

PHMA

Parameter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Albumin (g/dl)	3.95	3.73	3.88	3.78	3.87	3.90	3.82	3.75	3.92	3.78
Alk Phosphatase (U/L)	37.1	26.16	26.20	29.57	34.42	35.83	34.42	34.50	31.75	52.85
ALT (U/L)	29.66	28.33	32.20	30.42	34.28	26.50	34.42	25.33	27.25	31.42
AST (U/L)	12.83	10.33	15.80	12.57	14.85	10.00	17.14	11.66	12.00	11.14
Total Bilirubin (mg/dL)	0.27	0.21	0.27	0.22	0.22	0.23	0.23	0.24	0.24	0.22
Calcium (mg/dL)	15.36	14.68	15.40	14.80	15.41	15.40	15.31	15.01	16.12	14.95
Cholesterol (mg/dL)	23.00	32.66	25.60	33.85	22.14	40.83	22.14	32.50	23.00	34.85
CPK (U/L)	492	191	555	227	593	211	549	340	343	242
Creatinine (mg/dL)	1.18	1.30	1.28	1.30	1.28	1.43	1.31	1.33	1.25	1.37
BUN/Creatinine	15.61	14.66	12.12*	13.61	12.15*	13.95	11.75*	12.85	14.15	12.21
GGT (U/L)	6.0	7.16	6.4	6.71	6.85	7.66	7.14	6.0	6.5	8.14
Globulin (g/dL)	2.30	2.11	2.14	2.20	2.30	2.31	2.32	2.33	2.30	2.18
Glucose (mg/dL)	143	136	144	143	145	147	150	141	137	146
LDH (u/L)	46.66	24.33	93.40	25.71	106.28	19.00	33.57	38.83	21.25	18.71
Phosphorus (mg/dL)	3.61	3.11	3.60	3.04	3.64	3.18	3.71	3.08	3.60	3.22
Potassium (mmol/L)	5.26	4.61	5.22	4.65	5.21	4.85	4.94	4.40	5.32	4.50
Sodium (mmol/L)	145	142	146	144	146	145*	146	144*	146	144
Total Protein (g/dl)	6.25	5.85	6.0	5.9	6.1	6.2	6.1	6.0	6.2	5.9
TG (mg/dL)	159	85	121	91	110	103	82*	111	134	115
Urea nitrogen (mg/dL)	18.3	19.0	15.2*	17.7	15.4*	19.8	15.4*	17.3	17.7	16.5
Uric acid (mg/dL)	0.3	0.28	0.32	0.27	0.31	0.26	0.27	0.25	0.27	0.24

\*Statistically significant

No statistically significant trend was reported for the blood chemistry parameters.

Organ weight:

Three month sacrifice:

Data on the organ weight relative to the body weight are shown below (Percent organ/final body weight).

Parameter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Adrenals	0.0089	0.01	0.0087	0.0086	0.0093	0.0091	0.0098	0.0078	0.0099	0.0083
Brain	0.0062	0.0069	0.0049	0.0046*	0.0057	0.0049*	0.0059	0.0057	0.0059	0.0048*
Gonads	0.20	0.0083	0.14	0.0093	0.17	0.0069	0.16	0.0075	0.15	0.0066
Heart	0.24	0.24	0.20*	0.21	0.22	0.21	0.24	0.23	0.22	0.21
Kidney	0.59	0.53	0.55	0.52	0.55	0.52	0.57	0.52	0.56	0.49
Liver	3.7	3.0	3.7	3.0	3.5	2.9	3.3	3.2	3.4	2.9

\*Statistically significant

The relative weight of the brain showed significant differences from the untreated control as shown in the above table. The absolute organ weight (g) data for brain is presented in the following table.

Parameter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Brain	10.24	10.77	10.42	9.8	9.68	9.88	10.80	10.63	10.24	9.67*

The absolute data suggest that only the high dose animals (female) showed a decrease in the weight of the brain compared to the untreated control. Individual data for the weight of brain and body weight for the untreated control (female) and group 5A (female) are presented below.

Animal	Gr 1, B.W (kg)	Gr 1, Brain Wt (g)	Animal	Gr 5, B.W (Kg)	Gr 5, Brain Wt (g)
L6376F	3.6	11.26	L6369F	4.8	9.17
L6346F	4.8	11.28	L6377F	4.9	10.40
L6362F	4.0	10.29	L6403F	4.2	10.09
L6389F	3.5	10.59	L6366F	4.3	9.77
L6400F	4.2	10.43	L6391F	4.2	8.94

Three out of 5 animals showed lower weight of the brain. The data need to be compared to the long-term exposure data following one-year treatment. The lower weight of the brain at 2.5% S (-) betaxolol treated female rats compared to the control was considered to be incidental because the extended treatment for 12 months did not show similar changes in the absolute weight of the brain in male and female animals.  
One year data:

Relative organ weight data (G/BW X 100) are shown in the following table.

Para Meter	Gr 1, M	Gr 1, F	Gr 2, M	Gr 2, F	Gr 3, M	Gr 3, F	Gr 4, M	Gr 4, F	Gr 5, M	Gr 5, F
Adre Nals	0.017	0.0090	0.013	0.0094	0.013	0.010	0.015	0.0095	0.015	0.0085
Brain	0.0040	0.0045	0.0050	0.0039	0.0042	0.0038	0.0051	0.0040	0.0050	0.0042
Gonads	0.12	0.013	0.15	0.011	0.14	0.03	0.15	0.011	0.16	0.010
Heart	0.20	0.20	0.22	0.19	0.21	0.22	0.22	0.18	0.23	0.20
Kidney	0.50	0.40	0.51	0.41	0.48	0.41	0.48	0.40	0.46	0.40
Liver	2.86	1.88	2.73	2.03	2.48	2.12	2.42	2.01	2.69	2.05

Above data suggest that S (-) betaxolol treatment did not affect the organ weight relative to the body weight for above organs.

Gross pathology:

Three-month sacrifice:

There were no gross changes related to the treatment except incidental changes that are shown in the following table.

Group	Animal #	Remarks
Group 2	L6399 (F)	Small ovary and uterus
	L 6367 (F)	Kidneys pitted (holes)
	L6282 (M)	Lungs appeared mottled
Group 3	L6347 (F)	Fluid filled nodules on upper left uterine horn
Group 4	L6332 (F)	Fluid filled cyst in right ovary
Group 5	L 6300 (M)	Small testes

Twelve-month sacrifice:

Group	Animal #	Remarks
Group 1	L6383 (F)	Hairball in the stomach, Nodule in the left uterine horn.
	L 6361 (F)	Kidneys appeared discolored, small areas of satellite spleen tissue adjacent to the spleen.
	L 6357 (F)	Liver mottled and discolored, large intestine appeared dark, stomach filled with hairball and lining of stomach was sloughing.
Group 2	L 6271 (M)	Enlarged gall bladder, mottled liver, large hairball in stomach, red ness in the stomach and caecum lining.
	L 6368 (F)	Kidneys appeared discolored
	L 6371 (F)	Satellite area of splenic tissue
	L 6312 (M)	Pitted kidneys, stomach and intestine appeared red.
Group 3	L6335 (F)	Swollen submandibular salivary gland, dark area in the anterior lobe of left lung, trachea appeared red, white foci in kidneys, swollen liver and mesenteric lymph node, hairball in stomach, nodular appearance in mucosal surface of stomach, hemorrhage in pancreas.
	L6402	Mottled kidneys.
Group 4	L 6401 (F)	Hair ball in stomach.
	L6395 (F)	Small gall bladder and right kidney.
	L 6288 (M)	Hemorrhagic area in stomach
	L 6378 (F)	Nodules on right side of oviduct, dark appearance in left lung and liver, hole in diaphragm and stomach.
Group 5	L 6317 (M)	Enlarged gall bladder, hair ball in stomach, hemorrhage in stomach lining.
	L 6307 (M)	Fur in stomach, fluid filled colon.
	L6344 (F)	Small petechiae (hemorrhage) in the stomach lining.
	L 6322 (M)	Pitted kidneys.
	L 6325 (M)	Enlarged gall bladder, mottled liver, hairball in stomach.

Above changes were considered to be incidental. The sponsor has not provided the possible reasons for the appearance of hairball in the stomach. Presence of hairball in the stomach was not observed in rabbits sacrificed after three months of dosing.

Histopathology:

Three-month sacrifice:

There were no treatment related histopathological changes in the ocular tissues observed in male and female animals dosed for three months. Female rabbits showed higher incidences of lung edema and thymus involution than the untreated control as shown in the table below.

