** The sponsor has not completed dermal carcinogenicity studies for this drug product. The Agency's previous findings regarding the potential carcinogenic effects of diclofenac sodium, as delineated in the referenced product (Voltaren) provides adequate characterization of the potential systemic effects of diclofenac on this drug product; however, these studies are not adequate to address the higher concentrations of drug that will be found in the dermis via this route of administration. Although dermal carcinogenicity studies have been completed for a different drug product, Solaraze®, a dermal diclofenac drug product approved for the treatment of actinic keratosis, the highest tolerated dose of diclofenac in these studies was significantly lower than the clinical dose. From this experience, it is likely that the maximum tolerated topical dose of diclofenac that could be obtained with the PENNSAID formulation that could be used in nonclinical species will also not approach the topical dose proposed in

the clinical formulation due to the increased sensitivity of the nonclinical species to the systemic effects of diclofenac. However, the sponsor has not completed dose range-finding studies, nor any dermal toxicology studies for this drug product to support such a conclusion for PENNSAID. The sponsor should assess the feasibility of such studies using the clinical formulation. In the absence of an adequate dermal carcinogenicity assessment, the drug product label would have to clearly note that the referenced oral carcinogenicity data does not adequately address the potential dermal carcinogenic effects of the drug product.

5) Inadequate characterization of the photosafety of the drug product. The sponsor has not provided an assessment of the potential photocarcinogenicity of the drug product. There are data in the literature that indicate that diclofenac can be phototoxic. As per the photosafety guidance document, this drug product could be labeled adequately to address this issue.

6) Inadequate characterization of the photostability of the drug product. Dr. Leshin notes that DMSO is not stable in light. It is been reported that one breakdown product of DMSO in the presence of UV light is formaldehyde. The sponsor has not provided an assessment of the photostability of the drug product and should provide data to characterize the potential for photodegradation, identify any photodegradation products, and adequately characterize the potential toxicity of any photodegradation products as per ICHQ3B. Until this characterization is completed, the potential clinical significance of exposures under the conditions of use are not known and clear information regarding storage and use of the product can not be included in the label.

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this page is the manifestation of the electronic signature.**

/s/

R. Daniel Mellon
12/21/2006 03:55:47 PM
PHARMACOLOGIST
Pharmacology Toxicology Supervisor DAARP

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA Number: 20-947
Review Number: One
Date of Submission: August 9, 2001
Sponsor: Dimethaid International Inc.
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Appleby Gardens
St. James, Barbados
Phone: 905 415 1446 ext. 230
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Contact: George E. Markus

Reviewer: Hamid R. Amouzadeh, Ph.D.
Division: Division of Anti-inflammatory, Analgesic and Ophthalmic Drug Products
HFD: 550
Review Completion Date: July 12, 2002

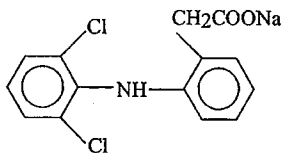
Drug:

Class: NSAID
Chemical Names: Acetic acid, O-(2,6-dichloroanilino)phenyl-, monosodium salt
Generic Names: Diclofenac sodium
Proprietary Name: PENNSAID®
CAS Registry Number: 15307-79-6
Structural Formula: $C_{14}H_{10}Cl_2NO_2.Na$
Molecular Weight: 318.13
Manufacturer: Active Ingredient: _____

b(4)

Drug Product: Dimethaid Manufacturing Inc., Quebec, Canada

Chemical Structures:



Relevant DMFs, INDs and NDAs: IND 42,773 and DMF _____
Indication: Osteoarthritis
Clinical Formulation:

b(4)

Component	Amount		Standard
	mg	%	
Diclofenac sodium	15	1.5	USP
Dimethyl Sulfoxide	455	45.5	USP
Glycerin	_____	_____	USP
Propylene Glycol	_____	_____	USP
Alcohol	_____	_____	USP
Purified Water	_____	_____	USP

b(4)

Route of Administration: Topical, dermal

Proposed Dose: _____

b(4)

Disclaimer: The Sponsor's materials were used in this review.

Key Words: Osteoarthritis, NSAID, diclofenac, pain, DMSO, PENNSAID®

Abbreviations: NSAID = non-steroidal anti-inflammatory drug, USP = United States Pharmacopeia, DMSO = dimethyl sulfoxide, PDR = Physician's Desk Reference

Executive Summary

I. Recommendations

- A. Recommendation on Approvability:** Based on the non-clinical information provided in this NDA and the previous review, the drug is approvable.
- B. Recommendation for Non-clinical Studies:** None.
- C. Recommendations on Labeling:** The label should essentially contain the information on the approved diclofenac label (VOLTAREN® and CATAFLAM®). The sponsor has provided information on a report indicating that ophthalmic administration of diclofenac exacerbated the asthma in an asthmatic patient (Szczeklik A, et al., 1977). Also, concomitant administration of PENNSAID® with systemic NSAIDs should be done cautiously because an arthritic patient who was being treated with sulindac for 6 month developed peripheral neuropathy after initiation of topical application of 90% DMSO (Reinstein L, et al., 1982). These findings should be incorporated into the label. In addition, the label should contain similar information to that found in the label for the approved DMSO product (RIMSO®-50). In particular, information on the potential for ocular toxicity of DMSO should be included.

The label for RIMSO®-50 indicates that *“since lens changes were noted in animals, full eye evaluation, including slit lamp examination, are recommended prior to and periodically during treatment.”* Although the exposure to DMSO through PENNSAID® may not be as high as that through RIMSO®-50, inclusion of this recommendation should be considered in the PENNSAID® label.

The followings are the suggested changes (*in italic*) to the label submitted by the sponsor.

Precautions:

Add the following:

DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically. In humans, daily topical administration of 80% DMSO for 12 weeks, or instillation of 7.5-60% DMSO in tetrahydrolazine for 1-15 months for tretment of intraocular inflammatory diseases did not cause ocular toxicity.

Concomitant administration of PENNSAID® with systemic NSAIDs should be done cautiously because an arthritic patient who was being treated with sulindac for 6 month developed peripheral neuropathy after initiation of topical application of 90% DMSO.

Carcinogenesis, Mutagenesis, Impairment of Fertility:

b(4)

Suggested Changes: None.

Suggested Changes: DMSO (100%) was not carcinogenic when applied to hamster oral pouch, or administered cutaneously or subcutaneously to mice for 76 to 94 weeks. DMSO was not mutagenic in bacterial reverse mutation assay (Ames). However, chromosomal damage was observed when rats were treated with 1-100% DMSO intraperitoneally. The effects of DMSO on fertility have not been studied.

Pregnancy:

Teratogenic Effects:

b(4)

Suggested Changes:

Diclofenac Sodium: Pregnancy Category C. Diclofenac sodium was not teratogenic when administered up to 20 mg/kg/day (60 mg/m²/day) in mice and up to 10 mg/kg/day in rats and rabbits (60 mg/m²/day for rats and 80 mg/m²/day for rabbits). Dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival were observed in rats at maternally toxic doses.

DMSO: DMSO was teratogenic when administered intraperitoneally to hamsters, rats and mice at 2.5 to 12 g/kg. In contrast, oral or topical administrations did not affect reproduction. In one study, terata were observed in rabbits treated with topical DMSO (5 g/kg for two days, then 2.5 g/kg, for eight days). However, in another

study, no effects were observed in rabbits treated with topical DMSO (1.1 g/kg from days 3 to 16 of gestation).

II. Summary of Non-Clinical Findings

- A. **Brief Overview of Non-clinical Findings:** Because the active ingredient is an approved drug, the sponsor did not provide any non-clinical studies. Instead, a number of published papers and drug monographs were provided.

The non-clinical toxicology profile of diclofenac is typical of NSAIDs. Its main toxicity is gastrointestinal damage. Diclofenac is not mutagenic or carcinogenic. It did not affect fertility, reproduction and development in mice, rats and rabbits. Diclofenac is classified as Pregnancy Category B (PDR Online, 2002).

DMSO is not very toxic in animals as evidenced by the lack of significant toxic manifestation at milligram per kilogram body weight doses. The results of the majority of studies on the mutagenic potential of DMSO are negative. However, a few studies reported positive findings in bacterial reverse mutation and chromosomal aberration assays. DMSO (100%) was not carcinogenic when applied to hamster oral pouch, or administered cutaneously or subcutaneously to mice for 76 to 94 weeks. It was teratogenic when administered intraperitoneally to hamsters, rats and mice at 2.5 to 12 g/kg. DMSO is classified as Pregnancy Category C (PDR Online, 2002).

DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically.

- B. **Pharmacologic Activity:** Diclofenac is an NSAID with anti-inflammatory, analgesic and antipyretic properties. The sodium (VOLTAREN®) and potassium (CATAFLAM®) salts of diclofenac are approved for osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, analgesia and primary dysmenorrhea in immediate-release, delayed release or extended release forms. The pharmacological effects of the diclofenac are thought to be mediated through inhibition of cyclooxygenase enzymes.

Because of its unique ability to readily penetrate the skin, DMSO is a suitable carrier for other substances. PENNSAID® contains — DMSO. Apparently, DMSO is being used only as a carrier and penetration enhancer in PENNSAID® because the sponsor did not claim any pharmacological efficacy for DMSO in this drug product. However, numerous pharmacological activities have been attributed to DMSO. Studies have shown that DMSO has anti-inflammatory and analgesic properties. Because of its free radical scavenging ability, DMSO can protect against damages caused by radiation and ischemia. DMSO affects the function of a number of enzymes. It has anti-microbial and anti-coagulative effects and is used for cryopreservation and as a solvent routinely.

b(4)

- C. **Non-Clinical Safety Issues Relevant to Clinical Use:** Because DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically, the possibility of occurrence of such effects in human exists. However, in humans, daily topical administration of 80% DMSO for 12 weeks did not have any ocular toxicity (Hull et al. 1969). Moreover, instillation of 7.5-60% DMSO in tetrahydrolazine for 1-15 months for treatment of intraocular inflammatory diseases did not cause ocular toxicity (Gordon, 1967).

III. Administrative

Reviewer's Signature:

Hamid R. Amouzadeh, Ph.D.

Date

Supervisor's Signature:

Concurrence:

Josie Yang, Ph.D.

Date

CC List:

NDA 20-947/Original NDA
NDA 20-947/Division File
HFD-550/PM/Halonen
HFD-550/MO/Witter
HFD-550/Pharm/Tox/Amouzadeh

TABLE OF CONTENTS
PHARMACOLOGY AND TOXICOLOGY REVIEW

I.	PHARMACOLOGY	7
II.	SAFETY PHARMACOLOGY	7
III.	PHARMACOKINETICS/TOXICOKINETICS	8
IV.	GENERAL TOXICOLOGY	9
V.	GENETIC TOXICOLOGY	14
VI.	CARCINOGENICITY	17
VII.	REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY	20
VIII.	SPECIAL TOXICOLOGY STUDIES:	22
IX.	DETAILED CONCLUSIONS AND RECOMMENDATIONS	22
X.	APPENDIX/ATTACHMENTS:	27
XI.	REFERENCES:	27

PHARMACOLOGY/TOXICOLOGY REVIEW

INTRODUCTION AND DRUG HISTORY: According to the sponsor, the active ingredient of PENNSAID® is diclofenac. Diclofenac is an NSAID with anti-inflammatory, analgesic and antipyretic properties. The sodium (VOLTAREN®) and potassium (CATAFLAM®) salts of diclofenac are approved for osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, analgesia and primary dysmenorrhea in immediate-release, delayed release or extended release forms. The pharmacological effects of the diclofenac are thought to be mediated through inhibition of cyclooxygenase enzymes.

Because of its unique ability to readily penetrate the skin, DMSO is a suitable carrier for other substances. PENNSAID® contains 45% DMSO. Apparently, DMSO is being used only as a carrier and penetration enhancer in PENNSAID® because the sponsor did not claim any pharmacological efficacy for DMSO in this drug product. However, numerous pharmacological activities have been attributed to DMSO. Studies have shown that DMSO has anti-inflammatory and analgesic properties. Because of its free radical scavenging ability, DMSO can protect against damage caused by radiation and ischemia. DMSO affects the function of a number of enzymes. It has anti-microbial and anti-coagulative effects and is used for cryopreservation and as a solvent routinely.

PENNSAID® was originally submitted under the name _____ on June 8, 1993 for osteoarthritis and was reviewed under IND 42,773 by Dr. S. R. Joshi. Dr. Joshi recommended a clinical hold because of the lack of non-clinical toxicity data. The sponsor provided these data in an amendment on August 17, 1993, which was reviewed by Dr. A. W. Coulter and found inadequate. On September 16, 1993 the sponsor was sent a letter detailing the deficiencies, which prevented the clinical trials from proceeding. Subsequently, in a letter dated June 7, 1995, the sponsor was allowed to proceed with the clinical trials. An NDA was submitted on December 15, 1997 and was withdrawn on October 26, 1998 because of incomplete pre-approval inspection issues. Finally, the NDA was resubmitted on August 7, 2001 under the name PENNSAID®. A number of materials, new and previously submitted, were included in the resubmission.

b(4)

Materials Submitted in this NDA: Dr. Almon W. Coulter reviewed many of the following materials in the initial submission of this NDA (review completed on July 16, 1998). These materials, which are identified by a check mark (✓), were not reviewed here. The remaining materials were reviewed if considered relevant.

Citation	Title	Location
	<i>Pharmacology</i>	
		✓
		✓
		✓

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		✓
		✓
		✓
		✓
		✓
		✓
<i>Pharmackinetics/Toxicokinetics</i>		
		✓
		✓
		✓
		✓
		✓
		✓
		✓
		✓
		✓
		✓
Tox. Appl. Pharmacol. 16: 606-612 (1970)	Toxicity of DMSO in primates	Vol. 1.6 pp. 131-138
		✓
		✓
<i>Genetic Toxicology</i>		
		✓
Mut. Res. 136: 137-145 (1984)	Mutagenic activity of some clastogenic chemicals at the hypoanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells	Vol. 1.7A pp. 15-24
Mut. Res. 457:15-30 (2000)	Induction of chromosome aberrations <i>in vitro</i> by phenolphthalein: mechanistic studies	Vol. 1.7A pp. 25-41

b(4)

b(4)

Mut. Res. 496: 105-115 (2001)	Protective effects of vitamins and selenium compounds in yeast	Vol. 1.7A pp. 42-53
Cancer Lett. 112: 11-16 (1997)	Mutagenic, carcinogenic and cocarcinogenic activity of cashew nut shell liquid	Vol. 1.7A pp. 54-60
Mut. Res. 303: 127-133 (1993)	Dimethyl sulfoxide (DMSO) is mutagenic for bacterial mutagenicity tester strains	Vol. 1.7A pp. 61-68
Mut. Res. 114: 283-385 (1983)	Cell transformation by chemical agents- a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program.	Vol. 1.7A pp. 69-74
Mut. Res. 168:69-240 (1986)	The <i>Salmonella typhimurium</i> /mammalian microsomal assay. A report of the U.S. nvironmental Protection Agency Gene-Tox Program	Vol. 1.7A pp. 75-82
Int. J. Radiat. Oncol. Biol. Phys. 47:1371-1378 (2000)	Dimethyl sulfoxide protects against thermal and epithermal neutron-induced cell death and mutagenesis of Chinese hamster ovary cells	Vol. 1.7A pp. 83-91
Mut. Res. 87: 17-62 (1981)	Sister-chromatid exchanges: a report of the GENE-TOX program	Vol. 1.7A pp. 92-137
Mut. Res. 88: 343-350 (1981)	Compatibility of organic solvents with the <i>Salmonella</i> /microsome test	Vol. 1.7A pp. 138-146
Mut. Res. 455: 29-60 (2000)	The Ames <i>Salmonella</i> /microsome mutagenicity assay	Vol. 1.7A pp. 153-185
J. Agric. Food Chem. 29: 1089-1091 (1981)	Natural occurring 7A/186 levels of dimethyl sulfoxide in selected fruits, vegetables, grains and beverages	Vol. 1.7A pp. 186-189
Mut. Res. 412: 251-260 (1998)	Chemicals mutagenic in <i>Salmonella typhimurium</i> strain TA1535 but not in TA100	Vol. 1.7A pp. 190-200
Ann. N.Y. Acad. Sci. 826: 348-374 (1997)	Amyloid is not a tombstone - a summation - the primary role for cerebrovascular and CSF dynamics as factors in Alzheimer's disease (AD): DMSO, fluorocarbon oxygen carriers, thyroid hormonal and other suggested therapeutic measures	Vol. 1.7A pp. 201-228
J. Clin. Oncol. 2: 227-241 (1984)	Polar solvents: a novel class of antineoplastic agents	Vol. 1.7 pp. 343-359
Mut. Res. 88: 397-410 (1981)	Induction of chromosomal aberrations and sister-chromatid exchanges in Chinese hamster cells in vitro by some proximate and ultimate carcinogenic arylamide derivatives	Vol. 1.7A pp. 229A-229O
Mut. Res. 402: 321-329 (1998)	Janus carcinogens and mutagens	Vol. 1.7A pp. 230-239
Radiat. Res. 124: 73-78 (1990)	Radioprotective effects of dimethyl sulfoxide in golden hamster embryo cells exposed to gamma rays at 77K. II. Protection from lethal, chromosomal and DNA damage	Vol. 1.7A pp. 240-246
FEBS Lett. 412: 603-609 (1997)	Granulocytic differentiation of HL-60 cells results in spontaneous apoptosis mediated by increased caspase expression	Vol. 1.7 pp. 360-366

b(4)

b(4)

Zeiger, E. (1994)	Personal Communication with Dr. R. Willer, Gaylord Chemical Corporation	Vol. 1.7A pp. 256-258
Env. Mol. Mutagen. 19: 2-141 (1992)	<i>Salmonella</i> mutagenicity tests: V. Results from the testing of 311 chemicals	Vol. 1.7A pp. 259-271
Carcinogenesis 10: 441- 445 (1989)	Effect of dimethyl sulfoxide on the genotoxicity and metabolism of benzene in vivo	Vol. 1.7A pp. 274-279

