IMPURITY QUALIFICATION

was found to be relatively non toxic (LD_{50}>2000 mg/kg). Five male and 5 female fasted rats were subjected to a single oral administration of at 2000 mg/kg. There were no deaths and no clinical signs of reaction to treated with No macroscopic changes were apparent during necropsy of the rats on day 15.

The LD_{50} of was approximated by graphical extrapolation to be 1850 mg/kg. No effects were observed following single dose treatment with 1000 mg/kg. Mortality was observed following treatment with 2000 mg/kg (3 deaths in 5 treated animals on days 2-7).

The LD_{50} of , not described in previous diclofenac oral formulations was determined in mice. Five males and 5 females were subjected to a single oral administration of 2000 mg/kg There were no clinical signs of any systemic toxic effect of the test article. Necropsy of mice surviving treatment revealed an abscess on the mucosal surface of the stomach of one male. No other macroscopic changes were apparent in any of the mice sacrificed on day 15. These results established that the acute minimum lethal oral dose level of in mice is greater than 2000 mg/kg body weight.

Summary of Studies for Qualification of Impurities

Assay Type Study number eCTD location GLP	Species/Cells	Dose	Finding
Bacteria Reverse Mutation Assay 63/50-D5140 GLP: yes	*S typhimurium*, TA98, 100, 1535, 1537 *E. coli* WP2 pKM101 *E. coli* WP2 *uvrA* pKM101	0 - 2000 µg/plate (TA98, TA100, TA1535, TA1537) 0 - 5000 µg/plate (E. coli strains) ± S9 (rat liver metabolizing enzymes)	**Negative** for mutagenicity with or without metabolic activation
Chromosome Aberration Assay 63/51-D5140 GLP: yes	Human peripheral blood Lymphocytes, *in vitro*	0- 700 µg/mL ± S9 (rat liver metabolizing enzymes)	**Negative** for clastogenicity with or without metabolic activation
Acute Oral Toxicology 63/52-D6144 GLP: yes	Rats/ M (Glx/BRL/Han)BR n=5/gender	Preliminary: 1200, 2000 mg/kg Main: 2000 mg/kg Single dose, oral gavage Vehicle: aqueous methyl cellulose 1% 15 days observation	Observed Minimum Lethal Dose (mg/kg) >2000 mg/kg LD50 >2000 mg/kg Observed Maximum Non-Lethal Dose (mg/kg) ND

Skin sensitisation 63/53-D6144 GLP: yes	Guinea pig, Dunkin-Hartley	Induction: intradermal injection (day 1), topical (day 8), 2 days duration of dosing Challenge: Topical (day 22), 24 hr duration of dosing 30% and 10% m/m in arachis oil Rechallenge: Topical (day 29), 24 hr duration of dosing 8% and 3% m/m in arachis oil	**Not sensitizing**
Acute Oral Toxicology 88-6185 GLP: yes	Rats/Tif:RAIf (SPF) n=5/gender	1000, 2000 mg/kg Single dose Oral Gavage Suspension in Na-CMC 0.5% 14 days observation	Observed Minimum Lethal Dose = 2000 mg/kg LD50 approximated by graphical extrapolation to be 1850 mg/kg Observed Maximum Non-Lethal Dose = 1000 mg/kg
Bacteria Reverse Mutation Assay 936002 GLP: yes	*S typhimurium*, TA98, 100, 1535, 1537 *E. coli*, WP2 uvrA	312.5 - to 5000 µg/plate ± Rat liver S9	**Negative** not mutagenic with or without metabolic activation
14-day repeated dose Toxicology 93-6004 GLP: yes	Rats, Tif:RAIf 6 to 8 weeks 141 to 191 g n=5/sex/dose	0, and 2 mg/kg/day by oral gavage for 14 days Vehicle: 0.5% Na-CMC solution	**no adverse effects were observed** **(only 1 dose tested)** no deaths no changes in body weight, food consumption, water consumption, hearing test, clinical observations
Bacteria Reverse Mutation Assay 63/40-1052 GLP: yes	*S typhimurium*, TA98, 100, 102, 1535, 1537	0 – 5000 µg/plate ± Rat liver S9	**Negative** not mutagenic with or without metabolic activation
Mouse lymphoma mutation assay 63/39-1052 GLP: yes	Mouse L5178Y cells, *in vitro* (Mutation at the thymidine kinase (tk) locus)	0 – 120 µg/mL ± Rat liver S9	**Negative** not mutagenic in the absence of metabolic activation, **Equivocal** in the presence of metabolic activation

Acute oral Toxicology 063/037 GLP: yes	Mouse, CD-1(ICR)BR Preliminary: n=1/gender Main: n=5/gender	Preliminary: 500, 1000, 1500, 2000 mg/kg Main: 2000 mg/kg Single dose, Oral gavage (in aqueous methyl cellulose) 15 days observation	**Preliminary:** Observed Minimum Lethal Dose (mg/kg) >2000 mg/kg Observed Maximum Non-Lethal Dose (mg/kg) : ND No deaths and no overt reactions to treatment. **Main:** Observed Minimum Lethal Dose (mg/kg): >2000 mg/kg Observed Maximum Non-Lethal Dose (mg/kg): ND LD50 >2000 mg/kg No clinical signs of any systemic toxic effect of the test article
Skin sensitization 063/038-D6144 GLP: yes	Guinea pig, Dunkin-Hartley	Induction: intradermal injection (day 1), topical (day 8), 2 days duration of dosing 0, 15%, 30% in Alembicol D Challenge: Topical (day 22), 24 hr duration of dosing	**Not Sensitizing**

Study title: [redacted] **Single dose oral toxicity study in the rat.**

Key study findings: A single oral dose of 2000 mg/kg of [redacted] administered to male and female rats resulted in no deaths, no clinical signs, weight loss in 1 of 5 males and 1 of 5 females, and no macroscopic changes when animals were examined 15 days after administration. The minimum lethal dose was >2000 mg/kg.

Reviewers Comment: The lack study of administration of a range of doses, of histopathology, hematology and clinical chemistry does not provide assurance of the potential safety of [redacted] Lethal dose studies are not very useful for toxicological assessment.

Study no.: 63/52-D6144
E-Location: CTD 4.2.3.7.6.1
Conducting laboratory and location: [redacted]

Date of study initiation: April 23, 1999; completed report Sept 13, 1999
GLP compliance: yes
QA report: yes
Drug, lot #, and % purity:
[redacted] Batch 9800031, Purity [redacted]
Vehicle: 1% (m/v) methyl cellulose

Preliminary Study

Methods: [redacted] was administered to fasted rats (n=1/gender/dose) at dose levels of 1200 and 2000 mg/kg.
Results: There were no deaths and no overt reactions to treatment. Necropsy of the rats on day 8 revealed no macroscopic changes.

Main Study

Methods: In the, five male and five female fasted rats [redacted] WI(Glx/BRL/Han)BR; n=5/gender; 9-11 weeks of age; males 264-281 g, female 187-203 g) were administered a single dose of [redacted] at 2000 mg/kg (gavage at a dose volume of 20 mL/kg). Clinical signs were checked at least once within half an hour of dosing and four times within the first four hours following administration, twice daily on days 2, 3 and 4 and once daily from the fifth to last day of the observation period. All animals were killed on day 15 and subsequently underwent a full necropsy. The necropsy procedure included inspection of external surfaces and orifices, all viscera and tissue within the abdominal, thoracic and cranial cavities, free-hand sectioning of the liver and kidneys and examination of representative sections of mucosal surfaces of the stomach, small and large intestines.

Results: There were no deaths and no clinical signs of reaction to treatment with —— All rats gained body weight throughout the observation period. The sponsor mentioned small weight losses were noted in one male rat during the first week of the study and one female during the second week, but the weight loss is actually from day -1 to day 1 in which all animals lost weight which was rapidly regained, and one female maintained a relatively constant weight during week 2. Necropsy did not reveal any macroscopic changes. The Sponsor stated there was no histological assessment was made since there were no macroscopic changes observed. The acute minimum lethal oral dose of —— to rats was greater than 2000 mg/kg.

Study title: —— **Reverse mutation in four histidine-requiring strains of Salmonella typhimurium and two tryptophan-requiring strains of Escherichia coli**

Key findings: —— did not induce mutations *Salmonella typhimurium* strains at doses up to 2000 ug/plate and up to 5000 ug/plate *Escherichia coli* strains in the presence or absence of metabolic activation system (S9).

Study no.: 63-50-d5140
E-location: CTD 4.2.3.7.6.5
Conducting laboratory and location: ——

Date of study initiation: April 14, 1999
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
——, Batch 9800031, Purity ——
Vehicle: dimethyl sulfoxide (DMSO)

Methods

Strains/species/cell line:

Organism	Strain	Type of mutation	Mutant gene
S. typhimurium	TA98	frame-shift	histidine
S. typhimurium	TA100	base-pair substitution	histidine
S. typhimurium	TA1535	base-pair substitution	histidine
S. typhimurium	TA1537	frame-shift	histidine
E. coli	WP2 pKM101	base-pair substitution	tryptophan
E. coli	WP2 uvrA pKM101	base-pair substitution	tryptophan

Doses used in definitive study:

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentration (µg/plate)
Mutation Experiment 2 (TA98, TA100 and TA1537)	– and +	0.2048*	20.48
		0.512*	51.2
		1.28*	128
		3.20*	320
		8.00*	800
		20.00*	2000
Mutation Experiment 2 (TA1535, WP2 pKM101 WP2 uvrA pKM101)	– and +	1.28*	128
		3.20*	320
		8.00*	800
		20.00*	2000
		50.00*	5000

Basis of dose selection:

An initial toxicity range-finder experiment was carried out in strain TA100 only, using final concentrations of — at 8, 40, 200, 1000 and 5000 µg/plate, plus negative (solvent) and positive controls. Evidence of toxicity occurred at 2000 and 5000 µg/plate, indicated by a thinning of the bacterial lawn following treatments. Precipitation of the ' — ' was present on all plates treated at the maximum test dose.

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentration (µg/plate)
Range-finder Experiment	– and +	0.08	8
		0.40	40
		2.00	200
		10.00	1000
		50.00	5000
Mutation Experiment 1	– and +	0.016	1.6
		0.08	8
		0.40	40
		2.00	200
		10.00	1000
		50.00	5000

Negative controls: DMSO

Positive controls:

Chemical	Source	Stock* concentration (µg/mL)	Final concentration (µg/plate)	Use	
				Strain(s)	S-9
2-nitrofluorene (2NF)		50	5.0	TA98	–
Sodium azide (NaN_3)		20	2.0	TA100	–
				TA1535	–
9-aminoacridine (AAC)		500	50.0	TA1537	–
4-nitroquinoline 1-oxide (NQO)		100	10.0	WP2 pKM101	–
-		20	2.0	WP2 uvrA pKM101	–
2-amino-anthracene (AAN)		50**	5.0	At least one strain	+

* With the exception of NaN_3, which was prepared in water, all stock solutions were prepared in sterile anhydrous analytical grade dimethyl sulphoxide (DMSO). NaN_3, 2NF, AAC and AAN were stored in aliquots at 1-10°C in the dark, and NQO in aliquots at −80°C in the dark.

** Concentration of stock solution used for the Experiment 2 pre-incubation positive control treatments with AAN was twice that stated above, in order to permit treatments at the final concentration stated, whilst volume additions were reduced to 0.05 mL for these pre-incubation treatments.

Incubation and sampling times:

Bacteria cultures were tested in two separate experiments, at the concentrations indicated above using triplicate plates without and with S-9. For all assays, bacteria were cultured for 10 hours at 37°C in nutrient broth (containing ampicillin for strains TA98, TA100 and Escherichia coli. A total of six test article doses were tested such that sufficient non-toxic data could be obtained for mutagenicity assessment. Negative (solvent) controls were included in each assay, in quadruplicate without and with S-9. Since the results of the first experiment were negative, treatments in the presence of S-9 in the second set of experiments included a pre-incubation step of 1 hour to possibly increase the range of mutagenic chemicals that cauld be detected in the assay. Volume additions for experiment 2 preincubation treatments were reduced to 0.05 mL due to the solvent (DMSO) employed in this study. This, and some other organic solvents, are known to be near to toxic levels when added at volumes of 0.1 mL in this assay system when employing the pre-incubation methodology. By reducing the addition volume to 0.05 mL per plate, it was hoped to minimize or eliminate any toxic effects of the solvent. The liver post-mitochondrial fraction (S-9) used for metabolic activation was prepared from male Sprague Dawley rats induced with Aroclor 1254 and obtained from

Batches of

Results

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

In each experimenf, bacterial strains were treated with diagnostic mutagens in triplicate in the absence of S-9. Colonies were counted electronically using a — , Colony Counter and the background lawn inspected for signs of toxicity. Some plate were counted manually where confounding factors (such as split agar or the preseace of microcolonies/test article precipitate) affected the accuracy of the automated counter. Individual plate counts from both experiments were recorded separately ard the mean and standard deviation of the plate caunts for each treatment were determined. The m-statistic was calculated to check that the data were Poisson-distributed and Dunnett's test was used to compare the counts of each dose with the control. The presence or absence of a dose response was checked by linear regression analysis.

The assay was considered valid if the following criteria were met:

1) the mean negative control counts fell within the normal ranges as defined by historical control data.
2) the positive oontrols induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation.
3) no more than 5% of the plates were lost through contamination or some other unforeseen event.

The test article was considered to be mutagenic if:

1) the assay was valid
2) Dunnett's test gave a significant response ($p < 0.01$) and the data set(s) showed a significant dose correlation
3) the positive responses described above, in 2, were reproducible

Study Outcome:

No — treatments of the test strains, either in the absence or in the presence of S-9, resulted in a statistically significant increase in revertant numbers. Thus, there was no evidence for — mutagenic activity.

