treated animals (see Table 5), and the weight gains in surviving 50 mg/kg "treated" pups was also less than that observed in the vehicle control group

Table 5 Group Mean Pup Body Weight (g) of the F1 Generation

		Day Post Partum			
Treatment (mg/kg)	Gender	1	4	14	21
Vehicle Control	Male	6.0 ± 0.6	9.0 ± 1.7	33.5 ± 4.6	55.2 ± 7.7
0.5		6.0 ± 0.5	9.3 ± 0.9	34.8 ± 2.8	57.0 ± 4.2
5.0		5.1 ± 0.5*	8.3 ± 0.7	31.6 ± 2.7	50.8 ± 5.2
50.0		3.9 ± 0.2***	4.7 ± 1.2***	18.8 ± 3.2***	34.4 ± 9.3**
100.0		3.5 ± 0.0***			
Vehicle Control	Female	5.6 ± 0.5	8.4 ± 1.6	31.4 ± 3.6	51.4 ± 5.2
0.5		6.0 ± 0.6	9.4 ± 1.1	34.6 ± 3.2	55.3 ± 4.5
5.0		4.8 ± 0.4*	7.8 ± 0.6	29.1 ± 2.6	47.4 ± 5.3
50.0		3.8 ± 0.2***	4.5 ± 0.9***	15.0 ± 4.9***	29.4 ± 11.4***
100.0		3.3 ± 0.0***	a	a	a

a = no animals with live litters
*= significantly different from control, $p<0.05$
**= significantly different from control, $p<0.01$

Both males and females gained weight at a decreased rate in a dose dependent fashion when compared to control and there is also a concomitant decrease in food consumption.

Conclusion: Interpretation of the results of this dose range-finding study has to be tempered by the failure of the sponsor and the contractor to reliably prepare a dosage form. However, the chemical analysis of the higher doses was more consistent than the lower doses and the data

demonstrate an apparent dose-response relationship. The results of this experiment suggest that a dose of 50 mg/kg would be a maximally tolerated dose in rat reproductive studies.

Study:	Rat Fertility and General Reproductive Study
Study Number:	PTC/50/90
Study Date:	November 1990
Test Article:	Batch Number 208
Species/strain:	Rat/OFD-SD (IOPS-Caw) Sprague-Dawley
Weights:	Males 180-199g (7 weeks of age) Females 60-79g (4 weeks of age)
Number of animals:	30 per sex per dose
Route:	p.o.
Dose volume:	10 ml/kg
Doses Evaluated:	Vehicle, 0.5, 5.0 and 50 mg/kg for males Vehicle, 0.5, 5.0 and 25 mg/kg for females
Dosing Schedule:	The F0 generation males were dosed once daily for 60 days prior to mating, through the mating period and until the day prior to necropsy on day 95. The F0 generation females were dosed once daily for 14 days prior (except the five replacement control females which were dosed for 7 days prior to mating) to mating and until the day prior to necropsy on either day 20* of pregnancy or day 21** postpartum. * Day 0 of pregnancy is the day on which sperm plug or sperm positive vaginal smear is observed. ** Day 0 post partum is the day of completion of littering.
Vehicle:	1% w/v methyl cellulose
Method and frequency of test article formulation:	A suspension was prepared daily for each treatment group by weighing the test article and mixing with a small quantity of vehicle to form a smooth paste using a mortar and pestle. More vehicle was added slowly, while continuously mixing to make up to final volume. For most of the study the 0.5 mg/kg group (group 2) formulation was prepared by dilution from the group 3 (5.0 mg/kg) formulation.

For the last few days of the female dosing period, test article designated by was used in error for formulation.

Accuracy of test article formulation:

The concentration of the formulation was variable and percentages of theoretical ranged from In fact they use "mill chips" to dose females for the "last few days of ...dosing..."

Stability of test article formulation:

References report PTC/49/89 which declares the formulation to be satisfactory does not provide any data to substantiate this claim.

Procedures and observations for F0 generation:

All animals were examined daily for clinical signs of toxicity.

Male body weights were recorded at weekly intervals throughout the study. Female body weights were recorded weekly during the pre-mating dosing periods and daily thereafter; body weights on days 0, 7, 14 and 20 of pregnancy and days 1, 7, 14 and 21 post partum only have been reported for the daily weighing period.

At the end of 60 days males were paired with females from the same dose group. The males were removed on the day of mating and the female was caged individually. If a female had not mated within seven days, the male was removed and another from the same group substituted. Pairing continued for up to fourteen days.

On day 20 of pregnancy approximately 15 females from each dose group were killed by CO_2 asphyxiation. The thoracic and abdominal cavities were opened and the following observations were made:

1. Abnormalities of major maternal organs
2. Number of corpora lutea
3. Number and distribution of implantation sites. The implantations were classified as early resorptions, late resorptions, dead fetuses or live fetuses.
4. Live fetal weights
5. Fetal gender
6. External abnormalities of fetuses.

Tissues showing major macroscopic abnormalities were preserved.

The remaining FO generation females were allowed to litter and rear their offspring.

Fetus processing and examination:	Two thirds of the live fetuses from each litter were fixed in 70% alcohol. They were skinned, dissected and the viscera were examined. They were then eviscerated and the carcasses processed for staining skeletons. The bones of each fetus were identified and examined for normality with respect to shape, size and the extent of ossification. The remaining fetuses were fixed in Bouin's fluid. When fixation was complete the fetuses were examined by a combined sectioning/dissections technique. Serial sections of the head were made and them examined. The thorax and abdomen were examined by microdissection. This included detailed sectioning of the heart and kidneys and inspection of all major organs and blood vessels. Structural congenital abnormalities that impair or potently impair the survival or fitness of the fetus were regarded as major abnormalities. Other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification from that expected of a day 20 gestation fetus together with common variations in the extent of renal cavitation and ureter dilation were recorded as variants.
Procedures and observations for F1 generation:	The litter size and pup sex were recorded as soon as possible after birth and daily thereafter for 21 days. Clinical signs were recorded daily. On day 4 post partum, all litters containing more than eight pups were culled to eight. Culled pups were killed and the major organs in the abdominal and thoracic cavities were macroscopically examined. Pup body weight, malformations and development were observed and recorded. After weaning, 15 male and 15 female pups from each treatment group were selected for rearing to sexual maturity. Pups not selected were killed and the major organs were examined macroscopically. After day 21 post partum pup's eye examined with an ophthalmoscope following ocular instillation of homatropine HBr and evaluated for auditory function and E-maze learning. Mating of animals was as described above as were observations during lactation.
Proof of absorption:	Not specified

Statistical analysis: Where significance was achieved using analysis of variance, each treated group was compared to the control group using Student's t test. Where significance was achieved using Kruskal-Wallis test, each tested group was compared to the control group using Dunn'/s multiple comparison test.

Results:

F0 generation: Five/30 males in the highest dose (50 mg/kg) treated group were either killed or died (one male) between days 36 and 54 of dosing and one female/30 from the highest dose (25 mg/kg) group was killed on day 30 of dosing because of poor clinical condition. Weight gains, expressed as a perentage of control final weight, in male treated with both 5.0 and 50 mg/kg were statistically significantly lower than vehicle control treated males during the 92 days of observation (fig. 3). Weight gain in females was statistically significantly reduced at all dose levels, 0.5, 5.0 and 25 mg/kg (fig. 4). In general, observation of statistically significant weight reduction was accompanied with statistically significant decreases in food consumption for both genders.

Figure 3 The effect of LE on final weight in males treated for 3 months.

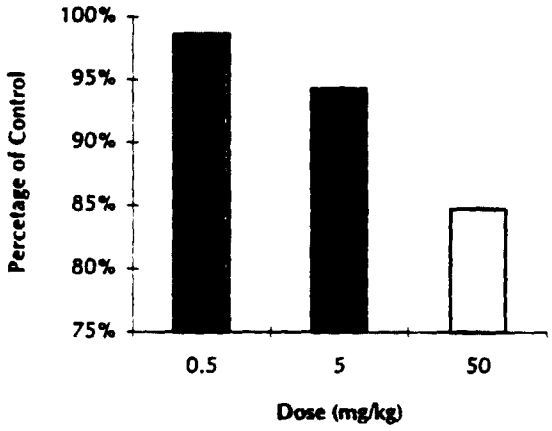

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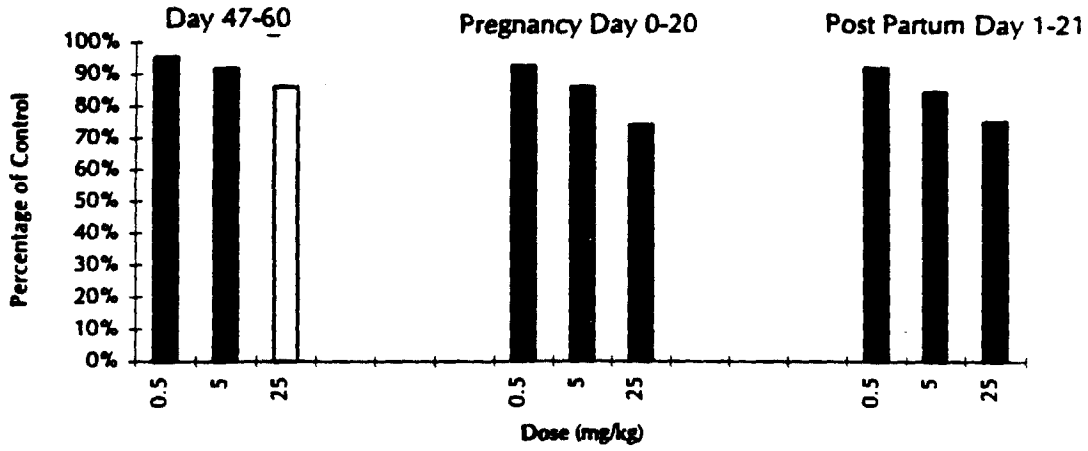
Figure 4 The effect of LE on final weight in females.