✓

b(4)

Teratog. Carcinog. Mutagen. 1: 141-145 (1980)	Mutagenicity of dimethylsulfoxide (DMSO): <i>in vivo</i> cytogenetics in the rat	Vol. 1.7A pp. 290-295
Mut. Res. 455: 155-166 (2000)	<i>In vivo</i> rodent micronucleus assay: protocol, conduct and data interpretation	Vol. 1.7A pp. 296-308
Mut. Res. 123: 183-279 (1983)	The sex-linked recessive lethal test for mutagenesis in <i>Drosophila melanogaster</i> . A report of the U.S. Environmental Protection Agency Gene-Tox Program	Vol. 1.7A pp. 309-318
Mut. Res. 40: 383-388 (1976)	Lack of proof of induction of somatic recombination and mutation in <i>Drosophila</i> by methyl-2-benzimidazole carbamate, dimethyl sulfoxide and acetic acid	Vol. 1.7A pp. 319-322
Eviron. Mutagen. 5: 273-277 (1983)	The solvent dimethyl sulfoxide (DMSO) does not induce aneuploidy in oocytes of <i>Drosophila melanogaster</i>	Vol. 1.7A pp. 323-328
Mut. Res. 134: 61-88 (1984)	Chromosome mutation tests for mutagenesis in <i>Drosophila melanogaster</i> . A report of the U.S. Environmental Protection Agency Gene-Tox Program	Vol. 1.7A pp. 329-357
Mutagenesis 8: 57-81 (1993)	Performance of 181 chemicals in a <i>Drosophila</i> assay predominantly monitoring interchromosomal mitotic recombination	Vol. 1.7A pp. 358-383
<i>Carcinogenicity</i>		
Arch. Path. 83: 293-297 (1967)	Dimethyl sulfoxide and experimental oral carcinogenesis in the hamster pouch	Vol. 1.7 pp. 129-134
Ann. N.Y. Acad. Sci. 141: 214-220 (1967)	The effect of dimethyl sulfoxide on the induction of breast cancer in the rat	Vol. 1.7 pp. 135-145
Gann 69: 599-600 (1978)	Induction of renal and hepatic tumors in mice by ochratoxin A, a mycotoxin	Vol. 1.7 pp. 143-145
Arch. Surg. 121: 1455-1459 (1986)	Polar solvents in the chemoprevention of dimethylbenzanthracene-induced rat mammary cancer	Vol. 1.7 pp. 146-151
Cancer 62: 944-948 (1988)	Use of polar solvents in chemoprevention of dimethylhydrazine-induced colon cancer	Vol. 1.7 pp. 151-157
Arch. Path. 87: 637-642 (1969)	Chemical carcinogenesis of hamster mucosa	Vol. 1.7 pp. 158-164
Science 236: 933 -941 (1987)	Prediction of chemical carcinogenicity in rodents from <i>in vitro</i> genetic toxicity assays	Vol. 1.7 pp. 165-174
Ann. N. Y. Acad. Sci. 411: 150-160 (1983)	Mechanism of the synergistic effect of oral dimethyl sulfoxide on anti-neoplastic therapy	Vol. 1.7 pp. 175-186
J. Natl. Cancer Inst. 39: 1217-1228 (1976)	Carcinogenicity of epoxides, lactones, and peroxy compounds. VI. Structure and carcinogenic activity	Vol. 1.7 pp. 187-199

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Tox. Appl. Pharmacol. 16: 606-612 (1970)	Chronic toxicity of DMSO in primates	Vol. 1.7 pp. 208-215	
Ann. N.Y. Acad. Sci. 243: 194-208 (1975)	Potentiation of anti-neoplastic compounds by oral dimethyl sulfoxide in tumor-bearing rats	Vol. 1.7 pp. 216-231	
Int. J. Urol. 6: 87-92 (1999)	Transmurally absorbed intravesical chemotherapy with dimethyl sulfoxide in an animal model	Vol. 1.7 pp. 232-238	
Regul. Toxicol. Pharmacol. 28: 85-95 (1998)	Identification of rodent carcinogens and non- carcinogens using genetic toxicity tests: premises, promises and performance	Vol. 1.7 pp. 239-250	
Environ. Mol. Mutagen. 6: 1-14 (1990)	Evaluation of four <i>in vitro</i> genetic toxicity tests for predicting rodent carcinogenicity: confirmation of earlier results with 41 additional chemicals	Vol. 1.7 pp. 251-265	
<i>Reproductive and Developmental Toxicology</i>			
Ann. N.Y. Acad. Sci. 141: 110-125	Limits of toxic and teratogenic tolerance of dimethyl sulfoxide	Vol. 1.6 pp. 335-351	
Ciba-Geigy Canada	Voltaren® (diclofenac sodium) – product monograph	Vol. 1.6 pp. 291-323	
J. Appl. Toxicol. 12: 49-56 (1992)	Teratogenic assessment of four solvents using the frog embryo teratogenesis assay-Xenopus (FETAX)	Vol. 1.7 pp. 22-30	
			✓
16: 49-54 (1966)			
Proc. Soc. Exp. Biol. Med. 125: 567-569 (1967)	Effect of maternal administration of dimethyl sulfoxide on the development of rat fetuses	Vol. 1.7 pp. 40-43	
			✓
			✓
<i>Special Toxicology</i>			
			✓
			✓
<i>Other Materials</i>			
			✓
			✓
			✓
			✓
USP Drug Information 1: 1263-1265 (2000)	Dimethyl sulfoxide	Vol. 1.8 pp. 60-64	
			✓

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Cornell Vet. (76): 61-90 (1986)	Dimethyl sulfoxide (DMSO)	Vol. 1.8 pp. 165-195
Ophthalmol. 115: 294-295 (1997)	Exacerbation of asthma by topical diclofenac	Vol. 1.8 pp. 196-197
		✓
		✓
J. Pharm. Sci. 76: 105-108 (1987)	<i>In vitro</i> protein binding of diclofenac sodium in plasma and synovial fluid	Vol. 1.8 pp. 233-237
		✓
Biochem. Biophys. Res. Commun. 98: 614-620 (1981)	The appearance of phospholipase and cyclo-oxygenase activities in the human promyelocytic leukaemia cell line HL-60 during dimethyl sulfoxide induced differentiation	Vol. 1.7 pp. 268-275
Biochem. Biophys. Acta 717: 217-225 (1982)	Synthesis of sialoglycoconjugates during dimethyl sulfoxide-induced erythrodifferentiation of Friend leukaemia cells	Vol. 1.7 pp. 276-285
Proc. Natl. Acad. Sci. 75: 2458-2462 (1978)	Terminal differentiation of human promyelocytic leukaemia cells induced by dimethyl sulfoxide and other polar compounds	Vol. 1.7 pp. 286-291
Oncology 39: 325-330 (1982)	Dimethyl sulfoxide induced alterations in the growth properties and protein composition of <i>in vitro</i> propagated murine hepatoma cells	Vol. 1.7 pp. 292-298
Cancer Detect. Prev. 8: 237-245 (1985)	Differentiation inducing effects of butyrate and DMSO on human intestinal tumor cell lines in culture	Vol. 1.7 pp. 299-308

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Prostate 18: 47-58 (1991)	Effect of dimethyl sulfoxide on the proliferation and glycosaminoglycan synthesis of rat prostate adenocarcinoma cells (PAII) <i>in vitro</i> : isolation and characterization of DMSO resistant cells	Vol. 1.7 pp. 309-321
Proc. Natl. Acad. Sci. 73: 1237-1240 (1976)	Dimethyl sulfoxide induced differentiation and hemoglobin synthesis in tissue culture of rat erythroleukemia cells transformed via dimethylbenz(a)anthracene	Vol. 1.7 pp. 322-326
Exp. Cell. Res. 162: 77-85 (1986)	Growth inhibition of murine melanoma by butyric acid and dimethyl sulfoxide	Vol. 1.7 pp. 327-336
Cancer Res. 44: 1648-1652 (1984)	Effects of sodium butyrate, dimethyl sulfoxide, and retinoic acid glycolipids on human rectal adenocarcinoma cells	Vol. 1.7 pp. 337-342
J. Clin. Oncol. 2: 227-241 (1984)	Polar solvents: a novel class of anti-neoplastic agents	Vol. 1.7 pp. 343-359
FEBS Lett. 412: 603-609 (1997)	Granulocytic differentiation of HL-60 cells results in spontaneous apoptosis mediated by increased caspase expression	Vol. 1.7 pp. 360-366
Prostate 6: 329-341 (1985)	Dimethyl sulfoxide-induced changes in rat prostate adenocarcinoma	Vol. 1.7 pp. 367-380

I. PHARMACOLOGY

Diclofenac: Diclofenac is a NSAID with anti-inflammatory, analgesic and antipyretic properties. The sodium (VOLTAREN®) and potassium (CATAFLAM®) salts of diclofenac are approved for osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, analgesia and primary dysmenorrhea in immediate-release, delayed release or extended release forms. The pharmacological effects of the diclofenac are thought to be mediated through inhibition of cyclooxygenase enzymes.

DMSO: DMSO is a solvent that has been studied extensively for a long period for its multiple pharmacological effects. The main characteristics of DMSO is its ability to readily penetrate the skin. This property makes DMSO a suitable carrier for other substances. Studies have shown that DMSO has anti-inflammatory and analgesic properties. Because of its free radical scavenging ability, DMSO can protect against damages caused by radiation and ischemia. DMSO affects the function of a number of enzymes. It has anti-microbial and anti-coagulative effects and is used for cryopreservation and as a solvent routinely.

II. SAFETY PHARMACOLOGY

Diclofenac: Diclofenac is an approved drug; therefore, its safety pharmacology is well-defined. The sponsor has provided information on a report indicating that ophthalmic administration of diclofenac exacerbated the asthma in an asthmatic patient.

DMSO: The safety pharmacology of DMSO has not been studied systematically.

III. PHARMACOKINETICS/TOXICOKINETICS

Diclofenac: Diclofenac is rapidly and almost completely absorbed and is distributed to blood, liver and kidneys when given orally under fasting condition. It is _____
_____ The plasma concentration is linear and the maximum is reached in about 2.5 hr and there is no accumulation at the therapeutic doses. About _____ of the administered dose is eliminated within 72 hrs mainly through _____ mainly as the _____ of diclofenac.

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DMSO: The following is the information on the pharmacokinetics/toxicokinetics of DMSO found in the PDR (RIMSO®-50, PDR, 2002, online). *“Dimethyl sulfoxide is metabolized in man by oxidation to dimethyl sulfone or by reduction in dimethyl sulfide. Dimethyl sulfoxide and dimethyl sulfone are excreted in the urine and feces. Dimethyl sulfide is eliminated through the breath and skin and is responsible for the characteristic odor from patients on dimethyl sulfoxide medication. Dimethyl sulfone can persist in serum for longer than two weeks after a single intravesical instillation. No residual accumulation of dimethyl sulfoxide has occurred in man or lower animals who have received treatment for protracted periods of time. Following topical application, dimethyl sulfoxide is absorbed and generally distributed in the tissues and body fluids.”*

The following summary of pharmacokinetics and metabolism of DMSO in humans was found in an article by Willhite and Kazt (J. Appl. Toxicol. 4: 155-160, 1984).

“Ingestion of 1 g/kg DMSO in humans resulted in a peak serum concentration within 4 h, with a $t_{1/2}$ = 20 h. The dimethyl sulfone concentration peaked between 72-96 h post-administration with $t_{1/2}$ = 72 h. At 120 h, 50% of the total dose was excreted in urine as DMSO and 9.6% as dimethyl sulfone. Within 480 h, 22% of the total dose was excreted as dimethyl sulfone. Cumulative urinary excretion of DMSO and dimethyl sulfone was linear and exponential, respectively, following 0.5 g/kg DMSO p.o. per day for 14 days.

Dermal application of 1 g/kg [^{35} S]-DMSO produced a maximum serum level between 4-8 h with $t_{1/2}$ = 11-14 h. The dimethyl sulfone peak serum concentration occurred between 36-72 h with $t_{1/2}$ = 60-70 h. At 48 h, 13.0% of the total dose was eliminated in the urine as DMSO, while 3.1 % of the dose was eliminated as dimethyl sulfone; 17% of the dose was excreted as dimethyl sulfone by 456 h. There was negligible excretion in the feces.

Eight hours after the cutaneous application of 2 g of 90% DMSO, 20% remained at the application site. The maximum concentration plateau in the blood occurred 4-6 h post-application with no change in the plateau for the next 1.5-3 days. Within 24 h, 10-15% of the total dose appeared in the urine. After 7 days, 40% was excreted in the urine. At 6 h and 24 h, 1% and 1.5-3.0% of the total dose, respectively, had been expired. Dimethyl sulfide expiration ceased after 24 h.

Topical application of 80% DMSO for 30 min per day for 8 days showed that 25-40% of the dose was absorbed per day. After 9 days, DMSO was not detectable in the plasma, while dimethyl sulfone was detectable until day 15; dimethyl sulfone $t_{1/2}$ = 2.5-3.0 days.

Administration of 2.0 g of 50% DMSO i.v. to a human resulted in the elimination of 80% of the total dose in 7 days; $t_{1/2}$ = 4 days. Negligible amounts were excreted in feces. Dimethyl sulfide expiration accounted for 3% of the total dose."

IV. GENERAL TOXICOLOGY

Diclofenac: The following information on non-clinical toxicology of diclofenac was found in the Voltaren® product monograph (Ciba-Geigy, 1989).

"Acute Toxicity: The symptoms include bradycardia and convulsions. The most frequent autopsy findings in animals that died were gastric irritation, perforation and their sequelae."

Species	Route	LD ₅₀ (mg/kg)	95% Confidence Limit (mg/kg)
Mouse	p.o.	389	197 – 595
	i.v.	133	126 – 140
Rat	p.o.	173	133 – 213
	i.v.	106	80 – 132
Guinea pig	p.o.	1110	950 – 1270
	i.v.	127	123 – 132
Rabbit	p.o.	194	151 – 259

"Long-term Toxicity Studies: Diclofenac sodium was given orally to male and female rats in doses of 0.25, 1.0 and 2.0 mg/kg/day from 59 weeks (high-dose groups) to 98 weeks (low- and intermediate-dose groups). High dose-related mortality rates resulted in termination of high-dose administration after 59 weeks; the high mortality rate was caused by severe dose-dependent ulceration of the gastrointestinal tract, with perforated ulcers leading to peritonitis and sequelae. Body weight gains and feed consumption of the treated groups were close to the controls. Hematologic patterns showing neutrophilic leucocytosis and anemia were seen in the high- and intermediate-dose groups, particularly females at weeks 52 and 98, respectively. Female animals tended to develop enlarged adrenals and eventually experienced depressed glucose and elevated alkaline phosphatase levels. Histology studies carried out on the tissues of the control, low- and intermediate-dose groups showed drug-related changes including mucosal ulceration of the small intestine, lymphangiectasis, lymphoid hypoplasia, and plasma cell hypoplasia of the mesenteric lymph nodes, foci of hepatocytic hyperplasia, adrenal cortical atrophy and prostatitis. No increase in tumor incidence was observed in the drug-treated groups as compared to the control group.

Diclofenac sodium was administered orally in gelatin capsules once daily to baboons (Papio spp.) at dose levels of 0, 5, 15 (reduced to 10 on day 254) and 50 (reduced to 30 on day 38) mg/kg/day for up to 52 weeks. At all dose levels studied, diclofenac caused ulceration of the gastrointestinal tract. Ulceration was confined to the colon in the low-dose group but was present in the stomach and small intestine also in the other two groups. Body weights were below controls. Constipation, with occasional episodes of diarrhea, was a marked feature. In all treated groups, there was a dose-related fall in serum albumin levels. Anemia and an increased ESR were observed in the high-dose group. In the recovery groups (control, low, and intermediate), no intestinal lesions were present. Food consumption and body-weight gains were within normal limits. Hematology parameters were comparable to controls and serum albumin levels returned towards normal values."

DMSO: The acute and chronic toxicity of DMSO has been summarized by Willhite and Kazt (J. Appl. Toxicol. 4: 155-160, 1984) in their toxicology update as follows:

Acute Toxicity: *"A single i.v. injection of DMSO at 2.5-10 g/kg in mice or rats resulted in decreased motor activity and myasthenia. Prior to death, tremors, myasthenia, dyspnea and convulsions were evident. Oral intubation of 10-40 g/kg resulted in immediate decreased motor activity, ataxia, myasthenia and bradypnea. Rats also showed polyuria and polydipsia. Parenteral administration of DMSO was associated with tail catatony and hypothermia in mice. Profuse lacrimal secretions and diuresis with some hemoglobinuria were observed in rats. Injection of rats with 1.2-4.8 mL/kg, i.p. DMSO produced a dose-related increase in periportal fatty infiltration. The maximum effect occurred 12 h after administration and regressed after 24 h. Administration of 14.5 g/kg, p.o. to rats caused hyperemia and eye inflammation. Inhalation of 1600, 2000 or 2900 mg m⁻³ for 4, 24 or 40 h respectively, by rats, caused elevated serum urea nitrogen levels in the 2900 mg m⁻³ group. Garlic breath and microscopic pulmonary edema were seen in all groups.*

Intradermal injection of 50 µl of 99.0% DMSO into guinea pig ear induced swelling, mast-cell degranulation and histamine release.

Rabbits administered 40% DMSO i.v. experienced a rise in arterial pressure and a decrease in heart rate. Dermal application of DMSO caused a slight erythema. A Draize eye test resulted in a slight conjunctivitis which disappeared after 24 h. Conquet et al. noted that ocular instillation of 0.1 ml of 100% DMSO in rabbits caused reversible irritation of conjunctivae, while Brown et al. failed to observe adverse effects.

