possibly associated with intestinal pathology but no other consistent changes. There was no effect on organ weights. Analysis of results from histopathology examinations identified only one significant positive trend, for thymic lymphomas in females. The incidence of primary malignant lymphoma was, however, not affected. Otherwise pathology examination confirmed chronic ulceration, especially of the small intestine, in males and females treated at 1 or 2 mg/kg/day. These changes were associated with increased incidences of amyloidosis and extramedullary hematopoiesis.

Based on frequent dietary analysis, dose results were between 103 and 110% of nominal except in high dose females, which received 127% of the nominal 2 mg/kg/day dose. Plasma diclofenac levels of 12-45 ng/mL in mice at the NOAEL (0.3 mg/kg/day) and 10-48 ng/mL in rats at the lowest dosage (0.25 mg/kg/day) were obtained. For comparison, the clinical studies of Voltaren Gel resulted in mean C_{max} concentrations of 15 and 54 ng/mL after 7 days of 4 times daily application of 160 mg diclofenac/day to 1 knee or 480 mg diclofenac/day to 2 knees and 2 hands, respectively.

Dermal Carcinogencity and Photococarcinogenicity

The dermal carcinogenic and photococarcinogenic potential of diclofenac were evaluated for approval of Solaraze, NDA 21-005, a topical diclofenac product approved in 2000, and referenced for this 505(b)(2) NDA. The topical application of Solaraze diclofenac gel did not result in an increased incidence of tumors. In the photococarcinogenicity study, exposure to UV radiation at weekly intervals also did not induce a significant increase in skin tumors although there was an earlier median time of onset of tumors in the UV-irradiated group. Refer to NDA 21-005 for additional information.

There was no evidence of diclofenac-related skin or systemic tumorigenic effects in the dermal carcinogenicity in mice. The diclofenac doses in this study were 0.035 % (2 mg/kg), 0.09% (5 mg/kg), and 0.18% (10 mg/kg). Groups administered 0.09% (5 mg/kg) and 0.18% (10 mg/kg) diclofenac gel exhibited mortality and gross pathological changes within the first month of treatment. The primary pathology consisted of perforation of the glandular portion of the stomach.

The photococarcinogenicity study results revealed an earlier median onset of tumors with UV radiation. In this study, groups of hairless mice were irradiated with simulated solar radiation, one at 600 RBU/week and the other at 1200 RBU/week. Five other groups of mice were treated with diclofenac gel at 0.0045% (0.36 mg/kg), 0.009% (0.72 mg/kg), 0.018% (1.4 mg/kg) or 0.035% (2.8 mg/kg) and irradiated at 600 RBU/week. The group treated with 1200 RBU exhibited high mortality and increase in tumors after week 30. No significant differences in mortalities were observed between the other groups. By week 40, with exposure to 600 RBU, 75% of animals dosed with 0.035% diclofenac gel had detectable tumors, while >50% of untreated control animals, < 50% of animals dosed with vehicle, 0.0045%, 0.009% and 0.018% diclofenac gel had detectable tumors. By week 45 tumor prevalence was similar between the untreated and high dose groups. A statistically significant decrease in median time to tumor onset was observed in animals receiving 0.035% diclofenac compared to the vehicle control group when both sexes were pooled. The reviewer concurs with the Sponsor's presentation and interpretation of the findings from the Solaraze studies.

REPRODUCTIVE AND DEVELOPMENTAL TOXICITY

These studies were submitted and reviewed in NDA 19-201. They were performed prior to the development of ICH guidances and GLP guidelines. There were no topically administered diclofenac reproduction studies. Since the current label for Voltaren lacks nonclinical information for the sections of pregnancy (section 8) and fertility (section 13) the relevant reproduction studies are briefly reviewed here to facilitate labeling.

Fertility

In fertility studies in rats found no effect of orally administered diclofenac on fertility of males or females at doses up to 4 mg/kg/day administered during premating, mating, gestation, lactation periods.

The sensitivity of the rats to the ulcerogenic effects of diclofenac sodium lead to increased maternal mortality at doses of 4 mg/kg/day or greater in the fertility study. This contrasts with the lack of any deaths among rats treated at 4 mg/kg/day in the oral gavage, repeat dose toxicity studies of up to 26 weeks duration. Reproductive effects at 2 and 4 mg/kg/day included increased fetal losses, reduced live births and reduced fetal weight. There was little evidence of significant maternal toxicity at 2 mg/kg/day in the fertility study. The fetal changes at 2 mg/kg/day are therefore considered to be associated with maternal toxicity. The lowest dosage investigated in the fetility studies was 2 mg/kg/day and therefore a NOAEL was not established for maternal toxicity.

Embryofetal Development

Oral treatment at up to 4 mg/kg/day in segment II studies in mice, rats and rabbits was without any consistent effect on fetal survival or development. At higher dosages (10 mg/kg/day) effects included reduced survival or viability and reduced ossification attributed to toxicity. There was no indication of any effect on organogenesis.

In an oral rabbit study, 5 mg/kg/day was a clear NOAEL for embryotoxicity; changes at 10 mg/kg/day included increased embryonic and fetal resorptions and reduced fetal ossification in three fetuses.

An intramuscular rat study indicated no changes in the fetuses at 10 mg/kg/day (NOAEL), despite maternal sedation (**Reviewer note:** perhaps a response to gastrointestinal adverse effects) and local injection site responses. An intramuscular rabbit study identified 3 mg/kg/day as the NOAEL with increased abortions and dead fetuses, reduced number of fully formed fetuses and reduced ossification, and reduced fetal viability at higher dosages, associated with maternal toxicity.

In a GLP compliant subcutaneous rat study, however, minimally reduced ossification was identified at 1.2 mg/kg/day; 0.4 mg/kg/day was the NOAEL dose.

The three studies just described, rabbit oral dosing, rat intramuscular and the rat subcutaneous dosing studies all had fetuses with reduced ossification. This could likely be a result of maternal toxicity as well as a pharmacodynamic effect of diclofenac. The Sponsor did not further investigate this phenomena, but it seemed to appear at the higher doses, which does not aid in ruling out one possibility or the other.

Postnatal development

In the pre-/postnatal development rat study, treatment with diclofenac sodium was associated with an increased duration of gestation and dystocia with consequent effects on perinatal survival. Post natal survival was not affected. In these studies, the sensitivity of the rats to the ulcerogenic effects of diclofenac sodium lead to increased maternal mortality at doses of at 2 mg/kg/day doses in the peri-/ post-natal study. The increased mortality associated with peritonitis in the postnatal study clearly indicates that 2 mg/kg/day was a maternally toxic dosage when given peri-natally. The fetal changes at 2 mg/kg/day are therefore considered to be associated with maternal toxicity. The lowest dosage investigated postnatal development studies was 2 mg/kg/day and therefore a NOAEL was not established for either fetal or maternal toxicity.

LOCAL TOLERANCE

Dermal Irritation

The results from the primary and 28-day repeated skin irritation studies performed in rabbits for the current NDA submission indicated that Voltaren Gel 1% is not a skin irritant. However, studies of cutaneous hypersensitivity and photosensitivity that involve similar initial procedures find that erythema does occur, although only of slight intensity. It does seem to depend on the type of drug or vehicle applied (which is not obvious due to conflicting names and terminology between most of the studies), and the amount applied.

Repeated Dose Dermal Toxicology

In one of the few studies submitted to IND 64,335 (SN-000), the local skin tolerance and the systemic toxicity of diclofenac sodium topical gel 1% (referred to as NCH927806 1% in the study) was investigated in rabbits by semi-occlusive dermal application for a period of 28 days. Each animal received a dosage of 0.5 ml per day on an administration surface of about 1.2 x 1.2 cm^2 for a period of 4 hours. Very slight erythema was seen at the 4 hour reading after 4 days which temporarily increased to a score 2 (well defined erythema after 8-12 days on 2 of the 6 animals. At the end of the 4-week period almost no erythema was observed. No edema was seen and no irritation at each of the 24 hour readings. Histopathological examination of the treated skin areas showed minor signs of inflammation, randomly distributed among control and treated skin samples. The treatment had no influence on survival, clinical signs, body weight and macroscopic findings. The slight erythema observed after 4 days was self-limiting and reversible. There was no evidence of systemic toxicity.

In the initial safety review of IND 64,335, there were no animal studies to support the safety of clinical studies longer than 4 weeks. It was recommended that the Sponsor compare and correlate the local toxicities of diclofenac sodium gel with dilofenac diethylammonium gel (Voltaren Emulgel, marketed in Europe). The Sponsor was informed that depending on the outcome of that analysis, additional animal toxicity studies may be required.

In response, the Sponsor submitted a 12-week dermal study in rabbits with Emulgel formulation to support the planned 12-week clinical trial with diclofenac sodium gel. They summarized the available data and comparisons:

- No serious adverse effects (minor skin reaction) in the 12- week Diclofenac Emulgel study
- 5-day skin irritation test with diclofenac diethylammonium gel showed only slight erythema after 3-5 day
- *In vitro* skin permeation study showed that diclofenac sodium gel and Diclofenac Emulgel penetrated equally
- Short term studies showed no significant difference between the two gels
- Reasonable to assume that the dermal effects of two gels are similar because they share the same active moiety
- Although there was no long term animal studies to directly compare the dermal effects of diclofenac sodium gel and Diclofenac Emulgel, human data existed for diclofenac sodium gel to compare its dermal effects with diclofenac Emulgel

The Division decided that no additional animal dermal studies with diclofenac sodium gel would be necessary. This 12-week dermal study appears to be an important link to the safety of Diclofenac Gel 1%, since the similarity of the data for Voltaren Emulgel 1.16% with Diclofenac Gel 1% is the underlying bases for not requiring additional diclofenac gel 1% dermal toxicology studies. It was not submitted to the NDA, but in response to our request, of June 2007, the Sponsor submitted the study in July, 2007. In response to our request for impurity levels in the study formulation, they stated it was not available, (which may mean it was never determined). The reviewer concurs with the findings of the 12-week dermal toxicology study and the above comparisons between Voltaren Emulgel and Voltaren Gel. However, it should be noted that this study would not likely be acceptable to support a 12-week clinical study in the United States, since it lacked multiple dose levels and toxicokinetic data.

Eye Irritation

The instillation of diclofenac sodium topical gel 1% into the rabbit eye resulted in mild/moderate, early-onset and transient ocular changes, such as reddening of the conjunctivae and sclerae, discharge and swelling. Slight corneal opacities, affecting up to the whole area of the cornea, were also observed in all animals. These effects were however reversible and were no longer evident 21 days after treatment, the last day of observation. No abnormal findings were observed in the iris. No corrosion was observed at any of the measuring intervals and there was no staining of the treated eyes by diclofenac. Diclofenac sodium topical gel 1% did not induce significant or irreversible damage to the rabbit eye. According to the EC classification criteria, diclofenac sodium was considered to be "not irritating" to the rabbit eye. However, this

reviewer thinks that this classification was in error, perhaps a misunderstanding of the EC critieria, and consider diclofenac sodium topical gel to be an eye irritant.

SPECIAL TOXICOLOGY STUDIES

Hypersensitivity

The results of skin sensitivity studies in guinea pigs suggest that diclofenac lacked sensitizing potential. The — excipient, carbomer —, which replaced ⟹ in Voltaren Emulgel, was also tested for dermal sensitization in guinea pigs. No skin reactions occurred upon contact challenge with — (w/w) carbomer – two weeks after induction with a 5% intradermal solution.

Phototoxicity and photosensitivity

Voltaren Gel 1% was not phototoxic when tested on guinea pigs exposed to UVB light. Results from photomutagenic studies indicated diclofenac lacked mutagenic potential when exposed to UVB light. The results from the first skin sensitization and photosensitization studies suggested that diclofenac sodium topical gel 1% may have a skin sensitizing potential. However, two subsequent sensitization and two photosensitization studies did not confirm these results, which is in accordance with the human sensitization test that did not show signs of a sensitizing potential. However the lack of parallel positive controls, differences in doses applied, and composition of the formulations, raise concerns about the negative nonclinical findings. The impurity — was not photosensitizing when tested in guinea pigs.

Photodegradants

Photodegradants were found in CMC stability studies for container protection and from studies of other NDAs for diclofenac products. As a 505(b)(2), the Sponsor will reference Solaraze (NDA 21-005) for dermal carcinogenicity and dermal-photococarcinogenicity safety data for diclofenac. However, the NDA lacked information about photodegradants that demonstrated relevance and appropriateness for referral (bridging) to the Solaraze label information. In response to our request for additional information, the Sponsor request additional clarification on June 26, 2007, and they proposed a functional dermal phototoxicity study comparing their product (1% diclofenac) with Solaraze (3% diclofenac) to be able to bridge to the Agency's findings of safety for Solaraze. After consulting with Dermatology, we notified Sponsor that this would not be acceptable and they need to compare the identities and amounts of photodegradants in their drug product relative to Solaraze.

Since the product can be labeled to avoid the sun, this will not be an approval issue if the Sponsor accepts that labeling. The comparative study of photodegradants will still be needed to demonstrate that reference to the Solaraze product is appropriate. It would only become an approval issue if the Sponsor will not label the product to avoid the sun and the degradants are greater than those found in Solaraze, which would be unexpected due to the lower concentration of diclofenac.

IMPURITIES/DEGRADANTS

As described in the section of clinical formulation in 2.6.1, there are impurities or degradants that exceed the release and/or stability threshold for qualification and these compound are incompletely qualified. These include

[redacted]. In studies that provide partial qualification according to current Agency recommendations, they were found to be relatively non-toxic and not mutagenic.

The Sponsor provided the following justification for the safe use Voltaren Gel 1% despite these excesses over current recommendations:

- Diclofenac sodium has been manufactured and ingested at higher doses with the same level of impurity control for approximately 20 years. There is no safety data on the prolonged continuous chronic use of this Voltaren Gel, or of the Euopean product Voltaren Emugel.
- Repeat oral dose animal studies and lifetime carcinogenicity studies with diclofenac do demonstrate pronounced toxicity with time, although maybe not carcinogenic effects. It was never determined if those were due to impurities or diclofenac sodium, all studies with diclofenac were confounded by the presence of these impurities.
- Exposure to impurities arising from topical diclofenac are expected to be comparable or lower than those from the oral Voltaren tablets. The long term dermal effects of these impurities has not been determined.

These responses from the Sponsor were expected. Unless CMC disagrees with the acceptance criteria for release and stability, or unless there is some clinical signal from the 20 year use of the product that would implicate further investigation, additional standard nonclinical dermal toxicological studies will not be necessary.

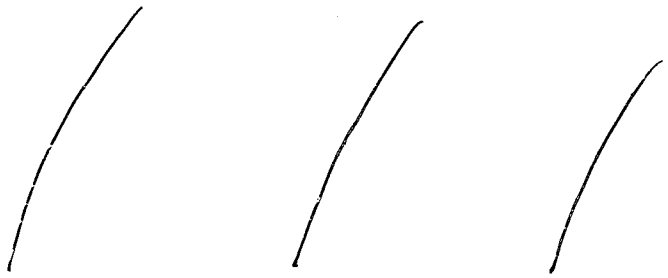
The Sponsor also acknowledged the newer guidance recommendations since the earlier (1988) product approvals and conducted additional studies to provide the missing genotoxicity information, to more adequately characterize these important toxicological characteristics, mostly concerning the clastogenic potential of the impurities.

Compound [redacted] is a novel impurity to this diclofenac product. It was not present in previous formulations. This compound exceeds the recommended stability threshold for qualification and has a structural alert component for genotoxicity, but genotoxic studies were negative for mutagenicity and clastogenicity. No specific studies were performed to determine its dermal toxicity.

Toward the end of the review process, Sept 18, 2007, the Sponsor was sent a request to accept the stability qualification threshold of [redacted] for [redacted], since an examination of clinical batch measurements indicated results far below the Sponsor's specifications. This was acceptable by the Sponsor (email of Sept 19, 2007) and no further qualification studies are necessary for this compound.

EXCIPIENT TOXICOLOGY

Cocoyl caprylocaprate
The Sponsor responded to our information request of June 15, 2007, by having the manufacturer send DMF — , Type IV for — to the Agency. —



It should be noted that in indicated patient population may be applying — of cocoyl caprylocaprate up to 4 times daily for a lifetime, therefore it is highly likely that over the course of years of use, the intradermal concentration and concentration within the hair follicles may occasionally accumulate and resulting in comedogenic effects. This may manifest itself also as skin infections, due to trapped bacteria. If this were to occur, it is readily detected and treatable, and does not present a nonclinical safety concern.

This excipient is is also present in many cosmetic products (aftershave lotions, bath oils, blushers, cleansing lotions, eye shadows and liners, hair conditioners, lipsticks, suntan preparations, etc) used as an emollient. —

— The concentration of this excipient in these various products was not able to be determined from available information, but its prevalence in and widespread use in various topical product have not signaled any safety concerns and provides reasonable assurance of its short-term safety.

There has been no determination of the carcinogenic potential of cocoyl caprylocaprate. The clinical experience, despite about 20 years of availability, is insufficient to characterize its carcinogenic potential. Furthermore, full qualification of this excipient which would also be necessary for inclusion in the Inactive Ingredient Database would require a dermal carcinogenicity study. As a component of Voltaren Gel, a product that could be applied daily for a lifetime, a dermal carcinogenicity study of should be conducted for cocoyl caprylocaprate to determine it carcinogenic potential.

The Sponsor responded to our information request of June 15, 2007, by having the manufacturer — end to the Agency the full proprietary composition. Perfume comprised only — w/w) of the drug product and was composed of — ompounds. The Sponsor supplied appropriate toxicological information for some of these compounds, while others were determined by this reviewer (through database searches) to be of minimal toxicological consequence (potentially allergenic) at the concentrations applied topically. Since it comprises less than — of the drug product, it is unnecessary to further characterize the perfume or its components.

2.6.6.2 Single-dose toxicity

These studies were reviewed for NDA 19-201.
Key findings are summarized in the table below.

Most of the single dose studies were carried out to establish the acute lethality of diclofenac sodium and the data are summarized in Table 2-1 below. Additional single dose studies with impurities/degradants are presented in the last table.

Table 2-1 Single dose toxicity studies (Data with diclofenac sodium, mg/kg)

Species	Route	LD_{50} (mg/kg)	Major Signs of Toxicity
Mouse	p.o.	185-541	Transient tonic convulsions, excitation, dyspnea, ataxia, ventricumbency, somnolence, roughening of the coat, latericumbency, poor general condition.
Mouse	i.v.	92-147	Dyspnea, convulsive movements of the tail, tonic-clonic convulsions, ataxia, ventricumbency, somnolence, opisthotonus, roughening of the coat, exophthalmos, rapid respiration, salutatory spasms, poor general condition, tremors, and convulsions.
Rat	p.o.	55-240	Reduced activity, exophthalmos, ptosis, diarrhea, salivation, piloerection, perineal staining, dyspnea, staxia, somnolence, ventrolatericumbency, tarry stool, paleness of the conjunctiva, persistent symptoms, roughening of the coat, poor general conditions.
Rat, 21-day	p.o.	131	Reduced activity, ptosis, piloerection, diarrhea, distended abdomen.
Rat	i.v.	97-161	Tonic and clonic convulsions, convulsive movements of the tail, exophtalmos, saltatory spasms, dyspnea, ataxia, ventricumbency, somnolence, tarry stool, paleness of the conjunctiva, roughening of the coat, diarrhea.
Rat neonatal	i.g.	142	Labored respiration, cyanosis, respiratory failure.
Guinea pig	p.o.	1110	Dyspnea, ventricumbency, somnolence, exaggerated startle reaction, roughening of the coat, latericumbency, tonic convulsions.
Guinea pig	i.v.	123-131	Tonic and clonic convulsions, dyspnea, reduction of spontaneous mobility, ventricumbency, somnolence, opisthotonus, ataxia.
Rabbit	p.o.	125-300	Dyspnea, ataxia, somnolence, tarry stool, bloody nose exudates, latericumbency, tonic convulsions.
Rabbit	i.v.	> 20	Dyspnea, occasional necrosis at injection site.
Rabbit	Dermal	2000 + 980	Body weight loss, anemia, nephrosis.
Dog	p.o.	> 800	Transient loss of appetite, diarrhea, superficial erosions on mucosa of the duodenum.
Monkey	p.o.	3200	Diarrhea, anorexia, emesis, loose stool, vocalization, salivation, ataxia, tarry stool, tremors, suspect drug in stool, rectal bleeding (ulcers at necropsy), coma before death.

