B. Body weights: 10-20% decrease in body weight gain was noted in male treated albino rabbits and male and female pigmented rabbits dosed 4 times daily. However, no treatment-related differences in body weights were noted, and no dose-response relationship was observed. Hence, the decrease in the body weight gain may not be toxicologically significant.

Body weight changes in rabbits after treated with ketotifen ophthalmic solution (kg)

Group	1	2	3	4	5	6
Dose	Control	25 µl, bid	25 µl, qid	Control	25 µl, bid	25 µl, qid
Males						
Week 0	2.38	2.53	2.40	2.93	2.85	2.90
Week 26	3.40	3.35	3.20	4.30	4.60	4.13
% of control at Week 26	100	98.5	94.1	100	107	96
Body weight gain (Wk 0-26)	1.02	0.82	0.80	1.37	1.75	1.23
% of control	100	80.4	78.4	100	127.7	89.8
Females						
Week 0	2.65	2.58	2.63	2.80	2.73	2.90
Week 26	3.53	3.50	3.88	4.68	4.70	4.53
% of control at Week 26	100	99.2	110	100	100.4	96.8
Body weight gain (Wk 0-26)	0.88	0.92	1.25	1.88	1.97	1.63
% of control	100	104.5	142	100	104.8	86.7

C. Food consumption: No drug-related differences in food consumption were observed.

D. Ophthalmology: No drug-induced irritation was noted. No treatment-related differences were noted. There was no difference between albino and pigmented rabbits.

E. Clinical pathology: No toxicologically significant findings were noted.

F. Urinalysis: No treatment-related changes were noted.

G. Organ weights: Several organ weight changes relative to the control animals were noted (see table below). The sponsor indicated that these changes were regarded as spontaneous findings, and were not drug-related.

Relative organ weight changes observed in animals treated with KFOS for 26 weeks (%)

Group	Adrenal♂	Adrenal♀	Gonads♀	Lungs♂	Lungs♀	Liver♂	Liver♀
1(Control)	0.0115	0.0121	0.0122	0.54	0.72		
2	0.0179	0.0132	0.0102	0.63	0.48		
3	0.0184	0.0092	0.0088	0.88	0.43		
4(Control)	0.0160	0.0144	0.0066	0.61	0.48	2.44	2.25
5	0.0240	0.0214	0.0119	0.76	0.61	2.82	2.33
6	0.0161	0.0134	0.0089	0.82	0.65	2.79	2.81

H. Gross pathology examinations: No treatment-related differences were noted in gross examinations.

I. Histopathology examinations: There was no difference between albino and pigmented rabbits. The lesions found in this study were regarded as spontaneous changes or mechanical changes caused by the daily instillation. However, the reviewing pharmacologist found that mild cervical lymph node hyperplasia appeared only in the treated animals (see table below). Since the changes were not dose-related, and the number of animals was small, the cause of the changes was unknown. It may involve local stimulation.

Cervical lymph node hyperplasia noted in Study 10868/97

Group	1	2	3	4	5	6
Males	0/4	0/3	1/4	0/3	3/4	2/4
Females	0/4	0/4	2/4	0/4	0/4	0/4

Fatty infiltration in liver was noted in all groups. The incidence was similar (see table below). Regarding the moderate to marked peripheral fatty infiltration in the hepatocytes in 3 animals at high dose, the sponsor believed that it was a coincidental finding.

Fatty infiltration in liver noted in Study 10868/97

Group (n=4/sex)	1	2	3	4	5	6
Single hepatocyte	2♂	3♂, 1♀		2♂, 2♀	2♂, 3♀	1♂, 2♀
	Minimal	Minimal		minimal	Minimal to mild	
Diffuse infiltration		1♀		1♂, 2♀	1♂	1♂, 1♀
		minimal		Mild/moderate	mild	
Peripheral			2♂			1♂
			Moderate/marked			Moderate/ marked

In conclusion: Albino and pigmented rabbits were topically treated with 0.025% ketotifen fumarate ophthalmic solution on the right eye for 26 weeks. No systemic or local changes related to the treatment were observed. The drug was well tolerated. Cervical lymph node hyperplasia was noted in treated animals with unknown cause. Peripheral fatty infiltration in hepatocytes was noted in high dose animals.

2. HC 20-511: An eye irritation study in rabbits. Vol. 7 [redacted]

Project Nº: Not indicated
Compound: A. HC 20-511 [redacted]
Route: Ocular, topical
Dose Level: 0.1 ml in one eye
Animal: New Zealand white rabbits, 3-4 months old, 2.5-3.2 kg
Study Site: [redacted]
Study Period: July 13 to 16, 1981
Report Time: January 5, 1982

GLP/QAU: Yes

The purpose of this study was to evaluate the ocular irritant effects of ketotifen fumarate using Draize's test. The eyes were examined 24, 48 and 72 hr after administration.

Results:

In both ketotifen eye drop treated animals and placebo treated animals, the irritation scores were 0, suggesting that HC 20-511 eye drop and its placebo cause no damage to the eye of rabbits in this study.

3. Ocular irritation study of ketotifen fumarate ophthalmic solution by one-time ocular instillation in rabbits. Vol. 7

Project Nº: NRILS 86-1927
Compound: Ketotifen fumarate ophthalmic solution
Route: Ocular, topical
Dose Level: 0.1 ml, single dose in right eye (left eye: physiological saline control)
Animal: Male New Zealand white rabbits, 2 months old, 2.03-2.41 kg, 5/group
Study Site:
Study Period: July 4 to November 14, 1986
Report Time: November 14, 1986
GLP/QAU: Yes

The purpose of this study was to evaluate the ocular irritant effects of ketotifen fumarate ophthalmic solution by using Draize's test in rabbits. The eyes were examined 1, 6, 24, 48 and 72 hr after dosing.

Results:

The findings and mean eye irritation score of each group after dosing are listed in the table below. The irritant responses were maximal from 1 to 3 hr after dosing, and disappeared or greatly decreased (below 0.4) by 6 hr after instillation.

Mean eye irritation score in rabbits following a single ocular administration of KFOS

Treatment	PSS	Vehicle	0.1%	0.2%	0.4%	0.8%
Score: 1 hr after dosing	0	0	0	1.2	1.6	2.8
Score: 6 hr after dosing	0	0	0	0.4	0.4	0
Fluid retention			1/5	2/5		
Chemosis 1°						2/5
Redness of conjunctiva 1°				3/5	2/5	5/5
Redness of conjunctiva 2°					1/5	

In conclusion: Single instillation of KFOS at the concentration of 0.1% produced no ocular irritation. At higher concentrations (0.2-0.8%), irritation responses were found that included grade 1 redness of conjunctiva and grade 1 chemosis or fluid retention. The incidence of irritation was related to the elevation of the local ketotifen concentration. The scores were classified as "practically nonirritating" (scores range: 0.5-2.5) at concentrations of 0.2 and 0.4% and "minimally irritating" (scores range: 2.5-15) at a concentration of 0.8%.
[**Reviewer's comments**: The interpretation of eye irritation in this and several other studies was based on the following table cited from *Interpretation of eye irritation tests* by Kay, JH and Calandry, JC (J. Soc. Cosmet. Chem., 13, 281-289, 1962).]

Classification of test article based on eye irritation properties

Rating	Range of mean score
Non-irritating	0.0 to 0.5
Practically non-irritating	> 0.5 to 2.5
Minimally irritating	> 2.5 to 15
Mildly irritating	> 15 to 25
Moderately irritating	> 25 to 50
Severely irritating	> 50 to 80
Extremely irritating	> 80 to 100
Maximally irritating	> 100 to 110

4. Eye irritation study in rabbits after repeated doses of eye drops containing ketotifen fumarate. Vol. 7 [redacted]

Project Nº: NRILS 86-1928
Compound: Ketotifen fumarate ophthalmic solution [redacted]
Route: Ocular, topical
Dose Level: 0.05 ml in right eye (left eye: physiological saline control)
Dosing Regimen: 15 times at 30 min intervals
Animal: Male New Zealand white rabbits, 2 months old, 2.13-2.7 kg, 5/group
Study Site: [redacted]
Study Period: July 18 to November 14, 1986
Report Time: November 14, 1986
GLP/QAU: Not indicated

The purpose of this study was to evaluate the ocular irritant effects of ketotifen fumarate ophthalmic solution by using Draize's test in rabbits receiving KFOS 15 times at 30 min intervals. The eyes were examined 1 and 3 hr after final instillation and daily for 7 days.

Results:

The irritation responses and mean rating scores are listed in the following table. The responses disappeared 1 to 5 days after dosing depending upon the concentrations.

Mean eye irritation score in rabbits following multiple ocular instillations of KFOS

Treatment	PSS	Vehicle	0.05%	0.2%	0.8%
Score: 1-3 hr after dosing	0	3.2	4.8	6.4	12.4
Score: 24 hr after dosing	0	1.2	2.8	2.0	6.0
Score: 72 hr after dosing	0	0	0	0.4	2.8
Chemosis 1°		4/5	5/5	4/5	
Chemosis 2°				1/5	2/5
Chemosis 3°					2/5
Chemosis 4°					1/5
Redness of conjunctiva 1°		3/5	5/5	4/5	
Redness of conjunctiva 2°				1/5	3/5
Redness of conjunctiva 3°					2/5
Discharge 1°		2/5	3/5	5/5	5/5
Lacrimation			2/5	3/5	5/5

In conclusion: The eye irritancy was studied by instilling 50 µl of ketotifen fumarate ophthalmic solution in the eyes of rabbits 15 times at 30 min intervals. Minimally irritating was seen at the concentrations from vehicle control to 0.8% evidenced by redness of conjunctiva and chemosis. The responses in the treated groups were stronger than those in the control group. All responses were classified as minimally irritating.

5. A four-week eye toxicity study on ketotifen fumarate eye drops in rabbits. Vol. 7

Project Nº: NRILS 86-1929
Compound: Ketotifen fumarate ophthalmic solution
Route: Ocular, topical
Dose Level: 0.05 ml in right eye, qid at 2 hr intervals for 4 weeks
Animal: New Zealand white rabbits, 2.5 months old, ♂: 2.16-2.55 kg, ♀: 2.13-2.55 kg
Study Site:
Study Period: August 14, 1986 to January 31, 1987
Report Time: January 31, 1987
GLP/QAU: No
Study Design:

Group	Dosing regimen	N/sex
Physiological saline solution (PSS)	0.05 ml in right eye, qid at 2 hr intervals for 28 days	5
Vehicle	0.05 ml in right eye, qid at 2 hr intervals for 28 days	5
0.05% ketotifen fumarate ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 28 days	5
0.2% ketotifen fumarate ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 28 days	5
0.8% ketotifen fumarate ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 28 days	5
Intal ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 28 days	5

The purpose of this study was to evaluate the ocular toxicity of ketotifen fumarate ophthalmic solution in rabbits following 4-week ocular administrations. Toxicity assessment is shown in the table below.

Toxicity assessment

Parameter	Procedure
General condition	Daily
Body weights	Twice a week
Food and water consumption	Weekly
Slit lamp	Twice daily (before the first dose and 1 hr after the last dose)
Funduscopy	Once every 2 weeks
Histopathology	At the end of the treatment, animals were euthanized. Right eyeballs and lacrimal glands were examined histopathologically.
Transmission and scanning electron microscopes	At the end of treatment, electron microscope examinations on ocular samples from 1 animal/sex/group (except for the low dose groups) were performed. The sites studied included corneal epithelial cells, corneal substantia propria, corneal endothelial cells, bulbal conjunctiva epithelial cells and goblet cells.

Results:

A. General condition, body weights, food intake and water intake: No toxicologically significant findings were noted.

B. Ocular irritation response: Mean weekly eye irritation scores obtained 1 hr after the last daily dose and 24 hr after the beginning of the first daily dose are summarized in the table below. Grade 1 redness of conjunctiva was observed from vehicle to high dose groups. Grade 2 redness was noted in mid and high dose animals. Grade 1 chemosis, lacrimation and discharge were found in all treated groups. Four times in males and 5 times in females, the scores exceeded 2.5 in high dose animals, which was minimally irritating level. The maximum group mean score was 4.4 in males and 3.6 in females.

Mean weekly eye irritation scores in rabbits treated with KFOS for 28 days

Group	PSS		Vehicle		0.05% ketotifen		0.2% ketotifen		0.8% ketotifen		Intal	
Males	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr
Weel 1	0	0	0.1	0.1	0.1	0.1	0.2	0	1.9	0.2	0.6	0.3
Week 2	0.1	0	0.5	0.4	0.7	0.2	0.7	0.4	1.6	0.4	1.5	0.5
Week 3	0.4	0.1	0.7	0.4	0.7	0.3	1.0	0.3	1.3	0.5	1.0	0.3
Week 4	0.3	0.2	0.5	0.1	0.7	0.2	0.7	0.3	2.3	1.2	0.9	0.6
Weeks 1-4	0.2	0.1	0.4	0.2	0.5	0.2	0.7	0.3	1.8	0.6	1.0	0.4
Females												
Weel 1	0	0	0	0	0.2	0	0.2	0	2.3	0.2	0.2	0
Week 2	0	0	0.2	0.1	0.8	0.3	0.8	0.2	2.1	0.2	0.6	0.2
Week 3	0.2	0.1	0.2	0.1	0.9	0.3	1.0	0.2	1.7	0.3	0.7	0.4
Week 4	0.1	0	0.5	0.2	0.6	0.4	1.4	0.3	1.9	0.8	0.5	0.2
Weeks 1-4	0.1	0	0.2	0.1	0.6	0.2	0.8	0.2	2.0	0.4	0.5	0.2

C. Ocular fundus studies: No treatment-related differences were observed.

D. Histopathological studies: No treatment-related differences in palpebral conjunctiva, cornea, scleral venous sinus, iris, ciliary body, retina, optical nerve, and lacrimal gland were observed.

E. Ultrastructural studies: No abnormal findings were observed.

In conclusion: rabbits were treated with ketotifen fumarate ophthalmic solution (0.05, 0.2 and 0.8%) for 28 days. No abnormal findings in general condition, body weights, and food and water consumption were noted. With respect to eye irritancy, practically nonirritating to minimally irritating effects were observed in the ketotifen treated animals in a dose-dependent manner. Histopathological and electron microscopic examinations revealed no abnormalities, suggesting that there should be no fundamental damage in the eye caused by ketotifen. There were no differences between male and female animals. The scores in low and mid dose animals revealed a practically nonirritating result, which was different from the results obtained in Studies NRILS 86-1928 and 87-2336. The differences may be due to the different interval length and total number of administrations that affected the drug accumulation, and irritating responses.

