SECTION III: SINGLE AND REPEAT ORAL TOXICOLOGY STUDIES

Summary Review of Previous Nonclinical Toxicity Studies with Diclofenac Administered Systemically, submitted to NDA 19-201 to support oral administration of Voltaran Tablets (Submitted by CIBA GEIGY Corporation, Food and Drug Administration, dated 1/10/85.)

Acute Systemic Toxicity: Calculated LD_{50} levels (mg/kg) determined following acute toxicity studies using various species and routes of administration:

Route	Mouse	Rat	Guinea Pig	Rabbit	Dog	Monkey
i.v.	92 - 147	97 - 161	123 - 131	>20	ND	ND
p.o.	185 - 541	55 - 240*	1110	125 - 300	>800	3200
i.g.	ND	142**	ND	ND	ND	ND
dermal	ND	ND	ND	2000 ± 980	ND	ND

* 131 after 21 days (consecutive) dosing in rats; ** neonatal rats.
ND = Not Done.

Major signs of toxicity following acute systemic administration of the lethal dose, maximum tolerated dose (MTD) or the highest dose tested included the following:

Mice: tremors and convulsions, ataxia, dyspnea, vetrolatericumbency, somnolence, exophthalmos, rapid respiration, saltatory spasms, rough coat, and general poor condition.

Rats: reduced activity, tremors, spasms and convulsions, ataxia, dyspnea, vetrolatericumbency, somnolence, exophthalmos, ptosis, diarrhea, tarry stool, paleness of the conjunctiva, salivation, piloerection, staining, rough coat, and general poor condition.

Guinea Pig: convulsions, opiathotonousataxia, dyspnea, vetrolatericumbency, somnolence, exaggerated startle reaction with reduction of spontaneous mobility, and roughening of the coat.

Rabbit: convulsions, ataxia, dyspnea, latericumbency, somnolence, tarry stool, bloody nose exudate.

Dog: transient loss of appetite, diarrhea, superficial erosions on mucosa of the duodenum.

Monkey: tremors, anorexia, emesis, diarrhea, loose and/or tarry stool, rectal bleeding (ulcers at necropsy), coma preceding death.

As can be seen, toxic effects observed across species are similar, and even acute doses are capable of causing gastrointestinal ulcerations in both rodents and mammals.

Repeat Systemic Toxicity: Studies ranging in duration from 7 days to 1 year have been conducted using various species and dosing routes. The primary effects observed included the following:

Rat - dosed by oral gavage for 4 weeks to 6 months with 0.25-40 mg/kg/day: GI toxicity, lymph node hypertrophy and hyperplasia (dose and duration dependent), anemia and changes in AST, ALT, AP and BUN.

Dog - dosed orally for 30 to 90 days with 0.5 to 2.5 mg/kg/day: GI toxicity, anemia, extramedullary hematopoiesis, and lymphadenitis.

Rhesus Monkey - dosed by oral gavage for 30 days to 6 months with 1-500 mg/kg/day: GI toxicity, anemia, degenerative kidney lesions (≥75 mg/kg/day for 6 mo.). The NOEL following 30 days of dosing was 50 mg/kg/day; a NOEL was not identified in the 6 mo. study.

Papio Baboon - dosed orally with gelatin capsules for 1 year with 5-50 mg/kg/day: Mortality (≥15 mg/kg/day), GI toxicity (reversible after 28 days), and anemia. A NOEL was not identified.

Overall, the findings from these studies indicate that diclofenac exhibits a toxicologic profile similar to other NSAIDs. The main target organ for toxicity is the gastrointestinal tract, accompanied by anemia (probably secondary to blood loss form the GI tract) and evidence of hepatic and renal effects.

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SECTION IV: SINGLE AND REPEAT TOPICAL TOXICOLOGY STUDIES

Study 4.1: A 7-Day Dermal Dose Range-Finding Study of Diclofenac —— Hyaluronan Gel in the Albino Mouse, Project No. 87161, Draft Report dated 10/3/95, in life 7/26/95 to 8/4/95, conducted by ——————, in accordance with Good Laboratory Practices regulations (21 CFR 58). *[Note: The quality assurance audit reports, GLP Assurance Statement, and pathology report were not signed. This draft report was previously submitted to IND —— Amendment N028 dated 3/15/96.]*

Study Design: This study was designed to determine if dose levels of 0.009, 0.018 and 0.035% diclofenac —— hyaluronate gel were suitable for use in dermal toxicity and/or carcinogenicity studies in Swiss Crl:CDr-1(ICR)BR mice. Animals were randomized to 15 mice/sex/group. The vehicle control or test article formulations of 0.2 ml/animal were administered to a shaved area of skin approximately 5 cm^2, for a maximum of 7 days (Approximate doses in mg/kg were based on group mean body weights recorded on days 1, 4 and 7. No post-dose removal of residual product was performed since each formulation appeared to be completely absorbed within approximately 1 hour post-administration. The first 5 animals/sex/group were euthanized 1 hour after treatment on Day 1, the second 5 animals/sex/group were euthanized 1 hour after treatment on Day 7, and all surviving animals were euthanized on Day 8, approximately 24 hours after last treatment. Animals were monitored for clinical signs of toxicity, body weight, food consumption, blood concentrations of diclofenac prior to euthanasia, and gross pathology, major tissues and organs were preserved, but not examined, and skin samples were analyzed for diclofenac levels.

Summary of Study Results: There were no deaths or adverse reactions were noted during the treatment period. There were no differences in body weight gain or food intake between control and treated groups. Calculated doses based on mean body weight per group are presented in the Table 5.

Examination of animals killed 1 hour after a single dose on Day 1 revealed no gross pathological findings. A scab was noted at the dose site of one female in the mid-dose (0.018% diclofenac) group on Day 7. Dark discoloration of digesta/ingesta associated in some animals with dark areas/foci in the stomach was seen in both control and treated animals killed on Day 8, with a higher incidence in females treated at 0.018 and 0.035% diclofenac. This appeared in a dose-response manner, but was not present in animals sacrificed on Day 7.

Table 4.1.1: Dose of diclofenac (mg/kg) achieved in mice following topical administration of gels formulated with 0.009, 0.018 and 0.035% diclofenac and — hyaluronate.

Day	Males			Females		
	0.009%	0.018%	0.035%	0.009%	0.018%	0.035%
1	0.7	1.4	2.7	0.8	1.6	3.1
4	0.7	1.3	2.6	0.8	1.5	3.1
7	0.6	1.3	2.5	0.8	1.6	3.0
Mean	**0.7**	**1.3**	**2.6**	**0.8**	**1.6**	**3.1**

[Note: Average diclofenac dose for a 60 kg human following administration of 2 grams of gel = 1.0 mg/kg/day.]

Although the protocol indicates that blood and skin samples were taken for determination of diclofenac concentration, this report does not include any toxicokinetic information.

Reviewer's Comments: As a dose range-finding study, this study was inadequate in both dose ranges and duration to determine adequate doses for long-term dermal toxicity. There was no mention in the Animal Management or Test Article Administration sections of this report on precautions taken to limit ingestion or removal of the test substance during grooming or rubbing against the cage.

Study 4.2 - Range-Finding Tolerance Test of Diclofenac Topical Gel Administered Topically to Hairless Mice. Protocol No. 808-001, Report dated 2/6/96, in life 7/26/95 to 8/4/95, conducted by ——————————, in accordance with Good Laboratory Practices regulations (21 CFR 58). [*Note: GLP Assurance Statement was not signed and quality assurance audit reports were not submitted.*]

Study Design: The purpose of this study was to determine: 1) whether the test article or vehicle interact with ultraviolet radiation (UVR) in the production of photo-biological responses (sensitization or protection); and 2) the test article concentrations that can be repeatedly administered to hairless mice either alone or in combination with UVR without inducing adverse effects such as inflammation that would preclude their use in a chronic photo-cocarcinogenicity study. Albino hairless Crl:SKH1(hr/hr)BR mice (3/sex/group) were randomly assigned to dosing groups. Formulations were prepared in the hyaluronate base gel at concentrations of 0.0, 0.09, 0.18, and 0.35 mg/ml diclofenac. 8-Methoxypsoralen (0.5 mg/ml) and SPF 4 sunscreen, — were used as controls. Three animals/sex were also assigned to an untreated control group. Doses were administered topically to lightly anesthetized mice at a constant volume of 0.2 ml/mouse. In part 1 of the assay, test preparations were spread onto the backs of the animals with the barrel of the syringe then covered with an aluminum foil mask with 6 holes. A single exposure of UVR (0 to 2.7 times the MED) was given to each of the six irradiation sites. The UVR source was a ——————arc

high intensity solar simulator with a 1 mm ——— glass filter coupled to an — light pipe. At approximately 24, 48 and 72 hours after irradiation, the mice were examined for signs of inflammation in the irradiated sites.

Having determined the best doses in part 1, part 2 of this study consisted of exposures to UVR for 8 weeks on the following schedule: on Monday, Wednesday and Friday of each week, animals received UVR exposure approximately 1 hour after administration; on Tuesday and Thursday of each week, UVR exposure occurred approximately 1 hour before administration. A 6.5 kilowatt xenon long arc, water cooled burner was vertically suspended within an octagonal metal frame holding one ———optical filter on each side. Racks were located approximately 2 meters from the UVR source and exposures were monitored by a ——— detector. Clinical observations were performed daily during the first week of exposures and weekly thereafter. Cutaneous responses were visually assessed using a modified Draize system. Mice were sacrificed and discarded after the completion of eight weeks of administration.

Summary of Study Results: In part 1, the average doses of diclofenac gel actually administered were 0.63, 1.23 and 2.37 mg/kg in the males, and 0.77, 1.60 and 3.13 mg/kg in the females following application of the 0.009, 0.018 and 0.035 % formulations, respectively. All mice survived treatment and no clinical observations, other than skin reactions in the irradiated sites, occurred in any mice during the study period. The average skin reaction thresholds in diclofenac treated animals were comparable to the vehicle control, indicative that the test article was neither phototoxic nor photo-protective. However, the mean MED was reduced in mice treated with vehicle (33%) and diclofenac gels (27%, 18%, and 23%, respectively) when compared to untreated animals.

In part 2, one high-dose male died during week 5 from a lesion in the anogenital area which was not considered to be dose-related. There were no other clinical signs of toxicity other than skin reactions within the administered areas which were considered to be dose-related. Body weights and body weight changes were comparable between dosing groups. The average mg/kg/day doses are presented in table 4.2.1.

Administration of 0.035 % Diclofenac Topical Gel for eight weeks to hairless mice caused grade 1 flaking of the skin (barely perceptible scales) in both UVR exposed (3/3 males and 3/2 females) and unexposed (3/3 males and 1/3 females). Flaking of the skin was observed only during week 1 with complete resolution by week 2 in all affected mice.

Table 4.2.1: Dose of diclofenac (mg/kg/day) achieved in mice following topical administration of gels formulated with 0.009, 0.018 and 0.035% diclofenac and — hyaluronate.

Dose in mg/ml (%)	Dose in mg/kg/day	
	Males	Females
0.009 %	0.50 - 0.57	0.60 – 0.67
0.018 %	1.00 – 1.07	1.23 - 1.37
0.035 %	1.85 – 2.03	2.37 – 2.63

[Note: Average diclofenac dose for a 60 kg human following administration of 2 g of the 3% diclofenac gel = 1.0 gm/kg/day.]

Based on the results of this study, concentrations of Diclofenac Topical Gel as high as 0.35 mg/ml, applied at a volume of 0.2 ml per mouse (~12.5 mg/m^2/day) were not phototoxic and deemed an acceptable high-dose for future photo-cocarcinogenicity studies in hairless mice, even though the gel formulations were shown to reduced the MED.

Study 4.3 - A 14-Day Dermal Range-Finding Toxicity Study of 3% Diclofenac in Minipigs. Project No. 54706. Report dated 11/13/96, *in life* 9/15/95 to 9/29/95, conducted by _____ in accordance with Good Laboratory Practices regulations (21 CFR 58). [*Note: Quality Assurance Statement and Pathology Report were not signed.*]

Study Design: The objective of this study was to determine the potential toxicity of 3% Diclofenac Gel (Lot No. VHD7, exp. Date 8/96) in Göttingen minipigs when administered by daily dermal application for 14 days. Two animals/sex were treated with 30 mg/kg/day in a constant volume of 1 ml/kg/day. The dose level of 30 mg/kg/day was selected based on a maximum practicable dose volume of 1 mg/kg in a single application and was the proposed high dose level for a subsequent 6 month dermal toxicity study. Two days prior to the first dose and as deemed appropriate during the remainder of the study, an area approximately 10 cm x 10 cm was shaved of hair on the dorso-thoracic region of each animal. The test article was applied once/day and spread using a glass rod. After 6 hours, any residue was wiped off using a clean gauze (on day-4 the residue was wiped off after 5 hours due to technical error). Animals were observed twice daily for mortality and given a detailed physical examination following each dose. Animals were weighed on days -7, -1, 7 and 14. Food consumption was monitored during the last 7 days of pretreatment and throughout the treatment period. Blood samples were collected for hematology and clinical chemistry predosing and on Day 14. Approximately 2 ml of blood was also obtained on day 1 and day 14 prior to dosing and approximately 1, 3 and 6 hours after dosing for toxicokinetic evaluation. At the time of necropsy, animals were examined for gross pathology and tissues were fixed and preserved in neutral buffered 10% formalin for possible future histopathological examination.