Organ	Group 1	Group 2	Group 5
Lung edema	1 (mod sev=1)	2 (slight=1, mod=1)	3 (slight=2, mod=1)
Thymus involution	1 (slight=1)	2 (minimal=2)	4 (minimal=3, moderate=1)

Animal # 6367 (F) in the group 2 and # 6403 (F) in-group 5 showed meningoencephalitis. The sponsor stated that the effect in the brain could be due to Encephalitozoon cuniculi infection. The changes in the lung and thymus could be incidental and similar changes were not observed after twelve months of the treatment.

Twelve-month sacrifice:

Histopathological data for eye tissues of both scheduled and unscheduled sacrifice are presented.

Tissue	Gr 1, R	Gr 1, L	Gr 2, R	Gr 2, L	Gr 3, R	Gr 3, L	Gr 4, R	Gr 4, L	Gr 5, R	Gr 5, L
Malignant lymphoma in cornea					1					
Keratitis					1 (mod)					
Malignant lymphoma in eyelid					1					
Malignant lymphoma in lacrimal tissue					1	1				
Multifocal dilatation in lacrimal tissue	4 (Minimal =3, slight=1)	6 (Minimal =3, slight=3)	5 (Minimal =3, slight=2)	3 (Slight =3)	7 (Minimal =4, slight=3)	7 (Minimal =2, slight=5)	12 (minimal =4, slight=8)	9 (minimal =2, slight=7)	9 (minimal =5, slight=4)	9 (minimal=4, slight=5)
Malignant lymphoma in nictitating membrane					1	1				
Malignant lymphoma in sclera/choroid					1	1				

Minimal to slight dilatation of the glandular elements of the lacrimal tissue was observed in right and left treated and untreated eyes, respectively. The change could be due to the aging process. However, increase incidences of dilatation of the lacrimal gland in the S (-) betaxolol treated eyes could be due to blockade of beta-adrenergic receptors in the lacrimal gland. The effect in the untreated left eyes could be due to bioavailability of the drug in the untreated eye following the treatment to the contralateral eyes. Female rabbit # 6335 showed generalized malignant conditions unrelated to the drug treatment. Histopathology data did not show drug-related changes in other organs after twelve months of the treatment.

Toxicokinetics:

Plasma S (-) betaxolol levels (ng/ml) are shown in the following table.

Group	Month 1		Month 3		Month 6		Month 12	
	M	F	M	F	M	F	M	F
3	4.6	2.7	6.0	9.2	6.3	4.6	3.8	4.1
4	5.6	7.0	7.5	8.4	10.3	9.8	6.9	5.6
5	22.8	16.7	13.7	11.9	19.3 *	9.3*	9.4	7.4

\*Samples lost during analysis

Data suggest that S (-) betaxolol given as ophthalmic drops are bioavailable dose dependently. However, the increase in the levels was not dose proportionate. The sponsor provided on Dec 2, 1999 the information on the drop sizes in the animal studies and clinical studies. Accordingly, the drop sizes of the pre-clinical studies were  $47 \pm 5.5 \mu\text{L}$ . The drop sizes for the clinical supplies were  $25.7 \pm 3.5 \mu\text{L}$ .

Reviewer's key study observations:

At 2.5% dose of S (-) betaxolol, there was an increased incidences of constipation in rabbits in the twelve month study. Also minimal conjunctival discharge was noted during several observation periods at 0.5-

2.5% S (-) betaxolol treated eyes. S (-) betaxolol treated animals showed higher incidences of dilatation of the glandular elements of lacrimal tissue. Above changes could be attributed to the beta-adrenergic blockade in the tissues.

Sponsor's summary and conclusions:

Daily observations for health and pharmacotoxic signs were generally limited to sporadic and transient instances of nasal and ocular discharge, diarrhea and little or no stool. Treatment with various test and control articles elicited no adverse effect on the growth of the animals for the duration of the study. Biomicroscopic evaluations revealed only minimal-moderate conjunctival congestion and a few sporadic instances of transient conjunctival discharge. No treatment related changes were observed during gross observations at necropsy. No treatment related histological changes were observed.

Based on the results of the one year evaluation, it is concluded that 0.5-2.5% S (-) betaxolol ophthalmic suspension does not possess a cumulative ocular irritation potential and does not demonstrate any apparent systemic toxicity in rabbits.

Toxicological study #2:

A rising dose tolerance toxicity study of S (-) betaxolol (levobetaxolol) and RS betaxolol administered orally by gavage to rats. Page 5-02420, vol 7. Protocol # N-98-140.

Conducting Laboratory: \_\_\_\_\_

Date of study initiation: June 15, 1998

Date of necropsy: June 30, 1998

GLP compliance: yes

QA report: Yes

Methods:

Sprague Dawley Cri:CD rats were used in the study. Total of 24 male and 24 female rats were used. Animals were 6-7 weeks of age and weighed 171-284 GM at the time of dosing. Dosing groups are shown in the following table.

Group	# Animal	Drug	Dose, mg/kg/oral
1	2M, 2F; 1M, 2M, 101F, 102F	Levobetaxolol	250
1	2M, 2F; 3M, 4M, 103F, 104F	RS- betaxolol	250
2	2M, 2F; 5M, 6M, 105F, 106F	Levobetaxolol	500
2	2M, 2F; 7M, 8M, 107F, 108F	RS- betaxolol	500
3	2M, 2F; 9M, 10M, 109F, 110F	Levobetaxolol	750
3	2M, 2F; 11M, 12M, 111F, 112F	RS- betaxolol	750
4	2M, 2F; 13M, 14M, 113F, 114F	Levobetaxolol	1000
4	2M, 2F; 15M, 16M, 115F, 116F	RS- betaxolol	1000
5	2M, 2F; 17M, 18M, 117F, 118F	Levobetaxolol	1250
5	2M, 2F; 19M, 20M, 119F, 120F	RS- betaxolol	1250
6	2M, 2F; 21M, 22M, 121F, 122F	Levobetaxolol	1500
6	2M, 2F; 23M, 24F, 123F, 124F	RS-betaxolol	1500

The drugs were administered once orally by gavage. The day of dosing was considered to be day 1 of the study for the animal. Animals were evaluated for clinical changes, general appearance, behavior and mortality. Animals were observed at least once daily prior to the dosing. Animals were observed frequently after dosing on day 1 and in the morning on day 2.

All survival animals were sacrificed by injecting sodium pentobarbital and discarded without necropsy. Animals that found dead or sacrificed in moribund conditions were necropsied for gross pathological examinations.

The body weight of the animal was recorded at arrival and on day 1 before dosing.

The lot # of levobetaxolol used in the study was 7231-020 and that of betaxolol was 97-04471. Drug substance was dissolved in deionized water at 25, 50, 75, 100, 125 and 150 mg/ml concentrations.

Results:

Mortality and moribund conditions:

Animal	Drug	Dose, mg/kg/oral	Day	Observation and necropsy findings
Male #22	Levobetaxolol	1500	1	Salivation, tremors, reduced activity and dyspnea and discoloration of glandular stomach.
Male #23	RS-betaxolol	1500	2	Salivation reduced activity and tremors, red foci in lungs and slight dilatation of pelvis of left kidney.

Highest dose tolerated without mortality was 1250 mg/kg. The no effect dose in the study is 250 mg/kg.

Clinical signs were reduced activity, tremors, ataxia, reduced breathing and excessive salivation. Most of the clinical signs were started within 30 minutes after dosing.

It is concluded that levobetaxolol single dose was tolerated up to 1250 mg/kg in rats. Clinical Signs of overdose toxicity were salivation, tremor, reduced activity and dyspnea.

Sponsor's summary and conclusions:

There were no clinical signs of systemic toxicity observed in both the 250 mg/kg S (-) betaxolol and RS-betaxolol dose groups. All animals treated at 500, 750, 1000, 1250 and 1500 mg/kg for both test materials exhibited adverse systemic effects. One or more of the following clinical signs of toxicity were observed in these treatment groups and included tremors, difficulty in breathing, reduced activity, excess salivation, ataxia, prostration, reduced breathing rate, ptosis, dyspnea, piloerection, hypothermia, hunched stance, morbidity and mortality. Morbidity and mortality were noted at 1500 mg/kg S (-) betaxolol and RS betaxolol dose groups.

Based upon the results, maximum tolerated dose for both S (-) betaxolol and RS betaxolol was considered to be 1250 mg/kg.

