

Toxicokinetics Non-compartmental analysis, two animals per time point

Dose No.	Timepoint relative to dosing									
	Predose	+2 h	+4 h	+8 h	+12 h	+24 h	+2d [day 3]	+4d [day 5]	+8d [day 9]	+14d [day 15]*
1	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
3	(5)									
4	(5)									
5	(5)									
6	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)

Timepoints are described relative to Day 1 as the first day of dosing ie +2d = +48 h after dosing with samples taken on study day 3.

() number of animals/sex sampled.

*On Day 15, the sample was taken prior to administration of Dose 2 and 7, respectively.

Histopathology Adequate battery

Results

Mortality – Five mice died prior to scheduled necropsy; the following table from the study report documents this mortality. All animals were from the high dose group. The investigators attribute two of these deaths to abscess at the injection site and one to dry eye all in the TK group. The deaths of two HD males appear to be dose related so the total mortality in the HD group is 10% making this dose a putative LD₁₀.

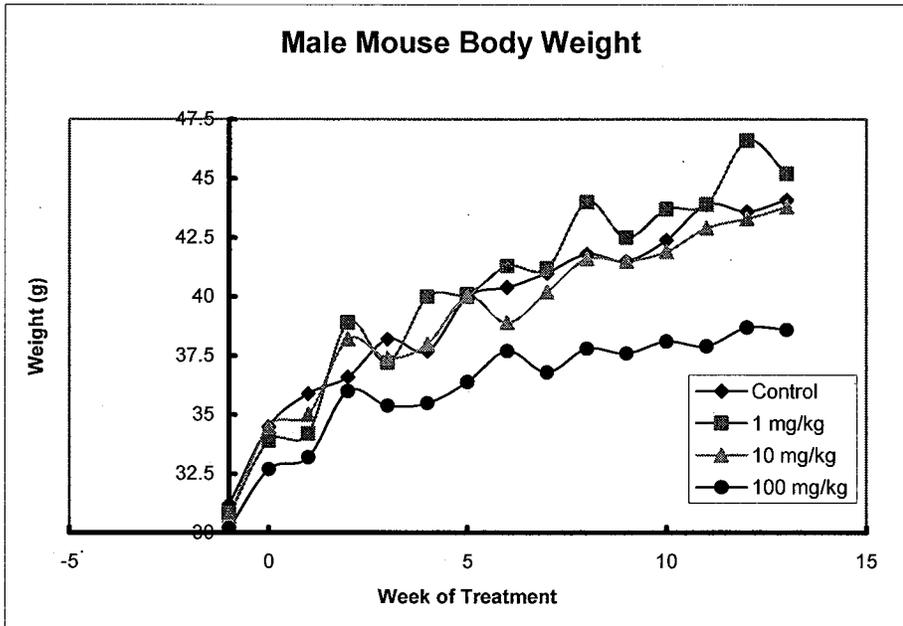
Group	Dose (mg.kg ⁻¹ .2weeks ⁻¹)	Animal Number/Sex	Week of Death	Fate	Main Clinical observations Prior to Death
4	100.0	96♂ TK	4	KP	Firm large swelling at right dorsal shoulder, ruptured with thick discharge evident
		185♀ M	11	FD	NAD
		186♀ M	5	KP	Subdued, rolling gait, hunched, piloerection, pale ears
		202♀ TK	2	KP	Eye dry
		204♀ TK	4	KP	Small swelling, ruptured with thick white discharge evident

KP = killed prematurely
 FD = found dead
 NAD = no abnormality detected
 TK = TK group
 M = Main study group

Clinical signs – Injection site damage in the HD animals included swelling in all animals. Other findings of swollen dorsal neck, thorax or abdomen were observed in all males and all but 2 females. This swelling was probably the result of rough handling during dosing.

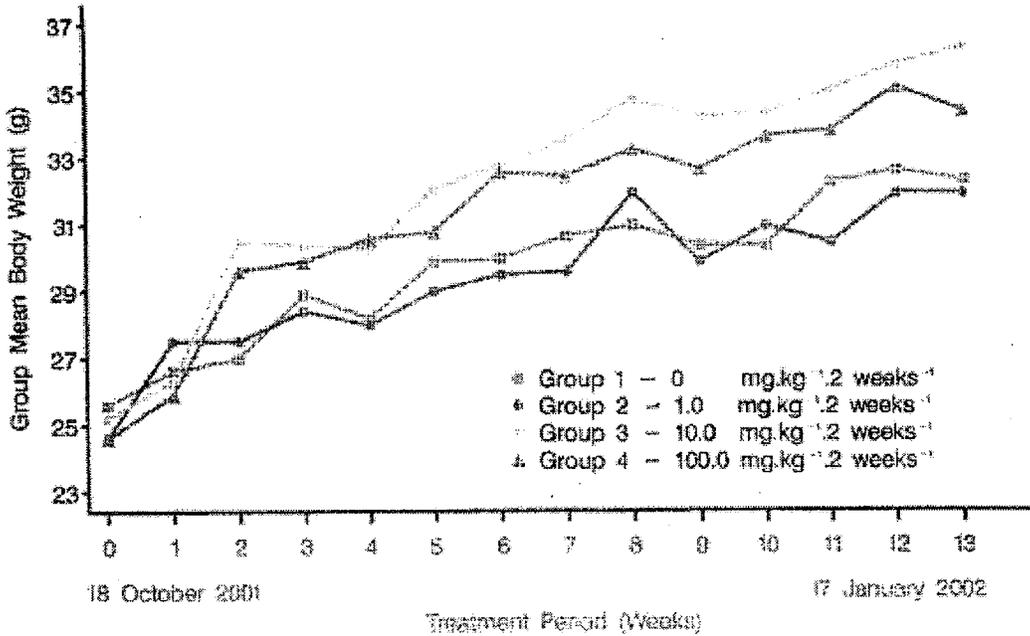
Body Weight – The following graph shows that male in the low and mid dose groups gained weight comparable to the control group but the high dose group gained significantly less weight from treatment week 1 onward.

Appears This Way
 On Original



The following graph from the study report shows that mid and high dose female mice gained more weight than controls throughout the dosing period.

FE200486
13 Week Toxicity Study in Mice with Subcutaneous Administration
Group Mean Body Weights: Main Study - Females



Food cons. – Decreased relative to controls in both mid and high dose males and females

Hematology – The following table from the study report shows that hemoglobin, MCV and MCHC were diminished in the high dose group while white cell parameters were increased. This increase in white cell parameters may be the result of an inflammatory response at the injection site.

FE200486
13 Week Toxicity Study in Mice with Subcutaneous Administration
Table 6 **Haematology: Main Study - Terminal**
Group Mean Values: Males

Group/Dose Level mg.kg ⁻¹ .2weeks ⁻¹		Hb	RBC	Hct	MCH	MCV	MCHC	WBC	Neut	Lymp	Mono	Eos	Baso	LUC
1 (0)	Number	9	9	9	9	9	9	9	9	9	9	9	9	9
	Mean	13.9	8.71	0.399	16.5	45.8	36.2	4.79	0.80	3.69	0.12	0.11	0.01	0.05
	SD	0.7	1.43	0.068	4.0	1.8	9.6	1.29	0.30	1.03	0.03	0.09	0.01	0.03
2 (1.0)	Number	8	8	8	8	8	8	8	8	8	8	8	8	8
	Mean	14.0	9.02	0.406	15.5	45.0	34.4	4.70	0.60	3.73	0.12	0.19	0.01	0.05
	SD	1.2	0.66	0.034	0.8	1.8	1.1	0.95	0.19	0.78	0.03	0.06	0.01	0.03
3 (10.0)	Number	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	13.9	9.02	0.417	15.4	46.2	33.3	6.25	0.60	5.35	0.13	0.17	0.02	0.08
	SD	0.6	0.52	0.020	0.7	1.4	1.1	0.98	0.14	0.88	0.04	0.06	0.01	0.04
4 (100.0)	Number	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	12.8	8.92	0.389	14.4	43.6	33.0	11.36	3.40	6.81	0.43	0.40	0.04	0.28
	SD	1.0	0.69	0.035	0.7	2.1	1.6	4.04	2.20	2.42	0.28	0.25	0.02	0.23
	Prob.	**			**	*	***	***	***	***	**	***	**	***

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

A similar pattern was seen in female mice.

FE200486
13 Week Toxicity Study in Mice with Subcutaneous Administration
Table 7 **Haematology: Main Study - Terminal**
Group Mean Values: Females

Group/Dose Level mg.kg ⁻¹ .2weeks ⁻¹		Hb	RBC	Hct	MCH	MCV	MCHC	WBC	Neut	Lymp	Mono	Eos	Baso	LUC
1 (0)	Number	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	15.1	9.07	0.430	16.7	47.5	35.2	5.98	0.69	4.91	0.10	0.19	0.02	0.08
	SD	0.7	0.66	0.029	0.9	1.2	1.3	3.19	0.25	2.86	0.05	0.19	0.02	0.09
2 (1.0)	Number	10	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	14.7	9.17	0.436	16.0	47.5	33.7	8.10	0.93	6.60	0.15	0.32	0.02	0.09
	SD	0.5	0.47	0.015	0.7	1.3	0.7	2.44	0.46	1.84	0.04	0.40	0.01	0.08
3 (10.0)	Number	9	9	9	9	9	9	9	9	9	9	9	9	9
	Mean	14.5	9.37	0.420	15.6	44.7	34.9	7.97	0.52	7.01	0.16	0.18	0.02	0.07
	SD	0.8	0.76	0.041	1.3	1.4	3.6	1.95	0.08	1.87	0.04	0.09	0.01	0.04
4 (100.0)	Number	8	8	8	8	8	8	8	8	8	8	8	8	8
	Mean	14.0	9.29	0.421	15.1	45.3	33.3	12.48	2.83	8.67	0.27	0.32	0.05	0.35
	SD	0.6	0.34	0.022	0.7	2.4	0.8	4.37	1.77	2.99	0.18	0.21	0.02	0.34
	Prob.	**			**	**	**	***	**	**	***	**	**	**

Significantly different from the Control: * P<0.05, ** P<0.01, *** P<0.001

Clinical chem. – There were no toxicologically significant changes in males. In females, ALT was significantly lower in dosed animals and some other protein parameter were lower suggesting the possibility of mild hepatotoxicity.

Organ Weights – The weights of sex organs in males and females were significantly lower and these changes were dose proportional. The relative weight changes suggests splenic hypertrophy in males and females, pituitary atrophy in females, salivary atrophy in males and females, atrophy of the kidneys in both sexes, adrenal hypertrophy in males and atrophy in females. The relative heart and kidney weights are decreased. The brain weight was decreased in females.

	Control value	Change from control			Relative Change*		
		Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
Male							
Body Wt	44	43	42	39			
Adrenals	0.0052	-3.8%	78.8%	44.2%	0.984	1.874	1.627
Brain	0.52	0.0%	0.0%	-5.8%	1.023	1.048	1.063
Epididymides	0.1273	-57.7%	-76.2%	-77.5%	0.432	0.249	0.253
Heart	0.27	-11.1%	-22.2%	-25.9%	0.910	0.815	0.836
Kidneys	0.699	-24.0%	-35.3%	-37.3%	0.777	0.677	0.707
Liver	2.27	0.0%	-7.9%	-8.4%	1.023	0.965	1.034
Prostate	0.032	-34.4%	-65.6%	-75.0%	0.672	0.360	0.282
Spleen	0.11	15.5%	26.4%	178.2%	1.181	1.324	3.138
Salivary Gland	0.2665	-17.9%	-32.2%	-40.6%	0.840	0.710	0.670
Testes	0.27	-33.3%	-92.6%	-92.6%	0.682	0.078	0.084
Females							
Body Weight	31	30	34	33			
Adrenals	0.0112	-5.4%	-23.2%	-15.2%	0.978	0.700	0.797
Brain	0.52	0.0%	-5.8%	-5.8%	1.033	0.859	0.885
Heart	0.19	0.0%	-15.8%	-21.1%	1.033	0.768	0.742
Kidneys	0.446	-10.8%	-25.8%	-17.5%	0.922	0.677	0.775
Liver	1.77	-15.8%	-14.7%	-6.8%	0.870	0.778	0.876
Ovaries	0.0353	-57.2%	-90.1%	-83.9%	0.442	0.090	0.152
Pituitary	0.0025	-8.0%	-52.0%	-52.0%	0.951	0.438	0.451
Spleen	0.127	-8.7%	-14.2%	82.7%	0.944	0.783	1.716
Salivary Gland	0.1709	-9.0%	-25.7%	-29.0%	0.940	0.678	0.667
Uterus	0.21	-19.0%	-85.7%	-85.7%	0.837	0.130	0.134

* (organ weight in control÷ body weight of control)÷(organ weight in treated group)÷(body weight in treated group). Values close to 1 show no change in organ weight relative to body weight.

Gross Pathology – Decrease size of sex organs and injection site damage

Histopathology –

- Injection site damage in all treated groups indicative of inflammation and necrosis
- Atrophy of both male and female sex organs increased in incidence and severity with increasing dose. In the testes, there was seminiferous epithelial degeneration, and atrophy and pigmentation of interstitial cells. In the epididymides, spermatozoa were absent or reduced; and in the ovaries, corpora lutea were absent in some animals.
- Variable necrosis and polymorphonuclear leukocyte infiltration, described as pyogranuloma in the renal capsule
- Retention of the x-zone in the adrenal cortex of mid and high dose males
- Mammary gland atrophy in females from all treated groups
- Atrophy of the granular ducts of the submaxillary salivary gland in males
- In HD animals, there was extramedullary hemopoiesis in spleen, liver and lymph nodes and increased granulopoiesis in vertebral and sternal marrow; and lymphoid hyperplasia in lymph nodes and spleen.
- Extramedullary haemopoiesis and lymphoid hyperplasia correlated with the increase in size of these organs seen at necropsy.

Toxicokinetics –

The pharmacokinetic parameters from this study were highly variable. In most cases, apparent T_{max} was at 2 hours post dosing but some values ranged as high as 12 hours. Terminal elimination half-life ranged from 31 to 800 hours. Clearance (CL/F) ranged from 265 to 2100 mL/hr*kg. Volume for distribution ranged from 17.8 to 1021 L/kg. The very large volume of distribution results from the deposition degarelix in the compartment of the SC injection site.

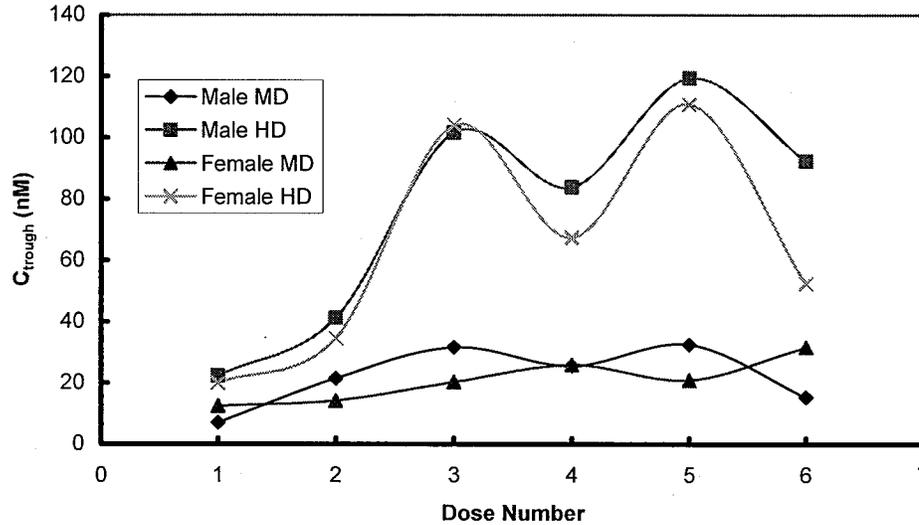
The following table shows that C_{max} is at least two orders of magnitude above k_i in humans (1.7 nM) on all but one occasion. The increase in AUC relative to the low dose is significantly less than dose proportional except in high dose females after dose 7. The increase in AUC relative to dose 1 demonstrates accumulation in the mid and high dose groups. Based on AUC_i and C_{max} data, males appear to have a higher exposure at each dose of degarelix when compared to females.

	Dose	Dose	C_{max}	Ratio to Low Dose	Ratio to Dose 1	AUC _i	AUC	Ratio to Low Dose	Ratio to Dose 1
	mg/kg	mg/m ²	μM			μM*hr	μM*hr		
Dose 1 male	1	3	0.25			2.3	2.3		
	10	30	0.28	1.1		8.7	11.0	4.7	
	100	300	0.54	2.1		26.7	29.8	12.9	
Dose 1 female	1	3	0.11			1.6	1.6		
	10	30	0.24	2.1		7.0	21.5	13.6	
	100	300	0.45	4.0		19.3	29.0	18.3	
Dose 7 male	1	3	0.10		0.4	2.1	2.8		1.2
	10	30	0.23	2.3	0.8	13.1	16.3	5.8	1.5
	100	300	1.60	15.6	3.0	81.3	100.2	35.7	3.4
Dose 7 female	1	3	0.17		1.5	1.4	1.6		1.0
	10	30	0.24	1.4	1.0	15.1	39.2	24.6	1.8
	100	300	2.97	17.3	6.7	163.7	168.3	105.5	5.8

The following graph shows the trough concentrations for the mid and high dose groups. The graph does not include values for the low dose groups because in too many instances these values were below the limit of quantitation. At most times in the mid dose group and at all times in the high dose group the trough concentration is more than five fold greater than k_i (1.7 nM) demonstrating that these animals were constantly exposed to an inhibitory concentration of degarelix. The values appear to oscillate and the pattern is the same for males and females in the high dose group. This oscillation occurred in other studies (see below) in other species but the period between peaks varies. The data could be demonstrating some real physiological phenomena.

