CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-234

PHARMACOLOGY REVIEW

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION
Pharmacology Toxicology Supervisor's Review

NDA NUMBER:	**21-234**
SERIAL NUMBER:	**000 AZ (Second submission)**
DATE RECEIVED BY CENTER:	**27-Jul-2006**
PRODUCT:	**Flector® Patch (Diclofenac epolamine topical patch) 1.3%**
INTENDED CLINICAL POPULATION:	**The proposed indication is for the relief of pain due to strains, sprains and contusions**
SPONSOR:	**Institut Biochemique (IBSA)**
DOCUMENTS REVIEWED:	**N000 AZ 27-Jul-2006 N000 BZ 28-Dec-2006 N000 BZ 16-Jan-2007**
REVIEW DIVISION:	**Division of Anesthesia, Analgesia, and Rheumatology Products (HFD-170)**
PHARM/TOX REVIEWER:	**R. Daniel Mellon, Ph.D.**
PHARM/TOX SUPERVISOR:	**R. Daniel Mellon, Ph.D.**
DIVISION DIRECTOR:	**Bob A. Rappaport, M.D.**
PROJECT MANAGER:	**Lisa Basham, M.S.**

Date of review submission to Division File System (DFS): 22-Jan-2007

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability

From the nonclinical pharmacology toxicology perspective, NDA 21-234 may be approved, pending agreement on the proposed drug product labeling recommendations. If agreement on the recommended exposure margins can not be obtained, the product may still be considered for approval, if the sponsor agrees to conduct the pharmacokinetic studies described below and update the product label based upon the data obtained.

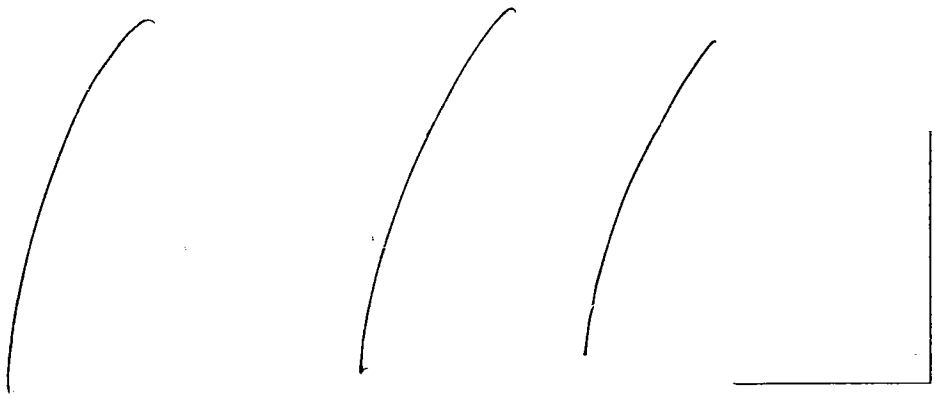
B. Recommendation for nonclinical studies

If the sponsor agrees to the proposed exposure ratios for the reproductive toxicology portions of the label, no further studies would be required. However, it may be in the Sponsor's interest to conduct pharmacokinetic studies that would provide exposure data in animal models to update the exposure margins for reproductive toxicity in the product labeling. The following studies are recommended, but not required.

1. The sponsor should obtain pharmacokinetic/toxicokinetic exposure data in both the rat and rabbit following oral administration to provide more accurate exposure margins for the drug product labeling.

C. Recommendations on labeling

NOTE: The following labeling recommendations have not yet been forwarded to the sponsor. The final agreed upon label may reflect subtle changes following discussion with the Sponsor and the review team. The blue text represents recommended additions to the label, the red crossed out text represents wording removed from the label.

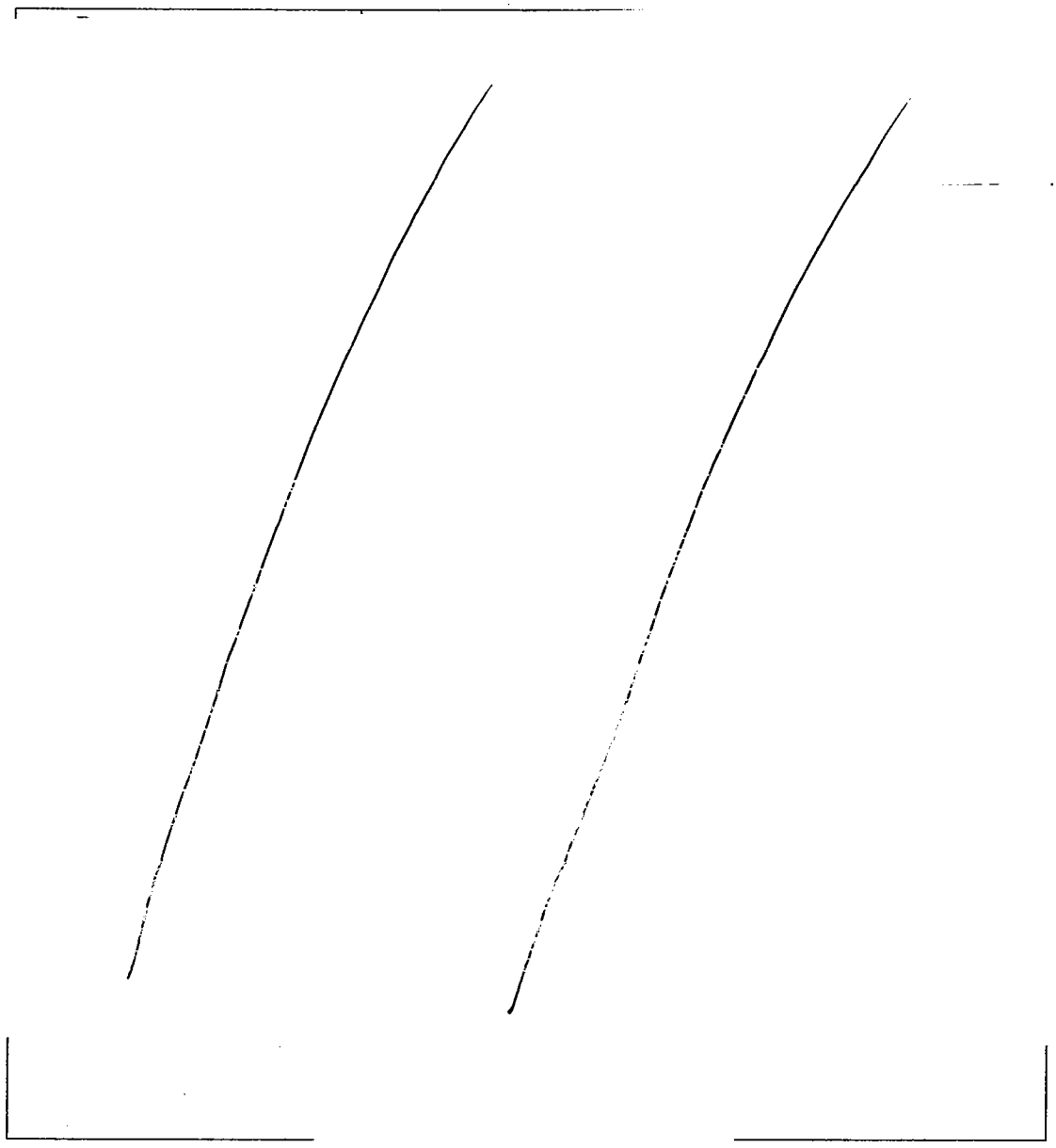


3 Page(s) Withheld

_____ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process



II. Summary of nonclinical findings

A. Brief overview of nonclinical findings

There were no nonclinical deficiencies listed in either the NA letter issued in 2001 or the incomplete response letter sent in 2003. The sponsor therefore did not submit any new nonclinical pharmacology or toxicology data in this submission.

Dr. Hamid R. Amouzadeh reviewed the nonclinical pharmacology and toxicology data submitted in the original NDA submission. Dr. Rőbert Osterberg was the pharmacology toxicology supervisor at that time. Drs. Amouzadeh and Osterberg made the following recommendation: "Based on the results of studies submitted in this NDA, there are no safety concerns with the approval of this drug." The summary of the nonclinical data submitted for diclofenac epolamine below was derived from Dr. Amouzadeh's NDA review and the original IND review completed by Dr. Asoke Mukherjee.

The sponsor completed acute toxicology studies for diclofenac epolamine via the oral route of administration in the dog and rat model. Repeat-dose toxicology studies submitted for diclofenac epolamine included a 4-week and 13-week oral toxicology study in the rat model and a 13-week oral toxicology study in the dog model. The toxicity noted in these studies was consistent with known effects of NSAIDS, including treatment related gastrointestinal toxicity.

A 28-day repeated skin irritation study was completed in the rabbit model with Flector Plaster. The results of the study indicate that the Flector Plaster was not irritating based on scoring for erythema and edema and gross pathology of the skin application site. Dr. Amouzadeh's review indicated that the composition of the Flector Plaster was not provided in the original NDA submission. The composition was requested during this review cycle. The sponsor provided the following information:

> The DEP formulation tested in 1992 (920629 and 920629A) was identical to that reported in the original NDA except that the amount of water was slightly decreased from [redacted] patch to [redacted] to allow for a corresponding increase in sodium carboxymethylcellulose and disodium edetate (see ***Attachment 1***). The current formula (proposed drug product) is the same as that reported in the NDA except disodium edetate was subsequently increased from [redacted] to [redacted] when the batch size was increased from [redacted] to allow for

Repeat-dose dermal toxicology studies were not completed by the sponsor. Although such studies would currently be required to support clinical studies with a novel topical drug product application, they were not required due to the existing clinical experience with the drug product in Europe.

A battery of genetic toxicology studies were completed for diclofenac epolamine, including the in vitro bacterial reverse mutation assay (Ames assay with *S. typhimurium* strains TA1535, TA1537, TA1538, TA100, and TA98), the in vitro mutagenicity in V79 lines of Chinese hamster cells, in vitro chromosome aberrations in human lymphocytes, and an in vivo micronucleus assay in rats. According to the review of these studies by Dr. Mukherjee, diclofenac epolamine was neither mutagenic nor clastogenic under the conditions of the assays.

Reproduction and developmental toxicology studies were completed to support the safety of diclofenac epolamine. The effects of oral diclofenac epolamine in fertility parameters was evaluated following drug treatment of both male and female Sprague Dawley rats. Although described as a segment I study (Fertility and Early Embryonic Development) the study contained additional animals that were treated out to postpartum day 21 and included an evaluation of post-natal growth, development, behavior, and reproductive performance of the F_1 animals. The potential teratogenic effects of diclofenac epolamine were evaluated in both the rat and rabbit models. According to Dr. Mukherjee's review of the rat teratogenicity study, oral administration of diclofenac epolamine did not result in clear evidence of treatment-related teratogenicity in the rat model; however, evidence of embryotoxicity (increased resorption of embryos) was evident at doses that did not produce maternal toxicity. According to Dr. Amouzadeh's review of the rabbit teratogenicity study, oral administration of diclofenac epolamine did not result in evidence of teratogenicity; however, evidence of embryotoxicity (increased late resorptions) was evident at doses that did not produce maternal toxicity. There were no toxicokinetic data obtained in any of the above oral reproductive toxicology studies. Therefore, the potential clinical relevance of the findings to the proposed drug product based on pharmacokinetic data is unknown. As the proposed drug product is intended for topical use, the systemic exposures obtained in the oral toxicology studies should be greater than that obtained clinically via topical application of the patch.

Long-term studies in animals to evaluate the carcinogenic potential of diclofenac epolamine were not completed nor required for an acute indication.

B. Pharmacologic activity

Diclofenac is a phenylacetic acid derivative that has analgesic, antipyretic, and anti-inflammatory activities. These effects are thought to be due to inhibition of cyclooxygenase.

C. Nonclinical safety issues relevant to clinical use

Inclusion of exposure margins in the product labeling provides useful information to the physician on the significance of the animal findings. Due to the absence of toxicokinetic data from the reproductive toxicology studies conducted with oral diclofenac epolamine, exposure margins based on toxicokinetic parameters can not be determined. Therefore, exposure margins must be made via a body surface area comparison. A worst case scenario of 100% absorption of the 180 mg per patch would be a gross overestimate of the total exposure clinically, since most of the drug remains in the patch. According to the sponsor's submission, based on analysis of residual diclofenac epolamine levels in patches following application, approximately

5% of the diclofenac epolamine is lost from the patch after topical application for a 24 hour application period. Since a patch contains 180 mg, and up to two patches could be applied per day for 12 hours, an individual could be exposed to a maximum of about 18 mg of diclofenac epolamine/day. Assuming 100% absorption of the 18 mg, this dose corresponds to 11.1 mg/m^2 based on body surface area for a 60 kg person. The oral doses used in the nonclinical reproductive toxicology studies can be compared to the clinical exposures based on body surface area comparisons. The rat oral dose of 6 mg/kg (36 mg/m^2) results in approximately 3.2-times the maximum human exposure based on body surface area comparisons. The rabbit dose of 6 mg/kg (72 mg/m^2) results in approximately 6.5-times the maximum human exposure based on body surface area comparisons. The table below summarizes the exposure margins obtained via the body surface area comparisons described above:

Species	Dose (mg/kg)	Dose (mg/m^2)	Exposure margin
Human[1]	18 mg/60 kg	11.1	--
Rat	3 mg/kg	18	1.6
	6 mg/kg	36	3.2
Rabbit	3 mg/kg	36	3.2
	6 mg/kg	72	6.5

[1] The maximum daily recommended dose in the human is based on predicted absorption of 5% of the diclofenac epolamine from a single patch application (9 mg) and a total of two patches applied per day (average 60 kg person).

It is recognized that these exposure margins are likely smaller than what would be obtained if pharmacokinetic data were available to provide a more accurate exposure comparison. Since clinical pharmacokinetic data following topical application exists, the sponsor would only have to obtain comparable pharmacokinetic data (C_{max} and AUC_{0-t}) in rats and rabbits following oral administration of 3 and 6 mg/kg diclofenac epolamine. Such data would likely result in greater exposure margins
However, since the data would not result in a change in the pregnancy category and would not likely result in a reduction in the exposure margins, these studies should be recommended but not required.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

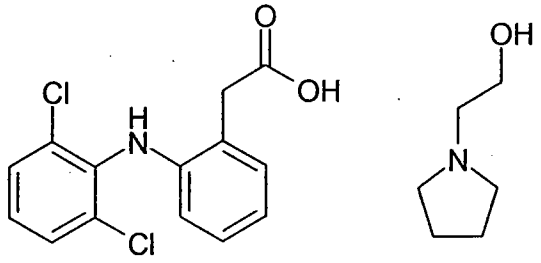
2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21-234
Review number: 2
Sequence number/date/type of submission: N000 / 27-Jul-2006 / AZ
N000 / 28-Dec-2006 / BZ
N000 / 16-Jan-2007 / BZ
Information to sponsor: Yes (X) No ( )
Sponsor and/or agent: Institut Biochemique SA (IBSA)
Campbell, CA
Manufacturer for drug substance:

Reviewer name: **R. Daniel Mellon, Ph.D.**
Division name: Anesthesia, Analgesia, and Rheumatology Products
HFD #: 170
Review completion date: 19-Jan-2007

Drug:
Trade name: **Flector® Patch**
Generic name: Diclofenac epolamine
Code name: **DIEP, DHEP**
Chemical name: 2-[2-[(2,6-dichlorophenyl)amino]phenyl]acetic acid; 2-pyrrolidin-1-ylethanol
CAS registry number: 119623-66-4
Molecular formula/molecular weight: $C_{14}H_{11}Cl_2NO_2 \cdot C_6H_{13}NO$ / 411.322

Structure:



Relevant INDs/NDAs/DMFs:

There are no other NDAs for diclofenac epolamine.

IND#	Drug Name	Status	Division	Indication	Stamp Date	Sponsor

49,459	Diclofenac epolamide patch 1.3%	Active	170		22-Jul-2004	Inst Biochem

DMF#	Subject of DMF	Holder	Status	Submit Date	Reviewer's Comment
	Diclofenac epolamine		Active	10-Apr-00	
			Actve	6-Mar-02	There are no toxicology data in DMF

Drug class: NSAID

Intended clinical population: As per the proposed product labeling:

> The Flector® Patch is indicated for the relief of pain due to strains, sprains and contusions It should be applied only to intact skin at the painful site.