6. A thirteen-week eye toxicity study on ketotifen fumarate eye drops in rabbits. Vol. 7

Project Nº: NRILS 87-2336
Compound: Ketotifen fumarate ophthalmic solution
Route: Ocular, topical
Dose Level: 0.05 ml in right eye, qid at 2 hr intervals for 91 days
Animal: Male New Zealand white rabbits, 2.5 months old, 2.13-2.73 kg
Study Site:
Study Period: July 20, 1987 to March 14, 1988
Report Time: March 14, 1988
GLP/QAU: No
Study Design:

Group	Dosing regimen	N
Physiological saline solution (PSS)	0.05 ml in right eye, qid at 2 hr intervals for 91 days	5
Vehicle	0.05 ml in right eye, qid at 2 hr intervals for 91 days	5
0.05% ketotifen fumarate ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 91 days	5
0.2% ketotifen fumarate ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 91 days	5
0.8% ketotifen fumarate ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 91 days	5
Intal ophthalmic solution	0.05 ml in right eye, qid at 2 hr intervals for 91 days	5

The purpose of this study was to evaluate the ocular toxicity of ketotifen fumarate ophthalmic solution in rabbits following 13-week ocular administrations. The day of the first administration was designated as Day 1. Toxicity assessment is listed in the table below.

Toxicity assessment

Parameter	Procedure
General condition	Daily
Body weights	Weekly
Food and water consumption	Weekly
Ophthalmological observations with Slit lamp	Twice daily (before the first daily dose and 1 hr after the last daily dose)

Parameter	Procedure
Funduscopy	After 4, 8 and 13 weeks
Histopathology	At the end of the treatment, animals were euthanized. Right eyeballs and lacrimal glands were examined histopathologically.
Transmission and scanning electron microscopes	At the end of treatment, electron microscope examinations on ocular samples from 1 animal/group (except for the low dose group) were performed. The sites studied included corneal epithelial cells, corneal substantia propria, corneal endothelial cells, bulbal conjunctiva epithelial cells and goblet cells.

Results:

A. General condition, body weights, food intake and water intake: No toxicologically significant findings were noted.

B. Ocular irritation response: The irritancy responses ranging from "practically nonirritating" to "minimally irritating" were noted in different groups (see table below). The incidence and frequency of the responses were concentration-dependent. Increased eye irritancy was noted with repeated instillation.

Mean eye irritation scores and responses in rabbits treated with KFOS for 91 days

Group	PSS		Vehicle		0.05% ketotifen		0.2% ketotifen		0.8% ketotifen		Intal	
	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr	1 hr	24 hr
Days 1-91	0.3	0.2	0.6	0.3	0.7	0.3	1.0	0.7	4.3	3.0	2.5	1.8
Range	0-1.2		0-2.4		0-2.0		0-2.8		2-6.0		0-5.6	
Day of first observation												
1° redness, conjunctiva	7		2		3		3		1		5	
1°Chemosis	70			65	74			40	4		19	
1°Discharge	40		2		4		3		1		9	
2° redness, conjunctiva									Rare		Rare	
2°Chemosis											Spora dically	
2°Discharge									Rare			
Rating	Practically nonirritating		Practically nonirritating		Practically nonirritating		Minimally irritating		Minimally irritating		Minimally irritating	

C. Ocular fundus studies: No treatment-related differences were observed.

D. Histopathological studies: No treatment-related differences in palpebral conjunctiva, cornea, scleral venous sinus, iris, ciliary body, retina, optical nerve, and lacrimal gland were observed.

E. Ultrastructural studies: No abnormal findings were observed.

In conclusion: Rabbits were treated with ketotifen fumarate ophthalmic solution (0.05, 0.2 and 0.8%) for 91 days. No systemic toxicities were noted. In eye irritancy test, practically nonirritating to minimally irritating effects were observed following repeated administrations in the ketotifen treated animals in a dose-dependent manner. Histopathological and electron microscopic examinations revealed no abnormalities. The

results suggested that ketotifen fumarate ophthalmic solution could cause weak eye irritation without fundamental ocular damage.

7. Quality test of ketotifen eye drops prepared in hospital. (2) The irritability test. Vol. 7

[Reviewer's comment: This is a paper published in Japanese Journal of Hospital Pharmacy, 10(3):177-181, 1984.]

Project Nº: Not indicated
Compound: Ketotifen fumarate ophthalmic solution
Isotonic vehicle solution
Route: Ocular, topical
Dose Level: 0.05 ml in right eye
Animal: Male New Zealand white rabbits, 2.5 kg
Study Site: Not indicated
Study Period and Report Time: Not indicated
GLP/QAU: Not indicated
Study Design:

Group	Dosing regimen	N
Vehicle	5 times at 30 min intervals	6
Vehicle	TID x 2 weeks	6
0.08% ketotifen fumarate ophthalmic solution	5 times at 30 min intervals	6
0.08% ketotifen fumarate ophthalmic solution	TID x 2 weeks	6

The purpose of this study was to evaluate the ocular irritant effects of ketotifen fumarate ophthalmic solution on cornea and conjunctiva by macroscopic and microscopic examinations.

Results:

No irritation responses in cornea and palpebral conjunctiva were noted under visual examination or under optical microscopic and electron microscopic examinations.

In conclusion: Neither ketotifen ophthalmic solution nor vehicle control produced any abnormal irritation in rabbit cornea palpebral conjunctiva under visual, microscopic and electron microscopic examinations.

8. 13-week local ocular tolerance and subchronic toxicity study of into the conjunctival sac of albino rabbits. Vol. 8, Page 178.

Report Nº: 10866/1/97
Compound: ketotifen fumarate ophthalmic solution

[**Reviewer's comment**: The study was to justify the toxicities of impurities in the drug product. The drug was degraded at elevated temperature to a level of impurities comparable to the highest anticipated over the shelf life of the drug.]

Route: Instillation into the conjunctiva sac of the right eye

Dosing Regimen: 25 µl/instillation, right eyes only, bid or qid x 13 weeks

Animal: New Zealand white rabbits, 3-month old, 2.0-2.74 kg for males, 2.28-2.72 kg for females

Study Site: [redacted]

Study Duration: May 26 to August 25, 1998

Date of Final Report: October 12, 1998

GLP/QAU: Yes

Study Design:

Groups	Number of 25 µl instillation/animal/day (right eye only)	N/sex
Albino rabbits		
1 (Vehicle control)	Qid at 2-hr intervals	8
2 (Heat-degraded KFSO)	Bid at 6-hr interval	8
3 (Heat degraded KFSO)	Qid at 2-hr intervals	8

The purpose of this study was to evaluate the local ocular tolerance and chronic toxicity of [redacted] following daily ocular administration for 13 weeks in the albino rabbit. The day of first dosing was designated as Day 0. Toxicity was assessed as shown below.

Toxicity assessment for Study 10866/1/97

Parameter	Procedure
Clinical observations	At least once daily
Body weights	Weekly
Food and water consumption	Daily
Ophthalmologic examinations	Twice daily for conjunctivae. Ophthalmoscopic and fluorescenin examinations were conducted on Days 0, 1, 15, 28, 56, 91 and 92. Examinations with slit lamp were performed on Days 0, 28, and 92.
Clinical pathology	Blood samples were collected prior to the first instillation on Days 0, 39 and 87 for hematology and clinical chemical examinations.
Urinalysis	Urine samples were collected before the treatment and on Days 39 and 87.
Gross pathology	On Days 92 and 93, all animals were euthanized. A complete gross pathology examination was conducted.
Organ weights	The following organs from each animal were weighed: adrenal, brain, heart, kidneys, liver, lungs, ovaries, pituitary, spleen, testes, thymus, thyroid.
Histopathologic examinations	The following organs from all animals in Groups 1 and 3 were examined histopathologically: adrenal gland, aorta, bone (femur), bone marrow, brain, caecum, epididymis, esophagus, eyes, gall bladder, gross lesions, heart, kidneys, large and small intestines, liver, lungs, lymph node (cervical and mesenteric), mammary gland, ovary, pancreas, parathyroid gland, pituitary gland, prostate gland, salivary gland, seminal vesicle, skeletal muscle, sciatic nerve, skin, spinal cord, spleen, stomach, testis, thymus, thyroid gland, tongue, trachea, urinary bladder, uterus and vagina.
Ocular histopathology	Left and right eyes from all animals in Groups 1 and 3 were examined histologically.

Results:

A. Clinical observations: No treatment-related mortality and clinical signs were observed.

B. Body weights: No treatment-related findings in body weights or body weight gain were noted.

C. Food consumption: No drug-related differences in food consumption were observed.

D. Ophthalmology: No drug-induced irritation or other treatment-related differences were noted.

E. Clinical pathology: No toxicologically significant findings were observed.

F. Urinalysis: No treatment-related changes were noted.

G. Organ weights: Several organ weight changes relative to the control animals were noted (see table below). The sponsor indicated that the adrenal weights were within the normal range of their historical background data. The liver changes that occurred in male high dose animals were correlated with diffuse fatty infiltration of hepatocytes. The changes in kidneys and gonads were not large, and there were no corresponding histopathological findings. Therefore, these changes may not be toxicologically significant.

Relative organ weight changes observed in animals treated with ketotifen ophthalmic solution for 13 weeks (%)

Group	Adrenals♂	Gonads♂	Gonads♀	Kidneys♂	Liver♂	Liver♀
1(Control)	0.0113	0.152	0.0132	0.46	2.93	2.08
2	0.0105	0.115	0.0123	0.48	2.94	2.59
3	0.0098	0.136	0.0071	0.52	3.29	2.57

H. Gross pathology examinations: No treatment-related differences were noted in gross examinations.

I. Histopathology examinations: No toxicologically significant findings attributed to the treatment were observed in animal eyes or other organs. The organ lesions were considered to be spontaneous changes or mechanical changes by daily instillation of the drug. Mild diffuse fatty infiltration of hepatocytes was observed in male animals at high dose (see table below). The sponsor indicated that this change was within physiological limits and could be caused by the stress during the daily drug administration. **[Reviewer's comment**: Since in control and female animals no diffuse fatty infiltration was observed, and no correlated adrenal gland changes were noted, the reviewing pharmacologist does not consider this change as a stress-induced response.

The fatty infiltration occurred with clear dose-dependence, and similar changes were noted in Study 10868/97. The reviewer is concerned because many hepatotoxins can cause fatty liver. The change was mild, there were not correlated clinical pathology changes, and only male animals were noted with this change, however, the toxicological relevance of this effect to human use is not known.]

Fatty infiltration of hepatocytes noted in Study 10866/1/97

Group	1	2	3
Males	1/8 (single hepatocyte)	2/8 (single hepatocyte) 1/8 (diffuse fatty infiltration)	1/8 (single hepatocyte) 5/8 (diffuse fatty infiltration)
Females	1/8 (single hepatocyte)	2/8 (single hepatocyte)	3/8 (single hepatocyte)

In conclusion: Albino rabbits were topically treated with 0.025% ketotifen fumarate ophthalmic solution [redacted] on the right eye for 13 weeks. Diffuse fatty infiltration was observed in treated groups. No other toxicologically significant systemic or local changes were observed. The drug was well tolerated.

9. Eye irritation study in rabbits after repeated doses of ketotifen fumarate eye drops of degraded quality. Vol. 7 [redacted]

Project Nº: FLS 88-3219
Compound: Ketotifen fumarate ophthalmic solution [redacted]
Ketotifen fumarate ophthalmic solution [redacted]
Route: Ocular, topical
Dose Level: 0.05 ml in right eye
Animal: Male New Zealand white rabbits, 3 months old, 2.18-2.68 kg
Study Site: [redacted]
Study Period: April 13 to September 21, 1988
Report Time: September 21, 1988
GLP/QAU: No
Study Design:

Group	Dosing regimen	N
Physiological saline solution (PSS)	15 times at 30 min intervals	5
Vehicle	15 times at 30 min intervals	5
Degraded ketotifen fumarate ophthalmic solution	15 times at 30 min intervals	5
Ketotifen fumarate ophthalmic solution	15 times at 30 min intervals	5

The purpose of this study was to evaluate the ocular irritant effects of degraded ketotifen fumarate ophthalmic solution by using Draize's test in rabbits. The eyes were examined 1, 3 and 24 hr after final instillation and daily for 7 days.

Results:

No treatment-related changes in general conditions were noted.

The irritation responses and mean rating scores are listed in the following table. The responses disappeared 1 to 3 days after dosing depending upon the concentrations.

Mean eye irritation score in rabbits following multiple ocular instillations of ketotifen fumarate ophthalmic solution

Treatment	PSS	Vehicle	Degraded	Ketotifen
Score: 1 hr after dosing	0	1.6	4.4	6.8
Score: 48 hr after dosing	0	0	0	0.4
Score: 72 hr after dosing	0	0	0	0
Chemosis 1°		1/5	3/5	3/5
Chemosis 2°				1/5
Redness of conjunctiva 1°		2/5	3/5	5/5
Discharge		1/5	5/5	4/5

In conclusion: The eye irritancy was studied by instilling 50 µl of degraded and nondegraded ketotifen fumarate ophthalmic solutions in the right eyes of rabbits 15 times at 30 min intervals. The responses in the vehicle group were rated practically nonirritating. Minimally irritating was seen in both ketotifen and degraded ketotifen groups evidenced by redness of conjunctiva and chemosis. Degraded ketotifen fumarate ophthalmic solution produced transient, mild irritancy under the current testing conditions, which might be caused by ketotifen fumarate.

10. Contact hypersensitivity to ketotifen base in albino guinea pigs. Vol. 7

Project N^{o}: 201600
Compound: Ketotifen base
(+) Control:
Animal: albino guinea pigs, 7-8-week old, 420-485 g for males, 371-466 g for females, 5/sex for vehicle, 10/sex for treatment group
Study Site:
Study Period: January 12 to February 26, 1988
Report Time: April 18, 1988
GLP/QAU: Yes

The purpose of this study was to evaluate the allergenic potential of ketotifen when administered to the skin of guinea pigs. The experiment procedure is as follows.

A. Induction: Three pairs of intradermal injections (0.1 ml/site) were made at an area of dorsal skin: 1) Freund's complete adjuvant 50:50 with propylene glycol:PSS. 2) 1% ketotifen solution. 3) Ketotifen (1%) in a 50:50 mixture of Freunds' complete adjuvant and the vehicle used in 2). One week later, a patch of filter paper saturated with 25% ketotifen base solution was placed over the injection sites for 48 hr.

B. Challenge: Two weeks later, 2 patches with 25% ketotifen solution or vehicle were placed on the skin for 24 hr. 24 and 48 hr after the patches were removed, erythema and edema were assessed.
C. Re-challenge: Two weeks later, the same procedure of "B" was repeated with the positions of ketotifen and vehicle switched.