Appears This Way
 On Original

Variation of C_{trough} as a Function of Increasing Dose in Male and Female Mice



2) 13 Week Toxicity Study in Rats with Subcutaneous Administration - RTD

Major findings:

- Body weight of female rats increased in a dose-dependent manner with degarelix.
- Degarelix-treated male rats had increased glucose, cholesterol and creatinine even at low dose (0.5 mg/kg). Female rats that received degarelix had increased alkaline phosphatase, cholesterol and triglycerides. At mid- and high-level (5 and 50 mg/kg) degarelix, glucose levels increased and albumin decreased in female rats.
- Degarelix treatment significantly decreased the size, weight and appearance of the epididymis, testes, prostate, and testis in males, and the ovary and uterus of females. Kidneys and liver absolute and relative weight decreased in males, while the pituitary and adrenal gland absolute and relative weight decreased in females. The thymus increased in weight in both male and female rats with degarelix-treatment.
- Elimination half-life ranged from 74 hrs to 844 hrs, with the half-life lengthening at higher doses. Increased doses of degarelix resulted in non-linear exposure of degarelix in the single-dose study and drug accumulation in the repeat-dose study.

Study number: FE200486DSTOX0112
project number: 455806
EDR filename: tox0112-nonclinical-data-1.pdf
Conducting laboratory:
Study date: September 21, 2001

b(4)

GLP compliance: Yes
QA report: Yes
Drug: Degarelix, Batch No. 0048262#PPL –FE4860001, 87.99% pure

Methods

Doses: 0, 0.5, 5 or 50 mg/kg (0, 3, 30, or 300 mg/m²)
Schedule: Fortnightly for 13 weeks (7 doses)
Species: Wistar rats
Number: 10 per sex per dose group
Route: Subcutaneous injection
Formulation: Mannitol solution in sterile water, 5% w/v
Volume: 5 mL/kg, the site of injection was different for repeated injections, based on evaluation of the site of injection
Toxicokinetics: Satellite group 10 per sex per dose group for TK
Age: 6 weeks old
Weight: Males (75-93 grams), Females (82-93 grams)

Mortality: Twice daily
Clinical signs: Once per week
Body weights: Once per week, more if deterioration was noted.
Food consumption: Weekly
Ophthalmoscopy: Performed on Week 5 and 12
EKG: Not measured
Hematology: Blood samples were acquired on Week 13 of treatment
Clinical chemistry: Samples were acquired on Week 13 of treatment
Urinalysis: Collected on Week 12 of the study over 4 hours
Gross pathology: Extensive pathological examination and organ weights from selected animals were obtained on Week 13.
Organ weights: Selected organs were weighed at Week 13 as is specified in the table supplied by the sponsor
Histopathology:
Adequate Battery: Yes
Peer review: Yes

Results

Mortality: No mortality was observed in any dose group throughout the study.

Clinical signs:

Swelling at the site of injection occurred at each injection site that received the highest dose, 50 mg/kg degarelix. The noted swelling led the sponsor to change of the site of injection based on an evaluation of the site prior to the next dose.

The sites of injection were as follows:

- 1.) the right scapular region (Dose 1, 2, 3, 5)
- 2.) left scapular region (Dose 7)
- 3.) left rump region (Dose 4)
- 4.) right rump region (Dose 6)

“Soft small swelling” or “soft large swelling” occurred with equal frequency in both sexes that received 50 mg/kg degarelix (see the following tables from the study report). Swelling at all sites showed partial reversibility and generally subside by the end of the study.

FE200486
13 Week Toxicity Study in Rats with Subcutaneous Administration
Selected Individual Clinical Signs for each Dose Site

Table 1

Dose Site 1 - Males

Finding	Dose 1		Dose 2		Dose 3		Dose 4		Dose 5		Dose 6		Dose 7
	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13
Soft small swelling	6	7	2	9	9	7	5	0	5	9	8	1	1
Soft large swelling	4	2	8	7	8	8	1	0	7	6	1	1	1
Firm small swelling	0	0	0	0	0	0	0	1	1	1	1	1	1
Firm large swelling	0	0	0	0	4	1	1	0	0	0	0	0	0
Number of animals in Group	10	10	10	10	10	10	10	10	10	10	10	10	10
No abnormalities detected	0	1	0	0	0	0	4	9	0	0	2	8	6

Group 4 (50 mg.kg⁻¹.2weeks⁻¹) only is presented in the above table, as these findings were not observed in any other treatment group, apart from a soft, small swelling which was seen in one Group 3 (5.0 mg.kg⁻¹.2weeks⁻¹) animal (43♂) in Weeks 5 and 6.

Dose Site 1 - Females

Finding	Dose 1		Dose 2		Dose 3		Dose 4		Dose 5		Dose 6		Dose 7
	Weeks 1	2	3	4	5	6	7	8	9	10	11	12	13
Soft small swelling	4	8	7	8	8	6	9	2	6	7	7	4	4
Soft large swelling	7	7	8	9	8	6	4	0	7	8	3	0	0
Firm small swelling	0	0	0	0	0	0	0	1	0	0	0	1	1
Firm large swelling	0	0	0	0	2	0	0	0	1	0	0	0	0
Number of animals in Group	10	10	10	10	10	10	10	10	10	10	10	10	10
No abnormalities detected	0	2	1	0	0	0	1	8	0	0	2	6	6

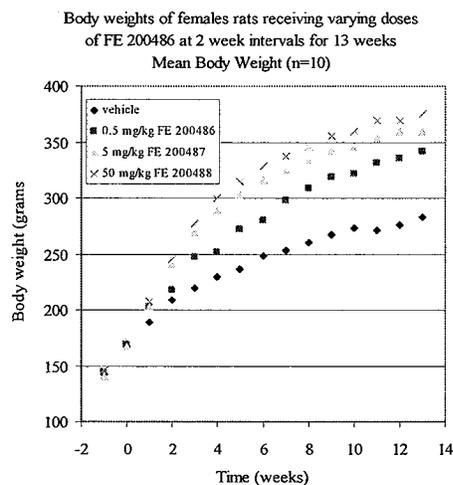
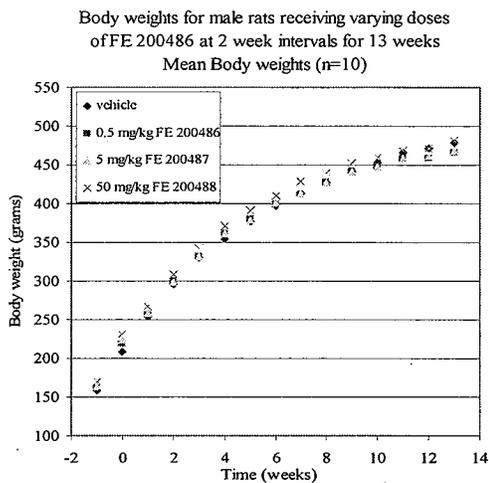
Group 4 (50 mg.kg⁻¹.2weeks⁻¹) only is presented in the above tables, as these findings were not observed in any other treatment group.
- = dose site not in use

The number of swelling events decreases over the course of the study and the number of mice with “no abnormalities detected” increases, thus this appears to be a reversible response to injection of degarelix. No other clinical signs were noted.

Body weights:

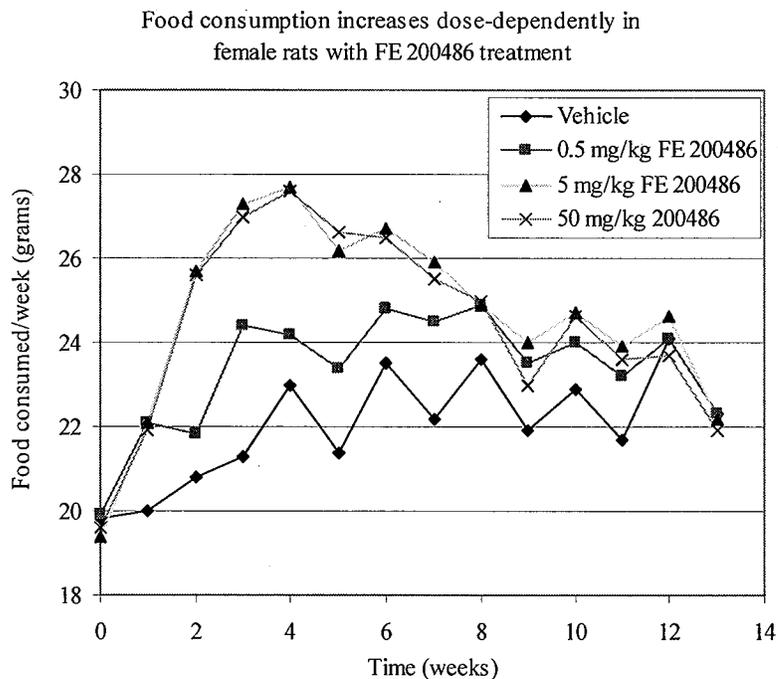
The body weights of male rats that received 0.5, 5, or 50 mg/kg degarelix were similar to the vehicle-treated group throughout the duration of the study. Female rats showed a dose-dependent increase in body weight that began one week after the first treatment with degarelix and maintained throughout the study.

Appears This Way
On Original



While there was no apparent effect of degarelix on the body weight of male rats, the standard deviation of male rat weights increased with degarelix treatment.

Food consumption:



Ophthalmoscopy:

The vehicle-treated group, 0.5 mg/kg, 5mg/kg and 50 mg/kg degarelix-treated groups had “mild diffuse central corneal opacity” or “small central cortical lens opacity.” Because these observation were made for both control and drug-treated groups, it is does not appear to be a drug-related response; however, rats that received any dose of degarelix had a marginal increase in rats with noted incidents of

corneal or lens opacity. The lens opacity may be caused by the vehicle, though this would only be confirmed with further study.

No. of rats (of 20 total)	Number of rats with noted events in the eye*			
	Vehicle	0.5 mg/kg	5mg/kg	50 mg/kg
	9	13	17	13

* Noted events are "mild diffuse central corneal opacity" or "small central cortical lens opacity"

Hematology:

Male rat blood cell counts that changed with Degarelix treatment

	RBC	MCV	lymphocyte	Eosinophil	PT
Vehicle	8.75	47.4	8.06	0.19	16
0.5 mg/kg Degarelix	8.12**	49.4**	9.96*	0.23	17**
5 mg/kg Degarelix	8.42	48.3	10.67**	0.27*	17**
50 mg/kg Degarelix	8.35*	48.3	9.76*	0.31***	17

Significant difference versus control: *P<0.05, ** P<0.01, ***P<0.001

Female rats that received 0.05 mg/kg degarelix (low dose) experienced an increase in hemoglobin, RBCs, hematocrit, white blood cells, lymphocyte, eosinophils and basophils. The moderate dose, 5 mg/kg degarelix, caused an elevation of neutrophils, monocytes and LUC. Finally, 50 mg/kg degarelix (high dose) caused an increase in reticulocytes. The increase in each of the white blood cell types was most dramatic in female rats and occurred even at the lowest dose of degarelix (0.5 mg/kg).

Female rat blood cell counts that changed with degarelix treatment

	Hb	RBC	Hct	Retic	WBC	Neut	Lympho	Mono	Eos	Baso	LUC
Vehicle	13.9	7.64	0.389	2	6.27	1.57	4.34	0.12	0.13	0.01	0.1
0.5 mg/kg Degarelix	14.8***	8.33**	0.415**	2	11.33***	1.89	8.82***	0.19	0.24*	0.03*	0.15
5 mg/kg Degarelix	14.5**	8.09**	0.41*	2.1	13.49***	3.04*	9.75***	0.21*	0.24*	0.04***	0.2**
50 mg/kg Degarelix	14.1	8.15*	0.4	3.7**	14***	4.33***	8.85***	0.27***	0.31***	0.05***	0.21***

Significant difference versus control: *P<0.05, ** P<0.01, ***P<0.001

Clinical chemistry:

Male rats that received 0.5 mg/kg degarelix had an increase in glucose, while high dose (50 mg/kg) degarelix increased glucose levels in female rats. An increase in glucose levels did not occur in other dosing groups of either sex. Cholesterol levels were elevated with degarelix treatment at all doses (0.5, 5 and 50 mg/kg) in both sexes. Low, moderate and high dose degarelix increased creatinine levels in male rats; however this was not evident in female rats. Degarelix treatment increased alkaline phosphates, potassium, albumin and triglycerides only in the female rat population. The elevated triglyceride response in female rats was the most dramatic of these responses in females and may be correlated to increased food intake that was observed with degarelix treatment.

Clinical chemistry data from males receiving Degarelix every 2 weeks for 13 weeks

Degarelix (mg/kg)	Glucose	Cholesterol	Creatinine
Vehicle	8.63	2.3	49
0.5	9.61**	2.8**	52**
5	8.77	2.8**	51*
50	8.65	3***	53***

Significant difference versus control: *P<0.05, ** P<0.01, ***P<0.001

Clinical chemistry data from females receiving Degarelix every 2 weeks for 13 weeks

Degarelix (mg/kg)	Glucose	Alkaline phosphatase	Potassium	Albumin	Cholesterol	Triglycerides
Vehicle	9.08	200	3.4	43	2.2	0.71
0.5	8.68	281**	3.8*	42	2.9**	1.46***
5	9.22	245	4.1**	41*	3.2***	1.37***
50	10.18*	244*	3.7	40***	3.5***	1.05**

Significant difference versus control: *P<0.05, ** P<0.01, ***P<0.001

Urinalysis: Unremarkable

Gross pathology:

Organ	General comments	Males				Females			
		FE200486 dose (mg/kg)				FE200486 dose (mg/kg)			
		0	0.5	5	50	0	0.5	5	50
	Number of animals necropsied	10	10	10	10	10	10	10	10
Epididymis	Small, both		9	9	8				
	Pale, both								1
	Dark, both					2			
Ovary	Small, both					9	9	9	10
Prostate	Small		10	10	8				
Seminal vesicle	Small, one/both horns		9	10	8				
	Lesion, both				1				
	Abnormal position, both		2	1	2				
Testis	Small, one/both		10	10	9				
	Dilated, with fluid, both horns					1			
Uterus	Small, one/both horns					9	9	9	10

Organ weights:

Organ weights affected by Degarelix treatment administered for 13 weeks at 2 week intervals

Male rats

Degarelix (mg/kg)	Adrenal glands	Epididymides	Kidneys	Liver	Prostate	Testes	Thymus
Vehicle	0.0615	1.3118	2.9	18.72	0.61	3.83	0.381
0.5	0.0853***	0.2096***	2.52**	15.6**	0.019***	0.37***	0.668***
5	0.0787***	0.1812***	2.46***	15.15***	0.023***	0.38***	0.564**
50	0.079**	0.1681***	2.43***	15.51**	0.026***	0.37***	0.607***

Significant difference versus control: *P<0.05, ** P<0.01, ***P<0.001

Female rats

FE 200486 (mg/kg)	Adrenal glands	Liver	Lung	Ovaries	Pituitary	Spleen	Thymus	Uterus
Vehicle	0.0946	10.55	1.43	0.111	0.015	0.7	0.278	0.72
0.5	0.0691***	10.67	1.53	0.03***	0.009***	0.83*	0.592***	0.2***
5	0.0684***	11.69	1.64***	0.025***	0.009***	0.84**	0.591***	0.15***
50	0.0699***	11.95*	1.67***	0.027***	0.009***	0.9***	0.562***	0.16***

Significant difference versus control: *P<0.05, ** P<0.01, ***P<0.001

Histopathology:

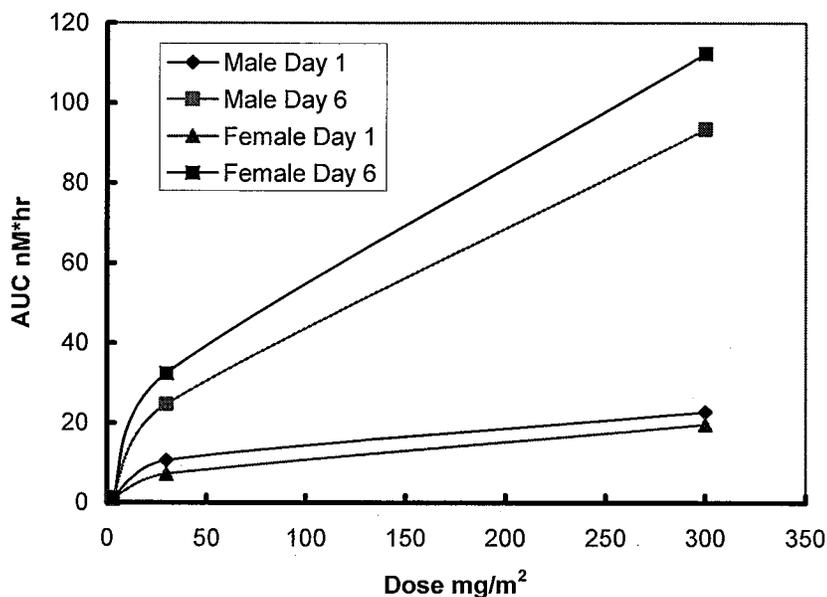
Organ	Macroscopic findings	Males				Females			
		Degarelix dose (mg/kg)				Degarelix dose (mg/kg)			
		0	0.5	5	50	0	0.5	5	50
Lung	No abnormality detected	9/10	1/1		10/10	7/9	2/2		9/10
	Alveolar foamy macrophage accumulation	1/10				2/9			1/10
	Agonal congestion/hemorrhage					1/9	2/2		1/10
Lymph node (mandibular)	No abnormality detected	3/10	0/5	0/4	1/10	4/10	0/2	0/1	8/10
	Reactive hyperplasia	7/10	5/5	3/4	9/10	6/10	2/2	1/1	2/10
	Lymphangiectasis	0/10	0/5	1/4	0/10	0/10	0/2	0/1	0/10
Spleen	No abnormality detected	9/10			5/10	10/10			10/10
	Hemopoiesis, increased								
	-Minimal	1/10			5/10	0/10			0/10
	-Total incidence	1/10			5/10	0/10			0/10
Thymus	No abnormality detected	10/10	1/1	1/1	10/10	7/10	1/1		10/10
	Tubular cystic hyperplasia	0/10				3/10		1/1	
	Agonal congestion/hemorrhage	0/10			1/10		1/1		

Toxicokinetics:

Exposure did not increase proportional to increasing dose as shown in the following graph.