Negative (solvent) and positive control treatments were included for all strains in both experiments. The mean numbers of revertant colonies on negative control plates all fell within acceptable ranges, and were significantly elevated by positive control treatments.

summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1537	TA1535	WP2 pKM101	WP2 uvrA pKM101
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 µl	51 ± 10	139 ± 9	12 ± 5 (M)	21 ± 4 (M)	47 ± 4	162 ± 15
—	20.48	53 ± 11	140 ± 16	12 ± 4 (M)	-	-	-
	51.2	48 ± 7	139 ± 6	11 ± 2 (M)	-	-	-
	128	50 ± 6	135 ± 2	14 ± 1 (M)	25 ± 4	55 ± 11	166 ± 9
	320	34 ± 7 (S)	133 ± 11 (S)	7 ± 1 (M+S)	21 ± 6	51 ± 11	152 ± 9
	800	28 ± 4 (S)	125 ± 12 (S)	4 ± 3 (M+V)	23 ± 4	39 ± 8	136 ± 5
	2000	21 ± 3 (S)	100 ± 12 (S)	5 ± 2 (M+V)	18 ± 4	37 ± 4	113 ± 32
	5000	-	-	-	19 ± 6 (M+Ppn)	36 ± 6 (M+Ppn)	88 ± 10 (M+Ppn)
Positive controls	Compound	AAN	-	-	-	-	AAN
	Dose Level	5 µg	-	-	-	-	5 µg
	Mean ± SD	760 ± 67	-	-	-	-	483 ± 21

SD Standard deviation

AAN 2-Aminoanthracene

S : Slight thinning of b/g lawn
Ppn : Precipitation of TA observed
V : Very thin background lawn
M : Plate counted manually

summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1537	TA1535	WP2 pKM101	WP2 uvrA pKM101
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	47 ± 5	121 ± 11	15 ± 4	19 ± 6	16 ± 4	169 ± 17
—	20.48	51 ± 11	122 ± 6	12 ± 1 (M)	-	-	-
	51.2	51 ± 4	116 ± 17	18 ± 3	-	-	-
	128	44 ± 3	133 ± 2	14 ± 6	21 ± 6	22 ± 8	142 ± 12
	320	39 ± 11	105 ± 8	15 ± 1	24 ± 3	11 ± 3	131 ± 4
	800	27 ± 3 (S)	111 ± 19 (S)	9 ± 3 (S)	22 ± 6	14 ± 2	122 ± 6
	2000	15 ± 4 (S)	74 ± 13 (S)	5 ± 0 (V)	20 ± 6	9 ± 6	74 ± 13
	5000	-	-	-	14 ± 2 (M+Ppn)	5 ± 2 (M+Ppn)	77 ± 10 (M+Ppn)
Positive controls	Compound	2NF	NaN3	AAC	NaN3	NQO	NQO
	Dose Level	5 µg	2 µg	50 µg	2 µg	10 µg	2 µg
	Mean ± SD	1019 ± 40	617 ± 28	426 ± 44	531 ± 11	826 ± 56	2027 ± 63

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
NQO 4-Nitroquinoline 1-oxide

S : Slight thinning of b/g lawn
Ppn : Precipitation of TA observed
M : Plate counted manually
V : Very thin background lawn

Study title: — **Induction of chromosome aberrations in cultured human peripheral blood lymphocytes**

Key findings:

Study no.: 63-51-d5140
E-location: CTD 4.2.3.7.6.6
Conducting laboratory and location: —

Date of study initiation: May 10, 1999
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— Batch 9800031, Purity —
Vehicle: Dimethyl sulfoxide (DMSO)

Methods

Strains/species/cell line:
Healthy, non-smoking volunteers were used in this study:

Experiment Donor Sex Donor Identity

Experiment	Donor Sex	Donor Identity
1, Trial 1	Female	
1, Trial 2	Female	
2	Female	

No donor was suspected of any virus infection nor had been exposed to high levels of radiation or hazardous chemicals. For each experiment and for each trial an appropriate volume of whole blood was drawn from the peripheral circulation within two days of culture initiation. Blood was stored refrigerated and **pooled prior to use**. Whole blood oultures were established in sterile disposable ccntrifuge tubes by placing 0.4 ml heparinised blood into 9.0 ml Hepes-buffered RPMI medium containing 20% (v/v) fetal calf serum and 50 pg/mL gentamycin. Phytohaemagglutinin (— reagent grade) was included at a concentration of approximately 10 pg per ml of culture to stimulate the lymphocytes to divide. Blood culturcs were incubated for approximately 48 hours at 37°C and rocked continuously.

Doses used in definitive study:

S-9	Treatment+ recovery (hours)	Vehicle Control	Concentration (µg/mL)	Positive Control
-	3+17	0*	320, 400 and 500	NQO, 2.5 µg/mL
+	3+17	0*	66.74, 118.7 and 210.9	CPA, 12.5 µg/mL

* Vehicle control was DMSO only

S-9	Treatment+ recovery (hours)	Vehicle Control	Concentration (µg/mL)	Positive Control
-	20+0	0[a]	448, 560 and 700	NQO, 2.5 µg/mL
+	3+17	0[a]	358, 448 and 700	CPA, 6.25 µg/mL

[a] Vehicle control was DMSO only

Basis of dose selection:
The highest dose level used in experiment 1 was 5000 µg/mL, was a concentration in excess of the solubility limit in culture medium. Following the results fom experiment 1, a top concentration of 700 pg/mL was chosen for experiment 2.

The highest dose for chromosome analysis from cultures sampled at 20 hours should be one at which at least 50% mitotic inhibition (approximately) has occurred or should be the highest dose tested Analysis of slides from highly cytotoxic concentrations is avoided, if possible. Slides from cultures treated with heavily precipitating doses are checked to confirm that the presence of precipitate does not preclude analysis. Slides from the highest selected dose and two lower doses, such that a range ef cytotoxicity from maximum to little or none is covered, are used for microscope analysis.

Negative controls: Dimethyl sulfoxide

Positive controls:

Chemical	Supplier	Concentration of treatment solution (mg/mL)	Final concentration (µg/mL)	S-9
4-nitroquinoline 1-oxide (NQO)	/ /	0.125	1.25	-
		0.250	2.50	-
		0.500	5.00	-
Cyclophosphamide (CPA)	/ /	0.3125	3.125	+
		0.625	6.25	+
		1.25	12.5	+

Incubation and sampling times:

Treatment	S-9	Number of cultures	
		3+17*	20+0*
Experiment 1			
Negative control	-	4	
	+	4	
Test article	-	2	
(all doses)	+	2	
Positive controls	-	2	
(all doses)	+	2	
Experiment 2			
Negative control	-		4
	+	4	
Test article	-		2
(all doses/doses as appropriate)	+	2	
Positive controls	-		2
(all doses)	+	2	

* Hours treatment + hours recovery

Whole blood oultures were established in sterile disposable ccntrifuge tubes by placing 0.4 ml heparinised blood into 9.0 ml Hepes-buffered RPMI medium containing 20% (v/v) fetal calf serum and 50 pg/mL gentamycin. Phytohaemagglutinin ' reagent grade) was included at a concentration of approximately 10 pg per ml of culture to stimulate the lymphocytes to divide. Blood cultures were incubated for approximately 48 hours at 37°C and rocked continuously. One set of quadruplicate cultures (A, B, C and D) for each of the treatment regimes was then treated with the solvent and one set of duplicate cultures with the test article. Cells were treated with colchicine 2 hours prior to harvest and after harvesting processed for cytological analysis.

Results:

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

Where possible, 100 metaphases from each code were analysed for chromosome aberrations. Only cells with 44-46 chromosomas were considered acceptable for analysis of structural aberrations. Any cell with more than 46 chromosomes, that is polyploid, endoreduplicated and hyperdiploid cells, was noted and recorded separately. Classifioation of structural aberrations was based on the scheme described by ISCN. Under this scheme, a gap is defined as a discontinuity less than the width of the chromatid and no evidence of displacement of the fragment and a deletion is defined as a discontinuity greater than the width of the chromatid and/or evidence of displacement of the fragment. Observations (summarised in Appendices 3 and 4) were recorded on raw data sheets with the microscope stage coordinates of any aberrant cell.
After completion of microscopic analysis, data were decoded. The aberrant cells in each culture were categorised as follows:
1) cells with struqtural aberrations including gaps
2) cells with structural aberrations excluding gaps
3) polyploid, endoreduplicated, or hyperdiploid cells.

The totals for category 2 in negative control cultures were compared with the current laboratory negative control (normal) ranges to detarmine whether the assay was acceptable or not. The proportion of cells in category 2 in test article treated cultures were also compared with normal ranges. The statistical significance of any data set was only to be taken into consideration if the frequency of aberrant cells in both replicate cultures at one or more concentration exceeded the normal range using the Fisher's exact test ($p<0.05$) The proportions of cells in categories 1 and 3 were also examined ia relation to historical control ranges.

The human lymphocyte assay is considered valid if the following criteria are met:

1) the binomial dispersion test demonstrates acceptable heteroganeity between replicate cultures,
2) the proportion of cells with structural aberrations (excluding gaps) in negative control cultures falls within the normal range,
3) at least 160 cells out of an intended 200 are analysable at each dose level,
4) the positive control chemicals induce statistically significant increases in the number of cells with structural aberrations.

The test article was to be considered as positive in this assay if:

1) the proportions of cells with structural aberrations at one or more ooncentration exeeeds the normal range in both replicate cultures,
2) a statistically signiGcant increase in the proportion of cells with structural aberrations (excluding gaps) occurs at such doses.

Increased incidence of calls with gaps or incraees in the proportions of cells with structural aberrations not exceedizg the normal range or occurring only at very high or very toxic concentrations were likely to be concluded as "equivocal". Full assessmant of the biological importamce of such increases is likely only to be possible with reference to data from other test systems. Cells with exchange aberrations or cells with greater than one aberration were to be considered of particular biological significance.

Study outcome:

Treatment of cultures with — in the absence and presence of S-9 (both experiments) resulted in frequencies of cells with structural aberrations that were similar to those seen in concurrent vehicle controls. The aberrant cell frequency fell within the historical negative (normal) control range in the majority of treatments. Two exceptions to this were observed at the lowest concentratian analysed (320 and 358.4 µg/mL) in the pulse treatments in the absence and present of S-9 respectively. However, although the number of cells with structural aberrations (excluding gaps) in each case exceeded the normal range, the increases were marginal, isolated and were not present in the replicate cultures. As such, these increases did not fulfill the criteria for a positive result and were considered spurious increases of no biological importance.

Therefore, [redacted] did not induce chromosome aberrations in cultured human peripheral blood lymphocytes when tested at a concentration of up to 700 µg/mL.

TABLE 3
20 hour treatment -S-9, 0 hour recovery (20+0), Experiment 2
Donor sex: female

Treatment (µg/mL)	Replicate	Cells scored	Cells with Aberrations Including gaps	Cells with aberrations excluding gaps	Mitotic index (mean)
Solvent	A	100	3	1	6.1
	B	100	2	0	5.8
	Totals	200	5	1	(6.0)
448	A	100	0	0	6.7
	B	100	4	1	8.1
	Totals	200	4	1	(7.4)
560	A	100	5	3	7.9
	B	100	3	1	10.0
	Totals	200	8	4	(9.0)
700	A	100	5	3	7.8
	B	100	4	1	8.9
	Totals	200	9	4	(8.4)
NQO, 2.5	A	100	[redacted]	[redacted]	
	B	100	[redacted]	[redacted]	
	Totals	200	42	37*	

Binomial Dispersiou Test g' = 4.05, not significant 'Statashcal sigruficanoe ps0.001 Numbers highlighted exceed historical negative coutrol range (Appandix 5)

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TABLE 4
3 hour treatment +S-9, 17 hour recovery (3+17), Experiment 2
Donor sex: female

Treatment (µg/mL)	Replicate	Cells Scored	Cells with Aberrations Including Gaps	Cells with Aberrations excluding gaps	Mitotic Index (mean)
Solvent	A	100	0	0	4.8
	B	100	1	0	4.3
	Totals	200	1	0	(4.6)
358.4	A	100	5	5	5.1
	B	100	0	0	5.5
	Totals	200	6	6	(5.3)
448	A	100	2	1	4.1
	B	100	2	2	2.6
	Totals	200	4	3	(3.4)
700	A	100	1	1	5.0
	B	100	7	2	2.2
	Totals	200	8	3	(3.6)
CPA, 6.25	A	100	33	27	
	B	100	27	20	
	Totals	200	60	47[a]	

Binomial Dispersion Test g' = 6.86, not significant 'Ststistical significance pc0.001 Number highlighted exceeds historical aegative control range (Appendix 5)

—

Study title: — **Acute oral toxicity study in rats.**

Key findings:

Study no.: 88-6185
E-Location: CTD 4.2.3.7.6.2
Conducting laboratory and location: CIBA-GEIGY Limited Pharmaceuticals Division, Pharma Toxicology, Basel, Svitzerland
Date of study initiation: Nov 17, 1988; Report dated Jan 11, 1989
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— , Batch 5, Purity > — (NMR)
Vehicle: Na-CMC 0.5%

Methods
A dose-range finding study was performed in rats, 1 male per dose level (Tif:RAIf (SPF); 105 to 125 g; 4-6 weeks of age),. An appropriate dose group was expanded to maximum 5 males to approximate a LD_{50} in one sex. Subsequently, a group of 5 males and then 5 females were dosed orally at 1000 mg/kg and another groups of males were dosed at 2000 mg/kg (10 mL/kg). They were followed for 14 days postdosing.

Results
The LD_{50} was approximated by graphical extrapolation to be 1850 mg/kg. There was no sex differences noted for the 1000 mg/kg dose. Three of the five males dosed at 2000 mg/kg died between days 2 and 7 postdosing. No females were given this higher dose. Body weight gain was not affected during the 14-day observation period in the survivors. There were no clinical signs in the 1000 mg/kg group. After 3 days postdose in the 2000 mg/kg group of males, ataxia, reduced spontaneous activity, arched backs, stiff movements, roughening of the coat, labored respiration, and cyanosis occurred. Also poor condition, inflated abdomen, or paleness occurred in one or two of the rats. After 6 days, survivors showed no apparent signs. Upon termination of the study, necropsy and gross examination of the major viscera revealed no abnormalities in the survivors. The animals that died showed autolytic changes.