[*Note: Analytical results of test materials and blood samples were not submitted by the Sponsor.*]

Summary of Study Results:

Clinical Observations: There were possible drug-related tremors and blue coloration of the muzzle in one female (#152) on day 14 of treatment. Reddening and swelling were also observed in the ventro-cervical region, which were attributable to the jugular vein blood sampling procedure.

Body Weight and Food Consumption: A reduction in food consumption was noted in both females on days 13 and 14 when compared with previous study days, resulting in proportionate weight losses (most significantly in female #152).

Hematology: On day 14, total WBC counts were significantly increased (2-3 fold) in 1 animal/sex and the absolute monocyte counts were increased (10-30 fold) in all 4 animals. Activated partial thromboplastin time was also increased in 1 animal/sex, together with giant platelets and toxic granulation in female #152. The most significant increases were observed in the female #152, which experienced tremors on day 14, and may or may not be drug-related. This animal also had moderate increases in all red cell indices.

Clinical Chemistry: Slight to moderate increases (2-3x) in BUN were noted in all animals. There was a marked increase (28x) in AST accompanied by 2 fold increases in total bilirubin and ALT in one male, however histological examination of the liver revealed no evidence of pathologic basis for these increases.

Gross Necropsy: Multiple adhesions between jejunal loops were observed in both females and was associated with multiple dark depressed areas in the mucosa of the jejunum or the stomach. Stress-related gastric ulcers are common in pigs but are usually restricted to the pars esophagea; jejunal ulceration and adhesions are uncommon and may be drug-related.

Study 4.4 - A 9-Week Dermal Range-Finding Toxicity Study of 3% Diclofenac in Minipigs. Project No. 54811. Report dated 11/13/96, *in life* 10/18/95 to 12/20/95, conducted by ———————————————, in accordance with Good Laboratory Practices regulations (21 CFR 58). [*Note: Quality Assurance Statement and Pathology Report were not signed.*]

Study Design: The objective of this study was to determine the potential toxicity of 3% diclofenac/ — HA gel in Göttingen minipigs when administered by daily dermal application for a minimum of 28 days. The duration of the study was extended by 35 days and the dose level increased due to a lack of definite treatment-related findings (as determined by gastric endoscopy) after 28-days dosing. The animals were approximately 5 months of age and ranged in weight from 7.3 & 8.5 kg for males and 6.8 & 7.3 kg in females. 1.0 or 1.5 ml 3% Diclofenac Gel/kg/day (lot no. VGD6, exp. Date 7/96) was administered in two equal applications, e.g., 15 or 22.5 mg/kg b.i.d., approximately 6 hours apart to a shaved area of skin (15 cm x 20 cm) in the dorso-thoracic region of each animal (Table 4.4.1). Residue product was wiped off prior to second application and again after approximately 6 hours exposure to the second dose. All animals were observed twice daily for mortality and clinical signs of toxicity, with a more detailed physical examination conducted weekly. Skin reactions were scored weekly for erythema and edema according to Draize. Body weight and food consumption were also recorded weekly prior to initiation and throughout the study period. Twice during the acclimation period and again during weeks 4 and 9, blood samples were collected for hematologic and clinical chemistry analysis. Blood samples were also collected prior to the first application and approximately 1, 3 and 6 hours after each dose on days 1, 28, 33 and 60 for toxicokinetic analysis. Gastric endoscopies were performed on fasted animals at the end of weeks 4 and 9 to evaluate the presence of drug-induced ulcerations. Complete necropsies were performed on fasted animals immediately after euthanasia. Tissues were preserved in neutral buffered 10% formalin for possible future evaluation.

Table 4.4.1: Dosing regiment for 9-week dermal range-finding study of 3% diclofenac /HA gel in minipigs.

Animal	30 mg/kg/day (1 ml/kg/day)	45 mg/kg/day (1.5 ml/kg/day)
Male #1001	Days 1-30	
Male # 1002	Days 1-32	Days 33-63
Female #1501	Days 1-33	Days 34-52
Female #1502	Days 1-32	Days 33-63

Summary of Study Results:

Mortality and Clinical Observations: Male no. 1001 was euthanized on day 30 due to a severe protrusion and swelling of the penis which occurred while under anesthesia for the gastric endoscopy on day 28.

Female no. 1501 was euthanized on day 52. Reduced food consumption was noted on day 43, and on day 51, the animal presented with a prolapsed rectum, diarrhea, moderate abdominal distension, moderately swollen eyelids, moderate to severe swelling of the cervical region, cold to touch and tremors. Hematology and clinical chemistry changes included slightly increased neutrophil count, markedly increased reticulocyte count, and a marked reduction in red cell parameters, total protein, albumin and A/G ratio consistent with gross pathological observations of gastro-intestinal ulcerations. Blood cell morphology revealed the presence of increased NRBCs and moderate toxic granulation.

There were no clinical signs of toxicity in the two animals surviving to study termination.

Skin Reactions: Very slight to well-defined erythema was noted in male no. 1001 and female no. 1501 from week 3 until euthanasia on days 30 and 52, respectively. Very slight erythema was noted in male no. 1002 only during week 8.

Body Weight and Food Consumption: A slight reduction in body weight was noted for male no. 1001 prior to euthanasia on day 30 and food consumption for female no. 1501 began progressively diminish starting around day 40 until her death on day 52. There were no other significant changes in body weight or food consumption.

Hematology: In addition to the changes observed in female no. 1501, female no. 1502 had slightly reduced red cell parameters and increased NRBC counts at week 9. There were no significant hematologic changes observed in males.

Clinical Chemistry: At week 4, there were slight increases in BUN in animals 1001 and 1501.

Gastric Endoscopy: Inflammation was observed in the stomach of all animals at week 4, with the presence of ulceration in female no. 1502 only. There were no findings in surviving animals 1002 and 1502 at week 9.

Gross Pathology: Treatment-related gross pathological findings observed in female no. 1501 consisted of multiple pale depressed areas in the mucosa of the stomach and colon associated with adhesion of the stomach to the liver. Emaciation and pallor of the carcass, swelling of the subcutaneous tissue, pancreas, cecum and rectum, prolapsed rectum and dilation of the intestines were considered secondary to the gastro-intestinal ulcerations.

There were no other notable gross pathological findings.

Toxicokinetics: Data not submitted.

Conclusion: Due to the complications observed in female no. 1501, the maximum dose level for a 26-week repeat dose study should not exceed 45 mg diclofenac/kg/day.

Study 4.5 - A 6-Month Dermal Toxicity of 3% Diclofenac Gel in Minipigs. Study no. 54658, Report dated 9/5/97, *in-life*: 10/18/95 to 4/18/96, conducted by [redacted], in compliance with Good Laboratory Practice Regulations (21 CFR 58).

This study was originally submitted and reviewed under IND [redacted] Amendment 48 dated 9/19/97. The review has been reproduced below.

Study Design: The objective of this study was to determine the potential toxicity of 3% diclofenac gel in Göttingen Minipigs when administered topically for 6 months. Diclofenac/[redacted]HA Gel (0, 1.5, 5.0, and 15.0 mg/kg) was applied to a shaved area approximately 15 cm x 20 cm on the dorsal-thoracic region of each animal b.i.d. At the start of treatment, animals were approximately 5 months of age and ranged in weight from 7.2 to 8.9 kg in males and 7.1 to 9.4 kg in females. After 4 weeks of treatment there were no signs of any treatment-related gastric ulceration, therefore, the high dose was increased to 45 mg/kg/day (22.5 mg/kg b.i.d.) for the remaining 22 weeks of the study. All doses were applied twice daily approximately 6 hours apart. Approximately 6 hours after each application, any residue was wiped off using clean gauze. Evaluations included clinical observations and food intake (daily); physical examinations, dermal scoring (Draize); and body weights (weekly); ophthalmoscopy, clinical laboratory, and toxicokinetic evaluations (weeks 0, 6, 13, 26); and bone marrow smears, skin biopsy, gross pathology, and organ weights (necropsy). All major tissues were prepared for histopathology analysis, however, only samples from animals in the control and high dose groups, gross lesions, duodenum, jejunum, liver, spleen and testes of low- and mid-dose animals, and animals euthanized before scheduled necropsy were routinely analyzed. All blood samples were collected from a jugular vein following an overnight deprivation of food and prior to dosing on the day of collection.

The data obtained were analyzed for homogeneity using Bartlett's test. The statistical significance of any differences in the data were assessed using Analysis of Variance, and the significance of any intergroup differences was determined using Dunnett's test for homogenous data or the Kruskal-Wallis test followed by Dunn's test for heterogeneous data.

Rationale for Dose Selection: The high-dose of 1 ml/kg or 30 mg/kg was considered the maximum practicable dose volume for application; the low-dose, 0.1 mg/kg or 3 mg/kg, is equivalent to the anticipated maximum human dose; and the mid-dose, 0.3 ml/kg or 10 mg/kg, was selected as the approximate log midpoint.

Summary of Study Results:

Mortality: Three animals were terminated early. One mid-dose male was euthanized due to deteriorating health on day 143 (week 21). The animal began losing weight and appetite about 2 weeks before its death. Clinical signs of illness were first noted 3 days prior to death and included reduced activity and intermittent hunched posture. On the day of euthanasia, the animal exhibited weakness, ataxia, labored breathing, evidence of emesis, and blue discoloration of the skin of the muzzle. Laboratory analysis revealed slight elevations in PT and APTT, a markedly elevated BUN, and slight reductions in ALT, alkaline phosphatase and chloride. Pathological examination of this animal revealed a moderate transabdominal ulcerative jejunitis associated with a moderate chronic active peritonitis.

One high-dose male was euthanized due to deteriorating health on day 159 (week 23). Clinical signs of illness were first observed on day 147 (week 21) and included marked weight loss and loss of appetite, tremors, reduced activity, pallor, cold to touch, lateral or sternal recumbency, ataxia, weakness and blue discoloration of the skin of the muzzle. Laboratory analysis revealed a marked increase in the WBC count due to a marked increase in absolute neutrophils (5 fold), a marked anemia including increased nucleated RBCs, and slight reductions in ALT and alkaline phosphatase. Pathological examination revealed a moderate subacute ulcerative gastritis associated with a mild chronic active peritonitis.

One control female was euthanized for humane reasons due to a suspected broken tibia on day 177 (week 26). The clinical data for this animal are included with those euthanized as scheduled upon completion of treatment.

Clinical Observations: With the exception of the clinical findings in the euthanized animals, the only treatment-related clinical findings were skin reactions at the site of application. These reactions were characterized by very slight to well-defined erythema and/or scab formation. Although there was no clear increase in severity with increasing dose, the onset of the reaction appeared to occur earlier in high-dose animals (beginning week 3). Severe reactions in 1 low-dose and 1 high-dose males resulted in discontinued drug treatment between weeks 14-16 and 16-20, respectively. However, following resumption of dosing the conditions quickly worsened.

Body Weight and Food Consumption: With the exception of the two euthanized animals, there were no treatment-related effects on body weight or food consumption.

Ophthalmoscopy: There were no dose-related ocular changes.

Local Skin Effects: Animals receiving placebo (8/8) showed no signs of dermal irritation at anytime during the study period. High-dose animals began showing signs of slight, sporadic erythema at the treatment site during study week 3, mid-dose animals in study week 7, and low-dose animals in week 9. Signs of edema were evident beginning in study week 12. Signs of slight to mild dermal irritation remained sporadic in most animals without progressing in severity. At the time of necropsy, 1 animal in each of the treated groups (6/24) presented with scabbed treatment sites.

One male in the low-dose group showed signs of moderate irritation between weeks 10 and 14. Another male in the mid-dose group showed signs of severe irritation between weeks 16 and 21. *(Note: This animal was euthanized during week 21.)* Large pustules containing gram positive cocci were observed in the stratum corneum of both of these animals. Other findings included slight to moderate perivascular/periadnexal superficial dermatitis, acanthosis, and parakeratotic hyperkeratosis.

Clinical Laboratory Values: There appeared to be a dose-related increase in the absolute neutrophil count, in surviving mid- and high-dose males and high-dose females. All animals showed slight reductions in red cell parameters, however, this appeared to be more prominent in mid- and high-dose females.

Signs of possible hepatotoxicity in 2/4 high-dose males included slight, dose- and time-dependent increases in total bilirubin, AST and ALT values. Slight decreases in albumin were also observed in both high-dose males and females.

Organ Weights: Dose-related changes in relative organ weights occurred in the kidneys, liver and ovaries (Table 4.5.1). In addition, 2 females, one from the mid-dose group and one from the high-dose group had reduced thymus/parathyroid weights of approximately 50% when compared to the other animals.

Table 4.5.1: Dose-related changes occurring in Minipigs following topical treatment with Diclofenac Gel for 6 months.

Organ	Weight Change	Sex	3 mg/kg/day	10 mg/kg/day	30/45 mg/kg/day
Kidney	Increase	M	3%	5%	18%
Liver	Increase	F	8%	2%	16%
Ovaries	Increase	F	23%	34%	66%

Macroscopic and Microscopic Effects (Table 4.5.2): There was a dose-related increase in the incidence of macroscopic lesions in the mucosa of the stomach, duodenum and jejunum. These lesions were comprised primarily of discolored or pale areas in the mucosa or serosa.

Microscopic examination of these lesions resulted in the following findings: slight erosive gastritis in 1/8 low-dose animals, slight focal acute necrosis of the fundic mucosa in 1/8 mid-dose animals, and slight to mild erosive gastritis or moderate ulcerative gastritis in 7/8 high-dose animals; slight erosive duodenitis in 2/8 high-dose animals; and mild to moderate ulcerative jejunitis in 1/8 mid-dose and 3/8 high-dose animals. Mild to moderate peritonitis was associated with the gastric and intestinal lesions in 1/8 mid-dose and 2/8 high-dose animals. Erosive esophagitis was also observed in 1/8 high-dose animals.