Clinical formulation: The drug product contains 1.3% diclofenac epolamine as the active ingredient, pplied to a non-woven cloth before covering with a polypropylene film. The nas the following composition:

Ingredient	CAS	Amount (mg/patch)	Amount (%)	Maximum Potency (Topical or transdermal) in the Inactive Ingredient Database)	Acceptability Status
Diclofenac salt (DHEP)	119623-66-4			N/A	N/A
D-Sorbitol solution				67.52%	Acceptable
Purified water	7732-18-5			N/A	Acceptable
1,3-Butylene glycol	107-88-0			8.12 mg	Acceptable
Sodium polyacrylate	9003-01-4			Unspecified	Acceptable
Sodium carboxymethylcellulose	9004-32-4			35 mg topical	Acceptable
Kaolin	1332-58-7			Unspecified	Acceptable
Propylene glycol	57-55-6			252 mg and 98.09%	Acceptable
Gelatin	9000-70-8			Unspecified	Acceptable
Polyvinylpyrrolidone (Povidone)	9003-39-8			506.5 mg	Acceptable
Titanium oxide (titanium dioxide)	13463-67-7			5%	Acceptable
Tartaric acid	87-69-4			Not listed in IIG	Acceptable
Dihydroxyaluminum aminoacetate (Aluminum glycinate)	13682-92-3			Not listed in IIG	Acceptable
Polysorbate 80	9005-65-6			72.18 mg	Acceptable

Edetate disodium (EDTA)	6381-92-6			1%	Acceptable
Methyl parahydroxybenzoate (methylparaben)	98-76-3			18%	Acceptable
Propyl parahydroxybenzoate (propylparaben)	94-13-3			10%	Acceptable
Fragrance (Dalin PH)	N/A			Novel Excipient	
Totals		**14000.0**	**99.9%**		

Each patch is 10 cm by 14 cm in size.

The levels of 1,3-butylene glycol used in the drug product formulation exceed those in the FDA's Inactive Ingredient's Database; therefore this ingredient is technically a novel excipient. However, 1,3-butylene glycol is GRAS as a direct food additive according to 21CFR §172.712. Although technically, the use of this compound at these levels via the topical route of administration is novel, it has been adequately characterized in terms of the potential local dermal toxicity via the clinical studies completed to date.

Sodium polyacrylate is listed in the FDA's Inactive Ingredient's Database; however, the maximum potency is not specified. Sodium polyacrylate is listed in 21CFR §173.73 as a secondary direct food additives permitted in food for human consumption. Based on the CAS number, sodium polyacrylate is also known as Carbomer 910 or Carbopol 910. Carbomers are a class of polymers of various viscosity that show a similar toxicology profile. The MW of Carbomer 910 is 750,000, and therefore is not likely absorbed to any appreciable extent. Several Carbomers are used in FDA approved drug products, including this one. Based on the topical route of administration, the large molecular weight, the previous experience with the class of polymers and the use of this polymer in a previously approved drug product, there are no significant safety concerns with the levels used in the Flector® Patch.

The levels of sodium carboxymethylcellulose used in the drug product formulation exceed those in the FDA's Inactive Ingredient's Database; therefore this inactive ingredient is technically a novel excipient. However, sodium carboxymethylcellulose is GRAS as a food product according to 21CFR §182.1745. Although technically, the use of this compound at these levels via the topical route of administration is novel, it has been adequately characterized in terms of the potential local dermal toxicity via the clinical studies completed to date.

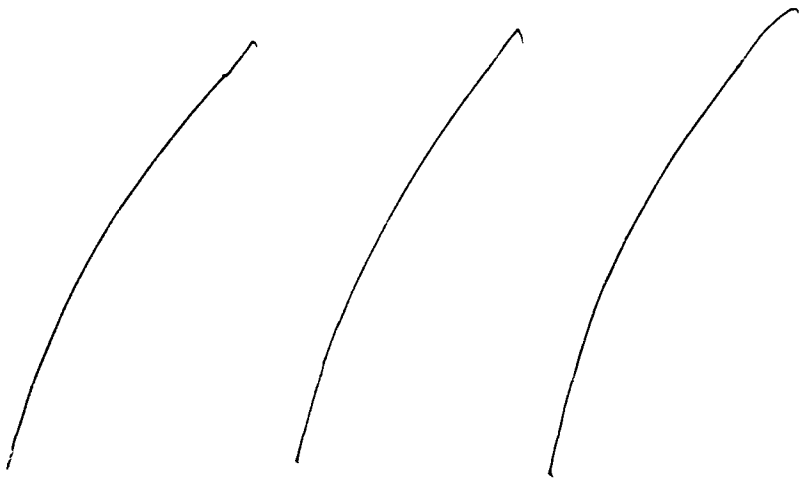
Kaolin is listed in the FDA's Inactive Ingredient's Database; however, the maximum potency is not specified. Kaolin, also called China clay, is hydrated aluminum silicate. According to the Household Products Database, this compound is found in paints and sealers, but also shampoos, conditioners, and cosmetics. Preparations of kaolin and pectin have been used to treat constipation (Kaopectate – currently reformulated so the active ingredient is bismuth subsalicylate). Although kaolin is not in the currently marked kaopectate preparation, it is generally recognized as safe and effective as OTC antidiarrheal drugs 21CFR §335.10. Kaolin is also generally recognized as safe and

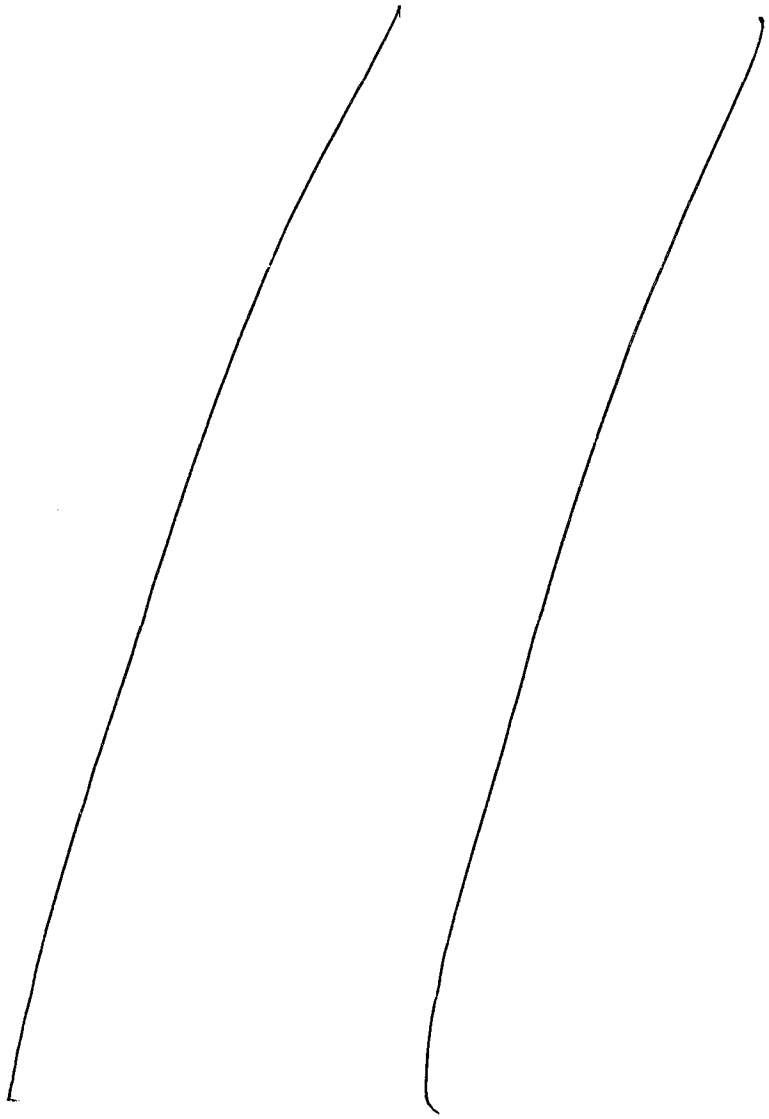
effective as an active ingredient in OTC skin protectant drug products for OTC human use at concentrations of 4 to 20% 21CFR §347.10. Kaolin is also generally recognized as safe and effective as an active ingredient in OTC anorectal drug products for OTC human use at concentrations of up to 50% 21CFR §346.14. There are no significant concerns regarding the safety of the levels this excipient in the drug product formulation.

Tartaric acid is currently not listed in the Inactive Ingredient's database; therefore, is technically considered a novel excipient in drug products. Tartaric acid, however, has been designated GRAS as a direct food additive. Although technically, the use of this compound via the topical route of administration is novel, it has been adequately characterized in terms of the potential local dermal toxicity via the clinical studies completed to date.

Dihydroxyaluminum aminoacetate (Aluminum glycinate) is also not listed in the FDA's Inactive Ingredient's Database. However, this compound is monographed as an active ingredient in antacid products for over-the-counter (OTC) human use 21CFR §331.11. Although technically, the use of this compound via the topical route of administration is novel, it has been adequately characterized in terms of the potential local dermal toxicity via the clinical studies completed to date.

The fragrance Dalin PH is not currently listed in the FDA's Inactive Ingredient's Database. The NA letter issued by the Agency in 2001 noted that the sponsor should provide a LOA for the DMF for this excipient. In this resubmission, the sponsor has obtained a LOA for DMF — DMF — describes the composition of the fragrance Dalin PH as follows (NOTE the following information is considered proprietary and is owned by the DMF holder – therefore should not be disclosed under an FOI requests):





As noted in the table above, all of the components of the Dalin PH fragrance that are not already found in FDA approved drug products are listed as GRAS as either a flavoring agent (21CFR §172.515) or an essential oil, oleoresin or natural extractive in foods (21CFR §182.20). Although technically, the use of these compounds via the topical route of administration is novel, they must be considered to adequately characterized in terms of the potential local dermal toxicity via the clinical studies completed to date. In addition, the fragrance was used in the 28-day dermal irritation study; therefore the local dermal irritation potential of the fragrance has been characterized in that study as well. Based upon the information available, there are no safety concerns with the use of this fragrance in the drug product for the proposed indication.

Route of administration: Topical

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

NOTE: This NDA was been submitted as a 505(b)(1) application. However, during the review of the current resubmission, the Agency determined that due to the reliance on information that was obtained from the published literature, the submission is actually a 505(b)(2) submission. There is no referenced drug product; rather the sponsor is relying on information in the published literature.

Data reliance: Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 21-234 are owned by Institut Biochemique or are data for which Institut Biochemique has obtained a written right of reference. Any information or data necessary for approval of NDA 21-234 that Institut Biochemique 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 Institut Biochemique 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 NDA 21-234.

Studies reviewed within this submission: No new nonclinical studies were submitted as part of this response to the nonapprovable letter of 10/18/2001 and the incomplete response letter of 4/16/203. All submitted nonclinical pharmacology/toxicology studies were previously reviewed by Dr. Hamid Amouzadeh during the first cycle submission. As noted in his review (attached in appendix A), several of the toxicology studies were reviewed by Dr. Asoke Mukherjee under the IND submission. The information from the DMF for Dalin PH fragrance was reviewed here (proprietary data owned by the DMF holder).

Studies not reviewed within this submission:

N/A.

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/s/

R. Daniel Mellon
1/22/2007 04:42:06 PM
PHARMACOLOGIST
Pharmacology Toxicology Supervisor, DAARP

First Cycle 9/26/01

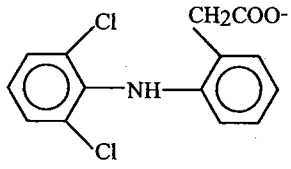
PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA Number:	**21-234**
Review Number:	One
Date of Submission:	December 12, 2000
Number of Volumes:	Seventeen
Sponsor:	Institut Biochimique SA (IBSA) 745-D Camden Ave. Campbell, CA 95008-4146 Phone: 408 871 7331 Fax: 408 374 0181 Contact: Larry J. Caldwell, Ph.D.
Reviewer:	Hamid R. Amouzadeh, Ph.D.
Division:	Division of Anti-inflammatory, Analgesic and Ophthalmic Drug Products
HFD:	550
Review Completion Date:	September 26, 2001

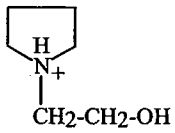
Drug Product:

Class:	NSAID
Chemical Names:	{[dichloro-2,6-phenyl)amino]-2-phenyl}-2-acetate(hydroxy-2-ethyl)-1-pyrrolidine
Generic Names:	Diclofenac epolamine
Synonyms:	(hydroxy-2-ethyl)-1-pyrrolidine diclofenac salt, DHEP, DIEP
Proprietary Name:	Flector®
CAS Registry Number:	119623-66-4
Structural Formula:	$C_{20}H_{24}Cl_2N_2O_3$
Molecular Weight:	411.3
Manufacturer:	[redacted]

Chemical Structures:



Diclofenac



Epolamine

Relevant DMFs, INDs and NDAs: IND 49,459

Indication: Treatment of [redacted]

Clinical Formulation:

Component	Amount (mg/patch)	CAS No.	Standard
Diclofenac salt (DHEP)	180	119623-66-4	-
D-sorbitol solution	[redacted]		JP
Purified water		7732-18-5	JPE
1, 3-butylene glycerol		107-88-0	JSCI
Sodium polyacrylate		9003-01-4	JP
Sodium carboxymethylcellulose		9004-32-4	JP
Kaolin		1332-58-7	JP
Propylene glycol		57-55-6	JP
Gelatin		9000-70-8	JP
Polyvinyl pyrrolidone (Providone)		9003-39-8	JP
Titanium oxide		13463-67-7	JP
Tartaric acid		87-69-4	JP
Dihydroxyaluminum aminoacetate		13682-92-3	JPC
Polysorbate 80		9005-65-6	JP
EDTA		6381-92-6	JP
Methyl parahydroxybenzoate		98-76-3	JP
Propyl parahydroxybenzoate		94-13-3	JP
Fragrance (Dalin PH)		-	-

Abbreviations are as follows: JP = Japanese Pharmacoepia, JPC = Japanese Pharmaceutical Codex; JSCI = Japanese Standards of Cosmetic Ingredients.

Route of Administration: Topical, dermal

Previous Clinical Experiences:

Study No.	Study Title	Major Findings
910195	Absorption and Excretion of DHEP after Cutaneous Repeated Applications of Two Different Dosage Forms: Plaster vs. Gel	- Drug release from plaster formulation was lower than gel - High inter-individual variability - T_{max} = 2-12 hr
PK 0033	Influence of Exercise on the Absorption of Diclofenac Epolamine (DHEP) in Healthy Male Volunteers Administered Epicutaneously Flector EP-Tissugel	AUC_{0-12} was 35% higher in exercising subjects than resting subjects
Giachetti et al.	Pharmacokinetics of Diclofenac Hydroxyethyl-pyrrolidine (DHEP) Plasters in Patients with Monolateral Knee Joint Effusion	Drug concentrations in synovial fluid (1.02 ng/mL) were lower than those found in plasma (3.74 ng/mL)
930499	^{14}C-EP Metabolism Study in Man after Single Oral Administration of the Drug	About 30 % of the administered radioactive dose was recovered in urine within the 0-72 hour
PK 9814	Single and Multiple Dose Pharmacokinetics of Epolamine in Male and Female Healthy Volunteers Administered Flector EP Granules According to a Three Daily Dose Regimen	- Rapid absorption - About 90% was eliminated in urine as epolamine *N*-oxide

Key Words: NSAID, Diclofenac, pain, [redacted]

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

Abbreviations: NSAID = non-steroidal anti-inflammatory drug, DIEP and DHEP = Diclofenac epolamine, EP = Epolamine, PCOL = Pharmacology, SF PCOL = Safety Pharmacology, PK = Pharmacokinetic, TOX = Toxicology, SP TOX = Special Toxicology, GEN TOX = Genetic Toxicology, REP TOX = Reproductive Toxicology, OECD = Organization for Economic Cooperation and Development, NOAEL = No Observed Adverse Event Level, GLP = Good Laboratory Practice, QA = Quality assurance, ANOVA = Analysis of Variance, MTD = Maximum Tolerated Dose, ICH = International Conference on Harmonization.

OVERALL SUMMARY AND EVALUATION:

In this NDA, the sponsor is seeking approval for an epolamine salt of diclofenac (DIEP) in a plaster form for the relief of pain [redacted]

A number of studies have been submitted in support of this NDA.

Pharmacology: The sponsor has provided only one pharmacology study. This study was not designed properly considering the indication of [redacted] Rats were treated with the patch prior to induction of inflammation by injection of carageenan into the joint. Because the model did not mimic the clinical condition and swelling was reduced by only 30%, the efficacy of the drug is questionable.

Two dose-range-finding studies and a study on the comparison of the gastrointestinal effects of diclofenac epolamine and diclofenac Na in rats and dogs by oral route were included under pharmacology studies. These studies were not categorized correctly by the sponsor. The first two studies were considered toxicology studies and the third study was considered a special toxicology study. Therefore, these studies were reviewed under appropriate sections.

Safety Pharmacology: The sponsor did not submit any safety pharmacology studies. The safety pharmacology of diclofenac is well-defined, as it has been used clinically for many years. However, no safety pharmacology study with EP was submitted.