Toxicology study #3

Sponsor's Original Title:

A three-month toxicity study of S (-) betaxolol (levobetaxolol) administered orally by gavage to rats.  
Vol 8, page 5-02464

Conducting Laboratory:

Date of study initiation: May 27, 1998

GLP compliance: Yes

QA Report: Yes (X), No 0

Methods:

Dosing information:

Species	#/sex/group	Age	Weight (g)
Sprague-Dawley, cri:CD rats	15	8 Weeks (approx)	191-375 (male) 174-250 (female)

Satellite groups used for toxicokinetics:

Group	#/sex	Test Article	Dose (mg/kg)	Concentration (mg/ml)
1	4	Vehicle	0	0
2	4	S(-) Betaxolol	10	1
3	4	S(-) Betaxolol	30	3
4	4	S (-) Betaxolol	100	10
5	4	RS-Betaxolol	100	10

Dosage groups in administered units:

Group	#/sex	Test Article (mg/kg)	Concentration (mg/ml)	Dose (ml/kg)
1	15	0 (vehicle)	0	10
2	15	10, S (-) Betaxolol	1	10
3	15	30, S(-) Betaxolol	3	10
4	15	100, S(-) Betaxolol	10	10
5	15	100, RS Betaxolol	10	10

Route and form:

Animals were treated by oral gavage daily for 90 consecutive days. The test article was dissolved in distilled water. The test article concentration was expressed as the free base. The dose volume for each animal was determined from the most recent body weight record.

Drug lot # and percent purity:

S (-) Betaxolol, Lot # 7231:020. The certificate of analysis is provided in page 5-02654, vol. 8.

RS-Betaxolol, Lot # 97-04471, a certificate of analysis was provided on page 5-02655, vol. 8. The certificate stated that the Alcon code of the lot was 7231:035. The concentration and the homogeneity of the solution were confirmed by the analytical method.

**Formulation and vehicle:**

The test article was dissolved in distilled water to achieve the required concentrations. The concentration of S (-) betaxolol or RS Betaxolol was determined prior to first day of dosing and monthly thereafter.

**Clinical signs:**

Animals were observed once daily prior to the dosing. During the dosing period, each animal was observed morning and afternoon for changes in the appearance and behavior.

**Body weight:**

Body weights of the animals were recorded at prestudy, on day 1 prior to dosing, once weekly and before the necropsy. Body weights were recorded on following days: Prestudy, days 1, 8, 16, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85 and 90.

**Food consumption:**

The food consumption was recorded at prestudy, days 8, 18, 22, 29, 36, 43, 50, 57, 64, 71, 78, 85 and 90.

**Ophthalmoscopy:**

Indirect ophthalmoscopic examinations were conducted prior to the first dose and before the sacrifice.

**Hematology:**

Blood samples were collected from the dorsal aorta at necropsy for hematology and serum chemistry. Animals were fasted overnight before blood collection. About 2 ml of the blood was collected from each animal. Following parameters were determined.

RBC counts, WBC counts and differential counts, hemoglobin, hematocrit, mean corpuscular volume (MCV) and hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) and platelet counts.

**Clinical chemistry:**

Blood samples were collected before necropsy for serum chemistry. Following parameters were analyzed.

Sodium, potassium, chloride, total bilirubin, alkaline phosphatase, LDH, AST, ALT, GGT, CPK, creatinine, calcium, phosphorus, glucose, urea nitrogen, total protein, albumin, globulin, albumin and globulin ratio, cholesterol and triglycerides.

**Urine analysis:**

Urine analysis was not conducted.

**Gross pathology:**

Animals were fasted overnight before terminal sacrifice. Animals were sacrificed by exsanguination under sodium pentobarbital anesthesia. Gross changes were noted at necropsy. Gross pathological changes were also conducted for animals that were killed before the scheduled termination.

Organ weights:

Organ weights were not recorded for animals found dead. Weights of following organs were recorded at scheduled sacrifice.

Adrenals, epididymides, kidneys, lungs, pituitary (post fixation), testes, thyroid and parathyroid (post fixation), brain, heart, liver, ovaries, spleen and thymus.

Histopathology:

Following tissues from animals that died prematurely or sacrificed at the end of the dosing period were fixed in neutral-buffered 10% formalin. Eyes and testes were fixed in 3% glutaraldehyde and Bouin's solution, respectively.

Aorta, heart, salivary gland, tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, pancreas, liver, trachea, lung, bone marrow (sternum), thymus, spleen, mandibular lymph node, mesenteric lymph node, kidneys, urinary bladder, testes, epididymides, prostate, seminal vesicles, ovaries, uterus, cervix, vagina, adrenals, pituitary, thyroid and parathyroid, skin/mammary gland, bone skeletal muscle, eyes with optic nerve, brain and spinal cord.

Slides were prepared for above tissues from groups 1, 4 and 5, stained with hematoxylin and eosin for microscopical examinations. Histological changes in the heart were examined for animals in-groups 1-5.

Toxicokinetics:

On days 1, 30 and 90 blood samples were collected from the satellite animals in-groups 2-5. Blood samples were collected prior to dosing and 30 min after dosing from the orbital plexus under light anesthesia induced by carbon dioxide. Plasma samples were separated for the analysis of the drug. [redacted] analyzed plasma samples. All animals from the toxicokinetic groups were exsanguinated under pentobarbital anesthesia and discarded without necropsy.

The concentrations of betaxolol enantiomers were determined using [redacted] methods.

Results:

Clinical signs:

Three animals from the main study group 5 on RS- betaxolol treatment died or sacrificed during the study as shown in the following table.

Animal #, group	Sex	Remarks
504, 5	male	Died on day 22 due to gavage error
508, 5	male	Unscheduled sacrifice on day 34, inflammation of kidney reported.
550, 5	female	Found dead on day 66, Pulmonary edema reported

Some of the clinical signs observed during the treatment period in the main study group are shown in the following table.

Observation	Control		10 mg/kg, S (-)		30 mg/kg, S (-)		100 mg/kg, S(-)		100 mg/kg, RS	
	M	F	M	F	M	F	M	F	M	F
Chromo dacryorrhea	2	0	1	1	2	1	1	0	1	4
Erythema/ scab/torn Pinna	4	5	5	5	5	1	10	5	5	11
Chromo rhinorrhia	6	3	6	3	9	2	7	3	7	7
Infrequent stool					1				1	1

Increased incidences of chromorhinorrhia (discharge of pigmented secretion from nose) and chromodacryorrhea (shedding of bloody tears) from eyes and the nose were noted in female animals that were treated at 100mg/kg racemic betaxolol. Chromodacryorrhea could be due to excessive secretion from harderian gland under the influence of the beta-blocker. Increased incidences in the pinna (erythema, torn and scab) were observed at 100 mg/kg S (-) betaxolol treated male animals and 100 mg/kg racemic betaxolol treated female animals.

Mortality or unscheduled sacrifice in the toxicokinetic groups is shown in the following table.

Group	No. of animal, sex	Remarks
2	226, M	Accidental death
4	471, F	Unscheduled sacrifice
5	572, F	Accidental death

Body weights:

The groups mean body weight (g) on days 1 and 90 are shown in the following table.

Sex	Group 1, Day		Group 2, Day		Group 3, Day		Group 4, Day		Group 5, Day	
	1	90	1	90	1	90	1	90	1	90
Male	295	567	292	624	301	524	294	519	293	517
Female	209	307	212	316	205	305	211	307	203	292

The weight gain (g) between day 1 and day 90 is shown in the following table.

Sex	Group 1	Group 2	Group 3	Group 4	Group 5
Male	272	332	223	225	224
Female	98	104	100	96	89

The sponsor stated that there was no statistically significant changes in the S (-) betaxolol or RS-betaxolol treated animals compared to the control. However, male animals in-groups 3-5 showed about 18% reduction in the weight gain between days 1 and 90 compared to the control. Female animals in the group 5 showed about 9% reduction in the weight gain during the same period.

Food consumption:

The mean daily food consumption (g/day) did not show treatment-related changes between the pretest and day 90-observation period. The data are shown in the following table.

Sex	Group 1		Group 2		Group 3		Group 4		Group 5	
	Pretest	day 90								
Male	27	30	26.4	27.9	28.1	27.5	25.9	27.2	27.3	27.9
Female	20	20.3	20.6	20.6	19.4	18.8	20.3	19.5	19.4	20.8

Ophthalmologic examinations:

Indirect ophthalmoscope examinations did not show any compound related changes. However, animal # 358F (30 mg/kg) showed retinal linear atrophy which was considered to be incidental.

Hematology:

Some of the hematological parameters are shown in the following table.