Renal lesions, characterized by slight to mild interstitial nephritis and tubular dilation, slight hyaline casts, mononuclear cell infiltrations and papillary degeneration were seen in some mid- and high-dose animals.

Atrophy of the testicular epithelium was observed in 4/4 high-dose, 3/4 mid-dose, 1/4 low dose, and 1/4 control males. This lesion was generally described as slight or mild, except in one low and high dose male, where it was described as moderate.

Slight to moderate hyperkeratosis and superficial dermatitis were observed in skin from the treatment site in almost all animals, including controls, increasing in severity with increasing dose in most cases. Mid- and high-dose animals also presented with hyperkeratosis and parakeratosis in skin taken from an untreated site (hind paw).

Other affected organs observed primarily in the mid- and high-dose groups included the spleen (congestion and extramedullary hematopoiesis), and liver (necrosis, vacuolization, extramedullary hematopoiesis). Hemorrhaging was noted in the thymus, larynx, thyroid and pericardium in almost all animals and was considered to be secondary to the experimental procedures involved in animal handling.

Table 4.5.2: Histopathology effects identified in Minipigs following topical treatment with Diclofenac Gel for 6 months.

	Males				Females			
Histopathology Finding	0 mg/kg	3 mg/kg	10 mg/kg	30/45 mg/kg	0 mg/kg	3 mg/kg	10 mg/kg	30/45 mg/kg
Esophagitis - erosive	0/4	-	0/1	1/4	-	-	-	-
Gastritis - ulcerative & erosive	0/4	0/4	0/4	4/4	0/4	1/4	1/4	3/4
Jejunitis - ulcerative	0/4	0/4	1/4	1/4	0/4	0/4	0/4	2/4
Duodenitis - erosive	0/4	0/4	0/4	0/4	0/4	0/4	0/4	2/4
Peritonitis	0/4	0/4	1/4	1/4	0/4	0/4	0/4	1/4
Liver Necrosis	0/4	-	0/1	1/4	1/4	-	-	0/4
Nephritis	1/4	-	1/2	2/4	0/4	-	-	1/4
Spleen - congestion	0/4	0/4	0/4	1/4	1/4	0/4	1/4	2/4
Testicular atrophy	1/4	1/4	3/4	4/4	-	-	-	-
Epididymis - oligo/asperemia	0/4	-	0/1	1/4	-	-	-	-
Dermatitis - Treated Site*	0/4	2/4	1/4	1/4	0/4	1/4	1/4	2/4

* Time to onset was dose-dependent, however, severity of reactions was independent of dose.

Toxicokinetic Analysis: Toxicokinetic analysis was performed at _____

The mean diclofenac plasma values for C_{max}, T_{max} and AUC_{0-24} derived from blood samples taken for a period of 24 hours following dosing on days 1, 85 and 176 are presented in Table 4.5.3. Samples were taken at 1, 3 and 6 hours following each daily dose and at 24 hours, just prior to the next days 1st dose (animals were dosed b.i.d, with 6 hours between doses).

Measurable diclofenac levels were found in all samples, including the vehicle control. Independent analysis of control lots by _____ revealed levels of diclofenac contamination (1.3 to 806 ng/ml) in the control gel. The highest possible dose with the contaminated vehicle was calculated to be 350 ng/kg/day. It was therefore concluded that this level of contamination could not account for the observed plasma levels.

Other possible explanations put forth by Hyal to account for the high diclofenac plasma levels observed in control animals include the following:

- administration of test article to control animals (dosing error)
- incidental exposure of control animals to test article
- incorrect plasma sample identification at testing facility
- contamination of blood samples at collection, centrifuging, or aliquot preparation
- incorrect sample identification or contamination of plasma samples at the analytical facility
- presence of a compound in minipig plasma masquerading as diclofenac (under investigation)

In addition, several animals from each group, 13/36 in all, had measurable pretreatment diclofenac plasma levels ranging between ——— ng/ml in blood sample collected on day 1. This information makes incorrect plasma sampling or contamination as the most likely sources of contamination.

Table 4.5.3: Selected pharmacokinetic parameters following dosing with 3% Diclofenac — Hyaluronate Gel in Minipigs at concentrations of 3, 10 and 30/45 mg/kg/day on days 1, 85 and 176.

PK Parameter	Day	0 mg/kg/day	3 mg/kg/day	10 mg/kg/day	30/45* mg/kg/day
C_{max} (ng/ml)	1	38.7 ± 42	30.7 ± 42	29.3 ± 9	143.9 ± 104
	85	146.6 ± 101	297.1 ± 243	245.6 ± 145	1190.0 ± 446
	176	284.6 ± 228	310.3 ± 192	597.7 ± 452	1816.3 ± 659
T_{max} (h)	1	10.6 ± 6.6	10.0 ± 6.0	16.4 ± 8.3	20.6 ± 6.3
	85	10.9 ± 9.2	10.9 ± 8.9	8.5 ± 7.3	4.1 ± 8.1
	176	10.4 ± 6.8	5.4 ± 4.3	8.3 ± 7.9	3.4 ± 3.2
AUC_{0-24} (ng.hr/ml)	1	359.8 ± 380	167.1 ± 112	492.0 ± 204	1851.5 ± 1237
	85	2016.7 ± 2017	4048.3 ± 2509	4038.1 ± 1216	17808.3 ± 5030
	176	2786.9 ± 2787	5380.2 ± 3708	8405.3 ± 6830	24594.8 ± 7712

* 30 mg/kg/day was administered from day 1 through day 28, thereafter, 45 mg/kg/day was administered to high-dose group animals.

Review Note: Without knowing the source of the contamination, all the samples must be suspect. Therefore little if any usable PK information can be salvaged from this study. Generalizations which might be made include the following:

- *Systemic absorption of diclofenac does appear to be dose- and time-related, with higher plasma levels observed both with increasing dose and over time.*
- *From the data collected, it appears safe to conclude that Cmax, Tmax and AUC are all dose- and time-dependent.*
- *Bioaccumulation appears to be occurring over time.*
- *Tmax may decrease with increasing plasma concentrations and time. This may be indicative of some form of inducible metabolism and/or plasma clearance mechanisms.*

Similar results were observed in PK analysis of preliminary data submitted from the 2-year dermal carcinogenicity study conducted in mice. Plasma samples were evaluated 1 hour post-dosing on day 1, and following weeks 13, 26 and 52. In this study, some animals in both the nontreated and placebo groups showed small amounts of diclofenac (——— ng/ml) in their plasma.

Conclusion: The 6-month minipig dermal toxicity study has been confounded by the presence of diclofenac in plasma samples collected across the entire study period. The information collected from this study is therefore suspect and should only be used when placed into context with other nonclinical and human data. In view of the fact that 1) the data demonstrates a dose- and time-related increase in diclofenac plasma concentrations following topical administration, and 2) the adverse effects reported in this study are similar to those reported following oral administration of diclofenac (namely the effects to the gastro-intestinal mucosa). A second non-rodent dermal toxicology study was not recommended since adequate exposures were achieved in the high-dose animals and it was unlikely that any new information would be collected in a repeat study.

Summary of Toxicology Studies

Acute Systemic Toxicity: Following administration of acute lethal and maximally tolerated doses of diclofenac signs of toxicity common across species included tremors and/or convulsions, signs of GI bleeding, anorexia and general poor conditions. In general rodents were much more sensitive to the acute effects of diclofenac with LD_{50} doses ranging between 55 to 541 mg/kg, compared to 125 to 300 mg/kg in rabbits, 1110 mg/kg in guinea pigs, >800 mg/kg in dogs and 3200 mg/kg in monkeys.

Repeat Systemic Toxicity: Studies ranging in duration from 7 days to 1 year were conducted in rats, dog, Rhesus monkeys, and Papio baboons. Doses were administered either by oral gavage, capsules, in the feed, or subcutaneous injection. Toxicological effects were observed to occur at lower doses following prolonged dosing regimens. The main target organ for toxicity in all species tested is the gastrointestinal tract, accompanied by anemia (probably secondary to blood loss form the GI tract). Evidence of hepatic and renal effects were also observed following prolonged (6 months) daily dosing.

Toxicity Following Acute Topical Application: LD_{50} following acute dermal application to rabbits was 2000 ± 980 mg/kg. In addition to clinical signs consistent with poor health, e.g., convulsions, ataxia, dyspnea, latericumbency, somnolence, loss of body weight, animals also exhibited tarry stools and nephrosis.

Toxicity Following Repeated Topical Application:

- *Dermal administration of formulations containing 0.009, 0.018 and 0.035% diclofenac hyaluronate to mice for 7 days (resulting in average mg/kg/day doses of 0.7, 1.3 and 2.6 in males and 0.8, 1.6 and 3.1 in females, respectively) produced clinical signs suggestive of gastric bleeding in the mid- and high-dose groups (more predominant in females). This is an expected side effect of cyclooxygenase inhibitors. No other adverse effects were noted.*

- *Administration of 0.035% Diclofenac Topical Gel for eight weeks to hairless mice caused grade 1 flaking of the skin (barely perceptible scales) in both UVR exposed (3/3 males and 3/2 females) and unexposed (3/3 males and 1/3 females) animals. Flaking of the skin was observed only during week 1 with complete resolution by week 2 in all affected mice. The mean MED was also found to be reduced by applications of both the vehicle and diclofenac gels when compared with untreated UV exposed animals. Based on the results of this study, concentrations of Diclofenac Topical Gel as high as 0.035 % (0.35 mg/ml), applied at a volume of 0.2 ml per mouse were otherwise not considered phototoxic and deemed an acceptable high dose for future photo-carcinogenicity studies in hairless mice.*

- *In a dose range-finding study in Göttingen minipigs, application of 3% Diclofenac Gel was administered for 14 days to achieve daily doses of 30 mg diclofenac/kg. Adverse effects noted included reduction in food consumption accompanied by proportionate weight loss, increased WBC counts, primarily in absolute monocyte counts, and slight to moderate increases in BUN. Multiple adhesions between jejunal loops were observed in both females and were associated with multiple dark depressed areas (ulcerations) in the mucosa of the jejunum or the stomach. No other histopathology was observed.*

This study was followed up with a second dose range-finding study in Göttingen minipigs in which the 3% diclofenac gel was administered at 30 mg/kg/day for the first 4 weeks and at 45 mg/kg/day for the remaining 5 weeks of the study. Signs of dermal erythema were noted as early as week 3. Inflammation was observed in the stomach of all animals at week 4, with the presence of ulceration (stomach and colon associated with adhesion to the liver) in one female who had to be euthanized on day 52. Laboratory evaluation revealed the presence of increased NRBCs and a slight reduction in red cell parameters in females, and as in the previous study, there were slight increases in BUN.

In the definitive 6-month dermal toxicity study in Göttingen Minipigs, diclofenac gel was applied b.i.d to simulate clinical use and achieve diclofenac doses between 1.5 mg/kg (3.0 mg/kg/day) and 22.5 mg/kg (45 mg/kg/day). Two animals had to be euthanized during the study due to complications associated with ulcerative GI lesions. These animals exhibited signs of toxicity similar to those in the acute studies, e.g., anorexia, weakness, ataxia, tremors, labored breathing, sternal recumbency, evidence of emesis, and blue discoloration of the skin of the muzzle. Laboratory analysis revealed increased WBC count, anemia including increased NRBCs, slight elevations in PT, APTT and BUN, and slight reductions in ALT, alkaline phosphatase and chloride. All other animals appeared normal except for skin reactions at the site of application which were characterized by very slight to well-defined erythema and/or scab formation. Although there was no clear increase in severity with increasing dose, the onset of reactions appeared to occur earlier in high-dose animals (beginning week 3). Histopathologic evaluation of skin lesions revealed slight to moderate perivascular/periadnexal superficial dermatitis, acanthosis, and parakeratotic hyperkeratosis. Skin reactions resolved with cessation of dosing.

Systemic effects included gastro-intestinal erosion and ulcerations which were observed in the stomach at doses >3 mg/kg/day, in the jejunum at doses >10 mg/kg/day, and in the duodenum at doses >45 mg/kg/day. The incidence and severity of these lesions increased with increasing dose and were similar to those observed following oral ingestion of diclofenac. Microscopic examination of these lesions resulted in erosive gastritis, erosive duodenitis, and ulcerative jejunitis. In some of the mid- and high-dose animals, mild to moderate peritonitis was associated with the gastric and intestinal lesions. Erosive esophagitis was also observed in one high-dose animal. There also appeared to be a dose-related increase in the absolute neutrophil count, in surviving mid- and high-dose males and high-dose females. This data correlated well with the increased incidences of gastro-intestinal inflammation and may therefore be secondary to the inflammation. Red cell parameters were also slightly decreased in all animals, including controls, but appeared to be more depressed in the mid- and high-dose females. Other organs which appeared to be adversely affected at the mid- and high-doses included the liver, kidneys and testicles. Testicular atrophy has not been previously reported following diclofenac administration and in the absence of abnormal histology or reproductive effects, its significance in the minipig is unknown. A NOAEL dose was not identified.

Special Studies: It does not appear that studies were performed to assess the sensitization potential or the ocular irritation potential of the diclofenac/hyaluronate gel; or that such studies were recommended by FDA.

SECTION V: GENOTOXICITY AND CARCINOGENICITY POTENTIAL

Genotoxicity: In previous studies... "Diclofenac sodium did not show mutagenic activity *in vitro* in point mutation assays in mammalian (mouse Lymphoma) and microbial (yeast, Ames) test systems and was nonmutagenic in several mammalian *in vitro* and *in vivo* test, including dominant lethal and male germinal epithelial chromosomal studies in mice and nucleus anomaly and chromosomal aberration studies in Chinese hamsters." (Cataflam and Voltaren Tablets Monograph dated August 1, 1997, PDR 1998).