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Study title: — **Salmonella and escherichia/livermicrosome test.**

Key findings: — was not mutgenic in strains of S. typhimurium and E. coli.

Study no.: 936002
E-location: CTD 4.2.3.7.6.7
Conducting laboratory and location: CIBA-GEIGY Limited, Basle, Switzerland, Laboratories of Genetic Toxicology
Date of study initiation: Feb 15, 1993
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— Batch Charge IV, Purity —
Vehicle: Dimethylsulfoxide (DMSO)

Methods

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

Doses used in definitive study:

Except for strain TA 1537, — was tested for mutagenic effects without metabolic activation at five concentrations in the range of 312.5-5000.0 µg/plate. Since the test substance exerted a growth-inhibiting effect on strain TA 1537, the experiment with this strain was performed with lower concentrations ranging from 19.5- 312.5 µg/plate. In the experiment carried out with matabolic activation five concentrations in the range of 312.5-5000.0 µg/plate were tested, except for strain TA 1537 in which concentrations ranged from 19.5- 312.5 µg/plate.

Basis of dose selection:

The concentration range of — to be tested in the mutagenicity test was determined in a preliminary toxicity test. A toxicity test was carried out with strains S. typhimurium TA 100 and E.coli WP2 uvrA without and with metabolic activation at six concentrations of the test substance and one negative control. The highest concentration applied was 5000 pg/plate. The five lower concentrations decreased by a factor of 3. The plates were inverted and incubated for about 48 hours at 37 + 1.5°C in darkness. Thereafter, they were evaluatad by counting the colonies and determininq the background lawn. One plate per test substance concentration, as well as each negative control was used.

Negative controls: dimethyl sulfoxide

Positive controls:

A) Experiment without metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	sodium azide	bidist. water	5.0 µg/ plate
TA 1535	sodium azide	bidist. water	5.0 µg/ plate
WP2 uvrA	4-nitroquinoline-N-oxide	DMSO	2.0 µg/ plate
TA 98	2-nitrofluorene	DMSO	20.0 µg/ plate
TA 1537	9(5)-aminoacridine	DMSO	150.0 µg/ plate

B) Experiment with metabolic activation:

Strain	Mutagen	Solvent	Concentration
TA 100	2-aminoanthracene	DMSO	2.5 µg/ plate
TA 1535	cyclophosphamide·H_2O	bidist. water	400.0 µg/ plate
WP2 uvrA	2-aminoanthracene	DMSO	50.0 µg/ plate
TA 98	2-aminoanthracene	DMSO	2.5 µg/ plate
TA 1537	2-aminoanthracene	DMSO	2.5 µg/ plate

Incubation and sampling times:

Each of the five concontrations of the test suhstance, a negative and a positive control were tested, using three plates per test substance concentration as well as each positive and negative control vith each tester strain. The highest concentration applied was determined in the preliminary toxicity test and the four lower concentrations were each decreased by a factor of 2. The plates were inverted and incubated for ahout 48 hours at 37 + 1.5 C in darkness. Thereafter, they were evaluated by counting the number of colonies and determining the background lawn.

Results

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

A test was considered acceptable if the mean colony counts of the negative control values of all strains are within the acceptable ranges and if the results of the positive controls meet the criteria for a positive response. In either case the final decision is based on the scientific judgement of the Study Director.

Criteria for a positive response: The test substance is considered to be mutagenic in this test system if one or both of the following conditions are met:

1) At least a reproducible doubling of the mean number of revertants per plate above that of the negative control at any concentration for one or more of the following strains: S. typhimurium TA 98, TA 1535, TA 1537 and E. coli WP2 uvrA.
2) A reproducible increase of the mean number of revertants per plate for any concentration above that of the negative control by at least a factor of 1.5 for strain S. typhimurium TA 100.

Generally a concentration-related effect should be demonstrable.

Study outcome:

In the mutagenicity test normal background growth was observed with all strains at all concentrations. The number of revertant colonies was reduced in strain TA 1537 at higher concentrations. With the other strains the test substance exerted no inhibitory effect on the grovth of the bacteria. The test substance formed precipitates after mixture with the top agar down to the concentration of 625 µg/plate.

In the original experiment performed without and with metabolic activation, none of the tested concentrations of — led to an increase in the incidence of either histidine- or tryptophan-prototrophic mutants when compared to with the negative control.

In the confirmatory experiment performed without and with metabolic activation, there was no increase in the incidence of either histidine- or tryptophan-prototrophic mutants when compared to the negative control.

— and its metabolites did not induce gene mutations in the strains of S. typhimurium and E. coli used.

TABLE 5

SUMMARY OF THE RESULTS (MEAN OF COLONY COUNTS)

Experiment without metabolic activation

Test number : 936002 Experiment : Original
Test substance : — Batch : Charge IV

Treatment/Strain	TA 100	TA 1535	WP2 uvrA	TA 98
Negative control	126.3	13.7	25.0	19.7
312.5000 ug/plate	83.7	14.3	20.7	18.3
625.0000 ug/plate	85.7	14.0	19.0	21.3
1250.0000 ug/plate	86.0	10.3	18.7	15.7
2500.0000 ug/plate	86.0	12.7	15.7	18.0
5000.0000 ug/plate	103.0	14.3	19.7	18.3
Positive controls:				
sodium azide	1304.3	1061.7	----	----
4-NQO	----	----	1004.3	----
2-nitrofluorene	----	----	----	1869.7

TABLE 6

SUMMARY OF THE RESULTS (MEAN OF COLONY COUNTS)

Experiment without metabolic activation

Test number : 936002 Experiment : Original
Test substance : — Batch : Charge IV

Treatment/Strain	TA 1537
Negative control	6.7
—	
19.5312 ug/plate	7.7
39.0625 ug/plate	4.7
78.1250 ug/plate	4.3
156.2500 ug/plate	2.7
312.5000 ug/plate	4.7
Positive controls:	
9-aminoacridine	2182.7

Study title: — **Pilot 14-day oral toxicity study in rats.**

Key findings: An oral dose of 2 mg/kg of — produced no toxicologic signs.

Study no.: 93-6004
e-location: 4.2.3.7.6.4
Conducting laboratory and location: CIBA-GEIGY Limited, Pharma Division, Toxicology/Pathology, Basel, Switzarland
Date of study initiation: Feb 11, 1993
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— , Lot IV, Purity —
Vehicle : 0.5% sodium carboxymethyl cellulose (Na-CMC) solution

Methods: — , was administered orally by gavage,once daily, to group of 5 male and 5 female Tif:RAIf (SPF) rats (6-8 weeks of age, 147 to 191 g of weight) at a dose of 2 mg/kg and at a volume of 10 mL/kg. A group of control animals, 5 males and 5 females, received a 0.5% Na-CMC solution at the same volume. Animals were dosed for 14 days.

The following investigations were performed: Mortality, Clinical Signs, Body Weight, Food Consumption, Water Consumption, Hearing Test, Clinical Biochemistry, Hematology, Urinalysis, Pathology: Organ Weights, Macroscopic and Microscopic Examination.

Organ Weights (*paired organs weighed together):
Adrenal glands*, axillary lymph nodes*, brain, epididymides*, heart, kidneys*, liver, lung, mandibular glands*, ovaries*, pituitary gland, prostate, seminal vesicles*, spleen, testes*, thymus, thyroid with parathyroid glands, and uterus.

Adrenal glands, aorta, bone with bone marrow: sternum/femur, brain, cecum, colon, duodenum, epididymides, esophagus, eyes with optic nerves, harderian glands, heart, ileum, jejunum, kidneys, knee joint, liver, lung, lymph nodes: axillary/mesenteric, mandibular glands, ovaries, pancreas, pituitary gland, prostate, rectum, sciatic nerve, seminal vesicles, skin with mammary area, spinal cord, spleen, stomach, testes, thigh muscle, thymus, thyroid with parathyroid glands, tongue, trachea, urinary bladder, uterus, vagina, and all other organs/tissues showing gross abnormalities during necropsy.

No toxicokinetics were performed.

Results: There were no mortalities, no clinical signs, no relevant body weight changes, no hearing changes, no changes in clinical biochemisty, hematology and urinalysis, and no changes in organ weights or macroscopic and microscopic findings.

Study title: — **: Reverse mutation in five histidine-requiring strains of Salmonella typhimurium**

Key findings: — was not mutagenic with or without metabolic activation at doses up to in *S.tyhimurium* and *E.coli* strains.

Study no.: 63/40-1052
E-Location: CTD 4.2.3.7.6.8
Conducting laboratory and location: —

Date of study initiation: Aug 15, 1997
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— Batch GK-97009, Purity —
Vehicle: Dimethyl sulfoxide (DMSO)

Methods

Strains/species/cell line: *Salmonella typhimurium*

Strain	Type of mutation in the histidine gene
TA98	frame-shift
TA100	base-pair substitution
TA1535	base-pair substitution
TA1537	frame-shift
TA102	base-pair substitution

Doses used in definitive study:

Experiment	S-9	Concentration of treatment solution (mg/mL)	Final concentratio (µg/plate)
Range-finder Experiment and Mutation Experiment 1	- and +	0.08	8
		0.40	40
		2.00	200
		10.00	1000
		50.00	5000
Mutation Experiment 2	-	0.256	25.6
		0.64	64
		1.60	160
		4.00	400
		10.00	1000
Mutation Experiment 2	+	0.512	25.6
		1.28	64
		3.20	160
		8.00	400
		20.00	1000
		50.00	2500

Basis of dose selection:

An initial toxicity range-finder experiment was conducted with TA100 only, using final concentrations of — at 8, 40, 200, 1000 and 5000 µg/plate, plus solvent and positive controls. Following these treatments, no evidence of toxicity (as would normally have bmn indicated by a thinning of the background bactcrial lawn and/or a marked reduction in revertant nurabers) was observed. However, precipitation of the test article was observed on all test plates treated at a concentration of 1000 µg/plate or above.

Negative controls: DMSO

Positive controls:

Chemical	Source	Stock* concentration (µg/mL)	Final concentration (µg/plate)	Use Strain(s)	Use S-9
2-nitrofluorene (2NF)		50	5.0	TA98	-
Sodium azide (NaN_3)		20	2.0	TA100	-
				TA1535	-
9-aminoacridine (AAC)		500	50.0	TA1537	-
Glutaraldehyde (GLU)		250	25.0	TA102	-
2-aminoanthracene (AAN)		**50	5.0	At least one strain	+

Incubation and sampling times:

In two separate experiments, cultures at each dose were set up in triplicate plates without and with S-9. Negative (solvent) controls were included in each assay, in quintuplicate without and with S-9. In each experiment, bacterial strains were treated with diagnostic mutagens in triplicate in the absence of S-9. The activity of the S-9 mix used in each experiment was confirmed by AAN treatments (in triplicate) of at least one strain in the presence of S-9. The mammalian liver post-mitochondrial fraction (S-9) used for metabolic activation was prepared from male Sprague Dawley rats induced with Aroclor 1254 and obtained from Plates were incubated for 3 days.

Colonies were counted electronically or manually, where confounding effects such as precipitation of test agent or poor quality top agar prevented an accurate automated count from being obtained. The background bacterial lawn of each test plate was inspected for signs of toxicity. Treatment of data individual plate counts from each experiment were recorded separately and the mean and standard deviation of the plate counts for each treatment were determined. The m-statistic was calculated to check that the data were Poisson-distributed and Dunnett's test was used to compare the counts of each dose with the control. The presence or otherwise of a dose response was checked by linear regression analysis.

Results

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

The assay was considered valid if the following criteria were met:

1) the mean negative control counts fell within the normal ranges
2) the positive control chemicals induced clear increases in revertant numbers confirming discrimination between different strains, and an active S-9 preparation
3) no more than 5% of the plates were lost through contamination or some other unforeseen event.

The compound was considered mutagenic if:

1) the assay was valid
2) Dunnett's test gave a significant response ($p \leq 0.01$), and the data set showed a significant dose-correlation
3) the positive responses described in 2) were reproducible

Study outcome:

In experiment 1 treatments of all strains were performed with the maximum treatment concentration of 5000 µg/plate and used the same dose range as the range-finder experiment. Evidence of toxicity (manifest as a slight thinning of the background bacterial lawn) was observed. These toxic effects occurred solely at the maximurn treatment concentration with strains TA98 and TA102 in the presence of S-9. Precipitation of the test article was also observed on a majority of test plates treated at the two highest treatment concentrations. s

In experiment 2 treatments were performed with the maximum treatment concentrations to the lower concentration at the limit of the toxic and/or precipitating range. A narrowad dose range was employed in order to more closely investigate those doses of — considered likely to to induce any mutagenic response. In addition, all treatments in the presence of S-9 employed a pre-incubation step. In this way, it was hoped to increase the range of mutagenic chemicals that could be detected in the assay. Evidence of toxicity was again observed, only at the maximurn treatrnent concentration, with strains TA98 ard TAl02 in the presence of S-9. precipitation of the test article was also observed on test plates treated at the higher treatment concentrations.

The mean numbers of revertant colonies on negative control plates were all comparable with acceptable ranges, and were significantly elevated by positive control treatments.