Parameter	Group 1		Group 2		Group 3		Group 4		Group 5	
	M	F	M	F	M	F	M	F	M	F
WBC ( $10^3/\mu\text{L}$ )	9.0	4.7	8.1	4.2	7.1*	4.9	7.0*	4.6	7.3*	4.4
Hemoglobin (g/dL)	15.3	14.8	15.1	14.8	15.0	14.6	14.8*	14.9	15.2	14.7
Lymphocyte (%)	76.6	81.6	74.2	81.4	79.8	81.2	75.7	77.9	76.6	80.4
Lymphocyte ( $10^3/\mu\text{L}$ )	6.89	3.83	6.0	3.4	5.6	3.9	5.2	4.6	5.5	3.5
Monocyte (%)	3.5	2.6	3.3	2.2	2.7	3.0	3.1	5.0*	3.4	4.2*

\*=Statistically significant

The monocyte (%) was increased at 100 mg/kg dose in S (-) and RS betaxolol-treated rats. However, 2-6% counts are considered to be within the normal range and the change is considered to be incidental. Male rats in-groups 3-5 showed statistically significant differences in the WBC counts compared to the control. Individual data for WBC counts for abnormal findings (counts that were not observed in gr 1 and gr 2 animals) are shown in the following table.

Animal #	Group	Dose (mg/kg)	WBC ( $10^3/\mu\text{L}$ )
304M	3	30	3.29
414M	4	100	4.77
505M	5	100 (RS)	3.98
514M	5	100 (RS)	4.71

Histology data for above animals did not show any changes in the bone marrow that would contribute to lower WBC counts. The changes in the WBC counts were considered to be incidental.

Clinical chemistry:

Some of the changes in the serum chemistry parameters are shown in the following table.

Parameter	Group 1		Group 2		Group 3		Group 4		Group 5	
	M	F	M	F	M	F	M	F	M	F
BUN (mg/dL)	16	16	14	14	15	16	16	25*	15	23*
Alk Phos (U/L)	50	23	55	48	28	48	24	51	32*	47
CPK (U/L)	469	287	405	256	305	217	271*	294	329	297
Phosphorus (mg/dL)	5.7	5.4	6.7	6.6	6.5	5.5	6.7	6.4*	6.4	6.7*
Total Protein (g/dL)	6.3	7.2	6.2	6.8	6.8	6.9	6.2	6.7*	6.3	6.6

\*=Statistically significant

Changes in AP, CPK and total protein were considered to be incidental since the individual data in the treated animals were within the range of the untreated rats. However, female rats at 100-mg/kg dose of S (-) or RS betaxolol showed changes in the serum BUN and phosphorus levels that were statistically significant. The individual data for BUN (mg/dL) and kidney histology findings in group 1,4 and 5 female rats are shown in the following table.

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BUN (female):

Group 1	Group 4	Group 5
150 F, 15, Min, focal mineralization of corticomedullary junction	450F, 21, normal	550F, 21, Min, focal subacute inflammation
151F, 16, Min, multifocal mineralization of corticomedullary junction	451F, 25, Min, focal mineralization in corticomedullary junction	551F, 23, Mild, focal subacute inflammation
152F, 14, normal	452F, 21, Min, focal basophilic tubules	552F, 24, normal
153F, 17, normal	453F, 21, Mild, focal mineralization in corticomedullary junction	553F, 23, normal
154F, 17, normal	454F, 16, Min, focal basophilic tubules	554F, 21, normal
155F, 16, Mild, multifocal mineralization of transitional epithelium	455F, 55, Mod, transitional epithelial hyperplasia, mod, multifocal basophilic tubules, pelvic dilatation, marked multifocal pyelonephritis	555F, 31, Mild, focal basophilic tubules; mild, focal subacute inflammation.
156F, 18, Mild, pelvic dilation; mod, subacute pelvic inflammation	456F, 23, Mild, multifocal transitional cell hyperplasia	556F, 18, normal
157F, 15, normal	457F, 23, Min, multifocal basophilic tubules	557F, 28, Min, focal basophilic tubules
158F, 15, Min, multifocal inflammation in corticomedullary junction	458F, 22, Mild corticomedullary mineralization	558F, 23, Min, focal mineralization of corticomedullary junction
159F, 17, Min, focal mineralization in corticomedullary junction	459F, 49, Marked, multifocal pyelonephritis; mod, multifocal transitional cell hyperplasia	559F, 27, normal
160F, 19, Min, focal basophilic tubules; mild, focal pelvic necrosis	460F, 21, normal	561F, 21, normal
161F, 13, Min, focal basophilic tubules; min, focal mineralization in corticomedullary junction	461F, 20, normal	562F, 16, Mild, multifocal basophilic tubules; mild, multifocal proteinaceous tubular filtrate
162F, 13, normal	462F, 21, Min, multifocal corticomedullary mineralization	563F, 19, Min, focal, subacute inflammation
163F, 15, normal	463F, 22, normal	564F, 22, normal
164F, 14, normal	464F, 15, Min, multifocal corticomedullary mineralization	

Phosphorus (female)

Group 1	Group 4	Group 5
150F, 6.2	450F, 5.0	550F, 6.1
151F, 4.0	451F, 7.4	551F, 6.2
152F, 5.1	452F, 7.7	552F, 7.2
153F, 6.5	453F, 7.2	553F, 7.0
154F, 6.1	454F, 7.0	554F, 8.5
155F, 5.3	455F, 7.4	555F, 5.4
156F, 4.9	456F, 6.2	556F, 5.3
157F, 4.7	457F, 5.5	557F, 6.5
158F, 5.2	458F, 6.1	558F, 7.2
159F, 5.7	459F, 6.8	559F, 6.8
160F, 6.0	460F, 5.4	561F, 6.4
161F, 5.5	461F, 5.4	562F, 6.7
162F, 4.4	462F, 6.7	563F, 6.9
163F, 5.0	463F, 7.0	564F, 6.3
164F, 5.9	464F, 5.9	

The sponsor stated that the changes in the BUN and phosphorus were related to the morphological changes in the kidney. However, comparisons with the clinical chemistry data with histopathology data of kidney suggest that changes in the BUN for animals # 455F and #459F could be related to the pathological changes in the kidney attributed to the treatment with S (-) betaxolol at 100 mg/kg.

Organ weight:

Statistically significant changes in the relative organ weight (g%, organ wt/body wt X100) data are presented in the following table.

Organ	Group 1		Group 2		Group 3		Group 4		Group 5	
	M	F	M	F	M	F	M	F	M	F
Brain	4.05	7.04	4.52*	6.73	4.37	7.01	4.38	7.09	4.42	7.25
Heart	3.05	3.24	3.08	3.07	3.08	3.40	3.06	3.29	3.11	3.60*
Liver	2.59	2.57	2.52	2.52	2.61	2.66	2.79*	2.97*	2.84*	3.10
Parathyroid/ Thyroid	0.042	0.078	0.051	0.072	0.059*	0.089	0.055*	0.082	0.058*	0.086
Testes	6.47		7.10		6.87		7.30*		7.06	

\*= Statistically significant

The weights of the brain in individual male animals at 10mg/kg dose were within the range observed in the control and treated rats at 30 and 100 mg/kg doses. Therefore, changes in the relative weight of the brain at 10 mg/kg are incidental.

The weight of heart of female animals in the control and RS-betaxolol treated groups are shown below.

Group 1, Control	Group 5, RS betaxolol
0.81	0.94
0.92	1.01
1.07	1.11
0.91	0.97
0.88	0.94
0.93	1.23
0.78	0.97
1.15	0.89
0.91	0.86
0.88	0.89
0.90	0.86
0.80	0.92
0.99	1.22
1.04	0.87
0.84	

Data suggest that animal # 555F and 563F had slightly higher weight of heart compared to the highest individual weight of heart observed in the control group. The changes in the weight of heart relative to the body weight at 100 mg/kg RS betaxolol treated animals were considered to unrelated to the treatment.

The individual data for the liver weights for animals in-groups 1, 4 and 5 are shown below.

Control, M	Control, F	Group 4, M	Group 4, F	Group 5, M	Group 5, F
15.0	7.93	13.49	8.08	16.67	8.33
11.69	7.29	18.65	7.99	14.77	8.09
12.85	8.71	13.65	9.21	16.46	8.97
16.53	7.70	12.10	8.34	13.72	8.53
15.32	6.23	15.27	9.41	11.99	8.49
13.72	7.63	11.61	9.28	15.73	8.50
12.15	5.92	15.51	8.42	10.94	9.38
16.23	10.18	11.21	8.49	15.46	8.49
13.70	6.91	14.89	7.77	10.53	7.58
13.68	7.03	11.73	9.01	16.13	7.84
15.25	5.99	13.58	7.69	14.45	8.53
12.24	5.37	16.38	8.53	12.74	7.73
14.55	7.99	14.43	8.98	12.72	9.03
13.21	7.71	10.83	8.17		7.87
12.39	7.60	14.55	7.77		

Above data suggest that most of the treated animals except M 402 in-group 4 had liver weights comparable to the control. Therefore, it is unlikely that the change in the relative weight of the liver in groups 4 and 5 animals was related to the treatment effect. Also there were no histological changes observed in the liver that could suggest possible effect of the drug on the weight of the liver.