Study 5.1 - Morphological Transformation of BALB/3T3 Mouse Embryo Cells Transformation Assay. Study Report No. TC722.304, dated 4/27/93, in life 1993, conducted by ———————————————, in accordance with Good Laboratory Practices regulations (CFR 21:58).

Study Design: Diclofenac was tested in BALB/3T3 cell transformation assay in the absence and presence of Aroclor-induced rat liver S-9 reaction mixture. Preliminary tests were conducted with diclofenac concentrations between 0.3 and 300 µg/ml. The final assays were conducted with a 3 day exposure in the nonactivated test system at dose levels of 11, 23, 45 and 90 µg/ml, and a 4 hour exposure in the S-9 activated test system at dose levels of 38, 75, 150 and 300 µg/ml. The maximum feasible concentrations was limited by the solubility of diclofenac powder in water. N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) was used as a positive control for the non S-9 and dimethylnitrosamine (DMN) was the positive control for the S-9 study.

Summary of Study Results: Relative to the solvent control, the cell survival was approximately 106%, 102%, 106% and 54% at 11, 23, 45 and 90 µg/ml, respectively in the nonactivated test system and approximately 110%, 98%, 90% and 80% at 38, 75, 150 and 300 µg/ml, respectively in the S-9 activated study. No statistically significant increases in transformation frequency were observed in either test system. Diclofenac was concluded to be negative in the BALB/3T3 cell transformation assay under these test conditions.

Study 5.2 - Morphological Transformation of BALB/3T3 Mouse Embryo Cells Transformation Assay. Study Report No. TC723.304, dated 4/27/93, in life 1993, conducted by ———————————————, in accordance with Good Laboratory Practices regulations (CFR 21:58).

Study Design: This study was conducted as described above using diclofenac/hyaluronate gel. Preliminary assays were performed at concentrations between 2 and 2400 µg/ml and definitive assays at concentrations between 300 and 2400 µg/ml gel (individual component concentrations are listed in Table 5.2.1).

Summary of Study Results: Relative to the solvent control, the cell survival was approximately 50% and 100% at the highest dose concentrations for the nonactivated test system and S-9 activated study, respectively. No statistically significant increases in transformation frequency were observed in either test system compared to the negative solvent (water) control. Diclofenac was concluded to be negative in the BALB/3T3 cell transformation assay under these test conditions.

Table 5.2.1: Concentrations of diclofenac and hyaluronic acid in gel formulations used in the *in vitro* 'Morphological Transformation of BALB/3T3 Mouse Embryo Cells Transformation Assay'

Gel concentrations (µg/ml)	Diclofenac (µg/ml)	Hyaluronic Acid (µg/ml)
2	0.06	
8	0.24	
24	0.72	
80	2.4	
240	7.2	
300	9	
600	18	
800	24	
1200	36	
2400	72	

Conclusion: Diclofenac sodium either as a solution or a solution of diclofenac/hyaluronate gel was not mutagenic in the in vitro mouse embryo cell assay wither with or without S9 metabolic activation at concentrations up to the limits of solubility.

Carcinogenicity Potential: Long-term oral carcinogenicity studies in rats and mice were performed and submitted to support the safety of oral formulations of diclofenac (Voltaren Tablets). "Rats given diclofenac at doses up to 2 mg/kg/day (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/m2/day) female rats (high-dose females had excessive mortality), but the increase was not significant for this common rat tumor. A 2-year carcinogenicity study in mice employing diclofenac 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." (Cataflam and Voltaren Tablets Monograph dated August 1, 1997, PDR 1998).

Under IND — the carcinogenic potential of dermally applied diclofenac/hyaluronate gel was assessed in a standard 2-year mouse study and a 1-year photo –cocarcinogenicity study in hairless mice. Study designs and results are presented below.

Study 5.3 - A 2-Year Dermal Carcinogenicity Study of Diclofenac Gel in the Albino Mouse. Report No. — 87000, dated August 12, 1998. In life: 1/3/96-2/16/98. Conducted by ———— in compliance with Good Laboratory Practice regulations (21 CFR 58).

Background: The proposed clinical formulation is 3% diclofenac — sodium hyaluronate. 3% Diclofenac could not be used in the chronic animal studies due to systemic effects (i.e., gastrointestinal ulcerations) suffered by the animals following oral consumption of the gel. The high-dose was selected based on the maximum diclofenac which could be tolerated following oral ingestion. Dose selection was based on a 7-day dermal dose range-finding study (No. 87161) which used 0.035% diclofenac as the highest dose tested. No other supporting documentation for dose selected was submitted.

The protocol for this study was first submitted to the FDA on November 20, 1995. Protocol changes were recommended and the Sponsor was asked to resubmit the protocol prior to evaluation by the CAC. A final protocol was never submitted and therefore never submitted to the CAC for concurrence. Areas of concern included lack of justification for doses, dosing regimen, and species selections. Hyal initiated the 2-year study on/or about 1/3/96. On 2/19/96, the Sponsor submitted a protocol revision for their 2-year dermal carcinogenicity study in mice. In the first 13 days of treatment 25% of the males and 30% of the females treated with 0.18% diclofenac — HA died or were killed in extremis. At necropsy most animals were found to have gastric perforations. An unspecified number of animals had also died in the 0.09% group. As a result, the above two treatment groups were terminated. Treatment groups receiving 0.009%, 0.018% and 0.035% diclofenac — HA treatment groups were continued and Hyal proposed adding two new groups at 0.0023% and 0.0045% diclofenac — HA. A third — HA group was also added. Once more, the Sponsor was urged to submit results from all studies, either interim or complete that would support the species and dose selection for the 2-year carcinogenicity study. They were informed of the advantages of submitting carcinogenicity study protocols to the Executive CAC for concurrence, but were advised that complete information was necessary before taking a protocol before the committee. This information was never submitted.

Study Design: This study was conducted in order to determine the carcinogenic potential of diclofenac gel when administered for a life time by dermal application to Swiss Crl:CD^{R}-1(ICR)BR mice. Groups of 60 mice/sex were administered topical doses of diclofenac — sodium hyaluronate gel (0.2 ml) to the shaved interscapular region, 7 days/wk for up to 104 weeks (Table 5.3.1). Mice were received from ———— aged approximately 26-29 days, weighting between 24.9 - 31.2 g for males and 20.3 - 26.3 g for females. Diclofenac concentrations of 0.009, 0.018, 0.035, 0.09 and 0.18% were initially selected by the Sponsor. However, as deaths and gross pathological changes consistent with administration of oral diclofenac were observed as early as week 1 in animals receiving 0.09 and 0.18%, these groups were terminated prematurely. Animals receiving 0.18% gel were euthanized after approximately 2 weeks of treatment and animals receiving 0.09% after approximately 4 weeks of treatment. Two lower dosage groups, 0.0023 and 0.0045% diclofenac, were added to the study design to ensure a no-effect level. Since treatment of these animals (Subset II) was initiated 7 weeks initiation of the main study (Subgroup I), an additional control group consisting of animals from the same shipment and the same age was also added to the study design. Terminal sacrifice was initiated on 1/5/98 for the main group of animals and on 2/16/98 for the second group.

Animals were housed individually, received ———— Laboratory Rodent Chow — and tap water *ad libitum*, and were randomized to treatment groups one week prior to initiation of the study (total acclimation time = 14-16 days).

Observations: Ten animals from each shipment were subjected to a complete physical evaluation prior to initiation of the study (groups 8 and 12), all other animals were examined once during the week prior to treatment initiation for abnormal clinical signs and weekly following treatment initiation. All animals were examined twice daily for mortality and clinical signs of ill health or reaction to treatment. From week 26 onwards, all animals were examined weekly for palpable masses. The site, size and appearance of these masses were recorded when first detected and monitored weekly. Masses were identified by a numerical designation according to order of

Table 5.3.1 - Dosage Groups

Group No.	Treatment (mg/kg/day Diclofenac)*	Dose Volume	Main Study (60/sex/group)		Satellite ** (20/sex/group)	
			Males	Females	Males	Females
Subset 1						
1	Control I — HA)	0.2 ml/day	1001-1060	1501-1560	1061-1080	1561-1580
2	Control II — HA)		2001-2060	2501-2560	2061-2080	2561-2580
3	0.009% (0.5 mg/kg)		3001-3060	3501-3560	3061-3080	3561-3580
4	0.018% (1.0 mg/kg)		4001-4060	4501-4560	4061-4080	4561-4580
5	0.035% (2.0 mg/kg)		5001-5060	5501-5560	5061-5080	5561-5580
6	0.09% (5.0 mg/kg)		6001-6060	6501-6560		
7	0.18% (10.0 mg/kg)		7001-7060	7501-7560		
8	Health Screen	-	8001-8010	8501-8510		
Subset 2						
9	0.0023% (0.125 mg/kg)	0.2 ml/day	9001-9060	9501-9560		
10	0.0045% (0.25 mg/kg)		1101-1160	1601-1660		
11	Control III — HA)		1201-1260	1701-1760		
12	Health Screen	-	1301-1360	1801-1860		

* Based on 35 g/animal, when administered at a constant dose volume of 0.2 ml,

** 5/mice/sex/group sacrificed after pharmacokinetic bleed on day 1, weeks 13, 26 and 52.

appearance. Weight and food consumption were monitored weekly prior to and throughout the treatment period.

Funduscopic (indirect ophthalmoscopy) and biomicroscopic (slit lamp) examinations were performed on all animals during acclimation, after 53-54 weeks of treatment and at study termination. The mydriatic used was 0.5% atropine sulfate. Laboratory investigations consisted of hematologic analysis of red and white cells (total, absolute and differential) and were performed on the animals designated for pretreatment health screening and on all mice (except group 7) in the main study either euthanized during the course of the study or at terminal necropsy.

Toxicokinetic Assessments: PK analyses were performed in the satellite animals (5 animals/sex/data set) on blood collected 1 hour post-dosing on day 1 and again during weeks 13, 26 and 52, and on all animals in Group 7 and 10 animals/sex in Group 6 at the time of euthanasia. Plasma samples were stored at –20°C prior to shipment to and analysis by the ——— (Report dated 7/24/98, GLP compliant).

Terminal Procedures: Animals were killed by exsanguination from the abdominal aorta after isoflurane anesthesia. Following necropsy, the following tissues and organs were fixed in neutral buffered 10% formalin and processed to the block stage (except Groups 6 and 7):

Abnormal tissues	Eyes	Optic nerves	Spleen
Animal identification	Gallbladder	Ovaries	Stomach
Adrenals	Heart	Panceas	Testes
Aorta (thoracic)	Ileum	Pituitary	Thymus
Bone and marrow (sternum)	Jejunum	Prostate	Thyroid/parathyroids
Brain	Kidneys	Salivary gland	Tongue
Cecum	Liver	Sciatic nerve	Trachea
Colon	Lungs	Seminal vesicles	Urinary bladder
Duodenum	Lymph nodes (mandibular and mesenteric)	Skeletal muscle	Uterus
Epididymides	Mammary gland	Skin (treated)	
Esophagus		Skin (untreated)	
		Spinal cord (cervical)	

In addition, each clinically observed mass together with the nearest identifiable drainage lymph node were preserved and labeled according to its numerical designation.

Histopathological examinations were performed on the tissues listed above as follows:

1) All animals in control groups 1, 2 and 11 and all animals in the highest dose group showing an acceptable survival at termination.
2) All animals in intermediate and low dose groups which died or were sacrificed before study termination.
3) All gross findings in all groups, with the exception of Groups 6 and 7.

Sodium hyaluronate (HA) gel ——, a colorless gel, was used both as the control and vehicle for test article formulations. All materials were supplied and characterized by Hyal Pharmaceutical. Batch information is presented in Table 5.3.2.