Appears This Way
On Original

Dose vs AUC



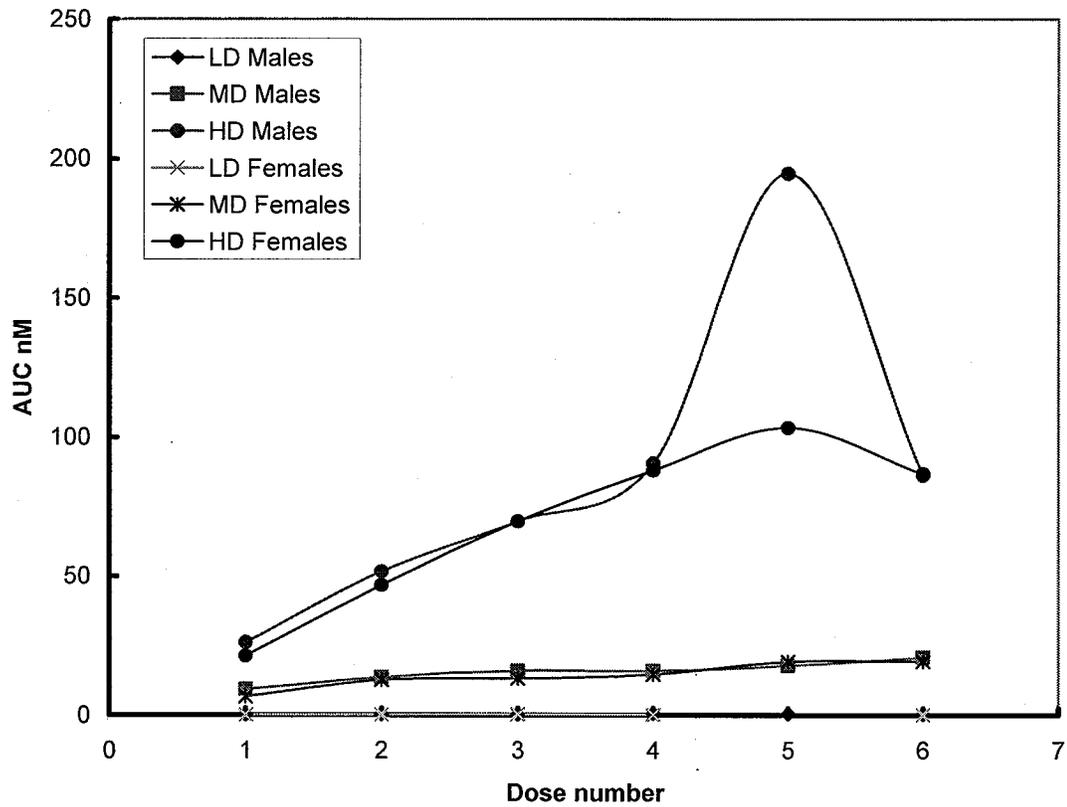
The following table shows the pharmacokinetic parameters determined in this study.

Gender	Dose (week #)	doses mg/kg	doses mg/m ²	C _{max} ng/g	C _{max} μM	AUC ng*hr/ml	AUC μM*hour	T _{max} hour	t _{1/2} hours
male	1	0.5	3	86	0.053	1530	0.94	2	77
male	1	5	30	129	0.079	17352	10.63	2	416
male	1	50	300	166	0.102	37104	22.73	4	227
female	1	0.5	3	95	0.058	1464	0.90	2	74
female	1	5	30	150	0.092	11785	7.22	2	223
female	1	50	300	208	0.127	32017	19.61	4	272
male	6	0.5	3	81	0.050	2134	1.31	2	85
male	6	5	30	115	0.070	40355	24.72	4	506
male	6	50	300	348	0.213	152600	93.49	48	466
female	6	0.5	3	86	0.053	2125	1.30	2	52
female	6	5	30	131	0.080	52962	32.45	4	844
female	6	50	300	321	0.197	183486	112.41	8	581

Values in bold type were determined from values of lambda z that the study pharmacokineticist considered unreliable.

The following graph shows that steady state was not reached in the mid and high dose groups until after the 5th dose. Except for one data point males and female values are remarkably consistent. The C_{trough} values for the low dose group fell below binding constant (1.7 nM) at all time points. The values in the mid dose group were about 10 fold greater than the binding constant demonstrating constant inhibitory concentrations in both the low and mid dose groups.

Dose vs C_{trough}



3) 2 Week Subcutaneous Toxicity Study in the Rat Followed by an 8 Week Treatment Free Period – WDM

Major Findings:

Two weeks of daily dosing with doses as high as 3 mg/kg (18 mg/m²) in male and female rats caused no dose related mortality. All doses levels caused significant atrophy of the sex organs in both males and females. Changes in clinical chemistry parameters, decreases in organ weight and hepatic perilobular vacuolization suggest the possibility of mild to moderate liver and kidney damage. In the heart there was a dose dependant increase in incidence of myocardial degeneration with interstitial fibrosis, though toxicologically significant this change did not reach statistical significance.

Study number	FE 200486 DS TOX 9802
_____ number	505:552
EDR filename	tox9802-nonclinical-data-1.pdf
Conducting laboratory	_____
Date of study initiation	May 1998
GLP compliance	Yes
QA reports	Yes
Drug	Degarelix, Batch 201 1-001-30, > 98% peptide purity Peptide content 86.44 %

b(4)

Methods

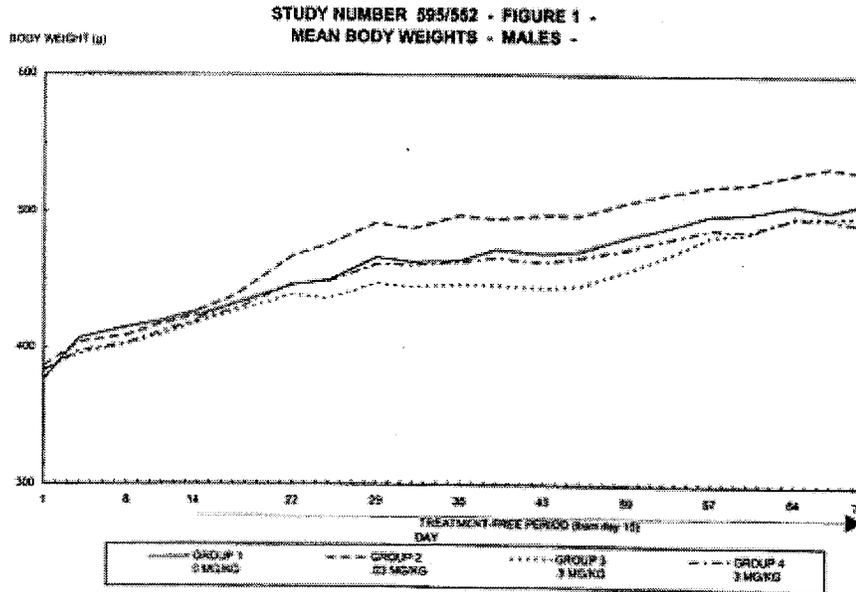
Animal	Sprague-Dawley rats
Doses	0, 0.03, 0.3 or 3 mg/kg, (0, 0.18, 1.8 or 18 mg/m ²) Expressed as peptide content Groups 1, 2, 3 and 4 respectively
N	15 (+9 satellite animals) per sex per dose group
Schedule	Daily for 14 days
Route	Subcutaneous injection
Dose volume	0.4 mL/kg
Formulation	Mannitol _{aq} 5% w/v (vehicle control)

Observations

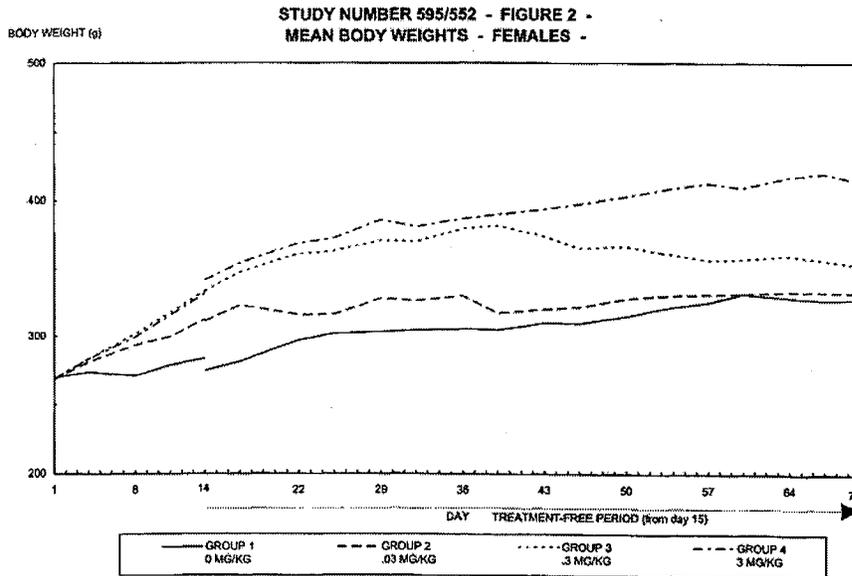
Mortality	Twice daily
Clinical signs	Twice daily
Body Weight	Twice weekly
Food cons	weekly
Hematology	Pretest, weeks 2, 4 and 10
Urinalysis	Pretest, weeks 2 and 10
Ophthalmology	Pretest, weeks 2
Deoxyuridine	Pretest, weeks 2, 6 and 10
Testosterone	Pretest, weeks 2 and 10
Toxicokinetics	Not reported
Necropsy	Week 2 (10 per group), Week 10 (5 per group)
Toxicokinetics	Days 1 and 15
Histopathology	Adequate battery

Results

Mortality – No unscheduled deaths
Clinical signs – Some injection site damage, some edema
Body Weight – dose related decreased weight gain in males, reversible. The graph below from the study report demonstrates these changes.



The graph below from the study report demonstrates the dose related increased weight gain in females.



- | | |
|-----------------|---|
| Food cons – | No dose related effects in males, dose related increased consumption in females |
| Ophthalmology – | No toxicologically significant effects |
| Estrus cycle – | Completely blocked at end of dosing in mid and high dose females; mid dose animals showed signs of recovery at 10 weeks but high dose females remained blocked |
| Hematology – | Numerous parameters were significantly deranged suggestive of a mild to moderate generalized inflammatory response and possibly decreased red cells production. White cell parameters remained elevated after 8 weeks recovery. |

Parameter	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
Hematology					
	Hb male Week 2	15.6	-1.3%	-2.6%	-5.1%
	PCV male Week 2	44.2	0.5%	-0.7%	-3.2%
	RBC male Week 2	8.12	-1.6%	-0.4%	-4.4%
	Platelet male Week 2	928	-3.6%	-6.4%	10.7%
	APTT male Week 2	21.7	-7.4%	-13.4%	-21.2%
	WBC male Week 2	8.6	3.5%	3.5%	1.2%
	Lymphocytes male Week 2	7.4	8.1%	2.7%	-6.8%
	Hb female Week 2	14.9	-0.7%	0.7%	-4.7%
	PCV female Week 2	43	0.7%	0.2%	-3.3%
	RBC female Week 2	7.7	-2.9%	-4.2%	-6.2%
	Platelet female Week 2	957	-5.7%	-4.0%	8.6%
	APTT female Week 2	17.6	-3.4%	-4.5%	-6.8%
	WBC female Week 2	5.1	51.0%	58.8%	66.7%
	Lymphocytes female Week 2	4.6	50.0%	56.5%	63.0%
	MCV male Week 10	50	4.0%	6.0%	8.0%
	WBC male Week 10	8.1	-6.2%	19.8%	33.3%
	Lymphocyte male Week 10	6	5.0%	33.3%	51.7%
	WBC female Week 10	5	36.0%	46.0%	84.0%
	Lymphocyte female Week 10	4.4	29.5%	40.9%	70.5%

Bone Marrow – Decreased erythroid cells in HD males and females consistent with decreased red count (slightly increased M/E ratio). These changes were also seen in one LD male.

Clinical chem. – Numerous parameters were significantly deranged suggestive of mild to moderate hepatic and renal damage. Some of these parameters did not completely normalize after 8 weeks recovery. Glucose was elevated in recovery animals but not at the end of dosing.

Clinical Chemistry	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
	Sodium male Week 2	143	-0.7%	-0.7%	-0.7%
	Potassium male Week 2	4.4	2.3%	6.8%	6.8%
	Cholesterol male Week 2	0.62	22.6%	29.0%	46.8%
	Albumin male Week 2	37	-2.7%	-5.4%	-5.4%
	Protein male Week 2	64	-4.7%	-3.1%	-1.6%
	Alk Phos male Week 2	297	-12.1%	-15.5%	-15.8%
	Potassium female Week 2	4.1	4.9%	4.9%	14.6%
	Cholesterol female Week 2	0.71	11.3%	-1.4%	18.3%
	Protein female Week 2	64	-6.3%	-6.3%	-7.8%
	Albumin female Week 2	39	-5.1%	-5.1%	-10.3%
	Alk Phos female Week 2	221	23.1%	32.6%	25.3%
	Cholesterol male Week 10	0.85	2.4%	-15.3%	24.7%
	Creatinine male Week 10	5.8	1.7%	8.6%	15.5%
	Glucose male Week 10	1.29	-4.7%	7.8%	7.0%
	Glucose female Week 10	1.3	6.2%	16.2%	16.9%
	Cholesterol female Week 10	0.86	15.1%	8.1%	37.2%

Urinalysis – Urinalysis indicated increased excretion of sodium, potassium, chlorine and calcium with a concomitant increase in urine volume. This is consistent with the possibility of mild to moderate kidney damage demonstrated above.

Urinalysis	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
	Volume male Week 2	13.2	28.0%	6.1%	21.2%
	Na male mEq/vol Week 2	0.73	47.9%	34.2%	54.8%
	K male mEq/vol Week 2	1.16	15.5%	8.6%	11.2%
	Cl male mEq/vol Week 2	0.65	38.5%	20.0%	44.6%
	Ca male mg/vol Week 2	0.27	159.3%	159.3%	414.8%
	Volume female Week 2	10.6	18.9%	42.5%	33.0%
	Na female mEq/vol Week 2	0.75	24.0%	41.3%	37.3%
	K female mEq/vol Week 2	0.92	16.3%	57.6%	33.7%
	Cl female mEq/vol Week 2	0.66	18.2%	57.6%	37.9%
	Ca female mg/vol Week 2	1.15	-13.9%	20.9%	-9.6%
	Na male mEq/vol Week 10	0.67	9.0%	-43.3%	-7.5%
	Cl male mEq/vol Week 10	0.45	6.7%	-46.7%	-2.2%
	Na female mEq/vol Week 10	0.64	10.9%	-48.4%	15.6%
	Cl female mEq/vol Week 10	0.55	-20.0%	-56.4%	-5.5%

Deoxyypyridinoline – Elevated in all treated animals at week 2 but this difference did not reach significance. It was significantly elevated at week 6 but showed signs of significant recovery at week 10. This indicates increased bone resorption.

Hormone Conc. – The following tables from the study report show that LH was decreased with concomitant decreases in estradiol and testosterone consistent with the proposed mechanism of the drug.