Only range-finder study treatments with strain TA100, in tbe presence of S-9, result in a statistically significant increase in revertant numbers ($p<1\%$ Dunnetts's test). The increase was very small in magnitude, occurred at the lowest treatment concentration, and could not be reproduced when considered alongside analogous treatments in experiment 1 and experiment 2, where no statistically significant increase occurred. None of the test treatments provided clear evidence of — nutagenic activity. Thus, it was concluded that — did not induce mutation in five strains of Salmonella typhirnurium (TA98, TA100, TA1535, TA1537 and TA102).

summary of mean revertant colonies (-S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	100 µl	35 ± 2	116 ± 11	14 ± 4	8 ± 3	397 ± 19
[redacted]	25.6	30 ± 2	118 ± 9	18 ± 2	8 ± 7	391 ± 15
	64	35 ± 4	111 ± 11	13 ± 5	5 ± 2	363 ± 16
	160	32 ± 3	117 ± 13	20 ± 1	8 ± 3	385 ± 37
	400	32 ± 3 (Ppn)	127 ± 7 (Ppn)	13 ± 3 (Ppn)	5 ± 5 (Ppn)	379 ± 19 (Ppn)
	1000	31 ± 1 (M+Ppn)	124 ± 23 (M+Ppn)	18 ± 4 (M+Ppn)	7 ± 3 (M+Ppn)	376 ± 90 (M+Ppn)
Positive controls	Compound	2NF	NaN3	NaN3	AAC	GLU
	Dose Level	5 µg	2 µg	2 µg	50 µg	25 µg
	Mean ± SD	538 ± 52	624 ± 19	391 ± 18	466 ± 94	699 ± 17

SD Standard deviation

2NF 2-Nitrofluorene
NaN3 Sodium azide
AAC 9-Aminoacridine
GLU Glutaraldehyde

Ppn : Precipitation of TA observed
M : Plate counted manually

APPEARS THIS WAY
ON ORIGINAL

— summary of mean revertant colonies (+S-9) - Experiment 2

Substance	Dose Level µg/plate	TA98	TA100	TA1535	TA1537	TA102
		Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD	Mean ± SD
DMSO	50 µl	40 ± 7	138 ± 11	17 ± 4	12 ± 3	482 ± 19
—	25.6	38 ± 5	149 ± 11	26 ± 4	10 ± 6	471 ± 11
	64	40 ± 6	132 ± 8	16 ± 9	10 ± 2	482 ± 2
	160	41 ± 8	141 ± 9	18 ± 3	16 ± 4	467 ± 8
	400	37 ± 8	145 ± 11	24 ± 6	11 ± 4	468 ± 2
	1000	34 ± 9 (M)	135 ± 7	21 ± 2	5 ± 1	487 ± 11
	2500	35 ± 9 (M+S +Ppn))	125 ± 9 (Ppn)	23 ± 4 (Ppn)	8 ± 6 (Ppn)	505 ± 26 (S + Ppn))
Positive controls	Compound	AAN	AAN			
	Dose Level	5 µg	5 µg			
	Mean ± SD	1101 ± 55	1766 ± 111			

SD Standard deviation

AAN 2-Aminoanthracene

Ppn : Precipitation of TA observed
S : Slight thinning of b/g lawn
M : Plate counted manually

Study title: — **Mutation at the thymidine kinase (tk) locus of mouse lymphoma L5178Y cells (MLA) using the echnique**

Key findings: — is not mutagenic in this test system m the absence of S-9. The results in the presence of S-9 were equivocal.

Study no.: 63/39-1052
E-location: CTD 4.2.3.7.6.9
Conducting laboratory and location: —

Date of study initiation: July 30, 1997
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— Batch GK-97009, Purity —

Vehicle: dimethyl sulfoxide

Methods

Strains/species/cell line: mouse lymphoma cells

Doses used in definitive study:
Experiment 1: 7.5 to 90 µg/mL, in the absence of S9
7.5 to 120 µg/mL, in the presence of S9

Experiment 2: 7.5 to 120 µg/mL, in the absence of S9
7.5 to 120 µg/mL, in the presence of S9

Basis of dose selection:
Doses were based on the result of a cyotoxic range-finding study. In the cytotoxicity range-finding experiment, six doses were tested, separated by two-fold intervals and ranging from 3.75 to 120 µg/mL limited by solubility). The top doses that survived treatrnent were 60 µg/mL in the absence of S-9 and 120 µg/mL in the presence of S-9. These doses yielded 15.32% and 11.31% relative survival, respectively. All doses were selected to determine viability and 5-trifluorothymidine (TFT) resistance 2 days after treatment. The top doses tested (90 µg/mL in the absence of S-9 and 120 µg/mL in the presence of S-9) yielded 40. 18% and 14.17% relative survival. In the second experiment, in the absence of S-9, the dose range was modified slightly to take account of toxicity observed in experiment 1. The dose range tested in the presence of S-9 was not modified. The top dose selected to determine TFT resistance was 120 µg/mL, which yielded 23.41% and 12.57% relative survival in the absence and presence of S-9, respectively.

Negative controls: DMSO diluted 100-fold in the treatment medium:

Positive controls:

compound	final concentration	S-9
4-nitroquinoline 1-oxide (NQO)	0.05	-
	0.10	-
Benzo(a)pyrene (BP)	2.0	+
	3.0	+

Incubation and sampling times:

Treatment of cell cultures for the cytotoxicity range-finding expcriment was the same as for the mutation experiments. However, single cultures only were used and positive controls were not included. Following treatment, cells were washed with tissue culture medium and then resuspended in 20 mL RPMI 10. Cell concentrations were adjusted to 8%ells0thL and, Oor°h dosep0.2°L was plated into each well of a 96-well micrr° plate. The plates were incubated at 37 C in a humidified incubator gassed with 5% v/v CO, in air for 7 days. Wells containing viable clones were identified by eye using backgraund illumination and counted.

—— was assayed for its ability to induce mutation at the tk locus (5-trifluorothymidine resistance) in mause lymphoma cells using a fluctuation protocol. The study consisted of a cytotoxicity range-finding experiment followed by two independent experiments, each conducted in the abseace and presence of metabolic activation by an Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9).

In the cytotoxicity range-finding experiment, six doses were tested, separated by two-fold intervals and ranging from 3.75 to 120 p,g/mL gimited by solubility). The top doses that survived treatrnent were 60 p.g/mL in the absence of S-9 and 120 pg/mL in the presence of S-9. These doses yielded 15.32% and 11.31% relative survival, respectively.

Accordingly, six doses were chosen for the first experiment, ranging from 7.5 to 90 p,g/mL, in the absence of S-9 and from 7.5 to 120 pg/rnL in the presence of S-9. All doses were selected to determine viability and 5-trifluorothymidine (TFT) resistance 2 days after treatment. The top doses tested (90 p,g/mL in the absence of S-9 and 120 p,g/mL in the presence of S-9) yielded 40. 18% and 14.17% relative survival (0.36 and 0.07 relative total growth [RTG]), respectively. In the second experiment, in the absence of S-9, the dose rarge was modified slightly to take accaunt of toxicity observed in Experiment 1. The dose range tested in the presence of S-9 was not modified. The top dose &lWted to determine TFT resistance was 120 pg/mL, which yielded 23.41% and 12.57% relative survival (0.21 and 0.08 RT5) in the absence and presencdbf S-9, respectively.

Statistical significance of mutant frequencies (total wells with clones) was carried out according to the UKEMS guidelines. Thus the control log mutant frequency (LMF) was compared with the LMF from each treatment concentration based on Dunnett's test for multiple comparisons, and secondly the data was checked for a linear trend in mutant frequency with treatment concentration using weighted regression. The test for linear trend is one-tailed, therefore negative trend was not considered significant. These tests required the calculation of the heterogeneity factor to obtain a modified estimate of variance.

Results

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

The assay was considered valid if all the following criteria were met:

1) the mutant frequencies in the negative (solvent) control cultures fell within the normal range (above 60 mutants per 10 viable cells but not more than three times the historical mean value)
2) at least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency (the difference between the positive and negative control mutant frequencies was greater than half the historical mean value)
3) the plating efficiencies of the negative controls from the mutation experiments were between the range of 60% to 140% on Day 0 and 70% to 130% on Day 2.

The test article was considered to be mutagenic if all the following criteria were met:

1) the assay was valid
2) the mutant frequency at one or more doses was significantly greater than that of the negative control ($p < 0.05$)
3) there was a significant dose-relationship as indicated by the linear trend analysis ($p < 0.05$)
4) the effects described above were reproducible.

Study Outcome:

Negative (solvent) and positive control treatrnents were included in each mutation experiment in the absence and presence of S-9. Mutant frequencies in negative control cultures were within the normal ranges, and clear increases in mutation were induced by the positive control chemicals 4-nitroquinoline 0-oxide (without S-9) and benzo(a)pyrene (with S-9). Therefore the study was accepted as valid.

In the absence of S-9, no statistically significant increases in mutant frequency were observed following treatment with — at any dose level tested, in Experiment 1 or 2.

In the presence of S-9, in Experiment 1, statistically significant increases in mutant frequency were observed following treatment with — , at the top three dose levels tested. A lincar trend was obtained. These increases in mutant frequency were all observed at highly toxic doses (between 0.22 and 0.07 relative total growth or TRG). Furthermore, although a linear trend was also obtained in Experiment 2, a statistically significant increase in mutant frequency was only observed at the toxic top dose level tested (120 µg/mL, yielding 12.57% relative survival (0.08 RTG). The increase in mutant frequency above control level at this dose was small (1.5 fold). Therefore, there was insufficient evidence of a reproducible effect to conclude that — was mutagenic in this test system.

TABLE 2

summary of results

Experiment 1

Treatment (μg/mL)	-S-9 %RS	RTG	MF§		Treatment (μg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	151.99		0	100.00	1.00	110.97	
7.5	105.79	0.93	137.99	NS	7.5	81.97	0.79	116.85	NS
15	83.73	0.91	137.32	NS	15	89.71	0.76	127.99	NS
30	92.26	1.10	128.96	NS	30	51.26	0.52	141.06	NS
45	76.05	1.15	129.48	NS	60	31.21	0.22	224.19	*
60	60.54	0.76	134.67	NS	90	23.33	0.14	233.56	*
90	40.18	0.36	164.06	NS	120	14.17	0.07	289.78	*
Linear trend				NS	Linear trend				***
NQO					BP				
0.05	91.28	0.80	542.70		2	33.46	0.29	932.68	
0.1	66.21	0.84	572.05		3	12.59	0.09	1127.94	

Experiment 2

Treatment (μg/mL)	-S-9 %RS	RTG	MF§		Treatment (μg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	92.86		0	100.00	1.00	86.39	
7.5	86.61	1.07	89.81	NS	7.5	114.04	0.98	65.85	NS
15	90.55	1.07	87.15	NS	15	85.30	1.01	72.65	NS
30	81.60	1.02	92.98	NS	30	67.57	0.61	77.20	NS
60	58.09	0.63	70.37	NS	60	47.89	0.33	100.93	NS
90	37.00	0.29	86.39	NS	90	41.51	0.25	90.81	NS
120	23.41	0.21	71.57	NS	120	12.57	0.08	131.89	*
Linear trend				NS	Linear trend				***
NQO					BP				
0.05	86.16	0.96	252.00		2	37.32	0.42	776.98	
0.1	75.96	0.73	498.87		3	46.63	0.33	969.36	

§ 5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS Percent relative survival adjusted by post treatment cell counts
NS Not significant
* Comparison of each treatment with control: Dunnett's test (one-sided), significant at 5% level
*, **, *** Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

Study title: Single dose oral toxicity study in the mouse (approximation of the minimum lethal dose level

Key findings: The acute oral toxicity of in the mouse exceeded 2000 mg/kg body weight.

Study no.: 063/037
E-location: CTD 4.2.3.7.6.3
Conducting laboratory and location:

Date of study initiation: Sept 9, 1997
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— , Batch GK-97009, Purity —
Vehicle: 1% m/v aqueous methylcellulose

Preliminary Study

Methods: In a preliminary study groups of fasted mice (CD-1(CR)BR strain; n=1/sex/dose) were administered a single oral dose of 500, 1000, 1500 and 2000 mg/kg of — at a dose volume of 20 mL/kg on Day 1.
Results: There were no deaths and no overt reactions to treatment. Necropsy of the mice on day 15 revealed no macroscopic changes.

Main study

Methods: Fasted mice (n=5/sex; 5-7 weeks of age, males 29 to 32 g, females 25 to 29 g) were administered a single oral dose of — at 2000 mg/kg (***reviewer comment***: *there was no vehicle control group*). Surviving mice were necropsied (only macroscopically) on day 15.

Results: There were no clinical adverse signs after dosing, and all surviving mice gained weight during the 14 day observation period (males: +6 to+7 g; females: +2 to +5 g). Necropsy of mice surviving treatment revealed no macroscopic changes except for one male mouse with an abscess on the mucosal surface of the stomach.

One male mouse that had shown no climical signs in the 24 hour period following dosing was found dead on day 2. Necropsy revealed a dark appearance of the lungs and abnormal contents within the thoracic cavity. The Sponsor attributed this to a technical error during dosing. *Reviewer concurs with this conclusion.*.

Study title: — **. Skin sensitization study in the guinea pig.**

Key findings: — did not elicit skin sensitisation when tested in guinea pigs.

Study no.: 063/038-D6144
e-location: 4.2.3.7.6.10
Conducting laboratory and location: —

Date of study initiation: Sept 10, 1997
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
— , Batch GK-97009, Purity
Vehicle: Alembicol D

Methods:

Species: Female guinea pigs (nulliparous, non-pregnant female Denkin-Hartley guinea pigs; 4 to 7 weeks of age and weighed 266 to 411 g) were used in these studies. The strain of guinea pig selected for this study has been subject to regular (six monthly) testing to confirm its susceptibility to a moderately strong skin sensitiser, 2-mercaptobenzothiazole or α-hexylcinnamaldehyde.

Screening studies
The dose levels for the main study were selected on the basis of screening studies. The maximum practical concentrations that could be achieved in the optimum vehicle for intradermal injection or topical administration were determined prior to conducting the screening studies.

In the first screening test (intradermal injection phase of induction) The vehicle and six formulations were selected. On Day 1, intradermal injeotions (0.1 mL per site), incorporating concentrations from 0.1% to 5.0% m/v — , in Alembicol D (up to the maximum practical concentration), were made into the scapular zone of the previously clipped dorsal region. Dermal reactions were individually assessed and recorded approximately 24 and 72 hours later.

In the second screening test (topical application phase of induction) four formulations, incorporating a range of concentrations from 10% to 60% m/m — in Alembicol D (up to the maximum practical concentration) were injected into two guinea pigs that had previously received two 0.1 mL intradermal injections of Freund's complete adjuvant emulsion (FCA) into the suprascapular dorsum at least five days prior to application of these second formulations. The animals were previously clipped and shaved then subjected to occluded, topical application of four 20 x 20 mm patches of Whatman No 4 filter paper each saturated with appraximately 0.2 mL of one of the test formulations and covered with an adhesive dressing and elastic bandage. At 48 hours after application, the treatment sites were washed with arachis oil and patch locations marked for assessment 21 and 96 hours after patch removal.