Table 5.3.2 – Test Lots

Formulation	Batch No.	Date of Receipt	Expiration Date
Control – [redacted] HA	VHE 5 *	12/29/95	09/96
	VHE 5	01/26/96	09/96
	XAE 12	05/09/96	09/96
	XPB 354	09/12/96	11/96
	XPB 370	10/31/96	01/97
	XPB 383	01/10/97	04/97
	XPB 389	03/11/97	06/97
	XPB 399	05/29/97	09/97
	XPB 411	08/12/97	11/97
	XPB 427	10/30/97	02/98
	DT 83	01/22/98	01/98
0.0023% Diclofenac [redacted] HA	XPB 313	02/07/96	08/96
	XPB 330	05/09/96	04/97
	XPB 376	10/15/96	01/97
	XPB 384	01/30/97	05/97
	XPB 400	05/29/97	09/97
	XPB 422	09/25/97	02/98
0.0045% Diclofenac/ [redacted] HA	XPB 314	02/07/96	08/96
	XPB 331	05/09/96	04/97
	XPB 372	10/14/96	01/97
	XPB 385	01/30/97	05/97
	XPB 401	05/29/97	09/97
	XPB 423	09/25/97	02/98
0.009% Diclofenac/ [redacted] HA	XPB 303	12/29/95	07/96
	XPB 332	05/09/96	04/97
	XPB 357	09/12/96	11/96
	XPB 373	10/14/96	01/97
	XPB 386	01/30/97	05/97
	XPB 402	05/29/97	09/97
	XPB 424	09/25/97	02/98
0.018% Diclofenac/ [redacted] HA	XPB 304	12/29/95	07/96
	XPB 333	05/09/96	04/97
	XPB 360	09/12/96	11/96
	XPB 374	10/14/96	01/97
	XPB 387	01/30/97	05/97
	XPB 403	05/29/97	09/97
	XPB 425	09/25/97	02/98
0.035% Diclofenac/ [redacted] HA	XPB 305	12/29/95	07/96
	XPB 334	05/09/96	04/97
	XPB 361	09/12/96	11/96
	XPB 375	10/14/96	01/97
	XPB 388	01/30/97	05/97
	XPB 404	05/29/97	09/97
	XPB 426	09/25/97	02/98
0.09% Diclofenac [redacted] HA	XPB 306	12/29/95	7/96
0.18% Diclofenac [redacted] HA	XPB 307	12/29/95	7/96

* *Traces of diclofenac [redacted] ng/g, <0.0005%) were found in control batch VHE 5.*

Summary of Study Results:

Mortality (See Figures I-II): During the first 13 days of treatment, 18/60 males (30%) and 15/60 females (25%) from Group 7 (0.18% Diclofenac gel) were found dead or euthanized for humane reasons. At necropsy, the primary pathology consisted of perforation of the glandular portion of the stomach. All surviving animals were euthanized on January 16-18 and 23, 1996 (study days 14-21). During the first month of treatment 6% (4 animals/sex) were found dead in Group 6 (0.09% Diclofenac Gel). Perforation of the glandular portion of the stomach was seen at necropsy for all females and ¾ males. In view of the early onset of the deaths this dosage was not considered to be suitable for long term administration and the remainder of Group 6 animals were euthanized on January 19-31, 1996 (Study days (17-29). Surviving males in Subset I were terminated during week 100 due to increasing mortality rates (>50%), all other animals (females and Subset II males) were terminated on study week 104. The mortality rate in males was dose-dependent (statistically significant trend with $p<0.0001$) whereas in females, there were no significant differences in mean survival between control and treated groups (Table 5.3.3).

Following 100 weeks of treatment average survival 42% for males and 53% for females. At the completion of the treatment period (104weeks) the average survival was 35% for males in Subset II (Groups 9-11) and 45% in females.

Table 5.3.3: Mortality at week 100 (or week 104)

Group No.	Treatment (mg/kg/day Diclofenac)	Males		Females	
		Mortality	%	Mortality	%
1	Control I — HA)	22/60	37	31/60 (35/60)	52 (58)
2	Control II — HA)	30/60	50	27/60 (33/60)	45 (55)
11	Control III — HA)	28/60 (32/60)	47 (53)	26/60 (29/60)	43 (48)
9	0.0023% (0.125 mg/kg)	34/60 (41/60)	57 (68)	27/60 (34/60)	45 (57)
10	0.0045% (0.25 mg/kg)	30/60 (37/60)	50 (62)	28/60 (34/60)	47 (57)
3	0.009% (0.5 mg/kg)	38/60	63	26/60 (32/60)	43 (53)
4	0.018% (1.0 mg/kg)	31/60	52	25/60 (28/60)	42 (47)
5	0.035% (2.0 mg/kg)	44/60	73	30/60 (37/60)	50 (62)

Clinical Signs: Clinically observed masses and swellings which were present at necropsy were noted in a similar proportion of animals from treated and control groups (2 to 13 in males and 2 to 9 in females). Their onset and distribution showed no indication of dose-dependence.

Other clinical signs of note recorded during the treatment period consisted of abdominal distension, associated in some cases with blue discoloration of the skin and yellow fur staining particularly around the urogenital region. In general the degree of incidence was not dose-related. No other treatment-related clinical signs were noted.

Body Weight and Food Consumption: In general, there were no significant differences in body weight, weight gain or food consumption between control and treated animals at doses up to 0.035% diclofenac at any time during the study period.

Figure I – Mortality in Subset I Males

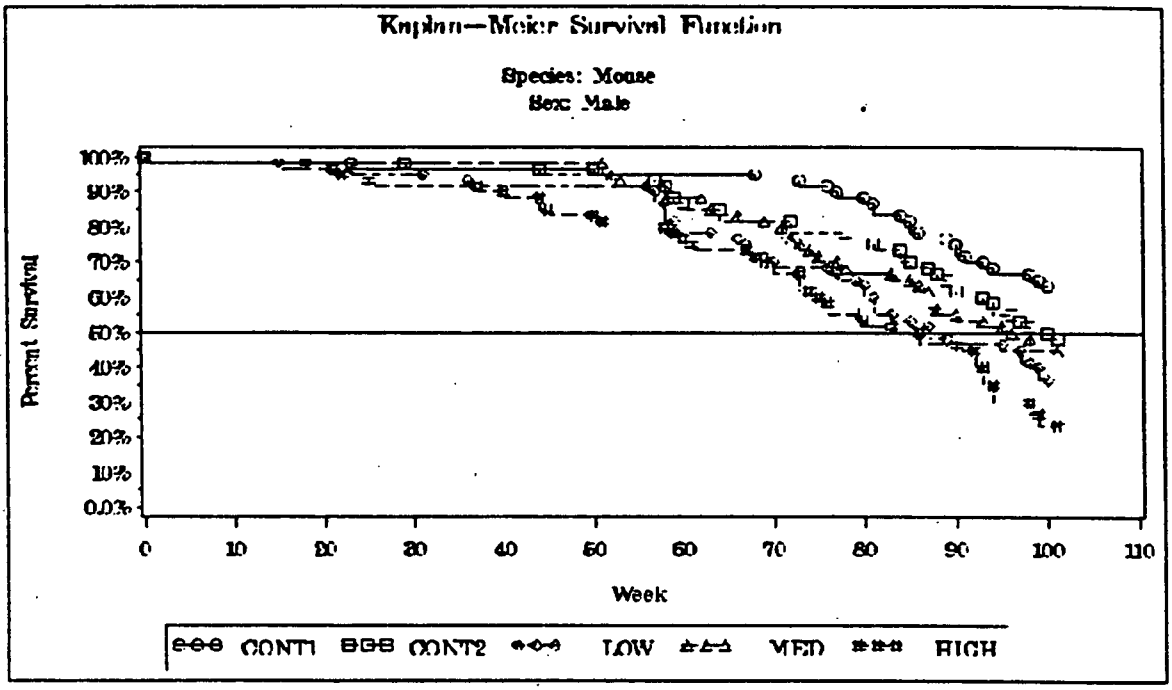
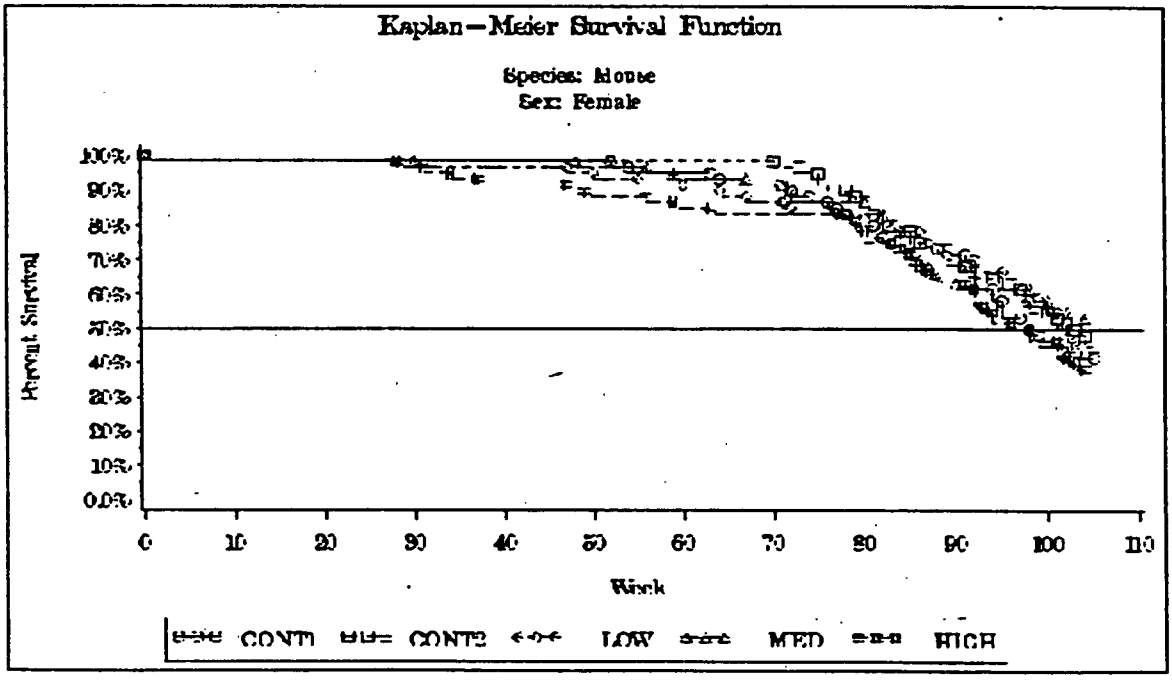


Figure II – Mortality in Subset I Females



Ophthalmoscopy: There was a high incidence of cortical cataracts at study end (considered age related), therefore the fundus could not be evaluated in most of the affected animals and the possibility of treatment-related retinal lesions could not be completely ruled out. There were no ocular changes noted during the first year of the study.

Toxicokinetic Analysis: Diclofenac plasma concentrations were measured using [redacted] analysis. Mean plasma concentrations increased with increasing dose at each time point. Consistent with PK studies performed with oral formulations, there was no apparent accumulation over time, with maximum plasma levels observed after 13 weeks of treatment. Interindividual variation led to fairly large ranges among each group. Mean plasma concentrations for Groups 1 through 5 are summarized in Table 5.3.5.

Table 5.3.5 – Mean diclofenac plasma concentrations following daily topical dosing.

Group No.	Treatment (mg/kg/day Diclofenac)	Mean Plasma Diclofenac (ng/ml)			
		Day 1	Week 13	Week 26	Week 52
Males					
1	Control I (— HA)	*	*	*	*
2	Control II (— HA)	*	1.3	1.1	*
3	0.09% (0.5 mg/kg)	109 ± 70	171 ± 140	22 ± 21	70 ± 18
4	0.018% (1.0 mg/kg)	199 ± 152	265 ± 91	47 ± 42	124 ± 39
5	0.035% (2.0 mg/kg)	636 ± 170	575 ± 181	78 ± 84	285 ± 44
Females					
1	Control I (— HA)	1.1	2.85 ± 2.47	*	*
2	Control II (— HA)	1.8	*	*	*
3	0.09% (0.5 mg/kg)	167 ± 56	201 ± 104	31 ± 33	38 ± 44
4	0.018% (1.0 mg/kg)	501 ± 559	802 ± 270	52 ± 48	116 ± 169
5	0.035% (2.0 mg/kg)	864 ± 446	1001 ± 552	300 ± 167	332 ± 670

* Below the limit of quantitation of — ng/ml

Gross Necropsy and Histopathology: No treatment-related findings were seen on treated skin samples. There was a slight increase in incidences of dermatitis in treated animals, especially preterminal males. However, wherever inflammation was observed at the treatment site, it was generally accompanied by a similar inflammation on other parts of the body.

Gross (macroscopic) examinations were performed on all animals. A complete list of the standard tissues and the numbers of animals/group examined histologically from Subset I can be found in Table 5.3.4. Tissues other than those listed in Table 5.3.4 in which gross lesions were observed and examined histologically are listed in Table 5.3.5. Other tissues in which gross lesions were noted in only 1-2 animals included the lacrymal gland, mesentery, penis, tail, vas deferens, meninges, pelvic cavity, pericardium, mediastinum, rib and urethra.

Due to the low incidence of both neoplastic and non-neoplastic lesions observed in the highest dose group (0.035%), a detailed review of Subset II will not be included in this review. With only a few rare exceptions, e.g., liver, prostate, ovaries and uterus, only tissues from all animals in the Subset I

controls and high-dose group were examined histologically. Therefore, no trend analyses could be performed on most of the tissues examined.

Non-neoplastic Findings: Other than gastrointestinal effects observed in preterminal Group 5 males, non-neoplastic lesions observed terminally were either degenerative or inflammatory and of the kind commonly reported for aging Swiss CD-1 mice. They were generally distributed in similar numbers in all groups and there was no evidence that they were associated with the administration of the test article. Non-neoplastic lesions which may have occurred more frequently in animals exposed to diclofenac are presented in Table 5.3.6. Comparisons for statistical purposes could only be made between the controls and animals exposed to 0.035% diclofenac get. However the number of occurrences observed in Subset I low- and mid-dose groups are also included for reference even though trend analysis could not be performed.

There was no significant increase in dermal reactions either at the site of treatment of at the preselected nontreated skin site. However, there were increases in the incidences of dermatitis observered at other skin locations (Table 5.4.7). There did not appear to be a pattern relative to these sites and there relevance to clinical use has not been determined.

For animals from Groups 6 and 7 terminated within the first month of treatment, notable findings recorded at necropsy were perforation of the glandular portion of the stomach (pyloric region) and/or thickening of the gastrointestinal tract, and masses in the liver (6/60 Group 7 males only). Microscopic evaluation of these masses revealed abscesses, some of which contained food particles and were centered around the hepatic capsule. Abscesses were considered to be most likely secondary to the gastric changes observed in these animals.

Inflammation, sometimes ulcerative and occasionally resulting in peritonitis, was noted in various parts of the gastrointestinal tract in a few animals, mainly from Group 5 (0.035%), that died or were euthanized early in the study. Euthanized and preterminal Group 5 males also presented with an increased incidence of erosions of the glandular stomach. These erosions were usually superficial and contained within the epithelial layer in dying animals. They were considered to be an agonal change and probably related to the higher incidence of mortality in this group. Increased myelopoiesis, mostly granulocytic, in the bone marrow of Group 5 of both sexes and increased hematopoiesis in the spleen or reactive changes in some lymph nodes were considered related to the increased incidence of various infections in this group.