Results:

One animal in the treatment group showed severe emaciation from Days 21 to 25 of test, and died "spontaneously" on Day 27. Macroscopic examination showed dark-red lung.

In the other animals, no drug-related local and systemic clinical signs were reported, and no body weight changes relative to control were noted. No drug-induced sensitizing effects were observed. In the positive control study in which 0.5% [redacted] was used for induction and 5% dilution was used for challenge, all animals showed positive reactions.

In conclusion: Albino guinea pigs were challenged twice in the contact hypersentivity study. No evident toxicities were observed in either treatment or control groups. Ketotifen base did not possess skin sensitizing potential in albino guinea pigs.

11. HC 20-511: Local tolerance in the rabbit to intravenous administration of the ampoule solution. Vol. 7 [redacted]

Project Nº: Not indicated
Compound: A. HC 20-511 [redacted]
B. HC 20-511 [redacted]
C. [redacted]
D. [redacted]
Route: Intravenous
Dose Level: 2 ml/site (0.5 ml/min)
Animal: Rabbits (mixed race), 2.98-4.43 kg
Study Site: [redacted]
Report Time: June 17, 1971
GLP/QAU: No

The purpose of this study was to evaluate the local irritant effects of ketotifen 24 hr, 48 hr and 7 days after the drug was administered to rabbit ear vein. A point system with the score from 0 to 4 was used to measure the inflammation, swelling, thrombosis and necroses in the ear (total score: 0-16).

Results:

The average scores are listed in the table below. HC 20-511's irritant responses were comparable to the placebo control's, and were much lower than those caused by Largactil-50®.

The scores of local irritant test (n = 4)

Test solution	Concentration (%)	24 hr	48 hr	7 days
HC 20-511	0.05	2.8	2.3	0.3
HC 20-511 (1:3 dilution)	0.017	2.8	2.0	1.5
Placebo		2.5	1.8	2.8
Largactil-50®(1:10 dilution)	0.25	7.0	6.5	5.5

In conclusion: Following IV injection, HC 20-511 (0.05%) had a local irritant effect comparable to that of the diluted solution (0.017%) and the placebo control. The effect of HC 20-511 was greatly reduced 7 days later.

Carcinogenicity study:

1. **HC 20-511: Carcinogenic-potential study in mice. Vol. 5**
2. **HC 20-511: Two years toxicity study in rats. Vol. 5**

These studies were reviewed by Dr. Terry S. Peters. Refer to Review and Evaluation of Pharmacology/Toxicology Data by Dr. Terry S. Peters dated 2/26/99 (Attachment 6).

Reproductive toxicity studies:

1. **HC 20-511 fertility study in male rats. Vol. 6**
2. **HC 20-511 fertility study in male rats. Vol. 6**
3. **HC 20-511 a teratological study in rats. Vol. 6**
4. **HC 20-511 a teratological study in rabbits. Vol. 6**
5. **HC 20-511 peri- and postnatal study in rats. Vol. 6**

[**Reviewer's comment**: The following five reproductive study reviews supersede the reviews by Dr. Gamil Debbas listed in Attachment 1.]

1. **HC 20-511 fertility study in male rats. Vol. 6**

Report N°: Not indicated
Compound: HC 20-511
Route: Oral by stomach tube
Animal: Albino rats, age 12-14 weeks, 250-350 g
Study Site:

Study Design: [redacted]

Dose (mg/kg/day)	Dosing volume (ml/kg)	N (male)	# of doses
Vehicle	5	25	Once daily x 10 wks prior to cohabitation; during cohabitation until sacrifice [app. 12 weeks].
2	5	25	
10	5	25	
50	5	25	

Study Duration: Not indicated
Report Time: September 19, 1975
GLP/QAU: Not indicated

Male rats were treated with the drug once daily for 70 days. On Day 71, 15 male rats per group were mated with untreated virgin females (One male rat was mated with 2 female rats). Treatment was continued until insemination occurred or, failing this, for a maximum of 2 weeks. The day on which sperm were detected was considered as Day 0 p.c. (post coitum).

After mating, half of the females were sacrificed on Day 13 p.c. and examined; the others were allowed to rear their young until Day 21 p.p. (post partum) before being terminated and examined together with the offspring.

Toxicity assessment

Parameter	Procedure
Clinical observations and mortality	Not indicated
Body weights	Males: Days 1, 36 and 71 of treatment Females: Day of mating, Days 13 or 20 p.c. and Days 4 and 21 p.p.
Dams killed on Day 13 p.c.	Uterine examinations: # of live and dead embryos, and # of resorption sites were counted. External anomalies were examined on all embryos.
Dams killed on Day 21 p.p.	Autopsy was performed on all dams and young. Implantation sites were counted . The young were examined for external and internal anomalies. X-ray and Alizarine-S were carried out for the examination of skeletal anomalies.
Macroscopic examinations	The following organs from all animals were examined macroscopically: sex organs, liver, kidneys, adrenals, spleen, lung, heart, stomach and intestines.

Results:

A. Males during pre-mating period:

1). Mortality and clinical observation: Treatment-related mortalities are summarized in the following table. No reasons of the deaths were given. In rats treated with the drug at 10 and 50 mg/kg/day, some clinical signs such as unhealthy coat and excitation were observed. No detailed information was provided. (**Reviewer's comment**: Although one death occurred in the control group, the deaths in the 10 and 50 mg/kg groups were considered treatment-related because [1] they exceeded the number of deaths in the control group by a considerable margin, and [2] there is a dose response.)

Male mortality observed during the pre-mating period (n=25)

Dose (mg/kg/day)	0	2	10	50
Mortality	1	1	6	9

2). Body weight: No significant differences were noted.

B. Mating results: Copulation index (♀ inseminated/♀ paired x 100) and fertility index (♀ pregnant/♀ inseminated x 100) were lower in high dose group than in control group (see table below). In males at high dose group, the second mating following 5-week recovery period produced normal copulation index and fertility index.

Copulation index and fertility index

Dose	Number of females			Copulation index	Fertility index
(mg/kg/day)	Paired	Mated	Pregnant	%	%
0	50	36	30	72	83
2	48	34	26	71	76
10	38	28	20	74	71
50	32	20	13	63	65

[Reviewer's comment: No historical control data were provided. The decrease in fertility at 10 and 50 mg/kg was viewed as real effects. However, it is recognized that these effects occurred at significantly toxic dose levels.]

C. Dam and litter data for Day 13 p.c.:
[**Reviewer's comment**: Females were not treated in this study.]

1). Body weight gain of dams: No differences were noted between control and treated groups.

2). Corpora lutea and implantations: The mean numbers of corpora lutea and implantation sites per dam were similar in all groups.

3). Litter size: No abnormal findings were noted.

4). Pre-implantation loss and resorptions: Pre-implantation loss and resorptions in rats of 50 mg/kg group were higher than control (see table below). After recovery period (second mating), the values returned to normal.

Pre-implantation loss and resorptions

Dose	Total						% of implantations	
(mg/kg/day)	Litters	Corpora lutea	implantations	Live embryos	Dead embryos	Resorptions	Pre-implantation loss	Resorptions
0	15	234	172	164	1	7	26.5	4.07
2	12	188	163	157	0	6	13.3	3.68
10	10	144	105	103	0	2	27.1	1.91
50	6	114	74	59	0	15	35.1	20.27

(**Reviewer's comment**: Although the increase in resorptions was 5-fold greater than control, it was not viewed as treatment-related because [1] it was primarily due to effects in 2 litters and [2] it was not observed in the animals

examined on Day 21 p.p. The increase in pre-implantation loss was not viewed as a treatment-related effect either because it was mainly due to the increase in one litter.)

5). Anomalies: No anomalies were detected.

D. Dam and litter data for Day 21 p.p.:
[Reviewer's comment: Females were not treated in this study.]

1). Body weight gain of dams, implantations and litter size: No significant differences in dam body weight gain, the mean number of implantation sites and the mean number of live pups at delivery were observed.

2). Pre- and perinatal mortality and postnatal loss: The table below summarizes the pre- and perinatal mortality and postnatal loss data. The increased post-implantation loss at 10 mg/kg was due to one dam that gave birth to 14 dead fetuses on Day 23 p.c., so the increase might not be a biologically relevant event. The relevance of the elevated postnatal loss at 50 mg/kg was questionable because there was no obvious mechanism for the treatment of males to result in an increased postnatal loss.

Pre- and perinatal mortality and postnatal loss in females (% of implantations or live pups)

Dose	Live pups	Post-implantation loss	Postnatal loss		
(mg/kg/day)	Day 0		Days 0-4	Days 4-21	Days 0-21
0	92.9	7.1	0	12.9	12.9
2	90.6	9.4	0	7.7	7.7
10	86.6	13.4*	2.7	4.7	7.3
50	98.8	1.2	1.2	20.2	21.2

* Due to one dam that gave birth to 14 dead fetuses

3). Body weight gain in pups, sex ratio and anomalies: No toxicologically significant findings were noted.

In conclusion, male rats were treated orally with ketotifen at 2, 10 and 50 mg/kg/day for 10 weeks followed by mating with untreated female rats. Treatment continued in the males until insemination occurred or, failing this, for a maximum of 2 weeks. Systemic toxicity was observed in male rats evidenced by the clinical signs (unhealthy coat and excitation) and increased mortality at 10 and 50 mg/kg/day. Treatment of male rats with oral doses of ketotifen $\geq$ 10 mg/kg/day for 70 days prior to mating resulted in a decrease in fertility. The pre-implantation loss and resorption rate were increased at 50 mg/kg group examined on Day 13 p.c., but these increases were primarily due to the effects in 1 or 2 litters. The data obtained on Day 21 p.p. showed that the post-implantation loss was normal. The increases in pre-implantation loss and resorptions on Day 13 p.c. were not viewed as treatment-related effects. NOAEL for both systemic toxicity and fertility was considered as 2 mg/kg/day.

2. HC 20-511 fertility study in female rats. Vol. 6

Report Nº: Not indicated
Compound: HC 20-511
Route: Oral by stomach tube
Animal: Albino rats, age 11 weeks, 200-250 g
Study Site:

Study Duration: Not indicated
Report Time: September 18, 1975
GLP/QAU: Not indicated
Study Design:

Dose (mg/kg/day)	Dosing volume (ml/kg)	N (female)	# of doses
Vehicle	5	30	Once daily x 14 days prior to cohabitation; during and after cohabitation until sacrifice.
2	5	30	
10	5	30	
50	5	30	

Female rats were treated with the drug once daily for 14 days. On Day 15, 30 female rats per group were mated with untreated males (2 females vs. 1 male) until insemination occurred or, failing this, for a maximum of 2 weeks. Treatment was continued until the females were killed. The day on which sperm were detected was considered as Day 0 p.c. (post coitum).

After mating, half of the females were sacrificed on Day 13 p.c. and examined; the others were allowed to rear their young until Day 21 p.p. (post partum) before being terminated and examined together with the offspring.

Toxicity assessment

Parameter	Procedure
Clinical observations and mortality	Not indicated
Body weights	Females: Days 1 and 15 premating treatment, Day of mating, Days 13 or 20 p.c. and Days 4 and 21 p.p.
Dams killed on Day 13 p.c.	Uterine examinations: # of live and dead embryos, and # of resorption sites were counted. External anomalies were examined on all embryos.
Dams killed on Day 21 p.p.	Autopsy was performed on all dams and young. Implantation sites were counted. The young were examined for external and internal anomalies. X-ray and Alizarine-S were carried out for the examination of skeletal anomalies.
Macroscopic examinations	The following organs from all animals were examined macroscopically: sex organs, liver, kidneys, adrenals, spleen, lung, heart, stomach and intestines.

Results:

A. Females during pre-mating period:

1). Mortality: No treatment-related mortalities were observed during the pre-mating period.

2). Body weight: Decreased body weight gain was noted in rats receiving HC 20-511 at 10 and 50 mg/kg/day (see table below). [**Reviewer's comment**: Although there was a marked, dose-dependent decrease in body weight gain during the pre-mating phase of treatment, this effect was not observed in the later stages of this study, suggesting a possible adaptation to the test material.]

Body weight changes (g) in female rats treated with HC 20-511 during the pre-mating period (n=30)

Dose (mg/kg/day)	Day 1	Day 15	Weight gain	% of control
0	207±16	228±21	21±12	100
2	210±15	229±15	19±10	90.5
10	211±18	225±16	14±11	66.7
50	213±15	223±16	10±10	47.6

B. Mating results: Two rats in control and 10 mg/kg groups were found dead after mating when the treatment continued. In 50 mg/kg group 3 rats died after insemination. These mortalities were not considered treatment-related due to deaths in the control group and low incidence at 50 mg/kg group relative to the control group. Copulation index (♀ inseminated/♀ paired x 100) and fertility index (♀ pregnant/♀ inseminated x 100) in treated animals were not lower than in control group (see table below).

Copulation index and fertility index in females

Dose	Number of females			Copulation index	Fertility index
(mg/kg/day)	Paired	Mated	Pregnant	%	%
0	30	17	14	56.7	82.4
2	30	18	16	60.0	88.9
10	30	25	24	83.3	96.0
50	30	20	17	66.7	85.0

C. Dam and litter data for Day 13 p.c.:

1). Body weight gain of dams: Decreased body weight gain was noted in the treated animals (see table below), but a dose-response was not apparent.

Body weight changes (g) from mating to Day 13 p.c. in female rats treated with HC 20-511 (n=6-9)

Dose (mg/kg/day)	Day of mating	Day of section	Weight gain	% of control
0	224±29	280±35	56±19	100
2	221±18	270±11	49±11	87.5
10	209±11	257±13	47±10	83.9
50	216±14	267±16	51±5	91.1

2). Corpora lutea, implantations and litter size: The mean numbers of corpora lutea, implantation sites per dam, and live embryos were similar in all groups.

3). Pre-implantation loss and resorptions: The following table summarizes the pre-implantation loss and post-implantation loss. There was no statistically

significant increase in these values. The changes in the treated animals were within the historical control range except for the pre-implantation loss at 2 mg/kg. In addition, in the treated animals, no dose-dependent relationship for the pre-implantation loss was observed. These changes were not considered biologically relevant.

Pre-implantation loss and resorptions in females

Dose	Total					% of implantations	
(mg/kg/day)	Litters	Corpora lutea	implantations	Live embryos	Post-implantation loss	Pre-implantation loss	Post-implantation loss
0	6	93	72	71	1	22.6	1.39
2	7	108	78	74	4	27.8*	5.13
10	9	126	102	96	6	19.1	5.88
50	7	103	93	89	4	9.7	4.30

* high than the historical control range of 2.7-22.6%

4). Anomalies: No anomalies were detected.