In the third screening test (topical application at challenge) four formulations, ranging from 30% to 60% m/m — in Alembicol D were chosen to identify the maximum non-irritant

concentration of test article after occluded, topical application to skin. Three guinea pigs were prepared by receiving two 0.1 mL intradermal injections of FCA into the suprascapular dorsum between 14 and 28 days prior to application of the test formulations. Each animal was subject to occluded topical application of four 12 mm Finn chambers each loaded with approximately 0.1 mL of one of the four selected test formulations. The Finn chambers were secured with adhesive adhesive dressing and elastic bandage. The dressings and chambers were removed after 24 hours and the treated areas of skin were washed with arachis oil. The location of each challenge site was marked and dermal reactions were assessed at 24 and 48 hours after removal of the chambers.

Criteria for the selection of the main study treatment regime included the following:

- Treatments should not cause systemic toxic effects.
- The highest concentration of which caused no more than moderate irritation (Grade 3 erythema) and no more than minute, central foci of necrosis or other marked tissue damage at sites of injection in the first screen was selected for the intradermal injection phase of induction.
- The highest concentration of which caused no more than moderate irritation (Grade 3 erythema) and no indication of necrosis or other marked tissue change at the test sites of the second screen was selected for the topical application phase of induction.
- The highest concentration of which caused no skin irritation in the third screen was selected for challenge.

Based on the results of the preliminary screening studies, the following formulations were selected:

Intraderrnal injection: 5 % m/v in Alembicol D and/or adjuvant
Topical induction: 60% m/m in Alembicol D
Challenge application: 30 and 15% m/m in Alembicol D

Main Study

The main study consisted of a test group of 20 female guinea pigs and a control group of 10 female guinea pigs (nulliparous, non-pregnant female Denkin-Hartley guinea pigs; 4 to 7 weeks of age and weighed 266 to 411 g). The dorsum overlaying the scapulae of each guinea pig was clipped on the day before treatment commenced. During the induction phase (day 1), 20 female test animals were treated by intradermal injection with FCA, 5% m/v in Alembicol D and 5% m/v in FCA. The control group was treated with FCA, Alembicol D and 50% v/v Alembicol D in FCA. One week after the intradermal treatment, the animals were pretreated with sodium lauryl sulfate and 60% m/m in Alembicol D (test animals) or Alembicol D alone (control) were applied topically with occlusion for 48h. Two weeks after the epidermal induction, the animals were challenged with 0.1 mL of 30% and 15% m/m in Alembicol D. Irritation or other dermal changes at the treated sites were recorded on day 2, day 11 and 24 and 38h after challenge with

Test and control guinea pigs were observed daily for clinical signs of reaction to treatment. The guinea pigs were weighed on Day 1 before induction commenced (initial body weights were less than 500g) and again on Day 25. Changes at the intradermal injection or occluded topical

application sites were assessed in a well-illuminated area. Dermal changes were recorded using the following scheme:

Dermal change	Record
No erythema	0
Slight erythema	1
Well defined erythema	2
Moderate erythema	3
Severe erythema (beet redness)	4
In-depth damage/eschar	E
Necrosis	N
Desquamation	Q
Discoloration	D
Induration	H

All other guinea pigs were killed by intraperitoneal injection of sodium pentobarbitone following completion of the challenge prooedures on Day 25. No tissue preservation or histopathological assessment of tissues was conducted.

Results

Main study:
Observations on day 2 showed that intradermal administration of the adjuvant emulsion alone, the vehicle alone or a mixture of vehicle and adjuvant emulation to control animals and of the test article in vehicle or adjuvant emulsion to test animals did not elicit dermal irritation. Slight erythema was apparent in test animals following occluded topical application of 60% m/m — in Alembicol D and control animals following application of Alembicol D on day 11. None of the animals developed a positive response indicative of skin sensitization following challenge application of — — Inconclusive dermal reactions were seen in 3 of the test guinea pigs. Sixteen of the 19 test animals showed no dermal reaction to challenge. The results indicated that — did not have the potential to cause skin sensitization.

No clinical observations of ill health or toxicity were noted during the study. One guinea pig was found dead on Day 6. It had shown no signs of ill-health prior to death and necropsy revealed no macroscopic changes. No cause of death was established. All guinea pigs gained weight during the course of the main study.

One guinea pig from the test group was found dead on Day 6. A full macroscopic necropsy was performed on the single decedent. The necropsy procedure included inspection of external surfaces and orifices, all viscera and tissue within the abdominal, thoracic and cranial cavities, free-hand sectioning of the liver and kidneys and examination of mucosal surfaces of the

stomach, small and large intestines. There was no findings that could be attributable to the treatment.

Induction reactions: A summary of reactions seeg in the induction phase is presented in Table 4.

TABLE 4
Summary of induction phase dermal reactions

Site	Intradermal injection		Topical application	
	Test animals	Control animals	Test animals	Control animals
Anterior	Slight erythema	No erythema	Slight erythema	Slight erythema
Middle	No erythema	No erythema		
Posterior	No erythema	No erythema		

Intradermal injection: Slight erythema was noted at the anterior injection sites of the test animals following intradermal injection of the emulsion of Freund's Complete Adjuvant. Intradermal administration of the adjuvant emulsion alone, the vehicle alone or a rnixture of vehicle and adjuvant emulsion to control animals and of the test article in vehicle or adjuvant emulsion to test animals elicited no dermal irritation.

Topical application: Slight erythema was apparent in test animals following occluded topical application of 60% m/m — in Alembicol D and in control animals following application of Alembicol D.

Challenge response: Individual control and test group reactions are presented below. No animal developed a positive response indicative of skin sensitisation (delayed contact hypersensitivity) following challenge application of — An inconclusive dermal reaction was seen in three of the test guinea pigs. Sixteen of the nineteen test animals subject to the procedure showed no dermal reaction to challenge.

Guinea pig number	Score						Results Positive[+] Inconclusive [±] Negative [-]
	24 hours			48 hours			
	A	P	C	A	P	C	
2246	0	0	0	1	0	0	±
2247	1	0	0	0S	0	0	±
2248	0	0	0	0q	0q	0	-
2249	0	0	0	0	0	0	-
2250	0	0	0	0q	0q	0q	-
2251	0	0	0	0q	0q	0q	-
2252	0	0	0	0q	0q	0	-
2253	0	0	0	0q	0q	0q	-
2254	0	0	0	0	0	0	-
2255	0	0	0	0	0	0	-

TABLE 5

Dermal reactions observed after challenge - controls

Guinea pig number	Score					
	24 hours			48 hours		
	A	P	C	A	P	C
2236	0	0	0	0	0q	0q
2237	0	0	0	0	0q	0q
2238	0	0	0	0	0	0
2239	0	0	0	0	0	0q
2240	0	0	0	0q	0q	0q
2241	0	0	0	0q	0q	0q
2242	0	0	0	0	0	1q
2243	0	0	0	0q	0q	0q
2244	0	0	0	0	0	0q
2245	0	0	0	0	0	0

Key:

A Anterior site exposed to — 30% m/m in Alembicol D
P Posterior site exposed to — , 15% m/m in Alembicol D
C Control site exposed to Alembicol D

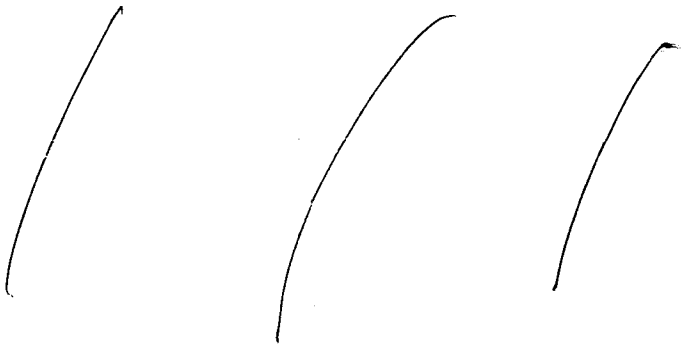
0 No erythema
1 Slight erythema
q Desquamation

Guinea pig number	Score						Results Positive[+] Inconclusive [±] Negative [-]
	24 hours			48 hours			
	A	P	C	A	P	C	
2256	0	0	0	1q	0q	0q	±
2257	0	0	0	0q	0q	0	-
2258	0	0	0	0q	0q	0q	-
2259	0	0	0	0q	0q	0	-
2260	0	0	0	0q	0q	0q	-
2261	0	0	0	0	0	0	-
2262	0	0	0	0q	0	0q	-
2263	0	0	0	0	0	0	-
2264	0	0	0	0q	0q	0q	-
2265	DEAD						

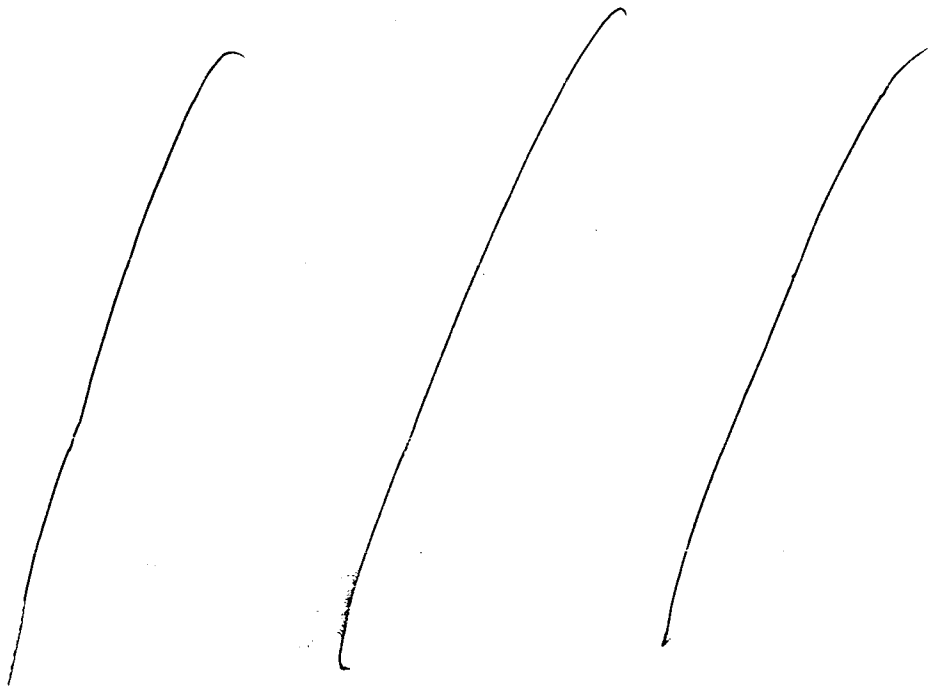
EXCIPIENT TOXICOLOGY

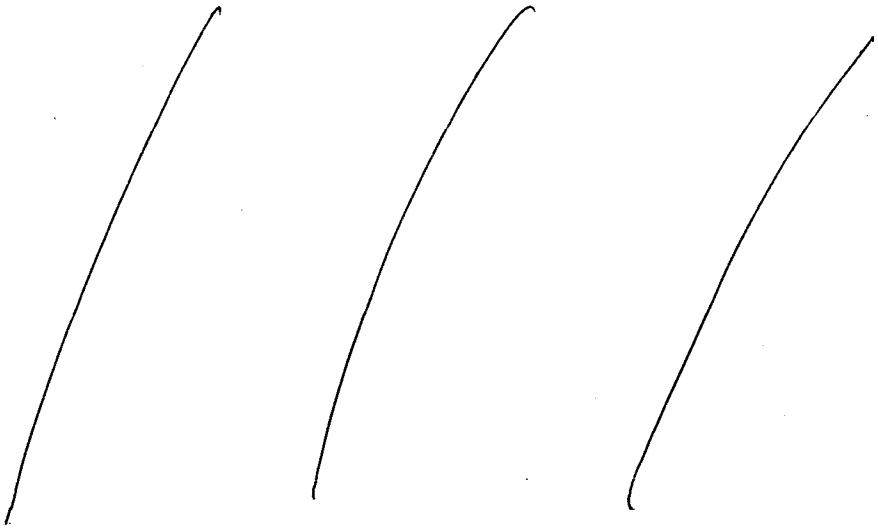
Cocoyl caprylocaprate

Cocoyl caprylocaprate is used [redacted] in Voltaren Gel, comprising [redacted] (w/w) of the drug product. Although it was listed in the European Pharmacopoeia, toxicology information was lacking. Upon our request for this information after the midcycle meeting, we received DMF [redacted] which contained the following [redacted]



Cocoyl caprylocaprate (CAS 95912-86-0. [redacted] is the caprylic/capric acid ester of saturated fatty alcohol C_{12-18} derived from [redacted]





Carbomer —

Study title: Carbomer — **Contact hypersensitivity in albino guinea pigs, maximization-test.**

Key study findings: Carbomer was not sensitizing in a cutaneous hypersensitivity study.

Study no.: 845093
E-Location: CTD 4.2.3.7.1.7
Conducting laboratory and location:

Date of study initiation: Aug 5, 2002 to Oct 10, 2002
GLP compliance: yes
QA reports: yes
Drug, lot #, and % purity:
Carbomer — Lot CC178CC398, Purity Unknown;
Vehicle: purified water

Methods
Female Himalayan spotted guinea pigs (Ibm: GOHI; SPF-quality; 5-7 weeks of age; 335-402 g, 15 females, 5 nulliparous and non-pregnant) were administered an intradermal injection in the nuchal region of a 5% dilution of the carbomer — in purified water and in an emulsion of Freund's Complete Adjuvant (FCA) / physiological saline. The epidermal induction of sensitization was conducted for 48 hours under occlusion with the test item at 15% in purified water one week after the intradermal induction. The animals of the control group 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 test animals were challenged by epidermal application of the test item at 0.5 % in purified water and

purified water alone under occlusive dressing. Cutaneous reactions were evaluated at 24 and 48 hours after removal of the dressing. Scoring of erythema, edema and other skin changes were assessed by the Magnusson and Klingman grading scale as follows:

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

Based upon the percentage of animals sensitized (24- and 48-hour reading), the test item was assigned to one of the following five grades of allergenic potency according to the Magnusson and Klingman ratings of allerenicity:

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

The concentration of carbomer used for the inductions and challenge were determined in a series of pretests. The concentrations selected for induction exposure was the highest concentration that caused mild-to-moderate skin irritation. The epidermal challenge concentration was the maximum tested non-irritant concentration.