The individual data for thyroid/parathyroid in male rats are shown in the following table.

Group 1, M	Group 2, M	Group 3, M	Group 4, M	Group 5, M
0.030	0.028	0.40	0.025	0.029
0.026	0.025	0.024	0.029	0.027
0.025	0.30	0.028	0.023	0.028
0.029	0.20	0.021	0.025	0.037
0.025	0.031	0.022	0.024	0.023
0.020	0.024	0.036	0.021	0.033
0.015	0.021	0.024	0.027	0.016
0.017	0.026	0.022	0.030	0.023
0.029	0.019	0.025	0.028	0.032
0.025	0.019	0.026	0.019	0.032
0.016	0.023	0.020	0.028	0.036
0.015	0.025	0.033	0.027	0.026
0.024	0.030	0.031	0.034	0.030
0.020	0.024	0.041	0.030	
0.024	0.031	0.032	0.029	

At 30mg/kg dose, the weight of thyroid/parathyroid for animal # 301 and #314 was 0.040 and 0.041 g, respectively. A comparative increase in the individual weight of thyroid/parathyroid was not noted in animals for groups 4 and 5. The effect of S (-) and RS betaxolol on the thyroid/parathyroid weight relative to the body weight was due to incidental changes that occurred to few treated animals.

The individual data for testes showed that the weight of testes between the untreated control and group 4 animals were comparable.

The average weight of thymus in female rats in-group 5 showed statistically significant decrease compared to the control. The lowest weight recorded in the control group was 0.15 for animal #154 F. The weight of thymus in animals #550F and 559F (gr 5) was 0.11 and 0.10 g, respectively. The weight of thymus in rest of the animals in-group 5 was within the observed range of the control. The change in the thymus was considered to be incidental.

Gross pathology:

Following gross changes were reported for animals that found dead

Animal, Sex	Group	Remark
504M	5	Esophageal perforation, fluid in thoracic cavity, reported death due to gavage accident
508M	5	Small spleen and thymus, white foci in the kidney cortex, firm prostate and red color in GI tract. Page 5-02537 indicated unscheduled sacrifice. However, page 5-02666 reported that the animal found dead.
560M	5	Red discoloration of thymus and lungs.

Macroscopical changes in male and female animals are attached in the attachment for pages 5-02669 and 5-02670, vol 8. As indicated in the table, the prevalence of microscopic changes were considered to be incidental in nature.

Histological changes:

Some of the histological changes in male animals are shown below.

Lesion	Group 1	Group 2	Group 3	Group 4	Group 5
Heart, chronic active inflammation	4/15 mild=3, mod=1	5/15 min=3, mild=2	3/15 min=1, mild=2	3/15 min=1, mild=1, mod=1	6/15 min=2, mild=4
Mesenteric lymph node, hemorrhage	0/15			3/15 min=2, mild=1	0/14
Lungs, ectopic bone	0/15			0/15	2/15 min=1, mild=1
Kidneys, tubular dilatation	0/15			0/15	2/15 min=1, mod=1

Microscopic changes in the female rats.

Lesion	Group 1	Group 2	Group 3	Group 4	Group 5
Heart, subacute inflammation	5/15* min=3, mild=1	6/15 min=5, mild=1	6/15, min	12/15 min=5, mild=6, mod=1	11/15** min=4, mild=3, mod=1
Lung, alveolar histiocytosis	6/15 min=5, mild=1			10/15 min=8, mild=2	5/15 min=5
Kidneys, basophilic tubules	2/15, min			4/15 min=3, mild=1	3/15 min=1, mild=2
Kidneys, subacute inflammation	1/15, min			1/15, min	5/15 min=3, mild=2
Kidneys, transitional epithelial hyperplasia	0/15			3/15 mild=2, mod=1	0/15

\* Individual data listing showed 4 animals out of 15

\*\* Individual data listing showed 8 animals out of 15

Increased incidences of subacute inflammation in the heart in female rats in-groups 4 and 5 were observed. Subacute inflammatory changes in heart were mostly focal origin with minimal severity in-groups 1-3. However, severity in-groups 4 and 5 were minimal to mild. Most of the lesions were multifocal. Data suggest that S (-) and RS betaxolol showed inflammatory changes in the heart at 100 mg/kg dose. At lower doses of S (-) betaxolol, the incidences were comparable to the control.

In male rats, subacute inflammation of the heart at 100 mg/kg doses of S (-) and RS betaxolol was comparable to the control, 10 and 30 mg/kg doses of S (-) betaxolol.

It is concluded that the inflammatory changes in the heart in female rats at 100 mg/kg dose of S (-) betaxolol was related to the treatment.

Increased incidences of mostly minimal alveolar histiocytosis in the lung were observed in female animals at 100 mg/kg dose of S (-) betaxolol.

Female rats at 100 mg/kg dose of S (-) betaxolol showed kidney lesions e.g. hyperplasia of transitional epithelial cells and basophilic tubules. RS betaxolol treated rats showed subacute inflammation in the kidney.

Toxicokinetics:

Page 5-04350, vol 12.

Predose (trough) levobetaxolol or betaxolol levels  $\pm$  SD (ng/ml) in the plasma are shown in the following table.

Group	Day 1		Day 30		Day 90	
	M	F	M	F	M	F
2. Levobetaxolol 10 mg/kg	BLQ	BLQ	BLQ	1.18 $\pm$ 0.50	BLQ	BLQ
3. Levobetaxolol 30 mg/kg	1.66 $\pm$ 2.32	1.71 $\pm$ 2.42	BLQ	3.35 $\pm$ 3.38	BLQ	1.90 $\pm$ 2.80
4. Levobetaxolol 100 mg/kg	3.18 $\pm$ 3.37	3.34 $\pm$ 3.82	18.2 $\pm$ 13.3	70.8 $\pm$ 82.1	25.6 $\pm$ 16.7	45.9 $\pm$ 16.4
5. Betaxolol 100 mg/kg	3.56 $\pm$ 5.11	1.42 $\pm$ 0.97	135 $\pm$ 115	99.4 $\pm$ 61.5	23.1 $\pm$ 25.5	71.2 $\pm$ 116

BLQ= Below limit of quantitation of 1 ng/ml

The post dose peak plasma levels ( $\pm$ SD) of levobetaxolol and betaxolol are shown in the following table. The sponsor referred the level as the peak level.

Group	Day 1		Day 30		Day 90	
	M	F	M	F	M	F
2. Levobetaxolol 10 mg/kg	343 $\pm$ 303	691 $\pm$ 127	288 $\pm$ 134	570 $\pm$ 100	664 $\pm$ 590	670 $\pm$ 141
3. Levobetaxolol 30 mg/kg	969 $\pm$ 656	1750 $\pm$ 420	878 $\pm$ 219	1220 $\pm$ 530	963 $\pm$ 103	1770 $\pm$ 490
4. Levobetaxolol 100 mg/kg	1520 $\pm$ 455	1680 $\pm$ 280	1530 $\pm$ 500	2440 $\pm$ 630	2260 $\pm$ 230	2970 $\pm$ 690
5. betaxolol 100 mg/kg	1220 $\pm$ 420	2600 $\pm$ 860	1480 $\pm$ 870	2520 $\pm$ 1060	3290 $\pm$ 1220	4090 $\pm$ 1050

The data suggest that the plasma levels of S (-) betaxolol were increased with the dose and a steady state level was achieved within 30 days. However, the increase in the plasma level was not dose-proportionate across all doses. The plasma levels for post dose samples in all groups among male and female animals were higher on day 90 compared to that on day 30. The sponsor stated that the reason for the increase in the plasma levels on day 90 compared to day 30 was unknown. There was also an increase in the peak to trough plasma levels. The difference is an indication of the short half-life of the drug. The plasma levels of racemic betaxolol at 100 mg/kg on day 90 were higher than levobetaxolol at 100 mg/kg. It is possible that the reduction of clearance and/or metabolism of R- enantiomer of racemic betaxolol on day 90 may contribute to the higher peak levels of racemic betaxolol on day 90. The sponsor has not addressed the possibility. The sponsor stated that the differences in the plasma levels on day 90 between groups 4 and 5 were not statistically significant.

Female rats showed higher exposure than male rats.

Reviewer's key study observations:

S (-) betaxolol was tolerated in rats up to 100 mg/kg/oral dose without mortality. Racemic betaxolol showed mortality at 100 mg/kg.

Both levobetaxolol and racemic betaxolol showed cardiac inflammation in female rats at 100 mg/kg.