Pharmacokinetics/Toxicokinetics: The sponsor did not provide adequate non-clinical pharmacokinetic/toxicokinetic information with DIEP. The results of submitted studies indicated that DIEP was not absorbed consistently and there was large inter-individual variation in the level of absorption. Similar inconsistencies in absorption and inter-individual variation were observed in humans. The metabolism and excretion profile of DIEP is similar to that of diclofenac sodium. EP is metabolized to epolamine *N*-oxide, which is mainly eliminated in the urine.

Toxicology: Single and repeated dose toxicity of DIEP, epolamine, 1-(2-hydroxyethyl) pyrrolidine-*N*-oxide (metabolite of epolamine) were studied in rats and dogs using mainly the oral route. Major findings of these studies are summarized below. The results of these studies indicate that DIEP has a similar toxicity profile to other salt forms of diclofenac. Epolamine was not toxic when administered to rats orally at 100 mg/kg/day (600 mg/m^2/day) for 14 days. This dose is much lower than the human exposure level of epolamine of about 60 mg (180 mg of DIEP) or 1 mg/kg (based on a 60 kg body weight) or 37 mg/m^2.

Study No.	Study Title	Dose Duration	GLP QA	Major Findings
9190090	EP: Dose-range finding study in dogs by oral route	10, 30, 90, 150 or 250 mg/kg/day, 3-7 days	Yes Yes	The dose of 90 mg/kg/day, buffered to pH 8, appeared clinically tolerated when administered for one week.
910226	DIEP: Dose-range finding study in dogs by oral route	1 or 3 mg/kg/day, 29 or 7 days	Yes Yes	No drug-related toxicity was found at 1 mg/kg/day. At 3 mg/kg/day, gastrointestinal bleeding was observed.

82258	Acute oral toxicity study with DIEP in rats	40, 100, 140, 200 or 450 mg/kg, Single dose	Yes Yes	The LD_{50} value for both sexes observed for a period of 22 days was 150 mg/kg.
644/502	DIEP: 4-week oral toxicity study in the rats	DIEP: 0, 3, 7 or 14 mg/kg/day and diclofenac Na: 3, 6 or 12 mg/kg/day for 4 weeks	Yes Yes	DIEP caused peritonitis at 7 mg/kg. Above this level, the toxic effects were similar to diclofenac Na. Females were more susceptible. The changes observed were typical of lesions caused by NSAIDs.
5821-644/2	DIEP: 13-week oral (gavage) subchronic toxicity study in the rat	DIEP: 0, 3, 7 or 14 mg/kg/day and EP: 20 mg/kg/day for 13 weeks	No Yes	No toxicity was observed at the highest dose for either DIEP or diclofenac Na. Maximum tolerated dose was not reached.
910091	DIEP: 13-week oral toxicity study in dogs	DIEP: 0, 0.5, 1 or 2 mg/kg/day and EP: 20 mg/kg/day for 13 weeks	Yes Yes	The 0.5 mg/kg dose was the highest dose tolerated. At higher doses gastrointestinal ulceration and bleeding, kidney damage and death occurred.
910172	Comparative single-dose toxicity study in rats by oral route	1000 – 3000 mg/kg, single dose	Yes Yes	NOAEL for non-buffered and buffered EP were < 1000 and 3000 mg/kg, respectively.
5673-644/1	EP: Pilot oral (gavage) toxicity study in the rat	50 – 1000 mg/kg, single dose and 100 mg/kg for 14 days	No Yes	No significant clinical changes up to 1000 mg/kg. Repeated treatment for 14 days at 100 mg/kg/day produced no evidence of toxicity.
910220	EP: 4-week oral toxicity study in the rat with 4 weeks of recovery	0, 25, 50 or 100 mg/kg/day, for 4 weeks	Yes Yes	No consistent effect was observed indicating that the maximum tolerated dose was not reached.
990012	1-(2-hydroxyethyl) pyrrolidine-N-oxide: 4-week intravenous toxicity study in rats	0, 0.7, 7 or 70 mg/kg/day for 4 weeks (followed by 2 weeks recovery)	Yes Yes	The 7 mg/kg/day dose could be considered NOAEL. At 70 mg/kg/day, ophthalmic toxicity was observed in some females.
990842	1-(2-hydroxyethyl) pyrrolidine-N-oxide: 4-week oral toxicity study in rats	0, 6, 18 or 30 mg/kg for 4 weeks (followed by 2 weeks recovery)		No consistent dose-related toxicity. Maximum tolerated dose was not reached.
644/501	DIEP: 14-day tolerance study by oral route in the rat	DIEP and Diclofenac Na: 90-245 mg/kg, single dose. DIEP: 0, 2, 6 or 20 mg/kg/day and Diclofenac Na: 1, 3 or 10 mg/kg/day for 15 days	Yes Yes	No toxicity at 6 mg/kg/day. 60-100% lethality at 20 mg/kg/day. NOAEL dose was between 6-20 mg/kg/day for DIEP.

Genetic Toxicology: Results of genetic toxicology studies submitted under IND 49,459 indicated that DIEP was not genotoxic in bacterial reverse mutagenesis, rat micronucleus, gene mutation (Chinese hamster lung cells), and chromosomal aberration (human lymphocyte) assays. Genotoxic potential of epolamine was tested using bacterial reverse mutagenesis, unscheduled DNA synthesis (HeLA cells), chromosomal aberration (human lymphocyte), and rat micronucleus assays. The results showed that epolamine was not genotoxic. However, there were a number of problems with genotoxic assays done with epolamine, which are detailed in the individual review of these studies.

Carcinogenicity: Because DIEP is intended for short treatment periods and was not genotoxic in a battery of genotoxicity tests, carcinogenicity testing, including photo-carcinogenicity, may not be needed (ICH Guidelines, S1A).

Reproductive and Developmental Toxicology: Reproductive toxicity studies were done with DIEP and EP in rats and rabbits. Teratogenesis, fertility and reproductive studies were done in rats with DIEP and EP. These studies were submitted under IND 45,459 and were reviewed previously. The results indicated that DIEP at 1, 3 or 6 mg/kg/day and EP at 50 mg/kg/day were not teratogenic and did not affect male and female fertility. Embryo resorption was observed at maternal non-toxic doses. In rabbits treated with 0, 1, 3 or 6 mg/kg/day of DIEP and 20 or 100 mg/kg/day of EP, DIEP increased the rate of early and late resorption, but EP increased only the rate of early resorption. This latter finding is in agreement with the results of a recently published study (Menegola et al., 1998) indicating that aminoalcohols such as EP cause early resorption in rats at 81 mg/kg (246 mg/m^2).

Special Toxicology Studies: Local toxicity of DIEP was studied in eye and skin irritation tests in rabbits and in a sensitization assay in guinea pigs. DIEP gel was slightly irritating to rabbit eyes, but it was not irritating to rabbit skin in either patch or gel form. DIEP was weakly sensitizing in guinea pigs. Adverse gastrointestinal effects of DIEP were compared to diclofenac sodium in rats and dogs. The results indicated that gastrointestinal effects of diclofenac epolamine were similar to diclofenac.

Evaluation: DIEP is an epolamine salt of diclofenac. Diclofenac is marketed in the United States as sodium salt (Voltaren® tablet and ophthalmic solution) and as potassium salt (Cataflam® tablet). Therefore, the pharmacologic and toxicologic profile of diclofenac is well-defined.

The epolamine salt of diclofenac was developed in [redacted] in an attempt to enhance solubility of diclofenac. The results of studies submitted under this NDA indicate that the pharmacologic and toxicologic profile of DIEP is very similar to other diclofenac salts. In addition, these results showed that EP was not toxic in animals at doses above that expected in humans, it was not genotoxic, and caused early resorption in rats and rabbits at higher doses.

Because this product is a patch and is applied locally, the expected plasma level of the drug should be much less than that of oral route. Therefore, the major concern is with the local tolerance of the drug product. The sponsor has provided local tolerance studies indicating that DIEP gel was slightly irritating to rabbit eyes, but it was not irritating to rabbit skin in either patch or gel form. DIEP was weakly sensitizing in guinea pigs. The studies with the gel form were not acceptable because the formulation and the form of the drug products were different from that used in clinical studies. The results of the 28-day studies with the patch using rabbit skin did not show any evidence of irritation. The interpretation of these results was confounded by the fact that the anti-inflammatory effects of diclofenac could mask the irritant effects of the patch.

All of the inactive ingredients are included in Japanese Pharmacoepia, Japanese Pharmaceutical Codex or Japanese Standards of Cosmetic Ingredients indicating that they are safe. Moreover,

according to Dr. Sue-Ching Lin, the chemist, "the inactive ingredients for which monographs exist in the USP/NF should comply with the requirements of the current USP/NF". Therefore, she asked that the sponsor "provide a revised section regarding the control of the inactive ingredients". The sponsor indicated that "the manufacturer, Teikoku Seiyaku Co., Ltd., controls all of the inactive ingredients at this time according to Japanese compendia. Teikoku is prepared to change the testing methods and acceptance criteria to the current USP/NF for all ingredients for which such monographs exist, as was done in the case of Lidoderm® Patch, prior to the completion of the review process". Dr. Lin indicated that "this issue remains a deficiency until the specifications of the inactive ingredients are revised". Once this deficiency is corrected, the inactive ingredients will be in compliance with USP/NF standards.

An additional evidence for the safety of the drug product is that it has been marketed in the patch form in other countries for many years indicating that a large number of humans have been exposed to the drug product, apparently without major toxicity. Therefore, the results of the studies submitted in this NDA provide adequate information about the safety of the DIEP patch.

RECOMMENDATIONS: Based on the results of studies submitted in this NDA, there are no safety concerns with the approval of this drug.

Reviewer's Signature: _____ _____
Hamid R. Amouzadeh, Ph.D. Date

Team Leader's Signature: _____ _____
Robert Osterberg, Ph.D. Date

STUDIES SUBMITTED IN THIS NDA:

Study Type	Study No.	Study Title	GLP QA	Location of Report
PCOL	-	Effects of DHEP plaster on carrageenan-induced acute inflammation in rats	-	Vol. 4A pp. 245-247
PCOL	9190090	EP: Dose-range finding study in dogs by oral route	OECD Yes	Vol. 4A pp. 51-107
PCOL	910226	DIEP: Dose-range finding study in dogs by oral route	OECD Yes	Vol. 4A pp. 108-176
SP TOX	870413 870414	Gastrolesivity of diclofenac hydroxyethyl pyrrolidine vs. diclofenac Na in rats and dogs	No No	Vol. 4A pp. 177-248
PK		Plasma levels of DHEP after topical applications in rats	No No	Vol. 4B pp. 1-2
PK	920443	DIEP: Comparison of bioavailability of an oral formulation of DIEP with an oral formulation of diclofenac-Na in the dog	No No	Vol. 4B pp. 3-59
PK	910096	^{14}C-EP: Whole body autoradiography in the rat after oral administration	OECD Yes	Vol. 4B pp. 59-97
PK	910097	^{14}C-EP: Pharmacokinetics study in the rat after single epicutaneous and oral administration and preliminary metabolism study after oral administration	OECD Yes	Vol. 4B pp. 98-212
PK	970335	^{14}C-EP: *In vitro* binding to rat, dog, human plasma proteins and to human serum albumin	No No	Vol. 4B pp. 213-258
PK	970336	^{14}C-EP: Pharmacokinetic after single and repeated oral administration in the dog	OECD Yes	Vol. 4B pp. 259-376
PK	970337	^{14}C-EP: Metabolism study in urine after single and repeated oral administration in the dog (with extension in plasma)	No No	Vol. 4B pp. 377-407
PK	930677	^{14}C-EP: Pharmacokinetic investigation in pregnant rabbits after single intravenous and oral administration	No No	Vol. 4B pp. 408-459 Vol. 7 pp. 440-491
TOX	082258	Acute oral toxicity study with DIEP in rats	OECD Yes	Vol. 5 pp. 197-250
TOX	644/502	DIEP: 4-week oral toxicity study in the rats	Yes Yes	Vol. 5 pp. 251-298
TOX	5821-644/2	DIEP: 13-week oral (gavage) subchronic toxicity study in the rat	No Yes	Vol. 5 pp. 299-346
TOX	910091	DIEP: 13-week oral toxicity study in dogs	OECD Yes	Vol. 5 pp. 347-410
SP TOX	902456	Local tolerance test in the rabbit, Cutaneous primary irritation test to evaluate the sensitizing potential by topical application in the guinea pig	Yes Yes	Vol. 5 pp. 411-504
SP TOX	920629	Flector® plaster: 28-day repeated skin irritation in rabbits	OECD Yes	Vol. 6 pp. 1-60
SP TOX	920629/ A	Flector® plaster: 28-day repeated skin irritation and absorption in rabbits – Proof of absorption	OECD Yes	Vol. 6 pp. 61-106
TOX	910172	Comparative single-dose toxicity study in rats by oral route	OECD Yes	Vol. 6 pp. 107-161

TOX	5673-644/1	EP: Pilot oral (gavage) toxicity study in the rat	No Yes	Vol. 6 pp. 162-190
TOX	910220	EP: 4-week oral toxicity study in the rat with 4 weeks of recovery	OECD Yes	Vol. 6 pp. 191-234
TOX	990012	1-(2-hydroxyethyl)pyrrolidine-N-oxide: 4-week intravenous toxicity study in rats followed by 2 weeks of recovery	OECD Yes	Vol. 6 pp. 235-278
TOX	990842	1-(2-hydroxyethyl)pyrrolidine-N-oxide: 4-week oral toxicity study in rats followed by 2 weeks of recovery	OECD Yes	Vol. 6 pp. 279-319
TOX	644/501	DIEP: 14-day tolerance study by oral route in the rat	No Yes	Vol. 6 pp. 320-387
GEN TOX	900178	Study of the capacity of the test article DIEP to induce gene mutation in strains of *Salmonella typhimurium*	OECD Yes	Vol. 6 pp. 388-423
GEN TOX	900179	Micronucleus induction in bone marrow cells of rats treated by oral route with test article DIEP	OECD Yes	Vol. 7 pp. 1-19
GEN TOX	900182	Study of the capacity of the test article DIEP to induce gene mutation in V79 Chinese hamster lung cells	OECD Yes	Vol. 7 pp. 20-47
GEN TOX	900183	Study of the capacity of the test article DIEP to induce chromosomal aberration in human lymphocyte *in vitro*	OECD Yes	Vol. 7 pp. 48-66
GEN TOX	910068	Study of the capacity of the test article N-(2-hydroxyethyl) pyrrolidine to induce gene mutation in strains of *Salmonella typhimurium*	OECD Yes	Vol. 7 pp. 67-103
GEN TOX	910069	Study of the capacity of the test article N-(2-hydroxyethyl)pyrrolidine to induce unscheduled DNA synthesis in cultured HeLA cells	OECD Yes	Vol. 7 pp. 104-129
GEN TOX	910070	Study of the capacity of the test article N-(2-hydroxyethyl)pyrrolidine to induce chromosome aberration in human lymphocytes cultured *in vitro*	OECD Yes	Vol. 7 pp. 130-150
GEN TOX	910132	N-(2-hydroxyethyl)pyrrolidine: micronucleus test in rat bone marrow	OECD Yes	Vol. 7 pp. 151-172
REP TOX	910092	EP: Teratogenesis study in rats by oral route	No No	Vol. 7 pp. 173-186
REP TOX	910093	EP: Fertility and reproduction study in rats by oral route	No No	Vol. 7 pp. 187-202
REP TOX	910227	DIEP: Teratogenesis study in rats by oral route	OECD Yes	Vol. 7 pp. 203-234
REP TOX	910228	DIEP: Fertility and reproduction study in rats by oral route	OECD Yes	Vol. 7 pp. 235-300
REP TOX	920505	DIEP in comparison with EP: Preliminary teratogenesis study in rabbits by oral route	OECD Yes	Vol. 7 pp. 301-328
REP TOX	920506	DIEP in comparison with EP: Teratogenesis study in rabbits by oral route	OECD Yes	Vol. 7 pp. 329-359
REP TOX	920507	EP: Fertility and reproduction study by oral route in rats	OECD Yes	Vol. 7 pp. 360-413
REP TOX	930240	EP: preliminary teratogenesis study in rabbit by oral route	OECD Yes	Vol. 7 pp. 414-439

Abbreviations: PCOL = Pharmacology, SF PCOL = Safety Pharmacology, PK = Pharmacokinetic, TOX = Toxicology, SP TOX = Special Toxicology, GEN TOX = Genetic Toxicology, REP TOX = Reproductive Toxicology, OECD = Organization for Economic Cooperation and Development

Studies Reviewed Under IND 49,459 (January 18, 1996):