Summary of single dose toxicity of diclofenac sodium
(From Sponsor's Tables 2.6.7.1.2 and 2.6.7.5.1)

Study Number / Location (eCTD Section)	Species and Strain	Doses (mg/kg) / Method of Administration / Duration of Observation	Findings
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Mouse, Tif MAGf n=10/sex/dose, except 600 mg/kg only males	10, 100, 300, 450, 600[a] Oral gavage in carboxy-methylcellulose (CMC) 2%, Observed for 15 days	Maximum Non-Lethal Dose: M 100, F 10 mg/kg Minimum Lethal Dose: M 300, F 100 mg/kg Acute oral LD_{50}: M 276, F 185 mg/kg Necropsy: **GI lesions**
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Mouse, Tif MAGf n=10/sex/dose, except 200 mg/kg only males	10, 100, 120, 150, 200[a] Intravenous in distilled water, Observed for 15 days	Maximum Non-Lethal Dose: M 10, F 10 mg/kg Minimum Lethal Dose: M 100, F 100 mg/kg Acute i.v. LD_{50}: M 92, F 97mg/kg Necropsy: **no gross organ changes**
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Rat, Tif Ralf n=10/sex/dose, except 450 mg/kg only males	10, 30, 60, 100, 150, 300, 450 Oral gavage, Observed for 15 days	Maximum Non-Lethal Dose: M 100, F 100 mg/kg Minimum Lethal Dose: M 150, F 150 mg/kg Acute oral LD_{50}: M 240, F 226 mg/kg Necropsy: **GI lesions**
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Rat, Tif Ralf n=10/sex/dose,	10, 100, 150, 170, 200 Intravenous in distilled water, Observed for 15 days	Maximum Non-Lethal Dose: M 100, F 100 mg/kg Minimum Lethal Dose: M 150, F 150 mg/kg Acute i.v. LD_{50}: M 151, F 161 mg/kg Necropsy: **GI lesions**
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Rat, Tif Ralf, neonatal n=10/sex/dose	0, 10, 30, 60, 100, 150, 200, 300 Intragastric, Observed for 24 hours	Maximum Non-Lethal Dose: 30 mg/kg Minimum Lethal Dose: 60 mg/kg Acute intragastric LD_{50}: 142 mg/kg Necropsy: **GI lesions**
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Rabbit, chinchilla (SPF) n=2/sex/dose	10, 30, 60, 100 Intravenous in distilled water, Observed for 15 days	Maximum Non-Lethal Dose: M 10, F 60 mg/kg Minimum Lethal Dose: M 30, F 100 mg/kg Acute i.v. LD_{90-100}: M 30-60, F 100 mg/kg Necropsy: M **pulmonary edema**, F **no organ changes**
22 Sept 1977 eCTD 4.2.3.1.1	Dog, beagle n=1/sex/dose	30, 60, 100, 300 Oral capsule, Observed for 21 days	Maximum Non-Lethal Dose: 100 mg/kg Minimum Lethal Dose:

GLP: No			300 mg/kg Acute oral LD_{90-100}: 300 mg/kg Necrospy: **inflammatory changes in the intestinal tract**
22 Sept 1977 eCTD 4.2.3.1.1 GLP: No	Dog, beagle n=1/sex/dose	10, 30, 100, 150 Intravenous, Observed for 15 days	Maximum Non-Lethal Dose: M 10, F 100 mg/kg Minimum Lethal Dose: M 30, F 150 mg/kg Acute i.v. LD_{90-100}: 30-100 mg/kg Acute i.v. LD90-100 30-100 Necrospy: **inflammatory changes in the intestinal tract** t
0289-70L eCTD 4.2.3.1.2 GLP: No Sept, 1970	rhesus monkeys {Macaca mulatta) n=9 male, 8 female	800, 1131, 1600, 2262, 3200 Oral gavage, Observed for 15 days	Oral LD_{50} (95% confidence limits) = 3200 (1600, 4720) mg/kg Diarrhea, anorexia, emesis, loose stool, vocalization, salivation, ataxia, tarry stool, tremors, suspect drug in stool, rectal bleeding (ulcers at necropsy), coma before death.

[a] highest dose administered to males only

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ON ORIGINAL

Summary of single dose toxicity of impurities/degradation products
(Modified from Sponsor Tables 2.6.7.1.3 and 2.6.7.5.2)

Study Number / Location (eCTD Section)	Degradant	Species and Strain	Doses (mg/kg) / Method of Administration / Duration of Observation	Findings
63/52-D6144 eCTD 4.2.3.7.6.1 GLP: yes	—	Rats/ WI(Glx/BRL/ Han)BR n-= 5/sex/dose	Preliminary: 1200, 2000 mg/kg; Main: 2000 mg/kg; Oral by gavage/Disper sion aqueous methyl cellulose 1% ; observed for 15 days	Maximal Non-Lethal Dose: not determined Minimum Lethal Dose: >2000 mg/kg LD_{50} >2000 mg/kg
88-6185 eCTD 4.2.3.7.6.2 GLP: yes	—	Rats/Tif: RAIf (SPF) n-= 5/sex/dose	1000, 2000 mg/kg; Oral by gavage/Suspe nsion in Na-CMC 0.5%; Observed for 14 days	Maximal Non-Lethal Dose: 1000 mg/kg Minimum Lethal Dose: 2000 mg/kg LD_{50} approximated by graphical extrapolation to be 1850 mg/kg
063/037-D6144 eCTD 4.2.3.7.6.3 GLP: yes	—	Mouse, CD-1(ICR)BR Preliminary n-= 1/sex/dose Main n-= 5/sex/dose	 Preliminary: 500, 1000, 1500, 2000 mg/kg Main: 2000 mg/kg; Oral gavage (in aqueous methyl cellulose); observed for 15 days	Maximal Non-Lethal Dose: not determined Minimum Lethal Dose: >2000 mg/kg Preliminary: No deaths and no overt reactions to treatment. Main: LD_{50} >2000 mg/kg No clinical signs of any systemic toxic effect of the test article

2.6.6.3 Repeat-dose toxicity

The non-dermal application studies were reviewed in NDA 19-201. Repeated dose toxicity studies from 2 to 26 weeks in duration were conducted with mice , rats, dogs, monkeys, and baboons. Key findings are summarized in the tables that follow.

Repeated dose dermal toxicity

The local skin tolerance and the systemic toxicity of diclofenac sodium topical gel 1% (referred to as NCH927806 1% in the study) was investigated by semi-occlusive dermal application for a period of 28 days. Young adult New Zealand White rabbits (3 male and 3 female), received 0.5 ml of diclofenac sodium topical gel 1% per day on an 1.2 x 1.2 cm^2 gauze surface for 4 hours period. The animals were examined daily for clinical signs and mortality/viability from the start of acclimatization to the end of the study. Local dermal signs were recorded daily from the start of treatment to the end of the study. Very slight erythemas were seen at the 4 hour reading after 4 days which temporarily increased to a score 2 (well defined erythema after 8-12 days on 2 of the 6 animals. At the end of the 4-week period almost no erythemas were observed. No edemas were seen and no irritation at each of the 24 hour readings. Histopathological examination of the treated skin areas showed minor signs of inflammation, randomly distributed among control and treated skin samples. The treatment had no influence on survival, clinical signs, body weight and macroscopic findings of treated and untreated skin. The results of the 4-week repeated dose dermal toxicity study showed that diclofenac sodium gel 1% is well tolerated. The slight erythema observed after 4 days was self-limiting and reversible.

Repeated Dose Oral Administration

In mice, no changes attributed to treatment was observed at a nominal dose of 0.1mg/kg/day. At 0.3 or 1.0 mg/kg/day, a small increase in liver weight was observed. Slight anemia and slight increases in liver and spleen weights, associated with increased extramedullary hematopoiesis, were observed. At the high dose (10mg/kg/day), mortality, associated with changes in the gastrointestinal tract were observed.

Rabbits and rats, respectively, tolerated from 2 to 4 mg/kg orally or up to 3 mg/kg subcutaneously (rats) for periods up to 4 weeks with no or minimal signs of toxicity. However, higher dose levels or the same dose administered for longer duration (up to 26 weeks in rats) resulted in dosedependent increases in gastrointestinal ulcerations, which frequently led to peritonitis, anemia, and deaths.

Dogs tolerated 5 mg/kg orally for 1 week, but experienced gastrointestinal ulcerations with peritonitis, anemia, and deaths at 10 mg/kg for the same interval. Similar findings were observed in another study in dogs given 2.5 mg/kg for 4 weeks. When medication was administered for 13 weeks at oral doses of 0.5-2 mg/kg, dogs suffered gastrointestinal ulcerations. At doses > 1 mg/kg, peritonitis, anemia, and deaths were observed. Oral chronic toxicity studies were conducted in rats for up to 24 months (carcinogenicity study)and in baboons for 52 weeks. Rats tolerated 0.25 and 0.5 mg/kg with scattered clinical chemistry and histopathological deviations. However, survival time was significantly reduced in males and females of the 1 and 2 mg/kg

groups primarily due to a dose-related increase in the incidence of gastrointestinal perforations. Incidence rates of tumors were within the range of historical or test controls.

Monkeys were least susceptible to intoxication by diclofenac sodium, being able to ingest up to 50 mg/kg/day for 13 weeks or 15 mg/kg for 26 weeks with only minimal evidence of gastrointestinal irritation (depressed hemograms and fecal blood). Monkeys endured doses as high as 150 mg/kg for 6-10 days or 75 mg/kg for 18-133 days before succumbing to the lethal sequelae induced by gastrointestinal ulcerations. Baboons tolerated 5 mg/kg, but developed some colon ulcers. At 10 mg and 30 mg/kg, respectively, mortalities rose to 36% and 94% due to sequelae of severe gastrointestinal ulcerations.

Study title: **NCH 927806 1%: 4-week repeated dose dermal toxicity study in the rabbit (semi-occlusive application)**

Key study findings: Topical application of NCH 927806 1% resulted in a persistent erythema together with slight to moderate scaling in all animals at the application site. This lasted for the first 15 days. At necropsy, 2 weeks later, signs of moderate inflammatory responses were present.

Reviewer's Comments: The reviewer agrees with the Sponsor's interpretation that they are observing a healing response to the irritation of NCH 927806 1%. The Sponsor termed the 14 days of erythema as transient, which the reviewer disagrees. Skin reactions were assessed 20 hours after removal of the occlusive bandage and the gauze containing NCH927806 1%, not at anytime between removal and 20 hours later, thus the erythema was at its minimum prior to each application, and did not recover until 2 weeks in the study. Day to day this would not be a transient condition. This study did not use the active compound of diclofenac sodium or the final product of this NDA, rather it was diclofenac diethylamine according to the origin of NCH 927806, but with this NDA the Sponsor states this is now diclofenac sodium. It is als unclear, which gel type is used, the Emulgel as stated, or the Voltaren Gel formulation.

Study no.: 843186
E-Location: CTD 4.2.3.6.2
Conducting laboratory and location: [redacted]

Date of study initiation: May 23, 2002
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
NCH 927806 1% (Voltaren 10 mg/g Emulgel), Batch DPH-030, Purity not provided. (Provided by the Sponsor in a semi-solid form, 0.5 ml (per animal) of NCH 927806 1% was measured with a syringe and applied undiluted as it was delivered by the sponsor.)

Methods
For 28 days, a daily application of a topical semi-occlusive application of 0.5 ml of NCH 927806 1% was placed on a 1.2 cm x 1.2 cm gauze patch which was then applied for 4 hours to the left flank of young adult New Zealand White rabbits (SPF, n=3/sex, males 10-12 weeks of age,

females 10-11 weeks of age). Skin reactions were assessed at least once daily immediately prior to application (***Reviewer comment***: which would be 20 hours after removal of the dressing from the previous day).
The application site was scored according to the following criteria:

ERYTHEMA
0 = no erythema
1 = very slight erythema (barely perceptible)
2 = well-defined erythema
3 = moderate to severe erythema
4 = severe erythema (beet redness) to slight eschar formation (injuries in depth)
EDEMA
0 = no edema
1 = very slight edema (barely perceptible)
2 = well-defined edema (area well defined by definite raising)
3 = moderate edema (raised approximately 1 mm)
4 = severe edema (raised more than 1 mm and extending beyond the area of exposure)
SCALING
0 = no scaling
1 = slight scaling
2 = moderate scaling

Representative tissue specimens were taken from all animals from the treated and untreated sites.

Results
There were no deaths and no clinical signs of systemic toxicity. The body weights of two animals (#1 and #4) exhibited a body weight loss of 3.8% and 0.9% respectively, during the first week, but then all gained weight. At necropsy, there were no gross organ abnormalities. Application site erythema (very slight to well-defined) occurred in all animals during the first 14 days of treatment together with slight to moderate scaling (desquamation of the skin) in 5 of 6 animals up to 15 days of treatment. One female (#6) had a wound present at the application sire drom day 11 to 15 that resulted in a scab on days 16 and 17. A minimal epidermal hyperplasia was seen in one male and two females and slight mononuclear foci were noted in another male and two females and also in the remaining female with less severity. The control skin showed minimal mononuclear foci in one male and one female. Overall, there was no difference in the skin reactions were noted between males and females.
The Sponsor considered the desquamation (scaling) of the skin as a healing process involving the release of intact keratinocytes after the degradation (irritation) of the principle structure responsible for corneocyte cohesion.

Study title: 3 Month Dermal Study in Rabbits with 1% Emulgel Formulation of GP 45840 G

This study was submitted in response to an information request of June 15, 2007. The study was previously submitted to the IND 64,334, in response to requesting additional toxicological information support clinical studies of 3 month or longer duration. Since this study uses the

Emugel formulation of diclofenac diethylamine, rather than the diclofenac sodium of this NDA, it was probably not originally submitted to this NDA.

Key study findings: Diclofenac diethylamine (GP 45840 G, Emugel formulation) was applied to the shaved skin of rabbits (back and flanks) daily for 3 months. There were no systemic effects. There was a slight reduction in hair growth at the application site. This appeared to recover after 1 month without treatment.

Study no.: 83-5084
Conducting laboratory and location: CIBA-GEIGY Pharmaceuticals Division, Cheshire, UK
Date of study initiation: May 18, 1983
GLP compliance: no
QA report: yes
Drug, lot #, and % purity:
Voltaren Emulgel (GP45840)11.6 mg/g (1.16%) gel (equivalent to 1% diclofenac sodium,
Vehicle/formulation: Voltaren Emulgel Placebo

Methods

New Zealand White rabbits (n=6/gender; 12-14 weeks of age) received a daily topical application with Voltaren® Emulgel™ 11.6 mg/g Gel at 400 mg gel/kg/day at a concentration of 10 mg/cm² (400 mg/40 cm^2 skin/kg using a template) or placebo Emulgel. The treated site was covered with occlusive bandages for six hours after each application. Two males and two females from each group were retained for a one-month recovery period. Parameters examined included clinical signs, including any local responses, body weight, food intake, ophthalmoscopy and hearing (high pitch noise observing animals reaction) examinations . Blood chemistry, hematology, and urine analyses were carried out monthly. On completion, animals were necropsied, organ weights obtained, histopathology performed. Organ weights were obtained from adrenal, brain, heart, kidney, liver, ovaries, pituitary, spleen, testes, thymus and thyroid gland.

Study Design

Group	Dose	No. of Rabbits	Duration
1	Control	4 ♂ + 4 ♀	3 months
	Article	2 ♂ + 2 ♀	3 months + 1 month recovery
2	Test	4 ♂ + 4 ♀	3 months
	Article	2 ♂ + 2 ♀	3 months + 1 month recovery

On completion, animals were necropsied, organ weights obtained, histopathology performed. Organ weights were obtained from adrenal, brain, heart, kidney, liver, ovaries, pituitary, spleen, testes, thymus and thyroid gland. Tissues for histopatholgy are listed below.

Adrenal Glands
Aorta
Bone Marrow (Sternum)
Brain
Eyes/Optic nerves
Gross Lesions
Heart
Kidneys
Large Intestine (Caecum, Colon)
Liver
Lungs
Lymph Nodes (Popliteal, Mesenteric)
Oesophagus
Ovaries/Uterus/Mammary Area
Pancreas
Peripheral Nerve
Pituitary Gland
Salivary Gland
Skeletal Muscle
Skin (Control/Application Site)
Small Intestine (Duodenum, Jejunum, Ileum)
Spinal Cord
Spleen
Stomach
Testes/Epididymides/Prostate Gland/Seminal Vesicle
Thymus Gland
Thyroids/Parathyroids
Trachea
Urinary Bladder

Results

There were no deaths. There was no effect on body weight, food consumption, hearing, ophthalmoscopy, hematology, clinical chemisty, urinalysis, or organ weights.

Effects that were related to treatment only occurred at the treatment sites. A reduction in hair growth was observed in all animals, treated with the test product or with placebo. The severity tended to be greater in animals receiving the test article. Pathological analysis performed on animals at the end of treatment indicated that the reduction probably resulted from a depression in the normal cycle of hair growth. Other changes were seen at the treatment sites in both treated and control animals. These included erythema, edema and papules, some with scabbing, which were worse in controls and flaking skin and cracking of the epidermis which were worse in treated rabbits. The Sponsor attributed these changes to the daily application of gel and occlusive dressings and not to treatment with Voltaren Emulgel 11.6 mg/g Gel.

TABLE: COMPARISON OF SIGNS (APPLICATION SITE) BETWEEN CONTROLS AND TREATED ANIMALS

Sign	Duration	Severity	Incidence	Recovery
ERYTHEMA	Equal	Worse Control	Worse Control	Most animals
OEDEMA	<----------	Worse Control	---------->	Some animals
PAPULES AND SCABS	<----------	Controls only	---------->	All animals
FLAKING SKIN	Worse Treated	Equal	Worse Treated	All animals
CRACKING EPIDERMIS	<----------	Worse Treated	---------->	All animals
REDUCED HAIR GROWTH	Equal	Worse Treated	Equal	No animals

Control = Animals receiving control article.
Treated = Animals receiving test article.

Main Study

Erythema was seen in a few animals from week 1, most animals from week3 and all animals from week 6. Higher scores occurred in control animals. Edema occurred n some animals from both groups during the second half of the study, the severity, incidence and duration was greater in control animals. Papules occurred in most control animals and 1 male and 1 female treated with Voltaren Emulgel. Scabs formed only on papules of the control animals..
Flaking skin occurred in most animals in both groups. The inicidence and duration was greater in Voltaren Emulgel treated animlas, and more frequently in females. Cracking of the epidermis occurred in most animals treated with Voltaren Emulgelbetween week 1 and 5 and in control animals during week 3 and 4, and in one animal during week 13. A reduction in hair growth occurred in in all animals of both groups from week 5 until termination of the study. The severity seemed greater in animlas receiving Voltaren Emulgel.

Recovery Groups

Erythema (Draize scores 1-2) occured in most animals up to week 16 and in 2 control females, and on female that received Voltaren Emulgel until week 17. Edema occured in control females during week 14 and most animals administered Voltaren Emugel from week 14, and persisted

until week 17 in one male and one female. One Voltaren Emulgel treated female developed edema during the recovery phase , but while treated with Voltaren Emulgel in the main study phase. Papules occurred on on control male during week 14.

Histopathology

In 7 of 8 rabbits treated with Voltaren Emulgel, there was a reduction in the number of adnexal and trichoid elements. Epithelial hyperplasia occurred in 7 of 8 Voltaren Emulgel animals and all control rabbits. Acanthosis coccurred in one animal. Changes were absent by the end of the recovery period.

Group	1				2			
Dose	Control Article				Test Article			
No of rabbits	4♂	4♀	2♂R	2♀R	4♂	4♀	2♂R	2♀R
Skin (Application site)								
Epithelial hyperplasia	4	4			3	4		
Acanthosis	1							
Adnexal/trichoid atrophy					3	4		

Gastric lesions occurred in 2 males treated with Voltaren Emulgel. Ulceration of the gastric mucosa was also seen in one treated male rabbit. This was thought to be related to ingestion following accidental removal of the occlusive dressing. There is no other mention of such incidents in the report although wounds (scratches) at the treatment site are mentioned for two females, one of which was not treated for three days because of the severity of these wounds. One male (2-003) had changes in the mucosa which consisted on focal ulcerations of the cardial mucosa and hydropic degeneration in the esophageal mucosa of the cardio-esophageal sphincter accompanied by local cellulites and mucosal edema. Male (2-0010 had minute focal gastric hemorrhage that could not be confirmed microscopically. (**Reviewer:** probably did not cut through appropriate tissue area)

Group	1				2			
Dose	Control Article				Test Article			
No of rabbits	4♂	4♀	2♂R	2♀R	4♂	4♀	2♂R	2♀R
STOMACH								
Ulcer					2 003♂			
Haemorrhage					2 001♂			
ADRENALS								
Nodules		1 004♀			2 001♂			
					2 005♂			
HEART								
Hypertrophy	1 007♂							

Other hisptopathological changes include nonsuppurative encephalitis thought to be caused by the parasitic disease encephalitozoon. The spleen of all animals had varying degrees of protozoan parasites, resulting in reactive lymphoid hyperplasia. In some animals chronic myocarditis and endocarditis were present, also signs of encephalitozoonosis. One animal had cardiac hypertrophy and dilatation with valvular fibrosis of unknown etiology.

A summary of the findings is presented in the following table:

Daily dose (mg/kg)	0 (Control – Group 1)		(Group 2) >400 (=4mg/kg diclofenac)	
Number of animals	M: 6	F: 6	M: 6	F: 6
Noteworthy findings				
Toxicokinetics: AUC	*No data*			
Died or sacrificed moribund	0	0	0	0
Body weight (%[a])	3.77kg	4.01kg	102	101
Food consumption (%[a])	1143g	1241g	101	102
Clinical observations[b]				
Erythema, edema, papules	Control > treated			
Flaking, cracking, reduced hair regrowth	Treated > control			

[a] = at the end of dosing period. For controls, group means are shown. For treated groups, percent of control is shown.

b=all findings were observed in both treated and control groups; flaking was more frequent in females but no other gender differences.