S (-) betaxolol at 100 mg/kg showed increased incidences of transitional epithelial hyperplasia and basophilic tubules in the kidney in female rats.

Racemic betaxolol (100 mg/kg) showed subacute inflammation of the kidney in female rats and tubular dilatation in male rats.

Male and female rats up to 30 mg/kg doses of levobetaxolol did not show treatment related pathological changes.

Female rats showed higher plasma levels of levobetaxolol and racemic betaxolol than male rats

Sponsors summary and conclusions:

There were no significant adverse effects on mortality, clinical signs, body weight, food consumption and eyes observed in the study. Hematology data showed higher values for differential counts of monocyte in females at 100 mg/kg S (-) betaxolol and betaxolol dose groups and large unstained cell values for high dose S (-) betaxolol. These higher values were most likely associated with the subacute myocardial inflammation in these groups and were considered biologically relevant. Clinical chemistry effects (elevated blood urea nitrogen and phosphorus) were noted for both female high dose groups. These effects primarily confined to the kidney function. These effects were considered not a direct toxic effect of the test or reference substance. Microscopic evaluation of the tissues revealed an increase in incidence and severity of subacute myocardial inflammation of both female high dose groups. Based on the results, treatment related effects of both test articles were confined to the high dose groups. In general both the test and reference articles were comparable. The no observed effect was determined to be 30 mg/kg of S (-) betaxolol.

Systemic exposure to levobetaxolol in plasma was demonstrated in rats dosed orally with levobetaxolol for 3 months. A sex related difference was demonstrated with female animals having somewhat higher post dose plasma drug concentrations than males. Steady state was achieved within first month of the treatment for levobetaxolol at all dose levels as well as for racemate. Mean steady state plasma drug levels for the 100-mg/kg-racemate-treatment group were not significantly different from the corresponding values for the 100 mg/kg per day levobetaxolol group.

Overall toxicology summary by the reviewer:

Rabbit study:

Male and female albino rabbits were treated at 0.5, 1.0 and 2.5% S (-) betaxolol ophthalmic suspensions. Rabbits were treated twice a day in the right eye with two drops of the suspension. Sham control and vehicle control groups were employed in the study. The duration of the treatment was 12 months. Separate groups of animals were treated for 3 months.

Treatment related mortality was not noted following 12 months of the treatment. Rabbits at 2.5% dose for 12 months showed sign of constipation. Conjunctival discharge of minimal severity was observed from the treated eyes at 0.5-2.5% dose.

Indirect ophthalmoscopic examinations did not show treatment related changes in the eyes following 12 months of the treatment at 0.5 to 2.5% S (-) betaxolol.

Histopathology report after 3 months of the treatment show that there was incidental changes in the lung and thymus in group 5 female rabbits. However, there were no histological changes in the eyes after three months of the treatment.

Treatment for twelve months showed minimal to slight dilatation of lacrimal gland in both eyes at 1-2.5% S (-) betaxolol. There were no other drug-related changes in the eye or other organs reported.

It is concluded that chronic treatment with S (-) betaxolol ophthalmic suspensions showed an increase conjunctival discharge at 0.5-2.5% dose two drops twice a day. At 2.5% S (-) betaxolol, dilatation of lacrimal tissues and constipation were observed. Ophthalmic doses were bioavailable in the systemic circulation.

Oral Rat study:

Oral single dose toxicity of levobetaxolol and racemic betaxolol were studied in Sprague-Dawley rats. Both levobetaxolol and racemic betaxolol were tolerated up to 1250 mg/kg dose. Clinical signs of overdose toxicity were salivation, reduced activity, tremors and difficulty in breathing.

Oral toxicity to levobetaxolol and racemic betaxolol was evaluated in Sprague Dawley rats. The sponsor has not mentioned any other oral toxicity study of S (-) betaxolol for the support of dose selection in the three-month study.

Rats were treated with levobetaxolol at 10, 30 and 100 mg/kg and, with betaxolol at 100 mg/kg doses. Mortality and moribund conditions were observed at 100mg/kg dose of RS-betaxolol. Increased incidences of chromodacryorrhea in the eyes and chromorhinorrhia in the nose were observed from female rats treated at 100mg/kg dose of RS-betaxolol. Indirect ophthalmological examinations did not show drug related ophthalmic changes. Blood chemistry data showed increase in BUN in female rats at 100 mg/kg dose of S (-) betaxolol and RS betaxolol. The changes could be due to the effect of the drug in kidneys in at least two female rats (455F and 459F) treated with 100 mg/kg S (-) betaxolol.

Male and female rats tolerated up to 100 mg/kg oral doses of levobetaxolol. At 100 mg/kg dose, female rats showed inflammation in the heart, lung, hyperplasia of transitional epithelium and basophilic tubules of kidney. Female rats showed greater toxicity than male rats.

Racemic betaxolol at 100mg/kg dose showed mortality. Male rats showed tubular dilatation in kidneys and female rats showed inflammation in heart and kidney.

Subacute inflammation in the kidney was observed in racemic betaxolol treated rats at 100 mg/kg. Similar changes were not observed in levobetaxolol treated rats at 100 mg/kg. The R enantiomer of betaxolol could contribute to the inflammation of kidney.

Both S (-) and racemic betaxolol treated animals showed higher inflammatory changes of the heart in females than male rats. Possibly the difference is due to higher exposure of the female rats at similar oral doses.

The no effect dose of levobetaxolol is not established in the study. Histopathology data in organs other than heart at low and mid doses were not evaluated.

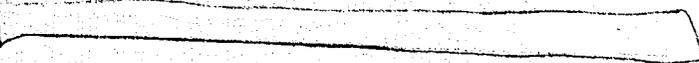
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Reproductive Toxicology:

Key words: RTX, ICH 413, and TERAT

Page 5-02932 vol 9, Protocol N-97-135

Title: Developmental toxicity study of S (-) betaxolol administered by gavage to rabbits.

Testing facility: 

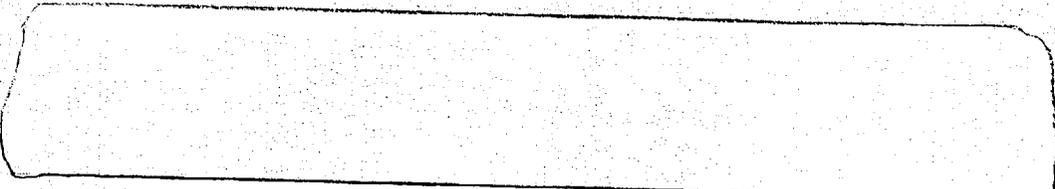
GLP: Yes, report audited

Protocol: not reviewed by the Division

Lot and batch number of product: S (-) betaxolol lot # AG-869  
- betaxolol lot # 16007

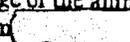
RS

Formulation:



Formulation # for S (-) betaxolol: A8599701, A8599703 and A8599706

Formulation # for RS betaxolol: A8599702, A8599704 and A8599705

Species: Albino SPF-NZ rabbits (HRA: NZ-SPF). The age of the animals was approximately 22 weeks and weighed 3-4 kg. Time mated animals were obtained from  and the supplier provided the insemination dates.

First day of dosing, Sept 14, 1997.

Terminal cesarean sections were conducted between Oct 7-10, 1997.

Doses employed and route of administration:

Study design:

Each animal received the test or control substance orally once a day by gavage from gestation days 6 through 19. The day of mating (insemination) was considered as day 0.

The dose groups are shown below.

Group I	# of Animals	Test article	Dose mg/kg/day	Dose volume, ml/kg	Drug Concentration, mg/ml	Number Terminated on gestation day 29
1	20	Vehicle	0	1	0	20
2	20	S (-) Betaxolol	4	1	4	20
3	20	S (-) Betaxolol	12	1	12	20
4	20	S (-) Betaxolol	36	1	36	20
5	20	RS-betaxolol	36	1	36	20

Parameters and end points evaluated:

Animals were evaluated for clinical signs, food consumption and body weight during the experimental period.

Clinical signs were observed and recorded twice a day.

The body weights were recorded before dosing and on days 6, 9, 12, 15, 18, 21, 24 and 29 of gestation.

Food consumption: Food consumption was measured on alternating days at predose to the necropsy.

Postmortem parameters:

On day 29 of gestation, animals were euthanized by overdose of i.v. Sodium pentobarbital and exsanguinated (it is not clear whether pentobarbital would kill the live fetuses). Gross necropsy was performed and the pregnancy status was determined. Thoracic, abdominal, pelvic cavities and the organs were examined for macroscopic changes at necropsy. Abnormalities were recorded and tissues were preserved in 10% neutral-buffered formalin.