There were no differences in the number of successful copulations and subsequent fertility rates for either males or females treated with 0.5, 5.0 or 50 mg/kg (25 mg/kg for females).

The effects on the number of fetuses delivered and the weights of the fifteen females per treatment group that were killed after 20 days of gestation are reported in Table 6.

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Table 6 The effects on the day 20 of gestation fetuses of orally administered LE to F0 generation rats during mating and pregnancy

Observation	Dose (mg/kg)				Reference Vol:pg
	Vehicle	0.5	5	25	
Number pregnant	14	14	11	11	13:203
Corpora lutea (Mean ± S.D.)	17.1 ± 1.8	18.4 ± 2.5	20.5 ± 3.7*	15.5 ± 2.5	13:203
Implantations (Mean ± S.D.)	15.5 ± 2.2	16.5 ± 2.6	17.7 ± 2.7	12.6 ± 4.7*	13:203
Live fetuses (Mean ± S.D.)	15.0 ± 2.4	16.1 ± 2.8	16.7 ± 2.9*	9.8 ± 5.2***	13:203
Fetal weight (g) (Mean ± S.D.) - males	3.82 ± 0.31	3.58 ± 0.19	3.39 ± 0.27**	3.23 ± 0.54**	13:204
Fetal weight (g) (Mean ± S.D.) - females	3.66 ± 0.31	3.42 ± 0.21	3.17 ± 0.22**	3.03 ± 0.58**	13:204
Fetal external and visceral examination - major abnormalities	0	0	0	2[a]	Suppl.. No. 3, Tab 4 Table 9

a. Number of litters with umbilical hernias.
* = significantly different from control, $p<0.05$
** = significantly different from control, $p<0.01$
*** = significantly different from control, $p<0.001$

The major visceral abnormalities were two litters with umbilical hernias.

During nursing the no differences between control and treated groups for the F1 generation were observed for the following:

- Ear opening on day 3
- Righting reflex on day 5
- Startle response on day 15
- Eyes opening on day 15
- Pupillary reflex on day 21

Following weaning there were no biologically meaningful differences between loteprednol treated F1 generation weanlings and vehicle treated F1 weanlings in the following observations:

- E-Maze performance
- Ophthalmoscopic evaluation
- Auditory function

The effects on the number of fetuses delivered and their weights of the fifteen F1 females per treatment group that were allowed to deliver are reported in Table 7.

Table 7 The effects on the F1 generation of orally administered LE to F0 generation rats during mating and pregnancy.

Observation	Dose (mg/kg)				Reference Vol:pg
	Vehicle	0.5	5	25	
Number F0 generation pregnant	14	15	13	15	13:210
Duration of gestation (Mean ± S.D.)	21.9 ± 0.3	21.7 ± 0.5	22.0 ± 0.3	22.5 ± 0.6*	13:210
Number of females delivering live pups	14	15	13	13	13:210
Live pups (Mean ± S.D.)	15.3 ± 2.1	16.5 ± 1.4	15.2 ± 2.0	12.0 ± 5.2**	13:210
Pup weight (g) (Mean ± S.D.) at birth - males	6.5 ± 0.4	6.2 ± 0.4	5.6 ± 0.3**	4.7 ± 0.5**	13:211
Pup weight (g) (Mean ± S.D.) at birth - females	6.1 ± 0.4	5.7 ± 0.4	5.4 ± 0.5***	4.3 ± 0.6***	13:211
Body weight at 18 wks of age (Mean ± S.D.) - males	571 ± 54	535 ± 45	507 ± 55***	459 ± 43***	13:218
Body weight at 13 wks of age (Mean ± S.D.) - females	271 ± 19	267 ± 19	255 ± 11*	238 ± 17***	13:219

* = significantly different from control, $p<0.05$
** = significantly different from control, $p<0.01$
*** = significantly different from control, $p<0.001$

The results of the exposure on the F2 generation are shown in Table 8. As with the F1 generation the following observations were different from the vehicle treated group:

- Ear opening on day 3
- Startle response on day 15
- Eyes opening on day 15
- Pupillary reflex on day 21

However, both the 0.5 and the 5.0 mg/kg treated pups had statistically significantly increased startle responses at 15 days.

Table 8 The effects on the F2 generation of orally administered LE to F1 generation rats that were exposed *in utero* and during nursing.

Observation	Dose (mg/kg)				Ref. Vol:pg
	Vehicle	0.5	5	25	
Number F1 generation paired - males	15	14	15	15	13:220
Number females pregnant	13	13	11	14	13:220
Number F1 generation paired - females	15	15	15	15	13:220
Number F1 generation pregnant	13	14	11	14	13:220
Duration of gestation (Mean ± S.D.)	21.8 ± 0.4	21.9 ± 0.3	21.9 ± 0.7	21.4 ± 0.5	13:222
Number of females delivering live pups	13	14	11	14	13:222
Pups born (Mean ± S.D.) (% live births)	14.5 ± 2.4 (99.4)	14.6 ± 1.4 (96.2)	13.6 ± 2.1 (98.3)	13.0 ± 1.6 (97.3)	13:222
Pup weight (g) (Mean ± S.D.) at birth - males	6.0 ± 0.3	6.3 ± 0.5	6.3 ± 0.5**	5.9 ± 0.4	13:222
Pup weight (g) (Mean ± S.D.) at birth - females	5.7 ± 0.2	6.1 ± 0.5*	6.3 ± 0.5**	5.8± 0.3	13:223

* = significantly different from control, $p<0.05$
** = significantly different from control, $p<0.01$
*** = significantly different from control, $p<0.001$

Conclusion:

Oral administration of loteprednol caused a dose-dependent decrease in body weight in both males and female of the F0 treated animals. This decrease was accompanied by a decrease in food consumption. Decreases in implantations and live fetuses were observed in the litters of females killed after 20 days of gestation following treatment with the highest doses of loteprednol. Dose dependent decreases in fetal weights were observed in both male and female fetuses of females in the high dose treated group of

females killed at 20 day of gestation. There were two litters in this same group that presented with umbilical hernia.

In F0 females that were allowed to go to parturition there was a dose-dependent increase in the duration of gestation and a statistically significant dose dependent decrease in pup weight at birth which continued into breeding age.

Following pairing of F1 males and females from similarly treated F0 dams there appeared to be no impairment in reproductive capacity nor were there decreases in mean pup weights.

Treatment with 5.0 mg/kg (intermediate) and 25 mg/kg (high) for females and 50 mg/kg (high) for males had adverse effects in F0 males and females and F1 offspring. These adverse effects include decreased body weight gain for males and females in the F0 generation and lower fetus and pup body weight. The F1 generation failed to regain the lost body weight, but were able to mate and produce offspring without any adverse effect. In these F1 animals in the highest dose group there were umbical hernias noted. Even at the low dose, 0.5 mg/kg, there was a nonstatistically different decrease in fetal and pup body weight and this continued throughout the period of observation. No adverse effects were observed in the F2 generation.

Study:	Rat Teratology Study
Study Number:	PTC/49/89
Study Date:	June 1990
Test Article:	Batches 137 and 156
Species/strain:	Rat/OFA-SD(IOPS-Caw) Sprague-Dawley
Weights:	Females 218 to 290g
Number of animals:	24 sex per dose
Route:	Oral
Dose volume:	10 ml/kg
Concentration Evaluated:	Vehicle, 0.5, 5.0, 50.0, and 100.0 $mgkg^{-1}day^{-1}$
Dosing Schedule:	Once daily from day 6 to 15
Vehicle:	1% w/v methyl cellulose

Method and frequency of test article formulation:

A suspension was prepared daily for each treatment group by weighing the test article and mixing with a small quantity of vehicle to form a smooth paste using a mortar and pestle. More vehicle was added slowly, while continuously mixing to make up to final volume.