D. Dam and litter data for Day 21 p.p.:

1). Body weight gain of dams, implantations and litter size: A slight body weight gain decrease was noted in treated dams (see table below). No significant differences in the mean number of implantation sites and the mean number of live pups at delivery were observed.

Litter data and body weight changes during pregnancy in female rats treated with HC 20-511 (n=7-11)

Dose	Litter data (per litter)			Body weight gain (g)			
(mg/kg/day)	Litters	Implantations	Live pups	Day 0 p.c.	Day 20 p.c.	Weight gain	% control
0	7	12±4	12±4	216±20	347±35	131±31	100
2	9	13±3	13±3	226±16	356±34	130±23	99.2
10	11	12±4	10±4	225±14	349±29	124±24	94.7
50	7	11±3	10±5	221±11	335±44	114±36	87.0

2). Post-implantation loss and postnatal loss: The table below summarizes the post-implantation loss and postnatal loss data. Although the increased post-implantation loss was seen at 10 mg/kg and 50 mg/kg, there was no dose-dependent relationship, and the value at 50 mg/kg, 9.3%, was within the historical control range (0-11%). In addition, the post-implantation loss in the rats sacrificed on day 13 p.c. was low. Therefore, the increase of post-implantation loss might not be a biologically relevant event. The elevated postnatal loss in treated rats lacked dose-response, and was within the historical control range (4.3-18.6%).

Post-implantation loss and postnatal loss in females (% of implantations or live pups)

Dose	Live pups	Post-implantation loss	Postnatal loss		
(mg/kg/day)	Day 0 p.p.		Days 0-4	Days 4-21	Days 0-21
0	100	0	1.16	10.6	11.6
2	98.3	1.7	1.69	11.2	12.7
10	85.7	14.3	1.75	0.9	2.6
50	90.7	9.3	1.47	16.4	17.6

3). Body weight gain in pups, sex ratio and anomalies: No toxicologically significant findings were noted.

In conclusion, female rats were treated orally with ketotifen at 2, 10 and 50 mg/kg/day for 14 days followed by mating with untreated male rats. Treatment continued in the female rats until Day 13 p.c. or Day 21 p.p. Systemic toxicity was evidenced by a decrease in body weight gain in female rats at 10 and 50 mg/kg in the pre-mating period. No changes in fertility and copulation indices were observed. The increase in post-implantation losses and postnatal loss lacked dose-dependence, and was located within the historical control ranges. Hence, these changes were not biologically relevant.

3. HC 20-511 a teratological study in rats. Vol. 6

Report Nº: Not indicated
Compound: HC 20-511
Route: Oral by gavage
Animal: Albino rats, age 13 weeks, 210-285 g
Study Site:
Study Duration: Not indicated
Report Time: March 14, 1972
GLP/QAU: Not indicated
Study Design:

Dose (mg/kg/day)	Dosing volume (ml/kg)	N	# of doses
Vehicle	5	30	Once daily from Days 6 to 15 post coitum (p.c.)
10	5	30	
30	5	30	
56	5	30	
100	5	20	

Two female rats were mated with one male rat. The day on which sperm were detected was considered as Day 0 p.c. (post coitum). On Day 21 p.c., the dams were sacrificed, and fetuses were delivered by cesarean section.

Toxicity assessment

Parameter	Procedure
Body weights	Days 0, 6, 15 and 21 p.c.
Macroscopic examinations	The following organs from all dams were examined macroscopically: uterus (including live and dead fetuses, the number of fetal and embryonic resorption sites), ovaries (including corpora lutea), liver, kidneys, adrenals, spleen, lung, heart, stomach and intestines. All fetuses were sexed, weighed and examined for external, internal and skeletal anomalies.

Results:

A. Findings in dams:

1). Mortality: Seven of 20 animals treated with HC 20-511 at 100 mg/kg/day died during the study period (see table below). The pregnancy rates were comparable across the groups.

Mortality and pregnancy rates in animals treated with HC 20-511

Dose		Dead		Survived		Pregnancy rate
(mg/kg/day)	N	pregnant	Not pregnant	pregnant	Not pregnant	%
Vehicle	30	0	0	25	5	83.33
10	30	2	0	25	3	90.00
30	30	0	0	25	5	83.33
56	30	0	0	24	6	80.00
100	20	5	2	12	1	85.00

2). Body weight: Decreased body weight gain was noted in rats receiving HC 20-511 at 56 and 100 mg/kg/day (see table below).

Body weight changes in female rats treated with HC 20-511 (g)

Dose		Start of the	End of the	Body weight	Body weight gain
(mg/kg/day)	N	treatment	treatment	change	(% of control)
Vehicle	30	254.4±16.5	286.6±25.8	32.6±15.8	100
10	30	253.6±15.9	284.0±17.5	30.4±8.5	93.2
30	30	259.0±16.5	289.4±17.0	30.4±19.2	93.2
56	30	259.4±11.8	285.0±15.5	25.6±7.7	78.5
100	20	252.1±20.1	275.0±18.1	22.9±10.1	70.2

B. Litter data: No toxicologically significant differences in the numbers of corpora lutea, implantation sites, litter size, and pre-implantation loss were observed. Prenatal mortality was slightly increased in all treated groups relative to the control group (see table below). Since the values were within the historical control range, they were not considered toxicologically significant.

Prenatal mortality data

Dose	Number of	Total	Total	Total resorption as	Prenatal mortality
(mg/kg/day)	litter	implantation	resorption	% of implantation	per litter
Vehicle	25	303	5	1.65	0.2
10	25	326	9	2.76	0.36
30	25	323	13	4.02	0.52
56	24	304	19	6.58	0.79
100	12	158	8	5.06	0.67
Historical control				1.34-13.5	0.16-1.5

C. Fetal data:

1). Fetal weights and sex distribution: A slight decrease in fetal body weights was noted (see table below). These changes were within the historical control levels. No significant differences in sex distribution were noted between control and treated animals.

Fetal body weight and sex data

Dose (mg/kg)	Fetal weight (g)	Sex distribution Male/female
Control	5.27	1.020
10	5.18	1.099
30	5.11	0.834
56	5.04	1.119
100	4.99	0.948
Historical control range	**4.83-5.27**	**0.80-1.30**

2). Anomalies: In Segment II studies (including the rabbit study), the sponsor classified all anomalies according to the time of their occurrence during intrauterine development. Three types of anomalies were distinguished. This was not a standard classification system. We do not review these studies based on this classification system.

Type A (retardations): Anomalies developing during the late fetal phase of maturation (e.g. inhibition or retardation of skeletal ossification). All minor variations in skeletal development fall into this group.

Type B (anomalies): Anomalies occurring during the early fetal phase of organ differentiation and fetal growth, comprising minor anomalies such as bipartite or bifurcated sternebrae.

Type C (malformations): Anomalies developing during the organogenetic phase. This type comprises genetically determined variations as well as major abnormalities.

In the supplement dated June 7, 1999, the sponsor clarified that "sternebra missing" did not mean that the sternebra was absent from the skeleton, but instead that it was not ossified.

The anomalies in rats are summarized in the table below. The sponsor did not provide detailed litter information on specific anomalies. No historical data on specific anomalies were provided. At the reviewing pharmacologist's request, the sponsor will submit the litter data as soon as possible. From the data available, no statistically and toxicologically significant differences were found across the groups.

Number of fetuses with anomalies

Dose (mg/kg)	**Control**	**10**	**30**	**56**	**100**
Total litter number	**25**	**25**	**25**	**24**	**12**
Total fetus number	**298**	**317**	**310**	**285**	**150**
Type A anomalies					
Number of fetuses with anomalies	50	35	43	67	31
Number of litters with anomalies	20	16	16	21	9
Sternebra missing, rudimentary, dumbbell-shaped, cleaved	44	29	39	66	30
Vertebral arches ossified incompletely	0	1	0	0	0
Metacarpal and matatarsal bones not ossified yet	0	2	0	0	0
Vertebral bodies missing, rudimentary, dumbbell-shaped, cleaved	8	12	9	10	9

Dose (mg/kg)	Control	10	30	56	100
Total litter number	25	25	25	24	12
Total fetus number	298	317	310	285	150
Type B anomalies					
Number of fetuses with anomalies	2	1	0	3	2
Number of litters with anomalies	2	1	0	3	2
Sternebra fractions displaced or fused	2	2	0	2	2
Vertebral arches fused unilaterally	0	1	0	0	0
Vertebral bodies displaced	0	1	0	0	0
Ribs thicker than normal, forked or fused	0	1	0	0	2
Omphalocele	0	1	0	1	0
Type C anomalies					
Encephaly	0	1	0	0	0
Fusion of 9th and 8th ribs right, fusion of thoracic vertebrae 1 and 2	0	1	0	0	0

In conclusion, pregnant rats were treated orally with ketotifen at 10, 30, 56 and 100 mg/kg/day from Day 6 p.c. to Day 15 p.c. Systemic toxicity in dams was noted at 100 mg/kg with decreased body weight gain and increased mortality. Decreased body weight gain was also found in the dams at 56 mg/kg/day. No differences in the numbers of corpora lutea, implantation sites, litter size, and pre-implantation loss were observed. The slightly increased prenatal mortality rate and decreased fetal body weights were within historical control ranges, and were not considered toxicologically significant. With respect to teratogenic effects, this study had some deficiencies: the sample size was too small (at high dose) compared with ICH guideline's recommendations; the classification system for anomalies was not standard; and the data presented were not complete (including litter and historical control data). Examinations on the fetuses did not reveal any biologically relevant teratogenic effects. Hence, based on the data available, HC 20-511 at the doses up to 100 mg/kg was neither embryolethal nor teratogenic in rats.

4. HC 20-511 a teratological study in rabbits. Vol. 6

Report N°: Not indicated
Compound: HC 20-511
Route: Oral by gavage
Animal: Yellow silver rabbits, age 5-6 months, 2.0-3.0 kg
Study Site:
Study Duration: Not indicated
Report Time: March 14, 1972
GLP/QAU: Not indicated
Study Design:

Dose (mg/kg/day)	Dosing volume (ml/kg)	N	# of doses
Vehicle	1	14	Once daily from Days 6 to 18 post coitum (p.c.)
5	1	14	
15	1	13	
45	1	14	

[Reviewer's comment: In ICH guideline, 16 to 20 litters were recommended in reproductive toxicity evaluation. In this study the number of litters was too small.]

One female rabbit was mated with one male rabbit. The day on which sperm were detected was considered as Day 0 p.c. (post coitum). On Day 29 p.c., the dams were sacrificed, and fetuses were delivered by cesarean section.

Toxicity assessment

Parameter	Procedure
Body weights	Days 0, 6, 18 and 29 p.c.
Macroscopic examinations	The following organs from all dams were examined macroscopically: uterus (including live and dead fetuses, the number of fetal and embryonic resorption sites), ovaries (including corpora lutea), liver, kidneys, adrenals, spleen, lung, heart, stomach and intestines. All fetuses were sexed, weighed and examined for external, internal and skeletal anomalies.

Results:

A. Findings in dams: No mortality occurred. The pregnancy rates in all groups were similar. No toxicologically significant findings were noted.

B. Litter data: No toxicologically significant changes in the number of corpora lutea, litter size, and prenatal mortality were observed. Implantation sites were decreased in high dose group due to increased pre-implantation loss (see table below). The increased pre-implantation loss could be attributed to the small number of litters because the pre-implantation loss was found very high (78%) in one litter. In addition, no dose-dependence was noted in the treated animals. Hence, the increased pre-implantation loss at high dose was not considered treatment-related.

Litter data

Dose (mg/kg/day)	Number of litter	Total corpora lut.	Total implantation	Total resorption	Pre-implantation loss	Pre-implantation loss % of corpora lutea
Vehicle	12	86	80	12	6	7
5	12	85	66	10	19	22
15	12	86	73	6	13	15
45	12	89	61	2	28	32
Historical control					1.34-13.5	2.9-28.6

C. Fetal data:

1). Fetal weights and sex distribution: No biologically relevant findings were noted.

2). Anomalies: The anomalies in rabbits are summarized in the table below. No anomaly information was provided regarding the incidence per litter for the findings that the sponsor classified as Type A. No historical data were provided. At the reviewing pharmacologist's request, the sponsor will submit the litter data as soon as possible. Regarding the observed 5th sternebra missing, the sponsor clarified that it did not mean that the sternebra was

absent from the skeleton, but instead that it was not ossified. Based on the data available, HC 20-511 revealed no teratogenic effects.

Number of fetuses with anomalies

Dose (mg/kg)	Control	5	15	45
Total litter number	**11**	**11**	**11**	**12**
Total fetus number	**68**	**56**	**67**	**59**
Type A anomalies				
Number of fetuses with anomalies	23	16	20	30
Number of litters with anomalies	9	7	8	8
5th sternebra rudimentary	20	15	20	22
5th sternebra missing	2	0	0	6
5th sternebra misshapen	1	0	0	1
5th cervical vertebral body misshapen	1	0	0	0
5th sternebra cleaved	0	1	0	1
Type B anomalies				
Number of fetuses with anomalies	4	2	0	1
Number of litters with anomalies	3	1	0	1
Sternebra fused	3	2	0	0
Runt	1	0	0	0
Thoracic vertebral bodies reduced and displaced	1	0	0	0
Cervical bodies reduced and displaced	1	0	0	0
5th sternebra cleaved and displaced	0	0	0	1

In conclusion, inseminated rabbits were treated orally with ketotifen at 15, 30 and 45 mg/kg/day from Day 6 p.c. to Day 18 p.c. No systemic toxicity in dams was noted. With respect to teratogenic effects, this study had some deficiencies: the sample size was too small compared with ICH guideline's recommendations; the classification system for anomalies was not standard; and the data presented were not complete (including litter and historical control data). The reviewing pharmacologist has requested the sponsor submit detailed historical control data and current litter data. The sponsor was also asked to address the concerns at pre-NDA meeting, but they did not do so. Although the deficiencies are of concern, the concern is lessened by the magnitude of the rabbit dose/human dose (The high dose, 45 mg/kg, was 30,000 times the proposed clinical dose). Based on the data available, HC 20-511 revealed no embryolethal and teratogenic effects. However, the final conclusion will be made when the data requested are evaluated.

5. HC 20-511 peri- and postnatal study in rats. Vol. 6

Report Nº: Not indicated
Compound: HC 20-511
Route: Oral by gavage
Animal: Albino rats, age 11 weeks, 160-230 g
Study Site:

Study Duration: Not indicated
Report Time: October 8, 1975

GLP/QAU: Not indicated

Study Design:

Dose (mg/kg/day)	Dosing volume (ml/kg)	N	# of doses
Vehicle	5	30	Once daily from Days 15 post coitum (p.c.) to Day 21 post partum (p.p.).
2	5	30	
10	5	30	
50	5	30	

Two female rats were mated with one male rat. The day on which sperm were detected was considered as Day 0 p.c. (post coitum). On Day 21 p.p., the dams and their young were sacrificed and examined.