At scheduled termination, ovaries and uteri were removed and weighed. Number of corpora lutea, implants, early or late resorptions, live and dead fetuses were recorded. If the uterus appeared non-gravid, it was opened and placed in a 10% ammonium sulfide solution and examined for very early resorptions. Each placenta was examined for gross changes.

Each fetus was weighed and examined for external changes. External abnormalities in the eyes, palate, shape of the head, trunk and extremities were recorded.

All live fetuses were euthanized by an i.p injection of sodium pentobarbital and examined for visceral changes. Sex for each fetus was determined. Following visceral examinations each fetus was eviscerated and skinned, and fixed in 95% isopropyl alcohol. The soft tissues of the fetuses were further digested in 2% potassium hydroxide. The skeleton was stained with Alizarin red and examined for skeletal abnormalities. Upon completion of skeletal examinations, the fetuses were preserved in 50% glycerin and 50% isopropyl alcohol.

If an animal aborted or delivered early, the time of early delivery was estimated and recorded. Animal was necropsied as soon as possible. Animal that appeared in poor condition was euthanized and necropsied immediately.

Statistical evaluations:

The incidences or means and standard deviations of maternal and fetal observations were calculated. Statistical analysis of fetal parameters was performed using the litter as the unit of analysis. The number of corpora lutea, implantation, live, and dead fetuses, resorptions, fetal sex ratios and gravid uterine weights were calculated as the total number for each group divided by the number of litters evaluated. Individual fetal weights were used to determine the mean fetal weight for each litter. The litter means were used to determine the mean for each group.

Following parameters were calculated as follows:

Preimplantation loss % =  $\frac{(\text{number of corpora lutea} - \text{number of implantations})}{\text{Cor. Lut.}} \times 100$

Postimplantation loss % =  $\frac{(\text{\#implantations} - \text{\#live fetuses})}{\text{\#implantations}} \times 100$

Total implantation loss % =  $\frac{(\text{\#cor. Lut.} - \text{\#live fetuses})}{\text{\# corpora lutea}} \times 100$

Statistical comparisons for maternal body weights, gravid uterus weights, food consumption were made by one way analysis of variance and by pair-wise comparisons of significant trend by Dunnett's test.

The number of corpora lutea, implantations, resorptions, live or dead fetuses, the percent preimplantation loss, percent post implantation loss and total implantation loss were compared by Kruskal-Wallis test and by pair-wise comparison using Mann-Whitney U test. Male to female sex ratios was analyzed by Chi-square test.

Incidences of malformations and variations were compared by Fisher's exact test with litter as the experimental unit.

Data for animals that delivered early were not included in the statistical analysis.

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ON ORIGINAL

Results:

Frequently observed clinical signs, stain on the cage bedding, premature delivery and abortion are shown in the following table.

Observation	Group 1	Group 2	Group 3	Group 4	Group 5
Alopecia/thin hair	5	2	5	6	2
Infrequent stool	2	3	3	5	5
Red/Brown stain on pad	2	3	8	14	13
Loose stool	2	7	3	5	5
Fetal/placenta tissue on the pad, #, day	0	0	1, #56, day 26	16, #61, Day 23*; #63, day 28*; #64, day 22*; #65, day 26*; #66, day 26*; #67, day 24*; #68, day 24*; #69, day 27*; #70, day 23*; #71, day 29*; #73, day 26*; #74, day 23*; #75, day 26*; #78, day 16*; #79, day 23*; #80, day 22*;	9, #83, day 27*; #84, day 24*; #89, day 22*; #90, day 25*; #91, day 27*; #94, day 24*; #95, day 22*; #96, day 24*; #98, day 25*;

\*Unscheduled sacrifice

Above data suggest that at 36-mg/kg dose of levobetaxolol or racemic betaxolol, there were increased incidences of spontaneous abortion/premature delivery as evident with increased incidences of fetal/placental tissues in the padding of the cage. A similar observation was made in one animal at 12 mg/kg dose of S (-) betaxolol. The sponsor stated that abortion was defined as delivering fetal/placental material before gestation day 25 whereas premature delivery was defined as delivering fetal/placental material from gestation days 25-29. All other animals survived to the scheduled cesarean section on gestation day 29.

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ON ORIGINAL

Mortality:

All animals other than that sacrificed due to abortion or premature delivery survived through the end of the study.

The summary of the pregnancy status is shown in the following table.

Observation	Gr 1, control	Gr 2, S (-) betaxolol, 4 mg/kg	Gr 3, S (-) betaxolol, 12 mg/kg	Gr 4, S (-) betaxolol, 36 mg/kg	Gr 5, RS- betaxolol, 36 mg/kg
# animals	20	20	20	20	20
Dead/euthanized for premature delivery and abortion	0	0	1 (5%)	16 (80%)	9 (45%)
Examined at cesarean section	20	20	19	4	11
Non-gravid uterus	1 (5%)	0	1 (5%)	1 (5%)	1 (5%)
#animals with total resorption at cesarean section	0	0	0	0	0
#animals with live fetuses at cesarean section	19	20	18	3	10
# pregnant (%)	19 (95)	20 (100)	19 (95)	19 (95)	19 (95)

Above data suggest that there was embryo-fetal toxicity in animals that belonged to groups 3, 4 and 5. The sign of the toxicity was abortion and premature delivery. The non-gravid uterus did not show any sign of implantation after ammonium sulfide treatment.

Body weight (kg):

Observation	Gr 1 Predose D 29		Gr 2 Predose D29		Gr 3 Predose D29		Gr 4 Predose D29		Gr 5 Predose D29	
B.W.	3.6	4.1	3.5	4.0	3.5	4.0	3.5	3.8	3.5	3.8
B.W. gain	0.5		0.5		0.5		0.3		0.3	

Above data suggest that the weight gain of animals was affected at 36 mg/kg dose of S (-) betaxolol or RS betaxolol by about 40% compared to the untreated control.

Food consumption (g/day):

Gr 1	Gr 2	Gr 3	Gr 4	Gr 5	
Day 2	Day 28	Day 2	Day 28	Day 2	Day 28
124.2	123.3	119.1	108.4	118.2	116.9
115.1	126.2	119.2	126.2	119.2	126.2

Above data suggest that the treatment did not have an adverse effect on the food consumption of the animals.

Toxicokinetics in parental animals: No data attached to the report.

Embryo-fetal development:

No gross abnormality was reported for animals that underwent cesarean section on day 29.

Findings for animals sacrificed before the schedule necropsies on day 29 are shown in the following table.

Animal	Group	Dose, mg/kg	Day sacrificed	Findings in the uterus and cage bedding
56	3	12 (S-betaxolol)	26	2 late resorptions and 1 early resorption
61	4	36 (S-betaxolol)	23	4 late resorptions and 2 early resorptions
63	4	36 (S-betaxolol)	28	4 fetal/placental resorptions
64	4	36 (S-betaxolol)	22	3 early resorptions
65	4	36 (S-betaxolol)	26	1 early resorption, 4 late resorptions
66	4	36 (S-betaxolol)	26	1 late resorption
68	4	36 (S-betaxolol)	24	7 early resorptions
69	4	36 (S-betaxolol)	27	4 resorptions
70	4	36 (S-betaxolol)	23	Bilateral multiple black foci in ovary, 4 early resorptions, 3 late resorption
71	4	36 (S-betaxolol)	29 (Before scheduled necropsy)	4 early resorptions and 2 late resorptions
73	4	36 (S-betaxolol)	26	7 late resorptions
74	4	36 (S-betaxolol)	23	6 late resorptions
75	4	36 (S-betaxolol)	26	7 late resorptions
78	4	36 (S-betaxolol)	16	No abnormality but fetal/placental tissues found in the cage
79	4	36 (S-betaxolol)	23	Small amount of clear fluid present; 5 early resorptions and 2 late resorptions
80	4	36 (S-betaxolol)	22	9 early resorptions
83	5	36 (Rsbetaxolol)	27	6 late resorptions
84	5	36 (Rsbetaxolol)	24	Sign
89	5	36 (Rsbetaxolol)	22	8 early resorptions
90	5	36 (Rsbetaxolol)	25	Scab on right pinna; 8 early resorptions
91	5	36 (Rsbetaxolol)	27	Premature delivery, otherwise no abnormalities reported
94	5	36 (Rsbetaxolol)	24	5 early and 5 late resorptions
95	5	36 (Rsbetaxolol)	22	Single black focus in right kidney, 4 early resorptions and one late resorption
96	5	36 (Rsbetaxolol)	24	5 Fetal/placental tissues found in the cage
98	5	36 (Rsbetaxolol)	25	8 late resorptions

Above data suggest that fetal/placental tissues found in females that aborted or delivered prematurely showed early and late resorptions.