Appears This Way
 On Original

Plasma LH (% difference compared with controls)

Sex Group	Dose level (mg/kg/day)	Day 14								
		15 min.	1 h	4 h	8 h	12 h	24 h	W 4	W 6	W 10
M2	0.03	-100 %	10 %	-28 %	-34 %	-38 %	-11 %	-14 %	-24 %	-1 %
M3	0.3	-23 %	-3 %	-21 %	-26 %	-27 %	-12 %	-39 %	-39 %	22%
M4	3	-27 %	12 %	-32 %	-49 %	-37 %	-12 %	-39 %	-45 %	-25 %

Sex Group	Dose level (mg/kg/day)	Day 14								
		15 min.	1 h	4 h	8 h	12 h	24 h	W 4	W 6	W 10
F2	0.03	-6 %	-18 %	-14 %	-40 %	-60 %	-2 %	1 %	19 %	-4 %
F3	0.3	-45 %	-16 %	-21 %	-69 %	-60 %	-17 %	-8 %	-29 %	-19 %
F4	3	-2 %	-10 %	-21 %	-61 %	-53 %	-2 %	-12 %	-18 %	-43 %

Plasma oestradiol (% difference compared with controls)

Sex Group	Dose level (mg/kg/day)	Day 14								
		15 min.	1 h	4 h	8 h	12 h	24 h	W 4	W 6	W 10
F2	0.03	-66 %	-100 %	-100 %	-100 %	-100 %	-100 %	-33 %	-78 %	-9 %
F3	0.3	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-53 %	-39 %
F4	3	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %

Plasma testosterone (% difference compared with controls)

Sex Group	Dose level (mg/kg/day)	Day 14								
		15 min.	1 h	4 h	8 h	12 h	24 h	W 4	W 6	W 10
M2	0.03	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-32 %	-20 %	-12 %
M3	0.3	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	139 %
M4	3	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %	-100 %

Sperm Count – Sperm count was significantly decreased in all treated animals as was sperm motility.

Group number	1		3		4	
	Terminal	Recovery	Terminal	Recovery	Terminal	Recovery
EPIDIDYMIS						
Total sperm count	667.8	1415.4	306.8	84.4	339.1	163.8
Motile sperm (%)	68.1	61.3	22.0	0.0	22.6	0.0
Average path velocity (VAP) (µm/sec.)	110.4	103.8	93.4	0.0	94.3	0.0
TESTIS						
Sperm heads count (from testis)	52.6	61.2	27.8	11.4	26.6	1.6
10 ⁶ sperm per gram of testis	125.2	133.8	107.9	41.0	101.4	25.1

Organ Weights – After two weeks of dosing the following tables from the study report demonstrate the changes in organ weights as percentages. *Abs.: Differences in the absolute organ weights when compared with controls (as a percentage). * *Rel.: Differences in the relative (to body weight) organ weights when compared with controls (as a percentage)

Male Sex Organs

Organ Dose level (mg/kg/day)	R.Epididymis		R.Testis		L.Testis		Prostate		Sem.Vesicles	
	Abs*.	Rel.**	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
0.03	-39	-38	-21	-21	-22	-22	-66	-66	-82	-82
0.3	-55	-55	-39	-39	-39	-38	-74	-73	-84	-83
3	-56	-56	-46	-46	-37	-37	-74	-74	-83	-83

Female Sex Organs

Organ Dose level (mg/kg/day)	Ovaries		Uterus	
	Abs*.	Rel.**	Abs.	Rel.
0.03	-39	-45	-54	-58
0.3	-32	-41	-72	-76
3	-25	-34	-74	-77

Male Kidneys

Organ Dose level (mg/kg/day)	Kidneys	
	Abs*.	Rel.**
0.03	-13	-13
0.3	-18	-17
3	-20	-20

Female Liver and Spleen

Organ Dose level (mg/kg/day)	Liver		Spleen	
	Abs*.	Rel.**	Abs.	Rel.
0.03	+11	+1	+21	+10
0.3	+15	0	+31	+14
3	+15	+1	+37	+20

Female Pituitary

Organs Dose level (mg/kg/day)	Pituitary gland	
	Abs*.	Rel.**
0.03	-23	-30
0.3	-7	-19
3	-12	-22

Organ Weights – After eight weeks recovery

Male Sex Organs

Organs Dose level (mg/kg/day)	R.Epididymis		R.Testis		L.Testis		Prostate		Sem.Vesicles	
	Abs*.	Rel.**	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.	Abs.	Rel.
0.03	-4	-8	-5	-10	-1	-6	2	-4	-23	-27
0.3	-44	-44	-38	-38	-41	-40	-28	-28	-26	-26
3	-78	-78	-84	-84	-86	-85	-87	-87	-93	-93

Female Sex Organs

Organ	Ovaries		Uterus	
	Abs*.	Rel.**	Abs.	Rel.
Dose level (mg/kg/day)				
0.03	-5	-6	-6	-8
0.3	-7	-14	-42	-46
3	-56	-66	-77	-83

Other Organs

The mean absolute and relative kidney weights in males treated at 3 mg/kg/day ($p < 0.01$ and $p < 0.001$, respectively) and in females ($p < 0.001$) treated at the same dose level were statistically significantly decreased when compared with the controls. The percentage differences were respectively -20 % and -18 % in males and -22 % for the relative weight in females. The mean relative weights of the pituitary gland were statistically significantly lower in males treated at 0.03 mg/kg/day ($p < 0.05$, 16 % lower) and in females treated at 3 mg/kg/day ($p < 0.01$ with a percentage difference of 34 %). The absolute weight showed a non-significant lower mean weight. The mean relative adrenal weight was statistically significantly lower in females treated at 3 mg/kg/day ($p < 0.05$ with a percentage difference of 25 %) than in controls.

Gross Pathology – Decreased sex organ weight size

Histopathology – Dose dependant atrophy of the sex organs
 Injection site damage and inflammation

The following table shows that erythropoietic tissue in the sternal bone marrow decreased in males and that in the spleen increased in females. In the lung, there was an apparent increase in pneumonitis and hemorrhage in males.

Week 3 Organ and Pathology	Male				Female			
	0	0.03	0.3	3	0	0.03	0.3	3
Heart – myocardial degeneration with interstitial fibrosis	1	0	2	3	0	0	0	2
Thymus - Hemorrhage	1		4	4	2	0	2	1
Liver - Perilobular microvacuolization	4	6	5	7	8	10	10	10
Lung - Alveolar foamy macrophages	2	0	3	5	1	0	0	1
Lung - Interstitial pneumonitis	4	0	4	5	0	0	1	1
Lung - Hemorrhage	1	0	5	4	0	0	1	2
Lung - pneumonitis	2	0	3	4	1	0	0	0
Sternal marrow - apparent decrease in erythropoietic tissue	0	0	0	4	0	0	0	0
Spleen - apparent increase in erythropoietic tissue	0	0	0	3	0	6	8	9

The following table from the study report details the severity of vacuolization in the lung.

SEX	Males				Females			
	0	0.03	0.3	3	0	0.03	0.3	3
DOSE LEVEL (mg/kg/day)								
Number of animals examined	10	10	10	10	10	10	10	10
Perilobular microvacuolization								
Graded as minimal	4	5	4	5	8	5	2	2
Graded as slight	0	1	1	2	0	5	7	7
Graded as moderate	0	0	0	0	0	0	1	1
Total incidence	4	6	5	7	8	10	10	10

This toxicity did not completely resolve in females after 9 weeks of recovery.

4) 2 Week Subcutaneous Toxicity Study in the Cynomolgus Monkeys Followed by an 8 Week Treatment-Free Period - WDM

Major Findings:

A dose of 3 mg/kg/day for two weeks caused injection site damage in all animals tested. This dose also caused atrophy of the sex organs in both males and females. The interpretation of the changes in male animals was confounded by the relative immaturity of the animals. At this dose level there was some indication of elevated urinary deoxy pyridinoline levels in both sexes suggesting bone resorption. Dosing at 0.3 mg/kg/day caused similar but less severe changes. These changes were not completely reversible after 8 weeks recovery. The dose levels in this experiment were not high enough to fully characterize the toxicity of degarelix on this schedule in monkeys.

Study number FE 200486 DS TOX 9808
_____ study number 5951553
EDR filename tox9808-nonclinical-data.pdf
Conducting laboratory _____
Date of study initiation May 1998
GLP compliance Yes
QA reports Yes
Drug Degarelix, Batch 201 1-032-30
Methods
 Animal Male and female Cynomolgus monkeys
 2 to 3 years old
 Males 1.9 to 2.8 kg
 Females 1.7 to 2.6 kg
 Doses 0, 0.03, 0.3 or 3 mg/kg, (0, 0.36, 3.6 or 36 mg/m²)
 N 5 per sex per dose group
 Schedule Daily for 14 days
 Route Subcutaneous injection
 Dose volume 0.4 mL/kg
 Formulation Mannitol_{aq} 5% w/v

Observations
 Mortality Twice daily
 Clinical signs Twice daily
 body weight Weekly
 Cardiovascular Pretest and week 2
 Hematology Pretest, weeks 2 and 10
 Urinalysis Pretest, weeks 2 and 10
 Ophthalmology Pretest, weeks 2 and 10
 Deoxy pyridinoline Pretest, weeks 2 and 10
 Testosterone Pretest, weeks 2 and 10
 Toxicokinetics Not done
 Necropsy Week 2 (3 per group), Week 10 (2 per group)

b(4)

Histopathology Adequate battery

Results

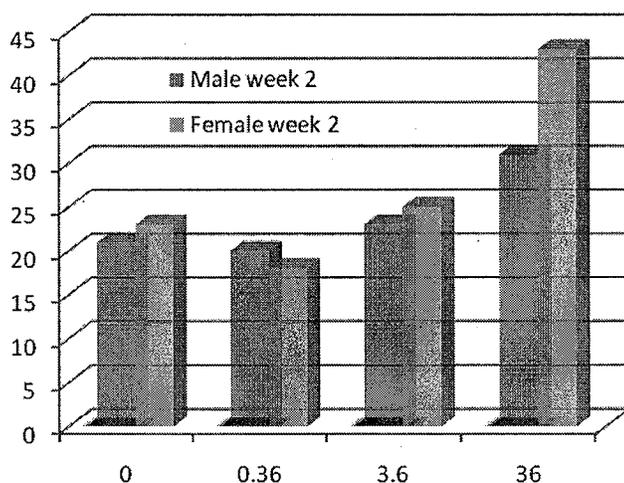
- Mortality – One HD female died on day 11, the investigators attributed this death to anesthesia during the ophthalmic examination
- Clinical signs – Lesions at the injection site (induration or tumefaction) at the high dose
- Body Weight – Minor decrease in males, minor increase in males
- Food cons – No dose related effects
- Ophthalmology – No toxicologically significant effects
- Hematology – No toxicologically significant effects
- Bone Marrow – No toxicologically significant effects
- Clinical chem. – No toxicologically significant effects
- Urinalysis – No toxicologically significant effects
- Hormone analysis – The following tables from the study report suggest that even at the high dose plasma testosterone concentrations were not at castrate levels in through much of this experiment.

Appears This Way
On Original

TABLE 2: Testosterone in plasma in nmol/L
% variation compared to controls

sex	N°	D14								W4	W6	W10
		Before	15min	1H	4H	8H	12H	24H				
GROUP 2 0.03 mg/kg/day	M 2361	-44%	-28%	-34%	-44%	-4%	-99%	-26%	-	-	-	
	M 2362	-39%	-49%	-61%	-44%	3%	-100%	-46%	-	-	-	
	M 2363	-51%	-10%	5%	13%	-12%	-96%	3%	-	-	-	
	M 2364	-62%	-52%	-56%	-56%	-85%	-98%	-26%	47%	108%	33%	
	m	-51%	-38%	-39%	-29%	-15%	-98%	-25%	4073%	132%	21%	
GROUP 3 0.3 mg/kg/day	M 2371	-17%	38%	71%	-40%	106%	-99%	13%	-	-	-	
	M 2372	-35%	-18%	-9%	-19%	-63%	-98%	27%	-	-	-	
	M 2373	-39%	-31%	-14%	-7%	-41%	-97%	3%	-	-	-	
	M 2374	-28%	-26%	-17%	9%	-19%	-98%	8%	53%	156%	65%	
	m	-35%	-10%	6%	-23%	-12%	-98%	2%	43%	112%	21%	
GROUP 4 3 mg/kg/day	M 2381	-73%	-71%	-56%	-80%	-78%	-99%	-36%	-	-	-	
	M 2382	-46%	-10%	-28%	5%	-19%	-99%	18%	-	-	-	
	M 2383	-17%	-28%	-23%	-44%	-19%	-96%	72%	-	-	-	
	M 2384	30%	1%	5%	13%	113%	-92%	47%	67%	108%	-9%	
	m	-24%	60%	46%	49%	-41%	-95%	96%	240%	196%	30%	

Deoxypridinoline – Deoxypridinoline/creatinine ratios were elevated in all treated animals at week 2 but this difference did not reach significance ($p < 0.05$ ANOVA). Values showed signs of significant recovery at week 10. This indicates increased bone resorption. The graph below shows the dose related increase in values at the end of dosing. Values at the high dose are significant by pairwise comparison to controls.



Organ Weights – The following table from the study report shows the significant changes in body weight in males

**ORGAN WEIGHTS AND BODY WEIGHTS
 PERCENTAGE DIFFERENCE COMPARED WITH CONTROL GROUP
 WITH STATISTICS
 SELECTED PARAMETERS
 WEEK 2**

Sex/ Group	TERMINAL		THYROID(S)		R. EPIDIDYMIS		L. EPIDIDYMIS	
	BODY WT (g)		(g)	(%)	(g)	(%)	(g)	(%)
M 2	-6		-26	-20	-40	-38	-24	-13
M 3	-6		-22	-17	-36	-32	-29	-26
M 4	-8		-30	-24	-45	-40	-24	-18

Sex/ Group	R. TESTIS		L. TESTIS		PROSTATE	
	(g)	(%)	(g)	(%)	(g)	(%)
M 2	-21	-17	-25	-22	-6	-5
M 3	-38	-34	-40	-36	-17	-15
M 4	-39	-35	-44	-40	-38	-34

Organ Weights – The following table from the study report shows the significant changes in body weight in females

**ORGAN WEIGHTS AND BODY WEIGHTS
 PERCENTAGE DIFFERENCE COMPARED WITH CONTROL GROUP
 WITH STATISTICS
 SELECTED PARAMETERS
 WEEK 2**

Sex/ Group	TERMINAL		THYROID(S)		OVARY(IES)		UTERUS	
	BODY WT (g)		(g)	(%)	(g)	(%)	(g)	(%)
F 2	3		8	5	-3	-6	-15	-17
F 3	8		12	4	-28	-33	-33	-38
F 4	7		-13	-17	-5	-11	-17	-22

Gross Path – Injection sight damage

Histopathology – The following table from the study report shows the microscopic pathologies observed in this study

MICROSCOPIC FINDINGS

Number of animals affected

WEEK 2

	Dose (mg/kg/day)					
	0.03		0.3		3	
	M	F	M	F	M	F
Microscopic lesions :						
Testicular and epididymidal immaturity	0	-	1	-	2	-
Apparent decrease in the number of ovarian follicles	-	0	-	0	-	1
Endometrial atrophy	-	2	-	3	-	2
Cervical atrophy	-	1	-	3	-	2
Vaginal atrophy	-	1	-	2	-	2
Granulation tissue at injection site 1	0	0	1	1	2	2
Granulation tissue at injection site 2	0	0	1	1	3	0
Granulation tissue at injection site 3	0	0	0	1	3	2
Granulation tissue at injection site 4	0	0	3	1	3	2
Granulation tissue at injection site 5	0	0	1	0	3	2
Granulation tissue at injection site 6	0	0	0	1	3	2
Granulation tissue at injection site 7	0	0	0	0	2	1
Granulation tissue at injection site 8	0	0	0	0	1	0

Changes in male sex organs were difficult to interpret because the animals were not completely sexually mature. After 10 weeks of recovery all the changes listed above were not completely resolved.

5) 26 Week Subcutaneous Toxicity Study in the Rat - WDM

Major Findings:

In a 26 week study of subcutaneous degarelix in rats, 13 fortnightly doses of degarelix as high 600 mg/m² caused no dose related mortality in rats. All male dose groups gained less weight than controls but all female groups gained more. RBC decreased in dosed males (<7%) but increased in females (as much as 6%). MCH and MCV decreased in both sexes. White cell count, neutrophils, monocytes and APTT increased with dose. The percent of lymphocytes decreased with dose. Pituitary and adrenal weights decreased with dose in females. Cholesterol and globulin increased; the latter shifting the A/G ratios significantly lower. The decrease in relative liver weight was greater in males (30% < controls) than females correlated with decreased ALT and AST. There was also possibly some dose dependant kidney damage; relative kidney weight decreased with dosing particularly in females while creatinine increased in males. Alkaline phosphatase increased with increasing dose to as much as

236% in females. Sex organs were severely atrophied in both males and females consistent with the primary pharmacology of the drug.