Daily dose (mg/kg):	0 (Control – Group 1)		(Group 2) (Group 2) >400 (=4mg/kg diclofenac)	
Number of animals:	M: 6	F: 6	M: 6	F: 6
Noteworthy findings:				
Ophthalmoscopy	-	-	-	-
Hearing test	-	-	-	-
Hematology:	-	-	-	-
Clinical biochemistry:	-	-	-	-
Urinalysis:	-	-	-	-
Organ weights	-	-	-	-
Gross pathology:	-	-	Stomach ulcer[c]	-
Histopathology: No. Examined	4	4	4	4
Atrophy, trichoid elements [d]	0	0	3	4
Focal necrotizing gastritis	0	0	1[e]	0
Additional examinations:				
Postdose evaluation: Number evaluated	2	2	2	2
Atrophy, trichoid elements [d]	1	0	0	0

- = no noteworthy findings, + = mild, ++ = moderate, +++ = marked

c =in one rabbit; a second had a focal hemorrhage

d =or reduction, at the application site

e=the pathologist states "possibly related to the accidental ingestion of the test article"

* $p<0.05$, ** $p<0.01$

Table 3-1 Repeat-dose toxicology studies

Diclofenac sodium. Reference is made to NDA 19-201 (Studies cited as indicated by their study no.).

Species	Sex	Route of admin.	Daily dose	Comments : special findings and/or observations	Study No.
Rat	M, F	oral intu-bation	0.5, 1, 2, 4, 8 or 16 mg/kg for 1 month	4-16 mg/kg = hypertrophy of mesenteric lymph nodes; 8 mg/kg = 10% male and 40% female mortality rates and small intestinal mucosal hemorrhage; 16mg/kg = 100% mortality in both sexes; weight loss; reduced food intake; hypertrophy of spleen; peritonitis; and gastrointestinal ulceration.	FR2020-0006
Rat	M, F	oral intu-bation	0.25, 0.5, 1, 2 or 4 mg/kg for 6 months	2-4 mg/kg = hypertrophy of mesenteric lymph nodes; 4 mg/kg = necrosis of cecal mucosa	FR2020-0006
Rat	M, F	oral intu-bation	0, 1.25, 2.5, 5, 10, 20 or 40 mg/kg for	1.25 mg/kg = small intestinal ulcer; > 5 mg/kg = decreased RBC;	A70-423, 774, 775, 776, A70-

Species	Sex	Route of admin.	Daily dose	Comments : special findings and/or observations	Study No.
			4 weeks, 6 days/ week; aqueous soln. with gum Arabic	> 10 mg/kg = depressed body weight gains; peritonitis and gastrointestinal ulceration. 10 mg/kg = 80% male & 20% female mortalities. 20 mg/kg = 80% male & 40% female mortalities. 40 mg/kg = 80% male & 60% female mortalities.	415
Rat	M, F	oral intu-bation	0, 0.5, 1, 2 or 4 mg/kg for 15 weeks, 7 days/ week; aqueous gum arabic suspension	4 mg/kg = 20% male & 30% female mortalities; decreased hemoglobin, hematocrit, albumin, and alpha$_1$ globulins; increased reticulocytes, alpha$_2$ and alpha$_3$ globulins; peritonitis; and gastrointestinal ulceration.	798, A70-425, 797
Rat	M, F	oral intu-bation	0, 0.5, 2 or 6 mg/kg for 90 days, 7 days/ week; suspen-sion in gum acacia	6 mg/kg = 20% male & 20% female mortalities. Increased reticulocytes, segmented neutrophils, alpha$_2$ and beta globulins; and decreased hemoglobin, hematocrits, total proteins, and gamma globulins at all sampling intervals.	6804080
Rat Adult	M, F	Oral, mixed to pelleted food	0, 0.3, 0.6, 1 or 2 mg/kg for 28 days	No toxicological effects observed under conditions employed.	428978
Rat Juvenile	M, F	Oral, mixed to pelleted food	0, 0.3, 0.6, 1 or 2 mg/kg for 28 days	No toxicological effects observed under conditions employed.	444878
Rat	M, F	s.c.	0, 0.6, 2 or 6 mg/kg for not less than 28 days	No toxicological effects observed under conditions employed.	4R03133/74/SL
Rat	M, F	s.c.	0, 3, 6 or 10 mg/kg for 1 month, in normal saline at 5 ml/kg	10 mg/kg = 13.3% female mortality rate with peritonitis; slight anemis (reversible); 6-10 mg/kg = ulcers (cecal and small intestine in females – reversible); 3 mg/kg = minimal effect.	78R14, 709178, 29/79/SL
Rabbit	M, F	oral	0, 2, 6 or 12 mg/kg for 4 weeks, 7 days/week ; suspension in gum arabic	2 mg/kg = 75% female mortality; 6-12 mg/kg = gastrointestinal ulceration; 6 mg/kg = 75% male and 25% female mortality; 12 mg/kg = 25% male mortality; (Erratic mortality rates attributed to fortuitous distribution of infectious lung diseases.)	6907140, A70-427
Dog	M, F	oral, caps	5 mg/kg (days 1-8) and 10 mg/kg (days 9-16) in gelatin capsule	5 mg/kg = No effects; 10 mg/kg = 50% (1/2) male & 0% (0/2) female mortality. Peritonitis and gastrointestinal ulceration. Decreased body weights, hematocrits, hemoglobins, total proteins, and albumins. Increased ESR, WBC, BUN alpha $_{1+2}$ and beta $_{1+2}$ globulins and reticulocytes.	A70-428, 133

Species	Sex	Route of admin.	Daily dose	Comments : special findings and/or observations	Study No.
Dog	M, F	oral, caps	2.5 mg/kg, 7 days/week , in gelatin capsule	Gastrointestinal ulceration and extramedullary hematopoiesis. Decreased RBC, hematocrits, hemoglobins, total proteins, albumins, and body weights. Increased reticulocytes, ESR, WBC, and alpha $_{1+2}$ and beta $_{1+2}$ globulins.	A70-429, 135
Dog	M, F	oral, caps	1 mg/kg, 7 days/week , in gelatin capsule	Minimal gastrointestinal erosion. Decreased hemoglobins, hematocrits, and albumins. Increased reticulocytes, ESR, WBC, and globulins.	A70-430, 136
Dog	M, F	oral, caps	0, 0.5, 1 or 2 mg/kg, 7 days/ week for 90 days, in gelatin capsule	0.5-2 mg/kg = gastrointestinal ulceration. 1 mg/kg = 33.3 mortality in each sex. 2 mg/kg = 100% mortality in each sex. 1.0-2.0 mg/kg = peritonitis; extramedullary hematopoiesis (splenic); dose-related decreases of hemoglobins, hematocrits, total proteins, and albumins; and concurrent increases in reticulocytes, platelets, WBC, and beta globulins.	6804161, A70-431
Rhesus Monkey	M, F	oral intuba-tion	1 mg/kg for 1 day; 50 mg/kg for 30 days; 150 mg/kg for 6-10 days; or 500 mg/kg for 1 day, suspension in 0.25% methyl-cellulose	1 mg/kg (acute) = unremarkable; 50 mg/kg (subacute) = diarrhea and transient weakness; 150 mg/kg (subacute) = emesis, bloody diarrhea, and 100% mortality after 10 days. Blood and inflammation along gastrointestinal tract; 500 mg/kg (acute) = emetic episodes during 5-hour post dosing period; Oral LD_{50} > 500 mg/kg.	0121-68-L A70-386
Rhesus Monkey	M, F	oral gavage (day 1-22), intuba-tion (day 23-94)	0, 5, 15.8 or 50 mg/kg for 94 consecutive days, suspension in 0.25% methyl-cellulose	> 5 mg/kg = dose-related depression of hemoglobins and RBC. > 15.8 mg/kg = dose-related depression of hematocrits and increase in reticulocytes. 50 mg/kg = depressed body weight gains and thymus weights; elevated relative liver weights.	68-315 A70-385

Species	Sex	Route of admin.	Daily dose	Comments : special findings and/or observations	Study No.
Rhesus Monkey	M, F	oral intuba-tion	0, 5, 15 or 75 mg/kg for 26 weeks	5.0-75.0 mg/kg = fecal occult blood (tarry stools at 75.0 mg/kg); 15.0-75.0 mg/kg = depressed thymus weights; 75.0 mg/kg = 100% male and 75% female mortality rates; gastrointestinal ulceration; facial edema; anorexia; body weight loss; depressed total proteins, hematocirts, hemoglobins, and RBC; and increased BUN and WBC. Primary lesions were inflammatory changes, erosions and ulcerations of the gastrointestinal tract and additional changes were secondary in nature.	68-315, A70-385

NOAEL is underlined.

APPEARS THIS WAY
ON ORIGINAL

Repeated dose toxicity of diclofenac sodium
(Modified from Sponsor Tables 2.6.7.1.4 and 2.6.7.6.1)

Study Number / Location (eCTD. Section)	Species and Strain	Doses (mg/kg/day)[a] / Method of Administration / Duration of treatment	Findings
840321 eCTD 4.2.3.2.1	Mouse, Tif:MAGf (SPF) n=10/sex/dose	0, 0.1, 0.3, 1, 3, 10 Oral gavage, for 28-29 days	NOAEL = 1 mg/kg All animals in 10 mg/kg dose died
FR-2020-0006 4.2.3.2.2	Rat, Sprague-Dawley, n=10/sex/dose	0, 0.5, 1, 2, 4, 8, 16[b] Oral gavage, for 31 days	NOAEL = 2 mg/kg 4-16 mg/kg = hypertrophy of mesenteric lymph nodes; 8 mg/kg = 10% male and 40% female mortality rates and small intestinal mucosal hemorrhage; 16mg/kg = 100% mortality in both sexes; weight loss; reduced food intake; hypertrophy of spleen; peritonitis; and gastrointestinal ulceration.
6804080 eCTD 4.2.3.2.3	Rat, Wistar CFE n=10/sex/dose	0, 0.5, 2, 6 Oral gavage, for 13 weeks	NOAEL = 2 mg/kg Results for 6mg/kg group reflect the presence of inflammatory and hemorrhagic changes
FR-2020-0006 eCTD 4.2.3.2.2	Rat, Sprague-Dawley, n=10/sex/dose	0, 0.25, 0.5, 1, 2, 4[b] Oral gavage, for 26 weeks	NOAEL = 1 mg/kg 2-4 mg/kg = hypertrophy of mesenteric lymph nodes; 4 mg/kg = necrosis of cecal mucosa
A70-423, 774, 775, 776, A70-415 eCTD 4.2.3.2.4	Rat N=10/sex/dose	0, 1.25, 2.5, 5, 10, 20, 40 mg/kg oral intubation (aqueous soln. with gum Arabic) for 4 weeks (6 days/week)	1.25 mg/kg = small intestinal ulcer; > 5 mg/kg = decreased RBC; > 10 mg/kg = depressed body weight gains; peritonitis and gastrointestinal ulceration. 10 mg/kg = 80% male & 20% female mortalities. 20 mg/kg = 80% male & 40% female mortalities. 40 mg/kg = 80% male & 60% female mortalities
798, A70-425, 797 eCTD 4.2.3.2.9	Rat n=?	0, 0.5, 1, 2 or 4 mg/kg oral intubation (aqueous soln. with gum Arabic), for 15 weeks (7 days /week)	NOAEL = 2 mg/kg 4 mg/kg = 20% male & 30% female mortalities; decreased hemoglobin, hematocrit, albumin, and alpha1 globulins; increased reticulocytes, alpha2 and alpha3 globulins; peritonitis; and gastrointestinal ulceration.
428978 eCTD 4.2.3.2.5	Rat, Adult n=?	0, 0.3, 0.6, 1 or 2 mg/kg oral administered to pelleted food, for 28 days	NOAEL = 0.3 mg/kg No toxicological effects observed under conditions employed

444878 eCTD 4.2.3.2.6	Rat, Juvenile n=?	0, 0.3, 0.6, 1 or 2 mg/kg oral administered to pelleted food for 28 days	NOAEL = 0.3 mg/kg No toxicological effects observed under conditions employed
4R03, 133/74/S.L eCTD 4.2.3.2.7	Rat n=?	0, 0.6, 2 or 6 mg/kg , S.C., for >28 days	NOAEL = 0.6 mg/kg No toxicological effects observed under conditions employed.
78R14, 709178, 29/79/S.L. 4.2.3.2.8	Rat n=?	0, 3, 6 or 10 mg/kg S.C. in normal saline at 5 ml/kg, for 1 month	10 mg/kg = 13.3% female mortality rate with peritonitis; slight anemis (reversible); 6-10 mg/kg = ulcers (cecal and small intestine in females – reversible); 3 mg/kg = minimal effect.
6907140, A70-427, 4.2.3.2.10	Rabbit n=?	0, 2, 6 or 12 mg/kg Oral (suspension in gum Arabic), for 4 weeks (7 days /week)	2 mg/kg = 75% female mortality; 6-12 mg/kg = gastrointestinal ulceration; 6 mg/kg = 75% male and 25% female mortality; 12 mg/kg = 25% male mortality; (Erratic mortality rates attributed to fortuitous distribution of infectious lung diseases.)
A70-428 133 eCTD 4.2.3.2.11	Dog n=?	5 mg/kg for days 1-8 and 10 mg/kg for days 916 Oral, gelatin capsule	NOAEL = 5 mg/kg 5 mg/kg = No effects; 10 mg/kg = 50% (1/2) male & 0% (0/2) female mortality. Peritonitis and gastrointestinal ulceration. Decreased body weights, hematocrits, hemoglobins, total proteins, and albumins. Increased ESR, WBC, BUN alpha 1+2 and beta 1+2 globulins and reticulocytes
A70-429 135 eCTD 4.2.3.2.12	Dog n=?	2.5 mg/kg, Oral, gelatin capsule, for 30 days	Gastrointestinal ulceration and extramedullary hematopoiesis. Decreased RBC, hematocrits, hemoglobins, total proteins, albumins, and body weights. Increased reticulocytes, ESR, WBC, and alpha 1+2 and beta 1+2 globulins.
A70-430 136 eCTD 4.2.3.2.13	Dog n=?	1 mg/kg, Oral, gelatin capsule , for 3 months	Minimal gastrointestinal erosion. Decreased hemoglobins, hematocrits, and albumins. Increased reticulocytes, ESR, WBC, and globulins.
6804161, A70-431 eCTD 4.2.3.2.14	Dog n=?	0, 0.5, 1 or 2 mg/kg Oral, gelatin capsule, for 90 days	0.5-2 mg/kg = gastrointestinal ulceration. 1 mg/kg = 33.3 mortality in each sex. 2 mg/kg = 100% mortality in each sex. 1.0-2.0 mg/kg = peritonitis; extramedullary hematopoiesis (splenic); doserelated decreases of hemoglobins, hematocrits, total proteins, and albumins; and concurrent increases in reticulocytes, platelets, WBC, and beta globulins.

0121-68-L, A70-386 eCTD 4.2.3.2.15	Rhesus Monkey n=?	1 mg/kg oral intubation (in 0.25% methyl-cellulose suspension), for 1 day	NOAEL = 1 mg/kg 1 mg/kg (acute) = unremarkable;
0121-68-L A70-386 eCTD 4.2.3.2.15	Rhesus Monkey n=?	50 mg/kg oral intubation (in 0.25% methyl-cellulose suspension), for 30 days	50 mg/kg (subacute) = diarrhea and transient weakness;
0121-68-L A70-386 eCTD 4.2.3.2.15	Rhesus Monkey n=?	150 mg//kg oral intubation (in 0.25% methyl-cellulose suspension), for 6-10 days	150 mg/kg (subacute) = emesis, bloody diarrhea, and 100% mortality after 10 days. Blood and inflammation along gastrointestinal tract;
0121-68-L A70-386 eCTD 4.2.3.2.15	Rhesus Monkey n=?	500 mg//kg oral intubation (in 0.25% methyl-cellulose suspension), for 1 day	500 mg/kg (acute) = emetic episodes during 5-hour post dosing period; Oral LD50 > 500 mg/kg.
68-315, A70-385	Rhesus Monkey n=?	0, 5, 15, or 75 mg/kg, Oral intubation, For 26 weeks	5.0-75.0 mg/kg = fecal occult blood (tarry stools at 75.0 mg/kg); 15.0-75.0 mg/kg = depressed thymus weights; 75.0 mg/kg = 100% male and 75% female mortality rates; gastrointestinal ulceration; facial edema; anorexia; body weight loss; depressed total proteins, hematocirts, hemoglobins, and RBC; and increased BUN and WBC. Primary lesions were inflammatory changes, erosions and ulcerations of the gastrointestinal tract and additional changes were secondary in nature.
68-315 A70-385	Rhesus Monkey n=?	0, 5, 15.8 or 50 mg/kg oral gavage (day 1-22), intubation (day 23-94) in 0.25% methylcellulose suspension, for 94 consecutive days	≥ 5 mg/kg = dose-related depression of hemoglobins and RBC. ≥ 15.8 mg/kg = dose-related depression of hematocrits and increase in reticulocytes. 50 mg/kg = depressed body weight gains and thymus weights; elevated relative liver weights.

151/172 SL eCTD 4.2.3.2.16	Baboon, n=?	0, 5, 15 (reduced to 10 on day 254) or 50 (reduced to 30 on day 38)[c] Oral capsule, for 52 weeks	All doses showed gastrointestinal ulceration (low dose in colon only), constipation, diarrhea, occasional vomiting, body weights below controls, and dose related decrease in serum albumin; Peritonitis, reduced food intake; fecal blood and abdominal pain observed in animals in mid and high dose groups 28.6% male and 42.9% female mortality observed in mid dose 87.5% male and 100% female mortality observed in high dose with reduced hematocrits and hemoglobins, and increased ESR, reticulocytes, and WBC (females). In recovery groups (during 4 weeks following treatment in low and mid dose), no clinical or histopathological signs of toxicity after cessation of medication.

2.6.6.4 Genetic toxicology

Most of the genetic toxicology studies were reviewed in NDA 19-201.
Key findings are summarized in the tables below. Additional studies to qualify degradants and impurities present Voltaren Gel 1% are also presented.

The genotoxic potential of diclofenac sodium and some major metabolites (either in pure form or in urine and bile) were extensively evaluated in a battery of assays. There were no clearly positive genotoxic results in any of the studies. These studies included: mutation studies with Salmonella typhimurium and mouse lymphoma cells, *in vitro* chromosomal aberration studies in Chinese hamster ovary cells, *in vivo* Chinese hamster somatic (bone marrow), and dominant lethal studies in mice, and analysis of male germinal epithelium cells from mice.

Three additional GLP compliant studies are submitted with this NDA. The photomutagenicity and photo chromosome aberration studies indicated that diclofenac did not have photogenotoxic potential. An in vivo chromosome aberration study in bone marrow of rats confirmed that diclofenac did not induce chromosomal aberrations.