Neoplastic Lesions: There was no evidence of drug-related skin or systemic tumorigenic effects. The sponsor's statistical analysis of the data concluded that there was a statistical significant trend (Tarone's test for trend with $p<0.05$) for benign mast cell tumor in the skin from the treatment site of a terminal Group 5 male, however since this was based on a single tumor, it was considered to be incidental and unrelated to treatment. Tarone's trend test also revealed an age adjusted dose-related linear trend for lymph node lymphosarcomas for preterminal males only (incidences were 1, 1, 1, 2, 3 for preterminal males and 2, 1, 1, 2 and 0 for terminal males in groups 1 through 5, respectively). The incidence of all other tumors was generally low and distributed similarly between groups. The histologic types were consistent for aging Swiss CD-1 mice.

Table 5.3.4: Tissues and the numbers of animals in each Subset I group examined histologically in the 2-year rat dermal diclofenac/hyaluronate gel carcinogenicity study.

	Males					Females				
Tissue	C-1	C-2	0.009	0.018	0.035	C-1	C-2	0.009	0.018	0.035
Adrenals	60	59	39	34	60	60	60	34	28	60
Aorta	60	60	37	33	60	60	60	32	28	60
Bone Marrow	60	59	38	33	60	60	60	32	28	60
Brain	60	60	38	32	60	60	60	32	28	60
Cecum	60	60	45	44	60	60	60	37	32	60
Colon	60	60	38	33	60	60	60	32	28	60
Duodenum	60	60	38	33	60	60	60	31	28	60
Epididymis	60	60	40	33	60					
Esophagus	60	60	38	33	60	60	60	32	28	60
Eye	60	60	49	51	60	60	50	50	46	60
Gall Bladder	60	56	38	34	56	58	60	31	29	59
Heart	60	60	43	43	60	60	60	35	32	60
Ileum	60	58	38	36	60	60	60	38	32	60
Jejunum	60	60	39	34	60	60	60	34	28	60
Kidneys	60	60	42	44	60	60	60	39	36	60
Liver	**60**	**60**	**52**	**52**	**60**	**60**	**60**	**50**	**54**	**60**
Lungs	60	60	46	42	60	59	60	40	37	60
Lymph Nodes – Mandibular and Mesenteric	55 60	57 60	38 47	30 48	54 60	57 59	58 60	36 46	31 43	58 60
Mammary Gland	59	59	36	33	59	60	60	32	28	60
Opitic Nerves	60	60	38	31	59	59	60	31	27	60
Ovaries						**60**	**60**	**53**	**53**	**60**
Pancreas	**60**	**60**	**59**	**55**	**60**	60	60	32	31	60
Parathyroid	40	41	22	19	27	32	38	22	16	38
Pituitary	60	60	38	33	60	58	60	35	30	60
Prostate	**59**	**59**	**56**	**55**	**60**					
Salivary Gland	60	60	38	33	60	59	60	32	28	60
Sciatic Nerve	60	60	37	33	60	60	60	32	27	60
Seminal Vesicles	59	60	40	37	60					
Skeletal Muscle	60	60	37	33	59	60	57	32	28	59
Skin, Treated	60	60	41	37	60	60	60	34	28	60
Skin, Untreated*	2	2	3	3	2	60	60	32	28	60
Spinal Cord	60	60	38	33	60	60	60	32	28	60
Spleen	60	60	42	38	60	60	60	50	36	60
Sternum	60	59	38	33	60	60	60	32	28	60
Stomach	60	60	41	38	60	60	60	41	31	60
Testis	60	60	42	40	60					
Thyroid	60	60	38	33	60	60	60	32	29	60
Thymus	60	58	41	36	60	59	60	41	33	59
Tongue	69	60	38	33	60	59	60	32	28	60
Trachea	9	8	8	14	6	59	59	31	28	60
Urinary Bladder	59	60	37	34	60	60	60	32	28	59
Uterus						**60**	**60**	**59**	**59**	**60**

Table 5.3.5: Tissues and the numbers of animals in each Subset I group with gross lesions examined histologically in the 2-year rat dermal diclofenac/hyaluronate gel carcinogenicity study.

	Males					Females				
Tissue	C-1	C-2	0.009	0.018	0.035	C-1	C-2	0.009	0.018	0.035
Abdominal Cavity	0	0	0	1	3	0	0	0	1	1
Blood vessels	2	0	0	0	1					
Bone - Miscellaneous	1	1	0	1	2	3	0	0	0	1
Bulbourethral Gland	3	2	3	9	5					
Fat	1	0	1	1	1	4	3	1	1	1
Harderian Gland	1	2	1	1	0	1	2	3	0	0
Lymph Nodes –Misc.*	17	23	22	25	32	27	26	27	29	34
Penis (Hemorrhage)	2	4	5	4	5					
Preputial Gland	4	2	2	3	3					
Rectum	0	0	0	0	1					
Skin - Miscellaneous**	8	11	2	18	16	6	6	6	11	12
Subcutaneous Tissues	3	10	7	9	11	11	14	14	10	13
Trachea	9	8	8	14	6					
Vagina						1	2	0	0	0

* Lymph nodes other than mandibular and mesenteric.

** Skin lesions observed at sites other than the treatment site and the lumbar site pre-selected for untreated skin sampling.

Table 5.3.6: Incidence of selected non-neoplastic lesions which may be related to life-time administration of diclofenac in rats.

	Males					Females				
Tissue	C-1	C-2	0.035	*0.018*	*0.009*	C-1	C-2	0.035	*0.018*	*0.009*
Bone Marrow – ↑ Myelopoiesis	19/60 32%	14/59 23%	36/60 60%	*17/33*	*15/38*	6/60 10%	9/60 15%	22/60 37%	*7/28*	*7/32*
Eye – Keratitis	4/60 6%	1/60 2%	9/60 15%	*9/51*	*6/49*					
Eye – Lenticular Degeneration	6/60 10%	8/60 13%	10/60 17%	*11/36*	*4/39*					
Kidney – Nephropathy *	11/60 18%	4/60 7%	18/60 30%	*10/36*	*7/39*	11/60 18%	4/60 7%	18/60 30%	*10/36*	*7/39*
Kidney – Mononuclear Infiltration						2/60 3%	1/60 2%	7/60 12%	*0/36*	*0/39*
Spleen – Capsulitis	0/60	0/60	4/60 7%	*3/38*	*0/42*					

* Nephropathy was also observed in the two low-dose groups in Subset II at the following incidence: Control – 9/60 (15%); 0.0023% - 13/60 (22%); and 0.0045% - 12/60 (20%).

Table 5.4.7: Incidence of gross signs of adverse dermal effects in animals treated over a life time with diclofenac/hyaluronate gel versus control animals exposed only to hyaluronate.

Group	Treated Skin		Untreated (Lumbar) Skin		Miscellaneous Skin Sites	
	Males	Females	Males	Females	Males	Females
Subset I:						
Control 1	3/60 (5%)	1/60 (2%)	2	0	8/60 (13%)	6/60 (10%)
Control 2	3/60 (5%)	3/60 (5%)	2	0	9/60 (15%)	6/60 (10%)
0.009%	5/60 (8%)	5/60 (8%)	3	0	22/60 (37%)	6/60 (10%)
0.018%	7/60 (12%)	2/60 (3%)	3	0	18/60 (30%)	11/60 (18%)
0.035%	4/60 (7%)	7/60 (12%)	2	0	16/60 (27%)	12/60 (20%)
Subset II:						
Control	1	8/60 (13%)	0	0	11/60 (18%)	4/60 (7%)
0.0023%	3	7/60 (12%)	0	0	19/60 (32%)	8/60 (13%)
0.0045%	1	5/60 (8%)	0	0	14/60 (23%)	10/60 (17%)

[*Review Note: The Sponsor should be asked to examine histologically all skin samples from all treated animals, including the 0.009 and 0.018% groups.*]

FDA Statistical Analysis: Steve Thomson, Biometrics IV, dependently performed analyses on the survival/tumorigenicity data. For survival data, the methods of Cox (1972) and Gehan (1965) were used. The tumor data were analyzed using the techniques described by Peto, et al (1980), with p-values computed from an exact permutation test or a pooling of approximate test when both fatal/observable and incidental tumors are found. (*See Pre-clinical Statistical Consult, filed 5/20/99.*) Age adjusted statistical evaluations revealed no statistically significant differences in tumor incidence in male mice due to dose. In female mice, a statistically significant trend in alveolar/bronchiolar adenomas ($p \leq 0.013$) was observed. However, when analyzing alveolar/bronchiolar adenomas and carcinomas, there was no increase in tumor incidence. Furthermore, since the lungs from all animals in the low- and mid-dose groups were not examined histologically, trend analysis was considered inappropriate for these tissues.

The incidence of all other tumors was generally low and distributed similarly between groups. The histologic types were consistent for aging Swiss CD-1 mice. These results are consistent with results from previously conducted 2-year systemic (oral) carcinogenicity studies in rats and mice.

Study Conclusion: Treatment of Swiss CD-1 mice with diclofenac/ — HA gel by dermal administration at concentrations up to 0.035% (2 mg/kg or 6 mg/m^2) over a life time, revealed no obvious treatment-related findings or evidence of carcinogenicity. However, there were several flaws in the performance of this study. First, according to the study protocols, histopathology should have been performed on all animals in the highest dose group showing acceptable survival at termination. Unfortunately, the Sponsor only performed histological evaluations on the high-dose group (#5), which did not in my estimation demonstrate acceptable survival (males = 25% compared to 50% in controls) at study end. Therefore all animals in the next highest dose group (#4 = 48% survival) should have also been examined histologically. Only part of the animals in this group were examined, therefore statistical analysis could not be performed for this group. A recommendation

that, at the minimum, all the skin (treated and untreated) samples from groups #3 and #4 should be examined microscopically. Second, I could not find where the untreated skin samples from male animals in groups 1 through 5 were examined histologically, except for a few samples with gross lesions. Histological examination of these samples should also be performed and a report filed which includes histological results of treated and untreated skin from all animals in groups 1 through 5 should be filed. Third, the day prior to beginning treatment (and after randomization of dosing groups), a number of animals in all the dosing groups were replaced. The Sponsor should submit a rational for this action. Fourth, an untreated control should have been included in this study to evaluate the oncogenic potential of sodium hyaluronate. However, combined with the evidence from oral carcinogenicity studies, the photo-cocarcinogenicity study, and any convincing evidence that diclofenac may be tumorigenic. This should not be considered a non-approvable issue.

The statistically significant dose-related mortality in male animals combined with a high, early incidence of death at 0.09%, suggesting that a higher dose could not have been tolerated in females, is indicative that a maximum tolerated dose was achieved.

Non-neoplastic lesions observed primarily in pre-terminal treated animals were consistent with a down-regulation of the immune system, i.e., increased myelopoiesis, extramedullary nematopoesis, lymph node congestion and hyperplasia, dermatitis, pulmonary histiocytosis, jejunitis, peritonitis, arthritis. Significant increases in non-neoplastic lesions observed in high-dose animals compared with controls included the following: increased myelopoiesis in the bone marrow (males and females), ocular keratitis and lenticular degeneration (males only), nephropathy (males and females), renal mononuclear infiltration (females only), and splenic capsulitis (males only).

Review Note: Review of this 2-year dermal carcinogenicity study in mice was presented to CDER's Executive Carcinogenicity Assessment Committee on June 15, 1999. The Exec CAC concurred with the following review findings:

- *There was no evidence of diclofenac-related skin or systemic tumorigenic effects at the highest concentration (0.035%) tested.*
- *The study was found to be adequate based on similar weight gain patterns between groups and acceptable survival up to approximately 88-90 weeks.*

Study 5.4 - A 12-Month Study to Determine the Influence of Diclofenac Topical Gel on Photocarcinogenesis in Hairless Mice. Study No. 808-002. *In life:* 3/4/96-3/7/97. Conducted by [redacted] in compliance with GLP regulations (21 CFR 58).

The protocol for this study was submitted to the FDA on March 15, 1996. It was presented to the CDER Carcinogenicity Assessment Committee (CAC) and approved in June, 1996. The final study report was submitted to IND [redacted] Amendment N057 dated February 2, 1998. The Review dated June 2, 1998 has been reproduced below.

Study Design: The purpose of this study was to determine the potential of Diclofenac Topical Gel to influence the development or growth of skin tumors in hairless Crl:SKH1(hr/hr)BR mice exposed

to 600 RBU/week simulated solar ultraviolet radiation (UVR). Mice were treated and irradiated once daily, 5 days/week for 52 weeks (except holidays)*. The light source was a 6.5 kilowatt xenon long arc solar simulator, vertically suspended within an octagonal metal frame holding one ——— optical filter on each side. During exposure, the cages were located approximately 2.25 meters from the UVR source and were monitored by a ——— detector. Study groups (36 mice/sex/group) consisted of 2 control groups which received radiation alone (600 and 1200 RBU/Week), 1 vehicle control group, and 4 groups treated with gel containing 0.0045, 0.009, 0.018 & 0.035% diclofenac to achieve doses of 0.36, 0.72 1.44 or 2.80 mg/kg, respectively (Table 5.4.1).

Table 5.4.1: Group designation and dose for hairless mice (36 mice/sex/group) in 12-month photococarcinogenicity study.