Toxicity assessment

Parameter	Procedure
Body weights	Days 15 and 20 p.c. and Days 0, 4 and 21 p.p.
Autopsy	On Day 21 p.p., autopsy was performed on all dams and young. All fetuses were sexed, weighed and examined for external, internal and skeletal anomalies.

Results:

A. Findings in dams: The mortality data are summarized in the table below. No reasons for the deaths in the rats in 2 and 50 mg/kg groups were given. One rat at 10 mg/kg was injured during handling and died on day 20 p.c. All these rats were pregnant and showed normal litters. The pregnancy rates in all groups were similar. Decreased body weight gain was noted in rats receiving the drug at 50 mg/kg during both pregnancy period and lactation period (see table below).

Mortality and body weight changes in dams treated with HC 20-511

Dose		mortality		Body weight gain (pregnancy)		Body weight gain (lactation)	
(mg/kg/day)	N	Died	Time	(g)	% of control	(g)	% of control
Vehicle	30	0		61±19	100	20±6	100
2	30	1	Day 21 p.c.	62±22	100	24±13	100
10	30	1*	Day 20 p.c.	63±25	100	20±10	100
50	30	3	Days 20-22 p.c.	55±17	90.2	17±16	85

* due to injury during handling

B. Findings in offspring: No toxicologically significant changes in the number of implantation sites, litter size, and pre- and perinatal loss were observed. In rats treated with HC 20-511 at 50 mg/kg, a significant increase in postnatal loss was found, which also exceeded the historical control levels (see table below).

Postnatal loss in rats treated with HC 20-511

Dose	Number of	Live pups			
(mg/kg/day)	litter	Day 0	Day 21	Loss	Loss of pups (% of live pups on day 0)
Vehicle	24	263	223	40	15.2
2	25	285	250	35	12.3
10	23	233	208	25	10.7
50	20	218	165	53	24.3
Historical control					4.3-18.6

Regarding body weights of offspring during lactation period, pups in 50 mg/kg group showed reduced body weight gain during the first 4 days of life. The data recorded on Day 21 p.p. showed normal body weight gain in these animals (see table below). No biologically relevant findings in sex ratio and anomalies were noted.

Mean body weight changes (g) in offspring

Dose	Day 0	Day 4	Day 21	Body weight gain		BW gain as % of control	
(mg/kg/day)	p.p.	p.p.	p.p.	Days 0-4	Days 0-21	Days 0-4	Days 0-21
Vehicle	5.97	9.36	39.6	3.39	33.6	100	100
2	6.17	9.41	39.5	3.24	33.3	95.6	99.1
10	6.11	9.63	40.8	3.52	34.7	100	100
50	6.02	8.88	38.9	2.86	32.9	84.4	98.2

In conclusion, pregnant rats were treated orally with HC 20-511 at 2, 10 and 50 mg/kg/day from Day 15 post coitum to Day 21 post post partum. Systemic toxicity evidenced by decreased body weight gain was observed in dams of high dose group. The deaths noted in the same treatment group were also considered treatment-related. Pups' body weight gain at the first 4 days of life was reduced. An increase in postnatal loss of pups was noted in 50 mg/kg group. Up to 10 mg/kg, HC 20-511 did not affect the peri- and postnatal development of the offspring.

6. HC 20-511 prolonged toxicity in rats during postnatal development (days 10-30 post partum). Vol. 6

This study was reviewed by Dr. Gamil Debbas (HFD-160) on January 18, 1980. Refer Review and Evaluation of Pharmacology and Toxicology Data, Amendment dated January 18, 1980 (Attachment 4), Page 4.

Genotoxicity studies:

Studies reviewed:

1. HC 20-511 Mutagenicity evaluation using Salmonella typhimurium. Vol. 6
2. HC 20-511: Mutagenicity evaluation using Salmonella typhimurium. Vol. 6
3. Mutagenicity study of ketotifen fumarate in the Salmonella typhimurium reverse mutation assay (in vitro). Vol. 6
4. HC 20-511: Dominant lethal test using male mice for evaluation of mutagenic potential. Vol. 6
5. Micronucleus test of ketotifen fumarate in bone marrow cells of the mouse by intravenous injection. Supplement submitted March 4, 1999.
6. HC 20-511 Test for the induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures. Vol. 6
7. HC 20-511 Evaluation of the induction of chromosomal aberrations using V79 Chinese hamster cells in vitro. Vol. 6

8. [redacted] (HC 20-511): Mutagenicity evaluation in V79 Chinese hamster cells (HGPRT-test). Vol. 6 [redacted]

Review:

1. HC 20-511 [redacted] Mutagenicity evaluation using Salmonella typhimurium. Vol. 6 [redacted]

Report N°: Not reported
Compound: HC 20-511 [redacted]
Concentration: 0, 1, 10, 100 and 1000 µg/plate
(+) Control: [redacted]
(-) Control:
Bacteria: *Salmonella typhimurium* strains TA-1535, TA-1537, TA-1538, TA-98 and TA-100
Study Site: [redacted]
Report Time: February 6, 1979
GLP: No

The mutagenic potential of ketotifen was assessed in the presence of metabolic activation. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (w/S9)	Dose µg/plate (w/S9) 2nd test
Salmonella typhimurium	TA-1535	6-aminochrysene 0.03 or MNNG 3.0	6-aminochrysene 0.1 or MNNG 10
	TA-1537		
	TA-1538		
	TA-98		
	TA-100		

Results:

HC 20-511 showed no cytotoxicity. The doses tested were 1, 10, 100 and 1000 µg/plate in the presence of S9 activation. In both initial mutagenicity assay and confirmatory assay, HC 20-511 did not increase the numbers of revertant of *Salmonella typhimurium*. Therefore, HC 20-511 was not mutagenic under the present testing conditions.

[**Reviewer's comment**: Since no cytotoxicity was shown, the concentrations used in this study were not high enough.]

2. HC 20-511: Mutagenicity evaluation using Salmonella typhimurium. Vol. 6 [redacted]

Study N°: Mut. Bakt. 3/89

Compound: HC 20-511
Concentration:
(+) Control:

(-) Control:
Bacteria: *Salmonella typhimurium* strains TA-1535, TA-97, TA 98, TA-100 and TA-102
Study Site:

Study Period: January 5, 1989 to February 6, 1989
Report Time: April 18, 1989
GLP: Yes

The mutagenic potential of ketotifen was assessed in the presence and absence of metabolic activation. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (with S9)	Dose µg/plate (without S9)
Salmonella typhimurium	TA-1535	AA 3.0	MNNG 3.0
	TA-97	AA 10.0	9-aminocridine 100.0
	TA-98	AA 3.0, Benzo(a)pyrene 3.0	NF 2.0
	TA-100	AA 10.0	MNNG 3.0
	TA-102		Mitomycin C 0.5

Results:

HC 20-511 showed significant bacteriotoxicity at 12500 and 6000 µg/plate in all tester strains. The doses tested were 125, 1250, 12500, 600, 2000 and 6000 µg/plate in the presence and absence of S9 activation. In both initial mutagenicity assay and confirmatory assay, the positive control compounds showed an increase in the numbers of revertant colonies, while HC 20-511 did not increase the numbers of revertant of *Salmonella typhimurium* (strains TA-1535, TA-97, TA-98, TA-100 and TA-102). Therefore, HC 20-511 was not mutagenic under the present testing conditions.

3. Mutagenicity study of ketotifen fumarate in the Salmonella typhimurium reverse mutation assay (in vitro). Vol. 6

Report Nº: 10523/97
Compound: Ketotifen fumarate
Concentration:
(+) Control:

(-) Control:

Bacteria: *Salmonella typhimurium* strains TA-1535, TA-1537, TA 98, TA-100 and TA-102

Study Site:

Study Period: June 12, 1997 to July 20, 1997
Report Time: August 25, 1997
GLP: Yes

The purpose of this study was to evaluate the mutagenic potential of ketotifen fumarate in the presence and absence of metabolic activation. The table below shows the treatment protocol of positive control.

Treatment protocol of positive control

Bacteria	Strain	Dose µg/plate (with S9)	Dose µg/plate (without S9)
Salmonella typhimurium	TA-1535	2-aminoanthracene 2.0	Sodium azide 10.0
	TA-1537		9-aminocridine 100.0
	TA-98		2-nitro-9H-fluorene 10.0
	TA-100		Sodium azide 10.0
	TA-102		MMS 100.0

Results:

HC 20-511 showed complete cytotoxicity at 3160 and 10000 µg/plate without S9 activation, and at 10000 µg/plate with S9 activation. The doses tested were 10, 31.6, 100, 316, 1000, 3160 and 10000 µg/plate in the presence and absence of S9 activation. In both initial mutagenicity assay and confirmatory assay, the positive control compounds showed an increase in the numbers of revertant colonies, while HC 20-511 did not increase the numbers of revertant of *Salmonella typhimurium* (strains TA-1535, TA-97, TA-98, TA-100 and TA-102). Therefore, HC 20-511 was not mutagenic under the present testing conditions.

4. HC 20-511: Dominant lethal test using male mice for evaluation of mutagenic potential. Vol. 6

Report Nº: Not indicated
Compound: HC 20-511
Route: ip with a dosing volume of 25 ml/kg
Dose Level: 0, 25 or 100 mg/kg (for toxicity screen: 100, 125, 160 and 200 mg/kg)
Dosing Regimen: Single dose, males only
Animal: mice, 10-14 weeks old, 25-42 g
Study Site:

Experimental period: October 1 to 14, 1985

Report Time: August 30, 1977
GLP/QAU: No

After being treated with HC 20-511, the male mice (40/dose) were mated with untreated virgin females. The mating period was divided into 8 1-week intervals with 2 different females at each interval to determine the drug's effects on different stages of spermatogenesis after dosing. Female mice were sacrificed on Day 12-15 of gestation and uterine analysis was performed. The dominant lethal effects were evaluated from total implants, living implants and dead implants per pregnant female. The dominant lethality (%) was determined by the formula listed below.

(1 – living implants per pregnant female (treated group)/living implants per pregnant female (untreated group))X 100

Results:

A. Mortality in toxicity screen: The following table shows the deaths in animals treated with HC 20-511 during the toxicity screen in this study. LD_{50} was calculated as 141.2 mg/kg.

Mortality of the mice treated with HC 20-511

Dose (mg/kg)	N	Number of death	%
100	10	0	0
125	10	1	10
160	10	9	90
200	10	10	100

B. Dominant lethal test: The values of calculated dominant lethal mutation were within the control variation in sponsor's laboratory. In addition, no drug's effects on fertility were detected.

In conclusion: At 25 and 100 mg/kg, HC 20-511 exhibited no dominant lethal effects on any stage of spermatogenesis after treatment of precopulation germ cells of male mice.

5. Micronucleus test of ketotifen fumarate in bone marrow cells of the NMRI mouse by intravenous injection. Supplement submitted March 4, 1999.

Report Nº: 11688/98
Compound: Ketotifen fumarate [redacted]
Dose level: 0, 3, 6 or 12 mg/kg
Route: Intravenous
Dosing Regimen: Single dose
Animal: NMRI mice, ♂: 23 days old, 20-25 g; ♀: 24 days old, 17-22 g
Study Site: [redacted]

Study Period: January 12 to 14, 1999
Report Time: February 23, 1999
GLP/QAU: Yes

Treatment protocol

Group	Compound	Dosage (mg/kg)	N/sex	24 hr sampling	48 hr sampling
1	Vehicle	0	10	5/sex	5/sex
2	Ketotifen fumarate	3	5	5/sex	
3	Ketotifen fumarate	6	5	5/sex	
4	Ketotifen fumarate	12	10	5/sex	5/sex
5	Cyclophosphamide (Positive control)	27 ip	5	5/sex	

The purpose of this study was to evaluate the mutagenic potential of ketotifen fumarate in mouse micronucleus assay. The bone marrow was harvested about 24 or 48 hr after dosing. The frequency of micronucleated cells was expressed as percent micronucleated polychromatic erythrocytes (MNPCE). The ratio of polychromatic erythrocyte over normochromatic erythrocyte (PCE/NCE) was also calculated.

Results:

A. Preliminary study: 75% mortality was noted in mice at 18 mg/kg (iv). At 12 mg/kg (iv), mice exhibited slightly reduced motility, slight ataxia and dyspnea.

B. Main study: At 12 mg/kg (iv), mice exhibited slightly reduced motility, slight ataxia and dyspnea. No abnormal signs were noted in mice receiving 3 or 6 mg/kg of ketotifen fumarate. The results of mouse micronucleus assay are shown in the table below. NCE = normochromatic erythrocyte. PCE = polychromatic erythrocytes. MNPCE = micronucleated PCE.

Results of micronucleus assay

Group	Compound	Dosage	PCE/NCE	MNPCE/PCE (%)
24 hr		(mg/kg)		
1	Vehicle	0	0.68	0.28
2	Ketotifen fumarate	3	0.81	0.23
3	Ketotifen fumarate	6	0.62	0.19
4	Ketotifen fumarate	12	0.68	0.29
5	Cyclophosphamide (+ Control)	27 ip	0.70	2.34
48 hr				
1	Vehicle	0	0.79	0.23
4	Ketotifen fumarate	12	0.74	0.27

In conclusion: Mice treated with ketotifen fumarate showed no decrease in the PCE/NCE ratio, and no increase in the number of micronucleated PCEs compared to the vehicle control. Therefore, ketotifen fumarate was not clastogenic under the present testing conditions.

6. **HC 20-511 Test for the induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures. Vol. 6**

Study Nº: HPC/UDS/A 1/89
Compound: HC 20-511
(+) control:

Concentration:
Animal: Male Wist rats 200-300 g
Study Site:

Study Period: January 6 1989 to March 21, 1989
Report Time: September 20, 1989
GLP/QAU: Yes

The purpose of this study was to assess the genotoxic potential of HC 20-511 by determining the induction of DNA repair synthesis (UDS) in rat hepatocyte primary cultures. Hepatocytes were isolated from rats and cultured. Serum-free WME containing 10 μCi/ml ^{3}H-thymidine and HC 20-511 at different concentrations were incubated with the cells for 18-20 hr. The DNA repair synthesis was quantified by determining the silver grains produced by the decay of ^{3}H-thymidine incorporated into DNA.

Results:

In 2 tests with the concentrations ranging from 0.32 to 50 μg/ml, the drug did not influence the net nuclear count values. Hence, under the present testing conditions, ketotifen fumarate showed no genotoxic potential.