Genotoxicity Studies with Diclofenac Sodium
(modified from tables 2.6.7.7.1 and 2.6.7.7.2

Name Study reference	Cells	Dose	Solar simulator light	Metabolic Activation	Result
MUTAGENIC POTENTIAL					
Bacterial Reverse Mutation Assay					
21 July 1977	S. typhimurium TA98, 100 1535, 1537	0.1, 1, 10, 100, 1000 µg/plate	No	No	**Negative**
				Yes (rat S9)	**Negative**
2 March 1978	S. typhimurium TA98, 100 1535, 1537	25, 75, 225, 675, 2025 µg/plate	No	No	**Negative**
				Yes (rat S9)	**Negative**
2 March 1978	S. typhimurium TA98, 100 1535, 1537	25, 75, 225, 675, 2025 µg/plate	No	No	**Negative**
				Yes (Rhesus monkey liver S9)	**Negative**
44050/02	S. typhimurium TA98, 100 1535, 1537; *Escherichia coli* WP2, *uvrA*		No	No	**Negative**
			Yes (0-3000 mJ/cm^2)		**Negative**
Mouse lymphoma L5178Y cells, Mutagenic Assay					
31872077 eCTD 4.2.3.3.1.12	Mouse lymphoma L5178Y cells	221.41 µg.mL (18h) or 400 µg/mL (4 h)	No	No	**Negative** (but 80% toxicity observed after 4 hour incubation at 400 µg/mL or 18 h incubation with 221

					µg/mL)
CLASTOGENIC POTENTIAL					
Chromosomal Aberation Assay					
V4402/02	Chinese Hamster Ovary cells, *in vitro*	0.1, 0.31, 0.93, 2.78, 8.33 and 25 µg/ml	No	No	**Negative**
			Yes	No	**Cytotoxic at low diclofenac concentrations coupled with UV-light**
				25 µg/ml ZY-17727B was clastogenic following 16 minutes of simulated solar UV irradiation but was not clastogenic following irradiation for lower lengths of time. Lower concentrations of ZY-17727B did not induce significant increases in chromosomal aberrations, following simulated solar UV irradiation for the different lengths of time.	
V 4402/14	In vivo rat bone marrow	25, 50 100 mg/kg, oral gavage, single administration	-	-	**Negative**
20 March 1973	*In vivo*, Chinese hamster, bone marrow;	0.8, 1.6, 3.2 mg/kg; Oral gavage; daily for 2 days; Metaphase analysis	-	-	**Negative**
31 May 1974	*In vivo*, Chinese hamster, bone marrow;	0.8, 1.6, 3.2 mg/kg; Oral gavage; daily for 2 days; Nucleus anomaly	-	-	**Negative**
40700677	*In vivo*, Chinese hamster, bone marrow;	0.138, 0.275, 0.55 mg/kg; Oral gavage; 3 times per week for 12 weeks; metaphase analysis	-	-	**Negative**
30180476	Chromosomal; aberration assay; NMRI mouse, spermatogonia	17, 34, 68 mg/kg Daily for 5 daysOral gavage Metaphase analysis	-	-	**Negative**
3271060D	Dominant lethal assay; NMRI mouse, spermatogozoa	65 130 mg/kg Single dose Oral gavage	-	-	**Negative** Mating and implantation ratios, resorption rates, comparable to controls Number of dead embryos within range of historical controls

Studies with Metabolites of Diclofenac (no clastogenic assays were conducted)

Study /Location (eCTD)	Species	Dose	Metabolic Activation (Rat liver S9)	Findings
CGP 13294 = 3'-hydroxydiclofenac				
Bacteria Reverse Mutation Assay				
1-Dec-1977a 4.2.3.3.1.3 Pre-GLP	*S typhimurium* TA98, TA100 TA1535 TA1537	25, 75, 225, 675, 2025 µg/plate	No	**Negative**
			Yes	**Negative**
Mouse Lymphoma Mutation Assay				
78-2307 4.2.3.3.1.13 Pre-GLP	Mouse lymphoma L5178Y cells	200 µg/mL (18h) 300 µg/mL (4h)	No	**Negative**
GP 47766 = 4'-hydroxydiclofenac				
Bacteria Reverse Mutation Assay				
14-Dec-1977a 4.2.3.3.1.5 Pre-GLP	*S typhimurium* TA98, TA100 TA1535 TA1537	25, 75, 225, 675, 2025 µg/plate	No	**Negative**
			Yes	**Negative**
Mouse Lymphoma Mutation Assay				
78-2309 4.2.3.3.1.16 Pre-GLP	Mouse lymphoma L5178Y cells	75 µg/mL (18h) 175 µg/mL (4h)	No	**Negative**
GP 47852 = 5'-hydroxydiclofenac				
Bacteria Reverse Mutation Assay				
14-Dec-1977b 4.2.3.3.1.6 Pre-GLP	*S typhimurium* TA98, TA100 TA1535 TA1537	25, 75, 225, 675, 2025 µg/plate	No	**Negative**
			Yes	**Negative**
Mouse Lymphoma Mutation Assay				
78-2310 4.2.3.3.1.15 Pre-GLP	Mouse lymphoma L5178Y cells	125 µg/mL (18h) 250 µg/mL (4h)	No	**Negative**
CGP 14217 = 4'-5-dihydroxydiclofenac				
Bacteria Reverse Mutation Assay				
1-Dec-1977b 4.2.3.3.1.4 Pre-GLP	*S typhimurium* TA98, TA100 TA1535 TA1537	25, 75, 225, 675, 2025 µg/plate	No	**Negative**
			Yes	**Negative**
Mouse Lymphoma Mutation Assay				
78-2308 4.2.3.3.1.14 Pre-GLP	Mouse lymphoma L5178Y cells.	200 µg/mL (18h) 300 µg/mL (4h)	No	**Negative**

Study title: **Photomutagenicity test in Zy-17727B active substance on induction of reverse mutations in bacteria**

Key findings: Zy-17727B (diclofenac sodium) was not photomutagenic in the bacterial reverse mutagenicity assay with strains of *S. typhimurium* and *E. coli.*

Reviewer Comments: The problems with this study included the use of only 2 doses of diclofenac, the transmission of UV light was through the dish top and agar of the culture plates and the positive control of strain 1535 did not demonstrate a positive response. Therefore the study may not be an accurate test of photomutagenitic potential of Zy-17727B.

Study no: V4405/02
E-Location: CTD 4.2.3.3.1.8
Conducting laboratory and location: [redacted]

Date of study initiation: Feb 13, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Zy-17727B diclofenac sodium/DS 01, batch number 1000076000, [redacted] purity,
(impurity: [redacted] was [redacted],
Vehicle: dimethylsulfoxide (DMSO), final concentration was 1%

Methods

Strains/species/cell line:
Salmonella typhimurium TA 98, 100,1535, 1537
Escherichia coli WP2 *uvrA*

Strain	Amino acid mutation	LPS	UV-repair	R-factor
TA 1535	His G46	rfa$^-$	uvrB$^-$	-R
TA 1537	His C3076	rfa$^-$	uvrB$^-$	-R
TA 98	His D3052	rfa$^-$	uvrB$^-$	+R
TA 100	His G46	rfa$^-$	uvrB$^-$	+R
WP2 *uvrA*	Trp	rfa$^+$	uvrA$^-$	-R

[1] rfa : this mutation causes partial loss of the lipopolysaccharide (LPS) barrier that coats the surface of the bacteria; it increases the permeability to large molecules, e.g. crystal violet
uvrB/A : these mutations comprise a deletion of a gene coding for the DNA excision repair system, which results in greatly increased sensitivity in detecting many mutagens including UV irradiation
R-factor : the R-factor strains contain the plasmid pKM 101, which increases chemical and spontaneous mutagenesis by enhancing an error-prone DNA-repair system normally present in *S. typhimurium*. It carries an ampicillin resistance gene

Doses of Zy-17727B used in definitive study:

5 and 1.67 mg Zy-17727B per ml of bacteria suspension
(The actual concentrations of the test substance in the test solutions were not determined.)

Doses of UV irradiation:
Four doses of UV irradiation ranging from 15 to 6000 mJ/cm^2.

Basis of dose selection:
Zy-17727B:

UV-radiation:
The highest UV irradiation dose was chosen to result in a weakly mutagenic response in the negative control compared to the cultures without UV irradiation, as evidenced by a more than 2-fold increase in the mean number of revertants.

Negative controls: DMSO 1%

Positive controls:

Positive control substances	
Strain	**Substance**
TA 1535, 1537, 98 and 100	chloropromazine: 10 µg/ml
WP 2 *uvrA*	8-methoxypsoralen: 3 µg/ml

Incubation and sampling times:

The bacterial suspensions were exposed to solar simulated UV irradiation through the lids of Petri dishes in the presence and absence of the test substance or the positive control. The light source for the UV irradiation consisted of two [redacted] sunlamps [redacted], [redacted] s). The UV emission spectrum of the lamp and the transmission spectrum of the lids of the Petri dishes nre presented in Annex 2 (spectra supplied by [redacted], [redacted]). The UVA irradiation intensity was monitored with a calibrated radiometer ([redacted] and was 2.5 + 0.05 mW/cm^2.

After irradiation the bacterial reverse mutation test procedure was followed: to 2 ml molten top agar (Containing 0.6% agar, 0.5% NaCl and 0.05 mM L-histidine HCl, 0.05 mM biotin for the *S. typhimurium* strains, and supplemented with 0.05 mM tryptophan for the *E. coli* strain) maintained at 46°C, was added: 0.1 ml of the bacterial suspension (each test substance-UV-Combination). The mixture was poured onto minimal glucose agar plates. All determinations were made in triplicate. The plates were incubated for three days at 37°C. Negative controls (solvent) and positive controls were run simultaneously Thereafter his' and trp' revertants were counted.

There were no tests with an S9 metabolic activation preparation.

1 Page(s) Withheld

✓ Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

Results

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

Stocks in the freezer of each strain were checked for histidine or tryptophan requirement and for sensitivity to ampicillin, crystal violet and UV irradiation

The mutagenicity test was considered valid if the mean number of revertant colonies of the control values of the strains are within accepiable ranges and if the mean number of revertant colonies of the positive controls with UV irradiation result in a two-fold or more increase compared to the concomitant cultures without UV irradiation.

Annex 3 Acceptable ranges for negative control data

Strain	revertant colonies per plate: negative control, acceptable range
TA 1535	13 - 70
TA 1537	4 - 30
TA 98	20-90
TA 100	100-220
WP2 *uvrA*	18-50

A test substance is considered to be photomutagenic if the mean number of revertant colonies on the plates with the test substance and UV-irradiation is increased two-fold or more compared to the plates without the test substanoe but with UV-irradiation, together with a demonstrable UV dose-related increase.

Cytotoxicity was defined as a reduction in the number of revertant colonies and/or a clearing of the background lawn of bacterial growth.

No statistical analysis is performed. Both numerical significance and biological relevance are considered together in the evaluation.

Study outcome:

In all strains except TA1537 the highest UV dose induced a 2.8-7.9-fold increase in the mean number of revertants in the negative control. In TA1537 phototoxicity, as evidenced by a decrease in the mean number of revertant colonies was observed at the highest UV dose in the presence of the test substance, but not in the presence of the unirradiated test substance, indicating that the UV dose was sufficiently high.

In strain TA 1535 and WP2 uvrA phototoxicity was observed with the combination of the highest UV dose and 5 and 1.67 µg/ml test substance, respectively. In strain TA 1537 phototoxicity was observed with the highest UV dose at 1.67 mg/ml, and with all UV doses at 5 mg/ml (except at 3000 mJ/cm^2, where no toxicity was observed; possibly the test substance was not added to the bacteria suspension). In addition, Zy-17727B active substance was toxic to TA 100, as evidenced by a decrease in the mean number of revertants compared with the negative control also in the absence of UV radiation. The observed phototoxicity indicated that the test substance concentration and UV doses were sufficient high.

In all strains, Zy-17727B did not cause an increase in the number of revertant colonies appearing in the test plates of cultures with UV radiation compared to the background spontaneous reversion rate observed with the concomitant cultures without UV radiation.

The positive controls gave the expected (at least 2-fold) increase in the number of his' and trp revertants at at least one UV dose.

It is concluded that the results obtained in this study demonstrated that under the conditions employed Zy-17727B active substance was not photomutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, and TA1537, or in the Escherichia coli strain WP2 uvrA.

TA 1535 (Mean ± sd)

UV (mJ/cm^2)	**Negative control DMSO**	**Zy-17727B**		**Positive control CPZ** (10 µg/ml)
		1.67 mg/ml	5 mg/ml	
0	14 ± 3	11 ± 4	12 ± 4	12 ± 2
38				19 ± 8
75				28 ± 4
150				33 ± 3
300				23 ± 3
375	16 ± 4	11 ± 6	11 ± 6	
750	21 ± 3	10 ± 5	13 ± 3	
1500	29 ± 6	9 ± 2	10 ± 4	
3000	40 ± 6	9 ± 3	1 ± 1	

TA 1537 (mean ± sd)

UV (mJ/cm^2)	**Negative control DMSO**	**Zy-17727B**		**Positive control CPZ** (10 µg/ml)
		1.67 mg/ml	5 mg/ml	
0	12 ± 2	6 ± 3	9 ± 6	13 ± 1
75				254 ± 24
150				342 ± 45
375				143 ± 38
750	13 ± 3	4 ± 0	0 ± 1	5 ± 1
1500	8 ± 2	4 ± 3	1 ± 1	
3000	14 ± 6	3 ± 2	15 ± 3	
6000	13 ± 2	0 ± 0	0 ± 0	

TA 98 (mean ± sd)

UV (mJ/cm^2)	Negative control DMSO	Zy-17727B		Positive control CPZ (10 µg/ml)
		1.67 mg/ml	5 mg/ml	
0	24 ± 7	16 ± 3	14 ± 6	22 ± 7
75				197 ± 26
150				326 ± 71
375	36 ± 2	20 ± 2	16 ± 4	75 ± 5
750	41 ± 5	28 ± 2	10 ± 3	9 ± 2
1500	54 ± 11	27 ± 4	18 ± 1	
3000	85 ± 11	16 ± 2	10 ± 3	

TA 100 (mean ± sd)

UV (mJ/cm^2)	Negative control DMSO	Zy-17727B		Positive control CPZ (10 µg/ml)
		1.67 mg/ml	5 mg/ml	
0	82 ± 18	61 ± 11	38 ± 4	92 ± 5
75	102 ± 1	67 ± 5	52 ± 9	388 ± 46
150	127 ± 8	56 ± 6	31 ± 4	566 ± 52
300	149 ± 13	71 ± 3	52 ± 6	341 ± 24
600	318 ± 19	55 ± 13	36 ± 5	36 ± 8

WP *uvrA* (mean ± sd)

UV (mJ/cm^2)	Negative control DMSO	Zy-17727B		Positive control CPZ (10 µg/ml)
		1.67 mg/ml	5 mg/ml	
0	34 ± 5	31 ± 8	17 ± 3	31 ± 3
15				38 ± 3
38				31 ± 1
75				16 ± 4
150				84 ± 9
375	45 ± 7	32 ± 6	21 ± 5	
750	75 ± 4	37 ± 4	18 ± 4	
1500	123 ± 18	31 ± 2	16 ± 1	
3000	269 ± 24	15 ± 3	19 ± 5	

Study title: **Photomutagenicity test with Zy-17727B active substance on formation of chromosomal aberrations in cultured Chinese hamster ovary cells**

Key Findings: Zy-17727B (diclofenac sodium) was photoclastogenic at 25 ug/mL with 16 minutes of exposure of simulated solar UV irradiation, but not at lowere doses of Zy-17727B or shorter times of UV radiation exposure.

Study no.: V4402/02
E-Location: CTD 4.2.3.3.1.17
Conducting laboratory and location: [redacted]

Date of study initiation: final report July 22, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Zy-17727B (Diclofenac sodium /DS 01), Batch 1000076000, Purity [redacted]
Vehicle: dimethylsulfoxide (DMSO)

Methods

Strains/species/cell line: Chinese hamster ovary cells (CHO K-1 line)

Doses used in definitive study: 0.1,0.31,0.93, 2.78, 8.33, 25 µg/mL with 0, 2, 4, 8, 16 minutes of simulated solar UV radiation

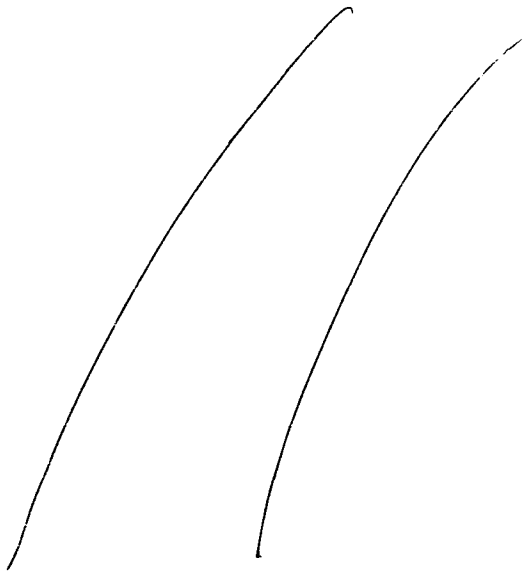
Basis of dose selection:
First pilot photomutagenicity test: A dose-range finding study consisted of three concentrations of Zy-17727B (750, 1500 and 3000 µg/mL) in combination with 5 doses (0, 8, 16, 32, and 64 minutes) of simulated solar UV radiation. In the 0 minutes and 64 minutes of UV radiation treatment groups, all cells were heavily damaged or dead at all doses used.
Dose Range Finding Test: Cells were incubated with Zy-17727B (12, 23, 47, 94, 187.5, 375, 750 µg/mL) for 15.5 hours, but not UV radiated. At 375 and 750 µg/ml, all cells were dead at the end of the incubation, and at 187.5 µg/mL, 50% of the cells were heavily damaged.
Second pilot photomutagenicity test: A second dose-range finding study consisted of six concentrations of Zy-17727B (6.25, 12.5, 25, 50, 100, 200 µg/mL) in combination with 5 doses (0, 8, 16, 32, and 64 minutes) of simulated solar UV radiation. Without UV radiation, all cells were dead at 50, 100 and 200 ug/ml concentrations. At 25 µg/mL, some dividing cells occurred. Cells incubated with 6.25, 12.5, and 25 ug/mL were used for mitotic index scoring. Chromosomal aberrations were observed at the highest concentration analysed (25 µg/mL) without UV radiation. In the 32 minutes and 64 minutes of UV radiation groups, all cells were dead 2 hours before harvesting. In the 16 minute UV radiation groups, all cells in the 100 and 200 µg/mL were dead, and cells 20% of the cells aof the 50 ug/mL were shriveled. Cells in the 6.25, 12.5, 25 and 50 ug/mL groups were scored and structural aberrations were observed at all these concentrations.

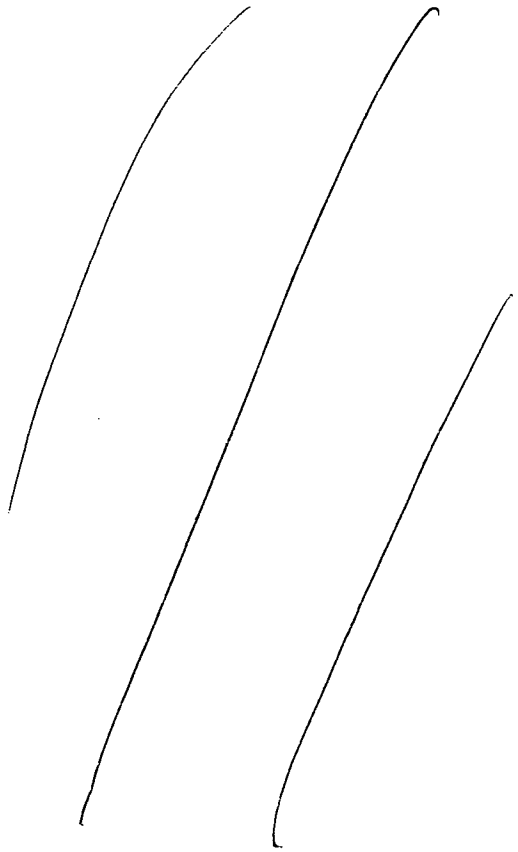
In the 8 minutes of UV radiation group, all cells were dead at the 200 ug/mL concentration. Some cells in the 100 ug/mL concentration were also affected. Doses of 6.25, 12.5, 25, 50 and 100 ug/mL were mitotic index scored. Chromosomal aberrations occurred at the 50 and 100 ug/mL concentrations.

Negative controls: dimethylsulfoxide (DMSO), 1% (v/v)

Positive controls: 8-methoxypsoralen (0.5 µg/ml) in combination with 3 doses of simulated solar UV radiation (1, 2, and 3 minutes in pilot studies, 1 minute in the main study)

Incubation and sampling times:
Duplicate cultures were incubated for 18 hours following treatment with or without UV radiation, then harvested and analysed for structural chromosomal aberrations. Before irradiation, culture medium was replaced with Hanks balanced salt solution containing Zy-17727B, positive, or negative controls, since it absorbs minimal UV light. Cultures were exposed to simulated solar UV radiation through the top of the culture flasks. Cells were washed with phosphate buffered saline and supplied with complete medium. The light source consisted of two [redacted] sun lamps. Two hours before the end of the total incubation period, the cells of the remaining cultures were arrested in the metaphase stage of mitosis by the addition of colcemid (final concentration: 0.1 mM medium), incubated an additional 2 hours then processed for cytological analysis.





Study Validity

The slides were coded by a qualified person not involved in scoring the slides, to enable "blind" scoring. At least 1000 nuclei in each culture were examined (500 on each slide) to determine the mitotic index (percentage of cells in mitosis). After the results of the mitotic index scoring and the observations with respect to the quality of the metaphases had been obtained, a selection of the concentrations of the test substance, to be analysed for chromosomal aberrations (main photomutagenicity test only), was carried out.

For each treatment group, 200 well-spread metaphases per concentration (100 metaphases per culture), each containing 20-22 centromeres, were analysed by microscopic examination for chromatid-type aberrations (gaps, breaks, fragments, interchanges), chromosome-type aberrations (gaps, breaks, minutes, rings, dicentrics) and other anomalies, such as interstitial deletions, endoreduplication, polyploidy and multiple aberrations (>10 aberrations per cell, excluding gaps), according to the criteria recommended by Savage (1975). If heavily damaged or endoreduplicated cells were observed, these cells were recorded but the cells were not counted and included in the 200 analysed cells.

The incidence of UV irradiation of the positive control substance, S-methoxypsoralen (0.5 µg/mL) resulted in a statistically significantly increase in the number cells with structural chromosomal aberrations compared to the concomitant UV irradiated vehicle. The negative controls were within the historical range.

The main criteria for a positive photomutagenic response are an increase in the percentage of cells with structural chromosomal aberrations of a treatment with the test substance and UV irradiation compared to the concurrent control without test substance but with UV irradiation (all in duplicate cultures), or a clear concentration- related or UV dose-related effect. Otherwise a test substance is considered to be negative in the photomutagenicity test.

Gaps (achromatic lesions) are recorded separately and not included in the final assessment of clastogenic activity.

Both statistical significance and biological relevance are considered together in the evaluation of the results.

Specific Methods and Results for Each Study

First Pilot Photomutagenicity Study

In the first pilot photomutagenicity test, three different concentrations of the test substance (750, 1500 and 3000 µg/mL) were examined in combination with five doses (0, 8, 16, 32 and 64 minutes) of simulated solar UV radiation. The vehicle dimethylsulfoxide (DMSO) was used as negative control and 8-methoxypsoralen, in combination with three doses (1, 2 and 3 minutes) of simulated solar UV radiation, was used as positive control and were run simultaneously with the test substance. In the treatment group combined with 64 minutes and in the treatment group combined without UV radiation, all cells were heavily damaged or dead, at all the concentrations used. Therefore, it was decided to stop and discard the first pilot photomutagenicity test and to perform a dose-range finding test first, prior to the start of the second pilot photornutagenicity test.