Accuracy of test article formulation:

The formulation was sampled 2 times early in the study. On the first occasion the range of concentrations found as a percentage of theoretical was , and on the second occasion the range was

Stability of test article formulation:

Not specified but declared satisfactory

Observation Times:

All rats were observed daily for clinical signs of toxicity.

Females: Body weight were recorded on days 0, 6 to 15 and 20 of pregnancy.

Food consumption was measured over the following periods: days 0 to 6, 6 to 11, 11 to 15 and 15 to 20 of pregnancy.

Mating:

A male was introduced into a cage containing two females. Mating was confirmed by the observation of sperm in vaginal smear.

Necropsy:

On day 20 of pregnancy all females were killed by CO_2 asphyxiation. The thoracic and abdominal cavities were opened by a ventral mid-line incision and the following observations were made:

- Number of corpora lutea
- Number and distribution of implantation sites. the implantations were classified as early resorptions, late resorptions, dead fetuses or live fetuses.
- Live fetal weight
- Fetal gender
- External abnormalities of fetuses
- Abnormalities of major maternal organs

Fetus processing and examination: Two thirds of the live fetuses from each litter were fixed in 70% alcohol. The were skinned, dissected and the viscera were examined. The were then eviscerated and the carcasses processed for staining skeletons. The bones of each fetus were identified and examined for normality with respect to shape, size and the extent of ossification.

The remaining fetuses were fixed in Bouin's fluid. When fixation was complete the fetuses were examined by a combined sectioning/dissections technique. Serial sections of the head were made and them examined. The thorax and abdomen were examined by microdissection. This included detailed sectioning of the heart and kidneys and inspection of all major organs and blood vessels.

Structural congenital abnormalities that impair or potently impair the survival or fitness of the fetus were regarded as major abnormalities. Other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification from that expected of a day 20 gestation fetus were recorded as variants.

Proof of absorption: Not determined

Results: There were no premature deaths observed, and body weight gain (fig. 5) and food consumption were decreased in a dose dependent manner following treatment with test material. The number of pregnancies, the mean number of corpora lutea, mean number of implantations, mean number of live fetuses, mean number of preimplantation loss and sex ratio were not different for any of the treatment groups. However, the mean number of post-implantation losses was statistically significantly increased by 11.4 % for females treated with 100 mg/kg.

The fetal weights for both male and female fetuses were decreased in a dose dependent manner with statistically significant decreases of 20 and 25% for doses of 50 and 100 mg/kg, respectively. Fetal examination (table 9) revealed no major abnormalities in the vehicle and the 0.5 mg/kg treated group. One litter with a major abnormality, absent of innominate artery, was observed in the 5 mg/kg treated group. the incidence of this abnormality increased with increasing dose. As can be seen from inspection of the incidence table other abnormalities were observed in the 50 and 100 mg/kg treated groups, however, the incidence dose not increase with increasing dose.

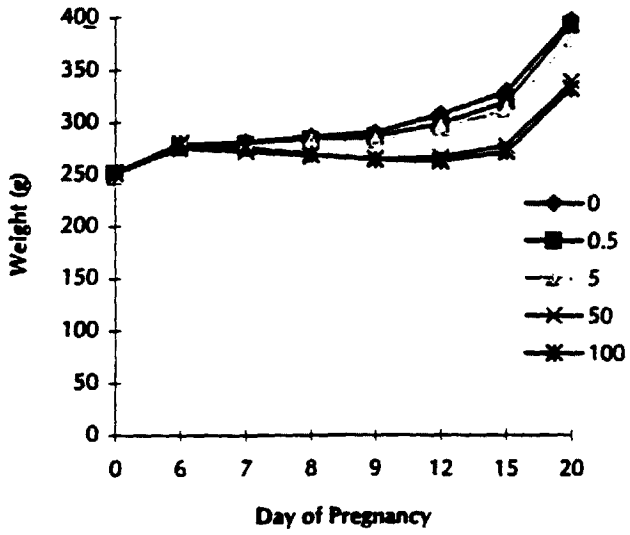

Figure 5 Weight gain in gravid rats treated with LE from day 6 to 15 of pregnancy.

The minor and variant abnormality observations were most often related to delayed ossification.

Conclusion: Oral administration of 50 and 100 mg/kg per day adversely effected both maternal and fetal weight. Doses of 50 mg/kg per day or greater were toxic to the dams, while a dose of 5.0 mg/kg per day cause absence of the innominate artery in the fetus, therefore, a dose of 0.5 mg/kg caused no fetal abnormalities or malformations.

The concentration of the suspension at the lower concentrations were not within 10% of expected and it was difficult to predict accurately the dose administered on any one day. However, if one assumes that any one dose could be as much as 80% of that anticipated then the lower doses would be 0.4 and 4.0 mg/kg instead of 0.5 and 5.0 mg/kg, respectively. Thus the no fetal effect dose is approximately 4 or 5 times the maximum anticipated human dose, or if only 5% is absorbed systemically, the dose could be as much as 100 times the human exposure.

Table 9. The Incidence and Percentage of Litters with Fetal Abnormalities

Parameter	Severity	Dose (mg/kg)									
		Vehicle		0.5		5		50		100	
		Incidence	%	Incidence	%	Incidence	%	Incidence	%	Incidence	%
Litters		23		23		22		22		23	
Cleft Palate	major	0	0	0	0	0	0	4	18	3	13
Agnathia	major	0	0	0	0	0	0	1	5	0	0
Situs inversus	major	0	0	0	0	0	0	0	0	1	4
Aortic arch: distended Pulmonary arch and ductus arteriosus: constricted (pulmonary valvular atresia	major	0	0	0	0	0	0	0	0	1	4
Innominate artery: absent	major	0	0	0	0	1	5	2	9	7	30
Aortic arch and right atrium: enlarged	major	0	0	0	0	0	0	1	5	0	0
Heart ventricles mis-shapen, groove accentuated	major	0	0	0	0	0	0	1	5	1	4
Ureter: uni or bilateral dilation	minor	12	52	15	65	10	45	2	9	2	9
Testes: uni or bilateral undescended	minor	0	0	0	0	0	0	4	18	7	30
Urinary bladder: distended	minor	0	0	0	0	0	0	4	18	4	17
Umbilical hernia	major	0	0	0	0	0	0	3	14	1	4
Inguinal hernia	major	0	0	0	0	0	0	0	0	1	4
Edematus	major	0	0	0	0	0	0	3	14	1	4
Runted fetus	minor	0	0	0	0	0	0	4	18	10	43

(Table 9. cont'd)	Dose (mg/kg)									
		Vehicle		0.5		5		50		100	
Parameter	Severity	Incidence	%	Incidence	%	Incidence	%	Incidence	%	Incidence	%
Skull: Parietals: retarded ossification	variant	1	4	0	0	1	5	5	23	10	43
Skull: Frontals retarded ossification	variant	0	0	0	0	0	0	5	23	8	35
Skull: Nasals retarded ossification	variant	0	0	0	0	0	0	2	9	2	9
Skull: Hyoid: not ossified	variant	0	0	4	17	2	9	1	5	2	9
Mandibles: reduced (agnathia)	major	0	0	0	0	0	0	1	5	0	0
Cleft palate	major	0	0	0	0	0	0	3	14	2	9
Vertebrae: Lumbar centra: one or more bilobed	minor	0	0	2	9	2	9	2	9	5	22
Vertebrae: Sacral: one or more ossified	minor	0	0	0	0	1	5	6	27	7	30
Vertebrae: Sacral: one or more retarded ossification	minor	1	4	0	0	0	0	6	27	9	39
One or more kinked ribs	minor	0	0	0	0	0	0	1	5	2	9
Sternebrae: 1st retarded ossification	minor	0	0	1	4	1	5	2	9	3	13
Sternebrae: 2nd retarded ossification	minor	0	0	1	4	1	5	6	27	11	48
Sternebrae: 3rd retarded ossification	minor	0	0	0	0	0	0	2	9	3	13
Sternebrae: 4th retarded ossification	minor	0	0	0	0	1	5	2	9	4	17