Single i.v. injection of 200 mg/kg DMSO into anesthetized cats produced apnea, transient hypotension and hemolysis with hemoglobinuria. Single doses of more than 400 mg/kg were fatal.

Injection of 5000-10,000 mg/kg i.v. in lightly anesthetized dogs caused a dose-dependent, transient tachycardia, tachypnea and hypertension.

In humans, topical and intradermal application of DMSO produced garlic breath, mast-cell degranulation, an increase in polymorphonuclear leukocytes and perivascular eosinophils, itching, and histamine-mediated and non-histamine-dependent whealing and erythematous flare. Two drops of > 50% DMSO in the eye caused a temporary burning sensation and vasodilation; concentrations of < 50% exhibited no effects."

The following table summarizes the LD₅₀ doses of DMSO in various species.

<i>Species</i>	<i>Route</i>	<i>LD₅₀ (g/kg)</i>
Mice	p.o.	21.4-28.3
	i.v.	3.1-9.2
	s.c.	15.3-25.6
	i.p.	17.7
Rat	p.o.	14.4-28.3
	i.v.	5.4-8
	s.c.	13.5-20.5
	i.p.	12.9
Guinea pig	p.o.	11
	i.p.	5.5
Dog	i.v.	2.5

Chronic Toxicity: *"Undiluted DMSO applied topically to mice twice a week for 30 weeks failed to produce dermal injury. Treatment of the Achilles tendon of mice between 0-21 days by rinsing the skin with 100 μ L of 70% DMSO decreased the mean separation force of the tendons 20.2%. After 3 weeks, the mean separation force underwent a net increase. After 4 weeks, the mean separation force decreased 9.4%.*

Chronic exposure of mice to DMSO by s.c. injection resulted in slightly irritated skin and mucosa, with pathological changes in the kidney and liver. Repeated doses of 2.5 g/kg, i.p. in mice caused tubulonephritis with diffuse necrosis of the intraperitoneal organs in the area of injection.

In mice and rats, chronic s.c. administration of DMSO produced muscle necrosis and inflammation with edema at the injection site. Mice developed liver adhesions, but rats failed to show consistent pathologic changes in any organ.

Oral intubation of 50% DMSO into rats at 5 g/kg for 45 days caused a slight loss in body weight, liver cell necrosis and inflammation with irritation of portal spaces. Daily intubation of 18 mL/kg 50% DMSO for 5 days per week for 18 months caused minimal changes in rat body weight, hematological values and the eye.

Rats injected i.p. with 0.5-8.0 g/kg DMSO 6 days per week for 4 weeks showed fibrinopurulent peritoneal exudate and peritoneal adhesions at the injection site. After the injections, all mice except those receiving the lowest dosage were restless, irritable and weak. The rate of growth was decreased in the 4-8 g/kg mice. The 8 g/kg mice showed reticulocytosis, anemia and increased mortality. Rats given 2 or 4 g/kg DMSO i.p., daily, for 28 days showed decreased weight gain and increased mortality. In vitro incubation and histological examination of rat kidney slices failed to show nephrotoxicity. Rats injected with DMSO daily at 7.5 mL/kg i.p. for 4 days showed fatty degeneration with congestion of the liver sinusoids, rounding of the liver margin, and fibrinous strands within the peritoneum.

Repeated inhalation of 200 mg m⁻³ DMSO, 7 h per day, 5 days per week for 6 weeks was not toxic in rats. Garlic breath was evident. Injury did not occur when DMSO was applied daily to the clipped backs of guinea pigs for 28 days.

Dermal application of 10 ml DMSO to the backs of rabbits for 15-20 days resulted in dry, cracked, scaly skin. At 1 month post-induction of the Dumonde Glynn model of arthritis in rabbits, the animals were treated with 80% DMSO at 1 g/kg topically to the knees, with one 0.5 ml injection of 80% DMSO at 1 g/kg to the knees, or with nothing for 5 days per week. After 3 months, knees treated with topical DMSO showed radiographic erosion, macroscopic cartilage loss, synovial hyperplasia, inflammatory infiltrate and increased joint circumference when compared to the control knees. Following 90 days of 50% DMSO at 8 mL/kg or 100% DMSO at 4 mL/kg to rabbits, lenticular changes were observed." (oral and dermal).

"Daily dermal or oral administration of 10 g/kg DMSO to rabbits caused lines of discontinuity in the lens at the end of the second week, haziness in the central nuclear region with continued administration, and by 5 weeks the lens became dense and myopic. After 10 weeks of continued administration, the lens cortex returned to normal and the nucleus was clear, but the lens was harder due to an increase in insoluble protein. Dermal application of 50% or 90% DMSO at the rate of 1.5 mL per kg per day of neat DMSO for 6 months to the normal or abraded skin of rabbits caused similar ocular disturbances.

Pigs required daily dermal application of 2.7 mL/kg DMSO to show ocular toxicity. Daily dermal application to pigs of 4.5 mL/kg 90% DMSO produced alterations of the lens.

In dogs, 0.4 g/kg DMSO i.v., daily, for 33 days and 1 mL/kg DMSO applied dermally to shaved backs 5 days per week for 15 months failed to induce toxicity. Dogs injected daily with 0.3-2.4 g/kg DMSO i.v. 6 days per week for 4 weeks showed perivascular inflammation and intravascular thrombi at the injection site. The dogs developed garlic breath, anemia, reticulocytosis, erythroid

hyperplasia in the bone marrow, hemoglobinuria and hematuria. In the liver, spleen, kidney and at the injection sites, an Fe-containing pigment collected. Cloudy swelling and granularity of the hepatic parenchyma were noted. Following 1.2 g per kg per day, biliary thrombi were seen, while at higher dosages, bilirubinuria was present. Serum transaminase values were slightly elevated. At 2.4 g/kg, both males died during the first injection, while the two females survived for another 3 weeks. Dermal application of 3.3-33 g per kg per week of 60-100% DMSO for 6 months to the shaved backs of dogs caused skin desquamation.

Three dogs were given daily doses of approximately 2.5, 5.0 or 10 g/kg p.o. 50% DMSO initially; then 25% DMSO after a few days. After 14 days, one dog died (average daily dose, 5.3 g/kg). Elevated hemoglobin, hematocrit, SGOT and SGPT were reported. Treatment continued with the second and third dogs receiving 5 g/kg for 35 days and 3.8 g/kg/day for 48 days, respectively. At the end of the treatment, faint anterior and posterior cortical bands in the lens of the eyes were present. Additional treatment of both dogs with 10 g/kg/day for 13 days produced myopia and lenticular changes. Dogs given daily oral doses of 5 g/kg DMSO 5 days per week for 9 weeks and 2.5 g/kg DMSO 5 days per week for 18 weeks exhibited myopia in the central region of the lens and hyperopia in the cortical region.

Oral administration of 50% DMSO to dogs 5 days per week for 2 years at 2-18 mL per day was associated with weight loss, hematological changes and diuresis. At the highest dose, myopia occurred after 5-10 weeks with transitory equatorial lens opacities by the 5th month. Similar changes occurred more slowly with lower doses. Early termination of DMSO administration led to a decrease in the myopia, but did not prevent opalescence. Decreased concentrations of soluble protein (α -crystallin), glutathione and water, with an increase in the amount of insoluble protein in the lens, were reported.

Instillation of 100 mL/kg of 50% DMSO into the bladder of dogs for 30 min, 8-10 times as frequently as in clinical practice, failed to induce local or systemic toxicity.

In monkeys, 0.4-4.0 g/kg DMSO i.v. or p.o. per day for 5 days or 4.0 g/kg DMSO i.v. per day, 5 days per week for 9.5 weeks, showed a dose related hemodilution and marked fluctuations in serum glutamic pyruvic transaminase, blood glucose, and platelet and leukocyte counts. No gross abnormalities were evident. Weekly cutaneous application of 33 g/kg 60-100% DMSO for 6 months to the shaved backs of monkeys resulted in skin desquamation. Monkeys orally administered 9 mL/kg DMSO daily for 14 weeks manifested lens changes, while monkeys receiving 5 g/kg/day for 100 days failed to show ocular toxicity. Daily i.v. doses of 3 g/kg 40% DMSO for 9 days caused diuresis, transient hemolysis, and central respiratory stimulation.

In humans, chronic dermal application of DMSO resulted in itching, whealing, erythema, eosinophilia, garlic breath, fatigue, headache, nausea, dizziness, burning or aching eyes, exfoliation, and pigmentation at the site of application. Volar skin surfaces of the knee and elbow, and skin of the face, neck and axillae were more sensitive to the local irritation caused by DMSO than other portions of the body. Topical application of 80% DMSO daily for 12 weeks failed to cause ocular toxicity in humans. Instillation of 7.5-60% DMSO in tetrahydrolazine for 1-15 months in patients with intraocular inflammatory diseases and ocular disorders failed to induce ocular toxicity. A review of papers concerned with topical application of DMSO revealed an absence of ocular toxicity in humans. Daily injection of 1 g/kg i.v. 10-40% DMSO resulted in dose-dependent hemolysis with hemoglobinuria and no sign of nephrotoxicity. Administration of DMSO i.v. has been reported to cause cerebral infarction and hepatic toxicity, but others have not considered DMSO the etiological agent."

V. GENETIC TOXICOLOGY

Diclofenac: The following is the information on the mutagenic potential of diclofenac found in the PDR (2002, online edition). *"Diclofenac sodium did not show mutagenic activity in in vitro point mutation assays in mammalian (mouse lymphoma) and microbial (yeast, Ames) test systems and was non-mutagenic in several mammalian in vitro and in vivo tests, including dominant lethal and male germinal epithelial chromosomal studies in mice, and nucleus anomaly and chromosomal aberration studies in Chinese hamsters."*

DMSO: From Willhite and Kazt (J. Appl. Toxicol. 4: 155-160, 1984). *"DMSO was not mutagenic in the Salmonella/mammalian microsome assay system. DMSO can, however, alter the mutagenic activity of other chemicals in Salmonella typhimurium by entering into chemical reactions."*

Male rats given 1-100% DMSO daily i.p. at 5 mL/kg for 5 days and examined 24 h after the last injection showed no significant differences in the number of chromosome breaks in bone marrow, but the numbers of chromatid breaks were elevated. The incidence of chromosome exchanges, rings and dicentric chromosomes was significantly increased following DMSO exposure. After 100% DMSO, there was a significant increase in the number of damaged cells. Overall, the incidence of aberrant cells increased from 10% at the 1% dose level to 68% at the 100% DMSO dose level."

The following table summarizes the findings of the literature that was submitted in this NDA and was reviewed. The result of the majority of these studies is negative. However, three studies reported positive findings in bacterial reverse mutation or chromosomal aberration assays.

Citation	Findings
Amacher, DE and Zelljadt I. Mutagenic activity of some clastogenic chemicals at the hypoxanthine guanine phosphoribosyl transferase locus of Chinese hamster ovary cells. <i>Mut. Res.</i> 136: 137-145 (1984)	DMSO was not mutagenic in CHO/HGPRT gene mutation assay at 2-16% (v/v) in the absence of metabolic activation.
Armstrong MJ et al. Induction of chromosome aberrations <i>in vitro</i> by phenolphthalein: mechanistic studies. <i>Mut. Res.</i> 136: 137-145 (1984)	DMSO was not clastogenic in CHO cells at 0.5-2%.
Bronzetti G et al. Protective effects of vitamins and selenium compounds in yeast. <i>Mut. Res.</i> 496: 105-115 (2001)	Irrelevant – No mention of DMSO.
Gorge, J. and Kuttan R. Mutagenic, carcinogenic and cocarcinogenic activity of cashew nut shell liquid. <i>Cancer Lett.</i> 112: 11-16 (1997)	No information on the genetic toxicity of DMSO – It was used as a solvent.
Hakura A. et al. Dimethyl sulfoxide (DMSO) is mutagenic for bacterial mutagenicity tester strains. <i>Mut. Res.</i> 303: 127-133 (1993)	DMSO (33%) was mutagenic.
Heidelberger C. et al. Cell transformation by chemical agents- a review and analysis of the literature. A report of the U.S. Environmental Protection Agency Gene-Tox Program. <i>Mutat. Res.</i> 114:283-385 (1983)	Incomplete paper. No cell transformation occurred at 0.0625-2% DMSO.
Kier LE et al. The <i>Salmonella typhimurium</i> /mammalian microsomal assay. A report of the U.S. Environmental Protection Agency Gene-Tox Program. <i>Mut. Res.</i> 168: 69-240 (1986)	Incomplete paper. No conclusion could be made.
Kinashi Y et al. Dimethyl sulfoxide protects against thermal and epithermal neutron-induced cell death and mutagenesis of Chinese hamster ovary cells. <i>Int. J. Radiat. Oncol. Biol. Phys.</i> 47:1371-1378 (2000)	Irrelevant – genotoxic potential of DMSO was not tested.
Latt et al. Sister-chromatid exchanges: a report of the GENE-TOX program. <i>Mutat. Res.</i> 87: 17-62 (1981)	DMSO (1.3×10^{-1} M) was not mutagenic in CHO cells in the absence or presence of metabolic activation.
Maron DM et al. Compatibility of organic solvents with the <i>Salmonella</i> /microsome test. <i>Mut. Res.</i> 88: 343-350 (1981)	Irrelevant – genotoxic potential of DMSO was not tested.
McCann et al. Detection of carcinogens as mutagens in the <i>Salmonella</i> /microsome test: Assay of 300 chemicals. <i>Proc. Natl. Acad. Sci.</i> 72: 5135-5139 (1975)	Illegible
Mortelmans K and Zeiger E. The Ames <i>Salmonella</i> /microsome mutagenicity assay. <i>Mut. Res.</i> 455: 29-60 (2000)	Irrelevant – genotoxic potential of DMSO was not tested.
Pearson TW et al. Natural occurring levels of dimethyl sulfoxide in selected fruits, vegetables, grains and beverages. <i>J. Agric. Food Chem.</i> 29: 1089-1091 (1981)	Irrelevant – genotoxic potential of DMSO was not tested.
Chemicals mutagenic in <i>Salmonella typhimurium</i> strain TA1535 but not in TA100. <i>Mut. Res.</i> 412: 251-260 (1998)	Irrelevant – genotoxic potential of DMSO was not tested.

Amyloid is not a tombstone - a summation - the primary role for cerebrovascular and CSF dynamics as factors in Alzheimer's disease (AD): DMSO, fluorocarbon oxygen carriers, thyroid hormonal and other suggested therapeutic measures. <i>Ann. N.Y. Acad. Sci.</i> 826: 348-374 (1997)	Irrelevant – genotoxic potential of DMSO was not tested.
Polar solvents: a novel class of antineoplastic agents. <i>J. Clin. Oncol.</i> 2: 227-241 (1984)	Irrelevant – genotoxic potential of DMSO was not tested.
Induction of chromosomal aberrations and sister-chromatid exchanges in Chinese hamster cells in vitro by some proximate and ultimate carcinogenic arylamide derivatives. <i>Mut. Res.</i> 88: 397-410 (1981)	DMSO (10%) caused chromosomal aberration in the presence of S9.
Janus carcinogens and mutagens. <i>Mut. Res.</i> 402: 321-329 (1998)	Irrelevant – genotoxic potential of DMSO was not tested.
Watanabe M et al. Radioprotective effects of dimethyl sulfoxide in golden hamster embryo cells exposed to gamma rays at 77K. II. Protection from lethal, chromosomal and DNA damage. <i>Radiat. Res.</i> 124: 73-78 (1990)	Irrelevant – genotoxic potential of DMSO was not tested.
Granulocytic differentiation of HL-60 cells results in spontaneous apoptosis mediated by increased caspase expression. <i>FEBS Lett.</i> 412: 603-609 (1997)	Irrelevant – genotoxic potential of DMSO was not tested.
Yee B et al. Biological effects of dimethyl sulfoxide on yeast. <i>Biochem. Biophys. Res. Comm.</i>	Irrelevant
	"It is highly unlikely that anyone would attempt to regulate a substance such as this on the basis of one isolated positive response in a bacterial test system"
Zeiger E et al. <i>Salmonella</i> mutagenicity tests: V. Results from the testing of 311 chemicals. <i>Env. Mol. Mutagen.</i> 19: 2-141 (1992)	DMSO (up to 10,000 µg/plate) was not mutagenic in TA 100, TA 1535, TA 97, TA 98 and TA 1537 strains.
Anwar W et al. Effect of dimethyl sulfoxide on the genotoxicity and metabolism of benzene in vivo. <i>Carcinogenesis</i> 10: 441- 445 (1989)	DMSO (1.25-12.5%, p.o.) was not clastogenic in mouse micronucleus assay.
Hrelia P et al. Dimethyl sulfoxide as modifier of the organospecific mutagenicity of metronidazole in mice. <i>Tertog. Carcinog. Mutagen.</i> 10: 263-271 (1990)	DMSO (5 g/kg, i.p.) was not mutagenic in a intrasanguineous host-mediated assay.
Kapp RW and Wventoff BE. Mutagenicity of dimethyl sulfoxide (DMSO): <i>In vivo</i> cytogenetics study in the rat. <i>Tertog. Carcinog. Mutagen.</i> 1: 141-145 (1980)	DMSO (1-100%, i.p., 5 days) caused significant increase in aberrant cells in bone marrow.
Krishna G and Hayashi M. In vivo rodent micronucleus assay: protocol, conduct and data interpretation. <i>Mut. Res.</i> 455: 155-166 (2000)	No mention of DMSO.
Lee WR et al. The sex-linked recessive lethal test for mutagenesis in <i>Drosophila melanogaster</i> . A report of the U.S. Environmental Protection Agency Gene-Tox Program. <i>Mut. Res.</i> 123: 183-279 (1983)	DMSO was not mutagenic in <i>Drosophila melanogaster</i> sex-linked recessive lethal test.

b(4)