Study Type	Study No.	Study Title	GLP QA	Location of Report
PCOL	-	Effects of DHEP plaster on carrageenan-induced acute inflammation in rats	-	Vol. 4A pp. 245-247
PK	-	Plasma levels of diclofenac after topical applications in rats	-	Vol. 4B pp. 1-2
SP TOX	-	Local tolerableness and PK profile of transdermal diclofenac in rabbits	-	-
PK	-	Plasma levels and tolerableness of topical diclofenac in adult human volunteers	-	-
TOX	-	Dose-range finding study of diclofenac hydroxypyrrolidine toxicity in dogs given orally	-	-
TOX	910091	DIEP: 13-week oral toxicity study in dogs	OECD Yes	Vol. 5 pp. 347-410
GEN TOX	900178	Study of the capacity of the test article DIEP to induce gene mutation in strains of *Salmonella typhimurium*	OECD Yes	Vol. 6 pp. 388-423
GEN TOX	900179	Micronucleus induction in bone marrow cells of rats treated by oral route with test article DIEP	OECD Yes	Vol. 7 pp. 1-19
GEN TOX	900182	Study of the capacity of the test article DIEP to induce gene mutation in V79 Chinese hamster lung cells	OECD Yes	Vol. 7 pp. 20-47
GEN TOX	900183	Study of the capacity of the test article DIEP to induce chromosomal aberration in human lymphocyte *in vitro*	OECD Yes	Vol. 7 pp. 48-66
REP TOX	910227	DIEP: Teratogenesis study in rats by oral route	OECD Yes	Vol. 7 pp. 203-234
REP TOX	910228	DIEP: Fertility and reproduction study in rats by oral route	OECD Yes	Vol. 7 pp. 235-300

Abbreviations: PCOL = Pharmacology, SF PCOL = Safety Pharmacology, PK = Pharmacokinetic, TOX = Toxicology, SP TOX = Special Toxicology, GEN TOX = Genetic Toxicology, REP TOX = Reproductive Toxicology, OECD = Organization for Economic Cooperation and Development

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INTRODUCTION AND DRUG HISTORY:

Diclofenac, a derivative of benzeneacetic acid, is a non-steroidal anti-inflammatory drug (NSAID) that has been on the market for many years. It is indicated for "acute and chronic treatment of signs and symptoms of osteoarthritis and rheumatoid arthritis" (PDR® Online). In the United States, the approved drug is in either sodium or potassium salt form. The epolamine salt of diclofenac was developed in in an attempt to enhance solubility of diclofenac. DIEP has not been approved in the US for any indication. In this NDA, the sponsor is seeking approval for DIEP in a patch form for the relief of pain

PHARMACOLOGY:

The sponsor has provided only one pharmacology study. In this study, effects diclofenac epolamine plaster on carrageenan-induced swelling of the hind paws were investigated. The plaster was applied to the hind paw of rats 4 hr prior to carageenan administration and swelling was measured 2, 3, and 4 hrs later. The result indicated that diclofenac epolamine plaster inhibited carageenan-induced swelling by 30%.

This study was not designed properly considering the indication of To determine the efficacy of diclofenac epolamine patch in conditions similar to clinical indication, DIEP patch should have been administered after carageenan administration. Because this model did not mimic the clinical condition and swelling was reduced by only 30%, the efficacy of the drug is questionable.

SAFETY PHARMACOLOGY:

The sponsor did not submit any safety pharmacology study. The safety pharmacology of diclofenac is well-defined as it has been used clinically for many years. However, similar information for epolamine was not provided.

PHARMACOKINETICS/TOXICOKINETICS:

Summary of Pharmacokinetic/Toxicokinetic Findings

Study No.	Study Title	Dose Duration	GLP QA	Major Findings
-	Plasma levels of DHEP after topical applications in rats		No No	Peak plasma concentration reached at 3 hr after application (Inferred from graph, this is only a 2-page study report)

920443	DIEP: Comparison of bioavailability of an oral formulation of DIEP with an oral formulation of diclofenac-Na in the dog	DIEP: 65 mg/kg DCF: 50 mg/kg Single dose	No No	DIEP: C_{max} (ng/mL) = 3696 ± 2035 t_{max} (hrs) = 6 ± 2.31 AUC_n (h.ng/mL) = 28385 ± 12806 F_n = 1.26 DCF: C_{max} (ng/mL) = 7158 ± 6140 t_{max} (hrs) = 9.25± 9.91 AUC_n (h.ng/mL) = 37179 ± 31377 High interindividual variation
910096	^{14}C-EP: Whole body autoradiography in the rat after oral administration	175 mg/kg, Single dose	OECD Yes	0.5 h: stomach and small intestine liver and kidneys > spleen, bone marrow and salivary glands, > heart and lungs 24 h: liver and kidneys (medulla) > thymus, lungs, brain, spleen, kidneys (cortex), bone marrow and salivary glands > gastrointestinal wall
910097	^{14}C-EP: Pharmacokinetic study in the rat after single epicutaneous and oral administration and preliminary metabolism study after oral administration	100 mg/kg	OECD Yes	**Parameter** / **EPI** / **PO** C_{max} (ng/mL) 2.25 20.81 t_{max} (hrs) 0.5 0.5 C_n (μg eq./mL): 1.67 2.49 t_n (h) 48 48 AUC_n (h.ng/mL): 74.53 234.5 MRT_n (μg eq./mL) 24.79 17.58 F_n 0.31 - One metabolite in urine
970335	^{14}C-EP: *In vitro* binding to rat, dog, human plasma proteins and to human serum albumin	1 and 10 mg/L	No No	Negligible binding (less than 6% in all cases).
970336	^{14}C-EP: Pharmacokinetic after single and repeated oral administration in the dog	50 mg/kg, single or repeated for 7 consecutive days	OECD Yes	**Parameter** / **Single** / **Multiple** C_{max} (ng/mL) 14.3 ± 3.5 20.1 ± 3.3 t_{max} (hr) 1.2 ± 0.3 0.85 ± 0.3 C_n (μg eq./mL) 3.3 ± 1.7 5.8 ± 3.4 t_n (h) 168.0 ± 0.0 168.0 ± 0.0 AUC_n (h.μg/mL) 855.4 ± 423.7 1366.2 ± 687.02 $t_{1/2}$ (hr) 95.4 ± 2.6 160.7 ± 54.3 AUC (h.μg/mL) 1302.0 ± 653.3 2890.2 ± 1874.5 MRT (hr) 151.9 ± 3.7 236.3 ± 77.9
970337	^{14}C-EP: Metabolism study in urine after single and repeated oral administration in the dog (with extension in plasma)	50 mg/kg, single or repeated for 7 consecutive days	No No	The GC-MS analysis showed the presence of two main peaks, the first with the characteristic ions of *N*-pyrrolidine acetaldehyde and the second corresponding to unchanged EP.

930677	^{14}C-EP: Pharmacokinetic investigation in pregnant rabbits after single intravenous and oral administration	100 mg/kg, Single dose	No No	**Parameter**	**IV**	**PO**
				C_{max} (ng/mL)	-	28.5 ± 4.24
				t_{max} (h)	-	1.0 ± 0.0
				C_n (μg eq./mL)	6.5 ± 1.3	6.6 ± 0.8
				t_n (h)	24.0 ± 0.0	24.0 ± 0.0
				AUC_n (h.μg/mL)	242.2 ± 21.9	230.4 ± 26.0
				MRT_n (h)	8.4 ± 0.7	9.4 ± 0.54
				AUC (h.μg/mL)	1187.2 ± 959.5	1182.4 ± 558.9
				MRT (h)	124.0 ± 109.9	141.15 ± 85.4
				$t_{1/2\beta}$ (h)	90.9 ± 76.23	101.5 ± 59.6
				F_n	-	0.94

TOXICOLOGY:

Study Title: **Epolamine (EP): Dose-Range Finding Study in Dogs by Oral Route**

Key Findings: *The dose of 90 mg/kg/day, buffered to pH 8, appeared clinically tolerated when administered for one week.*

Study Number: 9190090

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 4A, pp. 51-107

Study Period: April 22, 1991 – May 28, 1991

Species/Strain/Age/Sex/Weight: Dog, Beagle, 9-10 months old, ♂ and ♀, 8.8-10.4 kg

Test Substance: Epolamine, batch no. 176333, [redacted] pure

Vehicle: Deionized water

Treatment Scheme:

Phase I				Phase II*			
Dose	**n**		**Treatment**	**Dose**	**n**		**Treatment**
(mg/kg/day)	♂	♀	**Days**	(mg/kg/day)	♂	♀	**Days**
10	2	2	4	90	2	2	7
30	2	2	3	250	2	2	4
90	2	2	5	150	2	3	5

* Animals from Phase I were used after a 9-day washout period

Route of Administration: Oral (gavage)

Parameters Observed/Measured: Clinical signs, body weight, food consumption, hematology, clinical biochemistry, gross pathology, organ weight and histopathology.

Summary of Findings: There was no mortality. The 90 mg/kg/day oral dose level of EP solution (non-buffered, pH 12) was not tolerated by dogs, causing gastric intolerance. Oral doses of 150 and 250 mg/kg/day (buffered to pH 8) induced signs of general toxicity, with CNS involvement (muscular tremors and tonic-clonic convulsions), liver damage, and gastric intolerance (vomiting within 5-30

min of gavage). The oral dose of 90 mg/kg/day, buffered to pH 8, appeared clinically tolerated when administered for one week. A dose of 20-50 mg/kg (buffered to pH 8) could be used in prolonged (1-3 months) toxicity studies.

Reviewer's Comments: The reviewer concurs with the findings of the study. Because this was a preliminary study, the sponsor did not analyze many parameters in detail. However, for the stated objective of this study, i.e. dose-finding, the study is considered adequate.

Study Title: **DIEP: Dose-Range Finding Study in Dogs by Oral Route**

Key Findings: *No drug-related toxicity was found at 1 mg/kg/day. At 3 mg/kg/day, gastrointestinal bleeding was observed.*

Study Number: 910226

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 4A, pp. 108-176.

Species/Strain/Age/Sex/Weight: Dog, Beagle, 7-11 months old, ♂ and ♀, 6.7-9.2 kg

Test Substance: DIEP, batch no. 01.8.99, [redacted] ure

Vehicle: Deionized water

Route of Administration: Oral (gavage)

Treatment Scheme:

Dose (mg/kg/day)	n ♂	n ♀	Treatment Days
3	2	2	7
1	1	3	29

Parameters Observed/Measured: Clinical signs, body weight, food consumption, hematology, clinical biochemistry, gross pathology and organ weight.

Summary of Findings: There was no mortality. Dose of 3 mg/kg/day of DIEP administered for seven consecutive days by oral route to dogs was not tolerated as it induced severe adverse effects (mainly gastrointestinal bleeding) and consequent decrease in body weight and food consumption. The dose level of 1 mg/kg/day given for 29 consecutive days was tolerated, since no relevant adverse clinical signs were observed in any animal.

Reviewer's Comments: The reviewer concurs with the findings of the study. Because this was a preliminary study, the sponsor did not analyze many parameters in detail. However, for the objective of this study, i.e. dose-finding, the study is considered adequate.

Study Title: **Acute Oral Toxicity Study with DIEP in Rats**

Key Findings: *The LD_{50} value for the acute oral toxicity of DIEP in rats of both sexes observed for a period of 22 days was 150 mg/kg*

Study Number: 82258

Test Site: [redacted]

GLP Compliance: Yes

Quality Assurance: Yes

Location of Report: Vol. 5, pp. 197-250

Study Period: January 20, 1987-February 12, 1987

Species/Strain/Age/Sex/Weight: Rat, Wistar, 9-11 weeks, ♂ and ♀, 167-220 g

Animal Number: 5/sex/group

Test Substance: DIEP, batch no. 3, [redacted]

Dose/Route: 40, 100, 140, 200 or 450 mg/kg, oral (gavage)

Duration: Single dose

Observation Period: 22 days

Parameters Observed/Measured: Mortality, clinical signs and body weight.

Summary of Findings:

Dose (mg/kg)	Mortality (%)	Clinical Symptoms
40	0	Sedation, dyspnea, curved body position, ruffled fur
100	30	Sedation, dyspnea, rhinorrhea (♀), ataxia (♀), curved body position, emaciation, ruffled fur, anemia
140	20	Sedation, dyspnea, ataxia, curved body position, emaciation, ruffled fur, pale, anemia (♀)
200	80	Sedation, dyspnea, ataxia, ventral body position (♀), curved body position, emaciation, ruffled fur, pale, anemia
450	100	Sedation, dyspnea, ataxia, spasm (♀), curved body position, emaciation, ruffled fur, pale, anemia

Majority of sacrificed animals did not show any gross pathological changes. The LD_{50} value for the acute oral toxicity of DIEP in rats of both sexes observed for a period of 22 days was 150 mg/kg (185 mg/kg for males and 123 mg/kg for females). Death occurred between days 3-7.

Reviewer's Comments: The reviewer concurs with the findings of the study. Because this was a preliminary study to establish the toxicity profile of DIEP, the sponsor did not analyze many parameters in detail. However, for the objective of this study, i.e. dose-finding, the study is considered adequate. Certificate of analysis of the test substance should have been provided.

Study Title: **DIEP: 4-week Oral Toxicity Study in the Rats**

Key Findings: *DIEP caused peritonitis at 7 mg/kg. Above this level, the toxic effects were similar to diclofenac Na. Females were more susceptible. The changes observed were typical of the lesions caused by NSAIDs.*

Study Number: 644/502
Test Site: [redacted]
GLP Compliance: Yes
Quality Assurance: Yes
Location of Report: Vol. 5, pp. 251-298
Study Period: September 21, 1988-October 21, 1988
Species/Strain/Age/Sex/Weight: Rats, Sprague-Dawley, 6 weeks, ♂ and ♀, 145.5-198.7 g
Test Substance: DIEP, batch no. 4/TF, [redacted] pure and diclofenac Na. batch no. BT 510-C1, [redacted]
Vehicle: Distilled water
Treatment Scheme:

Treatment	Dose (mg/kg/day)	n ♂	n ♀
Vehicle	0	10	10
DIEP	3	5	5
DIEP	7	10	10
DIEP	14	10	10
Diclofenac Na	3	10	10
Diclofenac Na	6	10	10
Diclofenac Na	12	5	5

Route of Administration: Oral (gavage)
Duration: Once daily for 4 consecutive weeks
Parameters Observed/Measured:

Clinical Signs: Twice daily.

Body Weight and Food Consumption: Weekly.

Laboratory Investigation: At the end of treatment period.

Hematology parameters examined were: erythrocyte indices (MCV, MCH, and MCHC), hemoglobin, hemoglobin index, packed cell volume, platelet count, prothrombin time, red blood cell count, white blood cell count. Clinical Biochemistry parameters examined were: albumin, globulin, A/G ratio, alanine aminotransferase, alkaline phosphatase, aspartate aminotransferase, chloride, total cholesterol, creatinine, glucose, phosphorus, potassium, sodium, total bilirubin, total protein, blood urea nitrogen, calcium. Urinalysis parameters examined were: appearance, bilirubin, blood, glucose, ketones, microscopy of centrifuged deposit, pH, protein, specific gravity, urobilinogen, volume, and reducing substances. Bone marrow smears were prepared, but not read.

Gross Pathology: The following tissues were preserved using appropriate methods and fixatives for histological examination: gross lesions, adrenals, aorta (thorasic), bone (femur), bone marrow (rib), brain (fore, mid, hind), cecum, colon, duodenum, epididymides, esophagus, eyes, heart, ileum, kidneys, liver, lungs, lymph nodes (mesenteric and mandibular), mammary gland, ovaries, pancreas, pituitary, prostate, salivary gland (submaxillary), sciatic nerve, skeletal muscle (qudriceps), skin (mammary gland), spinal cord (cervical and lumbar), spleen, stomach, thymus, thyroid (with parathyroid), trachea, urinary bladder and uterus.

Organ Weight: The following organs were dissected free of fat and weighed: adrenals, thyroids, ovaries, testes, liver, brain, heart, kidneys and spleen.

Histopathology: All tissues were examined.

Statistical Analysis: Analysis of variance and Dunnett's test.

Summary of Findings:

Mortality: (killed moribund or found dead)

Treatment	Dose (mg/kg/day)	% Mortality ♂	% Mortality ♀
DIEP	3	0	0
	7	0	10
	14	20	100
diclofenac Na	3	0	0
	6	0	0
	12	0	100

Clinical Signs: No clinical signs or abnormal behavior were observed in surviving animals except fur staining in females at 6 mg/kg of DIEP.

Body Weight Gain: Body weight gain of surviving animals was lower at 14 mg/kg of DIEP and at 12 mg/kg of diclofenac from the first week of treatment compared to control. However, it was not greater that 10%.

Food Intake: No consistent dose-related changes were observed.

Hematology, Clinical Biochemistry and Urinalysis: Although some values in few individual animals were significantly changed, these changes were not consistent among animals of each group and sex. Blood was observed in urine at 14 mg/kg of DIEP (4/8 animals) and at 12 mg/kg of diclofenac (2/5 animals).

Gross Pathology: In surviving animals, no treatment related changes were observed at the macroscopic examination. Dead or killed animals had peritonitis secondary to perforation. In surviving animals 2/10 males at 14 mg/kg of DIEP and 1/10 females at 7 mg/kg of DIEP had peritonitis.