The Sponsor provided the following guide to interpretation of the results:
The results obtained from test animals following the challenge application were compared with the results seen in control animals. An allergic reaction was defined by visible reddening of the challenge site. If the dermal reactions of test animals following the challenge were more marked and/or persistent than those of the control animals, the animals were considered to show evidence of contact hypersensitivity. If the dermal reactions of test animals following the challenge were not clearly different from the reactions seen in the control group animals, the results for the test animals were considered "inconclusive". The test animals were considered to show no evidence of contact hypersensitivity if the dermal reactions to the challenge application were identical or less marked and/or persistent than the reactions observed in the control animals. By "maximizing" the exposure and enhancing allergenicity, some problems could arise, particularly in relation to specificity, especially the potential for false-positive reactions. An inflammatory response at challenge may not necessarily be due to allergenicity, but instead may be a false-positive irritant response caused by an inducing hyperirritability.

Results: There were no toxic symptoms were evident in the guinea pigs of the control or test group. No deaths occurred. None of the control or carbomer treated animals exhibited skin reactions after the challenge treatment at 0.5 % (w/w) in purified water.

Reviewer Comment: The positive control studies using alpha-hexylcinnamaldehyde, were not conducted simultaneous as the above study, but were conducted months earlier between mid Feb and April 2002. It resulted in the expected sensitization response.

2.6.6.9 Discussion and Conclusions

The nonclinical studies for Voltaren Gel relied mainly on studies originally submitted to support Voltaren (NDA 19-201), an oral tablet formulation of diclofenac sodium. Genetic, carcinogenetic, and reproductive toxicological studies were conducted previously for approval of Voltaren (NDA 19-201) and were not reviewed here, but briefly summarized for the purposes of labeling. Additional studies were submitted to support the topical route of administration. Several of these studies were conducted in the 1970's and 1980's prior to GLP implementation, but were not submitted to NDA 19-201. Other more recently conducted GLP studies included dermal and eye irritation, skin hypersensitivity, and dermal photosensitivity studies. With a few exceptions (indicated below when appropriate) they appear to be adequately conducted and interpreted. When the NDA was submitted, there a number of potentially problematic issues were revealed because the Sponsor had not previously provided sufficient information. These related to the toxicology of diclofenac-related impurities, novel excipients, and photodegradants.

Comment on types of studies, drug, and vehicle

The early studies were conducted with either diclofenac sodium or diclofenac diethylamine (diethylammonium) formulated in the Emugel vehicle. Later GLP studies conducted in the early 2000's were formulated in the Voltaren Gel vehicle. In developing the Voltaren Gel vehicle, the Emugel was modified to replace [redacted] with carbomer homopolymer Type C [redacted] as [redacted] strong ammonia solution [redacted]. These changes have potentially significant toxicological implication. The change in carbomer type is to one that theoretically [redacted]. Evidence for this was not presented, nor referenced. This carbomer is currently used in cosmetics and other dermatological formulation and listed in the Agency's Inactive Ingredient Database for use at a concentration greater than contained in Voltaren Gel. The other change in the vehicle removed the organic amine that may react with nitrous acid, nitrates or atmospheric nitrous oxides to form N-nitrosamines, known carcinogens. Given these improvements in the vehicle and change to diclofenac sodium salt, it is unfortunate that the nonclinical studies used so many different codes and names for the study drug and/or vehicle. Despite the clarification provided in the Chemistry section of the submission (see table 4-1, below), it was often not clear from comparison with the certificates of analysis and previous drug codes which salt form or vehicle was actually used in the study. Fortunately, due to the general lack of toxicity noted in the nonclinical studies, and expected greater safety of Voltaren Gel compared to Voltaren Emulgel (but not assessed in nonclinical studies), the type of active drug salt and type of vehicle used were not considered problematic for the overall conclusions.

Table 4-1 Cross reference of adopted product names during development

Product name	Other adopted product names
Diclofenac sodium topical gel 1%	(diclofenac sodium topical gel) 1%
	(diclofenac sodium topical gel), 1%
	DSG 1%
	DSG
	Voltaren
	Voltaren 1% Emulgel
	Voltaren Emulgel 1%
	Diclofenac sodium gel 1% (w/w)
	Voltaren 10 mg/g Emulgel
	Voltaren sodium gel 1%
	Voltaren sodium gel
	Voltaren gel 1%
	Voltaren gel

Results of Nonclinical Findings

Results from *in vitro* studies using hairless guinea pig skin indicated that skin penetration of diclofenac was similar for Voltaren Gel and Voltaren Emulgel and not affected by pH or viscosity in the rangeof the product specifications. These were based on 24 hour absorption profiles, but there was no kinetic analysis to characterize skin uptake or absorption parameters. In a potentially critical *in vivo* study, plasma concentrations of diclofenac were determined in hairless rats after topical application of diclofenac diethylamine (Voltaren® Emulgel™) to skin sites of induced sunburn. Unfortunately this GLP study lacked descriptive clarity, had inconsistencies in data presentation, and most animals lost weight over the study's 4-5 day duration. This made the determination of absorption and interpretation of the effect of sunburn problematic.

The absorption of ^{14}C-diclofenac (GP 45840) in the Emugel™ formulation was studied after application to guinea pig and rabbit skin (Botta 1985; Report B91/1984 from Ciba Geigy; buried in the literature reference section, rather than listed as a study report). For guinea pigs, 8% of the dose applied was absorbed when an occlusive bandage was also applied, and there was 5% absorption without the occlusive bandage. Importantly, if the cornified layer of skin was scrapped away, the absorption increased by 25%, to 10% of the applied dose. A single dose resulted in maximal blood radioactivity concentrations at 6-8 hours after application. Steady state blood concentrations in guinea pigs were obtained after 3 days of twice daily applications. An unusal finding of pharmacokinetics was observed in rabbits in which a pronounced gender effect was evident; in males, 16% and in females, 40%, of the applied dose was absorbed. This was not observed in other species. It was not invertigated further, but this reviewer suspects it may reflect differences in the metabolic efficacy of diclofenac between genders in the rabbit. There were no studies that examined the possibility of diclofenac depot formation at the site of application that may prolong exposure after washing of the surface.

Dermal irritation was minimal in acute studies in rabbits, expressed as slight erythema visible at 1 hour after a 4 hour semi-occlusive application, but not present 24 hours later. No corrosive effects or edema was observed. In a 4-week study in rabbits, repeated daily dermal application of diclofenac, the transient erythemic response was absent or attenuated within 2 weeks. Repeated dermal dosing was categorized as slightly irritating. Diclofenac applied to the rabbit

eye was classified as nonirritating according the Sponsor's interpretation of their findings using European assessment criteria. However, the observations of early onset reddening of conjuctiva and sclera with swelling, although reversible, lead this reviewer to conclude that it is a irritant. Skin sensitivity studies in guinea pig found no evidence for sensitizing ability of diclofenac. The [redacted] excipient, carbomer [redacted], of Voltaren Gel, which replaced [redacted] of the Voltaren Emulgel, was also tested for dermal sensitization in guinea pigs. No skin reactions occurred upon contact challenge with [redacted] carbomer [redacted] two weeks after induction with a 5% intradermal solution.

Photosenstivity studies in guinea pigs resulted in slight irritation in both UV-exposed and control skin sites, suggesting diclofenac was not photosensitive. Photosensitivity studies with diclofenac are difficult to judge, due to the fact that diclofenac absorbs UV wavelengths near the shorter wavelengths (290 nm) of the recommended testing spectrum (≥290-400 nm), and the intensity of these wavelengths is substantial less than longer wavelengths over the tested spectral range due to technical limitations of the solar simulated light equipment and filtering. Despite these limitations one can conclude that diclofenac was not photosensitizing, and that if photodegradants occurred, they did not induce signs of toxicity. Diclofenac is known to produced photodegradants upon sun exposure, but it is was not determined if photodegradants were formed on or in the skin in these studies. This is determination is not conducted in standard photosafety testing.

Other than the 4-week dermal repeated dose study in rabbits, there were no other repeated dosing studies. The Sponsor provided additional information pertaining to dermal toxicity when requested to provide support for a proposed 12-week duration clinical studies. it was not submitted to the NDA, but was submitted when requested after the midcycle review meeting. This was a 12-week dermal application study in rabbits that compared daily single application of Voltaren Emulgel (400 mg/kg/day at a concentration of 10 mg/cm^2) with placebo Emulgen formulation. For comparison, a human daily dose would consist of 4 applications of 320 mg diclofenac over an estimated area of 400 to 800 cm^2, corresponding to <4 mg/cm^2 (or ~200 mg/kg). Skin reactions included erythema, edema and papules, some scabs, flaking and cracking of the epidermis and a reduction in hair growth. However, these occurred in both groups, were reversible, and therefore attributed to the daily application and removal of occlusive dressings. There were no deaths, no changes in body weight, food consumption, hematology, clinical biochemistry, urinalysis, ophthalmogy, hearing, organ weights, gross and histopathology between treatment groups. There was one animal with an ulcerated gastric mucosa in the diclofenac treatment groups thought to be due to accidental removal of the occlusive dressing and ingestion of diclofenac or pieces of the dressing. There was no toxicokinetic data for this study. It is unlikely this study would have supported a 12 week clinical if submitted for that purpose today, lacking pharmacokinetic data and multiple dose levels.

When the NDA was submitted, there a number of potentially problematic issues were revealed because the Sponsor had not previously provided sufficient information. These related to the toxicology of diclofenac-related impurities, novel excipients, and photodegradants.

Diclofenac Impurities/Degradants

The Sponsor identified four impurities derived from either the synthesis of diclofenac or the degradation of diclofenac upon stability testing. Three impurities/degradants [redacted] exceed the ICH recommended threshold for qualification in stability tests at the proposed 36 month shelf life. Three impurities also had structural alerts for potential genotoxicity [redacted] since they [redacted] Collectively, these four impurities were only partially qualified in genotoxicity testing by mutation studies and by acute oral studies,, but lacked clastogenic assessment and were not studied in 3-month daily application dermal toxicology study. Voltaren Gel contains the same active pharmaceutical ingredient, diclofenac sodium, that has been injected in Voltaren and Voltaren-XR for approximately 20 years. If the dermal absorption of the impurities is similar to diclofenac, the systemic exposure (AUC) to these impurities from topical application is expected to be less than one fifth of that of oral Voltaren (refer to the Biopharm review).

Clinical studies did not reveal a safety concern with topical application of Voltaren Gel (refer to the Clinical Review) although this was at most 1-year duration of application. Unless there is some clinical signal that would warrant further toxicological investigation, this reviewer accepts that the current deficiencies in the toxicological characterization of these impurities, does not warrant further studies. As long as exposure to these impurities is expected to be substantially lower than currently approved product exposures, no further toxicological characterization is necessary and the reviewer defers to the CMC reviewer for decisions on the levels of impurities/degradants.

In our information request to the Sponsor in June 2007, we requested additional toxicological information, if available. The Sponsor had not conducted additional tests, but did acknowledge our comment that guidance and requirements change over the years [redacted]

There is one novel impurity, [redacted] not present in previous diclofenac formulations which also exceeds the stability qualification threshold and has a structural alert for genotoxicity. For this impurity, both mutagenicity (bacterial reverse mutation assay) and clastogenicity (chromosomal aberration assay) testing were negative for genotoxicity. Since no long term dermal toxicology studies were conducted with the Voltaren Gel or a 3-month bridging toxicity study with this specific compound, the compound is incompletely qualified and further studies would normally be requested. 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.

Novel Excipients

The novel excipients included cocoyl caprylocaprate and perfume [redacted] Cocoyl caprylocaprate was the only novel excipient comprising greater than [redacted] w/w) of the drug product, thus requiring toxicological characterization. Although cocoyl caprylocaprate was listed in the European Pharmacopoeia and was acceptable to Chemistry Reviewers as indicated

at an EOP2 meeting of June 1, 2005, we lacked toxicology information to support its safe use. Upon our request for this information after the midcycle meeting, we received DMF

Cocoyl caprylocaprate (CAS 95912-86-0, ") is the caprylic/capric acid ester of saturated fatty alcohol C_{12-18} derived from

The concentration of this excipient in Voltaren Emugel is the same as in Voltaren Gel, providing approximately 20 years of clinical experience with dermal application of this excipient. It 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.
In contrast to diclofenac and its related impurities, 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 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. Since this concern was not previously raised with the Sponsor, we recommend this to be conducted as a Phase 4 commitment.

Photodegradants and sun exposure of the treated sites
Voltaren Gel should be labeled to for patients to avoid sun exposure of the treated areas. It is known, from CMC stability studies for container protection of Voltaren Gel, that photodegradants of diclofenac readily developed with exposure to solar simulated light. Although all of the nonclinical photosafety studies of were negative, there is reason to suspect

the exposure intensity at 290 nm was inadequate. As revealed in exposure intensity-wavelength graphs, it is common for wavelength near the spectral boundaries to have attenuated intensities. This is due to do the light source and filtering. It is important for diclofenac photosafety studies, because the 290 wavelength is near diclofenac's absorption maximum and is comprises the lower boundary of the recommended range for ultraviolet radiation photosafety testing. Furthermore, it is not known if diclofenac photodegradants that are formed by exposure to simulated solar light or sunlight were actually formed in these phototoxicology studies, because this comparison is not required in standard testing and rarely, if ever, conducted. Thus, there is sufficient reason to doubt the adequacy of the standard photosafety studies, as a appropriate determination of diclofenac safety during sun exposure.