Mollet P. Lack of proof of induction of somatic recombination and mutation in <i>Drosophila</i> by methyl-2-benzimidazole carbamate, dimethyl sulfoxide and acetic acid. <i>Mut. Res.</i> 40: 383-388 (1976)	DMSO (1%) was not mutagenic.
Taut H. The solvent dimethyl sulfoxide (DMSO) does not induce aneuploidy in oocytes of <i>Drosophila melanogaster</i> . <i>Environ. Mutagen.</i> 5: 273-277 (1983)	DMSO (2%) did not cause aneuploidy.
Valencia R et al. Chromosome mutation tests for mutagenesis in <i>Drosophila melanogaster</i> . A report of the U.S. Environmental Protection Agency Gene-Tox Program. <i>Mut. Res.</i> 134: 61-88 (1984)	Negative
Vogel EW and Nivard MJ. Performance of 181 chemicals in a <i>Drosophila</i> assay predominantly monitoring inter-chromosomal mitotic recombination. <i>Mutagenesis</i> 8: 57-81 (1993)	Negative

On May 9, 2002, the sponsor provided a safety update on the genotoxicity and carcinogenicity of DMSO. This update is a review of literature and evaluation of the reports in literature by _____ whose curriculum vitae was provided as evidence of his expertise. _____ concluded that *“the preponderance of the evidence shows that DMSO does not produce gene mutation in the standard bacterial tester strains or mammalian cell lines, induce chromosome aberration or micronuclei in mammalian cells in vitro or in vivo, or sister chromatid exchange in mammalian cells in vitro. The weight of evidence from in vivo bone marrow studies in rats and mice also support the conclusion that DMSO would not produce genetic damage at non-toxic doses. In fact, there are a number of reports that DMSO protects against chromosomal damage induced by radiation and chemicals.”*

b(4)

VI. CARCINOGENICITY

Diclofenac: The following is the information on the carcinogenic potential of diclofenac found in the PDR (2002, online). *“Long-term carcinogenicity studies in rats given diclofenac sodium up to 2 mg/kg/day (or 12 mg/m²/day, approximately the human dose) have revealed no significant increases in tumor incidence. There was a slight increase in benign mammary fibroadenomas in mid-dose-treated (0.5 mg/kg/day or 3 mg/m²/day) female rats (high-dose females had excessive mortality), but the increase was not significant for this common rat tumor. A 2-year carcinogenicity study conducted in mice employing diclofenac sodium at doses up to 0.3 mg/kg/day (0.9 mg/m²/day) in males and 1 mg/kg/day (3 mg/m²/day) in females did not reveal any oncogenic potential.”*

DMSO: From Willhite and Katz (J. Appl. Toxicol. 4: 155-160, 1984). *“No evidence for carcinogenic activity attributable to DMSO administration in common laboratory animals was observed in cancer-related experiments. Topical application of pure DMSO to normal, pre-cancerous or malignant human cervical epithelial cells in situ failed to induce structural or cytochemical changes.”*

The following table summarizes the findings of the literature submitted in this NDA that was reviewed:

Citation	Findings
Elzay RP. Dimethyl sulfoxide and experimental oral carcinogenesis in the hamster pouch. <i>Arch. Path.</i> 83: 293-297 (1967)	Not carcinogenic (100% DMSO)
Fletcher WS and Dennis DL. The effect of dimethyl sulfoxide on the induction of breast cancer in the rat. <i>Ann. N.Y. Acad. Sci.</i> 141: 214-220 (1967)	No DMSO alone group. Therefore, not a test of carcinogenic potential of DMSO
Kanisawa M and Suzuki S. Induction of renal and hepatic tumors in mice by ochratoxin A, a mycotoxin. <i>Gann</i> 69: 599-600 (1978)	DMSO (0.1 mL/20 g, p.o., 50 weeks?) was not carcinogenic.
McCabe D et al. Polar solvents in the chemoprevention of dimethylbezanthracene-induced rat mammary cancer. <i>Arch. Surg.</i> 121: 1455-1459 (1986)	No DMSO alone group. Therefore, not a test of carcinogenic potential of DMSO.
O'Dwyer PJ et al. Use of polar solvents in chemoprevention of dimethyl hydrazine-induced colon cancer. <i>Cancer</i> 62: 944-948 (1988)	No DMSO alone group. Therefore, not a test of carcinogenic potential of DMSO.
Shkler G et al. Chemical carcinogenesis of hamster mucosa. <i>Arch. Path.</i> 87: 637-642 (1969)	No effects (DMSO 3x per week for 8-12 week, 0.5%?).
Tennant RW et al. Prediction of chemical carcinogenicity in rodents from <i>in vitro</i> genetic toxicity assays. <i>Science</i> 236: 933 -941 (1987)	No mention of DMSO
Thunning CA et al. Mechanism of the synergistic effect of oral dimethyl sulfoxide on anti-neoplastic therapy. <i>Ann. N. Y. Acad. Sci.</i> 411: 150-160 (1983)	No DMSO alone group. Therefore, not a test of carcinogenic potential of DMSO.
VanDurren BL et al. Carcinogenicity of epoxides, lactones, and peroxy compounds. VI. Structure and carcinogenic activity. <i>J. Natl. Cancer Inst.</i> 39: 1217-1228 (1976)	DMSO (100%, cutaneous, 3x per week for 662 days) was not carcinogenic in mice.
VanDurren BL et al. Carcinogenicity of isomers of epoxides and lactones: aziridine, ethanol, propane, sultene and related compounds. <i>J. Natl. Cancer Inst.</i> 46: 143-149 (1971)	DMSO (100%, subcutaneous, for 76 weeks) was not carcinogenic in mice.
Warren J et al. Potentiation of anti-neoplastic compounds by oral dimethyl sulfoxide in tumor-bearing rats. <i>Ann. N.Y. Acad. Sci.</i> 243: 194-208 (1975)	No effects (DMSO, 1-2%, p.o. in drinking water)
Yaman O et al. Transmurally absorbed intravesical chemotherapy with dimethyl sulfoxide in an animal model. <i>Int. J. Urol.</i> 6: 87-92 (1999)	No DMSO alone group. Therefore, not a test of carcinogenic potential of DMSO
Zeiger E. Identification of rodent carcinogens and non-carcinogens using genetic toxicity tests: premises, promises and performance. <i>Regul. Toxicol. Pharmacol.</i> 28: 85-95 (1998)	No mention of DMSO
Zeiger E et al. Evaluation of four <i>in vitro</i> genetic toxicity tests for predicting rodent carcinogenicity: confirmation of earlier results with 41 additional chemicals. <i>Environ. Mol. Mutagen.</i> 6: 1-14 (1990)	No mention of DMSO

[illegible]

On May 9, 2002, the sponsor provided a safety update on the genotoxicity and carcinogenicity of DMSO. This update is a review of literature and evaluation of the reports in literature by _____ whose curriculum vitae was provided as evidence of his expertise. _____ concluded that *“although none of these studies have been performed according to the currently accepted 2-year rat and mouse*

b(4)

carcinogenicity study, the combined weight of the animal studies, in conjunction with the report of DMSO's lack of mutagenicity, and the finding that it does not induce pre-neoplastic transformation in vitro, is sufficient to support the presumption that DMSO would not be carcinogenic in rodents.” “Overall, the mutagenicity, cell transformation, and carcinogenicity data are consistent, and support a determination that DMSO should be classified as not mutagenic or carcinogenic.”

Labeling Recommendations: The label should essentially contain the information on the approved diclofenac label (VOLTAREN® and CATAFLAM®). In addition, the label should contain similar information to that found in the label for the approved DMSO product (RIMSO-50®).

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY

Diclofenac: The following is the information on the reproductive and developmental toxicity of diclofenac found in the PDR (2002, online). *“Diclofenac sodium administered to male and female rats at 4 mg/kg/day (24-mg/m²/day) did not affect fertility. Reproduction studies have been performed in mice given diclofenac sodium (up to 20 mg/kg/day or 60 mg/m²/day) and in rats and rabbits given diclofenac sodium (up to 10 mg/kg/day or 60 mg/m²/day for rats, and 80 mg/m²/day for rabbits), and have revealed no evidence of teratogenicity despite the induction of maternal toxicity and fetal toxicity. In rats, maternally toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival. Diclofenac has been shown to cross the placental barrier in mice and rats.”* Diclofenac is classified as Pregnancy Category B (PDR, Online, 2002).

DMSO: From Willhite and Katz (J. Tox. Appl. Pharmacol. 4: 155-160, 1984). *“Oral dosing of mice with 5-12 g/kg 50% DMSO on days 6-12 of gestation failed to cause malformations in the fetuses. However, i.p. administration caused malformed limbs (4%), anencephalia (2%) and celosomia (1%) in surviving fetuses. The resorption rate was not affected.*

Daily oral administration of 5 g/kg DMSO to both male and female rats for 4 days prior to mating and to the females throughout gestation failed to interfere with either embryonic development or fertility.

An i.p. injection of 1.0 mL/kg 40% DMSO in saline in rats on day 15 of gestation was associated with 44% reduction in the weight of the ovaries and a 34% reduction in the weight of the genital tract of the female offspring at 6 months post-partum. A 9% reduction in body weight and a 3% reduction in the weight of the testes occurred in the male offspring, but there was no effect on the combined weights of the seminal vesicles and the coagulating gland at 6 months post partum.

An i.p. injection of 40% DMSO in saline at 1.0 mL/kg on day 13 of gestation in rats was not teratogenic. Offspring of rats given 8-10 g/kg of a 50% solution of DMSO on days

6-12 of gestation exhibited malformations of the abdominal wall, central nervous system, limbs, jaw and tail. The largest dose killed 4 of 14 dams.

Injection of rats with 10.25 g/kg DMSO s.c. on days 8, 9 and/or 10 of gestation failed to induce gross or skeletal anomalies in the offspring, but the number of resorptions was significantly increased.

Injection of 5.5 g/kg i.p. on day 8 of gestation in the hamster induced dilated embryonic intercellular spaces, cell shrinkage and enlarged peripheral vascular channels in the cephalic and axial non-segmental mesoderm. Administration of DMSO to pregnant hamsters at 2.5 or 5.5 g/kg i.v. or at 5.5 or 8.25 g/kg i.p. on day 8 of gestation resulted in fetal exencephaly, rib fusion, microphthalmia, limb abnormalities and cleft lip.

Administration of 5 g/kg of 50% DMSO p.o. or 4 g/kg of 50% DMSO s.c. on days 6-14 of gestation failed to alter significantly the fetal rabbit body weights or the abortion or malformation rates."

DMSO is considered a "Pregnancy Category C" agent. According to the label for RIMSO®-50 (PDR, 2002, online), "Dimethyl sulfoxide caused teratogenic responses in hamsters, rats and mice when administered intraperitoneally at high doses (2.5 to 12 gm/kg). Oral or topical doses of dimethyl sulfoxide did not cause problems of reproduction in rats, mice and hamsters. Topical doses (5 gm/kg first two days, then 2.5 gm/kg, last eight days) produced terata in rabbits, but in another study, topical doses of 1.1 gm/kg days 3 through 16 of gestation failed to produce any abnormalities."

The following table summarizes the findings in the literature that were submitted in this NDA and were reviewed:

Citation	Findings
Caujolle FME et al. Limits of toxic and teratogenic tolerance of dimehtyl sulfoxide. <i>Ann. N.Y. Acad. Sci.</i> 141: 110-125 (1967)	As 50% solution Mice: - 5-12 g/kg, 6-12th gestation day, p.o., i.p. - No effect p.o., 7/100 malformed ip Rats: - 5-10 g/kg, 6-12th gestation day, p.o., i.p. - ↑ % absorption and malformation, ↓ body weight, i.p. - 4/14 dead ♀ at 10 g/kg, i.p. Rabbits: - 5 g/kg p.o., 4 g/kg s.c., 6-14th gestation day - no effects, 1/83 with celosomia
Dresser TH et al. Teratogenic assessment of four solvents using the frog embryo teratogenesis assay-Xenopus (FETAX). <i>J Appl. Toxicol.</i> 12: 49-56 (1992)	0.25 – 2% dose-dependent increase in malformation such as ocular and gut abnormalities

Juma MB and Staples RE. Effect of maternal administration of dimethyl sulfoxide on the development of rat fetuses. <i>Proc. Soc. Exp. Biol. Med.</i> 125: 567-569 (1967)	10.25 g/kg, 3 days (8, 9, 10 th gestation), s.c. - 1 number of fetuses per litter - no gross or skeletal malformation except two umbilical hernia
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Labeling Recommendations: The label should essentially contain the information on the approved diclofenac label (VOLTAREN® and CATAFLAM®). In addition, the label should contain similar information to that found in the label for the approved DMSO product (RIMSO-50®).

VIII. SPECIAL TOXICOLOGY STUDIES: None.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS

The following is the summary and evaluation and recommendation parts of Dr. Coulter's review.

SUMMARY AND EVALUATION:

"PENNSAID™ (1.5% diclofenac sodium) Topical Lotion is indicated for initial use in topical treatment of _____

_____ The product is intended for external use only. PENNSAID™ is the product of Dimethaid Research Inc., a Canadian firm. PENNSAID™ contains, in addition to diclofenac sodium, dimethyl sulfoxide USP (DMSO), glycerin USP, propylene glycol USP, alcohol USP, and purified water USP. DMSO is present at 455 mg/g or 45.5% and is used as a skin penetrant for diclofenac sodium.

The recommended clinical dose is _____

No pre-clinical toxicity studies were submitted with this application. The submitted reprints and documents relate to the primary and secondary action, toxicity, reproduction, mutagenicity, and ADME studies for DMSO and diclofenac. As was agreed upon at the June 6, 1995 meeting with the company, human data generated in Canada with PENNSAID™ could replace a 90-day rat toxicity study. Pharmacology and toxicology data diclofenac sodium were obtained from several scientific literature reviews and documents.

Acute LD₅₀ toxicity of 100% DMSO in mice, with a 14-day observation period, was 5.7 g/kg iv and 21.4 g/kg po. The acute 24 hr LD₅₀ was 13.7 g/kg sq administered 50% DMSO. Values in rats were 5.4 g/kg iv and 28.3 g/kg oral. Twenty-four hour LD₅₀ data for 50% DMSO in mice were 11.0 g/kg, iv, 20.1 g/kg ip, and 16.0 g/kg for ip and sq administration were reported. Anesthetized dogs died from iv doses of 30-40 g of 50% DMSO.

In subacute and chronic toxicity studies with DMSO, Carworth CFN rats dosed four weeks with 100% DMSO at 0, 0.5, 1, 2, 4, and 8.0 g/kg ip developed anemia, WBC decreases, and decreased lymphocytes. Body weight gain was significantly reduced, and mortality occurred at 2 g/kg after 21 doses. Mice and rats have also been treated ip, sq, and po with 50% DMSO for 3 to 12 weeks. The sq route produced less tissue damage and was tolerated better than either iv or po administration. Weight loss occurred in mice at 5 g/kg po, followed by weight gain. In mice, tubulonephritis and diffuse necrosis of the intraperitoneal organs occurred following ip administration of 2.5 g/kg. Rats showed growth reduction and weight loss at 1 g/kg ip, but no weight loss was seen at 2 g/kg po. In rats, liver cell necrosis and inflammation of the portal spaces occurred with oral administration of 5 g/kg of 50% DMSO for 45 days.

Male dogs died at 2.4 g/kg during the first injection, with females dying later in the study. Toxicity included weight loss, anemia (decreased RBCs, Hb, and Hct), with hemoglobinuria, hematuria, and swelling and granularity of liver parenchymal cytoplasm seen at all doses. In dogs, perivascular inflammation and thrombi occurred at all doses (0.3, 0.6, 1.2, and 2.4 g/kg/day) when injected with, but could be reduced by diluting with 5% dextrose. Anemia, weight loss, increase in reticulocytes, hemoglobinuria, and hematuria were also observed. Yellow brown pigment was reported in the liver, spleen, kidneys and injection sites.

Oral and dermal administrations of 0, 1, 3, and 9 mL/kg/day or 90% aqueous DMSO (90% DMSO $\equiv$ 0.95 g/mL) were administered twice a day to rhesus monkeys in equally divided portions for 74 to 87 weeks. At 9 mL/kg po, anorexia and death occurred in some monkeys, and was attributed to emphysema and atelectasis that may have been the result of "regurgitation or tracheal inspiration of the test material." Topical application over the entire abdominal area produced scaling, flaking, and erythema. No significant changes were reported in BP, heart or respiration rate, temperature, water consumption, EKGs, or in neurological reflexes. Anemia was not observed in this study. Other hematology, blood chemistry, or urinalysis parameters, and organ weights were not affected. No evidence of lenticular changes was reported in these dogs. Dermal applications up to 9 mL/kg/day were well tolerated by these animals.

The teratogenic potential of DMSO has been studied in a) 129 Swiss mice (27 controls and 102 treated) administered at 0, 5, 8, 10 and 12 g 50% DMSO/kg/day po and ip from the 6th to the 12th day of gestation, b) in Wistar rats (59 controls and 91 treated) administered at 5 and 10 g 50% DMSO/kg/day po and at 5, 8, and 10 g/kg/day ip from the 6th to the 12th day of gestation, c) in 6 control and 10 treated Fawn Burgundy rabbits administered at 5 g/kg of 50% DMSO/kg/day po and 4 g/kg/day sc from the 6th to 14th day of gestation, and d) in golden hamsters administered at 50-8250 mg/kg iv on the 8th day of gestation.

No malformations were observed in mice dosed orally, but ip administration produced anencephaly, malformed limbs, and one case of celosomia in the treated mice. No malformations were reported in control mice treated with saline.

In rats, abortions were dose related with oral or ip administration of DMSO. Mortality occurred at 10 g/kg. Malformations (anencephaly, microphalia, celosomia) occurred at 5 and 10 g/kg po and at 8 g/kg ip. Eleven of the 729 live rat fetuses from dams injected ip developed malformations (1.5%). Only 1 of the 558 control rat fetuses (0.18%) was reported as showing "slight" celosomia.