Organ Weight: No treatment related changes were observed in both the absolute and the relative organ weights except a slight increase in spleen weight in females at 6 mg/kg of diclofenac Na (absolute: 0.54 ± 0.11 g in control vs. 0.61 ± 0.05 g in treated. Percentage of body weight: 0.2607 ± 0.012 in control vs. 0.2864 ± 0.018 in treated group).

Conclusions: DIEP caused a slight incidence of peritonitis at 7 mg/kg. Above this level, the toxic effects were similar to diclofenac Na. Females were more susceptible to the test articles. The changes observed were typical of the lesions provoked with this type of test article.

Reviewer's Comments: The reviewer concurs with the findings of the study. The test solutions should have been analyzed. Apparently, this was not done.

Study Title: **DIEP: 13-week Oral (gavage) Subchronic Toxicity Study in the Rat**

Key Findings: *No toxicity was observed at the highest dose for either DIEP or diclofenac Na. Maximum tolerated dose was not reached.*

Study Number: 5821-644/2

Test Site: [redacted]

GLP Compliance: No

Quality Assurance: Yes

Location of Report: Vol. 5, pp. 299-346

Study Period: January 25 – April 28, 1988

Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 28 days, ♂ and ♀, 134-230 g

Test Substances: DIEP, batch no, 3/TF and epolamine (EP), batch no. 176333984, — , pure

Vehicle: Distilled water

Treatment Scheme:

Treatment	Dose (mg/kg/day)	n ♂	n ♀
Vehicle	0	10	10
DIEP	1	10	10
DIEP	3	10	10
DIEP	6	10	10
EP	20	10	10

Route of Administration: Oral gavage

Duration/Frequency: Single dose, once daily for 13 weeks

Parameters Observed/Measured:

Clinical Signs: Once daily. Twice daily for mortality.

Body Weight: Before treatment, Day 1 and weekly thereafter.

Food Consumption: Weekly.

Samples Collected: Blood was collected from control and animals treated at 6 mg/kg/day during weeks 4 and 13. Urine was collected from control and animals treated at 6 mg/kg/day during weeks 4 and 12 for 17 hrs. Blood samples from all groups were collected during week 14 for measurement of plasma level of test substances.

Hematology, Clinical Chemistry and Urinalysis: Same as those described in study no. 644/502.

Gross Pathology, Organ Weight and Histopathology: Same as those described in study no. 644/502.

Ophthalmoscopy: Pre-treatment (all animals) and control and animals treated at 6 mg/kg/day in week 12.

Summary of Findings:

Mortality: No treatment-related death (one death due to injury).

Clinical Signs, Body Weight, Food Intake, Hematology, Clinical Biochemistry Ophthalmoscopy, Urinalysis, Gross Pathology, Organ Weight and Histopathology: No treatment-related effects.

Analysis of Test Solutions: Between — % of the nominal values.

Reviewer's Comments: The study report did not include the statement of compliance with Good Laboratory Practice (GLP) guidlines. It is not clear whether this study was performed according to GLP guidelines. The main flaw in this study is that at the highest dose administered, no toxicity was observed. Therefore, the maximum tolerated dose (MTD) was not reached. There are number of other problems with the study. However, because it is not clear whether the study is GLP-compliant and the MTD was not reached, this study was considered inadequate for safety assessment.

Study Title: **DIEP: 13-week Oral Toxicity Study in Dogs**

Key Findings: *The highest tolerated dose was 0.5 mg/kg. At higher doses gastrointestinal ulceration and bleeding, kidney damage and death occurred.*

Study Number: 910091

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 5, pp. 347-410

Study Period: August 29 – December 3, 1991

Species/Strain: Dog (Beagle), ♂ and ♀

Test Substances: DIEP, batch no. 01.8.99 [redacted]
pure and EP batch no. 176333 [redacted]
[redacted] pure

Vehicle: Distilled water

Route of Administration: Oral

Duration: Single does, once a day for 13 weeks

Treatment Scheme:

Treatment	Dose (mg/kg/day)	n ♂	n ♀
Vehicle	0	10	10
DIEP	0.5	10	10
DIEP	1	10	10
DIEP	2	10	10
EP	20	10	10

Reviewer's Comments: This study was reviewed under IND 49,459 by Dr. Asoke Mukherjee on January 18, 1996. The summary of the review is as follows: "*The results showed that 0.5 mg/kg dose was the highest dose tolerated. At 1 mg/kg dose, one out of eight dogs died in moribund condition after two months of treatment. The toxicities were occults blood, diarrhea, GI ulcers, tubular basophilia of kidneys. No histopathologically defined liver toxicity was observed.*"

Study Title: **EP (Buffered and Non-buffered Solutions) Comparative Single-Dose Toxicity Study in Rats by Oral Route**

Key Findings: *NOAEL for non-buffered and buffered EP were < 1000 and 3000 mg/kg, respectively.*

Study Number: 910172

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 6, pp. 107-161.

Study Period: May 15 – June 4, 1991

Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 5-6 weeks, ♂ and ♀, 100-125 g

Test Substance: Epolamine, batch no. 176333, [redacted] o pure

Vehicle: Deionized water and acidified deionized water

Treatment Scheme:

Treatment	pH	No. per Dose ♂	No. per Dose ♀	Dose (mg/kg)
Deionized water	-	5	5	-
Acidified deionized water	-	5	5	-
Epolamine (non-buffered)	12	5	5	1000, 2000 and 3000
Epolamine (buffered)	8	5	5	1000, 2000, 3000 and 5000

Route of Administration: Oral (gavage)
Duration: Single dose
Observation Period: 14 days

Parameters Observed/Measured:

Clinical Signs and Mortality: Twice daily. At 0.5, 1, 2, 4 and 6 hr after treatment on Day 1.
Body Weight: Before Treatment and on days 1, 3, 8 and 14.
Gross Pathology: On all animals at the end of observation period.
Histopathology: Was not done.

Summary of Findings:

Treatment	Mortality ♂	Mortality ♀	Total (%)
Epolamine (non-buffered):			
1000 mg/kg	0	0	0
2000 mg/kg	2	3	50
3000 mg/kg	5	5	100
Epolamine (buffered)			
1000, 2000, 3000 mg/kg	0	0	0
5000 mg/kg	2	4	60

The maximum non-lethal dose was 1000 mg/kg for EP non-buffered solutions and 3000 mg/kg for EP buffered solutions. Test article-related macroscopic changes were found in the gastrointestinal tract and were comparable in nature for both compounds, but were more severe in animals treated with the EP non-buffered solutions. The effects observed seem to be mainly due to the irritant properties of EP basicity rather than to an inherent toxicity.

Reviewer's Comments: The reviewer concurs with the findings of the study. The results indicate that the NOAEL for non-buffered and buffered EP were < 1000 and 3000 mg/kg, respectively. However, in the absence of histopathological examination, these values may not be accurate.

Study Title: **EP: Pilot Oral (Gavage) Toxicity Study in the Rat**

Key Findings: *No significant clinical changes were observed up to 1000 mg/kg. Repeated treatment for 14 days at 100 mg/kg/day produced no evidence of toxicity.*

Study Number: 5673-644/1
Test Site: [redacted]
GLP Compliance: No
Quality Assurance: Yes
Location of Report: Vol. 6, pp. 162-190.
Study Period: November 30 – December 23, 1987
Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 35 days, ♂ and ♀, 95-241 g
Test Substance: Epolamine, batch no. 1976333984, [redacted] pure
Vehicle: Distilled water
Treatment Scheme:

Treatment	Dose (mg/kg/day)	n ♂	n ♀	Duration
Epolamine	50	1	1	1 day
Epolamine	100	1	1	1 day
Epolamine	200	1	1	1 day
Epolamine	500	1	1	1 day
Epolamine	1000	1	1	1 day
Vehicle	0	5	5	14 days
Epolamine	100	5	5	14 days

Route of Administration: Oral (gavage)
Observation Period: 7 days for single dose and 14 days for multiple doses

Parameters Observed/Measured:
Mortality: Twice daily.
Clinical Signs: 0.25, 0.5, 1, 2, 4 hr post-dose and daily.
Body Weight: Days –1, 1, 8 and 14.
Gross Pathology: Only repeat-dose animals at the end of observation period.
Histopathology: Was not done.

Summary of Findings: The single dose administration of the test article to the rat at dose levels of up to 1000 mg/kg/day was non-lethal and produced no significant clinical changes (aside from transient piloerection) in appearance or body weight gain. The repeated administration of the test article to the rat for 14 days at a dose level of 100 mg/kg/day produced no evidence of toxicity.

Reviewer's Comments: As indicated in the title, this is a pilot study. Apparently, this study was not performed according to GLP guidelines. The main flaw in this study is that no toxicity was observed at the highest dose administered indicating that the MTD was not reached. The dosing solution should have been analyzed.

Study Title: **EP: 4-Week Oral Toxicity Study in the Rat with 4 Weeks of Recovery**

Key Findings: *No consistent effect was observed, indicating that the maximum tolerated dose was not reached.*

Study Number: 910220
Test Site: [redacted]

GLP Compliance: Yes (OECD)
Quality Assurance: Yes
Location of Report: Vol. 6, pp. 191-234.
Study Period: September 12 – November 8, 1991
Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 6 weeks, ♂ and ♀
Test Substance: Epolamine, batch no. 176333, [redacted] pure
Route of Administration: Oral (gavage)
Vehicle: Distilled water
Duration: Single dose, once daily for 4 consecutive weeks
Recovery Period: 4 weeks (5/sex from vehicle and high dose groups)
Treatment Groups:

Treatment	Dose (mg/kg/day)	n ♂	n ♀
Vehicle	0	10	10
Epolamine	25	5	5
Epolamine	50	5	5
Epolamine	100	10	10

Parameters Observed/Measured:

Clinical Signs: Daily.
Mortality: Twice daily.
Body Weight: Days 0, then weekly.
Food Consumption: Weekly.
Ophthalmologic Examination: Week –1, Days –7 and 26 for males and Days -8 and 27 for females.
Hematology, Clinical Chemistry and Urinalysis: Day 29, same as those described in study no. 910090.
Gross Pathology, Organ Weight and Histopathology: Same as those described in study no. 910090.
Analysis of Dosing Solution: Weeks 1 and 4.
Statistical Analysis: Initial test for homogeneity of variance followed by analysis of variance (ANOVA) or Kruskal-Wallis non-parametric ANOVA and Dunnett's multiple comparison test or Mann Whitney's "U" test.

Summary of Findings:

Mortality and Clinical Signs: None.
Body Weight: No treatment related effects with the exception of reduction in both absolute and body weight gain values in females at 100 mg/kg/day during week 2 of dosing.
Food Consumption, Hematology, Ophthalmoscopic Examination, Blood Chemistry, Urinalysis, Gross Pathology, Organ Weight and Histopathology: No treatment-related effects.
Analysis of Dosing Solution: Within acceptable range ([redacted] % of nominal values).

Reviewer's Comments: Aside from reduction in body weight and body weight gain in females at 100 mg/kg/day during week 2, and one females at 100 mg/kg/day with unilateral focal hemorrhage in vitreous body beginning at week 4 and present at week 8, no effects were observed indicating that the MTD was not reached in this study. Therefore, the results were of limited value in safety assessment.

Study Title: **1-(2-hydroxyethyl) pyrrolidine-*N*-oxide: 4-Week Intravenous Toxicity Study in Rats Followed by 2 Weeks of Recovery**

Key Findings: *The 7 mg/kg/day dose could be considered NOAEL. At 70 mg/kg/day, ophthalmic toxicity was observed in some females.*

Study Number: 990012

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 6, pp. 235-278.

Study Period: February 9 – March 24, 1999

Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 4 weeks, ♂ and ♀, 60-85 g

Test Substance: 1-(2-hydroxyethyl) pyrrolidine-*N*-oxide, batch no. HEP017/98, [redacted]

Route of Administration: Intravenous

Vehicle: 0.9% NaCl

Duration: Single dose, once daily for 4 consecutive weeks

Recovery Period: 2 weeks (10/sex from vehicle and high dose groups)

Treatment Groups:

		No. of Animals			
	Dose	Main		Satellite*	
Treatment	(mg/kg/day)	♂	♂	♀	♀
Vehicle	0	20	20	3	3
Test article	0.7	10	10	3	3
Test article	7	10	10	3	3
Test article	70	20	20	3	3

* Toxicokinetics

Parameters Observed/Measured:

Clinical Signs: Daily.

Mortality: Twice daily.

Body Weight: Days 0, then weekly.

Food Consumption: Weekly.

Ophthalmologic Examination: Days –3 and 42 for males and Days – 4 and 41 for females.

Hematology, Clinical Chemistry and Urinalysis: Day 29 and 42, same as those described in study no. 910090.

Blood Sample: Day 1 and Day 24 at 1, 3 and 8 hr.

Gross Pathology, Organ Weight and Histopathology: Same as those described in study no. 910090.

Statistical Analysis: Initial test for homogeneity of variance followed by ANOVA or Kruskal-Wallis non-parametric ANOVA and Dunnett's multiple comparison test or Mann Whitney's "U" test.

Toxicokinetics: Blood samples were collected on Days 1 and 24 at 1, 3 and 8 hr after treatment.

Summary of Findings:

Mortality: None.

Clinical Signs: No relevant clinical abnormalities were seen in the lower dose groups. No local changes in the treatment site were found in any animal.

Body Weight Gain and Food Consumption: No treatment-related effects.

Ophthalmoscopic Examination: Unilateral eye abnormalities were observed starting after two weeks of treatment in two females at 70 mg/kg/day, including intraocular darkness in one rat and intraocular darkness with an increase in bulbus volume, followed by intraocular whiteness and decrease in bulbus volume the another one. The abnormalities were present at the end of recovery period. One control male had unilateral whiteness of fundus.

Hematology, Blood Chemistry and Urinalysis: There were no dose-related findings at the end of treatment and recovery period.

Gross Pathology: There were no consistent findings at the end of treatment and recovery period, except unilateral eye modifications in some females at 70 mg/kg/day.

Organ Weight: No treatment-related changes were seen in organ weights at final and recovery periods.

Histopathology: There were no consistent findings at the end of treatment and recovery period. Few females (4 out of 20) at 70 mg/kg/day had unilateral hemorrhage confined to vitreous body causing secondary ocular modifications consistent with phthisis bulbi in one recovery animal.

Reviewer's Comments: The reviewer concurs with the findings of the study. However, the purpose of this study is not clear. 1-(2-hydroxyethyl) pyrrolidine-*N*-oxide is a metabolite of epolamine (Giachetti et al., 1996). The relevance of this study depends on the extent of exposure through cutaneous route and the possible *in situ* metabolism of the parent compound. Because the toxicokinetic data were not included in this study, it was difficult to compare the extent of exposure through different routes. The certificate of analysis should have been translated (Appendix) and dosing solution analysis should have been performed (pp. 251). The 7 mg/kg/day dose could be considered NOAEL.

Study Title:	**1-(2-hydroxyethyl) pyrrolidine-*N*-oxide: 4-week Oral Toxicity Study in Rats Followed by 2 Weeks of Recovery**
Key Findings:	*No consistent dose-related toxicity. Maximum tolerated dose was not reached.*
Study Number:	990842
Test Site:	[redacted]
GLP Compliance:	Yes (OECD)
Quality Assurance:	Yes
Location of Report:	Vol. 6, pp. 279-319.
Study Period:	October 13 – November 26, 1999
Species/Strain/Age/Sex/Weight:	Rat, Sprague-Dawley, 4 weeks, ♂ and ♀, 60-85 g
Test Substance:	1-(2-hydroxyethyl) pyrrolidine-*N*-oxide, batch no. HEP017/98, [redacted]
Route of Administration:	Oral (gavage)
Vehicle:	0.9% NaCl
Duration:	Single dose, once daily for 4 consecutive weeks
Recovery Period:	2 weeks (10/sex from vehicle and high dose groups)

Treatment Groups:

Treatment	Dose (mg/kg/day)	No. of Animals Main ♂	Main ♂	Satellite* ♀	Satellite* ♀
Vehicle	0	20	20	-	-
Test article	6	10	10	3	3
Test article	18	10	10	3	3
Test article	30	20	20	3	3

* Toxicokinetics

Parameters Observed/Measured: Same as those in study no. 990012.

Summary of Findings: There were no consistent and dose-dependent findings at the end of treatment and recovery period in any parameter.

Reviewer's Comments: The reviewer concurs with the findings of the study. However, the purpose of this study is not clear. 1-(2-hydroxyethyl) pyrrolidine-*N*-oxide is a metabolite of epolamine (Giachetti et al., 1996). The relevance of this study depends on the extent of exposure through cutaneous route and the possible *in situ* metabolism of the parent compound. Because the toxicokinetic data were not included in this study, it was difficult to compare the extent of exposure through different routes. In addition, the MTD was not reached. It is not clear why higher doses were not tested, especially because a maximum dose of 70 mg/kg/day was administered intravenously in a previous study (Study no. 990842). This study was of limited value for safety assessment. The certificate of analysis should have been translated (Appendix) and dosing solution analysis should have been performed (pp. 294).