Group Designation	1	2	3	4	5	6	7
Treatment (% Diclofenac)	0.0 % Untreated Control	0.0 % Untreated Control	0.0 % Vehicle Control	0.0045 %	0.009 %	0.0018 %	0.035 %
UVR Dosage (RBU/week)	1200	600	600	600	600	600	600

Gel applications and UVR exposures were performed as follows: on Mondays, Wednesdays and Fridays animals were treated then exposed to UVR approximately 1 hour after treatment; on Tuesdays and Thursdays animals were first exposed to UVR then treated approximately 1 hour later. Mice were approximately 45 days of age at commencement of the study with male mice weighing 25-33 g and female mice weighing 19-26 g. All mice were randomized to dosing groups and allowed food and water *ad libitum*. Mice were monitored daily for viability and weekly for clinical signs of toxicity, skin inflammation, body weight, and tumor formation. Treatment groups were monitored for tumor prevalence, median time to tumor onset, and tumor yield. Gross necropsies, consisting of examinations of all gross lesions, and the thoracic, abdominal and pelvic viscera, were performed on all mice which died or were euthanized during the study period and surviving mice terminated at the end of the study. Animal carcasses were preserved in formalin for possible future examination.

**In the original protocol, mice were to be exposed for only 40 weeks. However, during the first 39 weeks of the study the tumor response (in terms of time-to-tumor) was later than anticipated in the control animals due to a greater variance of UVR intensities associated with new exposure cage rack design. This protocol amendment was approved by the Sponsor with concurrence from this reviewer.*

Test articles and vehicle were used as supplied by the Sponsor (Table 5.4.2). Stability data and chemical analysis were performed and maintained by the Sponsor and submitted in IND —— Amendment N059 dated 6/17/98.

Data Tabulation: Tumors were divided into the following groups for analysis: < 1 mm (i.e. barely perceptible to perceptible tumors); at least 1 mm but less than 2 mm; at least 2 mm but less than 4 mm; and at least 4 mm or greater in maximum planar diameter. Statistical interpretation was based primarily on tumors of at least 1 mm in diameter. Mortality-Free Tumor Prevalence is defined as the proportion of mice that would have at least one tumor by the end of the week in question, in the absence of mortality. Median Tumor Onset is defined as the time at which 50% of the members of the groups have acquired one or more tumors 1 mm in size. "Biased" Median Tumor Onset data are

based on survivors, and "unbiased" are based on mortality-adjusted medians. Tumor Yield data include the total number of tumors in surviving mice, the number of surviving mice, and the average number of tumors per surviving mouse for each week from the time of first tumor in any group.

Table 5.4.2: Diclofenac Gel/ — Sodium Hyaluronate (HA) Gel Lot Numbers and Analysis for 12-month photo-cocarcinogenicity study.

— HA GEL	Vehicle	VHE 5	XPB 354	XPB 378
Diclofenac Gel	0.0045 %	XPB 309	XPB 356	XPB 379
	0.009 %	XPB 310	XPB 357	XPB 380
	0.018 %	XPB 311	XPB 358	XPB 381
	0.035 %	XPB 312	XPB 359	XPB 382
Date Delivered		2/5/96	8/21/96	12/19/96

Statistical Analysis: Group comparisons of tumor prevalence were based on two tailed probabilities using the methods of Peto, et al., for detection of carcinogenic trend. Each group was compared to every other group for statistically significant differences in the median time to tumor onset. Body weight and body weight change data were analyzed using Bartlett's Test of Homogeneity of Variances or Analysis of Variance, when appropriate (negative Bartlett's test). Dunnett's Test (Analysis of Variance p 0.05), Kruskal-Wallis Test (Bartlett p 0.05; 75% ties), Fisher's Exact Test (Bartlett p 0.05; > 75% ties), or Dunn's Method of Multiple Comparisons (Kruskal-Wallis Test p 0.05) were used to identify the significance of individual groups. Statistical comparisons between treatment groups are based on the entire time-based distribution of tumor response for each group and each sex.

Summary of Study Results:

Mortality: Survival was > 90% for all groups through week 31 of the study and > 90% in groups 2-7 through weeks 46, 47, 51, 46, 52 and 46, respectively. After week 30, survival declined precipitously in Group 1 primarily due to early terminations as a result of tumor burden (i.e. tumors >10 mm in planar diameter or total number of tumors). All surviving mice in group 1 were sacrificed during study week 44 (Table 5.4.3).

Table 5.4.3: Mortality following exposure to diclofenac gel and UVR in the 12-month photo-cocarcinogenicity study in hairless mice.

Mortality	Sex	1	2	3	4	5	6	7
Number Euthanized Due to Tumor Burden	M	34	2	3	1	2	4	2
	F	32	2	3	2	3	1	4
Number Found Dead or Terminated Moribund	M	2	6	4	2	8	1	11
	F	4	3	6	2	6	3	2
Scheduled Terminations at End of Study	M	0	28	29	33	26	31	23
	F	0	31	27	32	27	32	30

Clinical Observations and Body Weight Gain: There were no significant or biologically relevant changes in the health, behavior, or weight gain of treated animals when compared to vehicle or untreated controls receiving 600 RBU/week UVR.

Dermal Irritation Scores: There were no significant increases in skin irritation scores in males dosed 0.018% gel or in female mice dosed at 0.035% gel mg/kg when compared to untreated controls receiving 600 RBU UVR (Group 2). Male mice treated with 0.035% diclofenac showed a significant increase (p 0.01) in the number of animals with multiple, raised erythemic sites. However, these occurrences were infrequent and self-limiting. Dermal irritation scores were significantly reduced in all animals in groups 2-7 when compared to untreated controls receiving 1200 RBU/week (group 1). Diclofenac did not appear to enhance UVR induced dermal irritation when applied either before or after exposure to UV radiation, however, the skin dose in this study is approximately 100 times lower than the proposed dose in humans.

Dermal Toxicity: In this study dermal reactions other than those characteristic of UVR-induced erythema were not detected following application of test material containing 0.018 % diclofenac. However, it must be once again stressed that the diclofenac concentrations used in this study were 100 times lower that the proposed clinical formulation of 3.0%. At 0.035% diclofenac, there was an increase in male animals with periodic, multiple, raised erythemic sites which appeared to be drug related. Adverse dermal effects previously observed in minipigs treated with Diclofenac 3% Gel (doses 3 to 30 mg/kg/day) for 6-months consisted of skin lesions at the site of application characterized by very slight to well-defined erythema and/or scab formation. Histopathologic evaluation of lesions revealed slight to moderate perivascular/periadnexal superficial dermatitis, acanthosis, and parakeratotic hyperkeratosis.

Systemic Toxicity: Dose range-finding studies in mice demonstrated that 0.035% or 2.8 mg/kg/day diclofenac was the maximum tolerated dose for long term application. Administration of this dose for 12 months in this study, did not produce any apparent drug-related systemic effects. Systemic effects previously observed included gastro-intestinal erosion and ulcerations (presumably from concurrent oral ingestion of topical doses). These effects were observed in the guinea pig study at topical dosages containing >100 fold higher concentration of diclofenac than the highest dosage used in this study.

Non-neoplastic Pathology: Pathological effects observed at necropsy were not dose-dependent, were infrequent, and were consistent with findings common in this strain of mice, and therefore were not considered related to treatment with diclofenac.

Neoplastic Pathology:

Tumor Prevalence (Table 5.4.4): All UVR exposed, untreated control animals developed tumors while greater than 96 % of all surviving, treated animals developed perceptible tumors by the end of the study. Tumors were detectable in 100% of untreated control animals exposed to 1200 RBU by week 43 of exposure. By week 40, when >50% of the untreated control animals exposed to 600 RBU had detectible tumors, <50% of animals dosed with 0.0045 to 0.018% diclofenac gel had detectible tumors while 75% of both male and female animals dosed with gel containing 0.035% diclofenac had perceptible tumors (see table below). By week 45 tumor prevalence was similar between untreated control animals exposed to 600 RBU and high dose animals. When tumor

prevalence was analyzed by tumor size distribution (Table 5.4.5), diclofenac did not appear to have any significant influence on tumor progression.

Tumor Yield (Table 5.4.6): At week 44, the average number of tumors in untreated control animals exposed to 1200 RBU/week (group 1) was 6.9 tumors/survivor, compared with 1.4 for untreated control animals exposed to 600 RBU/week (group 2). The median tumor yield/surviving animal were also increased in high dose animals compared to both untreated animals exposed to 600 RBU/week, vehicle controls and animals treated with gel containing 0.018% diclofenac.

Median Time to Tumor Onset (Table 5.4.7): The data demonstrated a significantly decreased median time to tumor onset for exposed/untreated control animals receiving 1200 RBU/week (group 1) when compared to all groups receiving 600 RBU/week.

When comparing treated animals with untreated control animals exposed to 600 RBU/week, there were no dose-related, statistically significant differences in the median time to tumor onset for tumors >1 mm in either sex, for combined sexes, or when adjusted for mortality (unbiased).

When comparing diclofenac treated groups with the vehicle control group, a statistically significant decrease in median time to tumor onset was seen in animals receiving the 0.035% diclofenac gel only after combining the sexes. However, although the mean time to tumor was similar between untreated and vehicle controls, statistical significance was not found when comparing untreated and diclofenac treated animals. In addition, when tumors were grouped and analyzed by size, statistical significance was only observed for the two smaller tumor size categories (< 2 mm). Trend analysis supported an effect of diclofenac in males dosed with 2.8 mg/kg when compared to both vehicle and untreated controls.

Discussion: Topical application of gels containing 0.018% diclofenac did not appear to have any influence on UVR-induced skin tumor development in hairless mice. Administration of 0.035% diclofenac gel resulted in a slight increase in the mean tumor burden/animal and a reduction in the median time to tumor onset for UVR-induced skin tumors (sizes < 2 mm) when compared to the vehicle control, but not when compared to untreated, UVR exposed controls.

Although not statistically significant due to high variation in the time of tumor onset in individual animals, there does appear to be a dose-related trend toward earlier appearance of UVR-induced tumors in male mice dosed with diclofenac. The mean unbiased time to tumor onset in males treated with the 0.035% diclofenac gel was four (4) weeks earlier than was observed in either the untreated or vehicle control animals exposed to 600 RBU UVR. Peto trend analysis based on the number of animals with a first tumor by week 40 (historically the end of the exposure period), demonstrated statistical significance ($p<0.05$) for males treated with the 0.035% gel for first perceptible tumor and for first 1 mm tumor when compared to both untreated and vehicle controls (groups 2 and 3, respectively).

In females, the median unbiased time to tumor onset occurred at 40 and 43 weeks for untreated and vehicle control animals and at 40.5 weeks in the high-dose group. A statistically positive trend for early onset was only observed in females for tumors 1 mm. When median tumor onset in female groups was compared at week 40, a statistically positive trend was also observed in females for first 1 mm tumors, but only when compared to vehicle controls.

Table 5.4.4: Tumor prevalence in hairless mice treated with diclofenac gel in a 12-month Photo-cocarcinogenicity study.

Tumor Prevalence (combined sexes)	1 1200 RBU	2 600 RBU	3 Vehicle	4 0.0045%	5 0.009%	6 0.018%	7 0.035%
Week 40*	98.6 %	56.8 %	46.7 %	47.2 %	45.1 %	42.0 %	75.0%
Week 45	100 %	84.6 %	76.9 %	81.9 %	71.0 %	78.2 %	85.9 %
Week 52	100 %	100 %	98.5 %	98.6 %	94.9 %	98.5 %	98.2 %

[Note: Group 7 prevalence rates at week 40 were 75.0% for both males and females. Rates in other groups at both week 40 and week 45 were also similar for males and females.]

Table 5.4.5: Tumor prevalence analysis by size distribution in hairless mice treated with diclofenac gel in a 12-month Photo-cocarcinogenicity study.

Tumor Prevalence Week 52	1 1200 RBU	2 600 RBU	3 Vehicle	4 0.0045%	5 0.009%	6 0.018%	7 0.035%
Tumors 1 mm	100 %	100 %	94.8 %	97.0 %	91.3 %	95.7 %	97.4 %
Tumors 2 mm	98.6 %	93.5 %	82.0 %	80.6 %	73.1 %	76.8 %	83.6 %
Tumors 4 mm	95.6 %	52.9 %	55.9 %	46.9 %	39.1 %	49.3 %	53.2 %

Table 5.4.6: Tumor yield for tumors ≥1 mm in size in hairless mice treated with diclofenac gel and UVR for 45 weeks in a 12-month Photo-cocarcinogenicity study.

Tumor Yield Week 45 (Tumors ≥1 mm)	1 1200 RBU	2 600 RBU	3 Vehicle	4 0.0045%	5 0.009%	6 0.018%	7 0.035%
Median Tumors/Survivor	6.9*	1.7	1.6	1.9	1.2	1.9	2.7

*Tumor yield/animal at the time of sacrifice during study week 44.

Table 5.4.7: Median time to tumor onset in hairless mice treated with diclofenac gel and UVR in a 12-month Photo-cocarcinogenicity study.