One of the 83 rabbit fetuses was found with celosomia (1.2%). Weight changes or number of aborted embryos in the treated rabbits were indicated as not being remarkable when compared to controls.

Teratogenic effects in Golden hamsters were evaluated following intravenous administration of 50 to 8250 mg/kg DMSO on the 8th day of gestation. Dilutions were in saline. Intraperitoneal doses of 5500 and 8250 g/kg were also administered and studied. At ≥ 2500 mg/kg iv, non-closure of the neural tube, cleft lip and palate, and limb and rib abnormalities occurred. At 5500 mg/kg, the number of dead and malformed embryos increased. Exencephaly was the most common malformation. One other study in Golden hamsters with doses of 5 and 10 mL/kg DMSO (5.5 and 11 g/kg) administered on Day 7 or 8 of gestation produced exencephaly, fused ribs, cleft palate or hairlip, short or curved tail, and cranial pimple. Embryo toxicity also occurred at 5 mL/kg.

The injection of either Rhode Island or Leghorn chick embryos with 10.3 mg 50% DMSO in 0.9% saline/embryo produced a left torsion of the beak with reduction of the upper beak, celosomia, anophthalmia, and malformed limbs. Embryos were injected and followed for either 72 or 92 hours of incubation. Controls received an equal volume of 0.9% saline.

DMSO was reported as negative in the Ames Salmonella typhimurium assay that was published by Bruce Ames in 1975. The assay evaluated DMSO in strains TA 98, TA 100, TA 1535, and TA 1537 in the presence of S9. DMSO was positive in the in vivo cytogenic assay in rat bone marrow cells. All dose groups evaluated with 1% to 100% DMSO showed a statistically significant increase in cells with aberrant chromosomes.

RECOMMENDATIONS:

There are no objections to the approval of this drug, based on the pharmacology/toxicology of the data submitted to the NDA. There are several changes that will be required in the labeling.

- 1. Under Dimethyl sulfoxide in the Carcinogenesis, Mutagenesis, and Impairment of Fertility section, the statement should read: No carcinogenicity studies have been conducted with DMSO. DMSO was found to be non-mutagenic in the Ames test. Chromosome damage in rat bone marrow cells was significant at 1% to 100% DMSO when administered intraperitoneally.*

2. *Under the pregnancy section for Dimethyl Sulfoxide, the paragraph should read: High single, topical doses of 5-12 g/kg of 50 DMSO did not demonstrate any effect on the reproductive process of hamsters, mice or rats. However, in one rabbit oral study, administration of 5 mg/kg of 50% DMSO for 2 days followed by 2.5 g/kg for 8 days produced terata. DMSO was embryotoxic and teratogenic in hamsters administered 2.5 g/kg intravenously."*

Conclusions: In humans, application of 1 mL of PENNSAID® to the knee 4 times daily for 12 weeks resulted in peak serum level below 50 ng/mL for diclofenac and 200 ng/mL of DMSO. After oral administration of a 50 mg dose to humans, the maximum serum level of diclofenac was 1417 ng/mL (Diclofenac, Online PDR, 2002). The level of diclofenac after topical application is far below that resulting from systemic administration. Therefore, topical application of diclofenac is not expected to results in exposure to unsafe systemic levels of diclofenac.

Systemic exposure to DMSO from PENNSAID® (4 times daily for 12 weeks) was below 200 ng/mL. Results from a large number of published studies indicate that DMSO is not very toxic in animals as evidenced by the lack of significant toxic manifestation at milligram per kilogram body weight doses. DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically. In contrast, in humans, DMSO did not cause ocular damage when administered topically at 80% for 12 weeks, or when it was instilled at 7.4-60% in tetrahydrolazine for 1-15 months for treatment of ophthalmic inflammatory diseases (Hull et al., 1969 and Gordon, 1967). After PENNSAID® treatment, systemic exposure to DMSO (200 ng/mL) was far below that which could result from doses that caused toxicity in humans. The remaining concern with the safety of DMSO is with its potential for skin toxicity. According to the Dr. Coulter's review, the Division agreed that the clinical skin toxicity evaluation could replace the 90-day non-clinical study. Therefore, the medical officer's review should be consulted for the results of clinical skin toxicity evaluation.

Concomitant administration of PENNSAID® with systemic NSAIDs should be done cautiously because an arthritic patient who was being treated with sulindac for 6 month developed peripheral neuropathy after initiation of topical application of 90% DMSO (Reinstein et al., 1982).

General Toxicology Issues: None.

Recommendations: Based on the non-clinical information provided in this NDA and the previous review, the drug is approvable.

Labeling with Basis for Findings: The label should essentially contain the information on the approved diclofenac label (VOLTAREN® and CATAFLAM®). The sponsor has provided information on a report indicating that ophthalmic administration of diclofenac exacerbated the asthma in an asthmatic patient. This information should

be included in the label. In addition, the label should contain similar information to that found in the label for the approved DMSO product (RIMSO-50®). In particular, information on the potential for ocular toxicity of DMSO should be included.

The label for RIMSO®-50 indicates that “*since lens changes were noted in animals, full eye evaluation, including slit lamp examination, are recommended prior to and periodically during treatment.*” Although the exposure to DMSO through PENNSAID® may not be as high as that through RIMSO®-50, inclusion of this recommendation should be considered in the PENNSAID® label.

The followings are the suggested changes to the label submitted by the sponsor.

Precautions:

Add the following:

DMSO caused changes in refractive index and lens opacity in rabbits, dogs and monkeys at high doses (g/kg) when administered chronically. In humans, daily topical administration of 80% DMSO for 12 weeks did have any ocular toxicity. Instillation of 7.5-60% DMSO in tetrahydrolazine for 1-15 months for tretment of intraocular inflammatory diseases did not cause ocular toxicity.

Concomitant administration of PENNSAID® with systemic NSAIDs should be done cautiously because an arthritic patient who was being treated with sulindac for 6 month developed peripheral neuropathy after initiation of topical application of 90% DMSO.

Carcinogenesis, Mutagenesis, Impairment of Fertility:

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Suggested Changes: None.

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Suggested Changes: DMSO (100%) was not carcinogenic when applied to hamster oral pouch, or administered cutaneously or subcutaneously to mice for 76 to 94 weeks. DMSO was not mutagenic in bacterial reverse mutation assay (Ames). However, chromosomal damage was observed when rats were treated with 1-100% DMSO intraperitoneally. The effects of DMSO on fertility have not been studied.

Pregnancy:

Teratogenic Effects:

Suggested Changes:

Diclofenac Sodium: Pregnancy Category C. Diclofenac sodium was not teratogenic when administered up to 20 mg/kg/day (60 mg/m²/day) in mice and up to 10 mg/kg/day in rats and rabbits (60 mg/m²/day for rat and 80 mg/m²/day for rabbits). Dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival was observed in rats at maternally toxic doses.

DMSO: DMSO was teratogenic when administered intraperitoneally to hamsters, rats and mice at 2.5 to 12 g/kg. In contrast, oral or topical administrations did not affect reproduction. In one study, terata were observed in rabbits treated with topical DMSO (5 g/kg for two days, then 2.5 gm/kg, for eight days). However, in another study, no effects were observed in rabbits treated with topical DMSO (1.1 g/kg from days 3 to 16 of gestation).

X. APPENDIX/ATTACHMENTS: None.

XI. REFERENCES:

- Gordon, DM. Dimethyl sulfoxide in ophthalmology, with special reference to possible toxic effects. *Ann. N.Y. Acad. Sci.*, 141: 392-401 (1967).
Hull FW, Wood DC and Brobyn RD. Eye effects of DMSO, Report of negative results. *Northwest Med.*, 39-41 (1969).
Szczeklik A, Gryglewski, RJ, Czerniawaska-Mysik G. and Pieton R. *Br. Med. J.*, 23: 231-232 (1977).
Willhite, CC and Katz, PI. Toxicology Updates: Dimethyl sulfoxide. *J. Appl. Toxicol.*, 4: 155-160 (1984).

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this page is the manifestation of the electronic signature.**

/s/

Hamid Amouzadeh
7/17/02 02:24:33 PM
PHARMACOLOGIST

Josie Yang
7/17/02 03:20:22 PM
PHARMACOLOGIST

1. /
JUL 17 1998

THE DIVISION OF ANTI-INFLAMMATORY, ANALGESIC,
AND OPHTHALMIC DRUG PRODUCTS

PHARMACOLOGY/TOXICOLOGY REVIEW
ORIGINAL SUMMARY

NDA 20-947

DRUG: PENNSAID™ (1.5% diclofenac sodium) Topical Lotion

SPONSOR: Dimethaid Research Inc.
1405 Denison Street
Markham, Ontario, L3R 5V2 Canada

SUBMISSION: December 15, 1997

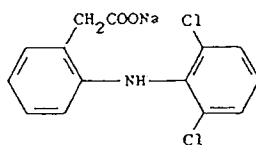
RECEIVED: December 16, 1997

REVIEWER: Almon W. Coulter, Ph.D.

REVIEW COMPLETED: July 16, 1998

DRUG CATEGORY: Anti-inflammatory

STRUCTURAL
FORMULA:



$\text{C}_{14}\text{H}_{10}\text{NCl}_2\text{O}_2\text{Na}$ Mol Wt: 318.13

Chemical Names:

- a) 2-[(2,6-Dichlorophenyl)amino]benzeneacetic acid monosodium salt
- b) [O-(2,6-Dichloroanilino)phenyl] acetic acid sodium salt
- c) Sodium [O-[(2,6-dichlorophenyl)amino]phenyl acetate

Established Name: Diclofenac Sodium USAN and USP

Proprietary Name: PENNSAID™

CAS Registry No: 15307-79-6

Solubility: Soluble in MeOH and EtOH, slightly soluble in acetone,
and sparingly soluble in water (2% at pH 7.7).

COMPOSITION:

INGREDIENT	CONCENTRATION (mg/g)
Diclofenac Sodium USP	15.0*
Dimethyl Sulfoxide USP	455.0
Glycerin USP	_____
Propylene Glycol USP	_____
Alcohol USP	_____
Purified Water USP	_____
Total	1000.0

PROPOSED MARKETING INDICATION:

PENNSAID™ is indicated for _____

RECOMMENDED CLINICAL DOSE:

RELATED DRUGS/INDs/NDAs:

INDs: _____ 42773
(Dimethaid Research Inc.), _____

NDAs: 19-201, 20-142, 20-254

PERTINENT MEMOS/CORRESPONDENCE:

The sponsor was informed of the following IND 42,773 clinical and preclinical deficiencies in a letter dated September 16, 1993.

Clinical

- a) Cadaver skin studies are needed in order to evaluate skin penetration.
- b) Preclinical toxicology data from the two active ingredients, as well as the drug product, should be provided prior to initiation of clinical efficacy studies (see below).

Preclinical

- a) If further development of this product is planned, it will be necessary to separate out the pharmacodynamic activity contributed by the drug diclofenac and DMSO, which has anti-inflammatory properties and therefore cannot be considered an inactive excipient as you propose. In addition, the following are required:
- b) A PK study of _____ in animals should be performed to determine the amount of diclofenac absorbed from _____. Other PK parameters should also be determined, such as elimination half-life and systemic distribution. The metabolic profile for diclofenac should be determined for this route of administration.
- c) A drug interaction study with DMSO should be conducted.
- d) A toxicity study will be needed to determine if diclofenac toxicity is increased by this route of exposure. The length of this study will depend on the proposed duration of human use. For chronic use in humans, a 90-Day study will be required.

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In addition, the following concerns were to be addressed as soon as possible:

- e) It was recommended that skin metabolism information be obtained from cell culture techniques.
- f) A rationale should be submitted to indicate there is no change in the systemic carcinogenicity or teratogenicity potential, or the potential to produce skin cancer, or mediate photocarcinogenicity by the intended route of administration. Animal PK data and metabolic profile can be the starting basis for the rationale.
- g) It should be determined if serum protein binding of diclofenac is altered by expected human serum concentrations of DMSO.

At our meeting with Dimethaid held on 6/6/95, the following information was exchanged:

- 1) FDA agreed the metabolic profile from cadaver skin could substitute for a cell culture profile to obtain skin metabolism data.
- 2) The potential for skin sensitization, skin phototoxicity, and photocontact allergy will be evaluated by Dimethaid in clinical studies.
- 3) Human transdermal bioavailability could be substituted for the recommended animal study.

- 4) Safety data from humans evaluated in Canada could replace a 90-day animal study.

PRECLINICAL STUDIES:

The nonclinical pharmacology/toxicology portion of this submission is found in Vols. 1.16 and 1.17. These two volumes contain documents or reprints related to the pharmacology/toxicology of DMSO, diclofenac, or diclofenac sodium. A complete list follows:

Primary Action:

- 1) Kadar D. Anti-inflammatory analgesics. In Kalant H, Roschlau WHE. Principles of Medical Pharmacology. Fifth ed. Toronto: BC Decker Inc., 1989, p. 325-326.
- 2) Menassé R, et al. Pharmacological properties of diclofenac sodium and its metabolites. Scand J Rheumatology 1978; 22 Suppl: 5-16.
- 3) Hiramatsu Y, et al. Assessment of topical nonsteroidal anti-inflammatory drugs in animal models. Arzneim.-Forsch./Drug Res 1990; 40(II)10:1117-1124.
- 4) Kyuki K, et al. Anti-inflammatory effect of diclofenac-sodium ointment (cream) in topical application. Japan J. Pharmacol. 1983; 33:121-132.

Secondary Action:

- 1) O'Brien WM. Adverse reactions to nonsteroidal anti-inflammatory drugs: Diclofenac compared with other nonsteroidal anti-inflammatory drugs. Am J Med 1986; 80 Suppl 4B: 70-80.
- 2) Szczeklik A, et al. Asthmatic attacks induced in aspirin sensitive patients by diclofenac and naproxen. Br Med J 1977 July 23: 231-232.
- 3) Kligman AM. Topical pharmacology and toxicology of dimethyl sulfoxide. JAMA 1965: 193; 10: 796-804(Pt 1); 11:923-28(Pt 2).
- 4) Allenby AC, et al. Mechanism of action of accelerants on skin penetration. Br J Derm 1969; Suppl. 4:47-55.
- 5) Vogin EE, et al. Chronic toxicity of DMSO in primates. Toxicology and Applied Pharmacology 1970: 16: 606-612.

Acute Toxicity Studies:

- 1) CIBA-GEIGY Canada Limited, VOLTAREN (diclofenac sodium) Product Monograph (Date of Revision Nov. 7, 1989).
- 2) Menassé R, et al. Pharmacological properties of diclofenac sodium and its metabolites. Scand J Rheumatology 1978; 22 Suppl: 5-16.

- 3) Wilson JE, et al. A toxicologic study of dimethyl sulfoxide. Toxicology and Applied Pharmacology 1965; 7: 104-112.
- 4) Caujolle FME, et al. Limits of toxic and teratogenic tolerance of dimethyl sulfoxide. Ann NY Acad Sci 1967: 141:110-125.

Subchronic/Chronic/Carcinogenicity Studies:

- 1) PDR 48th ed. Voltaren; p. 996-998.
- 2) Brobyn, RD. The human toxicity of Dimethyl sulfoxide. Ann NY Acad Sci 1975; 243:497-506.
- 3) Vogin EE, et al. Chronic toxicity of DMSO in primates. Toxicology and Applied Pharmacology 1970: 16:606-612.
- 4) Swanson BN. Medical uses of DMSO. Reviews in Clinical and Basic Pharmacology 1985; 5:1-33.
- 5) McCann J, et al. Detection of carcinogens as mutagens in the Salmonella microsome test: Assay of 300 chemicals. Proc Nat Sci USA 1975; 72(12): 5135-39.

Special Toxicity Studies:

- 1) Jobin F and Gagnon FT. Inhibition of human platelet aggregation by a dibenzazepine compound (GP 45296) and by N-(2,6-dichlorophenyl)-O-aminophenylacetic acid (GP 45840). Canadian Journal of Physiology and Pharmacology 1971; 49:479-481.
- 2) Ursin C, et al. Permeability of commercial solvents through living human skin. Am Ind Hyg Assoc j 1995; 56:651-660.

Reproduction Studies:

- 1) Montenegro MA and Palomino H. Induction of cleft palate in mice by inhibitors of prostaglandin synthesis. Journal of Craniofacial Genetics and Developmental Biology 1990; 10:83-94.
- 2) Ferm VH. Congenital malformations induced by dimethyl sulphoxide in the golden hamster. J Embryol exp Morph 1966; 16,1:49-54.
- 3) Robens JF. Teratologic studies of carbaryl, diazinon, norea, disulfiram, and thiram in small laboratory animals. Toxicology and Applied Pharmacology 1969; 15:152-163.

Mutagenicity Studies:

- 1) Kadatoni S, et al. Mutagenicity examination of several non-steroidal anti-inflammatory drugs in bacterial systems. Mutation Res 1984; 138:133-136.

- 2) McCann J, et al. Detection of carcinogens as mutagens in the Salmonella microsome test: Assay of 300 chemicals. PNAS USA 1975;72(12):5135-5139.
- 3) Yee B, et al. Biological effects of dimethyl sulfoxide on yeast. Biochem Biophys Res Commun 1972, Dec 4;49(5): 336-342.
- 4) Kapp RW Jr. and Eventoff BE. Mutagenicity of DMSO in vivo cytogenetics study in the rat. Teratog Carcinog Mutagen 1980;1(2):141-5.