(Table 9. cont'd)		Dose (mg/kg)									
		Vehicle		0.5		5		50		100	
Parameter	Severity	Incidence	%	Incidence	%	Incidence	%	Incidence	%	Incidence	%
Sternebrae: 5th not ossified	variant	10	43	13	57	9	41	17	77	19	83
Sternebrae: 6th not ossified	variant	0	0	1	4	3	14	16	73	16	70
Sternebrae: one or more bilobed, dipartite, misshapen or misaligned	minor	3	13	3	13	2	9	9	41	13	57
Pelvic girdle: Pubes: not ossified	minor	0	0	0	0	0	0	1	5	3	13
Pelvic girdle: Retarded ossification	minor	0	0	0	0	1	5	5	23	8	35
Pelvic girdle: Ischia: retarded ossificated	minor	0	0	0	0	1	5	3	14	4	17
Forelimbs: Metacarpals: one or more not ossified	variant	13	57	15	65	15	68	20	91	22	96
Forelimbs: Metatarsals: one or more not ossified	variant	0	0	0	0	1	5	7	32	8	35

Study:	Rat Peri and Post Natal Study
Study Number:	PTC/51/90
Study Date:	November 1990
Test Article:	Batches 137 & 156
Species/strain:	Rat/OFA SD (IOPS-Caw) Sprague-Dawley
Weights:	Females 222-272g
Number of animals:	20 per sex per dose
Route:	p.o.
Dose volume:	10 ml/kg
Doses Evaluated:	Vehicle, 0.5, 5.0 and 50.0 mg/kg
Dosing Schedule:	Females were dosed once daily from day 15 of pregnancy to day 20 post partum inclusively.
Vehicle:	1% w/v methyl cellulose
Method and frequency of test article formulation:	Test article was formulated daily by suspending in 1% w/v methyl cellulose. Separate formulations were prepared for each group. For 5.0 and 50 mg/kg groups a weighed amount of test article was mixed with a small quantity of vehicle to form a smooth paste using a mortar and pestle. More vehicle was added slowly, while continuously mixing, to make up to final volume. The 0.5 mg/kg formulation was prepared by dilution with vehicle from the 5.0 mg/kg formulation.
Accuracy of test article formulation:	The formulations were tested on two occasions and found to be within ' of theoretical.
Stability of test article formulation:	Found to be stable in study no. PTC/49/89 (ref. 13:1)
Proof of absorption:	not specified
Parturition observations:	The females were observed at 30 min intervals between the hours of 0600 and 2400, beginning at day 21 of pregnancy and ending when all pregnant animals littered.
Culling:	On day 4 post partum all litters were culled to 8. All culled pups were subjected to necropsy.

Pup observations: Body weight total weights of the female and male pups in each litter were recorded as soon as possible after birth and on days 4 and 14 post partum.
The development of the following characteristic was recorded for each litter:

- Day 3 Ears open (examined daily until occurrence)
- Day 5 Static righting reflex
- Day 15 Eyes open (examined daily until occurrence)
- Day 15 Startle response
- Day 21 Pupillary light reflex

Necropsy was conducted on all pups killed or found dead during lactation. All surviving pups were killed at weaning on day 21 post partum. The thoracic and abdominal cavities were opened and the major organs examined macroscopically. Any organ or tissue exhibiting abnormalities was removed and fixed in buffered formal saline.

Results: Three females in the 50 mg/kg treatment group were killed *in extremis* on days 6 or 15 of the post partum period, and one in the 5.0 mg/kg group was killed after being found to have a prolapsed uterus. There were also two females, one from the high and one from the intermediate dose group that were found upon palpation to have pups *in utero* after completion of parturition.

Treatment of females with doses of 5 and 50 mg/kg of LE caused a dose dependent reduction in weight gain (Table 10) and at the highest dose a concomitant reduction in food consumption.

Table 10 The Effect of Oral Administration of LE on Weight Gain in Rats During Pregnancy and Post Partum.

Dose (mg/kg)	Weight Gain Days 15 to 20 of Pregnancy (%)	Weight Gain Days 1 to 21 Post Partum (%)
Vehicle	18	18
0.5	14	18
5.0	10	18
50	3	5

The effects of treatment with 0.5, 5.0 or 50 mg/kg of LE from day 15 of pregnancy to day 20 post partum are shown in table 11.

Table 11 The effects orally administered LE to female rats once daily from day 15 of pregnancy to day 20 posts partum inclusively on pups.

Observation	Dose (mg/kg)				Ref. Vol:pg
	Vehicle	0.5	5	50	
Number pregnant	20	20	19	19	14:26
Duration of gestation (Mean ± S.D.)	21.6 ± 0.3	21.6 ± 0.1	21.8 ± 0.4	22.1 ± 0.5	14:26
Pups born (Mean ± S.D.)	14.3 ± 2.2	13.7 ± 2.5	13.7 ± 1.5	13.0 ± 3.3	14:26
Cumulative survival index (Mean)[a]	82.5	81.0	80.1	47.4*	14:26
Pup weight (g) at birth (Mean ± S.D.) - males	5.8 ± 0.4	5.7 ± 0.3	5.4 ± 5.4*	4.5 ± 0.4**	14:27
Pup weight (g) at birth (Mean ± S.D.) - females	5.5 ± 0.4	5.5 ± 0.6	5.1 ± 0.6*	4.3 ± 0.5**	14:27
Litters with incomplete gastrointestinal tracts	0	0	1	2	14:20
Umbical hernia and enlargement of pulmonary arch	0	0	0	1	14:20

a. [(no. of pups alive on day 21/no. of pup present after culling) x no. of pups alive on day 4]/total no. of pups born x 100

**= significantly different from control, $p < 0.01$

** = significantly different from control, $p < 0.001$

In the pups there was no differences between control and the 0.5 and 5.0 mg/kg treated groups were observed for the following:

- Ear opening on day 3
- Righting reflex on day 5
- Startle response on day 15
- Eyes opening on day 15
- Pupillary reflex on day 21

However, pups whose dams were treated with 50 mg/kg of LE had statistically significant decreased percentages of righting reflex, startle response and pupillary light reflex and statistically significant increased percentages eye opening at the days specified above.

Pups in 2 litters of the highest dosed dams were found to have incomplete gastrointestinal tract, thought to have resulted from an umbical hernia and another litter had an incidence of umbical hernia and an enlargement of the pulmonary and aortic arches. There was also a litter in the intermediate dose with an incomplete intestinal tract (Table 12).

In comparison to control litters, the 50 mg/kg group had a higher incidence of small, hypothermic, dead and missing pups.

Conclusion: Maternal treatment with orally administered LE during late pregnancy and lactation caused a dose dependent decrease in body weight gain with only slight decreases observed at 0.5 mg/kg and marked effects on body weight, food consumption and clinical condition at 50 mg/kg. In spite of these dramatic effects there were no effects on the onset or progress of parturition.

In the offspring, LE treatment elicited toxic changes at both 5.0 and 50 mg/kg treatments. In comparison to control pups, the high dose pups exhibited body weight and developmental retardation, poor survival, diminished clinical condition and the occurrence of umbical hernia. At the 5.0 mg/kg dose adverse effects were limited to retarded body weight at birth only and the observance of an umbical hernia in one litter.

This is one of the only studies performed by the sponsor in which the accuracy of the test article formulation at the low dose could be evaluated, and no adverse effects were observed for either the dams or the pups in the 0.5 mg/kg dose group.

Study:	Rabbit Teratology Dose Ranging Study
Study Number:	PTC/46/89
Study Date:	October 1990
Test Article:	The sponsor supplied three batches of P 5604 (loteprednol)
	Batch Numbers 125, 137 and 156
Species/strain:	Rabbit/New Zealand
Age:	Approximately 4 months
Number of animals:	5 non-mated rabbits in rising dose tolerance study 5 per dose for mated study from day 0 3 per dose for mated study from day 3
Route:	p.o.

Dose volume:	5 ml/kg	
Doses Evaluated:	Non-mated:	25, 50 and 100 $mgkg^{-1}day^{-1}$
	Mated day 0 of pregnancy:	Vehicle, 12.5, 25.0, 50.0, and 100.0 $mgkg^{-1}day^{-1}$
	Mated day 0 of pregnancy:	Vehicle, 0.1, 0.2, 0.4, 0.8, 1.5, 3.0 and 6.0 $mgkg^{-1}day^{-1}$
Dosing Schedule:	All animals were dosed from day 6 to day 18 inclusively.	
Vehicle:	1% w/v methyl cellulose	
Method and frequency of test article formulation:	Separate suspensions were prepared for each dose group A weighed amount of test article was mixed with a small quantity of vehicle to form a smooth paste using a pestle and mortar. More vehicle was added slowly, while continuously mixing to make up to final volume, Formulations for 0.1, 0.2, 0.4, 0.8, 1.5, 3.0 and 6.0 $mgkg^{-1}day^{-1}$ were, from the fourth day at dosing, prepared by dilution with vehicle of the group 6.0 $mgkg^{-1}day^{-1}$ formulation.	
Accuracy of test article formulation:	The range of % of theoretical for study number 1 - The range of % of theoretical for study number 2 - (The most variability occurred in the lowest concentration and was not reproducible from day to day. The analytical results for 3.0 and 6.0 mg/kg doses were, however, within theoretical.)	
Stability of test article formulation:	Not determined for this study but for similar formulations shelf lives have exceeded 2 years (NDA 20-583.002, pg 13).	
Observation times:	Maternal observation were recorded daily and body weight were recorded on days 0, 3, 6, 18, 22, 25 and 28 of pregnancy. Food consumption was recorded every two day beginning at day 0 for the animals mated at Toxicol and at day 4 for the animals bred at the rabbit supplier	
Proof of absorption:	No plasma levels were detected in any of the blood samples. The limit of detection was 0.5 μg/ml. No evidence of the presence of a metabolite was found.	
Mating:	There were two mating studies. In the first study female were exposed to two different mature males on consecutive nights. In the second study female were exposed to only one male (these rabbits were bred at the supplier. In both studies all females were given 25 IF of chorionic gonadotropin, i.v., with each mating.	