Study Title: **DIEP: 14-day Tolerance Study by Oral Route in the Rat**

Key Findings: *No toxicity at 6 mg/kg/day. 60-100% lethality at 20 mg/kg/day. NOAEL dose is between 6-20 mg/kg/day.*

Study Number: 644/501

Test Site:

GLP Compliance: Yes

Quality Assurance: Yes

Location of Report: Vol. 6, pp. 320-387.

Study Period: July 15 – August 25, 1988

Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 6 weeks, ♂ and ♀, 151-226 g

Test Substance: DIEP, batch no. 4/TF/ — , pure), and diclofenac Na, batch no. BT 51001/ — , pure), · ——— · (both)

Vehicle: Distilled water

Route of Administration: Oral (gavage)

Duration: Single dose (preliminary study) and once daily for 15 days

Treatment Scheme:

Treatment	Single Dose (mg/kg)	n ♂	n ♀	Multiple Dose (mg/kg/day)	n ♂	n ♀
Vehicle	-	2	2	0	5	5
DIEP	90	2	2	2	5	5
DIEP	125	2	2	6	5	5
DIEP	175	2	2	20	5	5
DIEP	245	2	2	-	5	5
Diclofenac Na	90	2	2	1	5	5
Diclofenac Na	125	2	2	3	5	5
Diclofenac Na	175	2	2	10	5	5
Diclofenac Na	245	2	2	-	-	-

Parameters Observed/Measured:

Clinical Signs: Daily (twice daily for mortality).
Body Weight and Food Consumption: Weekly.
Gross Pathology: Day 16. Full necropsy and collection of samples from macroscopic lesions.

Summary of Findings:

Percent Mortality*

Treatment	Single Dose (mg/kg)	♂	♀	Multiple Dose (mg/kg/day)	♂	♀
Vehicle	-	-	-	0	0	0
DIEP	90	0	0	2	0	0
DIEP	125	50	0	6	0	0
DIEP	175	100	0	20	60	100
DIEP	245	50	50	-	-	-
Diclofenac Na	90	50	0	1	0	0
Diclofenac Na	125	100	100	3	0	0
Diclofenac Na	175	100	100	10	0	80
Diclofenac Na	245	100	100	-	-	-

* Some animals were killed in moribund condition.

Clinical Signs: Seen only in moribund and dead animals. Only animals found dead or killed moribund in at high doses showed clinical signs: subdued behavior, hypothermia, dyspnea, piloerection, and abdominal mass. These clinical signs appeared after four administrations in all animals and persisted to death. No clinical signs were observed in any other animals except in one male at 2 mg/kg/day of DIEP, which had a distended abdomen after 12 days of treatment.

Body Weight: The body weight gains of surviving treated animals were comparable to the control values except at 20 mg/kg/day of DIEP, which a reduction in body weight (- 18 % at week 1 and - 21 % at week 2) was observed.

Food Consumption: The food consumption of surviving treated animals was comparable to the control values except for males at 20 mg/kg/day of DIEP, which had a reduced food consumption (about - 33 % at week 1 and - 20 % at week 2) and females at 10 mg/kg/day of diclofenac Na, which had a marked reduction in the first week (- 48 %).

Gross Pathology: No macroscopic abnormalities were observed on surviving animals except one male at 2 mg/kg/day of DIEP, which had a hepatomegalia and red liquid in the abdominal cavity.

Reviewer's Comments: Although the reviewer concurs with the findings, this study is considered inadequate because histological examination of tissues was not done. In addition, a NOAEL dose could not be estimated for DIEP because the dose was not increased gradually in the multiple treatment studies. At 6 mg/kg/day, no toxicity was observed

whereas at 20 mg/kg/day, 60-100% of the animals died indicating that the NOAEL dose is between 6-20 mg/kg/day.

GENETIC TOXICOLOGY:

The following genotoxicity studies were submitted and reviewed under IND 49,459 by Dr. Asoke Mukherjee on January 18, 1996 and were found adequate. Therefore, they were not reviewed here.

Study No.	Study Title	GLP QA	Result
900178	Study of the capacity of the test article DIEP to induce gene mutation in strains of *Salmonella typhimurium*	OECD Yes	Negative
900179	Micronucleus induction in bone marrow cells of rats treated by oral route with test article DIEP	OECD Yes	Negative
900182	Study of the capacity of the test article DIEP to induce gene mutation in V79 Chinese hamster lung cells	OECD Yes	Negative
900183	Study of the capacity of the test article DIEP to induce chromosomal aberration in human lymphocyte cultured *in vitro*	OECD Yes	Negative

Study Title:	**Study of the Capacity of the Test Article *N*-(2-hydroxyethyl) pyrrolidine to Induce Gene Mutation in Strains of *Salmonella typhimurium***
Key Findings:	*Epolamine was not genotoxic.*
Study Number:	910068
Test Site:	[redacted]
GLP Compliance:	Yes (OECD)
Quality Assurance:	Yes
Location and Date of Report:	Vol. 7, pp. 67-103
Study Period:	April 8-15, 1991
Test Substance:	Epolamine, batch no. 176333. [redacted] pure
Concentration of Test Substance:	1, 10, 100, 1000 or 10,000 μg/plate
Vehicle:	Water for injection
Tester Strains:	*Salmonella typhimurium* TA 1535, TA 1537, TA 98 and TA 100 (from B. Ames)
Positive Controls:	Hydrazine sulfate (500 μg/plate), 9-aminoacridine HCl monohydrate (40 μg/plate), doxorubicine HCl (4 μg/plate) and 2-aminofluorane (5 μg/plate)
Metabolic Activation System:	Liver S9 (10%, rats treated with Aroclor 1254, 500 mg/kg, single dose, 5 days prior to sacrifice) Tested using 2-aminofluorene (CASRN 153-78-6)
Test Replicate:	Two
Plate Replicates:	Three

Criteria for Valid Study: The test can be considered to have been conducted correctly if compliance with the following conditions has been achieved:

1. The sterility check must prove negative for bacterial growth.
2. The growth of all the strains must be inhibited by crystal violet; the growth of strains TA 1535, TA 1537, TA 1538 must be inhibited by ampicillin, while the growth of strains TA 98 and TA 100 must not.
3. The frequency of spontaneous reversion for each strain must fall within the range reported by literature and by our laboratory data.
4. The activity of the microsomal preparation must be confirmed by its capability to activate the positive control, which requires a metabolic transformation in order to explicate its mutagenic effect.
5. The number of colonies reverted owing to the mutagenic activity of the positive controls must be statistically greater than (Student's t-test) and at least double the number of spontaneously reverted colonies.

Criteria for Positive Result: The test article is considered positive:

1. If the number of reverted colonies is statistically significant increased in comparison with the number of control reversions (Student's t test).
2. If a dose-response can be verified, that is, a positive correlation between the number of reversions and the dose in an interval of at least 3 doses (linear regression test).

Data Evaluation: The mean and standard deviation were calculated for reversions in each dosage group. Comparison of the spontaneous reversions (in the negative control) with the ones in the test article plates and in the positive control plates were done by Student's t-test.

Summary of Findings: A slight reduction of the number of reversions/plate was observed at the highest concentration (10,000 μg/plate) mainly in the presence of metabolic activation, thus indicating a slight toxic effect on bacteria growth. No appreciable increase of the number of reversions in comparison with the negative control was evident in either experiments at any of the doses of *N*-(2-hydroxyethyl)-pyrrolidine for any strain, whether in the presence or in the absence of metabolic activation, while the reference mutagens induced a number of reverted colonies statistically greater than and at least double the mean number of spontaneous reverted colonies.

Reviewer's Comments: The assay was performed according to an established method and was adequately described. All criteria for a valid study were met as described in the protocol. The reviewer concurs with the findings of the study. However, a TAA base-pair substitution strain such as TA 102 or *E. coli* WP2*uvr*A should have been used and the conducting laboratory's historical controls should have been included. The values for positive control were within the expected range and were similar in replicate experiments with the exception of the values for doxorubicine in TA 98 in the absence of metabolic activation in experiment one (412.33 ± 67.5, Table 4, pp. 83) and in experiment two (918.67 ± 98.08, Table 14, pp. 93). The certificate of analysis of test substance and the results for the stability of the test solution should have been translated.

Study Title: **Study of the Capacity of the Test article *N*-(2-hydroxyethyl) pyrrolidine to Induce Unscheduled DNA Synthesis in Cultured HeLa Cells**

Key Findings: *Epolamine was not genotoxic.*
Study Number: 910069
Test Site: [redacted]
GLP Compliance: Yes (OECD)
Quality Assurance: Yes
Location and Date of Report: Vol. 7, pp. 104-129
Study Period: April 2-12, 1991
Test Substance: Epolamine, batch no. 176333. [redacted] pure
Test Scheme:

Experiment	Concentration (μg/mL)	Metabolic Activation
Preliminary Toxicity	0, 10, 100, 1000, 10000	With & without
1	0, 100, 300, 1000, 1500 or 3000	With & without
2	0, 100, 300, 1000 or 1500	Without
	0, 100, 300, 1000, 3000 or 5000	With

Vehicle: Water for injection
Tester Cells: HeLa cells from [redacted]
Positive Controls: Methylmethane sulfonate (1 mM) and cyclophosphamide (1.38 mM)
Metabolic Activation System: Liver S9 (10%, rats treated with Aroclor 1254, 500 mg/kg, single dose, 5 days prior to sacrifice)
Replicates: Three
Treatment Time: 2 hr

Criteria for Valid Study: On the basis of the laboratory data presently available and within the limits of this experimental model, the genotoxic activity of a substance can be identified unequivocally only if:

1. The T/C ratio gives an estimation of the toxicity of the test and positive control articles on the cells.
2. This ratio must not be lower than 0.4.
3. The mean cpm of the reference mutagen + hydroxyurea is statistically greater than that of the control + hydroxyurea.
4. The mean cpm of the test article + hydroxyurea is statistically greater than that of the control + hydroxyurea

Criteria for Positive Result: The test article is a mutagen when a statistically significant dose-effect correlation can be demonstrated.

Data Evaluation: Comparison of the mean cpm of the test article or the reference mutagen + hydroxyurea with the mean cpm of the control + hydroxyurea was performed by Student's t-test.

Summary of Findings: No increase of mean cpm of the test article in the presence of hydroxyurea was evident in comparison to that of the control, while the cpm of the reference mutagen in the presence of hydroxyurea was statistically greater than that of the control.

Reviewer's Comments: Because this assay is not on the ICH list of recommended assays for genotoxicity and a justification for using this assay as an alternate assay was not provided (as per ICH guideline), the report was not reviewed in detail.

Study Title:	**Study of the Capacity of the Test Article *N*-(2-hydroxyethyl) pyrrolidine to Induce Chromosome Aberration in Human Lymphocytes Cultured *in vitro***
Key Findings:	*Epolamine was not genotoxic.*
Study Number:	910070
Test Site:	[redacted]
GLP Compliance:	Yes (OECD)
Quality Assurance:	Yes
Location and Date of Report:	Vol. 7, pp. 130-150
Study Period:	April 9 – October 25, 1991
Test Substance:	N-(2-hydroxyethyl) pyrrolidine, batch no. 176333, [redacted]
Concentration of Test Substance:	*Experiment 1:* 1, 10, 100, 1000 and 10,000 μg/mL *Experiment 2:* 1, 10, 100 and 1000 μg/mL
Test Vehicles:	Water for injection
Positive Controls:	Mitomycin C, 2 μg/mL (without S9) and cyclophosphamide, 34.5 μg/mL (with S9)
Metabolic Activation System:	Liver S9 (6 %, rats treated with Aroclor 1254, 500 mg/kg, single dose, 5 days prior to sacrifice)
Test System:	Human lymphocytes collected from a healthy volenteer
Replicates:	Two
Duration of Exposure:	3 hr

Slide Preparation: Cells were collected and prepared approximately 20 hours after initiation of treatment. A sufficient amount of the suspension of the fixed cell was placed on a slide, stained with 5% Giemsa, air dried and permanently mounted.

Data Analysis: The CHI square method was used to compare the incidence of cells with aberrations occurring in the control incubations with the incidence of cells with aberrations in the incubations with the reference mutagen and with the test article, both including and excluding gaps.

Summary of Findings: None of the test article concentrations assayed induced an incidence of cells with chromosome aberrations statistically different from the control group in the presence or in the absence of metabolic activation.

Reviewer's Comments: This assay has a number of procedural problems. Apparently Experiment 1 was done as a preliminary toxicity test. In this experiment, the highest dose of 10,000 μg/mL was found to be toxic. Therefore, a maximum dose of 1000 μg/mL was used in Experiment 2. It is not clear why the recommended maximum dose of 5000 μg/mL was not tested. A measure of toxicity such as mitotic index should have been included. A continuous treatment test should have been done because the result of the 3-hr exposure was negative. This is the recommended approach by a working group on *in vitro* tests for chromosomal aberration (*Mutation Research*, 312: 241-261, 1994). Because the assay was done using cells from only one "normal" volunteer, it is difficult to make a reliable safety assessment especially because the conducting laboratory's historical controls were not

included. A better assessment could be made when rats are used for the assay because a reference base for comparison exists. In addition, the assay was not adequately described. The certificate of analysis of test substance and the results for the stability of the test solution should have been translated. Because of these deficiencies, the results of this assay could not be used for safety assessment.

Study Title: ***N*-(2-hydroxyethyl) pyrrolidine: Micronucleus Test in Rat Bone Marrow**

Key Findings: *Epolamine was not genotoxic.*
Study Number: 910132
Test Site: [redacted]
GLP Compliance: Yes (OECD)
Quality Assurance: Yes
Location of Report: Vol. 7, pp. 151-172
Study Period:
Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 5 weeks old, ♂ and ♀, 100-130 g
Test Substance: *N*-(2-hydroxyethyl) pyrrolidine, batch no. 176333, [redacted] pure
Positive Controls: Mitomycin C (8 mg/kg, i.p.)
Experimental Design:

Treatment	No. of Mice Treated		No. of Mice Used for Bone Marrow Collection					
			18 hr		42 hr		66 hr	
	♂	♀	♂	♀	♂	♀	♂	♀
Vehicle control (water, po)	15	15	5	5	5	5	5	5
N-(2-hydroxyethyl) pyrrolidine (3000 mg/kg, po)	15	15	5	5	5	5	5	5
Mitomycin C (8 mg/kg, ip)	5	5	-	-	5	5	-	-

Sample Collection and Slide Preparation: At the scheduled sacrifice times, the femurs were removed and the bone marrow was aspirated and washed in fetal bovine serum. The pellet was spread onto a clean glass slide. Two slides were prepared from each mouse. The slides were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted. For each animals, 2000 polychromatic erythrocytes were counted and scored for micronucleated cells.

Scoring for Micronuclei: The proportion of polychromatic erythrocytes to total erythrocytes was recorded per 1000 erythrocytes.

Evaluation of Test Results: Comparison of the frequency among groups was done with a non-parametric method (Mann-Whitney).

Summary of Findings: No cytotoxic effects on bone marrow cells were evidenced. On the basis of the results and the statistical analysis, there was no significant difference between the micronucleus frequency in the treated group in comparison with the control group at any sampling time. The group of animals treated with Mitomycin C showed a significantly higher number of micronucleated cells in comparison to the control group.

Reviewer's Comments: The assay was performed according to an established method and the reviewer concurs with the findings of the study. However, proof of exposure was not provided and could not be inferred because of the lack of toxicokinetic studies with epolamine. In addition, animals should have been randomized, the certificate of analysis of test substance and the results for the stability of the test solution should have been translated, and the conducting laboratory's historical controls should have been included. Because of these deficiencies, the results of this assay could not be used for safety assessment.

CARCINOGENICITY:

Carcinogenicity studies were not performed with DIEP. The following is the information in PDR® (Online) on carcinogenicity potential of diclofenac "Long-term carcinogenicity studies in rats given diclofenac sodium up to 2 mg/kg/day (or 12 mg/m^2/day, approximately the human dose) have revealed no significant 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/m^2/day) female rats (high-dose females had excessive mortality), but the increase was not significant for this common rat tumor. A 2-year carcinogenicity study conducted in mice employing diclofenac sodium at doses up to 0.3 mg/kg/day (0.9 mg/m ^{2} /day) in males and 1 mg/kg/day (3 mg/m^2/day) in females did not reveal any oncogenic potential". The carcinogenicity potential of epolamine is unknown. Because DIEP is intended for short treatment periods, carcinogenicity testing, including photo-carcinogenicity, may not be needed (ICH Guidelines, S1A).

REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

The following reproductive studies were submitted and reviewed under IND 49,459 by Dr. Asoke Mukherjee on January 18, 1996. In the summary, Dr. Mukherjee indicated that "*DIEP did not show adverse response to male and female fertility. However, resorption of embryo was observed at maternal non-toxic doses. DIEP is not teratogenic. However, on the basis of resorption data in the segment I study in rats, DIEP should be considered for pregnancy category C. This is a moot point for the IND because it will be used for* —

Study No.	Study Title	GLP QA	Dose	Duration
910227	DIEP: Teratogenesis study in rats by oral route	OECD Yes	0, 1, 3 or 6 mg/kg DIEP and 50 mg/kg EP*	Day 6 – 15 of gestation
910228	DIEP: Fertility and reproduction study in rats by oral route	OECD Yes	0, 1, 3 or 6 mg/kg DIEP and 50 mg/kg EP	♂ - 60 days before mating and during mating ♀ - 14 days before mating, during mating, and up to gestation day 19 and postpartum day 21

The following studies were not completed because of the large number of death.

Study No.	Study Title	GLP QA	Dose (mg/kg)
910092	EP: Teratogenesis study in rats by oral route	No No	0, 10, 50, 100 or 1000
910093	EP: Fertility and reproduction study in rats by oral route	No No	0, 10, 50, 100 or 1000

EP = Epolamine

Study Title: **DIEP in Comparison with EP: Preliminary Teratogenesis Study in Rabbits by Oral Route**

Key Findings: *No clinical signs, behavioral changes or compound-related deaths were observed at any dose.*

Study Number: 920505

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 7, pp. 301-328

Study Period: August 11 – February 9, 1993

Species/Strain/Age/Sex/Weight: Rabbit, New Zealand white, 14-18 weeks, ♂ and ♀, 2.7-3.2 kg

Number of Animals: 12 females/group (to end up with 6 gravid does/group)

Test Substance: DIEP, batch no. 01.8.99 and Epolamine, batch no. 176333, [redacted] pure

Vehicle: Deionized water

Dose/Route: 0, 1, 3 or 6 mg/kg/day DIEP and 5, 10 or 20 mg/kg/day of EP, oral (gavage)

Frequency/Duration: Daily from day 6 through day 18 of pregnancy

Summary of Findings: No clinical signs, behavioral changes or compound-related deaths were observed at any dose. The body weight gain and the mean daily food intake of the group treated with 6 mg/kg/day of DIEP was slightly lower than that of the control group during the treatment period. No important changes in these parameters were seen with the comparative test article EP. No embryotoxic effects were noted at any dosage of either compound.

Reviewer's Comments: Because this was a preliminary study, it was not reviewed in detail.

Study Title: **DIEP in Comparison with EP: Teratogenesis Study in Rabbits by Oral Route**

Key Findings: *DIEP increased the rate of early and late resorption. EP increased the rate of early resorption. The concurrent control value for early resporption is below historical control range. A NOAEL dose could not be identified.*

Study Number: 920506

Test Site: [redacted]
GLP Compliance: Yes (OECD)
Quality Assurance: Yes
Location of Report: Vol. 7, pp. 329-359
Study Period: December 21, 1993 – April 1, 1994
Species/Strain/Age/Sex/Weight: Rabbit, New Zealand white, 20-21 weeks, ♂ and ♀, 2.7-3.2 kg
Number of Animals: 20 females/group (to end up with 6 gravid does/group) and sexually mature males
Test Substance: DIEP, batch no. 01.8.99 [redacted] pure and Epolamine, batch no. 176333 [redacted] pure
Vehicle: Deionized water
Dose/Route: 0, 1, 3 or 6 mg/kg/day DIEP and 20 or 100 mg/kg/day EP, oral
Frequency/Duration: Daily from day 6 through day 18 of pregnancy

Mating: Each female found to be in estrus at an external examination was placed with one male for about 50 minutes. Coitus was verified by a vaginal smear examined at the microscope immediately after presumed coitus. The day on which spermatozoa were found was considered day 0 of pregnancy for that female.

Parameters Observed/Measured:

Clinical Signs: Daily.

Mortality: The female rabbits found dead were subjected to necropsy to detect the cause of death. Corpora lutea and implantations were counted, whenever possible. The organs with gross alterations were fixed in formalin for histologic examination, if necessary.

Abortion: Each doe with signs of abortion was left alive and necropsied on day 29 of gestation. Corpora lutea and implantations were counted, whenever possible.

Body Weight: Days 0, 6, 10, 14, 19, 23 and 29 of gestation.

Food Intake: Every two days during gestation.

Gross Pathology: On day 29 of gestation, the does were killed and were necropsied and the following parameters were recorded: gravid uterus weight, number of corpora lutea, number of implantations, number of resorptions (early: only placenta visible and late: placenta and embryo visible), number and sex of viable fetuses, number and sex of dead fetuses (fetuses without spontaneous movements and breathing), individual fetus weight, individual placental weight. A gross external examination was performed on all fetuses immediately. All the fetuses were kept in ethyl alcohol, until cleared, for any further skeletal examination that may be required. The dead fetuses were fixed after an external examination. Observations were classified as malformations, anomalies, and variants.

Data Evaluation and Statistical Analysis: The fertility index was expressed as the percent ratio of the number of females having evident signs of pregnancy compared to the number of females that had positive vaginal smear. The mean body weight of each group, with absolute body weight gain, was calculated from the weight of the gravid dams. Body weight gain was also calculated on the 1st day of treatment (day 6 of gestation). Calculation was also made of maternal body weight excluding gravid uterus. The mean food consumption of each group was calculated every two days in order to have the mean daily food intake. Litter weight, mean fetal weight and placental weight were calculated from individual fetal or placental weights. Fetal losses were subdivided into pre-and post-implantation and were counted per litter.

Pre-implantation losses = (No. corpora lutea) – (No. implantations) ÷ (No. corpora lutea) x 100

Post-implantation losses = (No. implantations) – (No. viable fetuses) ÷ (No. implantations) x 100

Group mean values were calculated from individual data in two ways: Mean A: calculated on all the surviving females having evident signs of pregnancy including those that presented 100% post-implantation losses. The abortions were included in Mean A for the mean value of corpora lutea, implantations and pre- and post-implantation losses. Mean B: calculated only on those females with viable fetuses at term.

Frequencies were compared using CHI square and Fisher's exact test. The trend test was also applied. All other parameters were tested initially for homogeneity of variance followed by ANOVA or Kruskal-Wallis non-parametric ANOVA and Dunnett's multiple comparison test or Mann Whitney's "U" test.

Dosing Solution Analysis: Stability and concentration were determined.

Summary of Findings:

Clinical Signs: No compound-related clinical signs or behavioral changes were observed.

Mortality: Three animals died during the study: two females of the control group died on day 13 and 14 of gestation, respectively, and one female at 100 mg/kg/day EP died on day 13 of gestation. Two females from control group died, one from gavage error and the other from spontaneous incidental pneumonia.

Abortion: None.

Body Weight and Food Intake: No treatment-related effects.

Effects on Reproduction and on Embryofetal Development: the sponsor indicated that there was only a treatment-related increase in late resorptions at 6 mg/kg/day of DIEP.

Summary of Reproduction Parameters

Parameter	Dose (mg/kg/day)					
	DIEP				EP	
	0	1	3	6	20	100
No. of positive smear	20	20	20	20	20	20
Fertility (%)	90	80	65	80	65	70
Pre-implantation death (%)	None					
Gravid death (%)	0	6	0	0	0	7
Non-gravid death (%)	0	25	0	0	0	6
Abortion (surviving + dead) (%)	None					
Incidental deliveries (%)	None					
Gravid (cesarean sectioned) (%)	100	94	100	100	100	93

Frequency of Embryofetal Development Parameters

Parameter	Dose (mg/kg/day)					
	DIEP				EP	
	0	1	3	6	20	100
No. of females	18	15	13	16	13	13
No. of corpora lutea	171	136	141	170	132	134
Implantations (%)	81	88	91	79	83	83
Early resorptions (%)	1.4	9.2	16.3	5.9	10.9	8.1
Late resorption (%)	1.4	4	0	19	1.8	1.8
Total resorptions (%)	3.0	13	16.3	25	12.7	9.9
Dead fetuses (%)	0	0.8	0	1.5	0	0
Live fetuses (%)	97	86	84	74	87	90
Live male fetuses (%)	48	45	49	50	44	44
Live female fetuses (%)	52	55	51	50	56	56

Live fetuses mean weight (g)	44.0 ± 5.6*	45.1 ± 6.8	42.4 ± 3.4	43.1 ± 7.6	43.7 ± 3.9	43.5 ± 4.9
Live placenta mean weight (g)	7.7 ± 1.6	7.5 ± 1.4	7.5 ± 1.3	7.1 ± 1.3	7.8 ± 1.0	7.7 ± 1.2

* Standard deviations.

Malformations, Anomalies and Variants: One fetus each with acrania, anencephaly and macroglossia was found in the 1 mg/kg/day DIEP group and in the control group. No other malformed fetuses were found in any treated group.

Frequency of Malformations, Anomalies and Variants

Parameter	Dose (mg/kg/day)					
	DIEP				EP	
	0	1	3	6	20	100
No. of viable fetuses	134	103	108	100	96	100
External malformations (%)	0.75	0.97	0	0	0	0
Skeletal malformations (%)	None					
Visceral malformations (%)	None					
External anomalies (%)	None					
Skeletal anomalies (%)	68	74	70	69	67	72
Visceral anomalies (%)	None					
Skeletal variants (%)	48	41	40	42	51	38
Visceral variants (%)	0	0	0	0	0	0
Malformations (%):						
Acrania	0.75	0.97	0	0	0	0
Anenecephaly	0.75	0.97	0	0	0	0
Macroglossia	0.75	0.97	0	0	0	0

Dosing Solution Analysis: Solutions were stable for up to 24 hr at room temperature. The concentrations of test solutions were within the acceptable range.

Conclusions: DIEP and EP, administered during the organogenesis period to pregnant rabbits, did not induce toxic effects in the does. An increase of late resorptions was found at 6 mg/kg/day of DIEP. No teratogenic effects were found in any treated group. The sponsor concluded that the no effect dose could be set at 3 mg/kg/day for DIEP and at least 100 mg/kg/day for EP.

Reviewer's Comments: The reviewer concurs with the findings of this study with the following exceptions. There is a problem with the historical control data with early resporption (summarized in the table below) that makes the findings questionable. The historical control percentage of early resorption is 24.3 ± 10.6 with a range of 5.6 – 43.0. The value for concurrent control in this study is 1.45%, which is below the historical control values. Comparing the values from treated groups to this concurrent control indicate a substantial increase in the incidence of early resorption for both compounds even though this increase is within historical control range. The sponsor did not consider this drug-related because the values were within historical control range and the incidence was not dose-related. In contrast, the incidence of late resorption was considered drug-related at 6 mg/kg/day even though the incidences were 4.17% and 0.0% at 1 and 3 mg/kg/day, respectively. These interpretations are contradictory. The results indicate that DIEP increased both early and

late resorption rates. Therefore, contrary to the conclusion of the sponsor, the dose of 3 mg/kg/day could not be considered the NOAEL dose.

Comparison of Resporption Frequency (%)

	Early	Late
Historical Control	24.3 ± 10.6 (5.6 – 43.0)	8.8 ± 9.0 (0.0 – 28.6)
This Study:		
Control	1.45	1.45
DIEP		
1 mg/kg/day	9.2	4.2
3 mg/kg/day	16.3	0.0
6 mg/kg/day	5.9	19.3
EP		
100 mg/kg/day	10.9	1.8
200 mg/kg/day	8.1	1.8

In addition, one fetus each with acrania, anencephaly and macroglossia was found in the 1 mg/kg/day DIEP group and in the control group (2/120). No other malformed fetuses were found in any treated group. This is higher than the background control frequency of 1/1491 of these 3 malformations (pp. 200). However, because it was found in control group and was not observed at higher doses in treated groups, it seems that it is not drug-related.

A translation of the certificate of analysis of the test substances should have been provided.

Study Title: **EP: Fertility and Reproduction Study by Oral Route in Rats (F_2)**

Key Findings: *Lower mean fetal weight was observed at 18 mg/kg/day. Increased resorptions and lower number of live born pups, associated with low maternal and fetal body weight, were observed at 50 mg/kg/day. The NOAEL was 18 mg/kg/day for parents and 9 mg/kg/day for the progeny.*

Study Number: 920507

Test Site: [redacted]

GLP Compliance: Yes (OECD)

Quality Assurance: Yes

Location of Report: Vol. 7, pp. 360-413

Study Period: August 7, 1992 – March 18, 1993

Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, 9-10 weeks, 200-225 g (♀), 4-5 weeks, 100-125 g (♂)

Number of Animals: 24/group

Test Substance: Epolamine, batch no. 176333 [redacted] pure

Vehicle: Deionized water

Dose/Route: 0, 3, 9, 18 or 50 mg/kg/day, oral (gavage)

Duration/Frequency: The test article was administered daily to the F_0 males from day 60 prior to the mating phase until the end of this phase and to the F_0 females for 14 days before the start of the mating period and throughout the same. Treatment continued during gestation up until day 19 for the females of subgroup F_t (teratogenesis) killed on day 20 of gestation and until the end of lactation (day 21 of lactation) for the mothers of subgroups F_f (fertility). The females with positive smear, which did not give birth, were treated until killing (presumed day 27 of pregnancy). The females without positive smear were treated up until 27 days after the end of the mating period.

Parameters Observed/Measured:

Clinical Signs: Clinical signs were observed and recorded daily from the start of treatment to the final killing of parent rats of the F_0 generation. The dams of the F_f subgroup were observed when possible during parturition. The newborn pups of the F_1 generation were observed daily from birth up until the sacrifice.

Mortality: The rats found dead were subjected to necropsy to detect the cause of death, whenever possible. Corpora lutea and implantations were counted, whenever possible. The organs with gross alterations were fixed in formalin for histologic examination, if necessary.

Abortion: The female rats presenting signs of abortion (vaginal bleeding) were left alive and necropsied at day 20 of gestation. Implantations were counted, whenever possible.

Body Weight: F_0 - All rats were weighed on a weekly basis during the pre-mating and mating periods. During gestation, the females were weighed on days: 0, 7, 14, 17, 20 and during lactation on days 0 (parturition day), 7, 14, 21. **F_1** - All the animals were weighed on days 0, 4, 8, 12 and 21 after birth and on a weekly basis up until the end of the mating period.

Food Intake: During the pre-mating period of the male and female F_0 generation, food was distributed in weighed amounts. The leftover amounts of the weighed food allocated per cage were recorded once a week in order to calculate food consumption in g/rat/day. Food consumption was then recorded on days 7, 14, 17 and 20 during the gestation period and on days 7 and 14 during the lactation period.

Observations on the F_t Subgroup: The F_0 females assigned to the F_t subgroup were killed on day 20 of pregnancy. Observations were made on the following parameters: gravid uterus weight, number of corpora lutea, number of resorptions (early: only placenta visible and late: placenta and embryo visible), number and sex of viable fetuses, number and sex of dead fetuses (fetuses without spontaneous movements and breathing), number of implantations, individual placental weight, individual fetus weight. A gross external examination was done on all fetuses immediately. Skeletal malformations, anomalies and variants were noted after clearing 50% of the fetuses of each litter. The remaining 50% of the fetuses of each litter were preserved in Bouin's fluid for examination by Wilson's technique. As far as possible, the distribution per litter for examination by clearing or by Wilson's technique was by equal number of sexes. Dead fetuses were fixed after the external examination.

Observations on the F_f Subgroup and F_1 Generation: The F_0 females assigned to the F_f subgroup were allowed to give birth. On day 0 the pups of each litter were identified. At birth and during lactation, the offspring were observed for physical appearance and tested using a battery of behavioral tests. On day 21 of lactation, the entire F_1 generation was killed except for one male and one female of each litter, selected so as to have a body weight as close as possible to the mean litter weight. The pups left alive were weighed weekly and checked daily for clinical signs and mortality. They were observed for descent of testes (daily from day 25 on) and for vaginal opening (daily from day 30 on). At weeks 7-8 from birth they were subjected to inclined plane and water Y-Maze behavioral tests.

Observations on F_2 Generation: At birth and during the lactation period, all the young were individually observed for external abnormalities at birth, live and stillbirths (ascertained by docimasia), mortality ensuing after live birth and ascertained daily, sex (at day 0), and pup weight (days 0, 4, 8, 12, and 21). At weaning (day 21 of lactation) all the pups were killed and necropsied with the mothers.

Gross Pathology Examination: All the animals were subjected to gross examination according to the following scheme:

F_0 Males: Killed at the end of the mating period. The following organs were removed and fixed for possible histological examination: testes, epididymides, seminal vesicles and prostate gland. The testes were weighed.