Absorption/Distribution/Metabolism and Excretion (ADME) Studies:

- 1) Riess W, et al. Pharmacokinetics and metabolism of anti-inflammatory agent Voltaren. Scand J Rheumatology 1978; Suppl 22:17-29.
- 2) Riess, et al. Percutaneous absorption of diclofenac. Pharma Res Dev 1986;3-7.
- 3) Hartway T, et al. In vitro percutaneous absorption of [¹⁴C]-Diclofenac sodium in human skin. Final Report San Francisco (CA): Surge Laboratory, Department of Dermatology, University of California; 1996 Dec. Study N° UCSF95SU03. Sponsored by Dimethaid Research inc.
- 4) Hewitt PG, et al. Bioavailability of [¹⁴C]-Diclofenac in vitro in human skin. Final Report San Francisco (CA): Surge Laboratory, Department of Dermatology, University of California; 1997 Oct. Study N° UCSF96SU05. Sponsored by Dimethaid Research Inc.

Other Articles Referenced in Package Insert:

- 1) USP Convention Inc. Anti-inflammatory drugs, Nonsteroidal, Systemic. In: Drug information for the health care professional. 17th ed. Rockville, MD; 1997; 1:379-426.
- 2) USP Convention Inc. Chemistry and Compendial Requirements: Diclofenac. In: Drug information for the health care professional 17th ed. Rockville, MD; 1997; 3:164.
- 3) Diclofenac Sodium. The Merck Index, 11th ed. 1989, p. 486.
- 4) Chandrasekaran SK, et al. Effect of dimethyl sulfoxide on drug permeation through human skin. Am Inst Chem Eng Journal 1977; 23(6):810-816
- 5) Cooper ER and Berner B. Penetration enhancers. In: Kydonieus AF and Berner B, editors. Transdermal delivery of drugs. Vol. II CRC Press, 1987:57-62.
- 6) Guy RH and Maibach HI. Drug delivery to local subcutaneous structures following topical administration. J Pharm Sci 1983; 72(12):1375-80.
- 7) Khan ZU and Kellaway IW. Differential scanning calorimetry of dimethyl sulphoxide treated human stratum corneum. Int J Pharm 1989; 55:129-134.

- 8) Kurihara-Bergstrom T, et al. Physicochemical study of percutaneous absorption enhancement by dimethyl sulfoxide: kinetic and thermodynamic determinants of dimethyl sulfoxide mediated mass transfer of alkanols. J Pharm Sci 1986;75(5):479-86.
- 9) Montes LF, et al. Ultrastructural changes in the horny layer following local application of dimethyl sulfoxide. J Invest Derm 1967; 48(2):184-96.
- 10) Sandborn EB, et al. The influence of dimethyl sulfoxide on cellular ultrastructure and cytochemistry. Ann NY Acad Sci 1975; 243:122-138.
- 11) Chan KK, et al. In vitro protein binding of diclofenac sodium in plasma and synovial fluid. J Pharm Sci 1987; 76(2):105-108.
- 12) Gabrielsen TØ, et al. Drug induced bullous dermatosis with linear IgA deposits along the basement membrane. Acta Dermatovener (Stockholm) 1981; 61:439-441.
- 13) Sulzberger MB and Cortese, Jr TA. Some effects of DMSO on human skin in vivo. Ann NY Acad Sci 1967; 141:437-50.
- 14) Roberts Pharmaceutical Canada Inc., RIMSO-50 (dimethylsulfoxide 50%) Product Monograph (September 21, 1979).

Other Articles Referenced in Pharmacologic Class, Scientific Rationale, Intended Use and Potential Benefits:

- 1) Montes LF, et al. Ultrastructural changes in the horny layer following local application of dimethyl sulfoxide. The Journal of Investigative Dermatology, 1967. 48(2):184-196.
- 2) Sandborn EB, et al. The influence of dimethyl sulfoxide on the cellular ultrastructure and cytochemistry. Ann NY Acad Sci, 1975. 243:122-138.
- 3) Sandborn EB and Bendayan M. The existence of a circulatory system in animal cells. Cell Vision, 1996. 3(3):180-190.

ABBREVIATIONS USED IN THIS REVIEW

DMSO = dimethyl sulfoxide W = week(s) DR = dose related
G = group(s) D = day(s) ↓ = decrease(d) ↑ = increase(d)

PHARMACOLOGY AND TOXICOLOGY OF DICLOFENAC SODIUM:

Pharmacological Properties of Diclofenac Sodium and its Metabolites:
Menasse R, et al. Scand J Rheumatol 1978;22 Suppl:5-16.
NDA Vol. 1.16, p. 14.

Anti-inflammatory potencies of NSAIDs and their inhibitory effect on prostaglandin synthetase in vitro are indicated in the following table (from

Table 2, p. 7 of reprint).

Preparation	Carrageen ED ₅₀ (mg/kg po ¹)	Kaolin ED ₅₀ (mg/kg po ¹)	Adjuvant Arthritis Reduction ED ₅₀ (mg/kg po ²)	Synthetase Inhibition IC ₅₀ μM/L
Diclofenac sodium	2.1	1.2	0.26	1.6
Acetylsalicylic acid	900	215	315	3300
Ibuprofen	170	63	>100 ³	39
Indomethacin	5.2	5.3	0.4	5.6
Naproxen	20	4.5	8	5.7
Phenylbutazone	50	42	25	490

¹ Determined by graphic interpolation from ≥3 doses.

² Calculated by the use of regression analysis.

³ 35% decrease in swelling at the highest dose level tested (100 mg/kg/day).

Analgesic and antipyretic potency of diclofenac sodium and other anti-inflammatory agents are indicated in the following table (from Table 3, p.8 of the reprint).

Preparation	Analgesic Potency			Antipyretic Potency
	Phenyl-p-Benzo- quinone Test Mouse ED ₅₀ mg/kg po	Acetic Acid Test, Rat ED ₅₀ mg/kg po	Ethacrynic Acid Test, Rat ED ₅₀ mg/kg po	Yeast Induced Fever, Rat mg/kg po ¹
Diclofenac sodium	4.3	2.5	1.4	0.5
Acetylsalicylic acid	165	65	88	185
Ibuprofen	44	10	nt ²	24
Indomethacin	2.7	2	2.5	1.2
Naproxen	22	nt ²	nt ²	55
Phenylbutazone	95	35	62	35

¹ Doses lowering body temperature by 1.5°C.

² Not tested.

- metabolites of diclofenac sodium had ED₅₀ >100 mg/kg compared to the diclofenac sodium value of 0.26 mg/kg po-
- LD₅₀ ♂ = 240 mg/kg po - ♀ 226 mg/kg po in rats-

Anti-inflammatory Effect of Diclofenac Sodium Ointment (Cream) in Topical Application. Kyuki K et al., Japan J Pharmacol 1983;33:121-132. NDA Vol. 1.16, p. 34.

These authors evaluated the anti-inflammatory effects of several

diclofenac sodium ointments in animal models. The ointments contained either a lipophilic base, an emulsion (cream) base, or a gel base. Of the three bases, the creams containing 1.0% or 1.5% diclofenac sodium gave optimum results and were said to be superior to other existing ointments. Inhibitory effects were evaluated on vascular permeability induced by histamine, carrageenin-induced edema, croton oil ear edema, paw edema in the rat, inhibition of UV erythema, and inhibition of proliferation of the granulation tissue.

Adverse Reaction to Nonsteroidal Anti-inflammatory Drugs.
O'Brien WM, Am J Med. 1986;80 Suppl 4B:70-80.
NDA Vol. 1.16, p. 48.

This article discusses the common and rare adverse reactions that have been attributed to NSAIDs and compared diclofenac with other anti-inflammatory drugs. The author states that the side effects from diclofenac may be lower than with other NSAIDs. Adverse reactions of NSAIDs that were known at the time this article appeared were discussed.

Three general types of adverse reactions were discussed. The first is that the elderly are particularly susceptible to toxicity from the NSAIDs that have long half-lives. Diclofenac was indicated as having about a 2 hour plasma half-life and "should be relatively safe" in this respect. The second type of adverse reaction is due to the effect of NSAIDs on prostaglandin synthesis. The third type of adverse reaction is the idiosyncratic events, with hepatocellular necrosis, aplastic anemia, and anaphylaxis being the most "feared" of these events. The idiosyncratic reactions were said to be the most serious.

The common side effects that were listed were: a) GI effects - diffuse gastritis and gastric and duodenal ulcers, b) renal effects - interstitial nephritis seems to be rare and is idiosyncratic, occurring suddenly, c) enterohepatic recirculation and diarrhea, and d) headache.

Rare adverse reactions include a) dermatologic (erythema multiforme and its variants - Stevens-Johnson syndrome), b) photosensitivity, c) musculoskeletal (aseptic necrosis), d) pulmonary (asthma), e) acute pulmonary edema, f) diffuse pulmonary infiltrates, g) possible reactivation of latent tuberculosis, h) anaphylaxis, I) febrile reactions, j) drug-induced lupus-like syndrome, and k) vasculitis.

PHARMACOLOGY AND TOXICOLOGY OF DMSO:

ACUTE TOXICITY OF DMSO:

Willson, J.E. et al., Toxicol Applied Pharmacol 1965; 7:104-112
NDA Vol. 1.16, p. 137.

Acute intravenous and oral toxicity of 100% DMSO was reported in mice and rats in this reprint. The results are indicated below.

Acute Toxicity of 100% DMSO, Density 1.1 g/mL - n=5/sex/dose

Species	Route	LD ₅₀ (g/kg)	95% Confidence	LD _{0.1} (g/kg)
Mouse	IV	5.75	4.65-7.10	2.74
Mouse	Oral	21.4	17.1-26.9	9.42
Rat	IV	5.36	4.27-6.72	2.35
Rat	Oral	28.3	-	-

- out of 5/sex/dose/route, all but one died within 24 hours-
- lethal iv signs: tremors, myasthenia, dyspnea, occasional convulsions-
- nonlethal iv signs: decreased motor activity and myasthenia-
- lethal po signs: ataxia, myasthenia, ↓ motor activity, and bradypnea-
- nonlethal po signs: decreased motor activity - polydipsia and polyuria noted in rats at 20 g/kg-

Acute Toxicity of DMSO:

Caujolle et al., Ann NY Acad Sci 1967:141(110-125).
NDA Vol. 1.16, p. 146.

These authors evaluated the acute toxicity of 50% DMSO in mice and rats. Rabbits and dogs were also studied in toxicity tests.

Acute Toxicity of 50% DMSO - n = 20 animals/group

Species	Route	LD50 at 24 hours (g/kg)
Swiss Mouse	PO	NE*
	IV	11.0 (9.6-13.7)
	IP	20.1 (18.5-23.7)
	SQ	16.0 (14.8-17.1)
Wistar Rat	PO	NE
	IV	NE
	IP	13.7 (13.3-14.2)
	SQ	13.7 (12.7-15.6)

* NE = evaluation unable to be made because of too great a volume administered to animals. This was never explained in the study.

- immediate but slight toxic effect-
- no difference in toxicity between sexes-
- mice: catatony of the tail (Straub tail), hypothermia-
- rats: lacrimal secretions, diuretic action, hemoglobinuria-
- 100% DMSO produced rapid changes in RBCs and coagulation (generates heat when mixed with blood or peritoneal fluid-

In chloralose anesthetized dogs, death was produced with IV doses of 30-40 g of 50% DMSO/kg. In rabbits, the mean survival time was 92 minutes following IV perfusion with 40% DMSO (90 mL/hr) - about 19.2 g/kg. The heart rate increased, with a slight rapid rise in BP.

SUBACUTE TOXICITY OF DMSO:

Four-Week Intraperitoneal Administration Of DMSO to Rats:

Willson, JE et al., Toxicol Applied Pharmacol 1965;7:104-112.

NDA Vol. 1.16, p. 137.

Carworth CFN rats (5/sex/group, 100-130 g) were administered 0, 0.5, 1.0, 2.0, 4.0, or 8.0 g/kg 100% DMSO/kg IP six times/week for 4 weeks, followed by a 4-week recovery period. The control was administered normal saline. The study included weekly body weight determinations, hematology (RBC/WBC counts, Hb, Hct, reticulocytes, platelet, and differential counts). Gross and microscopic examinations were done on half of the surviving animals, with the remaining animals allowed to recover for four weeks.

RESULTS

- > 0.5 g/kg/day: restlessness, irritability, depression, weakness
- mortality: control - 1/5 ♂ after 21 doses-
 - 2.0 g/kg/day - 2/5 ♀ after 21 and 22 doses-
 - 8.0 g/kg/day - 5/5 ♂ after 3, 13, 15, 18, 18 doses-
 - 3/5 ♀ after 1, 1, and 3 doses-
- body weight decrement: 4 g/kg/day ♂ ↓ 11% W4, with recovery by W6-
8 g/kg/day ♀ ↓ 38% W4 and ↓ 13% W8-
- ↓ RBC, Hb and Hct, WBC, lymphocytes, ↓ reticulocytes, neutrophils at 8 mg/kg/day-
- fibrinopurulent peritoneal exudate at injection sites - peritoneal adhesions were widespread-

Chronic Toxicity Studies in Mice and Rats:

Caujolle, FME et al., Ann NY Acad Sci 1967;141:110-125.

NDA Vol. 1.16, p. 146.

Mice and rats were treated ip, sq, or orally daily except Sunday

with 50% DMSO for 3 to 12 weeks. Mice (10 ♂ and 10 ♀) that received 33 doses of 2.5 g/kg ip or 34 doses of 2 g/kg sq had weight gain comparable to controls. Oral treatment of 5 g/kg over 20 days resulted in weight loss the first few days, followed by recovery. Tubulonephritis and diffuse necrosis of the intraperitoneal organs occurred with repeat ip administration of 2.5 g/kg. SQ doses of 2.5 g/kg were said to develop less tissue damage and were tolerated better.

Rats dosed ip with 1 g/kg for 45 doses showed no change in growth; however, at 5 or 10 g/kg ip, growth was reduced in young rats, and weight loss occurred with adult rats. With repeat ip injections, no marked lesion occurred at the injection site. Oral administration of 2 g/kg administered over 45 days did not affect weight gain or growth of young rats. With 5 g/kg po, necrosis of liver cells with inflammation and irritation of the portal spaces occurred.

Four-Week IV Administration of DMSO To Dogs:

Willson, JE et al., Toxicol Applied Pharmacol 1965;7:104-112.

NDA Vol. 1.16, p. 250.

Mongrel dogs (3 or 4/group) were dosed at 0.3, 0.6, 1.2, and 2.4 g/kg/day, 6 times/week at 1 mL/minute for a total of 24 IV injections. Undiluted DMSO was used for the first 10 injections; however, perivascular inflammation increased injection resistance, and gradual vein occlusion necessitated diluting 2:1 with 5% dextrose for the 0.6 and 1.2 g/kg groups. This was further diluted to 1:1 with 5% dextrose for the 0.3 g/kg group. Dose of 2.4 g/kg were administered without dilution throughout the study. Two survivors were sacrificed 24 hours after the last dose and necropsied. All remaining dogs were sacrificed and necropsied after a 3 to 4 week recovery period. Body weight, food/water consumption, blood chemistry, hematology, and urinalysis were evaluated. Microscopic examinations were conducted on selected tissues.

RESULTS

- discomfort during injection with 100% DMSO, less with diluted DMSO - straining with defecation, mucus feces with blood at times-
- mortality- both ♂ 2.4 g/kg treated dogs died during first injection - 2.4 g/kg ♀s survived through 17 and 21 injections-
- most dogs lost weight-
- anemia (↓ RBCs, Hb, Hct), DR ↑ reticulocytes, histologically normal bone marrow, but indicated erythroid hyperplasia with Wright's stain-
- DR hemoglobinuria and hematuria seen frequently-
- serum transaminases (mostly AST) slightly elevated at 2.4 g/kg/day-

- cloudy swelling and granularity of liver parenchymal cytoplasm at all doses - some biliary thrombi at 1.2 g/kg/day - yellow/brown pigment in liver, spleen, kidney, and injection sites in treated groups-
- perivascular inflammation and thrombi at all dose levels-

In another series of 24 IV injections using dilute DMSO, no thrombi or perivascular inflammation were noted. The above study indicated a sex difference in toxicity at the high dose. It was suggested the anemia was probably due to intravascular hemolysis.

Chronic Toxicity of DMSO in Primates:

Vogin, EE et al., Toxicol Applied Pharmacol 1970;16:606-612.

NDA Vol. 1.16, p. 209.

Rhesus monkeys (3 to 6/group) were daily administered control (water), 1, 3, 9 mL/kg of 90% (v/v) aqueous solutions of pharmaceutical grade DMSO by intubation. A parallel group was administered the same dermal doses to the abdomen. All animals received half of each dose in the morning and the other half in the afternoon. Surviving monkeys were treated for 74 to 87 weeks.

The study included water consumption, EKGs, neurologic (reflexes), heart rate, body weight, BP, body temperature, respiratory rate, and ophthalmologic examinations. Complete blood counts, blood chemistry, ALT, alk phosphatase, SUN, glucose, BSA retention, creatine clearance, and urinalysis were also done.

RESULTS

- 5/6 (9 mL/kg) died from treatment (W15-W53), 1 died accidentally-
- topical DMSO produced scaling and flaking of skin, some erythema-
- oral administration produced ptialism and emesis - anorexia occurred at 9 mL/kg-
- marked ↓ in body wt at 9 mL/kg po-
- no change in BP, heart/respiration rate, temperature, EKGs, water consumption, or neurological reflexes with po or topical dosing-
- no indication of lenticular changes - 1/3 G2 dermal with unilateral complete retinal detachment and syneresis of vitreous humor-
- no significant change in hematology, blood chemistry (no anemia), urinalysis, or organ weights in dermal or po treated animals-
- no gross changes-
- animals that died had emphysema and atelectasis-
- bone marrow smears showed no adverse erythropoietic activity-

The authors concluded by stating- "rhesus monkeys can tolerate

DMSO administered daily for approximately 18 months at doses up to 9 mL/kg dermal or 3 mL/Kg oral, without frank evidence of intolerance." The 90% DMSO aqueous solution, is about 0.95 g/mL.

DMSO AS A SKIN PENETRATION ENHANCER FOR DRUGS:

Effect of Dimethyl Sulfoxide on Drug Permeation Through Human Skin: Chandrasekaran SK, et al., Am Inst Chem Eng J 1977;23(6):810816. NDA Vol. 1.17, p. 174.