Study number ADR0029
 Sponsor reference number FE200486DSTOX0401
 EDR filename tox0401-nonclinical-data.pdf
 Conducting laboratory _____
 Date of study initiation May 2004
 GLP compliance Yes
 QA reports Yes
 Drug Degarelix, Batch 04D14-01
 Methods
 Animal Male and female rats (HsdBrlHan:WIST)
 Doses and N

b(4)

Group number	N males	N Females	Dose mg/kg/2 weeks	Dose mg/m ² /2 weeks	Designation
1	20	20	0	0	Main Study
2	20	20	10	60	Main Study
3	20	20	50	300	Main Study
4	20	20	100	600	Main Study
5	10	10	0	0	Toxicokinetics
6	10	10	10	60	Toxicokinetics
7	10	10	50	300	Toxicokinetics
8	10	10	100	600	Toxicokinetics
9	3	3	50	50	Preliminary study single dose
10	3	3	75	75	Preliminary study single dose
11	3	3	100	100	Preliminary study single dose

Preliminary phase animals were dosed only once and monitored for injection site damage for 8 weeks

Schedule Fortnightly (13 total doses), no recovery period
 Route Subcutaneous injection
 Dose volume 5 mL/kg
 Dose concentration 0, 1.22, 6.126, and 12.2 mM nominal
 Formulation Mannitol_{aq} 5% w/v

Observations

Mortality Twice daily
 Clinical signs weekly
 Body weight weekly
 Hematology 2 days after doses 7 and 13, weeks 13 and 24
 Clinical chem 2 days after doses 7 and 13, weeks 13 and 24
 Urinalysis 2 days after doses 7 and 13, weeks 13 and 24 (10 males and 10 females)
 Ophthalmology Pretest and week 26 group 1 and 4 only
 Toxicokinetics Blood samples for toxicokinetic evaluation were taken from satellite animals predose, 2, 4, 8, 12, 24, 48, 96, 192 and 336 hours after Doses 1, 7 and 13. In addition predose samples were taken before Doses 3, 4, 5, 6, 9, 10, 11 and 12. Two animals per sex per time point. Non-compartmental analysis.
 Necropsy Week 26
 Histopathology Adequate battery

Results

Mortality – The following includes deaths in the satellite groups. The investigators did not consider any of these deaths dose related

Dose mg/kg	Killed because of injection site damage	Killed because of a mass	Found dead	Killed because of damage due to procedure
0				1f
10			1f nephroblastoma	
50	2f	1m		
100	6m, 1f		1f unknown cause	2 m, 1f

Clinical signs – Swelling and inflammation at the injection site, occasional scabbing
 Body Weight – Main study males showed a dose dependant decrease in weight, females showed an increase. The following two graphs are from the study report.

Figure 1 - Bodyweights (g) - group mean values - males - main study

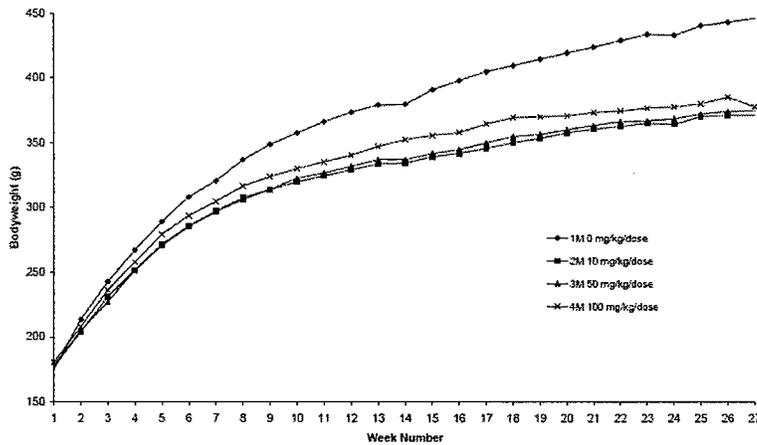
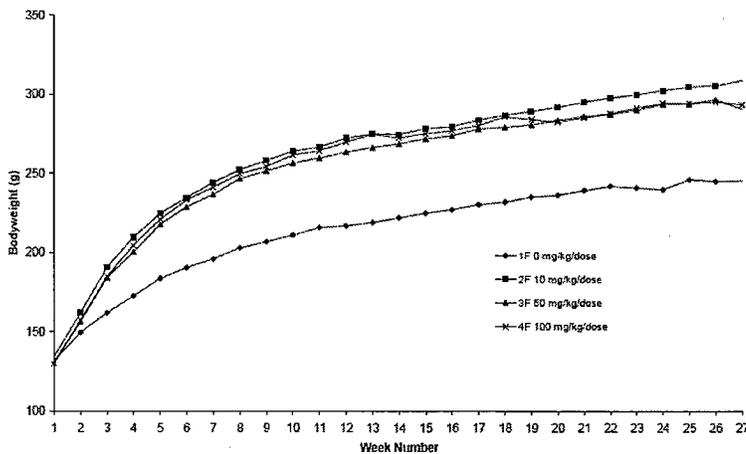


Figure 2 - Bodyweights (g) - group mean values - females - main study



Food cons – Males ate less than controls throughout the study (1 to 4 g/day). Females ate amounts comparable to controls.

Ophthalmology – No toxicologically significant changes
 Hematology – In the table below the values in bold are significantly different from controls by the study investigators calculations.

Parameter	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
Hematology					
	Hb male Week 25	16.3	-4.9%	-3.7%	-10.4%
	PCV male Week 25	47.5	-4.6%	-2.3%	-8.4%
	RBC male Week 25	8.83	-6.3%	-1.1%	-5.7%
	MCH male Week 25	18.5	1.1%	-3.2%	-5.4%
	MCV male Week 25	53.8	1.9%	-1.1%	-2.8%
	Platelet male Week 25	836	9.8%	7.3%	22.0%
	APTT male Week 25	18.9	-14.3%	-13.2%	-22.2%
	PT male Week 25	17	0.0%	-2.4%	0.6%
	WBC male Week 25	7.54	5.3%	37.3%	28.2%
	Neutrophils% male Week 25	19.7	9.6%	72.1%	115.7%
	Lymphocytes% male Week 25	75	-4.5%	-21.5%	-33.1%
	Monocytes% male Week 25	2.6	34.6%	30.8%	50.0%
	Hb female Week 25	15.4	0.0%	-1.9%	-1.3%
	RBC female Week 25	8.1	2.1%	4.6%	6.2%
	MCH female Week 25	19	-2.1%	-6.3%	-7.4%
	MCV female Week 25	55.9	-1.1%	-2.9%	-5.0%
	Platelet female Week 25	910	-3.4%	6.4%	7.4%
	APTT female Week 25	18.8	-13.8%	-8.0%	-17.0%
	PT female Week 25	16	5.0%	11.3%	5.0%
	WBC female Week 25	3.83	50.1%	124.3%	138.9%
	Neutrophils% female Week 25	15.8	49.4%	165.8%	154.4%
	Lymphocytes% female Week 25	78.5	-11.5%	-35.8%	-33.6%
	Monocytes% female Week 25	2.7	18.5%	48.1%	63.0%
	Basophils female Week 25	0.03	33.3%	133.3%	200.0%

Clinical chem. –

Appears This Way
 On Original

Clinical Chemistry	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
	Creatinine male Week 25	0.35	28.6%	22.9%	25.7%
	Creatinine female Week 25	0.38	7.9%	2.6%	2.6%
	I Phos male Week 25	5.6	-14.3%	-17.9%	-10.7%
	I Phos female Week 25	4.4	2.3%	11.4%	15.9%
	Potassium male Week 25	3.9	-7.7%	-2.6%	5.1%
	Potassium female Week 25	3.6	2.8%	2.8%	5.6%
	Chloesterol male Week 25	69	60.9%	59.4%	49.3%
	Cholesterol female Week 25	77	29.9%	31.2%	19.5%
	Protein male Week 25	6.9	0.0%	2.9%	-1.4%
	Protein female Week 25	7.6	-14.5%	-14.5%	-11.8%
	Alk Phos male Week 25	61	-4.9%	1.6%	14.8%
	Alk Phos female Week 25	22	168.2%	236.4%	200.0%
	Globulin male Week 25	2.6	7.7%	23.1%	26.9%
	Globulin female Week 25	2.2	9.1%	27.3%	31.8%
	Albumin male Week 25	4.3	-4.7%	-9.3%	-16.3%
	Albumin female Week 25	5.3	-22.6%	-30.2%	-28.3%
	ALT male Week 25	41	-12.2%	-14.6%	-22.0%
	ALT female Week 25	51	-23.5%	-25.5%	-37.3%
	AST male Week 25	61	11.5%	9.8%	3.3%
	AST female Week 25	105	-32.4%	-41.9%	-40.0%
	A/G ratio male Week 25	1.7	-11.8%	-23.5%	-35.3%
	A/G ratio female Week 25	2.4	-25.0%	-41.7%	-45.8%
	Bilirubin male Week 25	0.1	0.0%	10.0%	0.0%
	Bilirubin female Week 25	0.12	-16.7%	-16.7%	-8.3%

Urinalysis –

Urinalysis	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
	Volume male Week 25	1.7	-17.6%	-23.5%	88.2%
	Volume female Week 25	1	50.0%	20.0%	20.0%
	SG male Week 25		0.0%	0.1%	-0.6%
	SG female Week 25		0.0%	0.1%	0.0%

Organ Weight –

Appears This Way
 On Original

Organ weights	Control value	Absolute Change			Relative Change *		
		Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
male body weight	447.7	371.5	372	383.4			
Heart male	1.19	-14.3%	-16.0%	-6.7%	1.033	1.011	1.089
Kidney male	2.2	-26.8%	-24.5%	-17.7%	0.882	0.908	0.961
Liver male	13.7	-30.3%	-29.6%	-23.5%	0.840	0.848	0.893
Lung male	1.74	-9.2%	-11.5%	-5.2%	1.094	1.065	1.107
Epididymides	1.46	-86.3%	-82.2%	-83.6%	0.165	0.214	0.192
Prostate	0.43	-95.3%	-95.3%	-95.3%	0.056	0.056	0.054
Seminal Vesicles	1.63	-94.5%	-95.1%	-94.5%	0.067	0.059	0.064
Testes	3.62	-90.9%	-90.6%	-90.1%	0.110	0.113	0.116
Pituitary male	0.008	-25.0%	-25.0%	-12.5%	0.904	0.903	1.022
Thymus male	0.327	23.9%	19.3%	48.3%	1.493	1.435	1.732
Salivary gland male	0.6	-16.7%	-11.7%	-13.3%	1.004	1.063	1.012
Thyroid male	0.021	-9.5%	-23.8%	-14.3%	1.090	0.917	1.001
Female body weight	243.1	304	293	293			
Ovaries	0.073	-74.0%	-67.1%	-74.0%	0.208	0.273	0.216
Uterus	0.67	-82.1%	-85.1%	-83.6%	0.143	0.124	0.136
Pituitary female	0.014	-57.1%	-57.1%	-57.1%	0.343	0.356	0.355
Thymus female	0.278	52.2%	30.2%	47.5%	1.216	1.081	1.222
Spleen female	0.52	7.7%	11.5%	17.3%	0.861	0.926	0.972
Adrenals female	0.058	-8.6%	-19.0%	-10.3%	0.730	0.673	0.743
Liver female	7.76	-3.5%	-6.1%	-2.6%	0.772	0.780	0.807
Kidney female	1.51	-4.6%	-6.6%	-1.3%	0.762	0.775	0.818

(organ weight in control÷ body weight of control)÷(organ weight in treated group)÷(body weight in treated group)
Values close to 1 show no change in organ weight relative to body weight

Gross Pathology – Except for the notable decrease in size of sex organs in both males and females and frequent injection site damage there were no notable gross lesions.

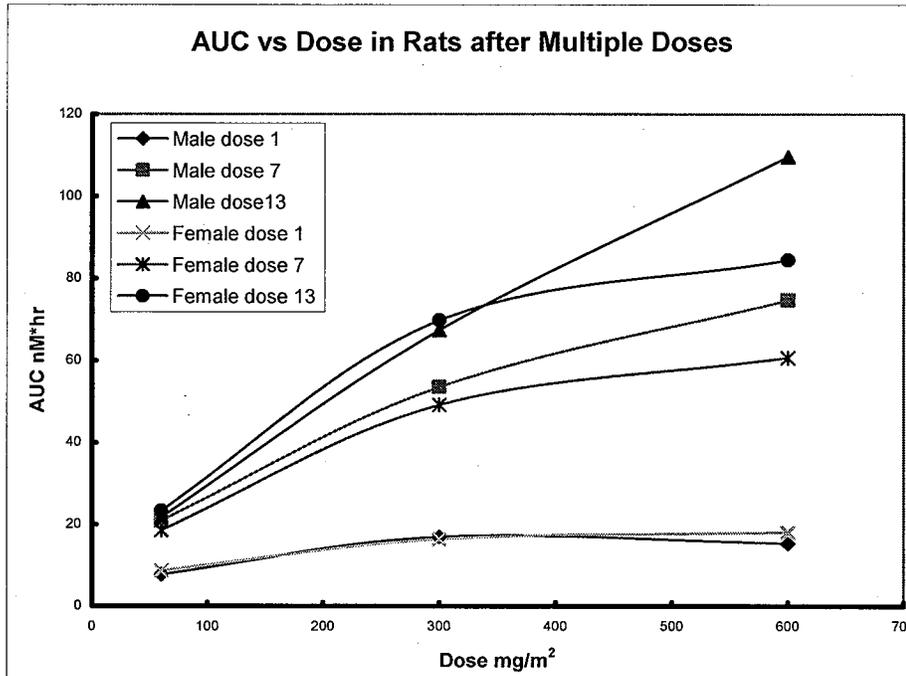
Histopathology – There was notable atrophy of the sex organs in both males and females. Injection site damage was frequent and severe in some cases. Some animals showed signs of a dose related increase in incidence of adipocytes in the bone marrow. There was a small decrease in extramedullary hematopoiesis in the spleen of high dose males and females which may be within the limits of normal variation.

Toxicokinetics - The following table from the study report shows the pharmacokinetic parameters determined in this study.

Text Table 9 : Toxicokinetic Parameters of FE200486 in rat plasma

Group/ sex	Dose (mg/kg/dose)	C _{max} (ng/mL)			T _{max} (h)			C _{trough} (ng/mL)			AUC _{0-τ} (ng.hr.mL)		
		Dose			Dose			Dose			Dose		
		1	7	13	1	7	13	1	7	13	1	7	13
6M	10	207	327	254	4	4	8	28.6	55.2	73.0	12800	34200	35500
6F	10	200	309	223	2	8	12	17.0	60.1	77.7	14300	30400	38100
7M	50	267	499	509	2	4	24	48.1	188	295	27800	87400	110000
7F	50	287	432	688	2	4	24	41.8	181	195	26800	80300	114000
8M	100	296	649	733	4	24	24	40.5	308	431	25100	122000	179000
8F	100	219	533	605	4	8	24	33.2	162	264	29500	99100	138000

The following graph shows that the increase in exposure was less than dose proportional. It also shows considerable accumulation over the course of the experiment.



C_{trough} values demonstrated consistent exposure throughout the experiment.

6) 12 month repeated dose toxicity study in the Cynomolgus monkey by the subcutaneous route with a 6 month interim necropsy and followed by a 6 month treatment-free period

Major Findings:

In a study of subcutaneous degarelix in monkeys dosed monthly for a year (7 doses in interim kill group and 13 in the main study) one HD male in poor condition was killed humanely on day 245 primarily because of neurological (ataxia) and gastrointestinal (diarrhea). On examination this animal had interstitial pneumonia and lesions in the stomach. No other monkeys showed these signs so it is not clear that this animal's condition was dose related.

All dosed monkeys showed signs of injection site damage but there were few other clinical signs. Male monkeys lost a significant amount of weight relative to controls. High dose males weighed as much as 32% less than controls and did not gain weight over the six month recovery period. Treated females had body weights comparable to controls throughout the study but were frequently amenorrhic. There were minor sporadic variations in arterial blood pressure. Hematological changes were transient and small. Changes in clinical chemistry parameters included elevations in serum gamma glutamyl transferase (GGT) in treated females, mild elevations in cholesterol in HD males and elevations in creatinine in HD females. Testosterone and estradiol decreased with increasing dose, but the concentrations of these hormones were not consistently below castration levels. Sex organs in males and females were atrophic in males

and females by six months and sperm count was very low. In some treated monkeys, degarelix appeared to retard epiphyseal closure.