7. **HC 20-511 Evaluation of the induction of chromosomal aberrations using V79 Chinese hamster cells in vitro. Vol. 6**

Study Nº: Z. 10
Compound: HC 20-511
Concentration:
(+) control:

(-) Control:
Indicator cell: V79 Chinese hamster cells
Study Site:

Study Period: January 9 1989 to September 5, 1989
Report Time: October 9, 1989
GLP/QAU: Yes

The purpose of this study was to evaluate the clastogenic activity of ketotifen fumarate by measuring the frequency of chromosomal aberrations in V79 Chinese

hamster cells with or without S9 activation. Cells were treated with HC 20-511 for 3 hr and then incubated for 4, 13 or 23 hr before sampling.

Results:

At concentrations of 180 µg/ml (non-activated) and 600 µg/ml (activated) or higher, HC 20-511 showed dose-related toxicity evidenced by a decrease in the cell survival rate. HC 20-511, with or without S9 activation, did not induce increases in the incidence of aberrations in V79 Chinese hamster cells. The positive control chemicals increased the number of cells with chromosomal aberrations. Hence, HC 20-511 was classified as non-clastogenic under these experiment conditions.

8. Zaditen (HC 20-511): Mutagenicity evaluation in V79 Chinese hamster cells (HGPRT-test). Vol. 6

Study Nº: Mut V79 3/89
Compound: HC 20-511
Concentration:
(+) control:
(-) Control:
Indicator cell: V79 Chinese hamster cells
Study Site:
Study Period: January 8 1989 to September 14, 1989
Report Time: October 8, 1990
GLP/QAU: Yes

The purpose of this study was to evaluate the mutagenic potential of ketotifen fumarate in V79 Chinese hamster cells with or without S9 activation. Cells were treated with HC 20-511 for 3 hr and then incubated for 6 days before sampling.

Results:

There was no concentration-related increase in mutant frequency. The highest cytotoxicity in the presence of metabolic activation was approximately 65% as opposed to 80% as specified in the ICH guidelines. Therefore, this study is deficient; however, the sponsor can use the results from the chromosomal aberration study to satisfy the ICH recommendation for in vitro assessment of DNA damage in mammalian cells.

LABELING REVIEW:

Original version:

Carcinogenesis, Mutagenesis, Impairment of Fertility:
Ketotifen fumarate demonstrated no carcinogenic effects in lifetime studies in mice and rats at dietary doses more than 70,000 times and 59,000 times the maximum recommended ocular human use level of 0.0012 mg/kg/day for a 50 kg adult respectively. Ketotifen fumarate was determined to be non-mutagenic in a battery of in vitro tests including: a bacterial mutation (Ames) test, a bacterial reverse mutation (Ames) test, a mammalian chromosome aberration test and a mutagenicity test in V79 Chinese hamster cells. In addition, the following in vivo tests were performed: a mouse dominant lethal test, a mouse micronucleus test and a Chinese hamster chromosome aberration test on bone marrow cells. There was no evidence of impaired fertility or reproductive capability in male rats at 8,330 times and in female rats at 41,000 times the maximum recommended ocular human use level.

Pregnancy: Pregnancy Category B
Teratology and peri- and post-natal studies have been conducted with ketotifen fumarate in rats and rabbits. At 80,000 times and 37,000 times the maximum recommended ocular human use level, ketotifen fumarate was shown not to be teratogenic in rats and rabbits respectively and no effects on peri/post-natal development were observed in rats at 37,000 times the maximum recommended ocular human use level. There are, however, no adequate and well controlled studies in pregnant women. Because animal studies are not always predictive of human response, this drug should be used during pregnancy only if clearly needed.

Revised version:

Carcinogenesis, Mutagenesis, Impairment of Fertility:

Carcinogenicity studies are still under assessment.

Ketotifen fumarate was determined to be non-mutagenic in a battery of *in vitro* and *in vivo* mutagenicity assays including Ames test, *in vitro* chromosomal aberration test with V79 Chinese hamster cells, *in vivo* micronucleus assay in mouse, and mouse dominant lethal test.

Treatment of male rats with oral doses of ketotifen $\geq$ 10 mg/kg/day orally [6,667 times the maximum recommended human ocular dose of 0.0015 mg/kg/day on a mg/kg basis (MRHOD)] for 70 days prior to mating resulted in mortality and a decrease in fertility. Treatment with ketotifen did not impair fertility in female rats receiving up to 50 mg/kg/day of ketotifen orally (33,333 times the MRHOD) for 15 days prior to mating.

Pregnancy: Pregnancy Category B. Oral treatment of pregnant rabbits during organogenesis with up to 45 mg/kg/day of ketotifen (30,000 times the MRHOD), and oral treatment of rats during organogenesis with up to 100 mg/kg/day of ketotifen (66,667 times the MRHOD) did not reveal any biologically relevant teratogenic effects.

Oral treatment of pregnant rats (up to 100 mg/kg/day or 66,667 times the MRHOD) and rabbits (up to 45 mg/kg/day or 30,000 times the MRHOD) during organogenesis did not result in any biologically relevant embryofetal toxicity. In the offspring of rats that received ketotifen orally from Day 15 of pregnancy to Day 21 post partum at 50 mg/kg/day (33,333 times the MRHOD), a maternally toxic treatment protocol, the incidence of postnatal mortality was slightly increased, and body weight gain during the first 4 days post partum was slightly decreased.

SUMMARY AND EVALUATION:

Pharmacology:

Ketotifen is a specific antihistaminic agent with very weak antiserotonin and anticholinergic activities. Ketotifen demonstrated antianaphylactic effects and clear antihistaminic activities. However, the antianaphylactic and antihistaminic activities may depend on different mechanisms.

Regarding the effects of ketotifen on histamine release from mast cells, several in vivo and in vitro studies provided conflicting results. One in vitro study showed that ketotifen could inhibit histamine release from human leukocytes.

No differences regarding antianaphylactic and antihistaminic activities were noted in (+) and (-) antipodes of HC 20-511. The metabolites of ketotifen, N-oxide and nor-ketotifen, were less active than ketotifen in anti-PCA (3 to 5 times) and antihistaminic (50-200 times) activities.

In the ocular pharmacology studies conducted in rats and guinea pigs, ketotifen fumarate 0.05% and 0.025% were effective in reducing ocular hypersensitivity responses induced by either 48/80 or immunization with ovalbumin. The drug was as potent as Patanol™ and Livostin™, both of which were approved H_1-receptor antagonists.

In cardiovascular studies in cats, ketotifen had no significant effects except at high dose (18.7 mg/kg, iv) at which the isoproterenol-induced tachycardia and carotid occlusion responses were inhibited. In mouse study, ketotifen also presented antiarrhythmic activity. In dog study (5 mg/kg, iv), ketotifen produced a mild increase in heart rate, contractile force and aortic mean flow. In studies for renal actions in rats and dogs, ketotifen (0.01-3 mg/kg, sc or po) decreased urine volume and Na^+ and Cl^- excretion. At doses up to 10 mg/kg (po or sc), no CNS effects of ketotifen were observed in mice.

ADME:

Absorption:

Assessment of the pharmacokinetics was conducted in several species. In rats, dogs and monkeys, the drug was well absorbed following oral administrations. The

summary of the plasma PK parameters was presented in the following table. Rat studies indicated that about 30% drug and metabolites were reabsorbed in the bile, which may explain the multiphased decreases in blood total radioactivity in several species.

Plasma PK parameters in mice, rats, dogs, monkeys and humans

Species	Dose (mg/kg)	Route	Cmax (μg-eq/ml)	Tmax (hr)	$AUC_{0-\infty}$ (μg-eq•hr/ml)	T1/2 (hr)
Mouse (♂, n = 3)	1 (single dose)	po	0.122	0.5	0.62*	20
Mouse (♂, n = 3)	1 (single dose)	iv	0.207	5 min	0.808*	28
Rabbit (♂, n = 3)	0.5/eye single dose	Eye drop	0.096	1	0.819	
	0.5/eye17 doses	Eye drop	0.129	0.5		
	0.5/eye single dose	Eye drop	0.087	1	0.366	1.475
Rat (♂, n = 5)	0.1/eye single dose	Eye drop	0.016	0.75		43
Rat (n = 16-20)	2 (single dose)	po	0.075	0.5	0.195	1.3
	15 (single dose)	po	0.402	1.0	3.742	5.3
	100 (single dose)	po	2.050	1.0	30.939	5.2
Rat (n = 2-4)	15 (qdx2w)	po	0.385	1.0	2.045	4.2
Rat (♂, n = 4-6)	2 (qd x 2years)	po	0.044	2.0	0.113 (0-4 hr)	
Rat (♂, n = 3-6)	15 (qd x 2years)	po	0.343	2.0	1.038 (0-7 hr)	
Rat (♂, n = 3-4)	65 (qd x 2years)	po	0.650	2.0	3.275 (0-6 hr)	
Rat (♂, n = 3)	1 (single dose)	iv	0.184	0.2	1.403	20
	1 (single dose)	po	0.093	2	1.246	36
Dog (n = 3)	0.5 (single dose)	iv	0.15	0.05	2.64	67
Dog (n = 3)	0.5 (single dose)	po	0.181	2	3.947	206
Monkey (♂, n=3)	0.5 (single dose)	iv	0.138	5	4.872	33
	0.5 (single dose)	po	0.11	7	2.974	45
Man						

*Blood concentration
#AUC_{0-24}

Tissue distribution:

In the distribution studies conducted in rabbits following ocular administration, cornea, conjunctiva and iris demonstrated high levels of radioactivity. After oral and intravenous administrations in rats and dogs, high levels of radioactivity were found in liver, lungs and kidneys.

Metabolism:

The following tables summarize the metabolites identified in urine and bile, and the relative amounts of these metabolites in urine in humans, dogs, monkeys, rabbits and rats. In rat, rabbit and monkey, demethylation was a major metabolic degradation.

Metabolites identified in urine and bile samples from different species

Metabolite #	Description	Man	Dog	Monkey	Rabbit	Rat
2	Parent drug		+	+	-	+
3, 4	Nor 20-511		+	+	-	+
5	Nor 20-511 sulfate		+	+	+	-
7, 8	Glucuronide of HC 20-511		-	+	+	-
9, 10	Hydroxylamine glucuronides of nor 20-511		+	+	-	+

Metabolite #	Description	Man	Dog	Monkey	Rabbit	Rat
12	Thiophene metabolites		+	+	-	-
13, 14	N-oxide		+	+	-	+
15	Amide of 11a		-	+	-	-
16			+	-	-	-
17			+	-	-	-
18			+	-	-	-
19			+	-	-	-
20			+	-	-	-
21			+	-	-	-
22			+	-	-	-
23			+	-	-	-
24			+	-	-	-
11, 11a	Tautomeric rearrangement products of 2-OH		-	+	-	-
12	Thiophene metabolites		+	+	-	-
13, 14	N-oxide		+	+	-	+
15	Amide of 11a		-	+	-	-
16			+	-	-	-
17			+	-	-	-
18			+	-	-	-
19			+	-	-	-
20			+	-	-	-
21			+	-	-	-
22			+	-	-	-
23			+	-	-	-
24			+	-	-	-

The Numbers represented different metabolites of ketotifen.

Relative amounts of urinary metabolites in man and animals after oral dose of ^{3}H-HC 20-511 (% of total radioactivity)

Metabolite #	Man	Monkey 1	Monkey 2	Dog 1	Dog 2	Rat 1	Rat 2	Rabbit
2		5.0	2.0	13.6	6.9	-	0.2	-
3, 4		19.6	9.7	2.4	0.4	38.3	65.4	-
5		7.2	2.6	2.9	2.9	-	-	33.9
7, 8			4.3	-	-	-	-	28.6
9, 10		11.5	14.7	8.8	10.4	1.4	2.6	-
11		-	-	-	-	-	-	-
12		±	-	-	-	-	-	-
13, 14		1.2	4.0	30.9	38.3	6.0	6.6	-
20		-	-	11.5	15.1	-	-	-
23		-	-	+	+	-	-	-
24		-	-	+	+	-	-	-
Total		44.5	37.3	70.1	73.7	45.7	74.8	62.5

Protein binding:

The binding of the drug to the serum protein in different species was presented in the table below. Human serum gave the highest degree of binding. However, there were 2 other studies indicating that the binding of the drug to human serum was about 70%.

Protein binding to HC 20-511 in various sera

	Human	Rabbit	Guinea pig	Cat	Dog	Monkey	Rat
Avg. protein binding (%)		82.24	81.1	77.5	78.7	78	73.6

Excretion:

Studies indicated that biliary/intestinal excretion played a very important role in the elimination of ketotifen. About 85-90%, 67%, 43-52% and 41-44% radioactivity was removed from the body by feces in rats, mice, dogs and monkeys, respectively. In the same species, about 9-10%, 26-27%, 31-35% and 36-43% radioactivity was found in urine. Urine was the major route of excretion (60-70% of total dose) in man.

Placental transfer and milk secretion:

The studies indicated that ketotifen could transfer to milk soon after oral administration with the radioactivity concentrations higher than plasma, and that ketotifen could pass the placental barrier easily.

Toxicology:

Acute toxicity studies:

Acute (single-dose) toxicity studies was assessed in mice, rats and rabbits. The results are summarized in the table below.

Summary of acute toxicity of ketotifen

Species (#/sex/group)	Dose (mg/kg), /route	Length of observation	Observation	NOAEL (mg/kg)
Mice, (10)	180-1440 /po	14 days	Drowsiness, convulsions and twitching, piloerection, recumbency, cramps, jumping, prone position, motoric unrest, and dyspnea. LD_{50} = 342mg/kg (LD_{50}=371, 749 and 390 mg/kg in (+), (-) and mixed HC 20-511).	
Mice (5)	100-1000 /po	7 days	Drowsiness, cramps, piloerection, forced breathing, flaccidity, and motor exicitation. LD_{50} for po: 365 mg/kg; for iv: 14 mg/kg	
	10-18/iv			
Mice (2)	10-24/iv	7 days	LD_{50}=18.0 mg/kg, drowsiness, flaccidity, convulsion, forced breathing	
Rats (5)	100-1800/po	7 days	Drowsiness, cramps, hyperreflexia, disturbed equilibration, forced and slow breathing, flaccidity, prone position. LD_{50}: 3.2 (iv) and 360 (po) mg/kg.	
	3.2-10/iv			
Rats (10)	0-600/po	14 days	Deaths, sedation, motor excitation, ataxia, hyperreflexia, acclerated breathing, cyanosis. Ten days old animals were most sensitive to HC 20-511.	40 (1-10 days) 80 (21-30 days)
Rats (5-10)	78-373/po	7 days	Deaths, decreased locomotor activity, ataxia, hypothermia, weak and loss of righting reflex	78
Rats (10)	0-820/po		Deaths, decreased locomotor activity Ten days old rats were most susceptible to the lethal effects of 20-511	60 (10-day) 101 (14-day) 170 (21-day)
Rabbits (3-5)	320-1800/po	7 days	Deaths, drowsiness, cramps, muscular fibrillation, opisthotonus, lateral decubitus, blinking, gasping, running motions, jerking, forced and accelerated breathing, tremor, and motor excitation. LD_{50} for po: 790 mg/kg, iv: 21.0 mg/kg	
	10-40/iv			

Subchronic toxicity studies:

Subchronic toxicity studies are summarized in the table below.