The Sponsor is referencing Solaraze for the dermal carcinogenicity and photococarcinogenicity studies as a 505(b)(2) NDA. Solaraze is labeled to avoid sun exposure, since the indication for Solaraze was for treatment of actinic keratosis in which patients should avoid the sun. Photodegradants were also identified in toxicological studies for Solaraze, but these were not further examined for toxicological effects. Being labeled to avoid sunlight rendered additional toxicological studies unnecessary. However, for the osteoarthritic patient population, unlike those with actinic keratosis, there is no clinical reason to avoid sunlight. The areas treated with Voltaren Gel, if exposed to sunlight, would be expected to result in high levels of uncharacterized photodegradants based on CMC stability studies. Furthermore, the photococarcinogenicity studies for Solaraze demonstrated an earlier occurance of tumors by the combination of diclofenac and UV radiation, than by just UV radiation alone. Diclofenac alone had no effect on tumor occurance or incidence at the low doses tolerated in those animal studies. The potentially toxic combination of diclofenac and sunlight can mitigated by labeling to avoid sun exposure of the Voltaren Gel treated skin surfaces.

2.6.6.10 Tables and Figures

See individual sections

2.6.7 TOXICOLOGY TABULATED SUMMARY

See individual sections

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions:

The nonclinical studies for Voltaren Gel relied mainly on studies originally submitted to support Voltaren (NDA 19-201), an oral tablet formulation of diclofenac sodium. Genetic, carcinogenetic, and reproductive toxicological studies were conducted previously for approval of Voltaren (NDA 19-201) and were not reviewed here, but briefly summarized for the purposes of labeling. Additional studies were submitted to support the topical route of administration. Several of these studies were conducted in the 1970's and 1980's prior to GLP implementation, but were not submitted to NDA 19-201. Other more recently conducted GLP studies included dermal and eye irritation, skin hypersensitivity, and dermal photosensitivity studies. With a few exceptions (indicated below when appropriate) they appear to be adequately conducted and interpreted. There were **no approval-related toxicological concerns with the studies reviewed.** A number of potentially problematic issues were revealed because the Sponsor had not previously provided sufficient information. These related to the toxicology of diclofenac-related impurities, novel excipients, and photodegradants.

Topical Toxicology: Minor skin irritation (slight to moderate erythema, some edema) was observed in dermal repeated-dosing toxicological studies. Skin hypersentivity reactions were not apparent in the guinea pig studies. Dermal photosensitivity reactions were also absent, although this may be a false negative as will be discussed below. The product is an eye irritant, although reversible, in contrast to the Sponsor's classification as a non-irritant. Repeated topical dose studies in rabbits of 1-month duration, not optimal for a chronic drug product indication) revealed no systemic toxicities and no dermal toxicity other than erythema, edema, some minor inflammation and epidermal hyperplasia. Due to the need to clip the animals hair at the site of application, the presence of a semiocclusive bandage to protect the area and the need to remove and replace it each day, the same findings were observed in the vehicle treated animals. If there were toxic effects of diclofenac, they were not greater or more severe than that of the vehicle formulation.

Impurities/Degradants: The Sponsor identified four impurities derived from either the synthesis of diclofenac or the degradation of diclofenac upon stability testing. Three impurities/degradants [redacted] exceed the ICH recommended threshold for qualification in stability tests at the proposed 36 month shelf life. Three impurities also had structural alerts for potential genotoxicity [redacted] since they [redacted] Collectively, these four impurities were only partially qualified in genotoxicity testing by mutation studies and by acute oral studies,, but lacked clastogenic assessment and were not studied in 3-month daily application dermal toxicology study. Voltaren Gel contains the same active pharmaceutical ingredient, diclofenac sodium, that has been injected in Voltaren and Voltaren-XR for approximately 20 years. If the dermal absorption of the impurities is similar to diclofenac, the systemic exposure (AUC) to these impurities from topical application is expected to be less than one fifth of that found with oral Voltaren indicated in the table below.

Pharmacokinetic Parameters and Comparison of Voltaren® to Oral Diclofenac Sodium Tablets After Repeated Administration in Human Subjects		
Treatment	**C_{max} (ng/mL)** (Mean ± SD) % of Oral	**AUC_{0-24} (ng•h/mL)** (Mean ± SD) % of Oral
Topical Voltaren® Gel 4 x 4 g per day **(=160 mg diclofenac sodium per day)**	15.0 ± 7.33 0.633%	233 ± 128 5.79%
Topical Voltaren® Gel 4 x 12 g per day **(=480 mg diclofenac sodium per day)**	53.8 ± 32.0 2.21%	807 ± 478 19.7%
Oral Diclofenac sodium tablets p.o. 3 x 50 mg per day **(=150 mg diclofenac sodium per day)**	2270 ± 778	3890 ± 1710

Clinical studies did not reveal a safety concern with topical application of Voltaren Gel (refer to the Clinical Review) although this was at most 1-year duration of application. Unless there is some clinical signal that would warrant further toxicological investigation, this reviewer accepts that the current deficiencies in the toxicological characterization of these impurities, does not warrant further studies. As long as exposure to these impurities is expected to be substantially lower than currently approved product exposures, no further toxicological characterization is necessary and the reviewer defers to the CMC reviewer for decisions on the levels of impurities/degradants.

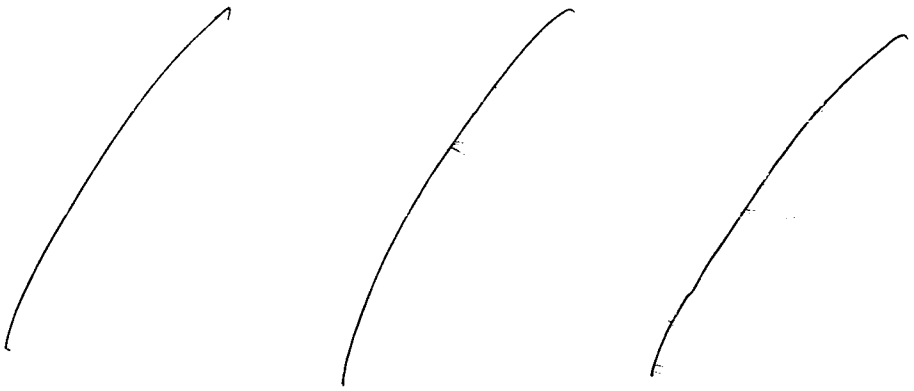
In our information request to the Sponsor in June 2007, we requested additional toxicological information, if available. The Sponsor had not conducted additional tests, but did acknowledge our comment that guidance and requirements change over the years

There is one novel impurity —, not present in previous diclofenac formulations which also exceeds the stability qualification threshold and has a structural alert for genotoxicity. For this impurity, both mutagenicity (bacterial reverse mutation assay) and clastogenicity (chromosomal aberration assay) testing were negative for genotoxicity. Since no long term dermal toxicology studies were conducted with the Voltaren Gel or a 3-month bridging toxicity study with this specific compound, the compound is incompletely qualified and further studies would normally be requested. Toward the end of the review process, Sept 18, 2007, the Sponsor was sent a request to accept the stability qualification threshold of — for —, 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.

Novel Excipients: The novel excipients included cocoyl caprylocaprate and perfume — —. Cocoyl caprylocaprate was the only novel excipient comprising greater than — (w/w) of the drug product, thus requiring toxicological characterization. Although cocoyl caprylocaprate was listed in the European Pharmacopoeia and was acceptable to Chemistry Reviewers as indicated at an EOP2 meeting of June 1, 2005, we lacked toxicology information to

support its safe use. Upon our request for this information after the midcycle meeting, we received DMF [redacted]

Cocoyl caprylocaprate (CAS 95912-86-0, [redacted] is the caprylic/capric acid ester of saturated fatty alcohol C_{12-18} derived from [redacted]



The concentration of this excipient in Voltaren Emugel is the same as in Voltaren Gel, providing approximately 20 years of clinical experience with dermal application of this excipient. It is also present in many cosmetic products (aftershave lotions, bath oils, blushers, cleansing lotions, eye shadows and liners, hair conditioners, lipsticks, suntan preparations, etc) and serves as an emollient, [redacted]. 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. In contrast to diclofenac and its related impurities, 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 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.** Since this concern was not previously raised with the Sponsor, we recommend this to be conducted as a Phase 4 commitment.

Photodegradants and sun exposure of the treated sites: Voltaren Gel should **be labeled to for patients to avoid sun exposure of the treated areas.** It is known, from CMC stability studies for container protection of Voltaren Gel, that photodegradants of diclofenac readily developed with exposure to solar simulated light. Although all of the nonclinical photosafety studies of were negative, there is reason to suspect the exposure intensity at 290 nm was inadequate. As revealed in exposure intensity-wavelength graphs, it is common for wavelength near the spectral

boundaries to have attenuated intensities. This is due to do the light source and filtering. It is important for diclofenac photosafety studies, because the 290 wavelength is near diclofenac's absorption maximum and is comprises the lower boundary of the recommended range for ultraviolet radiation photosafety testing. Furthermore, it is not known if diclofenac photodegradants that are formed by exposure to simulated solar light or sunlight were actually formed in these phototoxicology studies, because this comparison is not required in standard testing and rarely, if ever, conducted. Thus, there is sufficient reason to doubt the adequacy of the standard photosafety studies, as a appropriate determination of diclofenac safety during sun exposure.

The Sponsor is referencing Solaraze for the dermal carcinogenicity and photococarcinogenicity studies as a 505(b)(2) NDA. Solaraze is labeled to avoid sun exposure, since the indication for Solaraze was for treatment of actinic keratosis in which patients should avoid the sun. Photodegradants were also identified in toxicological studies for Solaraze, but these were not further examined for toxicological effects. Being labeled to avoid sunlight rendered additional toxicological studies unnecessary. However, for the osteoarthritic patient population, unlike those with actinic keratosis, there is no clinical reason to avoid sunlight. The areas treated with Voltaren Gel, if exposed to sunlight, would be expected to result in high levels of uncharacterized photodegradants based on CMC stability studies. Furthermore, the photococarcinogenicity studies for Solaraze demonstrated an earlier occurance of tumors by the combination of diclofenac and UV radiation, than by just UV radiation alone. Diclofenac alone had no effect on tumor occurence or incidence at the low doses tolerated in those animal studies. The potentially toxic combination of diclofenac and sunlight can mitigated by **labeling to avoid sun exposure of the Voltaren Gel treated skin surfaces.**

Unresolved toxicology issues (if any):

1) **Labeling to include language to avoid sun exposure of the Voltaren Gel treated areas.**
2) **Cocoyl caprylocaprate, an unapproved excipient of Voltaren Gel, lacks characterization of its dermal carcinogenic potential** as well as toxicological characterization in long-term repeated dosing studies in concordance with the chronic indication of this product. Since this was not previously raised with the Sponsor, a Phase 4 Commitment to determine the dermal carcinogenicity potential of cocoyl caprylocaprate is strongly recommended.

Recommendations: **Approve with**

1) **Labeling to include language to avoid sun exposure of the Voltaren Gel treated areas.**
2) **A Phase 4 dermal carcinogenicity study for the excipient cocoyl caprylocaprate.**

Suggested labeling: see Executive Summary

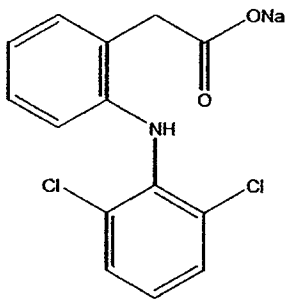
Signatures (optional):

Reviewer Signature _____
L.S. Leshin, DVM, PhD

Supervisor Signature_____ Concurrence Yes ___ No ___
A. Wasserman, PhD

Appendix 1: Diclofenac and Impurities

Diclofenac
GP 45840
Zy-17727B
NCH 927806



IMPURITIES/DEGRADANTS

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

/s/

Lawrence Leshin
9/27/2007 09:25:30 PM
PHARMACOLOGIST

Adam Wasserman
9/27/2007 10:51:15 PM
PHARMACOLOGIST
I concur with Dr. Leshin's review and recommendations. Please see Supervisory Pharmacologist Memo for further discussion of recommendations.

MEMORANDUM

Date: June 13, 2007

From: L.S. Leshin

Through: A Wasserman, PharmTox Team Leader

Subject: **Qualification of Excipients and Degradants**
NDA 22-122
Sponsor: **Novartis Consumer Health**
Drug: **Diclofenac Topical Gel, 1%**

Background

Diclofenac Sodium Topical Gel is a 1% diclofenac sodium formulation intended for the [redacted] such as the hands and knees. The NDA was submitted on Dec 20, 2006, and is currently just past the midcycle of the review.

Summary
An new excipient, cocoyl caprylocaprate, is used in Diclofenac Topical Gel. This is not listed in the inactive ingredient database and no toxicological information is provided to support its safe use. In addition, there was insufficient information submitted to qualify some of the diclofenac degradants, which exceed or may exceed the recommended qualification thresholds by the end of shelf life. The lack of nonclinical dermal toxicology studies to support clinical studies was the result of a decision based partly on a 12-week dermal application study in rabbits with Diclofenac Emulgel 1.16%, a different formulation than the proposed product. This was study was not submitted to the NDA, although it appears to be critical to their development program.

Cocoyl caprylocaprate

This compound is a mixture of esters of saturated C12 to C18 alcohols with caprylic (octanoic) and capric (decanoic) acids obtained by the reaction of these acids with vegetable saturated fatty alcohols. It is practically insoluble in water, but is miscible with mineral oil. [redacted] At the CMC End of Phase 2 meeting June 1, 2005, we informed the Sponsor that this compound was acceptable with reference to the European Pharmacopoeia monograph. However, it has since become apparent that it is a novel excipient for drug products marketed in the United States and therefore, its toxicology needs to be characterized. In the NDA, there was no information about its toxicological safety.

Excipient

Component	% of drug product	Amount applied/day at maximal daily dose*	Comments

Cocoyl caprylocaprate	—	—	**new excipient; no toxicological information**

* based on the intended maximal dose of diclofenac topical gel applied per day, 32 g of topical gel (0.32 g of diclofenac sodium)

— is listed as of the drug product, comprised of components listed as GRAS for oral use of food additives of synthetic flavoring substances and adjavants, or GRAS for oral use as essential oils, oleoresins and natural extractives. The Sponsor provided information about those components of that comprise ≥5% by weight of the parfum crème. This totals only 78% by weight of the composition. Thus, 22% of the composition of parfum crème is not identified.