Dose-Range Finding Study

In the dose-range finding test, the cells were treated continuously for 15.5 hours with seven different concentrations of the test substance (750, 375, 187.5, 94, 47, 23 and 12 µg/mL). The cells were not UV radiated. At the two highest concentrations used (750 and 375 µg/mL), all cells were dead at the end of the treatment period. At the concentration of 187.5 µg/mL 50 % of the cells were heavily damaged at the end of the treatment period. Based on the results of the dose-range finding test, six different concentrations of the test substance were selected for the second pilot photomutagenicity test.

Second Pilot Photomutagenicity Study

The second pilot photomutagenicity test was started with six different concentrations of the test substance (200, 100, 50, 25, 12.5 and 6.25 µg/mL) in combination with five doses (0, 8, 16, 32

and 64 minutes) of simulated solar UV radiation. DMSO was used as negative control in each treatment group and 8-methoxypsoralen (0.5 µg/ml, irradiated for 1, 2 and 3 minutes) was used as positive control. The negative and positive control substances were run simultaneously with the test substance.

In the group without UV radiation, all cells were dead at the three highest concentrations used (200, 100 and 50 µg/mLl). At the concentration of 25 µg/ml, the cells were affected and less dividing cells were observed. The three lowest concentrations (2S, 12.5 and 6.25 µg/mL), together with the negative and positive controls, were selected for mitotic index scoring. During the mitotic index scoring, chromosomal aberrations were observed at the highest concentration analysed (25 µg/mL).

In the 32 minutes and 64 minutes UV radiation groups, two hours before harvesting, all cells were dead. These groups were discarded from the test. In the 16 minutes UV radiation group, all cells were dead at the two highest concentrations used (200 and 100 µg/ml). At the concentration of 50 µg/ml, the cells were affected and 20 % of the cells were shrivelled. The four lowest concentrations (50, 25, 12.5 and 6.25 µg/mL), together with the negative controls, were selected for mitotic index scoring. During mitotic index scoring, structural chromosomal aberrations were observed at all the selected concentrations for mitotic index scoring.

In the 8 minutes UV radiation group, all cells were dead at the highest concentration used (200 µg/mL). At the concentration of 100 µg/ml, the cells were affected and less dividing cells were obbserved. Five concentrations (100, 50, 25, 12.5 and 6.25 µg/mL), together with the negative controls, were selected for mitotic index scoring. During mitotic index scoring, structural chromosomal aberrations were observed at the two highest concentrations (50 and 100 µg/mL).

Results obtained in the second pilot photomutagenicity test indicated that Zy-17727B was photoclastogenic to the cells at the concentration of 25 µg/mL without simulated solar UV irradiation, but this was not confirmed in the main photomutagenicity study.

Main Photomutagenicity Study

The main photomutagenicity test was started with six different concentrations of the test substance (25, 8.33, 2.78, 0.93, 0.31 and 0.1 µg/mL) in combination with five doses (0, 2, 4, 8 and 16 minutes) of simulated solar UV radiation. DMSO was used as negative control and 8-methoxypsoralen (0.5 µg/ml, irradiated for 1 minute) was used as positive control. The negative and positive control substance was run simultaneously with the test substance. Based on the results of the mitotic index scoring, three different concentrations of the test substance (25, 8.33 and 2.78 µg/ml), together with the negative and positive control cultures, were examined in combination with four doses (0, 4, 8 and 16 minutes) of simulated solar UV radiation, for the induction of chromosomal aberrations. Following treatment with or without UV radiation, the cells were incubated for 18 hours, harvested and analysed for structural chromosomal aberrations. All combinations were tested in duplicate cultures. The highest concentration analysed for the induction of structural chromosomal aberrations was based on toxicity of the Zy-17727B to the cells. In the main photomutagenicity test, at the concentration of 25 µg/ml, combined with 16 minutes UV radiation, a statistically significantly increase in the number of

cells with structural chromosomal aberrations was observed, when compared to the concurrent control without test substance but with UV radiation.

Results obtained in the main photomutagenicity test indicated that Zy-17727B was photoclastogenic to the cells at the highest concentration analysed (25 µg/mL) in combination with the highest possible dose of 16 minutes of simulated solar UV irradiation. However, Zy-17727B was not photoclastogenic at 8.33 µg/mL and lower concentrations at all UV doses tested. In addition, Zy-17727B was also not photoclastogenic up to 25 µg/mL at UV doses of 8 minutes or less.

Summary table of the results of the Main photomutagenicity test with Zy-17727B active substance

Number of cells showing structural chromosomal aberrations:positive/total (% positive of total)					
UV-radiation dose (minutes)	Negative control (DMSO)	Zy-17727B active substance (2.78 µg/ml)	Zy-17727B active substance (8.33 µg/ml)	Zy-17727B active substance (25 µg/ml)	Pos. control (8-MOP) (0.5 µg/ml)
0	1/200 (0.5)	4/200 (2.0)	1/200 (0.5)	0/200 (0.0)	2/200 (1.0)
1	1/200 (0.5)				136/200 (68.0)
4	2/200 (1.0)	1/200 (0.5)	3/200 (1.5)	1/200 (0.5)	
8	3/200 (1.5)	2/200 (1.0)	1/200 (0.5)	2/200 (1.0)	
16	1/200 (0.5)	2/200 (1.0)	4/200 (2.0)	19/200 (9.5)	

Study title: **In vivo (bone marrow) chromosomal aberration test in rats treated with Zy-17727B active substance**

Key Findings: Doses of Zy-17727B that induced clinical signs of toxicity were cytotoxic to bone marrow cells, but did not induce structural chromosomal aberrations.

Study no.: V4402/14
E-Location: CTD 4.2.3.3.2.8
Conducting laboratory and location:

Date of study initiation: July 2, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Zy-17727 (diclofenac sodium/DS 01), Batch 1000076000, Purity
Vehicle: corn oil
Methods

Strains/species/cell line: Male rats WI] WU BR; 8-10 weeks of age)
Because no sex differences were observed in an acute oral toxicity test, carried out by the sponsor, only male rats were used. **(Reviewer comment:** lack of gender differences in acute toxicity tests does not preclude the lack of gender differences in clastogenic responses).

Doses used in definitive study: 25, 50 and 100 mg/kg Zy-17727B

Basis of dose selection:

A range finding toxicity test determined the tolerable dose levels to be used in the main study. The acute oral toxicity for this compound in rats is 98 mg/kg (data provided by the sponsor). Therefore, the highest dose level for this range finding toxicity test was 100 mg/kg with three additional dose levels of 25, 50 and 75 mg/kg. After a fasting period, these four concentrations were administered (20 ml/kg) once by gavage to 3 males/dose. Observations included clinical symptoms (as a reaction to the treatment), and were recorded the first 4 hours post-treatment and at least once daily thereafter. Body weights were obtained on day 0 prior to dosing and on day 3.

Rats of the 100 mg/kg group exhibited sluggishness, a hunched back and piloerection, 1 and 4 hours after administration and weight loss. One animal was found dead on day 3. Rats of the 75 mg/kg group exhibited sluggishness, a hunched back and piloerection, 1 and 4 hours after administration and weight loss. There were no deaths. Rats of the 50 mg/kg group all lost weight. No other clinical abnormalities were observed in these rats. No clinical abnormalities were observed in 25 mg/kg group.

Negative controls: corn oil

Positive controls: mitomycin C.

Incubation and sampling times:

The study consisting of 5 groups of animals is presented in the table below. All animals were weighed prior to dosing and prior to sacrifice. The test substance and the vehicle (corn-oil) were administered to the rats once via the oral route (by gavage; 20 ml/kg).

Two hours prior to sacrifice, animals were injected intraperitoneally with colchicine (Aldrich Chemical, The Netherlands) at a concentration of 4 mg/kg to accumulate metaphase cells. At approximately 24 hours after administration, 5 rats of all treatment groups (A, B, C, D and E) were killed by decapitation. At approximatley 48 hours after administration, 5 negative control rats (group A) and 5 rats treated with the highest dose level of the test substance (group D; 100 mg/kg) were killed by decapitation. After sacrifice, bone marrow cells were removed from the femurs and processed for cytological analysis.

Summary of experimental design and killing schedule: Main study					
test substance: Zy-17727B active substance			vehicle: corn-oil		
route: orally (by gavage)			dosing volume: 20 ml/kg-bw		
Test substance	Dose level and dosing regime	group code/ colour code	scheduled killing after treatment (h)	number and sex of animals	coding of animals
negative control (corn-oil)	20 ml/kg-bw once orally	A/white	24h 48h	5 males 5 males	2, 4, 6, 8, 10 12, 14, 16, 18, 20
Zy-17727B active substance	25 mg/kg-bw once orally	B/blue	24h	5 males	22, 24, 26, 28, 30
Zy-17727B active substance	50 mg/kg-bw once orally	C/green	24h	5 males	42, 44, 46, 48, 50
Zy-17727B active substance	100 mg/kg-bw once orally *	D/red	24h 48h	5 males 5 males	62, 64, 66, 68, 70 72, 74, 76, 78, 80
Mitomycin C# (pos. control)	3.0 mg/kg-bw	E/yellow	24h	5 males	82, 84, 86, 88, 90

Derived from J503; lot no. 49H2508. Single intraperitoneal injection with 0.3 mg/ml mitomycin C (vehicle: saline; volume 10 ml/kg-bw; dose level 3.0 mg/kg-bw)

* At this dose level, three reserve males were included to replace any mortality

Results

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

The slides were randomly coded by a qualified person. This person was involved in analysing the slides. The slides were analysed "blind". Two slides per animal were examined. One thousend cells, 500 cells per slide, were examined to deternine the percentage of cells in mitosis (mitotic index). Of each animal 100 well-spread mataphases (50 metaphases per slide), each containing 40-42 centromeres, were analysed for chromatid-type (gaps, breaks, fragments, interchanges)

and chromosome-type (gaps, breaks, rings, dicentrics) aberrations and other anomalies (endoreduplication, polyploid cells, heavily damaged cells), according to the criteria recommended by Savage (1975). Heavily damaged or endoreduplicated cells that were observed, were recorded but the cells were not counted and included in the 100 analysed cells per animal. The Vernier readings of all aberrant metaphases scored were recorded.

Data were analysed statistically by the Fisher's exact probability test (two-sided) to determine significant diKerences between treated and control poups. Statistical significance was not the only determining factor for a positive response. Biological relevance of the results was considered first. Statistical methods were used as an aim in evaluating the test results. In general, there two criteria for determing a positive result, such as:

1. A clear dose-related increase in the relative number of cells with structural chromosomal aberrations over the concurrent control frequencies.
2. A clear statistically significantly increase ($p<0.05$) in the number of cells with chromosomal aberrations in a single dose group at a single sarnpling time.

An increase in polyploidy may indicate that the test substance has the potential to induce numerical chromosomal aberrations. An increase in endoreduplication may indicate that the test substance has the potential to inhibit cell-cycle progression. A test substance for which the results do not meet the above mentioned criteria is considered non-mutagenic in this test. Equivocal results will be clarified by further testing preferably using a rnodification of experimental conditions. Gaps (achromatic lesions) were recorded separately and not included in the final assessment of clastogenic activity.

Study outcome:

At both sacrifice times of 24 hours and 48 hours after treatment, no dose of Zy-17727B induced a statistically significant increase in the number of cells with structural chromosomal aberrations, when compared to the vehicle control value. The positive control substance mitomycin C induced the expected statistically significant increase in the incidence of structural chromosomal aberrations.

At the 24 hour post-treatment sacrifice there was no reduction of the mean mitotic index at any dose of Zy 17727B compared to the vehicle control rats. However at 48 hour post-treatment, the mean mitotic index of the 100 mg/kg group was reduced to 62 % of that of the mean mitotic index of the vehicle control rats, demonstrating that Zy-17727B reached the target cells of the bone marrow and resulted in cytotoxicity to the bone marrow cells.

Zy-17727B was cytotoxic to the bone marrow cells at the highest dose tested, but did not induce structural chromosomal aberrations in bone marrow cells. Thus, Zy-17727B was not clastogenic to the bone marrow cells of male rats.

Table 1 Chromosomal aberrations in bone marrow cells of male rats, treated with the test substance Zy-17727B active substance: summarized data
harvesting time: 24 hours after administration

treatment	dose level	group	N[1]	n	Number of cells with aberrations[2]				% cells with aberrations		Mitotic[3] index
					gaps	breaks	exchanges	multiple	incl. gaps	excl. gaps	
Vehicle control (corn-oil)	20 ml/kg-bw	A	500	5	1	0	0	0	0.2	0.0	7.9
Test substance	25 mg/kg-bw	B	500	5	2	0	0	0	0.4	0.0	8.0
Test substance	50 mg/kg-bw	C	500	5	1	0	0	0	0.2	0.0	9.9
Test substance	100 mg/kg-bw	D	500	5	2	0	0	0	0.4	0.0	9.5
Positive control* (mitomycin C)	3.0 mg/kg-bw	E	500	5	6	48	21	0	15.0***	13.8***	7.3

* the positive control mitomycin C was administered once intraperitoneally

[1] N = number of metaphases analyzed (100 metaphases per animal)

[2] gaps include chromatid and isochromatid (chromosome) gaps
breaks include chromatid and isochromatid (chromosome) breaks, interstitial deletions (minutes) and acentric fragments not associated with any obvious exchange process
exchanges include chromatid and chromosome inter- and intraexchanges
multiple aberrations more than 10 aberrations (excl. gaps) per metaphase

[3] mean percentage of metaphases determined in 1000 nuclei per animal

n = number of rats analyzed

Statistics: Fisher's exact probability test (two-sided) * P < 0.05; ** P < 0.01; *** P < 0.001

Table 2 Chromosomal aberrations in bone marrow cells of male rats, treated with the test substance Zy-17727B active substance: summarized data
harvesting time: 48 hours after administration

treatment	dose level	group	N[1]	n	Number of cells with aberrations[2]				% cells with aberrations		Mitotic[3] index
					gaps	breaks	exchanges	multiple	incl. gaps	excl. gaps	
Vehicle control (corn-oil)	20 ml/kg-bw	A	500	5	0	1	0	0	0.2	0.2	10.0
Test substance	100 mg/kg-bw	D	500	5	2	4	0	0	1.2	0.8	6.2

* the positive control mitomycin C was administered once intraperitoneally

[1] N = number of metaphases analysed (100 metaphases per animal)

[2] gaps include chromatid and isochromatid (chromosome) gaps
breaks include chromatid and isochromatid (chromosome) breaks, interstitial deletions (minutes) and acentric fragments not associated with any obvious exchange process
exchanges include chromatid and chromosome inter- and intrachanges
multiple aberrations more than 10 aberrations (excl. gaps) per metaphase

[3] mean percentage of metaphases determined in 1000 nuclei per animal

n = number of rats analysed

Statistics: Fisher's exact probability test (two-sided) * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$

2.6.6.5 Carcinogenicity

Two carcinogenic studies were submitted to NDA 19-201 for Voltaren. These were oral dosing studies in rats, mixing diclofenac in the feed. The earlier study (1976) was conducted prior to GLP regulations were developed and was not reviewed at the time of NDA 19-201 submission. The mouse study, also a dietary diclofenac study, was requested as second species study shortly after the development of the carcinogenicity guidance recommending two animal species be studied. This was submitted in 1989, after Voltaren's approval, and reviewed in 1993. The dermal carcinogenic and photococarcinogenic potential of diclofenac were evaluated for approval of Solaraze, NDA 21-005, a topical diclofenac product approved in 2000, and referenced for this 505(b)(2) NDA.

ORAL (DIETARY) CARCINOGENICITY STUDIES

The results of the carcinogenicity study with diclofenac sodium in mice and rats at doses of 0.1, 0.3, 1 and 2 mg/kg were similar with regards to mortality and gastrointestinal irritation. No tumorigenic effect of diclofenac sodium was observed. This study included dietary and plasma diclofenac determinations, signs, body weights, food and water intake, hearing and ocular examinations, hematology and blood chemistry, and a full pathology assessment. Achieved dosages, based on frequent dietary analysis results, were between 103 and 110% of nominal except in high dose females, which received 127% of the nominal 2 mg/kg/day dose. Mortality was increased at the nominal dosages of 1 and 2 mg/kg/day. At the 2 mg/kg/day dietary level, all but one surviving animal, a female, had died by Week 67. The primary pathology in these early deaths was perforation and/or ulceration of the small intestine with inflammation and adhesions in the peritoneum and adjacent organs. Body weight was reduced in both of these groups, but food intake was not affected. Water intake was initially decreased at the high dosage but increased in females as the study progressed. Water intake was not affected at lower dosages. There was no effect on hearing or on the eyes.

Hematology indicated anemia with reticulocytosis and leukocytosis in animals treated at 1 or 2 mg/kg/day from Week 27 onwards. At the terminal examination one male receiving 0.3 mg/kg/day was anemic. Blood chemistry indicated changes in plasma proteins and alkaline phosphatase activity at the high dosage that possibly associated with intestinal pathology but no other consistent changes. There was no effect on organ weights. Analysis of results from histopathology examinations identified only one significant positive trend, for thymic lymphomas in females. The incidence of primary malignant lymphoma was, however, not affected. Chronic gastrointestinal ulceration was confirmed especially of the in males and females treated at 1 or 2 mg/kg/day. These changes were associated with increased incidences of amyloidosis and extramedullary hematopoiesis. The predominant complications were endocardial thrombosis with thrombo-embolism, hemosiderosis and extramedullary hemopoiesis in the spleen, and centrolobular necrosis in the liver.

DERMAL CARCINOGENCITY AND PHOTOCOCARCINOGENICITY STUDIES

The information below is obtained from the Sponsor's summary of the studies submitted for approval of Solaraze™ (NDA 21-005). The Sponsor is referencing this information as a 505(b)(2) application and does not own this data as described in section 2.6.1 Introduction and Drug History, subsection: For (b)(2) Applications.

The carcinogenic effects of topical diclofenac sodium was evaluated in albino mice for Solaraze™ approval (NDA 21-005). Groups administered 0.09% (5 mg/kg) and 0.18% (10 mg/kg) diclofenac gel exhibited mortality and gross pathological changes within the first month of treatment. The primary pathology consisted of perforation of the glandular portion of the stomach. Groups administered 0.035 % (2 mg/kg) exhibited 73% mortality in males following 100 weeks treatment. The mortality in the females were similar to the control groups. No evidence of drug related skin or systemic tumorigenic effects were observed in any of the animals.

The influence of topically administrated diclofenac sodium on dermal carcinogenicity induced by simulated solar radiation was investigated in hairless mice also for Solaraze™ approval (NDA 21-005). Two groups of mice were irradiated with simulated solar radiation (UVR), one at 1200 RBU/week and the other at 600 RBU/week. Five other groups of mice were treated with diclofenac gel at 0.0045% (0.36 mg/kg), 0.009% (0.72 mg/kg), 0.018% (1.4 mg/kg) or 0.035% (2.8 mg/kg) and irradiated at 600RBU/week. The group treated with 1200 RBU exhibited high mortality and increase in tumors after week 30. No significant differences in mortalities were observed between groups treated with diclofenac gel 0.0045% (0.36 mg/kg), 0.009% (0.72 mg/kg), 0.018% (1.4 mg/kg), 0.035% (2.8 mg/kg) and 600RBU and 600RBU alone during the test period. By week 40, with exposure to 600 RBU, >50% of untreated control animals, <50% of animals dosed with vehicle, 0.0045%, 0.009% and 0.018% diclofenac gel, and 75% of animals dosed with 0.035% diclofenac gel had detectable tumors. By week 45 tumor prevalence was similar between the untreated and high dose groups. The tumor size distribution was similar in all groups exposed to 600 RBU. No dose-related, statistically significant differences in median time to tumor onset for tumor >1 mm was observed when treated animals were compared to untreated control animals exposed to 600 RBU/week. A statistically significant decrease in median time to tumor onset was observed in animals receiving 0.035% diclofenac when diclofenac group was compared to the vehicle control group. The decrease was significant only when data from both sexes were pooled.

Table 5-1: Summary of carcinogenicity tests with diclofenac sodium

Species, strain	Route	Weeks	Groups (m + f)	Dosages mg/kg/day	Year of study	Report number
Mouse, Tif MAGf	Oral, dietary	104	80 + 80[a]	0, 0.1, 0.3, 1, 2	1985-7	GU841110
Rat, Tif RAlf	Oral, dietary	104	90 + 90[b]	0, 0.25, 0.5, 1, 2	1978-80	GU785271
Rat, Sprague-Dawley	Oral, dietary	98	60 + 60[c]	0, 0.25, 1, 2	1971-3	8-76

[a] Includes 10+10 clinical pathology and 20+20 toxicokinetic satellites
[b] Includes 20+20 clinical pathology and 10+10 toxicokinetic satellites and 10+10 for a 52-week interim investigation
[c] Includes 10+10 for 52-week interim investigations.