Summary of subchronic toxicity of ketotifen

Species #/sex/group	Dose (mg/kg)	Duration and route	Findings	NOAEL (mg/kg)
Rats, 8/sex/dose	0, 1, 10 and 100 mg/kg	26-27 days Dietary or gavage	Decreased body weight gain was seen in both male and female rats at high dose. A slight ↑ or normal value in body weight gain was noted at low and mid doses. An increase in liver weights and total liver lipids were observed in high dose male animals. Mild to moderate periportal lipidosis in high dose animals was also noted in histopathological examinations.	10
Rats 7/sex/group	0, 1, 10 and 25 mg/kg/day	5 weeks Oral	Decreased body weight gain in female rats at 10 and 25 mg/kg/day. No changes in ECG were noted.	♂: 25 ♀: 10
Rats 4/sex/group	0-150 mg/kg/day	5 weeks Oral	Increased lipid accumulation was observed in liver and kidney at 60 and 150 mg/kg/day.	4
OFA IFFA CREDO SPF rats, 10/sex/dose	0, 10, 33 and 157 mg/kg/day	13 weeks Oral	All treated groups dose-dependently: ↑ serum cholesterol, ↑ liver weight, globoid cytoplasmic inclusions (liver). 33 mg/kg: hepatocyte swelling (4/10) 33 and 157 mg/kg: pulmonary lesions (grey/white or hemorrhagic flecks), liver discoloration, hepatic cytoplasmic (fat) vacuoles, ↑ incidence of lipid droplet in hepatocytes 157 mg/kg: agitation, ↓ body weight gain (43-49%) and food consumption, hepatocyte swelling (16/20), **degenerative changes in β cells in pancreas in 9/10 males**	10
Rats 5/sex/dose	0, 10, 33 and 157 mg/kg/day	13 weeks Oral	All treated groups: ↑ liver weight (Except low dose ♀), accumulation of lipids and cholesterol in hepatocytes, ↑ cytochrome P450 (except low dose) and N-demethylase. HC 20-511 was considered an enzyme inducer. The liver changes were considered as an adaptive response to the drug. **After 4 weeks recovery**: Relative liver weight was normal (33 mg/kg) or toward normal (157 mg/kg). Liver microscopy (hepatocytomegaly, globoid inclusions, large and small vacuoles): Returned to normal levels in 33 mg/kg group, while in 157 mg/kg group, liver microscopy showed a trend toward normal. The fatty changes in both doses were reduced. [**Reviewer's comment**: No control recovery rats were included in this study.]	
Rats 5 control, 10 treated	0 and 145 mg/kg/day	6 or 13 weeks Oral	Serum: ↑glucose, ALT, ↓cholesterol slightly **Liver samples under microscopic examination**: Cell enlargement, diffuse fat droplet infiltration, larger homogenous areas and nuclear-like inclusions, ↑fat content. **Liver samples under electron microscopic examination**: 6 weeks: A concentration of fat droplets in the peripheral area of the lobule. Cells were larger and contained many vesicles (proliferated smooth endoplasmic reticulum). 13 weeks: The membrane structures had increased further. There were clear membrane whorls (fingerprints) that often contained fat droplets in their centers. True necrosis was not present and cell kept its structure. More individual myelin figures were present.	
Pedigree beagle dogs 2/sex/group	0, 1.25, 5, 20 and 80 mg/kg/day	13 weeks Oral	Sedation and timidity 4/4, occasional convulsive seizures 1/4 at 80 mg/kg. Body weight gain and food consumption were increased in all treated groups. ↑ WBC, ALP and slight ↑ ALT, and ↑ albuminuria at 80 mg/kg. Twin-peaked T wave with the incidence ↑ with dosage. Mild ↑HR at 20 and 80 mg/kg. 80 mg/kg: prolonged QRS duration. ↓prostate weight without corresponding histological findings. ↑liver weight at 80 mg/kg. Liver under microscopy: slight to moderate fat droplets in single cells, hepatocyte swelling, and eosinophilic cytoplasmic inclusions at 20 (1-3/4) and 80 mg/kg (4/4). Ballooned hepatocytes and periportal inflammation at 80 mg/kg (2/4).	5

Degenerative changes in β cells in the pancreas in one study (at 157 mg/kg) did not cause an increase in blood sugar level, which might be due to that sufficient β cells remained intact. Similar changes were not observed in the other subchronic and chronic toxicity studies.

Chronic toxicity studies:

Dog:

Dogs were treated with HC 20-511 at 0, 0.1, 0.5, 5 and 50 mg/kg/day for 1 year. Reversible clinical signs observed at 50 mg/kg/day included slight disturbance of equilibrium and unphysiological position (2/4♀), slight clonic-tonic cramps (3/4♀), decreased pain reflex (5/8) and leg stretch reflex (3/8). Deaths occurred in 2 male dogs in Week 39 (5 mg/kg) of the treatment period, and in Week 5 of the recovery period (50 mg/kg), respectively. Urinary bladder concrements were considered the cause of the deaths. Slight increases in body weight gain and food consumption were noted in animals treated with the drug at ≥ 0.5 mg/kg/day, which might not be toxicological effects. Slight increases in plasma ALP and ALT activities were observed in the dogs at 50 mg/kg. The urinary excretion of K^+ was reduced since 26th week. In ECG examinations, dogs at high dose showed supraventricular extrosystole and increased twin-peaked T-waves, QT interval and QRS interval. Gall bladder stone was found in 6/8 of the high dose dogs, while control animals only had 1 case of gall bladder stone. Urinary bladder stone was found in all groups (2/8, 2/8, 2/8 and 3/8). At the end of the treatment, dogs at high dose exhibited increased liver, adrenal and ovary weights. Histopathological examinations indicated the following liver changes: liver cell hypertrophy (7/8) with increased granularity (8/8), inflammatory changes and bile duct proliferation (8/8), and increased pigment deposits in Kupffer cells (8/8).

One dog/sex/group was included in the recovery test. Following 8 weeks recovery period, all dosed groups showed a slight decrease in body weights without dose-dependence. The food consumption was normal. ECG and blood chemistry changes were normalized. Kidney, liver and heart weights were decreased in high dose dogs relative to the control animals. Postmortem tests revealed similar, but less pronounced histopathological changes in the livers of the 2 recovery animals at 50 mg/kg.

The no-toxic-effect level was set by the sponsor as 5 mg/kg/day in this study. With regard to body weight gain and food intake, 0.1 mg/kg was NOEL.

Urinary bladder concrements were distributed in all groups without dose-dependence, and the amount of HC 20-511 in these concrements was not more than 10 μg per 100 mg, suggesting that the formation of the concrements might not be treatment-related. In the supplement study, no signs of urolithiasis were present. The sponsor provided 2 factors that might contribute to the formation of concrements. One was microtrauma to the urethra and urinary bladder during catheterization for urine sampling; the other was food content (high pH, and high Mg^{2+} and vitamin D). In the supplement

study, the dogs were fed with the same food without producing concrements, indicating that food may not be that important, and microtrauma may be the cause.

Liver changes were found in both chronic and subchronic toxicity studies conducted in dogs and rats, which included increases in liver weight, total liver lipids and cholesterol levels, and serum ALT and ALP activities. Histopathological examinations showed liver cell hypertrophy, increased granularity, globoid eosinophilic cytoplasmic inclusions, hepatic cytoplasma vacuoles, fat droplet infiltration, and periportal lipidosis. Electron microscopy showed fat droplets, and proliferation of smooth endoplasmic reticulum, which corresponded to globoid eosinophilic cytoplasmic inclusion, and was known to occur with drugs that induce endoplasmic reticulum enzymes in livers. No necrosis was developed. Following 8- or 4-week recovery period, the microscopic changes were less than those in the main study, and globoid eosinophilic cytoplasmic inclusions were absent. In the livers of the rats treated with HC 20-511, cytochrome P450 and N-demethylase activities were increased. These data supported that the liver changes could be related to adaptive responses that were reversible, non-toxic, and a result of enzyme induction.

Monkey:

Monkeys were treated with HC 20-511 at 5 mg/kg/day for a year. An increase in body weight gain was observed in male monkeys. No other drug-induced effects were noted. In urinary bladders, there was no concrement formation. A dose of 5 mg/kg/day was considered as NOAEL.

An increase in body weight gain with increased food intake was observed in several chronic and subchronic toxicity studies, and in clinical studies under [redacted]. The sponsor indicated that these effects, which were also seen with other compounds of the same chemical class [redacted] were pharmacological effects. In nonclinical studies with ocular administration, such effects were not observed. In addition, the ocular doses in clinical administration will be very low (0.0015 mg/kg). Hence, this should not be a clinical concern.

Ocular toxicity studies:

Ocular toxicity studies are summarized in the table below. Similar to some subchronic and chronic toxicity studies, Slight liver weight increases and fatty infiltration in hepatocytes were observed in treated animals in a 13-week study and a 26-week study. The reviewer is concerned because many hepatotoxins can cause fatty liver. There were no clinical pathology changes correlated with the fatty infiltration. The changes were observed in only male animals. The toxicological relevance of this effect to human use is not known. The reviewing pharmacologist has informed medical officer of this issue. Cervical lymph node hyperplasia was noted in one study. Since the changes were not dose-related, and the number of animals was small, the cause of the changes was unknown. It may involve local stimulation.

Summary of ocular or local tolerance toxicity studies

Species	Treatment	Observations	Ocular toxicity	NOAEL
New Zealand white rabbits and Chinchilla Bastard rabbits (pigmented)	0.025% ketotifen fumarate ophthalmic solution 25 µl/right eye, bid or qid x 26 weeks	Cervical lymph node hyperplasia. Hepatocytes: Peripheral fatty infiltration in male animals at high dose (NZW:2/4; Bastard: ¼).	No	0.025% ketotifen fumarate ophthalmic solution 25 µl/right eye, qid at 2 hr intervals
New Zealand white rabbits	0.025% ketotifen fumarate ophthalmic solution (**heat degraded**) 25 µl/right eye, bid or qid x 13 weeks	Increased male liver weight (in Group 3) correlated with mild diffuse fatty infiltration of hepatocytes (0/8 control, 1/8 Group 2, 5/8 Group 3).	No	0.025% ketotifen fumarate ophthalmic solution (**heat degraded**) 25 µl/right eye, qid
New Zealand white rabbits, ♂	0.08% ketotifen fumarate ophthalmic solution 50 µl/right eye, 5 times at 30 min intervals, or tid x 2 weeks		Neither ketotifen ophthalmic solution nor vehicle control produced any abnormal irritation in rabbit cornea palpebral conjunctiva under the visual, microscopic and electron microscopic examinations.	
New Zealand white rabbits	0.1% ketotifen fumarate eye drop, 0.1 ml/one eye, single dose		No	0.1% ketotifen fumarate ophthalmic solution 100 µl/one eye, single dose
New Zealand white rabbits, ♂	0, 0.1, 0.2, 0.4 and 0.8% ketotifen fumarate ophthalmic solution, 100 µl/right eye, single dose		Score: 0 and 0.1%: 0 0.2%: 1.2, 0.4%: 1.6 (practically nonirritating) 0.8%: 2.8 (minimally irritating)	0.4% ketotifen fumarate ophthalmic solution 100 µl/right eye, single dose
New Zealand white rabbits, ♂	0, 0.05, 0.2 and 0.8% ketotifen fumarate ophthalmic solution 50 µl/right eye, 15 times at 30 min intervals		Score: PSS: 0; Minimally irritating was noted from vehicle to 0.8% KFOS	
New Zealand white rabbits	0, 0.05, 0.2 and 0.8% ketotifen fumarate ophthalmic solution 50 µl/right eye, qid at 2 hr intervals for 4 weeks	No	Highest weekly average score: PSS: 0.4♂, 0.2♀; vehicle: 0.7♂, 0.5♀; 0.05%: 0.7♂, 0.9♀; 0.2%: 1.0♂, 1.4♀; 0.8%: 1.9♂, 2.3♀ (practically nonirritating). No differences between ♂and ♀.	0.2% ketotifen fumarate ophthalmic solution, qid at 2 hr intervals for 4 weeks
New Zealand white rabbits, ♂	0, 0.05, 0.2 and 0.8% ketotifen fumarate ophthalmic solution 50 µl/right eye, qid at 2 hr intervals for 13 weeks	No	Average score: PSS: 0.3; vehicle: 0.6; 0.05%: 0.7; 0.2%: 1.0 (practically nonirritating) 0.8%: 4.3 (minimally irritating) Highest score: PSS: 1.2; vehicle: 2.4; 0.05%: 2.0 (practically nonirritating) 0.2%: 2.8; 0.8%: 6.0 (minimally irritating)	0.05% ketotifen fumarate ophthalmic solution, qid at 2 hr intervals for 13 weeks

Species	Treatment	Observations	Ocular toxicity	NOAEL
New Zealand white rabbits, ♂	Ketotifen fumarate ophthalmic solution (0.05%) (degraded and normal) 50 µl/right eye, 15 times at 30 min intervals	No	Score: PSS: 0 Vehicle: 1.6 (practically nonirritating) Degraded: 4.4; Ketotifen: 6.8 (minimally irritating)	
DUHA guinea pigs	Ketotifen base in propylene glycol:PSS (1:1 parts) intradermal injection (1%) and epidermal application (25%)	After 2 challenges, no differences between the treatment and control groups were noted		Ketotifen base possessed no skin sensitizing potential in guinea pigs.
Rabbits (mixed race)	HC 20-511 injectable ampoule solution (0.05 and 0.017%), 2 ml/site, iv	HC 20-511 (0.05% and 0.017%) had a local irritant effect comparable to that of the placebo control.		

Carcinogenicity studies:

The review of these studies is ongoing. According to the sponsor, "ketotifen fumarate demonstrated no carcinogenic effects in lifetime studies in mice and rats at dietary doses more than 70,000 times and 59,000 times the maximum recommended ocular human use level of 0.0012 mg/kg/day for a 50 kg adult respectively."