Study number 595/587
 Sponsor reference number FE200486DSTOX0126
 EDR filename tox0126-nonclinical-data.pdf
 Conducting laboratory _____
 Date of study initiation March 2004
 GLP compliance Yes
 QA report Yes
 Drug Degarelix, Batch PPL-FE4860001, (0048262)
 Purity: 99.66%, assumed to be 100% for dose calculation. Peptide content: _____ for the first three administrations, then _____ subsequently.

b(4)

Methods

Animal Cynomolgus monkey, (*Macaca fascicularis*)
 Doses and N

Group number	Dose mg/kg/2 weeks	Dose mg/m2/fortnight	6 month necropsy interim kill		12 month necropsy end of dosing		18 month necropsy recovery	
			Male	Female	Male	Female	Male	Female
1	0	0	2	2	4	4	2	2
2	0.5	6	2	2	4	4	2	2
3	5	60	2	2	4	4	2	2
4	50	600	2	2	4	4	2	2

Age Males: 37 to 60 months, females: 29 to 43 months.
 Sexual maturity was verified pretest
 Body weight Males: 3.34 to 6.69 kg, females: 2.20 to 3.30 kg.
 Schedule Once every 4 weeks (7 and 13 doses)
 Route Subcutaneous injection
 Dose volume 5 mL/kg
 Formulation Mannitol_{aq} 5% w/v

Observations

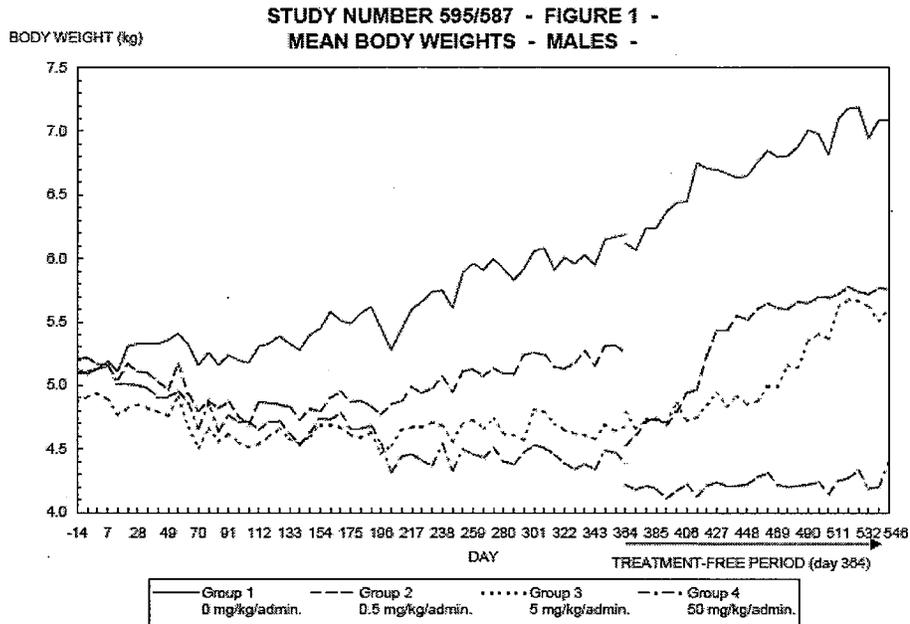
Mortality Twice daily
 Clinical signs Daily
 Body weight Weekly
 Food cons Estimated daily
 Cardiovascular Pretest, on day 1 (approximately 24 hours after the first dose), day 8, day 169, ay 337
 Hematology Pretest, for females day 0 before treatment, day 79 (week 12), day 191 (week 28), day 275 (week 40), day 359 (week 52), day 450 for females and 451 for males (week 65), day 544 for females and 545 for males (week 78)
 Clinical chem. Pretest, for females day 0 before treatment, day 79 (week 12), day 191 (week 28), day 275 (week 40), day 359 (week 52), day 450 for females and 451 for males (week 65), day 544 for females and 545 for males (week 78)
 Urinalysis Pretest (day -8), day 78 for males and 82 for females (week 12), day 191 (week 28), day 274 for males and 275 for females (week 40), day 359 (week 52), day

	450 for females and 451 for males (week 65), day 544 for females and 545 for males (week 78)
Ophthalmology	Pretest and during weeks 12, 28, 52 and 78.
Toxicokinetics	Blood samples at the 1st, 3rd, 7th and 13th dose (i.e. on days 0, 56, 168 and 336), before dosing, 1 hour, 4 hours, 8 hours, 24 hours then 2, 4, 7, 14, 21 and 28 days after dosing. Before the 5th, 6th, 9th, 10th, 11th and 12th dosing (i.e. on days 112, 140, 224, 252, 280 and 308) during weeks 56, 60, 65, 69, 74 and 78. Two animals per sex per time point. Non-compartmental analysis.
Hormone Conc.	Pretest (day -8), on day 7 (week 2), day 21 (week 4), day 49 for males and 50 for females (week 8), day 79 (week 12), day 191 (week 28), day 275 (week 40), day 359 (week 52), day 385 for females and 386 for males (week 56), day 413 for females and 414 for males (week 60), day 450 for females and 451 for males (week 65), day 476 for females and 477 for males (week 69), day 511 for females and 512 for males (week 74), day 544 for females and 545 for males (week 78).
Necropsy	28 days after the last dose for interim and terminal necropsy 18 months for recovery animals
Histopathology	Adequate battery

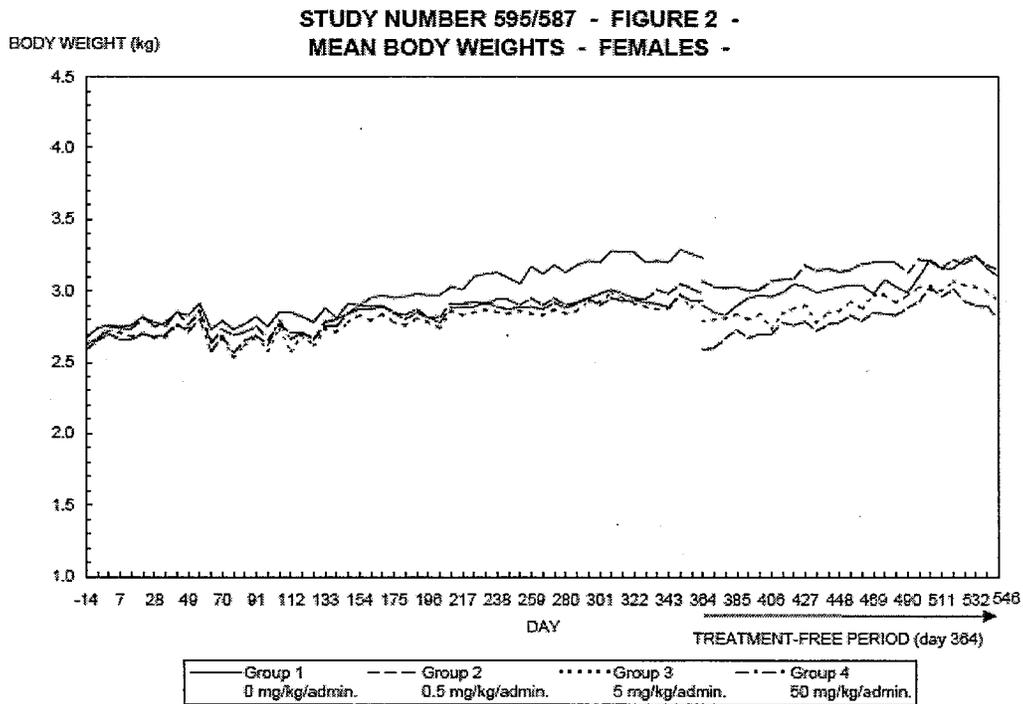
Results

Mortality –	One Group 4 male was in a poor condition from day 241 on. This animal was inactive, thin and had hair-loss, unsteady gait, hypothermia, liquid feces, yellowish mucous membranes, ataxia and a swollen abdomen. The investigators killed this animal humanely on day 245. This monkey weighed about 27 % less on day 245 compared with its weight on day 0. On gross examination, there were lesions in the gastrointestinal tract and lungs not seen in other animals. The abdomen was swollen, and the stomach and intestines full of material. The cecum and colon were distended with a large amount of clear material. The rectum was also distended with clear solid material and a dark raised area was noted with ulceration of the mucosa. Ulceration was seen in the skin and tail. In addition, there was interstitial pneumonia in the lungs. It is not clear that this death was related to dosing
Clinical signs –	Injection site damage in dosed monkeys
Body weight –	Male monkeys lost a statistically significant amount of weight compared to controls during the dosing period. This weight loss was dose dependant as shown by the graph below excerpted from the study report. This weight loss was directly related to the primary pharmacology of degarelix and the suppression of testosterone secretion. The graph shows that this decrease in body weight was not reversible in high dose males and only partially and slowly reversible in the low and mid dose animals.

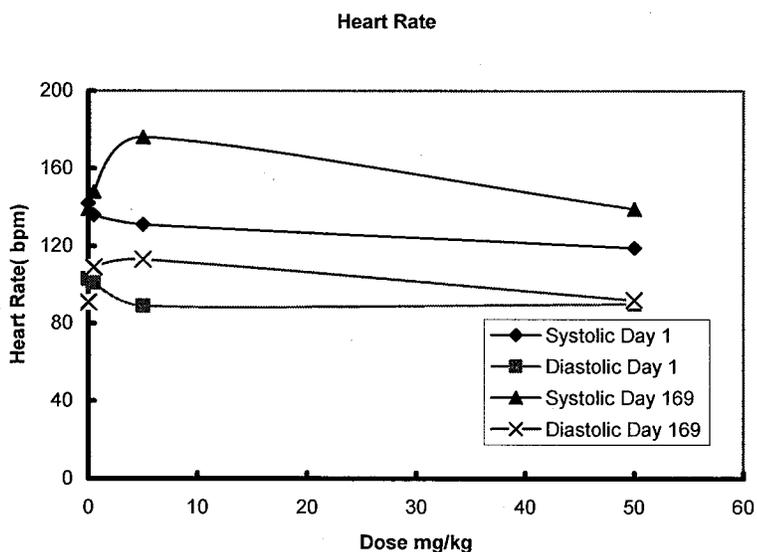
Appears This Way
On Original



The body weight of female monkeys was comparable to that of controls as the following graph from the study report shows.



- Food cons. – Both male and female monkeys ate amounts of food comparable to controls throughout the study
- Menstruation – All control females cycled normally throughout the study. Four of the eight low dose monkeys stopped cycling intermittently during the dosing period; one was amenorrhic for all but two weeks of the study. In the mid dose group, five of the eight females cycled irregularly while three were completely amenorrhic throughout the study. These three monkeys were also amenorrhic pretest. High dose monkeys cycled only sporadically if at all. These changes were reversible.
- Ophthalmology – No toxicologically significant changes
- Cardiography –
Arterial BP – Minor variations in arterial blood pressure were not statistically significant in males or females but in the context of the changes seen in dogs above these changes are toxicologically significant.

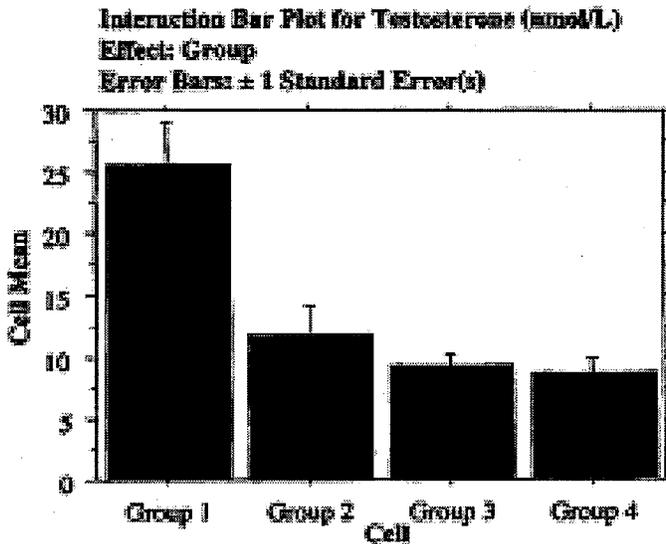


- Heart rate – No toxicologically significant changes
- Wave forms – No toxicologically significant changes
- Rhythm – No toxicologically significant changes
- Hematology – Small possibly dose dependant fluctuations in APTT in treated males
Transient sometimes statistically significant decreases in red cell parameters in mid and high dose males.
- Clinical chem. – Dosing caused transient fluctuations in serum albumin in MD and HD females. There were mild (to 1.7 times control) statistically significant elevations in serum gamma glutamyl transferase activities (GGT) in treated females compared with

control in both weeks 12 and 28. There were also transient minor elevations in cholesterol in HD males and transient elevations in creatinine in HD females.

Urinalysis – No toxicologically significant changes

Testosterone – The following tables and graphs from the report show that serum testosterone was significantly lower in a dose dependant manner in all treated monkeys. Testosterone remained similarly low throughout the treatment period. During the recovery period serum testosterone returned to values comparable to controls in the two low dose animals and in one mid dose and one high dose animal but values remained at castration levels in one mid dose and one high dose monkey.



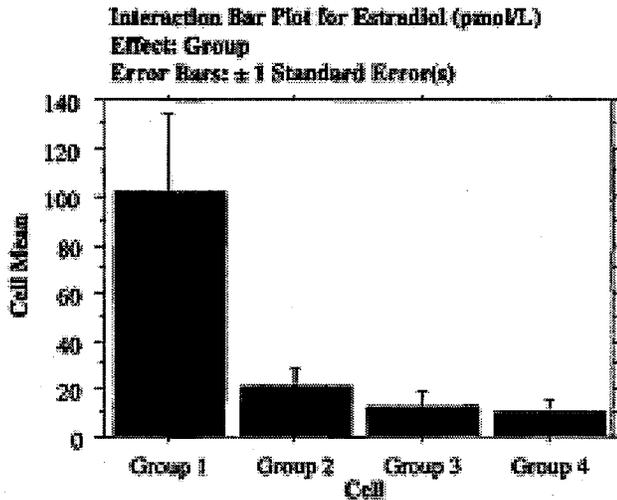
Best Possible Copy

Fisher's PLSD for Testosterone (nmol/L)
Effect: Group
Significance Level: 5 %

	Mean Diff	Crit. Diff	F-Value	
Group 1, Group 2	13.62	6.18	0.0001	\$
Group 1, Group 3	16.10	6.18	<0.0001	\$
Group 1, Group 4	16.75	6.18	<0.0001	\$

Best Possible Copy

Estradiol – The following tables and graphs from the report show that serum estradiol concentrations were significantly lower in a dose dependant manner in all treated monkeys. Estradiol remained similarly low throughout the treatment period and on occasions dropped to 0. During the recovery period serum estradiol did not completely recover in any of the dose groups except in one of the two low dose females.



Best Possible Copy

Fisher's PLSD for Estradiol (pmol/L)
Effect: Group
Significance Level: 5 %

	Mean Diff.	Crit. Diff.	P-Value	
Group 1, Group 2	80.96	48.64	0.002	S
Group 1, Group 3	89.84	48.64	0.0007	S
Group 1, Group 4	91.65	48.64	0.0004	S

Pathology after six months of treatment

Organ weights – Dose dependant relative and absolute low mean testes, epididymides, seminal vesicles and prostate weight, and low mean ovaries and uterus weight in the treated groups. There were dose dependant increases in mean relative and absolute thymus weights in all treated groups.

Gross Pathology – Injection site damage

Histopathology – Injection site damage
 Atrophy of the sex organs in males and females increased in incidence and severity with increasing dose.
 The total sperm count was so low that motility and morphology could not be evaluated in one low dose male and all mid and high dose males. One low dose male had a normal sperm count with normal motility and morphology.

Pathology after 12 months treatment

Organ Weights – There was a dose dependant decrease in the mean absolute and relative weights of the prostate gland, testes, seminal vesicles, epididymides in all male treated groups. These decreases were frequently statistically significant in the mid and high dose males.

The mean absolute and relative weights of the ovaries were lower in all female treated groups; these differences were statistically significant mid and high dose females.

The mean absolute and relative weights of the uterus were lower in all female treated groups; these differences were statistically significant in females treated with 5 mg/kg/administration and in mean absolute weight in females treated with 50 mg/kg/administration. Thymus weights were somewhat higher in some treated animals.

Gross Pathology – Injection site damage
Small atrophic sex organs in males

Histopathology – Most dosed animals had injection site damage. There were dose dependent atrophic changes in the sex organs of males and females. The epiphyseal plates of three males from the control group were not found, in addition, three females from the control group also had slight or severe decrease in the size of their epiphyseal plates. This indicated the completion (or near completion) of the maturation process of this growing structure. Conversely, the majority of treated monkey from both sexes had normal epiphyseal plates in the stifle joint which indicated that normal closure of the growth plates was possibly delayed.

Pathology at recovery

Organ Weights – In treated males, absolute and relative prostate, testis, seminal vesicle and epididymis weights remained low. In all treated females, mean absolute and relative uterus and ovary weight remained low.

Gross Pathology – Several instances of persistent injection site damage

Histopathology – There was some persistent injection site damage. One of the two HD males had tubular atrophy in the testes, atrophy of the seminal vesicles and epididymides and an apparent slight atrophy of the prostate. Tubular atrophy in the testes, seminal vesicles and epididymides also remained in one of two mid dose males. In females no Graafian follicles were seen in treated females and no corpora lutea were noted in females given 5 and 50 mg/kg/administration. In MD and HD females the endometrium was moderately or poorly developed and the evaluation of the menstrual cycle showed that these females were in anoestrus. The epiphyseal there was evidence of incomplete maturation in some treated animals.

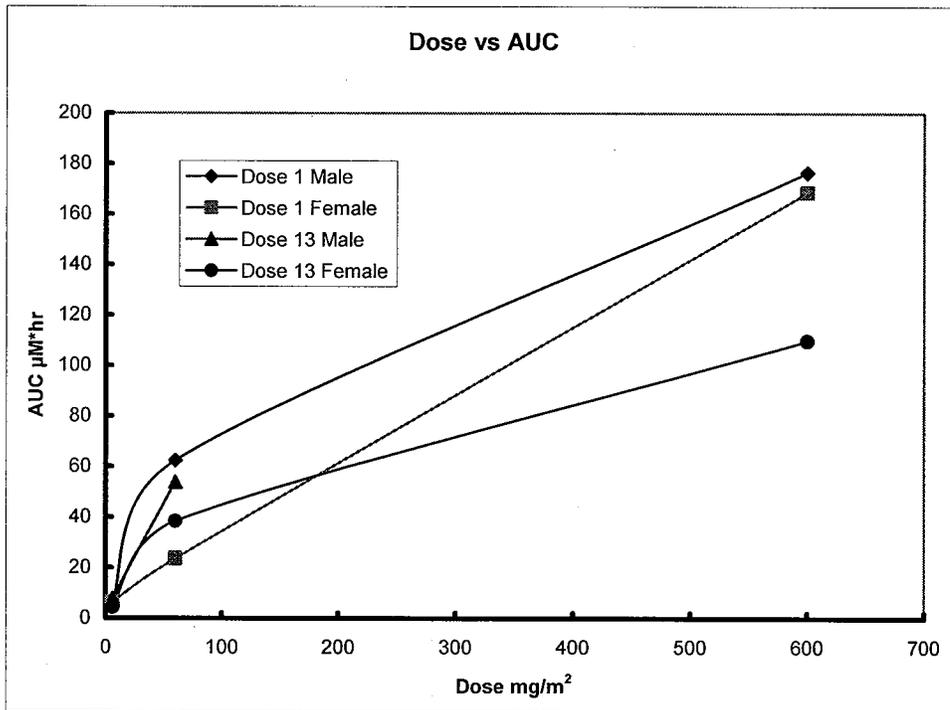
Toxicokinetics –

The following table from the study report shows the toxicokinetic parameters determined after the first dose.