This paper, the third in a series on the measurement and interpretation of the transport of relatively small biologically active molecules through the skin, discusses the effect of DMSO and aqueous mixtures of DMSO on the *in vitro* permeation rate of scopolamine in human skin. The reported results found that the *in vitro* skin permeability of scopolamine was elevated by DMSO, being dependent on the concentration gradient of DMSO and consistent with a passive diffusion model for drug transport.

Cooper RH and Berner B. Penetration enhancers. In: Kydonieus AF and Berner B, editors. Transdermal Delivery of Drugs. Vol. II CRC Press, 1987:57-62. NDA Vol. 1.17, p. 180.

The authors indicated that, with the possible exception of DMSO, the best known penetration enhancers are the surfactants, as was shown earlier in other studies that demonstrated soaps increase the flux of water, glucose, and sodium salicylate across human epidermal tissue.

The flux, J , of a drug is related to the diffusion coefficient, the concentration of the diffusing drug, and the position in the membrane. An osmotic gradient is apparently not sufficient to explain the penetrating effects of DMSO.

Drug Delivery to Local Subcutaneous Structures Following Topical Administration. Guy RH and Maibach HI, J Pharm Sci 1983; 72(12): 1375-80. NDA Vol. 1.17, p. 186.

This paper reported on data from several studies that demonstrated drug delivery to local subcutaneous structures by following topical administration. Several experimental observations were

summarized regarding drug penetration after topical administration, and it was suggested that the dermal route offers an alternate to systemic distribution by oral administration.

The anti-inflammatory effect of DMSO was reported by Gorog and Kovacs [Curr Ther Res 10, 485 (1968)] in a rat study in which carrageenan-induced paw edema was improved by both oral and topical administration. It was also reported that mycobacterium-induced arthritis was inhibited to a greater extent by dermal administration of DMSO rather than by oral administration. Similar inhibitory results were reported from topical administration of DMSO to rats with arthritis induced with 6-sulfanilamidazole.

Lang VW [Arzneim Forsch, 24, 71 (1974)] carried out experiments with ³H labeled escin applied to the backs of mice and rats. Radioactivity was monitored in back muscle, blood, liver, kidney, heart, lung, spleen, testes, brain, leg muscle, femur, and stomach lining. Their data in mice showed 20 to 100 times more radioactivity in back muscle than in other tissues. Results were similar in rats but not as pronounced, being 10-30 times greater in back muscle than in other tissues examined.

It was shown by Marty JP et al., 1974, 1975, 1976, 1980 in several publications that percutaneous absorption was accompanied by diffusion and retention in the connective and muscle tissues located beneath the applied area. Solutions of radiolabeled thyroxine, triiodothyronine, dexamethasone estradiol, and diisopropyl fluoro-phosphate, (cholinesterase inhibitor), were applied to the depilated skin on the abdomen or back of rats; the radioactivity was counted in plasma, liver, kidneys, subcutaneous tissue, muscle under the application zone, and paw muscle. It was generally shown that radioactivity accumulation in local subcutaneous tissue under the application area was many times greater than in plasma. Muscle under the application area was about 1/10 of the concentration in the subcutaneous tissue.

Results reported by Rabinowitz et al. [J Clin Pharmacol 22, 42 (1982)] demonstrated absorption of topically applied salicylate in dogs and compared these values to results from oral administration of salicylate. Concentrations in various tissues and fluids following oral ¹⁴C-aspirin (500 mg) were compared to concentrations obtained from topically applied 10 g triethanolamine ¹⁴C-salicylate cream to the animals shaved knees. Blood levels in dogs were 10-100 times lower

with the topical application. Topical application in humans produced 60% of the salicylate level in synovial fluid found after oral aspirin administration.

REPRODUCTION STUDIES WITH DMSO:

Caujolle, FME et al., Limits of Toxic and Teratogenic Tolerance of Dimethyl Sulfoxide.

Ann NY Acad Sci 1967;141:110-125. NDA Vol. 1.16, p. 323.

MICE:

Swiss mice were dosed orally or peritoneally from D6 to D12 of gestation with 0, 5, 8, 10, or 12 g 50% DMSO/kg and sacrificed 1 to 3 days before parturition. Controls were dosed with 25 mL/kg of physiological saline. The control contained 27 mice, with 102 being treated. There were said to be no differences in the number of abortions or the weight of survivors, compared to the control. IP administration resulted in 2 fetuses with anencephaly, 4 fetuses with malformed limbs, and 1 fetus with celosomia - a total of 7/100 fetuses with malformations. No malformations were reported for mice dosed orally. The reprint did not indicate the number of litters in the 12 g/kg group that had malformations. Results are indicated in Table 5 below (from the reprint).

RATS:

Mated rats were administered 0, 5, and 10 g 50% DMSO/kg orally by gavage, or 5, 8, and 10 g DMSO/kg ip from Day 6 to Day 12 of gestation. Fifty-nine control animals were treated with physiological saline and 91 were treated with DMSO. Sacrifice was 1 to 3 days before parturition. Fetuses were weighed, examined, and fixed in Bouin's solution. The report did not indicate the number of litters in which malformations were observed. The results are indicated in the following table.

TABLE 5
THE TERATOGENIC ACTION OF DMSO TOWARDS RATS AND MICE
 (From Caujolle et al., Ann NY Acad Sci 1967;141:110-125)

	ROUTE	DOSE (g/kg)	% OF ♀ GONE FULL TERM	% OF ABORTED EMBRYOS	N° OF MALFORMATIONS
MOUSE	DMSO ORAL	5	37.5	(*)	
		8	18.8		
		10	31.3		
		12	14.3		
	DMSO IP	5	36.4		1
		8	16.7		1
		10	31.3		1
		12	30.0		4
RAT	CONTROL ORAL	25	41.7		
	CONTROL IP	25	33.3		
	DMSO ORAL	5	73.7	3.1	4
		10	80.0	5.0	4
	DMSO IP	5	78.6	3.0	4
		8	78.6	18.3	
		10	50.0	17.0	
	CONTROL ORAL	10	86.2	3.5	1
	CONTROL IP	25	86.6	1.7	

(*) For the mouse, the very small number of treated ♀ going full term does not permit the evaluation of aborted embryos.

- 70-80% of rats went to full term - said to be comparable with control-
- aborted fetuses ! DR in rats treated IP with 8 and 10 g/kg DMSO-
- 4/14 10 g/kg ip DMSO treated rats died-
- body weight reduction- 15.4% at 8 g/kg ip and 28.5% at 10 g/kg ip-
- sex ratio was said to be normal with about 50% of each sex found-
- malformations: anencephaly, microphalia 2, limbs 2, jaw 1,
tail bud 1, abdominal wall (celosomia) 5, edema 1-
- slight celosomia seen in 1/558 control fetuses-
- author states the results give evidence of teratogenicity for DMSO-

RABBITS:

Ten Fawn Burgundy rabbits were dosed orally with 5 g/kg 50% DMSO or by sq injection of 4 g/kg from D6 to D14 of gestation. Six control rabbits were also treated. All rabbits were sacrificed between D20 to D24 of gestation. Table 6 follows from the reprint.

TABLE 6
ACTION OF DMSO ON PREGNANT RABBITS
(From Caujolle, FME et al., Ann NY Acad Sci 1967;141:110-125)

Substance Administered	Route	Dose	N° of Female Fertilized	Mean N° ♀ Full Term	Mean N° of Embryos
DMSO (g/kg)	Oral	5	5	3	5.3
	S.C.	4	5	3	10.0
Control (mL/kg)	Oral	10	3	3	9.7
	S.C.	8	3	3	6.0

- 1 of the 83 fetuses examined was found with celosomia-
- no unusual change in weight or the number of aborted embryos in treated rabbits vs controls-
- no additional data given-

FOWL:

Toxic doses of DMSO administered to Rhode Island chick embryos was shown to cause malformations when survival was 24 hours or greater. Treatment was with approximately the LD₅₀ (10.3 mg/embryo) at 72 hours of incubation or with 12.2 mg/embryo at 96 hours of incubation. The maximum number of malformations were observed when treatment occurred at 92 hours of incubation. A 50% DMSO solution was prepared with 0.9% physiological saline and aseptically injected above the omphalomesenteric vein in the 72 hr incubated embryos and above the allantois in the 96 hour incubated embryos. Control embryos received the same volume of 0.9% physiological saline.

Of the 18.5% malformed embryos from the 324 surviving embryos in the 72 hour incubation, 11.4% had left anophthalmia and left torsion of the beak. Anophthalmia and other eye malformations were observed in 16.9% of the total malformed embryos. Only two of the 324 embryos had limb malformations. In the 96 hour incubation group, 25.9% of the 436 surviving embryos were malformed, with the predominant malformations occurring in limbs (21.8%). Other reported observations were 4.6% malformed beaks, 3.9% malformations of the eye, 3.7% anourous embryos, and 5.5% celosomia. About 2% spontaneous malformations, such as anophthalmia and crossed beak with or without anencephalia, occur in untreated chick embryos.

Similar results were observed when these studies were conducted in Leghorn chick embryos. The toxicity was reported to be similar.

Ferm, VH, Congenital Malformations Induced by Dimethylsulphoxide in the Golden Hamster. J Embryol Exp Morph 16,1, pp. 49-54, August 1966. NDA Vol. 1.16, p. 339.

Teratogenic effects from iv or ip administration of DMSO were investigated in Golden hamsters. Animals were mated in the evening. The day following mating was considered the first day of gestation (D0). On D8 of gestation, various dilutions of DMSO in saline were injected with 50 to 8250 mg DMSO/kg at 0.5 mL/100 g body weight. Pregnant animals were sacrificed and the embryos recovered on D11.

RESULTS

At 5500 mg/kg undiluted DMSO/kg, the animals died within a few minutes with muscle spasms and hyperextension of the extremities. Muscle tremors lasting about one minute occurred at 2500, 3800, and 5500 mg/kg, and at 5500 and 8250 mg/kg IP, abdominal muscle rigidity lasting about one minute was observed. These animals did not show other adverse effects. Their weight gain was normal, and histologic examination of placentas indicated no obvious lesions.

Dose levels ≥ 2500 mg/kg resulted in non-closure of the neural tube, cleft lip and palate, limb abnormalities, and rib abnormalities. One case of conjoined twins was also observed. Exencephaly was the most consistent finding, with entire litters affected on three occasions. The following table indicates additional data.

Teratogenic and Embryocidal Effects of DMSO on Hamster Embryos
(From p. 51 of reprint)

Dose Level (mg/kg)	Route	Number Treated	Average Wt Gain (g)	Total N° Embryos	Total N° Dead	Abnormal Embryos
50	IV	5	13	66	2	1
250	IV	5	14	57	2	0
500	IV	5	18	77	4	2
1000	IV	5	12	66	3	1
2500	IV	5	14	67	2	4
5500	iv*	6	9	77	15	12
5500	ip	5	13	67	13	25
8250	ip	5	8	64	10	18

* Diluted to 1.0 mL/animal

Embryonic development in hamsters was affected, according to the author, only at levels of DMSO approaching 2500 mg/kg.

Robens, JF, Teratologic Studies of Carbaryl, Diazinon, Norea, Disulfiram, and Thiram in Small Laboratory Animals. Toxicology and Applied Pharmacology 1969; 15:152-163. NDA Vol. 1.16, p. 347.

Five Golden Syrian hamsters were dosed orally D7 of gestation with 5 mL/kg DMSO and 14 were dosed D7 or D8 with 10 mL/kg DMSO. The first day after mating was designated Day(0) of gestation. All animals were sacrificed D14 or D15 and the fetuses examined. The results are indicated in the following table.

Effects of DMSO In The Hamster
(From Table 4 of Robens, JF, p. 157)

Dose mL/kg	Day Treated	Dams Mortality	Fetuses		Total N ^o Implants	Fetal Mortality		Av. Fetal Weight (g)	Terata	
			Total	Per Litter		N ^o	%		N ^o	%
5	7	0/5	50	10.0	54	5	9.3	2.3	1	2.0
10	7	1/14	131	10.1	158	38	24.0	2.1	32	24.4
10	8	0/14	149	10.6	165	16	9.7	2.2	27	18.1

At 10 mL/kg, 1/14 hamsters treated D7 died. Fetuses from dams treated D7 and found dead at term were pale, slightly edematous, and had exencephaly and retarded bone development. Other dead fetuses, however, were said to be fully formed. Fetuses that were alive developed less severe anomalies than observed in dead fetuses.

Specific Anomalies From DMSO In The Hamster
(From Table 6 of Robens, JF, p. 160)

Dose mL/kg	Gestation Day Treated	N ^o Litters with Fetuses	N ^o Total N ^o Fetuses	Exen- cephaly	Cranial Pimple	Short- ended Maxilla or Mandible	Fused Ribs	Cleft Palate or Hairlip	Short or Curved Tail	Spina bifida
5	7	5	1/50	1	-	-	-	-	-	-
10	7	13	32/131	19	9	6	8	-	-	1
10	8	14	27/149	-	-	-	24	2	3	-

The results from this study show that DMSO was teratogenic as well as embryotoxic at 5 mL/kg. DMSO purity was not indicated.

MUTAGENIC STUDIES:

McCann J, et al., Detection of carcinogens as mutagens in the Salmonella microsome test: Assay of 300 chemicals. Proc Nat Sci USA 1975;72(12): 5135-5139. NDA Vol. 1.16, p. 364.

About 300 carcinogens and non-carcinogens were tested as mutagens in the Ames Salmonella/microsome test - details for the testing were

sketchy. Tester strains TA 100, TA 1535, TA 1537, and TA 98 were evaluated. The results for DMSO were <0.00001 revertants/nmol ($<70/5 \times 10^5$). The test was considered to be negative for DMSO in strains TA 98, TA 100, TA 1535, and TA 1537 in the presence of S9.

Yee B, et al., Biological effects of dimethyl sulfoxide on yeast. *Biochem Biophys Res Commun* 1972, Dec 4;49(5):336-342.
NDA Vol. 1.16, p. 369.

Saccharomyces cerevisiae strain MCC yeast cultures (10^7 cells/mL) were exposed to increasing concentrations of DMSO at various exposure times. Temporary exposure produced decreased cell viability above 20% (v/v) DMSO. Cell lysis was not reported.

Kapp, RW, Jr. and Eventoff, BE. Mutagenicity of Dimethylsulfoxide (DMSO): In vivo Cytogenetics Study in the Rat. *Teratogenesis, Carcinogenesis, and Mutagenesis* 1(2):141-145 (1980).
NDA Vol. 1.16, p. 376.

Sprague-Dawley CD[®] ♂ rats (10 ♂/group) were dosed IP (5 mL/kg) with 1%, 10%, 50% and 100% DMSO (Lab Grade-Lot #35359, J. T. Baker) for five consecutive days. Dilutions were made in distilled H₂O. Controls received 10 mL of distilled H₂O. Colchicine was administered IP on D6 at 2 mg/kg. Animals were sacrificed 2 hr after the last injection, and bone marrow aspirated from both femurs. The cells were mounted on glass slides, 3 slides/animal, and analyzed for cytogenetic aberration frequencies, including chromosome breaks, chromatid breaks, markers (rings, exchanges, and dicentrics), and for severely damaged cells. Wilcoxon nonparametric comparison of group means for total chromosome aberrations, chromatid breaks, chromosome breaks, markers, and severely damaged cells was evaluated. Results were compared to the negative water control. No positive control was used in the study, however.

Results

- 3 deaths prior to termination at 100%-
- 2 at 1%, 1 control, and 1 at 100% had no analyzable cells-
- this study indicated that significant increases occurred in chromosome aberrations in all DMSO groups dosed at 5 mL/kg with 1% to 100% DMSO-

TABLE I

Results of In Vivo Treatment of ♂ Rats With DMSO

From Kapp, RW, Jr. and Eventoff, BE, Teratogenesis, Carcinogenesis, and Mutagenesis 1:143, 1980

Treatment	N° of Survivors At Sacrifice	Animals with Analyzable Cells	N° Surviving At Sacrifice	N° Cells Analyzed	Mean Percent Aberrant Cells/Animal	Standard Deviation
Distilled Water	10/10	9/10	10	450	4.0	2.0
1% DMSO	10/10	8/10	10	400	10.0	6.05
10% DMSO	10/10	10/10	10	500	18.0	13.76
50% DMSO	10/10	10/10	10	500	19.2	6.68
100% DMSO	7/10	6/7	7	300	68.67	21.45

TABLE II

Results of Wilcoxon Nonparametric Comparison of Group Means for Chromosome Aberrations in Bone Marrow Cells

From Knapp, RW, and Eventoff, BE, Teratogenesis, Carcinogenesis, and Mutagenesis 1:143, 1980

Groups Compared	Total Chromosome Aberrations	Chromatid Breaks	Chromosome Breaks	Markers	Severely Damaged Cells
1 and 2	S	S	NS	S	NS
1 and 3	S	NS	NS	S	NS
1 and 4	S	S	NS	S	NS
1 and 5	S	S	NS	S	S

S = P<0.05

NS = not statistically significant

TABLE III

Results of Wilcoxon Nonparametric of Mean Number Severely Damaged Cells in Group 5 (100 % DMSO) and All Other Groups
From Kapp, RW, Jr., and Eventoff, BE, Teratogenesis, Carcinogenesis, and Mutagenesis 1:144 (1980)

Groups Compared	Results
1 and 5	S
2 and 5	S
3 and 5	S
4 and 5	S

S = P<0.05

The results showed that DMSO caused chromosomal aberrations to bone marrow cells when administered intraperitoneally to rats at 5 mL/kg doses 1% to 100% concentration. With the exception of the 10% DMSO dose, chromatid breaks were significantly increased at 1%, 50%, and 100% DMSO. Severely damaged cells, which included pulverized cells, cells in which

chromosomes were clumped together, and cells with more than 10 aberrations, were statistically significant only at 100% DMSO.