Necropsy — On day 16 the females were killed by an intravenous injection of sodium pentobarbital and necropsied. The thoracic and abdominal cavities were opened by a mid-line incision and the major organs were examined macroscopically.

Results:

The sponsor could not formulate reliable concentrations of test material at concentrations lower than those used to dose 3 mg/kg, therefore, only those doses of 3 mg/kg or higher can be used to evaluate the fetotoxicity of LE. Also no plasma levels were detected in any of the blood samples. The limit of detection was 0.5 μg/ml. No evidence of the presence of a metabolite was found.

In preliminary experiments in which non-mated female rabbits were treated for 5 days with 25, and then for 5 days with 50 and then for 5 days 100 $mgkg^{-1}day^{-1}$ food consumption and weight gain decreased as the dose of LE was increased.

Since none of the females died in this preliminary study, mated does were dosed with 12.5, 25, 50 and 100 mg/kg of LE from day 6 to day 18 of pregnancy and observed until day 28 of pregnancy. There was a dose dependent decrease in both body weight gain and increase in food consumption from day 10 to 18 followed by a decrease in food consumption. One female from the 100 mg/kg group died on day 20 and her death was attributed to a pulmonary infection. Between days 20 and 28 most animals were observed to have blood and/or macerated tissue in the feces tray on at least one day. One female in the 12.5 mg/kg group was found with an aborted fetus on day 20 and was killed. There was also an increase in the incidence of does with no live litters (Table 7). In the remaining litters arthrogryposis was observed in both treated and control litters and, therefore, is not believed to be a drug related effect. However, one dead fetus at 25 mg/kg was found with excencephaly and arthrogryposis.

Table 7 The Incidence of Pregnant Rabbits Treated With 12.5, 25, 50 or 100 mg/kg of LE From Day 6 to 18 of Pregnancy With Live Litters

Treatment (mg/kg)	No. of live Litters/No. of Rabbits Treated
Vehicle Control	5/5
12.5	3/4[a]
25	4/5
50	0/5
100	0/4[b]

a. 1/5 Aborted on day 20
b. 1/5 Died on day 20

Because of the reduced numbers of live litters another study in mated animals was done with doses of 0.1, 0.2, 0.4, 0.8, 1.5, 3.0 and 6.0 $mgkg^{-1}day^{-1}$. Following treatment with these doses the incidence of live litters was much higher (Table 8). In the 6 mg/kg group one female aborted on day 21 and one was observed with macerated tissue in the feces tray on day 21. The remaining female in this group had red vaginal staining on day 22. One female in the 3 mg/kg group was observed with red liquid in the feces tray on day 22. No discernable pattern food consumption and weight gain could be detected with these animals (fig. 6 & 7).

Table 8 The Incidence of Pregnant Rabbits Treated With LE From Day 6 to 18 of Pregnancy With Live Litters

Treatment (mg/kg)	No. of live Litters/No. of Rabbits Treated
Vehicle Control	2/2[a]
0.1	2/2[a]
0.2	3/3
0.4	3/3
0.8	3/3
1.5	3/3
3.0	3/3
6.0	1/1[b,c]

a. 1 Not pregnant
b. 1 Aborted on day 20 and 1 aborted on day 21
c. Only one fetus was present

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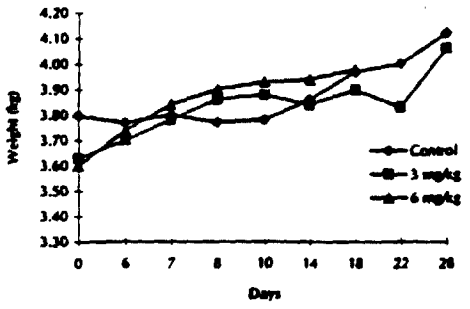

Figure 6 The effect of oral administration of LE from day 6 to 18 of pregnancy to rabbits on weight gain.

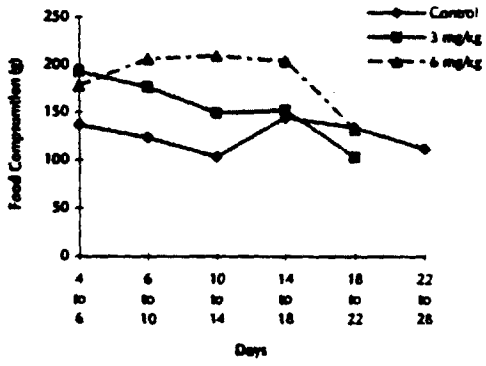

Figure 7 The effect of oral administration of LE from day 6 to 18 of pregnancy to rabbits on food consumption.

The number of females pregnant, mean number of corpora lutea, mean number of implantation, mean number of live fetuses, mean percentage of preimplantation loss, mean percentage of postimplantation loss, sex ratio and fetal weights were not different from vehicle control for animals receiving 3.0 $mgkg^{-1}day^{-1}$ or lower. However, one litter in this treatment group was observed to have one pup with umbical hernia and another with umbilical hernia, cleft palate and exencephaly.

Conclusion: The sponsor has not demonstrated proof of absorption of LE in these studies, however, because of the profound toxic effects to both the does and their fetuses following LE treatment, and the insensitivity of the assay (limit of detection 0.5 μg/ml) one can reasonable assume that orally administered LE is absorbed. Also because of the inability to formulate reliable concentrations of LE suspensions for doses of 1.5 mg/kg and lower this study shows that doses of 3.0 mg/kg administered orally from day 6 to day 18 of pregnancy are fetotoxic.

The pregnancy data for does treated with 12.5, 25, 50 or 100 $mgkg^{-1}day^{-1}$ show that LE was toxic to the dams and significantly reduces the incidence of live births, number of corpora lutea, preimplantation loss percentage and fetal weights in a dose dependent fashion. Doses of 3 and 6 mg/kg were not toxic to the dams.

Study:	Rabbit Teratology Study
Study Number:	PTC/67/90
Study Date:	November 1990
Test Article:	The sponsor supplied two batches of P 5604 (LE) Batch Numbers 137 and 156
Species/strain:	Rabbit/New Zealand
Age:	Approximately 4 months
Number of animals:	16 per dose
Route:	p.o.
Dose volume:	5 ml/kg
Concentration Evaluated:	Vehicle, 0.1, 0.5 and 3.0 $mgkg^{-1}day^{-1}$
Dosing Schedule:	All animals were dosed from day 6 to day 18 inclusively.
Vehicle:	1% w/v methyl cellulose
Method and frequency of test article formulation:	A suspension was prepared daily for 3.0 mg/kg dose concentration by weighing the test article and mixing with a small quantity of vehicle to form a smooth paste using a pestle and mortar. More vehicle was added slowly, while continuously mixing to make up to final volume. Concentrations for the two lower doses were prepared by dilution with vehicle.
Accuracy of test article formulation:	The range of % of theoretical for study number - '
Stability of test article formulation:	No data provided
Observation times:	Maternal observations were recorded daily and body weight were recorded on days 0, 3, 6, 18, 22,, 25 and 28 of pregnancy. Food consumption was recorded from day 3 to 6 and every two day.
Proof of absorption:	Not specified

Mating: The females were obtained from the supplier timed-mated. For mating, each female was mated with one male rabbit of the same strain and given an intravenous injection of 25 IU of chornioic gonadotropin to stimulate ovulation. The females were delivered to Toxicol on either day 1 or 2 of pregnancy.

Necropsy On day 28 the females were killed by an intravenous injection of sodium pentobarbital and necropsies the thoracic and abdominal cavities were opened by a mid-line incision and the major organs were examined macroscopically. Tissues showing severe abnormalities were removed and preserved in buffered formal saline

Fetal processing and examination: The live fetuses were killed by an i.p. administration of pentobarbital and placed in alcohol for fixation. Later the same day, the fetuses were skinned, dissected, the viscera examined and the gender recorded. After at least 12 hr fixation, a razor blade cut was made through the head along the frontal-parietal suture and the brain examined. The fetuses were cleared with KOH, stained with alizarin red S and examined for skeletal variants and abnormalities. The fetuses were stored in aqueous glycerol.