*Appears This Way
On Original*

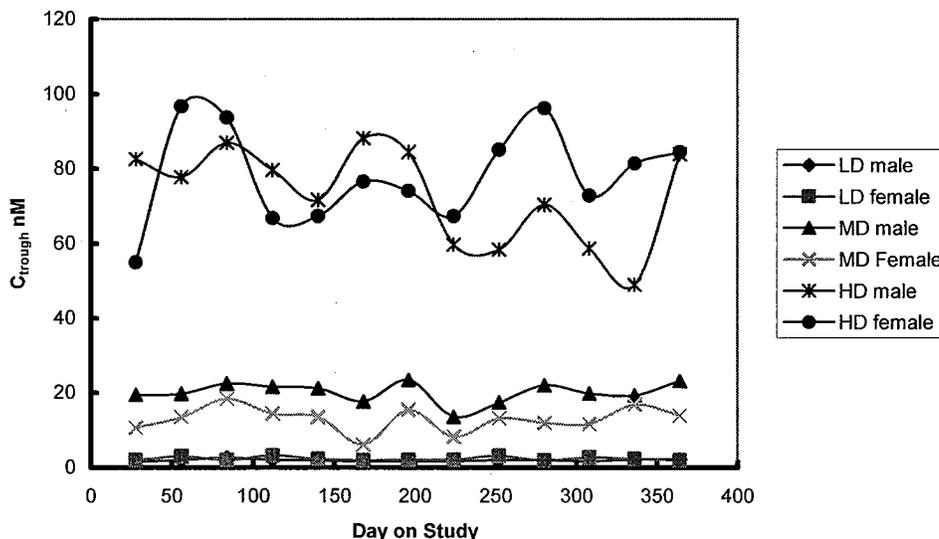
	Dose mg/kg	C _{max} ng/mL	T _{max} hour	AUC _t ng*hr/mL	AUC ng*hr/mL	t _{1/2} hour	Cl/F mL*hr/kg	Vz/F mL/kg
Dose 1								
Male	0.5	92.4	1	8029	10519		189	65
	5	276	24	49311	102003		221	49
	50	1106	24	169149	288028		177	174
Dose 1								
Female	0.5	112	1	8329	10551		226	67
	5	139	16	21804	38546		300	130
	50	923	24	157197	275309		287	198
Dose 13								
Male	0.5	42.4	1	8045	12891		162	84
	5	204	48	49723	88058		243	166
	50	773	24	171049				317
Dose 13								
Female	0.5	57.5	1	6061	7097		263	105
	5	124	24	29990	62716		264	199
	50	1831	24	292121	179184		288	221

The following graph shows that the increase in AUC is not proportional to increasing dose.



As the graph below shows, C_{trough} seems to oscillate in monkeys as it does in rodents. Values in the mid and high dose group demonstrate that plasma concentrations of degarelix remained well above k_i at all times during the experiment. Concentrations in the Low dose animals were on the same order as k_i. Concentrations in mid dose females were consistently lower than males as seen in other species but here concentrations in the high dose were comparable between males and females. Here again the values seem to oscillate.

Day on study vs C_{trough}



7) 4 week intravenous (bolus) dose toxicity study in the rat. WDM

Major Findings

Twenty-eight daily doses of degarelix of 18 mg/m² caused no mortality in rats. High dose males weighed significantly less than controls or other treatment groups while female rats gained more weight than controls as a function of increasing dose. Concentrations of testosterone in males were below those considered equivalent to castration in humans in mid dose rats and below the level of quantitation in high dose rats. Male rats had mild anemia without changes in MCV and both sexes had dose related elevations in white cell counts. Creatinine (to 85% in males), BUN (to 120% in males) and cholesterol (to 85% in males) were elevated in both sexes as a function of increasing dose. Alkaline phosphatase was elevated in females (to 25%). AST was mildly elevated (to 35% in females). Urine volume was increased and pH was decreased at the end of the study. Sex organs were atrophic in both sexes. Thymus and spleen weights were elevated as a function of increasing dose. There was aggregation of Kupffer cells in the liver and macrophages in the spleen. This correlated the finding of drug in Kupffer cells by immunological assay in high dose males and PAS positive staining in both Kupffer cells and splenic macrophages. This implies that these cells may be responsible for clearing the drug. There were microscopic signs of toxicity in the kidneys in high dose males and females.

Study number	5951602
Sponsor reference number	FE200486DSTOX0122
EDR filename	tox0122-nonclinical-data.pdf
Conducting laboratory	_____
Date of study initiation	December 2001
GLP compliance	Yes

b(4)

QA reports Yes
 Drug Degarelix, Batch 0048262 # PPL-FE4860001
 Purity 99.66%, Peptide content 87.99,

b(4)

Methods
 Animal Male and female Sprague-Dawley rats (ICO0: FA.SD. (IOPS Caw))
 males: 381.2 to 417.6 g, females: 230.9 to 275.2 g, 11 to 12 weeks
 Doses and N As shown in the table below from the study report.

Group/ Treatment	Dose level (mg/kg/day)*	Dose volume (ml/kg/day)	Dose concentration (mg/ml)*	Number of animals	
				Males ⁽¹⁾	Females ⁽¹⁾
1. Control	0	5	0	10	10
2. Low dose	0.03	5	0.006	10 (+6)	10 (+6)
3. Intermediate dose	0.3	5	0.06	10 (+6)	10 (+6)
4. High dose	3.0	5	0.6	10 (+6)	10 (+6)

* dose level/concentration expressed in mg of active ingredient.

Schedule Daily for 28 days males, 29 days females
 Route IV
 Dose volume 5 mL/kg
 Dose concentration 0, 0.006, 0.06, 0.6 mg/mL (nominal)
 Formulation Glucose_{aq} 5% w/v

Observations
 Mortality Twice daily
 Clinical signs Daily
 Body weight Twice weekly
 Food cons. Weekly
 Hematology At termination
 Clinical chem. At termination
 Urinalysis At termination
 Ophthalmology Pretest and termination, controls and HD only
 Toxicokinetics Satellite group sampled on days 1, 15 and 28 according to the schedule below (table from the study report). Non-compartmental analysis.

Number of animals per group	predose	Time after dosing ^(a)							
		+ 5 min.	+ 15 min.	+ 30 min.	+ 1 hour	+ 2 hours	+ 4 hours	+ 10 hours	+ 24 hours
2 males + 2 females	+			+			+		
2 males + 2 females		+			+			+(b)	
2 males + 2 females			+			+			+(b)

^(a) Time of sampling with respect to the end of dosing.

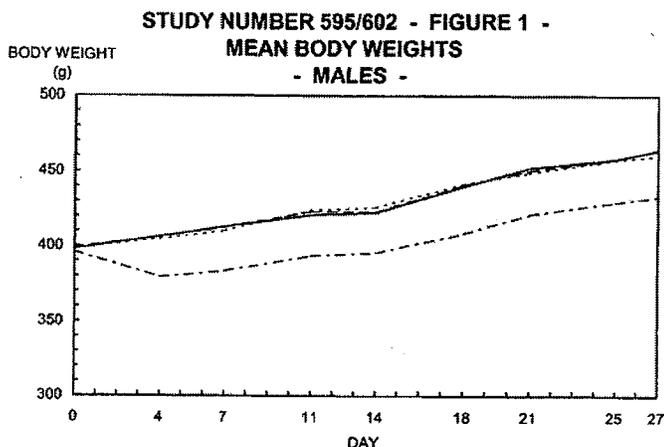
^(b) No samples were available for high dose male no. 96 on day 0 at the 10 hours post-dosing time-point (coagulated sample) or for low dose male no. 85 on day 0 at the 24 hours post-dosing time-point (unknown reason).

min.: minutes.

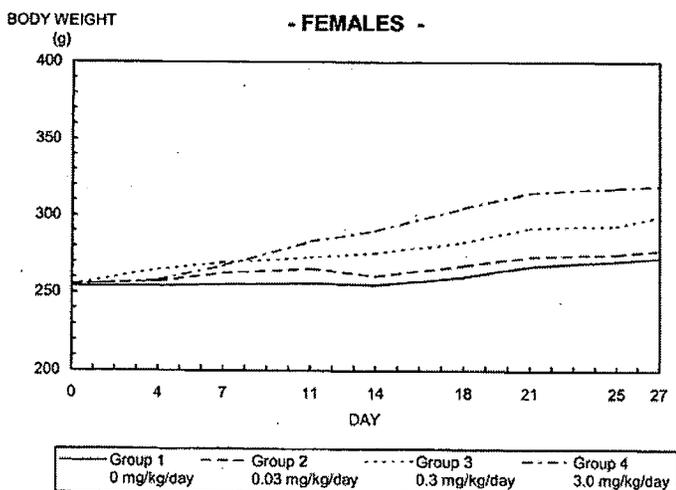
Necropsy Day 28 males, day 29 females
Histopathology Adequate battery

Results

Mortality – All animals survived to scheduled necropsy
Clinical signs – No toxicologically significant changes
Ophthalmology – No toxicologically significant changes
Body weight – High dose males weighed significantly less than controls or other treatment groups as shown in the following graph from the study report.



Female rats gained more weight than controls as a function of increasing dose as shown in the following graph from the study report.



Food cons. – HD males ate somewhat less than controls in the first week thereafter there were no toxicologically significant differences from control in any group.

Water cons. – Males and females in the HD group consumed significantly more water than controls throughout the study. This symptom was more pronounced in males.

Hormone Analysis – The following table shows the concentrations of the major affected hormones at the end of the study (day 28). The decrease in LH in the low does group establishes that there is no NOEL in this experiment. Clinically a concentration of testosterone below 1.7 nM results in a hormonal state equivalent to castration.

Hormone	Control	LD	MD	HD
Male Testosterone nM	10.1	11	0.4	LOQ
Female Estradiol nM	50.3	57.3	21.3	19.6
Testosterone ng/mL	16.5	18.0	0.7	LOQ
Estradiol ng/mL	82.1	93.5	34.8	32.0
Male LH ng/mL	0.38	0.17	0.03	0.01
Female LH ng/mL	0.19	0.3	0.14	0.02

Hematology – The following table shows that degarelix caused anemia in males and females that increased with increasing dose. MCV was unchanged. White cell counts increased with increasing dose in both sexes.

Appears This Way
On Original

Parameter	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
Hb male	Day 28 or 29	16.1	-0.6%	-0.6%	-11.8%
PCV male	Day 28 or 29	46.9	-2.8%	-1.9%	-13.2%
RBC male	Day 28 or 29	8.78	-4.6%	-3.5%	-13.3%
MCH male	Day 28 or 29	18.4	3.8%	2.2%	1.1%
MCV male	Day 28 or 29	53.5	1.7%	1.5%	0.0%
APTT male	Day 28 or 29	21.5	3.3%	-7.9%	-23.7%
Reticulocyte % male	Day 28 or 29		4.8%	0.0%	61.9%
PT male	Day 28 or 29	13.8	-0.7%	-5.1%	-0.7%
WBC male	Day 28 or 29	8.49	-8.2%	13.1%	53.8%
Neutrophils male	Day 28 or 29	17.7	-8.5%	-26.6%	-20.3%
Neutrophils% male	Day 28 or 29	1.48	-12.8%	-12.8%	22.3%
Lymphocytes male	Day 28 or 29	6.45	-7.4%	15.5%	60.8%
Lymphocytes% male	Day 28 or 29	75.7	1.5%	3.6%	4.8%
Monocytes male	Day 28 or 29	0.23	4.3%	39.1%	69.6%
Monocytes% male	Day 28 or 29	2.6	23.1%	26.9%	11.5%
Eosinophils male	Day 28 or 29	0.19	-5.3%	94.7%	52.6%
Eosinophils % male	Day 28 or 29	2.4	-4.2%	50.0%	-8.3%
Leukocyte male	Day 28 or 29	0.06	0.0%	50.0%	133.3%
Leukocyte % male	Day 28 or 29	0.8	0.0%	25.0%	25.0%
Hb female	Day 28 or 29	15.5	-2.6%	0.0%	-4.5%
PCV female	Day 28 or 29	44.1	-2.9%	0.0%	-4.8%
RBC female	Day 28 or 29	8.09	-4.4%	-0.6%	-5.8%
MCH female	Day 28 or 29	19.2	2.1%	1.0%	1.6%
MCV female	Day 28 or 29	54.5	1.7%	0.6%	1.3%
APTT female	Day 28 or 29	17.5	1.7%	-2.9%	-6.9%
Reticulocyte % female	Day 28 or 29	2.1	9.5%	4.8%	28.6%
PT female	Day 28 or 29	14.4	-2.1%	-1.4%	-0.7%
Fibrinogen female	Day 28 or 29	1.72	-4.7%	7.0%	53.5%
WBC female	Day 28 or 29	6.05	4.0%	18.3%	66.8%
Neutrophils female	Day 28 or 29	0.82	29.3%	12.2%	43.9%
Neutrophils% female	Day 28 or 29	13.8	18.8%	-6.5%	-14.5%
Lymphocytes female	Day 28 or 29	4.79	-1.0%	19.0%	70.4%
Lymphocytes % female	Day 28 or 29	79	-4.6%	0.9%	2.2%
Monocytes female	Day 28 or 29	0.17	35.3%	0.0%	64.7%
Monocytes% female	Day 28 or 29	2.8	35.7%	-14.3%	0.0%
Eosinophils female	Day 28 or 29	0.15	-13.3%	53.3%	73.3%
Eosinophils % female	Day 28 or 29	2.3	0.0%	30.4%	17.4%
Leukocyte female	Day 28 or 29	0.06	16.7%	16.7%	100.0%
Leukocyte % female	Day 28 or 29	1	10.0%	0.0%	20.0%

Clinical chem. – The table below the changes in clinical chemistry parameters in this study.

Clinical Chemistry	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
Creatinine male	Day 28 or 29	5.5	-3.6%	1.8%	50.9%
Creatinine female	Day 28 or 29	6.2	-4.8%	-3.2%	14.5%
Potassium male	Day 28 or 29	5	0.0%	-2.0%	0.0%
Potassium female	Day 28 or 29	4.5	1.1%	0.0%	6.7%
Urea male	Day 28 or 29	0.39	0.0%	-5.1%	120.5%
Urea female	Day 28 or 29	0.38	-2.6%	-2.6%	65.8%
Chloesterol male	Day 28 or 29	0.62	-4.8%	12.9%	85.5%
Cholesterol female	Day 28 or 29	0.76	22.4%	6.6%	31.6%
Protein male	Day 28 or 29	61	-1.6%	0.0%	-3.3%
Protein female	Day 28 or 29	61	8.2%	1.6%	-3.3%
Alk Phos male	Day 28 or 29	275	-1.5%	-12.0%	-10.2%
Alk Phos female	Day 28 or 29	193	-22.3%	9.3%	25.4%
Albumin male	Day 28 or 29	40	-2.5%	-2.5%	-5.0%
Albumin female	Day 28 or 29	36	11.1%	0.0%	-2.8%
AST male	Day 28 or 29	113	6.2%	-7.1%	15.9%
AST female	Day 28 or 29	99	4.0%	2.0%	35.4%
Bilirubin male	Day 28 or 29	1.6	0.0%	6.2%	12.5%
Bilirubin female	Day 28 or 29	1.9	10.5%	5.3%	-15.8%

Urinalysis – The following table shows the changes in urine parameters in this study

Urinalysis	Observation	Control value	Percent Change		
			Low Dose	Mid Dose	High Dose
Volume male	Day 28 or 29	12.6	16.7%	22.2%	19.0%
Volume female	Day 28 or 29	7.8	-9.0%	0.0%	9.0%
pH male	Day 28 or 29	7.4	0.0%	0.0%	-14.9%
pH female	Day 28 or 29	6.5	-3.1%	-1.5%	-12.3%

Organ weight – The following table shows the dose related changes in organ weights. The table also includes major organs that showed no change to allow comparison with studies of subcutaneous dosing.