Reproductive toxicity studies:

The results from reproductive toxicity studies are summarized in the table below.

Summary of reproductive toxicity studies

Animal species	Dose (mg/kg)	Duration of treatment	Observations	NOAEL (mg/kg)
Fertility studies				
♂ rats/	0, 2, 10 or 50	♂: 10 weeks prior to mating→insemination ♀: Untreated	10 and 50 mg/kg: ↑mortality rates in males, ↓fertility index. 50 mg/kg: ↓copulation index.	♂: 2
♀ rats/	0, 2, 10 or 50	♀: 2 weeks prior to mating→sacrificed (gestation Day 13 or Day 21 post partum) ♂: Untreated	10 and 50 mg/kg: ↓body weight gain. No embryo/fetal or postnatal findings	♀: 2
Teratological studies				
♀ rats/	0, 10, 30, 56 or 100	♀: Gestation Days 6-15	100 mg/kg: ↑mortality in dams. 56 and 100 mg/kg: ↓dam body weight gain. No embryo/fetal findings	♀: 30
♀ Rabbits/yellow silver	0, 5, 15 or 45	♀: Gestation Days 6-18	No toxicologically significant findings were noted.	♀: 45
Peri- and postnatal studies				
♀ rats	0, 2, 10 or 50	♀: Gestation Day 15→Day 21 post partum	50 mg/kg: 3/30 dead (dams), ↓dam body weight gain, ↓pups' body weight gain during the 1st 4 days, ↑postnatal loss.	♀: 10 Offspring: 10

Genotoxic studies:

Ketotifen was not genotoxic in Ames test, in vitro chromosomal aberration test with V79 Chinese hamster cells, and in vivo micronucleus assay in mouse.

RECOMMENDATION:

This application is approvable from a nonclinical perspective with some modifications of labeling as revised in the Carcinogenesis, Mutagenesis, Impairment of Fertility section and Pregnancy: Pregnancy Category B section.

/S/ 6-16-99
Zhou Chen, Ph.D.

Concurred by: /S/ 17 Jun 99
Andrea B. Weir, Ph.D.

cc:
NDA 21-066/Division File
NDA 21-066/Original NDA
HFD-550/CSO/Rodriguez
HFD-550/MO/Dunbar
HFD-550/TL Pharm/Weir
HFD-550/Pharm/ChenZ

MESSAGE TO BE CONVEYED TO THE SPONSOR

Modifications are made in the Carcinogenesis, Mutagenesis, Impairment of Fertility section and Pregnancy: Pregnancy Category B section of the labeling for ketotifen fumarate ophthalmic solution 0.025%. The following is the revised part.

Carcinogenesis, Mutagenesis, Impairment of Fertility:

Carcinogenicity studies are still under assessment.

Ketotifen fumarate was determined to be non-mutagenic in a battery of *in vitro* and *in vivo* mutagenicity assays including Ames test, *in vitro* chromosomal aberration test with V79 Chinese hamster cells, *in vivo* micronucleus assay in mouse, and mouse dominant lethal test.

Treatment of male rats with oral doses of ketotifen $\geq$ 10 mg/kg/day orally [6,667 times the maximum recommended human ocular dose of 0.0015 mg/kg/day on a mg/kg basis (MRHOD)] for 70 days prior to mating resulted in mortality and a decrease in fertility. Treatment with ketotifen did not impair fertility in female rats receiving up to 50 mg/kg/day of ketotifen orally (33,333 times the MRHOD) for 15 days prior to mating.

Pregnancy: Pregnancy Category B. Oral treatment of pregnant rabbits during organogenesis with up to 45 mg/kg/day of ketotifen (30,000 times the MRHOD), and oral treatment of rats during organogenesis with up to 100 mg/kg/day of ketotifen (66,667 times the MRHOD) did not reveal any biologically relevant teratogenic effects.

Oral treatment of pregnant rats (up to 100 mg/kg/day or 66,667 times the MRHOD) and rabbits (up to 45 mg/kg/day or 30,000 times the MRHOD) did not result in any biologically relevant embryofetal toxicity. In the offspring of female rats that received ketotifen orally from Day 15 of pregnancy to Day 21 post partum at 50 mg/kg/day (33,333 times the MRHOD), a maternally toxic treatment protocol, the incidence of postnatal mortality was slightly increased, and body weight gain during the first 4 days post partum was slightly decreased.

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: ketotifen fumarate; leukotriene inhibitor, H_1-receptor blocking activity
Reviewer Name: Terry S. Peters, D.V.M.
Division Name: DAIDP, HFD- 520
Review Completion Date:5/24/99
IND/NDA number: NDA 21066
Serial number/date/type of submission: Carcinogenicity studies in rats and mice
Sponsor (or agent): Ciba Vision Corporation
Manufacturer for drug substance: Novartis Riganskiddy Ltd., County Cork, Ireland

Drug:

Code Name: Ketotifen fumarate
Generic Name: Ketotifen fumarate
Trade Name: Unknown
Chemical Name: 4-(1-Methyl-4-piperidylidene)-4H-benzo[4,5]cyclohepta[1,2-b]thiophen-10(9H)-one hydrogen fumarate; 9,10-dihydro-4-(1-methyl-4-piperidylidene)-10-oxo-4H-benzo[4,5]cyclo-hepta[1,2-b]thiophene fumarate
CAS Registry Number: 34580-14-8

[redacted]

Drug Class: Selective H_1-receptor antagonist and mast cell stabilizer

Indication: Preventing ocular itching associated with allergic conjunctivitis

Clinical formulation: 0.345 mg ketotifen fumarate with glycerol, sodium hydroxide/hydrochloric acid, and purified water

Route of administration: Topical ophthalmic

Studies reviewed within this submission: Rat and mouse carcinogenicity studies

PHARMACOLOGY:

Mechanism of Action: Non-competitive histamine antagonist (H_1 receptor)
Drug Activity Related to Proposed Indication: Inhibits the release of inflammatory mediators with decreased chemotaxis and activation of eosinophils

CARCINOGENICITY:

Study Title: Cancerogenic-Potential Study in Mice
Study Number: HC-20-511
Volume Numbers: 35
Test Facility: [redacted]
Study Date(s): Report dated September 13, 1976. Actual study dates unknown
Date of Submission: 4/28/99
GLP Compliance/Quality Assurance: No information provided
QA Report- None provided
Study Type: Oral carcinogenicity study
Species/strain: mice
Number of animals per group; age at start of study: 50; aged 8 weeks at study initiation
Animal housing: Individually
Drug Lot/Batch number(s): Weeks 1-48: 73901; Weeks 49-74: 74901
Drug Purity / Stability / Homogeneity: None submitted
Doses: Weeks 1-52: 1.7, 13.5, or 88 mg/kg/d; Weeks 53- 74 (termination): 2.1, 16, or 93 mg/kg/d. Theoretical doses were 2, 15, and 100 mg/kg/d.

- Basis of Dose Selection: Not provided
- CAC Concurrence: None
- Route of Administration: In feed
- Frequency of Drug Administration: Continuous
- Dual Controls Employed: No
- Interim Sacrifices: None
- Satellite PK or Special Study Group(s): None
- Unscheduled Sacrifices or Deaths: Males: 19, 23, 20, and 31 for the control, low, mid and high dose groups.

  Females: 19, 25, 26, and 22 for the control, low, mid and high dose groups.
- Original Study Protocol: "It was decided at the onset that when the number of mice in either control or treatment groups was reduced to approximately 60% of their initial number, the study should be discontinued in order to permit adequate histological evaluation of sufficient animals to reach a reliable conclusion."

Study Results and Frequency of Monitoring:

- Clinical Observations: Daily for 1 week, weekly thereafter
- Mortality: daily for 1 week, weekly thereafter
- Body Weight: Weekly, but only graphic representation of data presented, no raw data provided. Body weight gain appeared to be reduced in the high dose animals. Increased mortality was reported for high dose males.

- Food Consumption: Not measured in this study
- Ophthalmoscopy: Not performed
- Hematology: First 10 survivors in each group and sex. All groups had a few animals with normochromic anemia and increased reticulocytes. In the high dose animals, there was a slight left shift without an increase in total number of cells
- Clinical Chemistry: None
- Organ Weights: Not performed
- Gross Pathology: All animals
- Histopathology: Tissues examined: lung (3 sections), liver (each lobe), thymus, mediastinal lymph node, stomach, spleen, mesenteric lymph nodes, stomach, testes, prostate, seminal vesicles, ovaries, uterus, urinary bladder, tumor-bearing tissues.

  Non-Tumor: No significant differences were reported between controls and treated animals.

  Tumor: Liver tumors (primarily adenomas) were more frequent in males, and lymphoreticular tumors more frequently in females. No biologically significant differences between controls and treated animals were presented.

Premature Decedents with Lymphoreticular Tumors*

Dose group	Control	Low	Mid	High
Male	12	10	8	11
Female	12	17	16	15

* No significant differences were found using the Chi^2 test.

Terminal Sacrifice Animals (Weeks 72-74) with Lymphoreticular Tumors

Dose group	Control	Low	Mid	High
Male	3	1	5	2
Female	7	5	4	2

Although the incidence of liver tumors was high in premature male decedents, they were reported across groups.

Males: 17, 13, 21, and 10 for controls, low, mid and high dose, respectively).

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model: Even considering the time frame of the study, pre-1976, one would expect a more complete presentation of the data and a GLP compliance statement.
- Evaluation of Tumor Findings: No significant differences were reported between controls and treated animals.

Summary Conclusions and Recommendations

- Major Tumor Findings: None reported
- Non-neoplastic Findings: None reported
- Biological Significance: None reported
- Potential Clinical Implications of Findings: Unknown
- Recommendations for Further Analysis: None

Study Title: Two Years Toxicity Study in Rats
Study Number: HC-20-511
Volume Number: 1
Test Facility: [redacted]
Study Date(s): Report dated December 16, 1976. Actual study dates unknown
Date of Submission: 4/28/99
GLP Compliance/Quality Assurance: No information provided
QA Report- None provided
Study Type: Oral carcinogenicity study
Species/strain: [redacted] rats, aged 8 weeks at study initiation
Number of animals per group; age at start of study: 50
Animal housing: Paired
Drug Lot/Batch number(s): 73901 and 74901
Drug Purity / Stability / Homogeneity: None submitted
Doses: 2, 16, and 71 mg/kg/d

- Basis of Dose Selection: Not provided
- Relation to Clinical Use: Unknown
- CAC Concurrence: None
- Route of Administration: In feed
- Frequency of Drug Administration: Continuous
- Dual Controls Employed: No
- Satellite PK or Special Study Group(s):
- Unscheduled Sacrifices or Deaths: Males: 26, 29, 23, and 31 for the control, low, mid and high doses Females: 22, 27, 32 and 35 for the same groups. Statistically significant increases were reached for the high dose females.
- Original Study Protocol: "It was decided at the onset that when the number of mice in either control or treatment groups was reduced to approximately 60% of their initial number, the study should be discontinued in order to permit adequate histological evaluation of sufficient animals to reach a reliable conclusion."

Study Results and Frequency of Monitoring:

- Clinical Observations: Daily for 1 week, weekly thereafter
- Mortality: Daily
- Body Weight: Weekly, but only graphic representation of data presented, no raw data provided. Body weight gain appeared to be reduced in the high dose animals. Increased mortality was reported for high dose males.
- Food Consumption: Decreased in the high dose animals, but only graphic representations of the information are presented
- Ophthalmoscopy: Weeks 53/54, and at study termination. No treatment-related lesions were reported.
- Hematology: 10 males/group at weeks 6, 13, 26, 52, 78, and 98, and 10 females/group at week 104. High dose females showed reduced mean hemoglobin and hematocrit values from Week 26.
- Clinical Chemistry: 10 males/group at weeks 6, 13, 26, 52, 78, and 98, and 10 females/group at week 104. Slightly elevated SGPT levels were reported for high dose males.
- Organ Weights: 10/sex/group: Heart, spleen, liver, kidneys, adrenals, testes, ovaries. Relative liver weights were increased in high dose males.
- Gross Pathology: All animals

- Histopathology: Tissues examined: For spontaneous deaths and premature sacrifices: liver, thymus, stomach, testes, prostate, ovaries, uterus, urinary bladder, pituitary, thyroid, adrenal, pancreas, stomach, thymus, and tumor-bearing tissues.

Ten additional (first 10 survivors) animals/dose/sex were examined at study termination: lung, heart, spleen, thymus, lymph nodes, bone marrow, stomach, small and large intestines, salivary gland, pancreas, liver, kidneys, adrenals, urinary bladder, prostate, testes, epididymides, uterus, ovaries, pituitary, thyroid, parathyroid, trachea, esophagus, eye, skeletal muscles, skin, aorta, brain, gross lesions.

Non-Tumor: Periarteritis of mesenteric vessels and pancreas were found in a few animals from all groups. Hepatocytes showed vacuolar changes with increased incidence with increasing dose. Sudan black staining was increased in hepatocytes (increased fat content), and slight bile duct proliferation was reported in increased incidence and severity in high dose males.

Tumor: Pituitary tumors were statistically increased in premature decedent high dose females when compared to controls, but no dose relationship was evident. Terminal sacrifice incidences were comparable across groups. Pituitary tumor incidence: 10/26, 11/29, 5/23, 17/31 for control, low, mid and high dose premature decedent males, and 14/24, 12/21, 15/27, and 13/19 for the terminal sacrifice males, respectively. Females: 2/22, 3/27, 7/32, and 11/34 for premature decedents and 15/28, 13/23, 11/18, and 7/15 for the terminal sacrifice animals, respectively.

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model: Even considering the time frame of the study, pre-1976, one would expect a more complete presentation of the data and a statement of GLP compliance.
- Evaluation of Tumor Findings: The sponsor reports that the compound is not carcinogenic in this model.

Summary Conclusions and Recommendations

- Major Tumor Findings: None
- Non-neoplastic Findings: Mild hepatic changes were reported.
- Biological Significance: None evident from the materials submitted
- Potential Clinical Implications of Findings: Unknown
- Recommendations for Further Analysis: None

RECOMMENDATIONS:

Internal comments: The data submitted for the rat study seems limited as only the first 10 survivors/sex/dose were examined histologically at study termination. Target tissues (with the marked exception of kidney) were examined for most animals on study. There were no apparent carcinogenic effects on rat liver or other histologically examined tissue.

The data submitted for the mouse study seems more complete than for the rat study. Target tissues (with the marked exception of the kidney) were examined for most animals on study. There were no significant carcinogenic effects on any histologically examined tissue.
External Recommendations (to sponsor): None

Reviewer signature /S/

Team leader signature [Concurrence/Non-concurrence]

/S/