Structural congenial abnormalities that impair or potentially impair the survival or fitness of the fetus were regarded as major abnormalities. Other defects were classified as minor abnormalities. Commonly observed variations in the degree of ossification form that expected of a day 28 gestation fetus, together with normal variations in the numbers of thoracic and lumbar vertebrae and ribs, were recorded as variants.

Results and conclusion: There were no mortalities considered to be treatment-related. One animal from the vehicle control group and one from 0.5 mg/kg group died on day 10 and 16, respectively, as a result of intubation failure. They both exhibited respiratory difficulty and had hemorrhages in the lungs.

There were no meaningful differences in the body weights or food consumption in treated animals, and there were no necropsy findings in surviving rabbits.

Fetal abnormalities were observed in the offspring of female rabbits treated orally with LE from days 6 to 18 (Table 9). While incidence of spina bifida, exencephaly and meningocele were observed following treatment with 0.1, 0.5 and 3.0 mg/kg from days 6 to 18 of pregnancy, respectively, there does not seem to be a dose-response relationship. However, meningocele was observed in two litters at the highest dose. The incidences of left carotid artery abnormalities and signs of delayed ossification are other observations that appear to increase in incidence as the dose is increased.

Table 9 Incidence of Litters with Fetal Abnormalities in Rabbits (n=16)

Parameter	Dose (mg/kg)			
	0	0.1	0.5	3
Litters	15	13	14	15
Exencephaly	0	0	1	0
Meningocele	0	0	0	2
Left common carotid artery: abnormal, arising directly from innominate artery	8	8	9	11
Spina bifida	0	1	0	0
Limbs: uni or bilateral flexure	3	0	0	6
Parietals: retarded ossification	1	1	2	3
Vertebrae - Number of caudal centra: < or + 14	5	8	8	13
Lumbar vertebrae: one or more misplaced or hemicentric. Lumbar neural arches: one or more: absent (scoliosis)	0	0	0	2
Pubes: retarded ossification	3	2	5	11

A dose of 0.5 mg/kg appears to be the highest dose that is well tolerated by the dams and does not induce fetotoxicity.

Pharmacokinetic Studies:

Study:	Pharmacokinetics, Metabolism and Excretion of a Soft Corticosteroid, Loteprednol Etabonate
Study Number:	PHA-34
Study Date:	April 28, 1993
Test Article:	Not specified
Species/strain:	Rat/Sprague Dawley
Weights:	200-250g (Gender not specified)
Number of animals:	3 per dose for intravenous study 5 per dose for biliary excretion study
Route:	i.v.
Dose volume:	8 ml/kg

Doses Evaluated:	1, 2, 5, 10 or 20 mg/kg for intravenous study 10 mg/kg for biliary excretion study
Dosing Schedule:	Injected into the tail vein of anesthetized rats.
Vehicle:	Dissolved in 50% HPCD (hydroxypropyl-β-cyclodextrin) aqueous solution
Method and frequency of test article formulation:	Not specified
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	Not specified
Observation times:	Various

Results and Conclusion: Pharmacokinetic parameters of LE were obtained by the methods of residuals according to a two compartment model fitting (see Table 10). For metabolism and excretions studies, bile juice and urine were collected. The results indicate that loteprednol was rapidly eliminated from the systemic circulation, and no parent compound was present in bile but significant levels of both the primary and secondary metabolism were present. The urinary excretion of parent compound and the primary metabolite were 1 and 2%, respectively, and no conjugated parent compound was detected. It should also be noted that at intravenous doses of 10 and 20 mg/kg the AUC increased proportionally greater than dose.

Table 10 Pharmacokinetic data from derived by using a two compartment model.

Pharmacokinetic parameters of LE after intravenous bolus administration in rats.

Dose, mg/kg	1	2	5	10	20
A, μg/ml	1.19±0.46	1.69±0.44	3.11±0.38	8.65±2.75	17.87±0.71
B, μg/ml	0.32±0.06	0.38±0.15	0.51±0.37	1.92±0.25	3.41±0.40
α, 1/min	0.561±0.265	0.468±0.117	0.635±0.357	0.280±0.150	0.191±0.014
ß, 1/min	0.044±0.003	0.041±0.004	0.024±0.000	0.014±0.006	0.014±0.000
$T_{1/2}$(ß), min	15.92±1.23	17.22±1.71	29.49±0.00	43.41±7.58	48.82±1.52
AUC, μg x min/ml	9.2±0.4	16.0±1.1	56.1±6.2	159.2±31.3	333.2±17.9
CL, ml/min/kg	108.53±4.47	125.76±9.01	90.28±9.98	67.35±11.62	60.35±3.09
K_{el}, 1/min	0.162±0.049	0.132±0.025	0.067±0.021	0.071±0.024	0.064±0.002
V_d, ml/kg	749±257	1004±258	1444±298	1092±264	944±45
MRT, min	17.95±0.95	18.34±0.80	31.98±0.78	48.72±8.95	51.79±1.70

* Each value represents the mean ± S.E. of three trials.
* The equation, $C = Ae^{-\alpha t} + Be^{-\beta t}$, was used for the two-compartmental analysis.
* $T_{1/2}$(ß), half live of the ß phase; AUC, area under the blood concentration-time curve; CL, total blood clearance; K_{el}, elimination rate constant; V_d, volume of distribution; MRT, Mean resident time.

Study:	Pharmacokinetics of Loteprednol Etabonate in Dogs
Study Number:	PHA-27
Study Date:	9/21/90 (Rev. Apr. 1994)
Test Article:	Not specified
Species/strain:	dog/mongrel
Weights:	Males 21.5 - 27kg
Number of animals:	4 per dose
Route:	i.v. followed by two weeks p.o.

Dose volume:	Not specified
Doses Evaluated:	5 mg/kg
Dosing Schedule:	Once
Vehicle:	i.v. - 150 mg of loteprednol was dissolved in a mixture of 3 ml of ethanol and 4 ml PEG 200 using a sonicator. The solution was filtered (0.2 μm) into sterile vials. p.o. - 150 mg was dissolved in a mixture of 3 ml of ethanol and 4 ml Tween 80 and 8 ml hydroxypropyl methycellulose using a sonicator.
Method and frequency of test article formulation:	Not specified
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	Not specified
Observation times:	Jugular blood samples: 0, 5, 10, 20, 30, 40, 50, 60, 80, 100, 150, 180, 210, 240, 300, 360, 420, 480, 540, 600 and 1440 min. after drug administration Urine samples: 0, 30, 60, 90, 120, 180, 300, 360, 420, 480, 540, 600 and 1440 min. after drug administration
Level of detection:	Not specified

Results:

Following intravenous administration the following parameters were determined:

	Non-Compartment Analysis	Compartment Analysis
Total Body Clearance	21.9 ± 5.7 L/hr	21.8 ± 5.3 L/h
Mean Resident Time	1.7 ± 0.3 h	2.0 ± 0.3 h
Volume of Distribution	37.2 ± 12.1 L	43.6 ± 10.1L
Terminal $t_{1/2}$	N.D.	2.8 ± 0.3 h

Volume of Distribution of Central Compartment	N.D.	21.3 ± 2.4 L
Volume of distribution During Elimination	N.D.	88.9 ± 23.4 L

N.D. Not determined

No LE was detected in urine and an average of 0.8 mg of PJ-91 (primary metabolite)was recovered from urine over 24 hr. This represents less than 1% of the dose administered.

After oral administration no parent compound could be detected in plasma.

Conclusion: Following both oral and intravenous administration of LE to dogs, extensive conversion of the drug to its primary metabolite PJ-91 was observed. The large volume of distribution observed is characteristic of a lipid soluble drug of this class. The half life of 2.8 hrs. is comparable to other steroids.

Study: Protein binding, erythrocyte partition and stability of the steroidal anti-inflammatory drug loteprednol etabonate in dog blood and plasma - Part 1 Stability of Loteprednol Etabonate in Dog Blood and Plasma

Study Number: PHA-27A

Study Date: 1990

Test Article: Not specified

Species/strain: Dog/mongrel - male

Number of animals: per sex per dose

Doses Evaluated: 1 μg/2 ml of blood

Observation times: Various times up to 300 min.

Results and conclusions: Half-life of LE in blood and plasma is 18 and 22 hr, respectively.