Study Number / Location (CTD. Section)	Species and Strain	Doses (mg/kg/day)[a] / Method of Administration / Duration of treatment	Findings
Oral Dosing Carcinogenicity Studies			
Rat			
8-76 eCTD 4.2.3.4.1.3 Pre-GLP	Rat Sprague-Dawley M=60 F=60	0, 0.25, 1, 2 GP45840 blended with powdered feed, oral administration for 98 weeks 2 mg/kg for 59 weeks due to mortalities	NOAEL= 0.25 mg/kg/day No increase in tumor incidence. 0.25 mg/kg = unremarkable 1-2 mg/kg =dose-related increases in mortality rates (females suffered higher mortality rates than males at equivalent doses); peritonitis, gastrointestinal ulceration; neutrophilic leukocytosis; and anemia
GU 785271 eCTD 4.2.3.4.1.2 GLP: yes	Rat Tif RAIf	0, 0.25, 0.5, 1, 2 GP4584 blended with powdered feed, oral administration for 104 weeks	NOAEL= not obtained? Increase in tumors within ranges of historical controls. 0.25 to 0.5 mg/kg = scattered clinical chemistry and histopathological deviations; 1-2 mg/kg = dose-related increases in mortality rates; decreased body weight and food consumption; clinical chemistry deviations- anemia, neutrophilia, hypoproteinenia, gastrointestinal perforation; endocarditis
Mouse			
GU 841110 eCTD 4.2.3.4.1.1 GLP: yes	Mouse Tif MAGf M=80 F=80	0, 0.1, 0.3, 1, 2 blended with powdered feed, oral administration For 104 weeks High dose based on: Study 785271 (24 months carcinogenicity – rats)	NOAEL= 0.3 mg/kg/day Mortality increased at 1 and 2 mg/kg/day. Primary pathology in dead animals were perforation and/or ulceration of small intestine, with inflammation of adhesions in the peritoneum and adjacent organs.

		and Study 840321 (28 day toxicity study in mice): maximum tolerated dose = 2mg/kg	No significant increases in tumors

Daily Dose (mg/kg)	0 (Control)		> 0.1 (Group 1)		> 0.3 (Group 2)		> 1(Group 3)		> 2 (Group 4)	
Gender	M	F	M	F	M	F	M	F	M	F
Toxicokinetics: AUC										
Number of Animals at Start*	80	80	80	80	80	80	80	80	80	80
Died/Sacrificed Moribund	39	38	36	39	42	39	53	41	59	59
Terminal Sacrifice	20	21	24	21	18	18	7	15	0	1
Survival (%) (of initial 80)	25%	26%	30%	26%	23%	20%	9%	19%	0%	1%
Body Weight							Slight decrease from week 25		Moderate decrease from week 20	Moderate decrease from week 20
Food Consumption									Food spillage in weeks prior to death	Food spillage in weeks prior to death
Water consumption									Decrease	Increase after 28 weeks
Hematology					Anemia 1M at week 105		From week 27, anemia with reticulocytosis and leuco-cytosis in some animals		From week 27, anemia with reticulocytosis and leucocytosis in some animals	
Clinical biochemistry									From week 27, variations in blood PROT and AP	

Cont.
* Each group of 80 mice included 50 for evaluation of carcinogenicity, 10 for hematological and biochemical evaluations and 20 for blood level determinations at month 6, 12, 18 and 24.

Daily Dose (mg/kg)	0 (Control)		> 0.1 (Group 1)		> 0.3 (Group 2)		> 1 (Group 3)		> 2 (Group 4)	
Number Evaluated	M: 59	F: 57	M: 60	F: 60	M: 60	F: 58	M: 60	F: 58	M: 60	F: 60
Number of Animals with Neoplastic Lesions: **none found to be drug-related.** Organ										
Primary site uncertain	1	1		4	1	1	1			
Skin		1		1	1	1				
Subcutaneous tissue			1	1				1		
Mammary gland		5		1		2				
Lymphoretic. Tissue	5	19	2	20	7	23	2	13		
Hemopoietic tissue	3	2				1		2		
Bone marrow				1						
Spleen				2	1	2	1			
Lymph node				1			1			
Mesenteric lymph node	3		1	2	1	1	1	1		
Axillary lymph node				1						
Bone								1		
Lung	10	15	14	9	9	6	3	2		
Pleura			1							
Myocardium					1					
Liver	35	7	35	13	28	8	16	1	1	
Pancreas, exocrine	1	2		1						

Daily Dose (mg/kg)	0 (Control)		> 0.1 (Group 1)		> 0.3 (Group 2)		> 1 (Group 3)		> 2 (Group 4)	
Number Evaluated	M: 59	F: 57	M: 60	F: 60	M: 60	F: 58	M: 60	F: 59	M: 60	F: 60
Intrapancreatic duct	1						1			
Stomach	1	1								
Large intestine	1		2		3		1			
Caecum			1		1					
Kidney					1					
Prostate	1									
Seminal vesicle	2			1						
Urinary bladder		1								
Vagina		1								
Uterus		3		5		3		2		
Ovary		1		1		2		3		
Pituitary gland		5		3						
Adrenal gland					1					
Adrenal cortex	1									
Adrenal medulla		1								
Thymus	1			1		2		2		
Pancreatic islet			1	1						
Harderian gland	15	5	18	6	15	9	15	3	2	2
Mediastinum	21									
Retroeritoneum	1			1						

Daily Dose (mg/kg)	0 (Control)		> 0.1 (Group 1)		> 0.3 (Group 2)		> 1 (Group 3)		> 2 (Group 4)	
Number Evaluated	M: 59	F: 57	M: 60	F: 60	M: 60	F: 58	M: 60	F: 59	M: 60	F: 60
Noteworthy findings										
Gross Pathology							Extensive and chronic perforation and/or ulceration of GI tract, esp. small intestine. Fibrinous and/or fibrous adhesions in abdominal cavity. Enlarged spleen +/or liver.		Extensive and chronic perforation and/or ulceration of GI tract, esp. small intestine. Fibrinous and/or fibrous adhesions in abdominal cavity. Enlarged spleen +/or liver.	
Histopathology – Non-Neoplastic Lesions							Chronic ulceration of GI tract, esp. small intestine.		Chronic ulceration of GI tract, esp. small intestine.	

505(b)(2) Studies (from NDA 21-005 for Solaraze, however, data reproduced here is taken information presented in NDA 22-122)	
Dermal Dosing Carcinogenicity Study and Dermal Dosing Photococarcinogenicity Study	
sba-nda-21-005.pdf (this was also submitted in Jan 1985 in support of NDA 19-201 for Voltaren Tablets; Ciba Geigy)	Section III Single and Repeat Oral Toxicology Studies of the Pharmacology/Toxicology review for NDA 21-005 Solaraze
Study 5.3 (87000)	2-year Dermal Carcingenicity Study of Diclofenac Gel in the Albino Mouse
Study 5.4 (808-002)	A 12-Month Study to Determine the Influence of Diclofenac Topical Gel on Photocarcinogenesis in Hairless Mice

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings
Mouse, albino	Dermal, 0.035% diclofenac gel *	104 weeks	0.125, 0.25, 0.5, 1, 2, 5, 10	M= 60, F =60	No evidence of drug related skin or systemic tumorigenic effects. Groups administered 0.09% (5mg/kg) and 0.18% (10 mg/kg) diclofenac gel exhibited mortality and gross pathological changes within the first month of treatment. The primary pathology consisted of perforation of the glandular portion of the stomach. Groups administered 0.035 % (2 mg/kg) exhibited 73% mortality in males following 100 weeks treatment. The mortality in the females were similar to the control groups.

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings
Mouse, hairless (SKH1(hr/hr)BR	Dermal, Diclofenac gel* and 600RBU UVR Or 600RBU, 1200RBU	52 weeks	0.36, 0.72, 1.44, 2.8.	M=36 F=36	Group treated with 1200 RBU exhibited high mortality and increase in tumors after week 30. No significant differences in mortalities were observed between groups treated with diclofenac gel 0.0045% (0.36 mg/kg), 0.009% (0.72 mg/kg), 0.018% (1.4 mg/kg), 0.035% (2.8 mg/kg) and 600RBU and 600RBU alone during the test period. By week 40, with exposure to 600 RBU, >50% of untreated control animals, < 50% of animals dosed with vehicle, 0.0045%, 0.009% and 0.018% diclofenac gel, and 75% of animals dosed with 0.035% diclofenac gel had detectable tumors. By week 45 tumor prevalence was similar between the untreated and high dose groups. The tumor size distribution was similar in all groups exposed to 600 RBU. No dose-related, statistically significant differences in median time to tumor onset for tumor >1 mm was observed when treated animals were compared to untreated control animals exposed to 600 RBU/week. A statistically significant decrease in median time to tumor onset was observed in animals receiving 0.035% diclofenac when diclofenac group was compared to the vehicle control group. The decrease was significant only when data from both sexes were pooled.

*(Solaraze™ formulation)

Photocarcinogenicity

Species/ Strain	Method of Administration (Vehicle/ Formulation)	Duration of Dosing	Doses (mg/kg)	Gender and No. per Group	Noteworthy Findings
Mouse, hairless SKH1(hr/hr)BR	Dermal, Diclofenac gel* and 600RBU UVR Or 600RBU, 1200RBU	52 weeks	0.36, 0.72, 1.44, 2.8	M=36 F=36	Group treated with 1200 RBU exhibited high mortality and increase in tumors after week 30. No significant differences in mortalities were observed between groups treated with diclofenac gel 0.0045% (0.36 mg/kg), 0.009% (0.72 mg/kg), 0.018% (1.4 mg/kg), 0.035% (2.8 mg/kg) and 600RBU and 600RBU alone during the test period. By week 40, with exposure to 600 RBU, >50% of untreated control animals, < 50% of animals dosed with vehicle, 0.0045%, 0.009% and 0.018% diclofenac gel, and 75% of animals dosed with 0.035% diclofenac gel had detectable tumors. By week 45 tumor prevalence was similar between the untreated and high dose groups. The tumor size distribution was similar in all groups exposed to 600 RBU. No dose-related, statistically significant differences in median time to tumor onset for tumor >1 mm was observed when treated animals were compared to untreated control animals exposed to 600 RBU/week. A statistically significant decrease in median time to tumor onset was observed in animals receiving 0.035% diclofenac when diclofenac group was compared to the vehicle control group. The decrease was significant only when data from both sexes were pooled.

*(Solaraze™ formulation)

2.6.6.6 Reproductive and developmental toxicology

These studies were submitted and reviewed in NDA 19-201. They were performed prior to the development of ICH guidances and GLP guidelines. There were no topically administered reproduction studies. Since the current label for Voltaren lacks nonclinical information for the sections of pregnancy (section 8) and fertility (section 13) the relevant reproduction studies are briefly reviewed here to facilitate labeling.

FERTILITY AND GENERAL REPRODUCTION (SEGMENT I)

In rats, at doses that were maternally toxic, there were no effects on fertility of males or females. Rats were exposed to 0, 2 or 4 mg/kg/day during pre-mating, mating, gestation and lactation period At 2 mg/kg/day diclofenac sodium only resulted in small reductions in food intake and body weight gain in the mothers during the lactation period. The number of live births and survival were also slightly reduced at this dose. At 4 mg/kg, there were clearly toxic effects on treated parents, resulting in reduced food intake and body weight gain and maternal deaths in 5 of 20 animals, and on offspring, resulting in reduced numbers and birth weight, but there was no effect on fertility of males or females. There was no assessment of the behavior of the offspring or of their reproductive capability.

EMBRYO-FETAL DEVELOPMENT (SEGMENT II)

Studies were conducted in mice, rats, and rabbits.

Three oral gavage studies in mice resulted in no significant treatment-related effects at dosages of 4 mg/kg/day or less. One study found an increase in resorptions at 2 and 4 mg/kg/day but this was the result of a single animal in each group and was not confirmed in the other two mouse studies. Toxic changes, including deaths, reduced implantations, reduced fetal weight and reduced ossification were seen in mice treated at 10 or 20 mg/kg/day from Day 0 to 17 of gestation. No malformations were observed.

Oral administration of 4 mg/kg/day in rats resulted in increased litter size and fetal weight with reduced resorptions. At the same dosage in a second rat study a small increase in fetal weight was reported that was not statistically significant. In a third study fetuses of rats treated at 4 mg/kg/day on days 0-20 of gestation had decreased weights. A fourth study found an increase in resorption sites in rats treated at 4 or 5 mg/kg/day from the Day 1 to 12 of gestation, but only at 5 mg/kg/day when treated on Days 8 to 14 of gestation. Collectively, the Sponsor interpreted this data as random variation, and that 4 mg/kg/day had no consistent effect on fetal development. Higher dosages (10 mg/kg, in two of these studies) resulted in maternal deaths due to gastrointestinal ulceration, and associated adverse effects on fetal development during the time of maternal survival.

When diclofenac was administered intramuscularly to rats, no fetal changes were identified even at 10 mg/kg/day, which resulted in maternal death, weight loss, low food intake and painful swollen injection sites in the dams. Subcutaneous administration at 1.2 or 4 mg/kg/day resulted

in a statistically significant and dose related increase in the number of unossified phalangeal nuclei. Three "pig tail" fetuses with multiple abnormalities, all from a single litter were identified in one study. The Sponsor stated that these were the only significant malformations seen among more than 1000 fetuses examined. The Sponsor concluded that these isolated findings was not associated with treatment with diclofenac sodium.

In rabbits, oral administration of 10 mg/kg/day resulted in reduced maternal weight and increased embryonic and fetal resorptions, low fetal weight and irregular ossification of vertebral centers in three fetuses. There were no significant findings at 5 mg/kg/day. In another rabbit study oral administration at doses up to 5 mg/kg/day on days 6-12 or 8-14 of gestation had no consistent effect on fetal development. Intramuscular administration produced no efffects at 3 mg/kg/day, but higher doses resulted in maternal toxicity and associated resorptions, fetal death, reduced fetal and placental weights, and delayed ossification.

The results of the above in vivo studies did not indicate a teratogenic potential of diclofenac. However, Chan et al., (2001) reported teratogenic effects of diclofenac when studied in the Whole-Embryo Culture (WEC) assay. Rat embryos obtained on day 9.5 were maintained *in vitro* as a whole embryo culture exposed to diclofenac during organogenesis and scored for growth and differentiation on 17 morphological features at 48 hours of culture (approximately 11.5 days of gestation). Diclofenac concentrations greater than 5.0 µg/mL (7.5 µg/ml or 15.0 µg/ml) diclofenac, resulted in indicators of abnormalities in organogenesis that included morphology of the caudal neural tube, flexion and hindlimb buds, a finding reported similar to that reported for aspirin (McGarrity et al., 1981). The author stated the concentrations of 1.5 and 2.5 µg/ml (low doses of the study) were the average peak plasma diclofenac concentrations after a single oral dose of 50 and 150 mg respectively of delayed-release (enteric-coated) diclofenac sodium tablets. Since the abnormalites were not specifically described in this study, it is not known if they would have resulted in malformations, although that was the underlying assumption of the study.

PERI-NATAL AND POST-NATAL DEVELOPMENT (SEGMENT III)

Groups of 20 pregnant rats were treated with diclofenac at oral doses of 0, 2 or 4 mg/kg/day from day 15 of gestation to day 21 of lactation. There was a dose-related increase in mortality in both diclofenac groups from gastrointestinal ulceration and peritonitis. Body weight and food intake were reduced at both dosages. The duration of gestation was prolonged at both dosages and two of the 4 mg/kg/day dosed animals were killed because of dystocia. There was a marked increase in still births and an additional increase in fetal or neonatal losses or both. Postnatal survival was unaffected but at the high dosage, birth weight was reduced and postnatal growth was retarded because of maternal toxicity.

Reproductive and Developmental Toxicity

(Modified from Sponsor Tables 2.6.7.10 and 2.6.7.11)

Study Number / Location (eCTD)	Species and Strain	Doses (mg/kg) / Method of Administration / Duration of Observation	Findings
FERTILITY AND EARLY EMBRYONIC DEVELOPMENT (SEGMENT 1)			
RAT			
1 Oct 1972a eCTD 4.2.3.5.1.1 Report dated 10/1/72 Pre GLP	Rat, Sprague-Dawley	Males: 0, 2 or 4 mg/kg by oral gavage, for 60 days prior to mating and during mating Females 0, 2 or 4 mg/kg by oral gavage, for 14 days prior to mating, during mating and throughout gestation and lactation periods to day 21 post partum	No effect on fertility of either sex. 2-4 mg/kg: reduced food consumption and body weight gain (males at 4 mg/kg, females during lactation); 4 mg/kg: 25% female mortality rate with peritonitis, and/or gastrointestinal ulceration, prolonged gestation and dystocia Embroyotoxicity was evident at 2 mg/kg (minimal) and 4 mg/kg as indicated by increased intrauterine resorptions, decreased number of pups in full term litters, and reduced mean weights of neonates. Postnatal growth and survival rates of pups derived from treated parentswere comparable to controls.
EMBRYOFETAL DEVELOPMENT (SEGMENT 2)			
MOUSE			
370, 371, A70-434 Pre-GLP	Mouse	10 mg/kg in 0.1%; In aqueous gum Arabic by oral gavage, from days 7-16 gestation daily except Sundays	10 mg/kg: 1 of 77 fetuses with multiple malformations of head. Number of offspring after birth and after 21st day reduced relative to controls.
2/68 Pre-GLP	Mouse	0, 2, or 4 mg/kg In aqueous susp. with gum Arabic by oral gavage, given on 1st to 18th post-coital day	2-4 mg/kg: embroyotoxicity as indicated by increased absorption quota and increased incidence of skeletal immaturity. **No indications of teratogenicity.** Dams tolerated both doses without overt toxic effects.
7034 (+ suppl) eCTD 4.2.3.5.2.2 Pre-GLP	Mouse, CFLP	0, 2, 4, 10 or 20 mg/kg In 1% gum Arabic used as vehicle Oral D 0 to d 17 gestation period,	2-4 mg/kg: No effect on dams or embroyos; 10-20 mg/kg: 30% and 20% dam mortality rates, resp. and stomach ulceration; Embroyotoxicity at 10-20 mg/kg indicated by reduced mean neonate weights and increased incidence of skeletal immaturity; and at 20 mg/kg-total number of implantations reduced. **No indications of teratogenicity**
M22 eCTD 4.2.3.5.2.1 Pre-GLP	Mouse, CF1-C	0, 2,or 4 mg/kg in aqueous soln by oral gavage on day 6 through day 15 of gestation	Evidence of embryotoxicity indicated by dose-related increase in intrauterine resorptions. **No evidence of teratogenicity**

Clin Rep 1972 (J) 6(8):41-49 ?	Mouse	0, 1 or 4 mg/kg in aqueous soln oral from days 7 to 12 of gestation	litter parameters, or fetal development. 1 mg/kg: Higher fetal loss, 1 rib adhesion 4 mg/kg: Higher fetal loss, reduced mean fetal weight, rib defects, temporary reduced body weight of offspring.
18 July 1974 eCTD 4.2.3.5.2.3	Mouse, Swiss	0,2,4 or 7.5 mg/kg; regimen indentical as that used for the rat Oral gavage 1-12, 8-14 of 0, 2, 4, 7.5 administered from before implantation, day 1 to 12; or after implantation on day 8 to14	No indications of any toxic effects on the adult mice, fertility, pregnancy or fetal development. 7.5 mg/kg: Increased rate of resorptions.
RAT			
Clin Rep 1972 (J) 6(8):41-49 ?	Rat	0, 1 or 4 mg/kg In aqueous soln oral on days 9-14 of gestation	No indications of any toxic effects on the pregnant rats, litter parameters or fetal development. 1 mg/kg: Higher fetal loss, reduced mean fetal weight 4 mg/kg: Higher fetal loss, reduced mean fetal weight, rib defects, temporary reduced body weight of offspring.
18 July 1974 eCTD 4.2.3.5.2.3	Rat, Wistar	of 0, 2, 4, 5 mg/kg/day by oral gavage in 1% tragacanth gum administered from before implantation, day 1 to 12; or after implantation on day 8 to14	No indications of any toxic effects on the adult rats, fertility, evolution of gestation, or fetal development.
347, 348	Rat	0 and 10 mg/kg susp. With gum Arabic; given from 1st to 21st day of gestation, excluding Sundays	Preliminary test results included as part of the study listed below.
53/67	Rat	0,2,4 or 10 mg/kg susp with gum Arabic, given from 1st to 21st day post coitum	10mg/kg (dams)= 40% mortality rate; peritonitis; and gastrointestinal ulceration; 10 mg/kg: Fetal weight decrease; 2-4 mg/kg: Increased resorption and decreased fetal weights. No indications of teratogenicity
70221	Rat	0, 2, 4, or 10 mg/kg oral susp with gum Arabic given throughout gestation period.	10mg/kg (dams): 75% mortality rate; stomach ulcerations; 10mg/kg: Increased fetal mortality and resorptions; 4-10mg/kg: Decreased fetal weights. **No indications of teratogenicity**

038-141	Rat	0,2,or 4 mg/kg aqueous Oral gavage solution from day 6 through day 15 of gestation	No evidence of maternal toxicity. Survival rates, growth rates and overall incidence of abnormalities among fetuses from treated and control dams were comparable. Three "pigtail" fetuses (one of which had urogenital malformations and the other 2 had skeletal malformations) were derived from one high dose dam and considered spontaneous in origin.
038-141	Rat	0, 4, or 6 mg/kg Oral gavage aqueous solution from day 6 through day 15 of gestation	**No evidence of teratogenicity**
RABBIT			
6-68 / 47-68 (+supp) A70-477 eCTD 4.2.3.5.2.9	Rabbit,Silver fawn	0, 5, 10 Oral gelatin capsule During 7-16 of gestation	10 mg: Effect level for mother rabbit; reduced body weight gain; 1 death. Fetuses showed dose-dependent changes that included increased resporption rate, diminished fetus weight, and increased findings after Alizarine Red stainings. 5 mg: Beginning effect level for fetuses. No indications of teratogenicity. 10 mg/kg: Reduced body weight gains of dams, decreased fetal weights, and increased resorptions. **No indications of teratogenicity**
18 July 1974 eCTD 4.2.3.5.2.3	Rabbit	0, 1.5, 3 or 5 mg/kg Oral gavage given between day 6-12 or day 8-14 of gestation	No indications of effects on pregnancy or fetal development. 3 mg/kg: Abortions in 2 of 14 rabbits 5 mg/kg: Abortions in 2 of 17 rabbits
PRENATAL AND POSTNATAL DEVELOPMENT (SEGMENT 3)			
RAT			
1 Oct 1972b eCTD 4.2.3.5.3.1	Rat, Sprague-Dawley	0, 2, 4 mg/kg/day In aqueous solution by Oral gavage once daily from day 15 of gestation through day 21 post partum. On days15 gestation – 21pp	2-4 mg/kg (dams): 15% and 60% mortality, resp. with peritonitis, gastrointestinal ulceration, reduced food intake and body weight gains, prolonged gestation and increased fetal and/or neonatal wasrage. 4mg/kg: dystocia, increased frequency of stillbirths and reduced weights of neonates.