Other excipients that include isopropyl alcohol, propylene glycol, mineral oil, and carbomer homopolymer Type C are present at concentrations below maximal topical (solution, gel) concentrations listed on the inactive ingredient database.

These degradants of the drug product exceed the recommended criteria for release and for stability at 36 months which is the proposed shelf-life for the drug product. — have structural alerts for potential genotoxicity. These three compounds, therefore, should be limited to a daily application amount of not more than — /day, unless they are qualified. The information provided in the NDA indicates inadequate qualification, lacking genotoxic characterization (mutation and/or clastogenicity) and/or toxicity characterization in a repeated dosing dermal application study of at least 3-months duration. Even with complete qualification, the compounds exceed the recommended stability criteria of — of the drug product at the proposed 36 month shelf-life, as indicated in the table below.

The toxicology studies used to characterize the degradants consisted of acute or short term repeated oral toxicology studies using high doses to determine a lethal dose, differed in the route of administration from the proposed topical route, and lacked histopathology. At the preIND meeting the proposed indication was for — which was later changed to osteoarthritis of the hand and knee, a chronic painful condition. At the Pre-NDA meeting of July 21, 2006, we acknowledged that the impurity limits were acceptable based on previous acceptance of oral diclofenac NDA 19-201 (approved July 28, 1988), but were unaware of the toxicological studies used for assessment at that time in which the product was indicated for —

Diclofenac sodium topical gel is a different route of administration. Diclofenac is photosensitive, yielding additional degradants (based on the 505(b)(2) referenced compound Solaraze, NDA 21-005, approved Oct 16, 2000). The toxicity of these compounds is has not been characterized. Solaraze is indicated for patients with actinic keratosis in which sun avoidance is recommended.

Degradants/Impurities of Drug Substance and Drug Product

Compound	Acceptance Criteria % of drug product (Amount applied/day at maximal daily dose)[1]		Structural Alert for Genotoxicity (conclusion from genotoxicity assays)	Comment	Missing Information
	Release	36 months (proposed shelf life)			
CGP 51553			Yes		
—	NMT —	NMT —	No Not mutagenic; Not clastogenic	Exceeds qualification threshold: > — at 36 months[3]	**Chronic Dermal Toxicity**
—	NMT —	NMT —	Yes (Genotoxicity not tested)	Exceeds qualification threshold: > — / at release and at 36 months[2] — at 36 months[3]	**Genotoxicity Assays** (Mutation and Clastogenicity) **Chronic Dermal Toxicity**
—	NMT —	NMT —	Yes Not mutagenic; (Clastogenicity not tested)	Exceeds qualification threshold: > —/day at release and at 36 month[2]	**Genotoxicity Assay** (Clastogenicity); **Chronic Dermal Toxicity**
—	NMT —	NMT —	Yes Not mutagenic; Not clastogenic	Exceeds qualification threshold: — at 36 month[3]	**Chronic Dermal Toxicity**

[1] intended maximal dose of diclofenac applied per day = 0.32 g (32 g of topical gel)

[2] greater than —) of the drug product, (Guidances for Industry: Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients, May 2005)

[3] threshold for application of maximum daily dose of 0.32 g of diclofenac, is — or — otal daily dose (termed total daily "intake" in guidance), (Guidances for Industry: ICH Q3B(R2) Impurities in New Drug Products, July 2006).

Missing Critical Dermal Application Study in Rabbits

It was note in the initial safety review of IND 64,335 SN-000 that there was no animal study to support the safety of clinical studies longer than 4 weeks. A 4-week skin irritation study in rabbits was the longest study conducted with diclofenac sodium gel. The sponsor was advised to compare and to correlate the local toxicities of diclofenac sodium gel and diclofenac diethylammonium gel from the existing animal data. Depending on the outcome of the analysis, additional animal toxicity studies may be required. The sponsor submitted on January 23, 2004, a 12-week dermal study in rabbits with Emulgel formulation to support the planned 12-week clinical trial with diclofenac sodium gel. The results of this study together with the following information resulted in a decision that no additional nonclinical repeated dosing studies needed to be conducted to support the clinical program.

The sponsor reported no serious adverse effects (including some skin reaction) in the 12-week Diclofenac Emulgel study. A 5-day skin irritation test with diclofenac diethylammonium gel also showed slight erythema after 3-5 days. An *in vitro* skin permeation study showed that diclofenac sodium gel and Diclofenac Emulgel penetrated equally well. Although there are no long term animal studies to directly compare the dermal effects of diclofenac sodium gel and Diclofenac

Emulgel, the available short term study showed no significant difference between the two gels. It is reasonable to assume that the dermal effects of two gels are similar because they share the same active moiety. There is also human data for diclofenac sodium gel to compare its dermal effect with Diclofenac Emulgel. It is felt that no additional animal dermal study with diclofenac sodium gel will be necessary.

The study seems 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 is also important to note that the above decision occurred before a change in the indication from —— to the chronic condition of osteoarthritis.

Internal Comments:

Obtaining toxicological information about the excipient, cocoyl caprylocaprate, may be problematic since we already accepted it as a component based on the European Pharmacopoeia. The individual components are listed in the European Pharmacopoeia (see table in the Appendix), and these can be found in the Toxnet web site. Some of these compounds have at least some associated toxicological information (see Appendix 1). However, the photodermal safety of these compounds is not known.

Likewise, obtaining additional information about the degradants may also be problematic since diclofenac was approved before more extensive impurity testing and qualifications were required. However, as indicated, it is a new route of administration with a new formulation than the approved product. Unfortunately, the indication changed after the preIND meeting —— to chronic use, and no follow-up from the nonclinical safety perspective was provided. Furthermore, as a 505(b)(2) they have referenced Solaraze for the dermal carcinogenicity and dermal photo-cocarcinogenicity studies. It is important to note that Solaraze is indicated for use in a patients population (actinic keratosis) that would avoid sun exposure. This is not the case with Diclofenac sodium gel 1%. Although Solaraze is a topical 3% diclofenac product, the Sponsor will need to determine how the types and quantities of degradants compare with the Solaraze product to be able to bridge to the Agency findings for Solaraze. If the Sponsor's product contains additional degradants or in greater amounts than in Solaraze, additional information or studies may be necessary.

External Comments to Sponsor

During the review of your product, Diclofenac Sodium Topical Gel, 1%, the following issues have been identified for which we are requesting further information:

1. Cocoyl caprylocaprate is a novel excipient for drug products marketed in the United States. As a novel excipient, the safety of cocoyl caprylocaprate will need to be established for use according to the following Guidance for Industry: ***Nonclinical Studies for the Safety Evaluation of Pharmaceutical Excipients*** which may be

found at http://www.fda.gov/cder/guidance. In particular, you will need to provide toxicological information about cocoyl caprylocaprate, especially genetic, dermal, photodermal, and systemic toxicology. Alternatively, provide support from published literature or the use of topical products containing similar or greater amounts of cocoyl caprylocaprate that this compound meets FDA criteria of safety.

2. The list of components of — comprises only those that are equal or greater than 5% of the total composition. This only totals to 78% of the — — composition by weight. There is no threshold for reporting excipients or excipient components. Provide the entire composition for —

3. Impurities and Degradants appear incompletely qualified and/or controlled. Since Voltaren® was approved in 1988, Agency chemistry and toxicology guidelines have been updated to ensure the safety of marketed products. In particular, these now include qualification of impurities containing structural alerts for genotoxicity [see FDA position paper *Regulation of genotoxic and carcinogenic impurities in drug substances and products* (McGovern and Jacobson-Kram; Trends in Analytical Chemistry, 2006)] and the qualification of degradants observed in stability testing as described below.

— are — and contain structural alerts for genotoxicity. According to current Agency policy, such impurities must be controlled to — total daily intake or be toxicologically qualified consisting of negative findings in two in vitro genotoxicity studies (point mutation and clastogenicity assays). — while not containing a structural alert, exceeds the threshold for qualification (NMT — on stability and requires a similar genotoxicity evaluation unless levels can be reduced. Review of submitted studies present in the NDA does not identify evaluations of these impurities in both assays. Furthermore impurities have not been toxicologically qualified in repeat-dose studies up to 3 months duration recommended to support a chronic indication. Provide further data and/or information to support the safety of the identified impurities at the levels proposed.

4. Provide information that the photodegradants for which you are relying on the Agency's prior findings of safety are the same and do not exceed the levels of those produced by the 505(b)(2) reference compound, Solaraze.

5. Submit to the NDA the 12-week rabbit dermal study using Voltaren Emulgel which was submitted to IND 64,334 in Jan 2004 to support the safety of clinical studies longer than 4 weeks. Also provide the concentrations of impurities in the formulation used in this study if available.

Appendix 1: Excipient and Degradant Information

Excipient Composition

COMPONENTS	% W/W of drug product	DOSE/DAY (32 g of gel (= 0.32 g diclofenac) maximum allowed for all joints (g)
Diclofenac sodium (1 g/100 g gel; or 10 mg diclofenac/1.0 g gel	**1.0**	**0.32**
Isopropyl alcohol		
Propylene glycol		
Cocoyl caprylocaprate		
Mineral oil		
Polyoxyl 20 cetostearyl ether		
Carbomer Homopolymer Type C (Carbomers)		
Strong ammonia solution		
Perfume		
Total		
Purified water		
Total	**100 g**	**32 g**

* total components of [redacted] 78% of [redacted] is accounted for by these components which are the main ones (comprising >5%); 0.078/0.1=0.78%; 0.78 x 0.032=0.025 g difference due to rounding error

Appendix 2: Degradant Information

Degradant Specifications

	Release	Shelf life limit (36 months)
	NMT	NMT
	NMT	NMT
	NMT	NMT
	NMT	NMT

Appendix 3: Relevant Previous Meetings and Communications

Review of IND 64,335 SN-000 submitted Nov 3, 2003, (In DFS 12/18/03)
Message to the Sponsor:
1. There is no animal study to support the safety of clinical study longer than 4 weeks. The sponsor is advised to compare and to correlate the local toxicities of diclofenac sodium gel and diclofenac diethylammonium gel from the existing animal data. Depending on the outcome of the analysis, additional animal toxicity studies may be required.

Memo for EOP2 Meeting (In DFS 7/7/04)

FDA response: Although there are no long term animal studies to directly compare the dermal effects of diclofenac sodium gel and Diclofenac Emulgel, the available short term study showed no significant difference between the two gels. It is reasonable to assume that the dermal effects of two gels are similar because they share the same active moiety. There is also human data for diclofenac sodium gel to compare its dermal effect with Diclofenac Emulgel. It is felt that no additional animal dermal study with diclofenac sodium gel will be necessary.

CMC End of Phase 2 meeting June 1, 2005, meeting minutes 7/1/05

2. **Does the FDA concur that adherence to the Ph. Eur. monograph is sufficient to support use of cocoyl caprylocaprate [redacted] in the drug product?**

   FDA Preliminary Response: Yes. It is acceptable. The specification and certificates of analysis for the excipient should be submitted in the NDA.

   Meeting Comments: In response to the preliminary response from the Agency, Novartis stated they would provide the specifications and certificates of analysis for the excipient.

3. **Does the FDA concur that sufficient information has been provided to support approval for use of [redacted] in the drug product? Is an authorization letter necessary to support use of this ingredient?**

   FDA Preliminary Response: It is stated in the 8/13/04 amendment that Carbomer [redacted] is the same polymer as [redacted] An authorization letter is not necessary as long as the structure of the polymer and how it is manufactured is provided in the NDA (as stated in the 8/13/04 amendment).

   Meeting Comments: Novartis accepted the response and no further discussion was required.

4. **Does the FDA concur that the information provided in this document along with that being collected in the ongoing clinical studies mentioned, will be sufficient to support approval for the use of [redacted] in the diclofenac sodium gel, 1 % product?**

FDA Preliminary Response: From the Pharm/Tox perspective, the information presented is adequate to support the NDA filing.

From the CMC perspective, the names of the ingredients should be provided in English, along with their CAS numbers.

Meeting Comments: Novartis stated they would provide submit to the IND the English translation of the ingredient names and CAS numbers for [redacted]

APPEARS THIS WAY
ON ORIGINAL

Pre-NDA meeting July 21, 2006, meeting minutes 8/21/2006

NCH Position for Question 3:

DSG is applied topically and in comparison to oral administration, results in lower systemic exposures. The proposed limits for the impurities for DSG are lower or similar to the specifications for diclofenac sodium delayed-release tablet ; , any individual impurity not more than sum of all impurities not more than ; in the USP Monograph. The diclofenac related compound not described in previous diclofenac oral formulations, has been qualified was found to be non-toxic (LD50 > 2,000 mg/kg), not mutagenic in the Ames assay and did not have potential to cause skin sensitization.

Question 3: With respect to impurities, the limits of ; not more than not more than not more thar not more than , any other individual impurity not more than and sum of all impurities not more than will be applied to DSG. Does the agency agree with our impurity limits for DSG?

FDA Response:

The proposed impurity limits are acceptable. However, we expect future manufacturing capability and experience may result in the reduction of these impurities and subsequent improvement of the drug product quality.

We encourage you to include toxicity study reports of these impurities, if available, even though these compounds may be within regulatory limits.

Additional CMC Comments

a. **Provide a well documented Pharmaceutical Development Report. Refer to ICH Q8 guideline**
b. **Provide adequate amount of stability data to cover the proposed expiration dating**
c. **Provide complete names, addresses and CFN numbers for all the sites involve in manufacturing, testing and packaging of the drug substance and the drug product**

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

/s/

Lawrence Leshin
6/14/2007 09:12:15 AM
PHARMACOLOGIST

Adam Wasserman
6/14/2007 10:01:29 AM
PHARMACOLOGIST
I concur with Dr. Leshin's comments to the Sponsor.

Ravi Harapanhalli
6/14/2007 04:25:43 PM
CHEMIST
The comments to be sent to the applicant are
on pages 4-5 entitled "External comments."