Median Time to Onset For Tumors ≥1 mm		1 1200 RBU	2 600 RBU	3 Vehicle	4 0.0045%	5 0.009%	6 0.018%	7 0.035%
Males (Median Week)	Biased	26.0	44.0	42.5	42.0	41.0	42.0	39.0
	Unbiased	26.3	44.0	44.0	43.5	43.0	43.0	40.0
Females (Median Week)	Biased	26.0	39.5	41.0	41.0	43.0	43.0	40.0
	Unbiased	26.0	40.0	43.0	41.0	44.0	43.5	40.5
Combined Sexes (Median Week)	Biased	26.0	42.0	42.0	42.0	43.0	43.0	40.0
	Unbiased	26.0	42.0	43.0	42.0	43.5	43.3	40.0

The Sponsor considers the reduced time to tumor and slight increase in the mean tumor burden/animals dosed with 0.035% diclofenac gel to be a marginal effect and has submitted the following points for consideration in support of this conclusion:

- the apparent increase in tumor development was not statistically significant in males or females alone;
- the apparent increase only occurred for the two smaller tumor size categories (< 1 mm and 1 mm to < 2 mm);
- statistical significance was only achieved in comparisons between high-dose animals (combined sexes) and vehicle controls, but not when compared to UVR exposed, untreated controls;
- although the median time to tumor onset was 4 weeks earlier in high-dose males when compared to controls, a 4 week difference was also observed between control (group 2) males and females
- Although of limited value, statistical significance was also demonstrated in all the analysis described above when the sexes were combined. The Sponsor has asked us to take into consideration that the 4 week difference in median time to tumor onset is a result of individual animal variation and uses the 4 week difference between male and female animals to support this point. However, this difference between male and female mice has been noted in other studies and forms the basis for analyzing the sexes separately.

The proposed clinical formulation contains 3.0% diclofenac and the daily dosage is estimated to be approximately 0.2 ml/application. Topical formulations in this study contained only 0.0045%, 0.009, 0.018 and 0.035 % diclofenac; the highest (0.035%) dose being only slightly greater than 1/100 of the proposed clinical product, and the apparent NOAEL (no observable adverse effect level) was set at 0.018% diclofenac, which is 100 times lower than the proposed clinical product. In view of the fact that there does appear to be a dose-related trend toward earlier appearance of UVR-induced tumors in mice dosed with diclofenac, and in view of the fact that this occurs at doses almost 100 fold lower than the proposed clinical dose, and in the absence of any data at the clinical dose, it would be prudent to avoid sun exposure with this product. It should also be pointed out that while the concentration of diclofenac was approximately 100 to 600 times lower, the concentration of hyaluronic acid in the vehicle, —— was the same as that proposed for the clinical product, effectively ruling out influences from increased concentrations of this excipient.

Summary of Genotoxicity and Carcinogenicity Results

Genotoxicity Potential: *Diclofenac sodium did not show mutagenic activity in vitro in point mutation assays in mammalian (mouse Lymphoma) and microbial (yeast, Ames) test systems and was nonmutagenic in several mammalian in vitro and in vivo test, including cell transformation assays in BALB/3T3 mouse embryo cells, dominant lethal and male germinal epithelial chromosomal studies in mice, and nucleus anomaly and chromosomal aberration studies in Chinese hamsters.*

Carcinogenicity Potential: *Two year carcinogenicity studies in rats given diclofenac up to 2 mg/kg/day (12 mg/m^2/day), the approximately the human oral dose, have revealed no significant*

increases in tumor incidence. A 2-year carcinogenicity study in mice 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. Treatment of Swiss CD-1 mice with diclofenac — hyaluronate gel by topical administration at concentrations up to 0.035% (2 mg/kg or 6 mg/m^2) over a life time, revealed no treatment-related findings or evidence of oncogenicity.

Photo-Cocarcinogenicity Potential: *Topically applied diclofenac, 0.035%, does appear to have some influence on UVR-induced carcinogenicity by decreasing the time of onset for UVR-induced tumors in male hairless mice at concentrations approximately 100 times lower than the proposed clinical formulation of 3%. Labeling for this product should reflect the possibility for an increased risk for UVR-induced tumors and advise patients to avoid excessive exposure to sunlight during treatment with this product on sun exposed areas.*

SECTION VI: Reproduction and Developmental Toxicity

Reproduction and Developmental Toxicity Assessment: Reproduction and developmental toxicity studies were conducted in, rats, and rabbits (in support of the registration of VOLTAREN® (10 mg/kg/day oral diclofenac (~370 mg/m^2/day). Maximum doses tested were 20 mg/kg/day (60 mg/m^2/day) in mice, 10 mg/kg/day (60 mg/m^2/day) in rats and 80 mg/m^2/day in rabbits. Diclofenac has been shown to cross the placental barrier in rodents. In rats, maternally toxic doses were associated with dystocia, prolonged gestation, and reduced fetal weights, growth, and survival. Oral products containing diclofenac are currently labeled as Pregnancy Category B drugs. No new reproductive or developmental studies were recommended or submitted for evaluation for this NDA.

Current Labeling (Cataflam and Voltaren Tablets Monograph dated August 1, 1997, PDR 1998): "In male and female rats, 4 mg/kg/day (24 mg/m^2/day) did not affect fertility. Reproductive studies have been performed in mice (up to 20 mg/m^2/day or 60 mg/m^2/day) and in rats and rabbits (up to 10 mg/kg/day or mg/m^2/day for rats or 80 m^2/day for rabbits) have revealed no evidence of teratogenicity despite induction of maternal toxicity and fetal toxicity. In rats, maternal toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival. Diclofenac has been shown to cross the placental barrier in mice and rats. It is listed as pregnancy category B.

The effects of diclofenac on labor and delivery in pregnant women are unknown. Because of the known effects of prostaglandin-inhibiting drugs on the fetal cardiovascular system (closure of the ductus arteriosus), use of diclofenac during late pregnancy should be avoided and, as with other nonsteroidal anti-inflammatory drugs, it is possible that diclofenac may inhibit uterine contraction."

OVERALL SUMMARY AND DISCUSSION

Diclofenac is a non-steroidal anti-inflammatory drug (NSAID) advocated for oral use in treating painful and inflammatory rheumatic and certain non-rheumatic conditions. Diclofenac has demonstrated anti-inflammatory, analgesic, and antipyretic activity. Its exact mode of action is unknown, however, it has been hypothesized to be related to its ability to inhibit cyclo-oxygenase activity, inhibit prostaglandin (PGE_2) synthesis, and/or to modulate arachidonic acid uptake and release. It also affects polymorphonuclear leukocyte function, thereby reducing chemotaxis, superoxide production and protease production. Diclofenac is currently marketed as Voltaren® in delayed release tablets containing 25, 50 and 75 mg diclofenac. Diclofenac is also currently available in a number of other administration forms which can be given orally, rectally and intramuscularly.

Following topically administration of an average daily dose of 2 g of the 3% Diclofenac/ —— hyaluronate Gel (contains 30 mg diclofenac/g of gel), the average 60 kg human would be exposed to approximately 1.0 mg/kg or 37 mg/m^2 diclofenac/day. The AUC, C_{max} and T_{max} in humans following a single dose of Voltaren XR tablets containing 100 mg diclofenac were 1079 ng.hr/ml, 417 ng/ml, and 5.25 hr, respectively. Voltaren XR is approved for chronic administration of daily doses up to 225 mg/day or approximately 140 mg/m^2. When administered orally, diclofenac is 100% absorbed from the GI tract, however only 50% of each dose is systemically available in humans due to first pass metabolism. When systemic exposures were compared in a 2-way crossover study in healthy volunteers between 3% diclofenac/ —— HA topical gel (2 g administered t.i.d. for 6 days) and 250 mg tablets (administered once daily for 6 days), bioavailability as measured by AUC was about 175 times higher with the tablet (~1600 ng.hr/ml vs 9.1 ng.hr/ml for the tablet and gel respectively) and C_{max} was approximately 70 times higher (316 vs 4.5 ng/ml, respectively).

Adverse effects observed in humans include gastro-intestinal, hepatic and kidney toxicity. Similar effects were observed in minipigs following topical application of 3% diclofenac gel (3 to 45 mg/kg/day or 105 to 1575 mg/m^2/day diclofenac) for 6 months. While the lowest dose in this study approximates the exposure expected in humans, the highest dose is 10 fold higher than the typical dose expected for an average adult. AUC values reached in high-dose animals were 1851, 17,808 and 24,594 ng.hr/ml following 1, 85 and 176 days of administration. Since it is almost impossible to limit oral ingestion during chronic topical studies in animals, bioavailability in animals is a combination of absorbed material from the dermis and GI tract. Therefore, although dermal absorption is low (estimated at 1-3% of the applied dose), systemic bioavailability in these animals was quite high, approximating that of humans following a single administration of the maximum daily oral dose. In humans, systemic exposures as measured by plasma concentrations, were extremely low (<100 fold of levels measured following oral administration).

The primary adverse effect observed in animals was GI tract ulcerations accompanied by secondary anemia and extramedullary hematopoieses resulting from GI bleeding. This is an expected side effect of cyclooxygenase inhibitors and correlated with both dose and duration of treatment. Also observed following administration of high doses or prolonged administration were adverse effects on the liver and kidney, resulting in elevations of BUN, ALT and alkaline phosphatase. Gastrointestinal effects and transient depression of renal function have been noted to occur frequently following administration of NSAIDs. No novel toxic effects, with the exception of possible adverse effects on

the eyes (1 keratitis and lenticular degeneration in mice) were observed in animals following chronic topical applications of diclofenac.

Diclofenac related skin reactions at the site of chronic applications in minipigs were characterized by very slight to well-defined erythema and/or scab formation. Although there was no clear increase in severity with increasing dose, the onset of reactions appeared to occur earlier in high-dose animals (beginning as early as week 3). Histopathologic evaluation of skin lesions revealed slight to moderate perivascular/periadnexal superficial dermatitis, acanthosis, and parakeratotic hyperkeratosis. Skin reactions resolved with cessation of dosing.

Previous studies have not demonstrated any evidence of mutagenicity or genotoxicity in multiple *in vitro* and *in vivo* transformation assays: point mutation assays in mammalian (mouse lymphoma) and microbial (yeast, Ames) test systems, dominant lethal and male germinal epithelial chromosomal studies in mice, nucleus anomaly and chromosomal aberration studies in Chinese hamsters, and morphological transformation assays in BALB/3T3 mouse embryo cells. Previous 2-year carcinogenicity studies in mice administered oral 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 and in rats administered oral doses of diclofenac up to 2 mg/kg/day (12 mg/m^2/day, a dose designed to approximate the average oral daily dose in humans) did not reveal any oncogenic potential. The 2-year dermal carcinogenicity study in albino mice administered formulations containing up to 0.035% diclofenac — hyaluronate (2.0 mg/kg/day or 6 mg/m^2/day diclofenac) revealed no identifiable increases in tumor incidence. In the 1-year photo-cocarcinogenicity assay in hairless mice, topical application of — hyaluronate gels containing 0.018% diclofenac did not appear to have any influence on UVR-induced skin tumor development. However, administration of 0.035% diclofenac resulted in a slight increase in the mean tumor burden/animal and a reduction in the median time to tumor onset for UVR-induced skin tumors (sizes < 2 mm) when compared to the vehicle control, but not when compared to untreated UVR exposed controls.

Reproductive studies have been performed previously using oral doses of diclofenac in mice up to 20 mg/m^2/day (60 mg/m^2/day), in rats up to 10 mg/kg/day (mg/m^2/day), and in rabbits at 80 m^2/day to support the oral formulations. Diclofenac has been shown to cross the placental barrier in mice and rats, however, these studies revealed no evidence of teratogenicity despite induction of maternal toxicity and fetal toxicity. In rats, maternal toxic doses were associated with dystocia, prolonged gestation, reduced fetal weights and growth, and reduced fetal survival. In fertility studies in male and female rats, doses up to 4 mg/kg/day (24 mg/m^2/day) had no affect on reproductive ability. Oral formulations for diclofenac are currently classified under pregnancy category B.

Unresolved Safety Issues:

1) ***Hyaluronate Sodium:*** To date, the source of the hyaluronate used in all pivotal nonclinical studies appears to be from ——— and it is unclear what the source is for the many of the clinical studies including the dermal sensitization and irritation studies. At some point in the development of the 3% diclofenac — hyaluronate gel, the source of the hyaluronate was changed from ——— to ——— While the specifications for hyaluronate itself appear to be reasonably similar, safety information regarding and impurities and — contaminants from the — hyaluronate product has not been submitted. Also, due to the presence of ——— there are concerns that this product may

be antigenic. Therefore, the following informational request was sent to the Sponsor on June 22, 1999:

(Please) provide material to assure the safety of the [redacted] hyaluronic acid (HA) and any potentially hazardous impurities which may result from the [redacted] process, such as [redacted] If data is not currently available to assure the safety of the [redacted] HA, a nonclinical toxicology study should be performed to bridge to the nonclinical studies performed with the HA [redacted] If the [redacted] HA was not used in formulations used for clinical sensitization studies, then the sensitization potential of the [redacted] HA should be evaluated nonclincially in an assay such as the Local Lymph Node Assay (LLNA). If further testing is necessary, we recommend submission of study protocols for review concurrence prior to initiation of studies.

2) ***Solarase Degradation Products or Any Other Issues Arising from the CMC Review:*** Any findings arising from the CMC review which may impact safety will be need to be addressed by the Sponsor.

Any information received after completion of this review will be addressed in an addendum.

From a Pharm/Tox perspective, until this information is received and reviewed, there is insufficient information to evaluate the safety of this product.

Labeling:

Labeling appears to be truthful and adequate with the following suggested changes:

WITHHOLD 1 PAGE (S)

Draft

Labeling

CONCLUSION

Until Hyal can provide information on the safety of the ———— hyaluronan proposed for use in the to-be-marketed Solarase 3% Gel, there is insufficient information to evaluate the safety of this product.

APPEARS THIS WAY
ON ORIGINAL

Lynnda Reid, Ph.D. Date
Pharmacologist/Toxicologist

cc:
IND ———
HFD-540
HFD-540/Pharm/Reid
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