2.6.6.7 Local tolerance

PRIMARY SKIN IRRITATION IN RABBITS

Study title: NCH 927806 1%: Primary skin irritation study in rabbits (4-hour semi-occlusive application

Key study findings: NCH 927806 produced a very slight erythema evident at 1 hour after removal of the applied undiluted dose, but not by 24 hours (next time examined). It is considered non irritating.

Study no.: 843178
E-Location: CTD 4.2.3.6.1
Conducting laboratory and location: [redacted]

Date of study initiation: April 4, 2002
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
NCH 927806 1% (Voltaren 10 mg/g Emulgel), Batch DPH-030, Purity not provided. (Provided by the Sponsor in a semi-solid form, 0.5 ml (per animal) of NCH 927806 1% was measured with a syringe and applied undiluted as it was delivered by the sponsor.)

Methods: A semiocclusive application of 0.5 ml of NCH 927806 1% was applied to the intact left flank of each of three young adult New Zealand White rabbits for 4 hours. The skin reactions were scored at 1, 24, 48 and 72 hours after removal of the dressing.

Results: The application of NCH 927806 1% to the skin resulted in mild signs of irritation, indicated by very slight erythema was observed at the test site of all animals 1 hour after removal of the dressing. There were no skin reactions at the application site of any animal at the 24-, 48- and 72-hour examinations (all scores 0). The individual mean score for erythema/eschar and oedema for each of the three animals was therefore 0. The test item caused no staining of the treated skin. No corrosive effects were noted on the treated skin of any animal at any of the measuring intervals. NCH 927806 did not induce significant or irreversible damage to the skin. It is considered not irritating.

PRIMARY EYE IRRITATION IN RABBITS

Study title: NCH 927806 1%: Primary eye irritation study in rabbits

Key study findings: The Sponsor classied NCH 927806 1% as non-irritating, the reviewer classifies it as an irritant, because the Sponsor only quantified eye responses after 24 hours, not at earlier times, and it took up to 21 days for any signs of irritation to clear.

Reviewer's Comment: This reviewer disagrees with this classification that is based on results 1 to 3 days after administration. The acute administration response indicates it is a definite irritant, and the prolonged time (weeks) for signs to completely reverse do not support a nonirritant classification.

Study no.: 843182
E-Location: CTD 4.2.3.6.4
Conducting laboratory and location: [redacted]

Date of study initiation: April 23, 2002
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:

NCH 927806 1% (Voltaren 10 mg/g Emulgel), Batch DPH-030, Purity not provided. (Provided by the Sponsor in a semi-solid form, 0.5 ml (per animal) of NCH 927806 1% was measured with a syringe and applied undiluted as it was delivered by the sponsor.)

Methods: NCH 927806 1% (0.1 mLof undiluted, semipaste consistency) was instill into the conjunctival sac of the left eye each of three young adult New Zealand White rabbits (n=1 male, 10 weeks of age; n=2 females, 11 weeksof age). The right eye was untreated and served as a reference control. Scoring of irritation effects was performed approximately 1, 24, 48 and 72 hours, as well as 7, 10, 14, 17 and 21 days after instillation. Irritation scores of each following parameters: corneal opacity (including the area affected, where applicable), iridic effects, conjunctival and scleral reddening and/or chemosis for each individual animal at all observation intervals. In addition, any lesions including the degree and nature of irritation, corrosion or reversibility, and any other toxic effects were observed. The Cumulative Scores for the Eye Irritation Scores represent the sum of all numerical scores, except area of corneal opacity and scleral reddening, for each animal at each time point. The resulting Mean Cumulative Eye Irritation Score was calculated for all animals at each time point. The primary eye irritation score was calculated by totaling the mean cumulative scores at 24, 48 and 72 hours and then divided by the number of data points.

Results: Slight swelling of the conjunctivae was apparent in all animals 1 hour after treatment. The severity of the swelling increased in all animals at the 24-hour examination and swelling with partial eversion of lids continued to be observed up to 48 hours and 72 hours (in one animal) after treatment. The severity of the swelling subsequently decreased and was no longer evident 14 days after treatment. Reddening was however present in all animals until day 17 of the observation. Assessment of the sclerae was not possible on a number of occasions due to swelling of the conjunctivae. Increased ocular discharge was noted in all animals 1 hour after treatment and persisted in two animals up to the 24-hour examination. Slight corneal opacities, affecting up to the whole area of the cornea, were also observed in all animals. No abnormal findings were observed in the iris. No corrosion was observed at any of the measuring intervals and no staining of the treated eyes by the test item was observed. The primary eye irritation score was 3.67 (max. 13). The eye reactions (mean values from 24 to 72 hours) consisted of grade 0.78 corneal opacity, grade 0.00 iris lesions, grade 1.11 redness of the conjunctivae and

grade 1.78 chemosis of the conjunctivae. Diclofenac sodium topical gel 1% was not found to induce significant or irreversible damage to the rabbit eye.

The body weights of all rabbits were considered to be within the normal range of variability. Based upon the referred classification (Commission Directive 2001/59/EC of August 06, 2001), NCH 927806 1% is considered to be "not irritating" to the rabbit eye (Table 3).

TABLE 1: EYE IRRITATION SCORES - INDIVIDUAL VALUES

Animal Number	Sex	Evaluation Interval	Corneal Opacity	Area of Corneal Opacity	Iris	Conjunctivae		Cumulative		Sclera
						Redness	Chemosis	Score	Mean	
85	M	1 hour	1	4	0	1	1	3.00	3.00	1
86	F		1	4	0	1	1	3.00		1
87	F		1	4	0	1	1	3.00		1
85	M	24 hours	1	4	0	1	2	4.00	4.33	1
86	F		1	4	0	2	2	5.00		n.a.
87	F		1	4	0	1	2	4.00		n.a.
85	M	48 hours	1	4	0	1	2	4.00	4.00	n.a.
86	F		1	4	0	1	2	4.00		n.a.
87	F		1	4	0	1	2	4.00		n.a.
85	M	72 hours	0	0	0	1	1	2.00	2.67	1
86	F		0	0	0	1	1	2.00		1
87	F		1	4	0	1	2	4.00		2
85	M	7 days	0	0	0	1	0	1.00	1.67	1
86	F		0	0	0	1	1	2.00		1
87	F		0	0	0	1	1	2.00		1
85	M	10 days	0	0	0	1	0	1.00	1.67	1
86	F		0	0	0	1	1	2.00		1
87	F		0	0	0	1	1	2.00		1
85	M	14 days	0	0	0	1	0	1.00	0.67	1
86	F		0	0	0	1	0	1.00		0
87	F		0	0	0	0	0	0.00		0
85	M	17 days	0	0	0	1	0	1.00	0.67	1
86	F		0	0	0	1	0	1.00		0
87	F		0	0	0	0	0	0.00		0
85	M	21 days	0	0	0	0	0	0.00	0.00	0
86	F		0	0	0	0	0	0.00		0
87	F		0	0	0	0	0	0.00		0

n.a. = not assessable due to swelling of the conjunctivae

TABLE 2: EYE IRRITATION SCORES – MEAN VALUES AFTER 24, 48 AND 72 HOURS

Animal Number	Sex	Corneal Opacity	N	Iris	N	Conjunctivae				Primary Eye Irritation Score
						Redness	N	Chemosis	N	
85	M	0.67	3	0.00	3	1.00	3	1.67	3	3.67
86	F	0.67	3	0.00	3	1.33	3	1.67	3	
87	F	1.00	3	0.00	3	1.00	3	2.00	3	
Mean score		0.78		0.00		1.11		1.78		

N = number of available data points

TABLE 3: EYE IRRITATION SCORES – ASSESSMENT ACCORDING TO EEC GUIDELINES

Evaluated intervals	Corneal Opacity	Iris	Conjunctivae	
			Redness	Chemosis
24 hours 48 hours 72 hours	Not Irritating	Not Irritating	Not Irritating	Not Irritating

EEC COMMISSION DIRECTIVE 92/69/EEC, JULY 31, 1992

Grading of Ocular Lesions

CORNEA

Opacity: degree of density (area most dense taken for reading)

No ulceration or opacity 0
Scattered or diffuse areas of opacity (other than slight dulling of normal luster), details of iris clearly visible 1
Easily discernible translucent area, details of iris slightly obscured 2
Nacreous area, no details of iris visible, size of pupil barely discernible 3
Opaque cornea, iris not discernible through the opacity 4

Area of cornea involved

Zero 0
One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

IRIS

Normal 0
Markedly deepened rugae, congestion, swelling, moderate circumcorneal hyperemia, or injection, any of these or combination of any thereof, iris still reacting to light (sluggish reaction is positive) 1
No reaction to light, hemorrhage, gross destruction (any or all of these) 2

CONJUNCTIVAE

Redness (refers to most severe reading of palpebral and bulbar conjunctivae when compared with control eye)

Blood vessels normal 0
Some blood vessels definitely hyperemic (injected) 1
Diffuse, crimson color, individual vessels not easily discernible 2
Diffuse beefy red 3

Chemosis: lids and/or nictitating membranes

No swelling 0
Any swelling above normal (including nictitating membranes) 1
Obvious swelling with partial eversion of lids 2
Swelling with lids about half-closed 3
Swelling with lids more than half-closed 4

Note: Reddening of the sclerae was assessed using the same scoring grades as conjunctivae.

PHOTOTOXICITY IN GUINEA PIGS

Study title: **NCH 927806 1%: Determination of phototoxicity in albino guinea pigs.**

Key study findings: NCH 927806 1% (diclofenac sodium topical gel 1%) had irritating effects (erythema and edema) but lacked photoirritating potency.

Study no.: 843180
E-location: CTD 4.2.3.6.3
Conducting laboratory and location: [redacted]

Date of study initiation: March 27, 2002
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:

NCH 927806 1% (Voltaren 10 mg/g Emulgel), Batch DPH-030, Purity not provided. (Provided by the Sponsor in a semi-solid form, 0.5 ml (per animal) of NCH 927806 1% was measured with a syringe and applied undiluted as it was delivered by the sponsor.)

Vehicle: purified water, NCH 927806 was not soluble in [redacted] but was readily soluble in purified water.

Methods: The cutaneous phototoxic potential of diclofenac sodium topical gel 1% (referred to as NCH 927806 1% in the study) was determined in 15 (10 test and 5 control) male Himalayan spotted guinea pigs. Animals anesthetized with 32 mg/kg of Pentobarbital (Narcoren, 160 mg Pentobarbital/ml, by intraperitoneal injection, 0.2 ml/kg). Thereafter, 4 test sites of 2 cm^2 were marked on the hair-clipped flanks by a circular stamp. Diclofenac sodium topical gel 1% was applied topically at concentrations of 100%, 75%, 50% and 25%, each, to skin areas of 2 cm^2 on both flanks. All animals were pretreated with 2% DMSO in ethanol to enhance the skin penetration of diclofenac gel or controls. Thirty minutes after application, the left flank of the animals was exposed to 20 J/cm^2 UV-A radiation. The light source produced UV-A radiation at 320 – 400 nm at a dose intensity of 20 J/cm^2 controlled by a time control device which switched off after reaching the required dose of 20 J/cm2. The distance between the light source and the backs of the animals (16 cm for UV-A) was adjusted in such a manner that the resulting irradiance was approximately 2.7 mW/cm^2. The right flank was not exposed to light after treatment and served as non-irradiated diclofenac treated reference site. Control animals were treated with the solvent (purified water), then exposed to UV-A radiation. Cutaneous reactions, were evaluated at 24, 48 and 72 hours after exposure.

Results: Erythema with or without edema were in animals treated with 100% diclofenac sodium topical gel 1%. Only erythema was observed at sites of some of the animals treated with 75% and 50% diclofenac sodium topical gel 1%. These effects were observed on both irradiated and non-irradiated test sites with no differences in the incidence of effects between irradiated and non-irradiated groups. No irritation was seen with the 25% dilution in water, with or without UV-irradiation, or in the animals of the control group treated with purified water only. Since in the control group neither the non-irradiated nor the irradiated site presented with any skin

reaction, it is concluded that NCH 927806 1 % showed no photoirritating potency but irritating potency at 100 %, 75 %, and 50 %.

Reviewers Comment: The Sponsor stated the sensitivity and reliability of the experimental technique used was assessed with 8-methoxypsoralene. However, this was not conducted using a parallel positive control group, but based the validity on positive control work performed 3 months earlier.

	CONCEN-TRATION	LEFT FLANK UV-A irradiated (20 J/cm^2)			RIGHT-FLANK non-irradiated		
	%	Hours after application			Hours after application		
		24 hrs	48 hrs	72 hrs	24 hrs	48 hrs	72 hrs
CONTROL GROUP	purified water	0/5*	0/5	0/5	0/5	0/5	0/5
TEST GROUP	100	7/10	8/10	6/10	6/10	8/10	5/10
(NCH 927806 1 %)	75	2/10	6/10	5/10	4/10	7/10	5/10
	50	2/10	6/10	5/10	2/10	4/10	3/10
	25	0/10	0/10	0/10	0/10	0/10	0/10

* Number of animals showing an erythema/total number of treated animals.

All animals were pretreated with 2 % DMSO in ethanol to enhance the skin penetration of the test item.

APPEARS THIS WAY
ON ORIGINAL

Summary of Local Tolerance Studies
(From Sponsor's Tables 2.6.7.1.1

Study Number/ eCTD location/ GLP/year of study	Species /Strain	Dose and Methods	Findings
Skin Irritation			
843178 eCTD 4.2.3.6.1 GLP: yes 2002	Rabbits, New Zealand white	0.5 ml diclofenac Emugel 1%, Single application on 4X4cm, semi-occlusive for 4 hour duration, assessment of reactions up to 72h	**Primary skin irritation** **Not irritating**
843186 eCTD 4.2.3.6.2 GLP: Yes 2002	Rabbits, New Zealand white	0.5 ml diclofenac sodium 1% on 1.2 x 1.2 cm^2 gauze patch; semi occlusive for 4h daily for 4 weeks clinical and dermal signs recorded up to 24h after treatment	**Cumulative skin irritation** **Well tolerated**
Eye Irritation			
843182 eCTD 4.2.3.6.4 GLP: Yes 2002	Rabbits, New Zealand white	One instillation into the eye 0.1 ml of 1% diclofenac sodium gel Scoring of eye irritation up to 21 days after treatment	**Primary eye irritation:** **Is an irritant** Transient, reversible reddening, Not irritating according to Sponsor, but was irritating by this reviewer
Skin Phototoxicity			
843180 eCTD 4.2.3.6.3 GLP: Yes 2002	Guinea pigs, albino	Pretreatment with DMSO/ethanol 1% diclofenac sodium gel topical (undiluted, 75%, 50% and 25% dilution) ± UV irradiation (20 J/cm^2) Assessment of skin reactions up to 72h after application	**Not phototoxic to skin**
Skin sensitization			
843179 eCTD 4.2.3.7.1.1 GLP: Yes	Guinea pigs, albino	Induction: intradermal injection with Freunds complete adjavant and diclofenac (undiuted) topical treatment with diclofenac after 1 week **Challenge: at 2 weeks later with 0.2 mL of 25% dilution of diclofenac** Observation: up to 48h after challenge	Slight redness in test animals but not in controls, "**sensitizing**"
V4413/09 eCTD 4.2.3.7.1.2 GLP: Yes	Guinea pigs, albino	Induction: intradermal injection with Freunds complete adjavant and diclofenac (3%); topical treatment with sodium lauryl sulfate or diclofenac 1 week later; **Challenge: at 2 weeks later with 30 uL of undiluted diclofenac**	**Not sensitizing**

		Observation: up to 48h after challenge	
844802 eCTD 4.2.3.7.1.3 GLP: Yes	Guinea pigs, albino	Induction: intradermal injection with Freunds complete adjavant and diclofenac (3%); topical treatment 1 week later; **Challenge: 2 weeks later with 0.2 mL of 10% dilution of diclofenac** Observation: up to 48h after challenge	**Not sensitizing**
Photoallergenicity			
843181 eCTD 4.2.3.7.1.4 GLP: Yes	Guinea pigs, albino	**Induction:** intradermal injection with Freunds complete adjavant Topical treatment with diclofenac and irradiation at 10 J/cm2 UV-A + 1.8 J/cm2 UV-B, 4 more topical treatments and UV irradiation during 2 weeks, **Challenge:** 2 weeks later with 5%, 10%, 15%, and 25% dilutions of diclofenac and irradiation UVA+B	**Signs of sensitization, but not photosensitization**
PH02/0247 eCTD 4.2.3.7.1.6 GLP: Yes	Guinea pigs, albino	**Induction:** intradermal injection with Freunds complete adjavant Topical treatment with diclofenac and irradiation at 10 J/cm2 UV-A + 1.8 J/cm2 UV-B, 4 more topical treatments and UV irradiation during 2 weeks, **Challenge:** 2 weeks later with 5%, 10%, 15%, and 25% dilutions of diclofenac and irradiation UVA+B	**Not sensitizing, Not photosensitizing**
845817 eCTD 4.2.3.7.1.5 GLP: Yes	Guinea pigs, albino	**Induction:** intradermal injection with Freunds complete adjavant Topical treatment with diclofenac and irradiation at 10 J/cm2 UV-A + 1.8 J/cm2 UV-B, 4 more topical treatments and UV irradiation during 2 weeks, **Challenge:** 2 weeks later with 5%, 10%, 15%, and 25% dilutions of diclofenac and irradiation UVA+B	**Not sensitizing, Not photosensitizing**

2.6.6.8 Special toxicology studies

CUTANEOUS HYPERSENSTIVITY

The cutaneous hypersensitivity potential of DSG was assessed in 3 Magnusson-Kligman maximization-tests using male albino guinea pigs. The results from the first sensitization study indicated that diclofenac sodium topical gel 1% may have sensitization potential. Two subsequent studies one performed by the same contract research organization and one by a different company did not confirm those results. This first sensitization study was also conducted by the same company that conducted the first photoallergenicity test, which also resulted in positive results suggestive of diclofenac-induced photohypersensitivity. However, these findings were not reproduced in subsequent testing by the same or a different company.

Reviewer Comment: It should be noted that the positive controls for these GLP studies were not run simultaneously with the main study but relied on studies conducted a few months earlier. The formulation, although it is indicated as Emulgel is not the same Emulgel apparently as in Voltaren Gel or Voltaren Emulgel and infact contains substances not contained in those formulations. Also, the NCH 927806 concentrations for induction and challenge were different between studies. Therefore, **the conclusions from this group of studies are problematic and will not be considered for the risk assessment of diclofenac sodium or the formulation.**

Study title: **NCH 927806 1%: Contact Hypersensitivity in albino guinea pigs, maximization-test**

Key study findings: NCH 927806 1% (undiluted) was sensitizing to later cutaneous exposure to a 25 % solution of NCH 927806 1%, resulting in slight irritation (redness, score of 1 or 2) upon later challenge.

Study no.: 843179
E-Location: CTD 4.2.3.7.1.1
Conducting laboratory and location: [redacted]

Date of study initiation: April 8, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:

NCH 927806 1% (diclofenac sodium), Batch DPH-030, Purity not provided
Formulation: in Emulgel (propylenglycol, isopropylic alcohol, and water)
Dilutions in physiological saline or purified water

Also:
Voltaren Emulgel 1% (diclofenac diethylammonium 1.16%), Lot WC038, Purity not provided
Formulation: in Emulgel as above

Methods: Male albino guinea pigs were divided into a test group of 10 animals and 3 control groups of 5 animals each. The induction of sensitization was an intradermal injection (0.1 mL/site) into the nuchal region of undiluted diclofenac sodium topical gel 1%, as an emulsion in Freund's Complete Adjuvant (FCA) in physiological saline. One week after the intradermal induction, an epidermal induction of sensitization involved treatment for 48 hours of diclofenac sodium topical gel 1% under an occlusive bandage. A control group consisted of animals that received an intradermally injection of purified water in FCA/physiological saline and then epidermally induced with purified water under occlusion. Two weeks after epidermal induction (3 weeks from initial intradermal injection) the control and diclofenac treated animals were challenged by an epidermal application of both a 25% solution of diclofenac sodium topical gel 1% in purified water and purified water alone under occlusive dressing. An identical second challenge was performed two weeks later. Also, at this time a second control group was challenged on naive skin sites with a 25% diclofenac sodium topical gel 1% in purified water. Sixteen days after the second challenge, a third challenge was performed in the same way as the second challenge to the original test and control groups, except that the test sites were left uncovered. A third control group was also challenged at this time. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing during the first and second challenge and at 24 and 48 hours after the application during the third challenge. Base on the percentage of animals sensitized (24- and 48-hour readings) diclofenac was assigned a allergenic potency classification.