Study: Protein binding, erythrocyte partition and stability of the steroidal anti-inflammatory drug loteprednol etabonate in dog blood and plasma - Part 2 Protein binding and Erythrocyte Partition

Study Number: PHA-27B

Study Date: 1990

Test Article:	Not specified		
Species/strain:	Dog/mongrel - gender not specified		
Number of animals:	Not specified		
Concentrations Evaluated:	Study	LE	(PJ-91)
	RBC partition	3.8,7.5, 15.1, 30.2, 45.3 & 67.9 μg/ml	2, 10 & 20 μg/ml
	Protein binding	6.1, 12.4 & 18.5 μg/ml	5, 10 & 15 μg/ml

Results and Conclusions: LE and its primary metabolite (PJ-91) are highly bound to protein, 95 and 73 %, respectively. LE has a erythrocyte partition coefficient of approximately 7.8 which is approximately 30 times greater than PJ-91.

Study:	Hydrolysis of loteprednol etabonate in plasma samples from rats, rabbits, beagles and humans and human liver In Vitro	
Study Number:	PHA-5A	
Study Date:	1990	
Test Article:	Not specified	
Species/strain:	Species	Tissue evaluated
	Human/healthy male volunteers	plasma & liver
	Rabbit/New Zealand	plasma
	Dog/Beagle	plasma
	Rat/Sprague Dawley male	plasma
Doses Evaluated:	8 μg	
Incubation times:	Plasma: 5, 10, 20, 30 or 60 min Liver: 5, 10 or 30 min	

Results and Conclusions:

LE following incubation for 60 min in human, dog or rabbit plasma is not hydrolyzed, however, rat plasma hydrolyzes LE completely within 30 min of incubation (table 16).

Homogenates of human livers hydrolyzes LE into two HPLC peaks, while evaluation of the ability of the liver to degrade LE was not done in the other species.

Based on the results from this study the pharmacokinetics of LE in humans is different than rats and humans may have greater body burdens than rats.

Table 16 The effects of plasma and liver homogenates on LE metabolism

Species	Plasma	Liver homogenate
Human	No hydrolysis	27% hydrolysis after 30 min of incubation
Rabbit	No hydrolysis	Not determined
Dog	No hydrolysis	Not determined
Rat	Rapid and complete within 30 min with the appearance of two new peaks	Not determined

Study:	A preliminary evaluation of the ocular absorption and distribution of ^{14}C-loteprednol etabonate in rabbits
Study Number:	PHA-25
Study Date:	May 25, 1990
Test Article:	^{14}C labeled in the 4 position was synthesized by The source and identity of the unlabeled loteprednol was unspecified.
Species/strain:	Rabbit/New Zealand
Weights:	2 to 2.5kg
Number of animals:	3 or 4 per dose (gender not specified)
Route:	Topically to the eye
Dose volume:	50 μl
Doses Evaluated:	3 μmoles or 26 μCi or 0.5 μmoles or 4.33 μCi
Dosing Schedule:	Administered to each eye 3 time within 5 min.
Vehicle:	Clinical formulation from
Observation times:	0.5, 1, 2, 4, 6 & 8 hrs after administration

Results: Ocularly applied ^{14}C-loteprednol etabonate results in distribution of parent compound into the conjunctiva, cornea, iris/ciliary body, and aqueous humor, and no detectable levels in blood. The peak concentrations were achieved within the first 0.5 to 1 hr after administration and diminished to the lowest concentration by 6 to 8 hr.

Concentration of metabolites were highest in the cornea and peak concentration were observed at 0.5 hr after administration.

Relative concentration of loteprednol and its metabolites are shown in Table 17.

Table 17 Concentration of loteprednol and its metabolites in the eye at 0.5 hr after administration.

	Concentration (nmoles/g)	
	Lotoprednol etabonate	Metabolites
Conjunctiva	30.5	3.5*
Cornea	3.8	4.9
Iris/Ciliary Body	1.9	0.4
Aqueous Humor	0.027	0.017

*N=3

The amount of radioactivity (dpm) in plasma, both in aqueous and in organic extracts, was not significantly higher than the background activity

Conclusion: Relatively small amounts of the administered dose in rabbit eyes are absorbed, and once absorbed are metabolized to putative metabolites by esterases.

Study:	Preliminary evaluation of Oral Absorption and Distribution of the Steroidal anti-inflammatory drug Loteprednol Etabonate in Rats
Study Number:	PHA-26
Study Date:	4/18/90
Test Article:	Not specified
Species/strain:	Rat/Sprague-Dawley
Weights:	Gender and weight not specified.
Number of animals:	5 per dose
Route:	p.o. by gavage following CO_2 sedation
Dose volume:	Not specified
Doses Evaluated:	5 mg/kg (50μCi/kg)
Dosing Schedule:	Once

Vehicle:	Suspended in 1% hydroxymethylcellulose
Method and frequency of test article formulation:	Not specified
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	not specified
Observation times:	0.5, 1, 2, 3, 4 and 5 hrs after dosing
Level of detection:	Not specified

Results and Conclusions: Loteprednol and its primary metabolite (PJ-91) partition based on pH and can be separated. Figure 8 show that the highest concentration of parent drug is found in the liver and concentrations of the primary metabolite PJ-91 are in the liver and kidney.

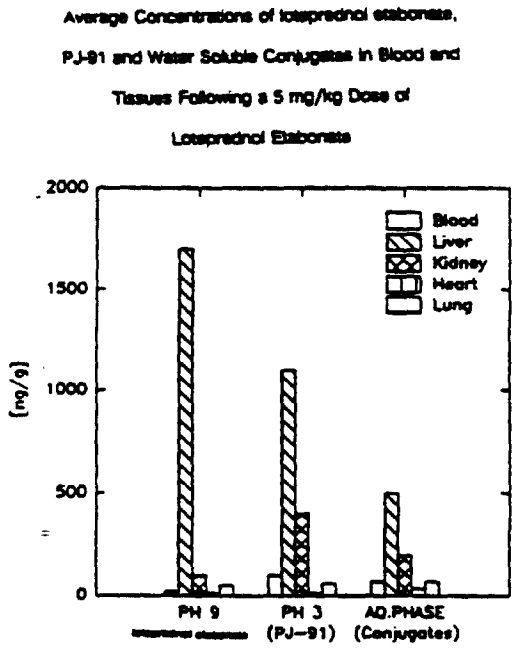

Figure 8 The mean concentration of LE and PJ-91 and water soluble conjugates in various tissues

Conclusion: Loteprednol is absorbed following oral administration. Concentration in plasma are low (<33 ng/ml) and highest in liver (up to 1.9 μg/g). It is slowly absorbed and extensively broken down to metabolites which reach a plateau in 2-3 hrs after administration.

Genotoxicity:

Study:	Mutagenicity Study of OPC-5604 by Ames Test
Study Number:	PTC/1431
Study Date:	11/25/82 to 12/9/82
Test Article:	Test article - Lot No.2J88M
Species/strain:	E. coli S. typhimurium
Concentrations Evaluated:	Test article - 0.5, 1, 5, 10, 50 and 100 μg/plate
Concentration rational:	Test article precipitates at 100 μg/plate
Vehicle:	Dimethyl sulfoxide (DMSO)
Method of test article formulation:	Test article was aseptically weighed and serially diluted with DMSO to obtained the desired concentration.
Accuracy and stability of test article formulation:	The homogeneity and stability of the test solutions were evaluated in Analytical Certificate No. 821115 (Data not included in the report).
Preparation of rat liver microsomal S-9 fraction:	Liver microsomal enzymes were induced in Sprague-Dawley rats by i.p. phenobarbital and β-naphthoflavone and prepared from the supernatant after homogenization and centrifugation at 9000x g.
Statistical Analysis:	None reported

Results and Conclusion: The validity of the test procedure was confirmed by the positive control substances. LE at the limit of solubility (between 10 and 50 μg/plate) did not induce gene mutations in the bacterial strains evaluated with and without S9 activation.

Study:	Metaphase Analysis of Human Lymphocytes Treated with P-5604 (Loteprednol)
Study Number:	PTC/10/M
Study Date:	March 1988

Test Article:	Batch 92-001
Species/strain:	Human lymphocytes - and - - The liver microsomal fraction was prepared according to the method of Ames et al (Mutation Research 31:347, 1975.) (Liver microsomal enzymes had been induced by pretreatment of the rats for 5 days
Doses Evaluated:	Test article: 6.25, 12.5, 25 and 50μg/ml. (50μg/ml was the limit of solubility)
Vehicle:	Dimethyl sulfoxide (DMSO)
Method and frequency of test article formulation:	Loteprednol was dissolved in 100 times the required concentration in DMSO. All solutions were prepared immediately before use.
Accuracy and stability of test article formulation:	Not specified
Statistical Analysis:	Compared using method of Chi Squared

Results and Conclusion: In the presence and absence of rat liver microsomal faction LE caused no statistically significant increases in chromosome aberration in human lymphocytes.