F_0 Females: F_t subgroup animals were killed on day 20 of pregnancy. F_f subgroup animals were killed after their respective offspring reached 21 days of age. The number of corpora lutea and implantations was recorded for the F_t dams, and the number of implantations was recorded for the F_f dams. The uterus of apparently non-pregnant females was stained using the Salewski method (Salewski, 1964) and examined for the presence of implantation sites. The ovaries were weighed and then fixed for histologic examination if necessary.

F_1 Generation: All animals with the exception of the pups chosen on day 21 of lactation, were killed at the age of 21 days and macroscopically examined for abnormalities. The pups chosen for behavioral tests and for F_1 mating were killed, as the F_0 parents were.

Data Evaluation and Statistical Analysis: To compare frequencies, the heterogeneity test (CHI square 2 x N) and Fisher's exact test were used. The Trend test was also used. All these tests were one-tailed. Log-rank test was used to compare the mating distribution during the time allowed to F_0 generation for mating. Other data were treated as that described in Study no. 920506.

Dosing Solution Analysis: Stability and concentration were determined.

Summary of Findings:

F_0 Generation: No significant drug-related effects were found in any of the measured parameters with the following exceptions. The body weight gain of the females treated with 50 mg/kg/day was slightly lower than that of the control females during the late gestation period and the first week of the lactation period. Significantly higher value of early resorptions and of post-implantation losses and a low number of live born pups were observed in the 50 mg/kg/day group where also a low mean fetal weight was noted. A low mean fetal weight was observed also in the 18 mg/kg/day treated group. One externally malformed fetus was observed in the 3 mg/kg/day group with anasarca, astomia and arhinia. Two visceral malformed fetuses were observed in the 50 mg/kg/day group, one with right anophthalmia and one with right aortic arch and right Botallus duct. A not dose-related slight increase of skeletal variants was noted in the treated groups.

F_1 Generation: No significant drug-related effects were found in any of the measured parameters

Dosing solutions were within the expected values.

Conclusions: EP given by oral route during the pre-mating, mating, gestation and lactation periods to male and female rats did not have any effect up to 9 mg/kg/day. At 18 mg/kg/day there was a lower mean fetal weight. A clear embryotoxic effect (increase of resorptions and lower number of live born pups), associated with low maternal and fetal body weight, was found at 50 mg/kg/day. The fertility index of the F_0 and F_1 generations and the postnatal development of their progeny were similar. The no observed toxic effects dose level was 18 mg/kg/day for parents and 9 mg/kg/day for the progeny when the test article was given during the entire reproductive cycle.

Reviewer's Comments: The reviewer concurs with the findings of this study. The study report indicated that an analysis of dosing solution was performed by the sponsor and "the results

were found to conform with the expected values" (pp. 382). The actual data should have been provided with the study reports for independent verification.

Study Title:	**EP: Preliminary Teratogenesis Study in Rabbit by Oral Route**
Key Findings:	*No embryotoxic or compound-related malformed fetuses were found at any dose.*
Study Number:	930240
Test Site:	[redacted]
GLP Compliance:	Yes (OECD)
Quality Assurance:	Yes
Location of Report:	Vol. 7, pp. 414-439
Study Period:	May 11, 1993 – June 25, 1993
Species/Strain/Age/Sex/Weight:	Rabbit, New Zealand white, 14-18 weeks, females, 2.7-3.2 kg
Number of Animals:	8 females/group with positive smears
Test Substance:	Epolamine, batch no. 176333 [redacted] pure
Vehicle:	Deionized water
Dose/Route:	0, 50 or 100 mg/kg/day EP, oral (gavage)
Duration/Frequency:	Daily from day 6 through day 18 of pregnancy

Parameters Observed/Measured: Clinical signs, mortality, abortion, body weight, food intake and reproductive parameters were assessed.

Summary of Findings: No clinical signs, behavioral changes, deaths or abortions were observed in any group. Slightly lower body weights gain and mean daily food consumption was found during the treatment period at the dose of 100 mg/kg/day. No embryotoxic or compound-related malformed fetuses were found at any dose.

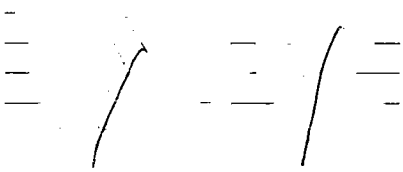
Reviewer's Comments: Because this was a preliminary study, it was not reviewed in detail.

SPECIAL TOXICOLOGY STUDIES:

Study Title:	**Local Tolerance Test in the Rabbits (Ocular Irritation and Cutaneous Primary Irritation)** **Test to Evaluate the Sensitizing Potential by Topical Application in the Guinea Pig**
Key Findings:	*DIEP was slightly irritant to rabbit eyes, non-irritant to rabbit skin, and weakly sensitizing to guinea pigs skin.*
Study Number:	902456
Test Site:	[redacted]
GLP Compliance:	Yes
Quality Assurance:	Yes

Location of Report: Vol. 5, pp. 411-504.
Study Period: November 28, 1988-January 20, 1989
Test Substance: Flector Gel (1%), batch no. 20/IB-22, white gel
Formulation of Test Substance:

Ingredient	Percentage
DIEP	1.32



Summary of Study Protocol and Findings

	Ocular Irritation	Cutaneous Irritation	Sensitization[A]
Species	Rabbit	Rabbit	Guinea pig
Strain	New Zealand white	New Zealand white	Dunkin-Hartley
Age	Adult	Adult	Young adult
Sex	Male	Male	Male and female
Weight	2.3-2.65 kg	2.3-2.55 kg	3.42-4.25 kg
n	6	6	10/sex
Pretreatment	—	—	Freund's adjuvant (0.1 mL) on Day 1, intradermal
Treatment	Flector gel (1%)	Flector gel (1%)	Flector gel (1%)
Dose	0.1 mL per animal (100 mg)	5 mL (0.5 g) per 6.2 cm^2	0.5 mL per 4 cm^2
Route	Ocular (right eye)	Cutaneous (intact and abraded)	Cutaneous (intact)
Duration of Exposure	One minute (rinsed afterward)	24 hr (occluded)	48 hr (Days 1, 3, 8, 10 and 15) or 72 hr (Days 5 and 12), occluded
Frequency	Single dose	Single dose	Days 1, 3, 5, 8, 10, 12 and 15
Control	Same animal - Untreated left eye	Same animal	Same animal
Rest Period	--	--	Days 17-29 (12 days)
Challenge	--	--	0.5 mL on Day 29 for 48 hr
Observation Period	1 hr after treatment and on Days 1, 2, 3 and 4	24 and 48 hr after application	6, 24 and 24 hr after removal of patch
Parameters Observed/ Measured:	Ophthalmoscopy of cornea, iris and pupils at 1 hr and on Days 1, 2, 3 and 4	Cutaneous examination	Cutaneous examination, 6 and possibly 24 hr after removal of patch
Findings	Maximum Ocular Irritation Index = 19.50 and Mean Ocular Irritation Index on day 4 = 0.0	Primary Cutaneous Irritation Index = 0.33	Possible sensitization
Histopathology	-	-	6, 24 and 48 hr
Classification	Slightly Irritant	Non-irritant	Weak (Grade I)
Protocol Reference	Journal Officiel de la Republique Francaise (Oct. 1984 and Feb. 1985)	Journal Officiel de la Republique Francaise (Feb. 1982)	AFNOR[B]: FD no. T03-300, 1982

A = A preliminary study was done under occlusion for 48 hr (0.25 or 0.5 mL, single dose, n = 2/sex)
B = AFNOR = Association Francaise de Normalisation.

Classification of Ocular Irritation According to Journal Officiel de la Republique Francaise (Feb. 1985)

Maximum Ocular Irritation Index	Ocular Irritation	Intensity of Ocular Irritation	Classification
< 5	At day 1 = 0	-	Non-irritant
≥ 5 and < 15	At day 2 ≤ 2	-	Very slightly irritant
≥ 5 and < 25	At day 4 ≤ 2	-	Slightly irritant
≥ 25 and < 50	At day 7 ≤ 20	≤ 60 in 6 rabbits and ≤ 30 in at least 4 rabbits at day 7	Irritant
≥ 50 and < 80	At day 7 ≤ 40	≤ 30 in 6 rabbits and ≤ 14 in at least 4 rabbits at day 7	Very irritant
> 80	-	-	Extremely irritant

Classification Dermal Irritation According to Journal Officiel de la Republique Francaise (Feb. 1982)

Primary Cutaneous Irritation Index	Classification
≤ 0.5	Non-irritant
0.5 < and ≤ 2	Slightly irritant
2 < and ≤ 5	Irritant
5 < and ≤ 8	Very Irritant
≥ 50 and < 80	Very irritant
> 80	Extremely irritant

Classification of Dermal Sensitization According to AFNOR (FD no. T03-300, 1982)

% Sensitized Animals	Grade	Classification
> 0 and < 10	I	Weak
11 to 25	II	Mild
26 to 50	III	Moderate
51 to 75	IV	Strong
76 to 100	V	Extreme

Reviewer's Comments: The reviewer concurs with the findings of these studies. The studies were conducted according to methods that are similar to that described in 16 CFR 1500.41 and 16 CFR 1500.42 for skin and eye irritation, respectively. However, these studies were not adequate to determine the safety and local tolerance of the drug product because a different formulation and form (gel) of the drug products than that proposed for clinical use (patch) were used. Therefore, other problems with these experiments were not detailed here.

Study Title:	**Flector® Plaster: 28-day Repeated Skin Irritation in Rabbits**
Key Findings:	*Flector® Plaster is non-irritant to rabbit skin.*
Study Number:	920629 and 920629A (Proof of Absorption)
Test Site:	
GLP Compliance:	Yes (OECD)
Quality Assurance:	Yes
Location of Report:	Vol. 6, pp. 1-106
Study Period:	August 18 – September 16, 1992
Species/Strain/Sex/Weight:	Rabbit, New Zealand white, male, 2.1-2.6 kg
Test Substances:	Flector® plaster placebo, batch 61/IB-22 and Flector® plaster, batch 62/IB-22

Route of Administration: Cutaneous
Duration/Frequency: Twice a day (8 hr apart) for 28 consecutive days
Treatment Scheme:

Treatment	DIEP	n	Sex
Untreated Control	0	10	♂
Flector® plaster placebo	0	10	♂
Flector® plaster	4.05 mg/kg	10	♂

Parameters Observed/Measured:

Clinical Signs: Daily.
Mortality: Twice daily.
Body Weight: Days -1, 0 and at weekly intervals.
Skin Reaction Evaluation: Before the first daily application. Scored erythema and edema, skin appearance and elasticity, fur growth, and cutaneous thickness.
Gross Pathology: Examined the treated areas of skin and collected sample for histological examination.
Proof of Exposure: Blood samples were collected before and 2, 4, 8 hr and day 14 and 28 from animals treated with placebo and DIEP. Right lumbar muscle below treatment area was collected from animals treated with placebo and DIEP. Diclofenac levels were determined.

Summary of Findings: No effects were found in any of the measured parameters in any group. A slight hair follicle atrophy observed in animals of Flector® plaster and placebo groups was not seen in untreated control rabbits and was ascribed to the daily stripping to the change of the adhesive plaster.

After 7 days of treatments, 4 rabbits showed no quantifiable levels of diclofenac in plasma at any sampling times, 3 rabbits showed very low amounts (1-7 ng/mL), one rabbit showed irregular profile, and only 2 rabbits showed a more consistent release of diclofenac. After 14 days of treatment, only 3 animals showed quantifiable amount of diclofenac in plasma. The plasma concentration levels of diclofenac were not quantifiable in other 7 rabbits. After 28 days of treatment, 2 rabbits showed a dramatic decrease of diclofenac plasma levels. Three other animals showed higher levels of diclofenac. The other 5 rabbits showed levels near the quantitation limit. The highest plasma level of diclofenac was — ng/mL, which was found after 14 days of treatment. Low concentrations of diclofenac were found in the muscles of all rabbits treated with DIEP, ranging from — ng/g. The minimum quantifiable level of diclofenac was 1 ng/mL for the plasma and 2 ng/g for the muscle. The precision of the assay was — , and the accuracy was — .

Conclusions: Flector® at the dose of about 4.05 mg/kg (b.i.d.) of DIEP and Flector® plaster placebo are considered "Non-Irritant".

Reviewer's Comments: The reviewer concurs with the findings of this study. The formulation of the test substance should have been provided. The absorption of diclofenac was highly variable among the animals and no absorption occurred in many animals indicating that the patch did not deliver the drug consistently. Therefore, the efficacy of the patch, in the absence and/or lack of consistent absorption of diclofenac, is questionable.

Study Title: **Gastrolesivity of Diclofenac Hydroxyethyl Pyrrolidine vs. Diclofenac Na in Rats and Dogs**

Key Findings: *DIEP caused gastrointestinal toxicity similar to diclofenac.*
Study Number: 870413 and 870414
Test Site: [redacted]
GLP Compliance: No
Quality Assurance: No
Location of Report: Vol. 4A, pp. 177-248
Study Period: November 11-18 (rats) and November 16-23, 1987 (dogs)
Species/Strain/Age/Sex/Weight: Rat, Sprague-Dawley, about 5 weeks, ♂ and ♀, 75-100 g
Dog, Beagle, about 7 months, ♂ and ♀, 7.2-8.1 kg
Test Substance: DIEP, batch no. 27/IB-9 ([redacted] pure) and diclofenac Na, lot no. BT 51001 ([redacted] pure), Voltaren® 25 mg (batch no. 044800)
Vehicle: Deionized water (DIEP) and 0.5% carboxymethylcellulose (diclofenac Na)
Route of Administration: Oral (gavage in rats and tablet in dogs)
Treatment Scheme:

Rats

Treatment (5 mg/kg/day)	n ♂	n ♀	Treatment Frequency
DIEP	6	6	7 days
Diclofenac Na	6	6	7 days

Dogs

Treatment (25 mg/kg/day)	n ♂	n ♀	Treatment Frequency
DIEP	2	2	7 days
Voltaren®	2	2	7 days

Parameters Observed/Measured:

Clinical Signs: Daily.
Body Weight: Before and after treatment.
Food Consumption: During treatment.
Gross Pathology: At the end of treatment period. In particular, stomach and intestine were carefully examined. Stomach, duodenum, jejunum, ileum, cecum, colon, rectum and gross modifications were preserved in 10% buffered formalin.
Histopathology: Two sections of stomach (including glandualar and non-glandular mucosa), duodenum and gross modifications were fixed

Summary of Findings: Administrations of DIEP or diclofenac Na at the oral doses of 5 mg/kg/day to rats and 25 mg/kg/day to dogs induced the expected untoward effects common to NSAIDs. In rats there were no clinical signs while in dogs diarrhea, blood in feces, vomiting and disorrexia were present in both the experimental groups and their severity increased in time from day 1 to 7 of treatment. Body weight decreased in dogs given DIEP only. In rats, no differences in gastrolecivity were seen for either of the test articles. Slight drug-related modifications of the gastric mucosa (congestion and/or focal ulcers) were seen in both groups. In dogs, both groups treated with DIEP or diclofenac showed drug-related gastrointestinal modifications without appreciable differences between the two compounds. The changes generally consisted of gastric pyloric mucosa ulcers and erosion with congestion or hemorrhage of the mucosa of the ileum and/or intestine.

Reviewer's Comments: The reviewer concurs with the findings of the study. This study should have been conducted according to GLP guidelines and the statement of quality assurance should have been provided.

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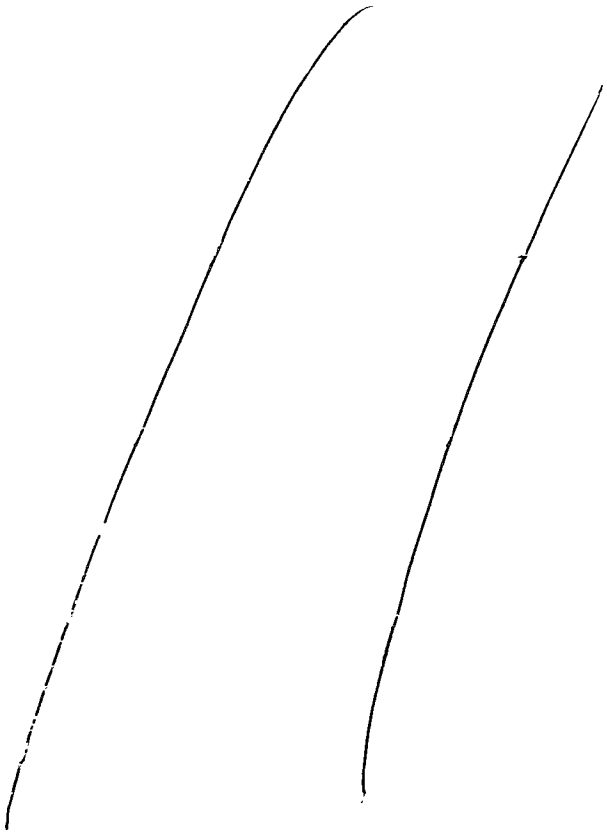

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