Magnusson and Kligman grading scale:

0 = no visible change
1 = discrete or patchy erythema
2 = moderate and confluent erythema
3 = intense erythema and swelling

Magnusson and Kligman allergenic potency classification

Sensitization Rate (%)	Grade	Classification
0 - 8	1	weak
9 - 28	2	mild
29 - 64	3	moderate
65 - 80	4	strong
81 - 100	5	extreme

Results: The initial challenge (day 22 post-induction) indicated that 89% of guinea pigs had been sensitized (very slight redness, score of 1) with diclofenac sodium topical gel 1% while no skin effects were noted in the control group. In an attempt to further clarify this unexpected finding, a second challenge was done two weeks later with the marketed Voltaren® Emulgel™ and, again, with a 25% solution of the diclofenac sodium gel 1%. Under these conditions, undiluted Voltaren® Emulgel™ was irritating to control and diclofenac sodium gel 1% group animals. No conclusions concerning sensitization could be drawn. The second challenge (day 36 post-induction) with diclofenac sodium gel 1% resulted, again in slight irritation in the diclofenac sodium gel 1% animals. A third challenge (day 52 post-induction), similar to the second but with open non-occlusive application gave similar results. For the second and third challenge, the original control animals (control group I) were exposed as well as naïve control

animals (control group II in the second challenge and control group III in the third challenge). The naïve control animals did not react, but slight redness was observed in the original control animals, who had been exposed to the test product during the first challenge. These findings indicated that diclofenac sodium gel 1% should be classified as a skin sensitizer.

The positive control, alpha-hexylcinnamaldehyde (induced with 10% solution in PEG 300 and challenged with 0.1% inPEG 300 or PEG alone) resulted in positive response only with alpha-hexylcinnamaldehyde was conducte at some time between February and April 2002, a few months before the main study. ***Reviewer comment:*** They were not run in parallel (simultaneously) with the main study.

Study title: **Sensitization study with NCH 927806 (1%) in guinea pigs (maximization test).**

Key study findings: NCH 927806 1% (at a 3% dilution) was non-sensitizing to later cutaneous challenge with undiluted NCH 927806 1%.

Study no.: V4413-09
E-Location: CTD 4.2.3.7.1.2
Conducting laboratory and location: [redacted]

Date of study initiation: June 18, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
NCH 927806 1%, Batch DPH-030, Purity [redacted]
Formulation/vehicle: Voltaren Emulgel 927806

Methods: In the second cutaneous hypersensitivity assay, induction of 10 male guinea pigs involved intradermal injection of 3% diluted diclofenac sodium topical gel 1% in Freund's Complete Adjavant (FCA)/physiological saline. During the pretest, higher concentrations had resulted in symptoms of necrosis at the injection site. A 3% dilution of diclofenac sodium topical gel was subsequently chosen for induction. One week after the intradermal treatment, the animals were pretreated with sodium lauryl sulfate and undiluted diclofenac sodium gel 1% was applied topically. Two weeks after the epidermal induction, the animals were challenged with patches soaked with 30 µL of undiluted diclofenac sodium gel 1%. Five control animals were treated identically, but with purified water replacing the test article during the induction phase.

The positive control was formaldehyde (37%) sensitized and then challenged with a 10% or 3% dilution. ***Reviewer's Comment:*** The positive control was conducted in March, 2002, not in parallel with the main study conducted in June 2002.

Results: The skin scores observed in all animals in the test and control groups following challenge with diclofenac sodium topical gel 1% were 0. These results indicated that there were no reactions in the test, nor in the control animals. Diclofenac sodium gel 1% was thus not found to be sensitizing, under these test conditions.

Study title: **NCH 927806 1%: Contact hypersensitivity in albino guinea pigs, maximization-test**

Key study findings: NCH 927806 1% (undiluted) was non-sensitizing to later cutaneous challenge with a 10% dilution of NCH 927806 1% with or without excipients perfume and

Study no.: 844802
E-Location: CTD 4.2.3.7.1.3
Conducting laboratory and location:

Date of study initiation: July 30, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:

NCH 927806 1%, Batch DPH-032, Purity
Formulation/vehicle: as below with and without perfume, and with (the formulation that is listed with still lists Carbomer in the formulation)

Diclofenac sodium	1%
Carbomer	
Isopropanol	
Propylenglycol	
Purified water	

Voltaren Emulgel 1% containing diclofenac diethylammonium 1.16%, and Emugel listed as propylenglycol, isopropylic alcohol, and water

Methods: In the third cutaneous hypersensitivity assay, undiluted diclofenac sodium topical gel 1% (referred to as NCH 927806 1%) in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline was again intradermally injected in the nuchal region. The epidermal induction of sensitization was conducted for 48 hours under occlusion with undiluted diclofenac sodium topical gel 1% one week after the intradermal induction. The control animals were intradermally induced with purified water and FCA/physiological saline and epidermally induced with purified water under occlusion. Two weeks after epidermal induction the control and diclofenac sodium gel 1% animals were challenged under occlusive dressing by epidermal application of 10% solution of diclofenac sodium topical gel 1% in purified water. To investigate if the perfume or one of the excipient, might play a role in the apparent sensitizing potential of the formulation (as observed in Maximization test I), challenges were also performed with the same formulation but not containing the perfume, the test formulation in which , Carbomer Homopolymer Type C) was replaced with (as in Voltaren® Emulgel™), and Voltaren® Emulgel™. The same highest non-irritating concentration was used for all four formulations, which was a 10% dilution in purified water. Five control animals were treated identically, but with purified water

replacing the diclofenac sodium gel 1% during the induction phase. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing.

Results: None of the control animals showed skin reactions after the challenge treatment with diclofenac sodium topical gel 1% applied at 10 % (w/w) in purified water. No reactions were seen in any of the test animals following challenges with any of the other test products, nor in the control animals. These results indicated that diclofenac sodium topical gel 1% is a non-sensitizer under the test conditions used. Neither the perfume nor was found to affect the potential cutaneous allergenicity of diclofenac sodium topical gel.

Reviewer comment: The positive control, alpha-hexylcinnamaldehyde, was not run stimultaneously with this study, but months earlier between February and April 2002. It appears to be the same data as in the first study (study number 843179).

PHOTOHYPERSENSITIVITY (PHOTOALLERGENICITY) STUDIES

The photoallergenicity of diclofenac sodium topical gel 1% was assessed in 3 studies using albino guinea pigs. The first photoallergenicity study gave a positive result, independent of UV light. Two subsequent studies, one performed in the same CRO and one in a different CRO did not confirm those results. The first photoallergenicity study was performed in the same CRO and at the same time as the seemingly positive first sensitization test, that also could was not reproducible.

Reviewer Comments: As with the cutaneous sensitivity studies, there is a lack of information about the formulation, a lack of simultaneous positive control study animals, and variable dose intensities of the light source bwteen studies with no indication of the light irradiance at the skin surface (in the second study).

Study title: **NCH 927806 1%: Determination of photoallergenicity in albino guinea pigs (including information about allergenicity, photoirritation and irritation)**

Key study findings: NCH 927806 1 % may have a senstizing potential, but the effect of UV irradiation is unclear.

Study no.: 845817
E-Location: CTD 4.2.3.7.1.5
Conducting laboratory and location:

Date of study initiation: Oct 15, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
NCH 927806 1%, Batch DPH-032, Purity not mentioned
Formulation/vehicle:

Diclofenac sodium	1%
Carbomer 980	
Isopropanol	
Propylenglycol	
Purified water	

Methods: The first photohypersensitivity study was conducted with diclofenac sodium gel 1% in female albino guinea pigs (Himalayan spotted; Ibm: GOHI; SPF-quality, 371-429 g, 5-7 weeks of age), using a maximization protocol. During the induction phase, 20 test and 10 control animals were treated with intradermal injections of Freund's Complete Adjavant (FCA) followed by topical treatment with the undiluted diclofenac sodium gel 1% (test animals only).

For the induction of sensitization of the test group the undiluted NCH 927806 1 % was applied epicutaneously to a nuchal skin area of 6-8 cm^2 (marked previously with 4 intradermal injections of Freund's Complete Adjuvant/physiological saline 1:1). The test sites were then exposed to 10 J/cm^2 UV-A irradiation and 1.8 J/cm^2 UV-B. This procedure was repeated 4 times within 2 weeks of the induction phase. Control animals were intradermally treated with FCA/physiological saline only. Three weeks after beginning of the induction a challenge was performed by treating the experimental animals (test and control) epicutaneously on both flanks with the test item at the concentrations of 25 %, 15 %, 10 % and 5 % (dilutions in purified water). Treated sites were then either exposed to 10 J/cm^2 UVA irradiation (left flank) or remained unirradiated (right flank). Cutaneous reactions, i.e. erythema and edema formation were evaluated at 24, 48 and 72 hours after the challenge exposure. The highest non-irritating concentration used for the challenge application was 25 %.

Results: One test animal died during the induction phase, which the Sponsor suggest was not related to the treatment. Defined erythema was observed on the irradiated flank of seven (at the 24-hour reading) and eight (at the 48- and 72-hour reading) out of 19 test animals after treatment with 25%cdiclofenac sodium gel 1% in purified water. The same skin reaction was seen at the 24-, 48- and 72-hour reading in three test animals after treatment with 15% solution of diclofenac sodium gel 1% and in two test animals treated with 10% solution of diclofenac sodium gel 1% as well as in one test animal at the 72-hour reading when treated with 5% solution of diclofenac sodium gel 1%. Defined erythema was also observed on the non-irradiated flanks of two (at the 24-hour reading) and four (at the 48- and 72-hour reading) out of 19 test animals after treatment with the 25% solution of diclofenac sodium gel 1%, in one (at the 24-hour reading) and two (at the 48- and 72-hour reading) test animals treated with 15% diclofenac sodium gel 1% and in one (at the 48-hour reading) and two (at the 72-hour reading) test animals when treated with 10% diclofenac sodium gel 1%. No skin reactions were observed on the both irradiated and non-irradiated flanks of the control animals treated with the any dilution of diclofenac sodium gel 1%. The reactions were slightly more frequent on the irradiated side, compared to the nonirradiated side. However, a case-by-case analysis showed that 4 test animals reacted on both irradiated and non-irradiated flanks with diclofenac sodium gel 1%, suggesting an hypersensitivity potential without UV, similar to the first maximized sensitization test conducted at the same time in this same CRO. The Sponsor suggested the additional irritation of UV-irradiation probably exaggerate the responses, and the reviewer concurs with this assessment. Diclofenac sodium topical gel 1% may have a sensitizing potential and the influence of UV irradiation was not clear.

Reviewer Comment: The positive control study used 3,3',4',5-Tetrachlorrosalicylanilide and resulted in a positive photosensitization response, but was not run in simultaneously with above study, rather it was conducted months earlier, between March and April 2002

Study title: **Assessment of photosensitization on the albino guinea pig**

Key study findings: NCH 927806 1% had nó photosensitizing potential.

Study no.: -PH-02/0247

E-Location: CTD 4.2.3.7.1.6
Conducting laboratory and location: [redacted]

Date of study initiation: Oct 14, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
NCH 927806 1%, Batch DPH-032, Purity not provided
Formulation/vehicle: not provided

Methods: A second study was conducted in which 21 test and 10 control female guinea pigs (Dunkin-Hartley; weight 318-470 g) were treated with intradermal injections with FCA followed by topical treatment with the undiluted test product (test animals only). The treated skin was irradiated with 6.5 J/cm^2 UV-A. This procedure was repeated without the FCA injections, 2 more times with 2 day intervals. Three weeks after the beginning of the induction, the animals were challenged with 0.5 mL undiluted diclofenac sodium gel 1% and 6.5 J/cm^2 UV-A light irradiation. Cutaneous reactions were evaluated 24 and 48 hours after the challenge exposure. All administrations of NCH 927806 1% were in an undiluted form.

Results: Three animals died during the study from causes that are probably not related to treatment with the test product, but the suggested cause was not provided. There were no cutaneous reactions attributable to photosensitization following the challenge phase in the test and control groups. This test indicated that diclofenac sodium topical gel 1% did not have photosensitizing potential.

Reviewer's Comments: There was no positive control mentioned in this study.

Study title: **NCH 927806 1%: Determination of photoallergenicity in albino guinea pigs (including information about allergenicity, photoirritation and irritation)**

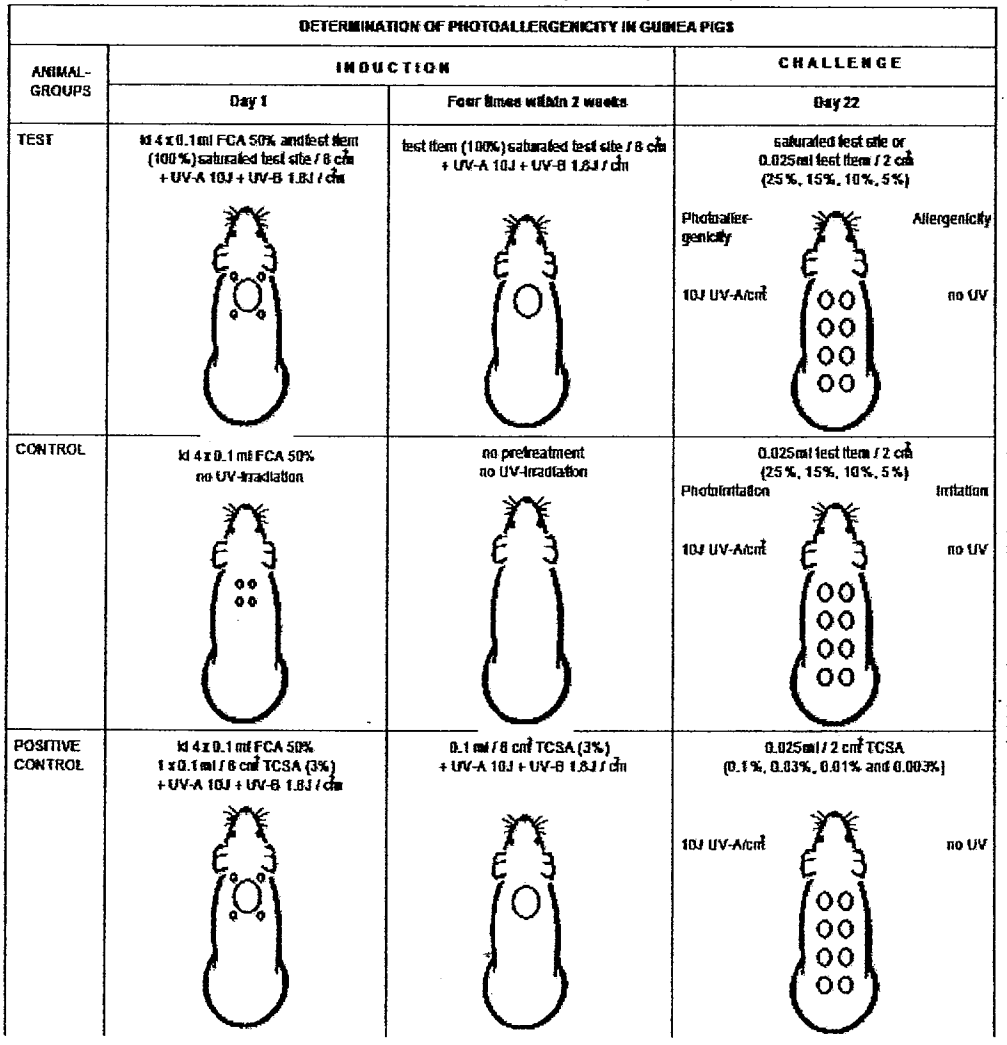
Key study findings: NCH 927806 1% was not photosensitizing.

Study no.: 843181
E-Location: CTD 4.2.3.7.1.4
Conducting laboratory and location: [redacted]

Date of study initiation: April 30, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
NCH 927806 1%, Batch DPH-030, Purity [redacted]
Formulation: Emulgel consisting of propylenglycol, isopropylic alcohol, and water
Vehicle: purified water
(The test item was not soluble in [redacted] but was readily soluble in purified water.)

Methods: A third photoallergenicity study was conducted in which, 20 test and 10 control male guinea pigs (Himalayan spotted; Ibm: GOHI; SPF-quality guinea pigs; 5-7 weeks of age; weight 267-445 g) were treated with intradermal injections of FCA followed by topical treatment with the undiluted diclofenac sodium gel 1% (test animals only). The treated skin was irradiated with 10 J/cm^2 UV-A and then with 1.8 J/cm^2 UV-B. The duration of the photoexposure was regulated by a time control device which switched off immediately after reaching the requested dose. The distance between the light source and the backs of the animals (16 cm for UV-A and 5 cm for UV-B) was adjusted in such a manner that the resulting irradiance was approximately 1.7-1.8 mW/cm^2 for UV-B and 2.6-2.7 mW/cm^2 for UV-A. This procedure, without the FCA injections, was repeated a 4 more times within the 2 weeks of the induction phase. Three weeks after the beginning of the induction, the animals were challenged with 25%, 15%, 10% or 5% diclofenac sodium gel 1% and UV-A and UV-B radiation. Cutaneous reactions were evaluated 24, 48 and 72 hours after the challenge exposure.

Scheme of the Photoallergenicity Study



SCHEME OF THE PHOTOALLERGENICITY STUDY

Results: One test animal died during the induction phase, probably not related to the treatment. None of the control and test animals showed skin reactions after the challenge treatment with any dilution of diclofenac sodium topical gel 1%. No toxic symptoms were evident in the guinea pigs of the control or test group. No deaths occurred. These results also suggested that diclofenac sodium topical gel 1% was not photosensitizing.

Reviewer Comment: The positive control study used 3,3',4',5-Tetrachlorrosalicylanilide and resulted in a positive photosensitization response, but this study was not run-in simultaneously with above study, rather it was conducted weeks earlier, between mid March and early April 2002.

Summary of Special Toxicology Studies

Study Number/ eCTD location/ GLP/year of study	Species /Strain	Dose and Methods	Findings
Skin sensitization			
843179 eCTD 4.2.3.7.1.1 GLP: Yes	Guinea pigs, albino	Induction: intradermal injection with Freunds complete adjavant and diclofenac (undiuted) topical treatment with diclofenac after 1 week **Challenge: at 2 weeks later with 0.2 mL of 25% dilution of diclofenac** Observation: up to 48h after challenge	Slight redness in test animals but not in controls, "**sensitizing**"
V4413/09 eCTD 4.2.3.7.1.2 GLP: Yes	Guinea pigs, albino	Induction: intradermal injection with Freunds complete adjavant and diclofenac (3%); topical treatment with sodium lauryl sulfate or diclofenac 1 week later; **Challenge: at 2 weeks later with 30 uL of undiluted diclofenac** Observation: up to 48h after challenge	**Not sensitizing**
844802 eCTD 4.2.3.7.1.3 GLP: Yes	Guinea pigs, albino	Induction: intradermal injection with Freunds complete adjavant and diclofenac (3%); topical treatment 1 week later; **Challenge: 2 weeks later with 0.2 mL of 10% dilution of diclofenac** Observation: up to 48h after challenge	**Not sensitizing**
Photoallergenicity			

843181 eCTD 4.2.3.7.1.4 GLP: Yes	Guinea pigs, albino	**Induction:** intradermal injection with Freunds complete adjavant Topical treatment with diclofenac and irradiation at 10 J/cm2 UV-A + 1.8 J/cm2 UV-B, 4 more topical treatments and UV irradiation during 2 weeks, **Challenge:** 2 weeks later with 5%, 10%, 15%, and 25% dilutions of diclofenac and irradiation UVA+B	**Signs of sensitization, but not photosensitization**
— **PH02/0247** eCTD 4.2.3.7.1.6 GLP: Yes	Guinea pigs, albino	**Induction:** intradermal injection with Freunds complete adjavant Topical treatment with diclofenac and irradiation at 10 J/cm2 UV-A + 1.8 J/cm2 UV-B, 4 more topical treatments and UV irradiation during 2 weeks, **Challenge:** 2 weeks later with 5%, 10%, 15%, and 25% dilutions of diclofenac and irradiation UVA+B	**Not sensitizing, Not photosensitizing**
845817 eCTD 4.2.3.7.1.5 GLP: Yes	Guinea pigs, albino	**Induction:** intradermal injection with Freunds complete adjavant Topical treatment with diclofenac and irradiation at 10 J/cm2 UV-A + 1.8 J/cm2 UV-B, 4 more topical treatments and UV irradiation during 2 weeks, **Challenge:** 2 weeks later with 5%, 10%, 15%, and 25% dilutions of diclofenac and irradiation UVA+B	**Not sensitizing, Not photosensitizing**