Summary of data on sex and fetal weights at cesarean section:

The table represents data on the pre and post implantation changes in rabbits.

Observations	Gr 1, control	Gr 2, 4 mg/kg S-betaxolol	Gr 3, 12 mg/kg S-betaxolol	Gr 4, 36 mg/kg S-betaxolol	Gr 5, 36 mg/kg RS betaxolol
#Gravid Females	19	20	18	3	10
# corpora lutea	9.4	9.9	10.0	10.0	9.5
# Implantation	9.1	9.5	9.3	9.0	9.0
%pre implantation loss	3.0	3.6	6.8	9.9	8.9
# Live fetuses	8.9	8.9	5.8*	2.3*	2.9*
#dead fetuses	0	0	0	0	0
#early resorptions	0.1	0.4	1.8*	5.7*	3.3*
#Late resorptions	0.1	0.3	1.8*	1.0*	2.8*
#Total resorptions	0.2	0.7*	3.6*	6.7*	6.1*
%post implantation loss	1.8	7.4*	36.1*	75.1*	65.6*
%total implantation loss	4.8	10.6	41.7*	76.8*	72.7*
Sex MF	4.7,4.3	3.9,5.0	3.2,2.6	1.7,0.7	1.4,1.5
Fetal Wt (g), M	43.79	41.41	45.68	45.56	49.40*
Fetal Wt (g), F	42.47	39.83	44.38	45.65	45.89
Fetal Wt, (g), litter mean	43.26	40.50	45.39	45.44	48.19*
Wt gravid uterus (g)	548.37	517.85	411.90*	234.56*	266.01*

\*Statistically significant

Increased % post implantation loss was observed at 12, 36 mg/kg doses of S (-) betaxolol and 36 mg/kg dose of racemic betaxolol. The sponsor stated that percent post implantation loss at 4 mg/kg dose was comparable to the historical control of 7.2%. Similarly, total resorptions at 4mg/kg dose were comparable to the historical control of 0.6. Above historical control data were obtained from the Mid Atlantic Reproduction and Teratology Association and Midwest Teratology Association. Data gathered in NZ rabbits during 1992-1994 are presented.

Live fetuses were also decreased at 12, 26 mg/kg doses of S (-) betaxolol and 36 mg/kg dose RS-betaxolol. The data reflected in the reduced weight of gravid uterus at 12, 36 mg/kg doses of S (-) betaxolol and 36 mg/kg dose of RS betaxolol.

Absolute data on fetal observations for malformation that show higher prevalence than the control are presented in the following table.

Observation	Gr 1, Fetuses/Litters	Gr 2, Fetuses/Litters	Gr 3, Fetuses/Litters	Gr 4, Fetuses/Litters	Gr 5, Fetuses/Litters
# examined externally	170/19	171/20	97/18	7/3	29/10
Number examined skeletally and viscerally	170/19	177/20	104/18	7/3	29/10
Vestigial pulmonary trunk/Bulbous aortic arch	1/1	0/0	0/0	0/0	0/0
Fused Sternebrae	0/0	10/4	0/0	0/0	0/0
Malaligned and fused sternebrae	0/0	2/2	0/0	0/0	0/0
Costal cartilage anomaly	1/1	1/1	0/0	0/0	0/0
Vertebrae anomaly	2/2	0/0	0/0	0/0	0/0
Total with malformation	4/4	12/5	0/0	0/0	0/0

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ON ORIGINAL

Absolute numbers of variations are shown in the following table.

Observation	Gr 1, Fetuses/Litters	Gr 2, Fetuses/Litters	Gr 3, Fetuses/Litters	Gr 4, Fetuses/Litters	Gr 5, Fetuses/Litters
#Examined externally	170/19	171/20	97/18	7/3	29/10
#Examined viscera and skeletally	170/19	177/20	104/18	7/3	29/10
Gall bladder Variation	13/6	6/5	4/3	0/0	0/0
Small spleen	0/0	1/1	0/0	0/0	0/0
Retrocaval Uterus	1/1	1/1	1/1	0/0	0/0
Hemorrhagic ring around iris	0/0	0/0	1/1	0/0	1/1
Major blood vessel variation	6/4	5/4	0/0	0/0	2/1
13 <sup>th</sup> Full rib	93/19	74/18	40/14	2/2	17/8
13 <sup>th</sup> Rudimentary rib	25/12	40/19*	18/13	0/0	3/3
Thickened rib	1/1	0/0	0/0	0/0	0/0
Hyoid arches	9/6	4/3	4/3	1/1	1/1
Slight to moderate malaligned and fused sternebrae	20/13	17/10	7/5*	0/0	4/3*
Unossified (5-6) sternebrae	21/13	24/11	8/6	0/0	5/4
25 Presacral vertebrae	0/0	1/1	0/0	0/0	0/0
27 Presacral vertebrae	36/13	16/11	17/7	0/0	10/5
Total fetuses/litters with variations	135/19	131/20	71/16	3/2	22/9

\*Statistically significant

Other than incidental changes, there were no treatment-related malformations that were observed in S (S) betaxolol and RS-betaxolol treated animals.

Statistically significant increase in number of litters that showed 13<sup>th</sup> rudimentary rib was reported at 4 mg/kg dose of S (-) betaxolol. Percent of 13<sup>th</sup> rudimentary rib was 14.7, 22.6, 17.3, 0, 10.3% at control, 4, 12 and 36 mg/kg doses of S (-) betaxolol, and 36 mg/kg dose of RS-betaxolol, respectively. The sponsor stated that the incidence of 13<sup>th</sup> rudimentary rib at 4 mg/kg dose of S (-) betaxolol was not treatment related due to absence of such incidences at higher doses of S (-) betaxolol. However, number of fetuses and litters examined at higher doses (12-36 mg/kg) were lower than that in the groups 1 and 2, which confounded the results.

Reviewer's summary and evaluation:

The effect of S (-) betaxolol and RS-betaxolol on developmental toxicity (segment II) was evaluated in rabbits. Maternal toxicity characterized by a reduction in the weight gain was observed at 36 mg/kg dose of S (-) betaxolol and RS-betaxolol. Embryo-fetal toxicity with increased resorptions were observed at 12, and 36 mg/kg doses of S (-) betaxolol and 36 mg/kg dose RS-betaxolol. At the schedule necropsy, a decrease in live fetuses was noted at 12, 36 mg/kg doses of S (-) betaxolol and RS-betaxolol. Data suggest that S (-) betaxolol showed increased resorption and postimplantation loss at 12mg/kg and above doses. The comparator RS-betaxolol also showed similar toxicity at 36 mg/kg. No treatment related malformation was observed at cesarean section. However, at 4mg/kg dose, variation in the fetal development e.g. rudimentary 13<sup>th</sup> rib was noted. Malaligned sternbrae were observed at 12-36 mg/kg doses of S (-) betaxolol and 36 mg/kg dose of RS-betaxolol.

The doses selected for the segment II study showed that maternal toxicity, embryo-fetal toxicity and skeletal variations during the development of the fetus. Results of the previous study reported in the package insert for RS-betaxolol ophthalmic suspensions showed increase post implantation loss at 12mg/kg dose in rabbits similar to that observed in the present study with S (-) betaxolol. Considering the similarity, it is considered that the effect of betaxolol and its S (-) isomer in the present study is reproducible when compared to the previous study with betaxolol in the rabbit model.

It is concluded that S (-) betaxolol is teratogenic in rabbits at 4 mg/kg/oral and above doses in NZ rabbits.

Labeling Recommendations: Pregnancy category C.

S (-) betaxolol has been shown to be teratogenic at oral doses of 4 mg/kg, embryocidal at 12 mg/kg and higher doses in New Zealand rabbits.

Sponsor's summary and conclusions:

Spontaneous abortion/premature delivery was included in the high dose (36 mg/kg) test article and reference article (36 mg/kg) groups. Embryotoxicity, as characterized by an increase in the number of early and late resorptions occurred in the mid (12 mg/kg), high dose test article and reference article groups. No evidence of teratogenicity was observed. Although a statistically significant increase in total resorptions was indicated for the low dose (4mg/kg) test article group, this number was within the historical range reported for control data. Therefore, the finding was not considered to be biologically meaningful. Accordingly, the no observable adverse effect level (NOAEL) for S (-) betaxolol developmental toxicity was considered to be 4 mg/kg/day.

Genetic Toxicology: APPEARS THIS WAY

Study Evaluation: ON ORIGINAL

Title: Mutagenicity test with levobetaxolol in the Salmonella-E. coli/mammalian microsome reverse mutation assay.

Page 5-03125, vol 9