It should be noted that by inspection the data appear to show no differences from control, and the statistical analysis does not appear to be appropriate for the comparisons that the sponsor has made.

Study:	Mutation in L5178Y Mouse Lymphoma Cells
Study Number:	PTC 24596
Study Date:	October 1990
Test Article:	Batch 137 and 156
Species/strain:	Mouse/L5178Y lymphoma cells
Concentrations Evaluated:	Test article - 0.075, 0.15, 0.75 and 1.5 μg/ml

Concentration rational:	1.5 μg/ml was the limit of solubility in the test media
Vehicle:	Dimethyl sulfoxide (DMSO)
Method and frequency of test article formulation:	Prepared fresh for each experiment
Accuracy of test article formulation:	not specified
Stability of test article formulation:	not specified
Duration of drug exposure:	Cells were exposed to test compounds for 4 hours before cells were centrifuged, washed and resuspended.
Duration of observation:	Minimum of 12 days.
Preparation of rat liver microsomal fraction:	Liver microsomal enzymes were induced in Fischer 344 rats by
Statistical analysis:	All individual mutation assessment points are compared to the controls, using the comparison of multiple treatments with the control described in "Statistical Evaluation of Mutagenicity Test Data" (Ed. D.J. Kirkland).

Results and Conclusion: The viability of the test system and the S-9 liver microsome fraction to metabolizing a genotoxic intermediate (BP) was established. In this system loteprednol was not found to induce a point mutations at the TK locus of L5178Y TK +/- cells with or without metabolic activation.

Study:	Mutagenicity Study of OPC-5604 (loteprednol)
Study Number:	PTC/1430
Study Date:	From 11/29/82 to 12/2/82
Test Article:	Lot N. 2J88M
Species/strain:	

Doses Evaluated:	Test article: 1.2, 12, 120, 1200 and 12,000 μg/disc. Positive control: Negative control:
Vehicle:	Dimethyl sulfoxide (DMSO)
Method and frequency of test article formulation:	Loteprednol was aseptically weighed, and qs with DMSO to prepare stock solution. This solution was serially diluted with DMSO
Accuracy and Stability of test article formulation:	Analytical certificate No. 821115

Results and Conclusion: , a protein synthesis inhibitor, inhibited the growth of both strains to a similar extent, while , DNA-damaging substance, inhibited only the growth of

Loteprednol at Log_{10} concentrations from 1.2 to 12,000 μg/disc had no effect on either strain of B. subtilis.

Study:	Mouse Micronucleus Test -Loteprednol Etabonate
Study Number:	PTC 24595
Study Date:	July 1990
Test Article:	No batch or lot specified
Species/strain:	Mouse/Charles River CD-1 outbred
Weights:	Males 31.2 ± 2.0 g Females 25.3 ± 1.8 g
Number of animals:	15 per sex per dose for test article 5 per sex per dose
Route:	Test article - p.o. Positive control
Dose volume:	p.o. - 10 ml/kg i.p. - 10 ml/kg
Doses Evaluated:	Test Article - 1, 2, and 4 g/kg
Dosing Schedule:	

Vehicle:	0.5 % carboxymethylcellulose saline
Method and frequency of test article formulation:	Loteprednol was suspended in 0.5% carboxymethyl cellulose. dissolved in saline.
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	Not specified
Observation times:	Test Article - 24, 48 and 72 hours after dosing : - 24 hour after dosing
Proof of absorption:	Not determined
Statistical analysis:	Assessment of whether numbers of micronuclei scored differed significantly between groups of control and treated animals was by the Mann-Whitney U test.

Results: Treatment with oral doses of 1, 2 or 4 g/kg of LE was not found to induce micronuclei in the bone marrow of mice.

Conclusion: In previous studies in mice the maximally tolerated dose of LE was determined to be 4 g/kg and this dose had no effect on the induction of chromosomal or spindle damage in developing erythrocytes in bone marrow.

Studies with PJ-90 (Secondary Metabolite):

Study:	PJ-90 Primary Eye Irritation Test in the Rabbit
Study Number:	A/E 40367
Study Date:	April 1994
Test Article:	Batch Number 165.98-M
Species/strain/sex:	Rabbits New Zealand White - female
Vehicle:	Not specified
Route:	Ocular in right eye
Dose volume:	0.1 ml
Number of animals:	1 in preliminary screen 5 for study

Dose:	0.1 ml of a 0.1% (w/v) - The rabbits are restrained in wooded stocks and the material is instilled into the right eye by pulling away the lower lid to form a cup into which the material was placed. The lid was then held shut for a few seconds and moved about to distribute the test material around the surface of the eye and lid.
Observation times:	One, 24, 48, and 72 hrs and 7 days after instillation the eyes were macroscopically assessed for damage or irritation to the cornea, iris and conjunctivae using the untreated eye as a control.
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	Not specified
Method and frequency of test article formulation:	Not specified
Accuracy of test article formulation:	Not submitted
Stability of test article formulation:	No data presented to support the stability of the formulation.

Results:

Slight conjunctival redness was observed after 1 hr which resolved by 24 hrs.

Study:	PJ90 Acute Subcutaneous Toxicity in the Rat
Study Number:	A/MISC/40368
Study Date:	May 1994
Test Article:	Batch 165-98-M
Species/strain:	Rat/Crl:CD(SD)BR (VAF plus)
Vehicle:	Test article was suspended in 50% w/v aqueous solution of 2-Hydroxypropyl-β-cylodextrin prepared on the day of dosing and mixed thoroughly before and during dosing.50% w/v aqueous solution of 2-Hydroxypropyl-β-cylodextrin prepared on the day of dosing and mixed thoroughly before and during dosing

Route:	Subcutaneous	
Dose volume:	10 ml/kg	
Number of animals:	Range finding	1 per sex per dose
	Maximal Tolerated Dose (MTD)	5 per sex
Dose:	Range finding	10, 30, 100 mg/kg
	MTD	100 mg/kg
Observation times:	Both studies on day 1	Continuously for first 30 minutes and then at 1, 2, and 4 hr after dosing.
	Range finding	At least once daily for 7 days following single oral dose
	MTD	At least once daily for 14 days following single oral dose. Animals were necropsied.
Weights:	Range finding & MTD	Approximately 100g and age 4-6 weeks

Results:

No deaths were observed in the 7 day range finding or the 14 day MTD studies and the weight gains were those expected. Proof of absorption was not determined.

Conclusion:

An acute subcutaneous dose of 100 mg/kg was tolerated.

Study:	PJ-90 (0.5%) 28 Day Ocular Tolerance Study in Rabbits
Study Number:	PTC/92/A
Study Date:	June - 1994
Test Article:	PJ-90 Lot No. 165.98M

Species/strain:	Rabbit/New Zealand
Weights:	Males; 2.4 to 3.0 kg Females, 2.5 to 3.1 kg
Number of animals:	6 per sex per dose
Route:	Topical in the right eye
Dose volume:	0.1 ml
Doses Evaluated:	Vehicle, 0.5%,administered once daily for 28 consecutive days
Vehicle:	50% w/v aqueous 2 hydroxypropyl-β-cyclodextrin
Method and frequency of test article formulation:	Formulation prepared weekly
Accuracy of test article formulation:	Not specified
Stability of test article formulation:	No data presented in the pharmacology information to support the stability of the formulation.
Observation times:	All rabbits were observed twice daily for changes in morbidity and mortality. All visible signs of reaction to treatment were recorded daily. The appearance of the eyes were assessed daily prior to administration of test material. Body weights were recorded the first day and weekly thereafter. Food consumption was recorded weekly throughout the pre-dose and treatment periods.
Proof of absorption:	Not determined
Histopathology:	The eyes, eyelids, nictitating membrane and optic nerves were preserved in Davidson's fluid, embedded in was, cut a nominal thickness of 5μm, stained with hematoxylin and eosin and examined microscopically.

All tissues from all animals were wax embedded, cut at a normal thickness of 5 μm, stained with hematoxylin and eosin and examined microscopically.

Statistical analysis:	Body weights were analyzed by analysis using the t-tests for each sex separately. Leven's test for homogeneity of variance was also performed for all variables.

Results:

The sponsor has not provided any data to support the reliability of the dosage form or its stability.

While the statistical method used to evaluate the effects of treatment on weight gain is not appropriate, there appear to be no treatment-induced changes in weight gain of either male or female rabbits.

Macroscopic and histologic examination of 0.5% PJ-90, the primary metabolite of LE, treated rabbit eyes showed no differences from the contralateral vehicle treated eyes.

Conclusion:

The 28-day rabbit eye irritation study of PJ-90, the primary metabolite of LE, is not adequate to support of the safety of the test material in an ocular irritation test, because no data was provided to support that the formulation could reliable deliver the specified dose of test compound.