ADME STUDIES:

Hartway T, Poblete N, Wester RC, Maibach HI. In vitro percutaneous absorption of [¹⁴C]-Diclofenac sodium in human skin. Final Report. San Francisco, CA: Surge Laboratory, Department of Dermatology, University Of California; 1996 Dec. Study N^o UCSF 95SU03. NDA Vol. 1.17, p. 1.

The objective of this study was to determine the skin absorption profile when diclofenac in _____ is applied *in vitro* to fresh human skin. This study was conducted by the Surge Laboratory, Dept. of Dermatology, University of California, Box 0989, San Francisco, CA. The study was indicated as being in compliance with GLPs. The QAU Statement was signed 12/17/96.

b(4)

¹⁴C-Diclofenac sodium was added to 1) PENNSAID Topical Lotion, Lot N^o 4063, 2) PENNSAID Placebo, Lot N^o 116701, and 3) PENNSAID Control Lotion, Lot N^o 40053. Human male or female viable thigh skin, 500 microns thick from four different sources, was taken from 32, 38, and 53 year old male and female cadavers. Skin (1 cm²) was mounted in glass cells of the flow-through design containing Eagles MEM in one reservoir. A 1-5 µCi/10 µL/cm² dose was used for the determinations. Cell contact time was 6 and 24 hours. The receptor fluid was assayed by ¹⁴C-TLC at 6 time points over 24 hours. The results are presented in Table 1. Formulations tested are indicated.

- A. 1000 ppm ¹⁴C-_____ Lotion (1.5% diclofenac sodium, 45% DMSO, 11% EtOH, 11% propylene glycol and water.
- B. Replicate of A.
- C. Base lotion without DMSO (1.5% 1000 ppm ¹⁴C-diclofenac sodium, 11% glycerin, 11% EtOH, 11% propylene glycol and water.
- D. Replicate of C.
- E. ¹⁴C-Diclofenac in 45% DMSO, 11% glycerin, 11% EtOH, 11% propylene glycol and water.
- F. Radiolabeled chemical to be used as positive control for skin metabolism-viability.

b(4)

TABLE 1
In Vitro Percutaneous Absorption of ^{14}C -Diclofenac Na in Human Skin
Percent of Dose Absorbed (Mean $\pm$ SD)
(From Vol. 1.17, p. 19)

	A	B	C	D	E
Receptor Fluid	1.34 $\pm$ 1.10	0.44 $\pm$ 0.06	1.02 $\pm$ 0.72	1.12 $\pm$ 0.62	0.72 $\pm$ 0.34
Tape Strips	4.41 $\pm$ 2.47	-	7.02 $\pm$ 3.63	-	-
Skin Digest	3.78 $\pm$ 2.60	-	2.61 $\pm$ 0.64	-	-
Absorption	9.53		10.65		
Surface Wash	<u>87.4$\pm$7.2</u>	<u>90.6$\pm$5.1</u>	<u>84.7$\pm$4.2</u>	<u>87.2$\pm$3.8</u>	<u>95.1$\pm$10.8</u>
Total	95.7 $\pm$ 4.5	91.1 $\pm$ 5.2	95.3 $\pm$ 1.8	87.4 $\pm$ 3.9	95.8 $\pm$ 11.1

Percent absorption was measured as accumulation in the receptor fluid (1.34 $\pm$ 1.10). The total absorbed in formulation A was 9.53% and 10.65% in formulation C over 24 hr. Receptor fluid diclofenac content increases up to 12 hr, decreased between 12 and 17 hr, then increased up to 24 hr. TLC detected some of the known metabolites. These data indicate the PENNSAID formulas A and C are absorbed through viable human skin.

Hewitt, PG, Wester, RC, and Maibach, HI. Bioavailability of ^{14}C -Diclofenac in vitro in Human Skin. Final Report. San Francisco, CA: Surge Laboratory, Department of Dermatology, University of California; 1997 Sponsored by Dimethaid Research Inc. Study N $^{\circ}$ UCSF96SU05. NDA Vol. 1.17, p. 91.

The purpose of this *in vitro* study was to determine the comparative bioavailability of diclofenac sodium in the PENNSAID formulation when compared to an aqueous solution of diclofenac sodium after topical application to viable human skin.

Human leg skin was obtained from five different cadavers. Skin thickness was 500 microns. ^{14}C -Diclofenac sodium (6 mg) of 99% radiochemical purity and 9.76 mCi/mmol was added to 400 μL of PENNSAID lotion, giving a 1.5% formulation. The 9 dose formulations are indicated below.

- A. 2 μL 1.5% (1000 ppm) ^{14}C -Diclofenac sodium in PENNSAID Lotion. Single dose.
- B. 5 μL 1.5% (1000 ppm) ^{14}C -Diclofenac sodium in PENNSAID Lotion. Single dose.
- C. 2 μL 1.5% (1000 ppm) ^{14}C -Diclofenac sodium in HPLC grade H_2O (+10% EtOH). Single dose.
- D. 5 μL 1.5% (1000 ppm) ^{14}C -Diclofenac sodium in HPLC grade H_2O (+10% EtOH). Single dose.

- E. 2 μ L 1.5% (1000 ppm) ^{14} C-Diclofenac sodium in PENNSAID Lotion. Multiple doses, at times 0, 4, 8, 12, 24, 28, 32, and 36 hr.
- F. 5 μ L 1.5% (1000 ppm) ^{14} C-Diclofenac sodium in PENNSAID Lotion. Multiple doses, at times 0, 4, 8, 12, 24, 28, 32, and 36 hr.
- G. 2 μ L 1.5% (1000 ppm) ^{14} C-Diclofenac sodium in HPLC grade water (+10% ethanol). Multiple doses, at times 0, 4, 8, 12, 24, 28, 32, and 36 hr.
- H. 5 μ L 1.5% (1000) ^{14} C-Diclofenac sodium in HPLC grade water (+10% ethanol). Multiple doses, at times 0, 4, 8, 12, 24, 28, 32, and 36 hr.
- I. Viability control (glucose metabolism to lactate).

The above Pennsaid Lotion contained 45% DMSO, 11% glycerin, 11% ethanol, 11% propylene glycol, and water. This diffusion study was comparable to the previous bioavailability study. Results are shown below.

Amount Absorbed Through/Into Human Cadaver Skin
(From Vol.1.17, pp. 120-121)

Single Dose	Formulation	Total Diclofenac Absorption (μ g)	
		Mean (SD)	Percent (SD)
A. 2 μ L PENNSAID Lotion (30 μ g/skin)		10.18 (6.70)	33.92 (22.32)
B. 5 μ L PENNSAID Lotion (75 μ g/skin)		26.26 (17.50)	35.01 (23.34)
C. 2 μ L Aqueous Diclofenac Na (30 μ g/skin)		8.33 (1.51)	27.76 (5.03)
D. 5 μ L Aqueous Diclofenac Na (75 μ g/skin)		12.25 (5.66)	16.34 (7.54)
Multiple Doses	Formulation	Total Diclofenac Absorption (μ g)	
		Mean (SD)	Percent (SD)
E. 2 μ L Pencil Lotion x8 (240 μ g/skin)		40.14 (17.58)	16.72 (7.32)
F. 5 μ L PENNSAID Lotion x8 (600 μ g/skin)		85.61 (41.36)	14.27 (6.89)
G. 2 μ L Aqueous Diclofenac Na x8 (240 μ g/skin)		9.44 (2.87)	3.94 (1.20)
H. 5 μ L Aqueous Diclofenac Na x8 (600 μ g/skin)		35.68 (18.97)	5.94 (3.16)

Total absorption included recovery from stratum corneum, recovery from skin, and recovery from receptor fluid. These data indicate that a greater amount of drug was absorbed/penetrated the cadaver skin with multiple applications. On the other hand, the percent of drug absorbed/penetrated from the total amount applied was less in the single dose. It is of interest that diclofenac sodium was also absorbed from an aqueous ethanol solution (C, D, G, H), but less than that from the PENNSAID Lotion.

The mean amount of drug absorbed in the stratum corneum was approximately 11.6% (2.0%-24.9% range) from a single application of the 30 μ g lotion, 11.8% (3.5-21% range) from a single application of 75 μ g lotion, 10.3% (4.7-18.5% range) from multiple applications of the

240 µg lotion, and 6.0% (2.4-13.1% range) from multiple applications of the 600 µg lotion.

LABELING:

The following sections of the labeling referring to preclinical information are in italics.

Second paragraph Under Distribution :

b(4)

This statement does not agree with the information in the Rimso-50 labeling for intravesical instillation of 50% DMSO for interstitial cystitis. It states "Changes in the refractive index and lens opacities have been seen in monkeys, dogs and rabbits given high doses of dimethyl sulfoxide chronically. Since lens changes were noted in animals, full eye evaluations, including slit lamp examinations, are recommended prior to and periodically during treatment."

Carcinogenesis, Mutagenesis, Impairment of Fertility

b(4)

This should be changed to: DMSO was found to be nonmutagenic

in the Ames test. In the cytogenetics study, chromosome damage in rat bone marrow cells was observed at 1% to 100% DMSO when administered intraperitoneally at 5 mL/kg body weight.

It is to be noted, however, that DMSO has been used as a negative control in the Ames assay, the L5178 TK+/- mouse lymphoma forward mutation assay, in the in vitro chromosomal aberration assay in CHO cells, and in other assays.

Pregnancy:

Teratogenic Effects: _____

b(4)

The sponsor has not done reproductive studies with diclofenac sodium. The labeling for the approved drug, VOLTAREN® (diclofenac sodium), states that up to 10 mg/kg/day has been administered to rats and rabbits with no evidence of teratogenicity.

b(4)

The sponsor has not conducted any teratology studies with DMSO. All data are taken from the literature or from the Ciba-Geigy product monograph dated 11/7/89.

It is important to note, that the earlier reproductive and mutagenic studies on DMSO do not meet the current methodology standards.

SUMMARY AND EVALUATION:

PENNSAID™ (1.5% diclofenac sodium) Topical Lotion is indicated for initial use in topical treatment of _____

_____ The product is intended for external use only. PENNSAID™ is the product of Dimethaid Research Inc., a Canadian firm. PENNSAID™ contains, in addition to diclofenac sodium, dimethyl sulfoxide USP (DMSO), glycerin USP, propylene glycol USP, alcohol USP, and purified water USP. DMSO is present at 455 mg/g or 45.5% and is used as a skin penetrant for diclofenac sodium.

b(4)

The recommended clinical dose is _____

b(4)

No preclinical toxicity studies were submitted with this application. The submitted reprints and documents relate to the primary and secondary action, toxicity, reproduction, mutagenicity, and ADME studies for DMSO and diclofenac. As was agreed upon at the June 6, 1995 meeting with the company, human data generated in Canada with PENNSAID™ could replace a 90-day rat toxicity study. Pharmacology and toxicology data for diclofenac sodium were obtained from several scientific literature reviews and documents.

Acute LD₅₀ toxicity of 100% DMSO in mice, with a 14-day observation period, was 5.7 g/kg iv and 21.4 g/kg po. The acute 24 hour LD₅₀ was 13.7 g/kg sq administered 50% DMSO. Values in rats were 5.4 g/kg iv and 28.3 g/kg oral. Twenty-four hour LD₅₀ data for 50% DMSO in mice were 11.0 g/kg iv, 20.1 g/kg ip, and 16.0 g/kg sq. In rats, values of 13.7 g/kg for ip and sq administration were reported. Anesthetized dogs died from iv doses of 30-40 g of 50% DMSO.

In subacute and chronic toxicity studies with DMSO, Carworth CFN rats dosed four weeks with 100% DMSO at 0, 0.5, 1, 2, 4, and 8.0 g/kg ip developed anemia, WBC decreases, and decreased lymphocytes. Body weight gain was significantly reduced, and mortality occurred at 2 g/kg after 21 doses. Mice and rats have also been treated ip, sq, and po with 50% DMSO for 3 to 12 weeks. The sq route produced less tissue damage and was tolerated better than either iv or po administration. Weight loss occurred in mice at 5 g/kg po, followed by weight gain. In mice, tubulonephritis and diffuse necrosis of the intraperitoneal organs occurred following ip administration of 2.5 g/kg. Rats showed growth reduction and weight loss at 1 g/kg ip, but no weight loss was seen at 2 g/kg po. In rats, liver cell necrosis and inflammation of the portal spaces occurred with oral administration of 5 g/kg of 50% DMSO for 45 days.

Male dogs died at 2.4 g/kg during the first injection, with females dying later in the study. Toxicity included weight loss, anemia (decreased RBCs, Hb, and Hct), with hemoglobinuria, hematuria, and swelling and granularity of liver parenchymal cytoplasm seen at all doses. In dogs, perivascular inflammation and thrombi occurred at all doses (0.3, 0.6, 1.2, and 2.4 g/kg/day) when injected with, but could be reduced by diluting with 5% dextrose. Anemia, weight loss, increase in reticulocytes, hemoglobinuria, and hematuria were also observed. Yellow brown pigment was reported in the liver, spleen, kidneys and injection sites.

Oral and dermal administrations of 0, 1, 3, and 9 mL/kg/day of

90% aqueous DMSO (90% DMSO $\approx$ 0.95 g/mL) were administered twice a day to rhesus monkeys in equally divided portions for 74 to 87 weeks. At 9 mL/kg po, anorexia and death occurred in some monkeys, and was attributed to emphysema and atelectasis that may have been the result of "regurgitation or tracheal inspiration of the test material." Topical application over the entire abdominal area produced scaling, flaking, and erythema. No significant changes were reported in BP, heart or respiration rate, temperature, water consumption, EKGs, or in neurological reflexes. Anemia was not observed in this study. Other hematology, blood chemistry, or urinalysis parameters, and organ weights were not affected. No evidence of lenticular changes was reported in these dogs. Dermal applications up to 9 mL/kg/day were well tolerated by these animals.

The teratogenic potential of DMSO has been studied in a) 129 Swiss mice (27 controls and 102 treated) administered at 0, 5, 8, 10 and 12 g 50% DMSO/kg/day po and ip from the 6th to the 12th day of gestation, b) in Wistar rats (59 controls and 91 treated) administered at 5 and 10 g 50% DMSO/kg/day po and at 5, 8, and 10 g/kg/day ip from the 6th to the 12th day of gestation, c) in 6 control and 10 treated Fawn Burgundy rabbits administered at 5 g/kg of 50% DMSO/kg/day po and 4 g/kg/day sc from the 6th to 14th day of gestation, and d) in golden hamsters administered at 50-8250 mg/kg iv on the 8th day of gestation.

No malformations were observed in mice dosed orally, but ip administration produced anencephaly, malformed limbs, and one case of celosomia in the treated mice. No malformations were reported in control mice treated with saline.

In rats, abortions were dose related with oral or ip administration of DMSO. Mortality occurred at 10 g/kg. Malformations (anencephaly, microphalia, celosomia) occurred at 5 and 10 g/kg po and at 8 g/kg ip. Eleven of the 729 live rat fetuses from dams injected ip developed malformations (1.5%). Only 1 of the 558 control rat fetuses (0.18%) was reported as showing "slight" celosomia.

One of the 83 rabbit fetuses was found with celosomia (1.2%). Weight changes or number of aborted embryos in the treated rabbits were indicated as not being remarkable when compared to controls.

Teratogenic effects in Golden hamsters were evaluated following intravenous administration of 50 to 8250 mg/kg DMSO on the 8th day of gestation. Dilutions were in saline. Intraperitoneal doses of 5500 and 8250 g/kg were also administered and studied. At $\geq$ 2500 mg/kg iv, non-closure of the neural tube, cleft lip and palate, and limb and rib abnormalities occurred. At 5500 mg/kg, the number of dead and malformed embryos increased. Exencephaly was the most common malformation. One other study in Golden hamsters with doses of 5 and 10 mL/kg DMSO

(5.5 and 11 g/kg) administered on Day 7 or 8 of gestation produced exencephaly, fused ribs, cleft palate or hairlip, short or curved tail, and cranial pimple. Embryo toxicity also occurred at 5 mL/kg.

The injection of either Rhode Island or Leghorn chick embryos with 10.3 mg 50% DMSO in 0.9% saline/embryo produced a left torsion of the beak with reduction of the upper beak, celosomia, anophthalmia, and malformed limbs. Embryos were injected and followed for either 72 or 92 hours of incubation. Controls received an equal volume of 0.9% saline.

DMSO was reported as negative in the Ames *Salmonella typhimurium* assay that was published by Bruce Ames in 1975. The assay evaluated DMSO in strains TA 98, TA 100, TA 1535, and TA 1537 in the presence of S9. DMSO was positive in the *in vivo* cytogenic assay in rat bone marrow cells. All dose groups evaluated with 1% to 100% DMSO showed a statistically significant increase in cells with aberrant chromosomes.

RECOMMENDATIONS:

There are no objections to the approval of this drug, based on the pharmacology/toxicology of the data submitted to the NDA. There are several changes that will be required in the labeling.

- 1) Under Dimethyl Sulfoxide in the Carcinogenesis, Mutagenesis, and Impairment of Fertility section, the statement should read: No carcinogenicity studies have been conducted with DMSO. DMSO was found to be non-mutagenic in the Ames test. Chromosome damage in rat bone marrow cells was significant at 1% to 100% DMSO when administered intraperitoneally.
- 2) Under the pregnancy section for Dimethyl Sulfoxide, the paragraph should read: High single, topical doses of 5-12 g/kg of 50 DMSO did not demonstrate any effect on the reproductive process of hamsters, mice or rats. However, in one rabbit oral study, administration of 5 mg/kg of 50% DMSO for 2 days followed by 2.5 g/kg for 8 days produced terata. DMSO was embryotoxic and teratogenic in hamsters administered 2.5 g/kg intravenously.

Almon W Coulter

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July 98

CC:

NDA 20-947

HFD-550/Division File

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/ACoulter

HFD-345

F/T by AWC 7/16/98