Organ weights	Control value	Absolute Change			Relative Change *		
		Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
male body weight	422.7	418.2	416.2	387.2			
Heart male	1.42	1.4%	-0.7%	-5.6%	1.025	1.008	1.030
Brain male		-0.9%	0.0%	-1.8%	1.002	1.016	1.072
Kidney male	2.96	0.3%	-8.1%	-2.0%	1.014	0.933	1.070
Adrenal male		4.0%	15.2%	30.2%	1.052	1.170	1.421
Liver male	11.79	-4.2%	-3.2%	-7.5%	0.969	0.983	1.009
Lung male	1.67	21.6%	3.0%	2.4%	1.229	1.046	1.118
Epididymides	1.45	-4.8%	-43.7%	-69.5%	0.962	0.571	0.333
Prostate	1.36	-19.1%	-67.1%	-80.8%	0.818	0.334	0.209
Seminal Vesicles	1.75	-20.0%	-81.6%	-88.0%	0.809	0.187	0.131
Testes	3.75	-2.7%	-36.0%	-74.5%	0.984	0.650	0.278
Pituitary male	0.01198	14.7%	11.7%	2.6%	1.159	1.134	1.120
Spleen male	0.68026	-1.3%	-3.2%	31.0%	0.998	0.983	1.430
Thymus male	0.38338	6.4%	51.5%	44.0%	1.075	1.538	1.572
Mandibular gland male	0.73281	-2.8%	-13.4%	-11.5%	0.982	0.879	0.967
Thyroid male	0.01926	19.9%	-2.3%	-10.2%	1.212	0.992	0.980
Female body weight	248.6	251	272	285			
Heart female	0.93882	3.0%	3.1%	8.6%	1.020	0.944	0.947
Brain female	2.07	0.0%	0.0%	1.4%	0.990	0.916	0.884
Kidney female	1.73	-0.6%	2.3%	17.3%	0.985	0.937	1.022
Adrenal Female	0.07413	7.7%	3.4%	4.9%	1.066	0.947	0.914
Liver female	6.49	4.9%	9.1%	16.9%	1.039	0.999	1.019
Lung female	1.35	3.7%	2.2%	3.0%	1.027	0.936	0.897
Ovaries	0.10805	-29.1%	-39.5%	-38.6%	0.702	0.554	0.535
Uterus	0.80423	0.9%	-44.3%	-77.4%	0.999	0.510	0.197
Pituitary female	0.01641	17.0%	-0.6%	-18.2%	1.159	0.910	0.713
Spleen female	0.44859	2.6%	16.4%	48.1%	1.016	1.066	1.291
Mandibular gland	0.54018	-1.5%	1.0%	-0.1%	0.976	0.925	0.871
Thymus female	0.29262	36.3%	85.0%	146.8%	1.350	1.694	2.150
Thyroid female	0.01508	7.1%	11.4%	6.8%	1.061	1.020	0.930

The following table shows the microscopic pathology associated with this treatment

Appears This Way
 On Original

Organ and lesion N unless otherwise indicated	Male			Female				
	Control 10	LD 10	MD 10	HD 10	Control 10	LD 10	MD 10	HD 10
Liver								
Lymphoid cell infiltration		6			4	2	1	2
Kupffer cell vacuolization				10				10
Kupffer cell aggregation			3	9				10
Liver PAS test positive in Kupffer cells				10				10
Liver IMM test positive in Kupffer cells				4/4	NT	NT	NT	NT
Liver IMM test positive background staining	3/4	3/4	3/4					
Spleen								
Aggregation of macrophages			3	10				8
Eosinophilic material in macrophages			1	10				8
PAS positive in macrophages				9				10
Lung								
Eosinophilic material in deposit				10				7
Granuloma formation			1	9				9
Kidney								
Tubular basophilia	4	3	1	10	2		1	10
Tubular degeneration and regeneration				9				10
Necrotic debris in the lumen				8				5
Tubular dilation				10			1	8
Peritubular fibrosis		2		10	1			10
Eosinophilic material in the tubules				9	1			10
Lymphoid cell infiltration	1	3		9			1	8

NT = not tested

IMM = immunohistochemical staining with antibodies to degarelix

PAS = Periodic Acid Schiff technique, indicating eosinophilic deposits

Toxicokinetics

The following table from the study report shows the results of the toxicokinetic analysis after the first dose.

Mean pharmacokinetic parameters of FE200486 after intravenous administration to Sprague Dawley rats on the first day of dosing.

Group	Gender	AUC h·ng/ml	AUC/dose NA	CL ml/h/kg	V _z ml/kg	V _{ss} ml/kg	t _{1/2} h
2	Female	115	0.0038	261	419	396	1.11
	Male	149	0.0050	202	487	420	1.67
	Mean	132	0.0044	232	453	408	1.39
3	Female	1319	0.0044	227	946	424	2.88
	Male	1586	0.0053	189	867	403	3.18
	Mean	1453	0.0049	208	907	414	3.03
4	Female	16782	0.0056	179	898	684	4.47
	Male	19942	0.0066	150	971	717	3.48
	Mean	18362	0.0061	165	935	701	3.98

The following table from the study report shows the results of the toxicokinetic analysis after the last dose.

Mean (±SD) pharmacokinetic parameters of FE200486 after intravenous administration to Sprague Dawley rats on the last day of dosing (day 27).

Best Possible Copy

Group	Gender		AUC h·ng/ml	AUC _{27/1} NA	AUC/dose NA	CL ml/h/kg	V _z ml/kg	V _{ss} ml/kg	t _{1/2} h
2	Female		108	0.9	0.0036	277	484	393	1.21
	Male		138	0.9	0.0046	217	501	460	1.60
		Mean	123	0.9	0.0041	247	493	427	1.41
3	Female		1055	0.8	0.0035	284	1083	587	2.64
	Male		1218	0.8	0.0041	246	616	558	1.73
		Mean	1137	0.8	0.0038	265	850	573	2.19
4	Female		15961	0.9	0.0053	188	844	551	3.11
	Male		8152	0.9	0.0061	165	853	563	3.58
		Mean	17057	0.9	0.0057	177	849	557	3.35

8) 13 Week Bridging Study in Rats with Subcutaneous Administration

Major Findings

This study was designed to demonstrate the toxicological similarities or differences among three different batches of drug product. One batch was prepared by a _____ and was relatively pure, _____-PB (Batch No. PPL-FE4860201A). One was prepared by the same method but contained a significant amount of degradation products and impurities that remained after synthesis, _____-IB (Batch No. 03H15-02). The third was a relatively pure batch prepared by a _____ (Batch No. RD293-04). All three drug products were given fortnightly for 7 doses at a dose level of 150 mg/m².

b(4)

One female rat given _____-IB died in week six, the investigators ascribed this death to pneumonia. Clinical signs were similar among dose groups and primarily consisted of injection site damage. Treated male animals all weighed less than controls and their weights were all comparable to each other. In females, all treated groups weighed more than controls but females receiving _____-IB (the batch containing significantly more impurity) weighed more than other treated animals. This is possibly due to an: _____

b(4)

The impure batch caused a smaller decrease in the size of the adrenals in females than did the pure batch, the change was comparable to that caused by _____. It caused more hepatomegaly than _____-PB but a smaller increase in cholesterol. It caused an increase in size of the mesenteric lymph node in females when compared to _____-PB but was again comparable to _____. It caused less reduction in size in the male kidney than either pure batch. _____ appeared to cause less hepatotoxicity and splenomegaly than either _____ products.

b(4)

Exposure to the parent was greatest with _____, and least with _____-IB but the exposure caused by _____-IB was somewhat less than one would expect relative to the _____. Other toxicokinetic parameters were comparable.

With the exception of an increase in weight of the mesenteric lymph node, the _____ drug product is either less toxic than the _____-PB drug product or the differences are toxicologically insignificant. The difference in toxicity at the mesenteric lymph node may be toxicologically significant. The increase

b(4)

b(4)

in body weight caused by — -IB demonstrates that it is clearly different from the pure batches toxicologically. This experiment does not qualify SSPS-IP as pharmacologically equivalent to the other to batches.

_____ number 457033
Sponsor reference number FE200486DSTOX0303
EDR filename tox0303-nonclinical-data.pdf
Conducting laboratory _____
Date of study initiation October 2003
GLP compliance Yes
QA reports Yes
Drug

b(4)

b(4)

- 1) _____ (PB) Batch No. PPL-FE4860201A, 91.4% free base),
- 2) Impurity batch, _____ -IB, (Batch No. 03H15-02; 84.4% free base), which contains degradation products and synthesis impurities
- 3) _____ (Batch No. RD293-04, 91.6% free base)

Methods

Animal Wistar rats — (WI)BR)

Doses

Group	Treatment* (mg.kg ⁻¹ .2 weeks ⁻¹)	Animal Numbers			
		Main Study		Toxicokinetic Study	
		Males	Females	Males	Females
1	Control 0	1-10	81-90	11-20	91-100
2	.PB 25	21-30	101-110	31-40	111-120
3	-IB 25	41-50	121-130	51-60	131-140
4	25	62-70, 161	141-150	71-80	151-160

b(4)

*Dose levels are expressed as 100% peptide content.

Schedule fortnightly for 13 weeks (7 doses)
Route SC
Dose volume 5 mL/kg
Formulation Mannitol_{aq} 5% w/v

Mortality: Twice daily
Clinical signs: Once per week
Body weights: Once per week, more if deterioration was noted.
Food consumption: Weekly
Ophthalmoscopy: Performed on Week 5 and 12
Hematology: Blood samples were acquired on Week 13 of treatment
Clinical chemistry: Samples were acquired on Week 13 of treatment
Urinalysis: Collected on Week 12 of the study over 4 hours
Gross pathology: Extensive pathological examination and organ weights from selected animals were obtained on Week 13.
Organ weights: Selected organs were weighed at Week 13 as is specified in the table supplied by the sponsor
Histopathology: Adequate battery but as this is a comparative study controls were not examined microscopically

The design of this study is the same as that of FE200486DSTOX0112 reviewed above.

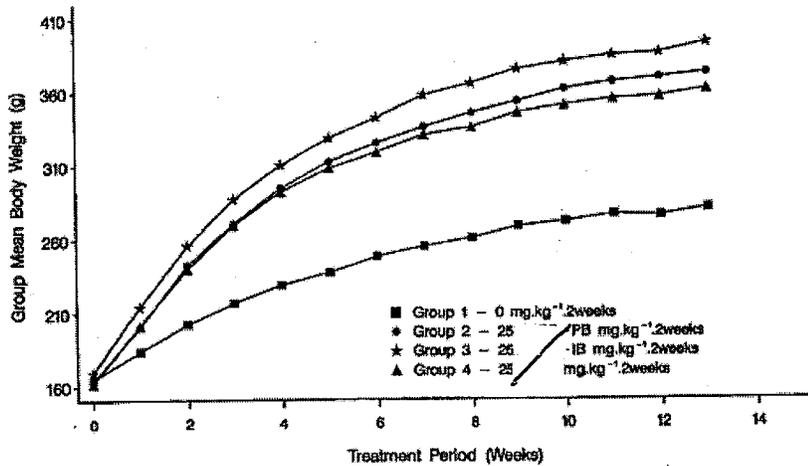
Mortality One female receiving — IB died in week six. The investigators ascribed this death to pneumonia.

Clinical signs Injection site damage comparable in all groups

Body Weight In males, all treated groups weighed less than controls and were statistically indistinguishable from each other. In females all treated groups weighted more than controls but females receiving — IB (the batch containing significantly more impurity) weighed more than other treated animals as the graph from the study report shows.

b(4)

FE200486
13 Week Bridging Study in Rats with Subcutaneous Administration
Group Mean Body Weights: Main Study - Females



b(4)

Food cons. All treated females ate more than controls. Males ate amounts comparable to controls.

Ophthalmology No toxicologically significant findings

Toxicokinetics The toxicokinetic parameters determined in this experiment were comparable to those seen for rats receiving the same dose in the carcinogenicity study. The exposure of rats in the — IB group appeared to be somewhat lower than the exposure of rats receiving the two purer drug products as the tables from the study report below shows.

b(4)

Appears This Way
On Original

Table A - Parameters Relating to Systemic Exposure to FE200486: Effect of Test Item in Males and Females (Dose 1)

Sex	Treatment 25 mg.kg ⁻¹ .2 weeks ⁻¹ [Group]	AUC (ng.h/mL)	AUC _t (ng.h/mL)	C _{max} (ng/mL)
Male	PB	63108*	22435	314
	IB	32341	17990	235
	[4]	160380*	25357	331
Female	PB	40457	22743	267
	IB	112541*	17715	233
	[4]	51623	24678	308

FE200486 made by
 FE200486 made by
 FE200486 made by
 * Parameter estimate derived from unreliable determination of lambda-z.

Parent Batch,
 Impurity Batch.

b(4)

Table B - Toxicokinetic Parameters: Effect of Test Item in Males and Females (Dose 1)

Sex	Treatment 25 mg.kg ⁻¹ .2 weeks ⁻¹ [Group]	t _{max} (h)	t _{1/2} (h)	CL/F (mL/h/kg)	V _{z/f} (mL)
Male	PB	4.00	682.64*	396.1*	390136*
	IB	2.00	338.92	773.0	377978
	[4]	4.00	1799.82*	155.9*	404757*
Female	PB	4.00	372.62	617.9	332194
	IB	4.00	1800.21*	221.9*	576422*
	[4]	4.00	441.95	454.3	308778

FE200486 made by
 FE200486 made by
 FE200486 made by
 * Parameter estimate derived from unreliable determination of lambda-z.

Parent Batch,
 Impurity Batch.

b(4)

The following table shows the parameters that differ among the three drug products. The columns marked Prob 1 gives the probability that the value for the drug product is different from that of — PB (the pure batch). The column marked Prob 2 gives the probability that — is different from — IB (the impure batch). The table includes values for both males and females when the parameter was significant in either sex. It also includes values that did not reach statistical significance but are toxicologically interesting.

b(4)

Appears This Way
 On Original

Parameter	Control value	Percent Change			Prob 1	Prob 1	Prob 2
		-PB	-IB				
Hematology							
WBC male	9.84	21.5%	28.5%	4.2%			0.05
Eosinophils male	0.19	57.9%	57.9%	10.5%		0.01	0.01
WBC female	7.05	53.6%	85.7%	65.0%			
Eosinophils female	0.2	30.0%	55.0%	45.0%			
Clinical Chemistry	Control value	-PB	-IB				
Creatinine male	51	7.8%	7.8%	7.8%			
Creatinine female	51	3.9%	11.8%	0.0%			
Chloesterol male	2.2	36.4%	31.8%	22.7%			
Cholesterol female	2.3	43.5%	26.1%	26.1%	0.05	0.01	
Protein male	67	0.0%	0.0%	-1.5%			
Protein female	66	1.5%	-1.5%	-3.0%		0.01	
A/G ratio male	2	-5.0%	-5.0%	0.0%			
A/G ratio female	2.6	-26.9%	-23.1%	-15.4%		0.05	0.05
Globulin male	23	4.3%	0.0%	0.0%			
Globulin female	19	21.1%	15.8%	5.3%		0.01	0.05
Bilirubin male	1.4	35.7%	28.6%	42.9%			
Bilirubin female	2.4	12.5%	-20.8%	-16.7%			
Urinalysis	Control value	-PB	-IB				
Volume male	2.8	-3.6%	-32.1%	-14.3%			
Volume female	1.7	-11.8%	-35.3%	11.8%			
pH male	8.6	3.5%	3.5%	-1.2%			
pH female	8.1	1.2%	-8.6%	8.6%			
Organ Weight		-PB	-IB				
Kidney male	3.17	-21.8%	-15.8%	-19.9%	0.05		
Kidney female	2.01	0.5%	5.5%	-2.5%			
Liver male	19.75	-28.3%	-27.1%	-29.5%			
Liver female	9.56	10.9%	29.2%	5.2%	0.01		0.001
Adrenals male	0.0667	15.0%	4.0%	15.7%			
Adrenals female	0.0899	-30.1%	-19.5%	-25.8%	0.01		
Spleen male	0.93	-6.5%	-4.3%	-6.5%			
Spleen female	0.65	18.5%	21.5%	6.2%			0.01
Pituitary male	0.012	-16.7%	-25.0%	-16.7%			
Pituitary female	0.013	-23.1%	-15.4%	-30.8%			0.05
Mesenteric Lymph Node male	0.1674	-11.1%	10.9%	-2.0%			
Mesenteric Lymph Node female	0.1302	-12.0%	25.7%	27.0%	0.05	0.01	

b(4)

Gross Pathology – no toxicologically significant differences
 Histopathology – no toxicologically significant differences

RTD reviewed the following toxicology studies in the tabular summary below

- 9) 13-Week Subcutaneous Toxicity Study in the Male Rat
- 10) Toxicity Study by Subcutaneous Administration to DC Rats for 26 Weeks Followed by a 26 Week Recovery Period
- 11) A 14-Day Intravenous Toxicity Study in Rats
- 12) 2-Week Intravenous (bolus) Toxicity Study in the Cynomolgus Monkey
- 13) 4-Week Intravenous (bolus) Toxicity Study in the Cynomolgus Monkey

2.6.6.4 Genetic toxicology

The sponsor submitted six standard Ames studies of degarelix

- 1) **MUT9801-FE200486 Ames Test – WDM**
- 2) **MUT0201-FE200486 Ames Test – WDM**
- 3) **MUT0301-FE200486 Ames Test – WDM**
- 4) **2357-FE200486 Batch No 06G18-01 Ames Test – WDM**
- 5) **2368-FE200486 Batch No 06G18-02 Ames Test – WDM**
- 6) **2369-FE200486 Batch No 06H09-01 Ames Test – WDM**

The following table shows that all these GLP tests were negative:

Study	Study Number	GLP/Audit	Batch	Doses µg/plate	Mutagenicity without activation	Mutagenicity with activation	Toxicity	Precipitation
Ames March-1998	FE200486	yes/yes	201 1-001 -30	0	none	none		
				5	none	none		
				15	none	none		
				50	none	none		
				150	none	none		
				500	none	none	minor	
Ames October-2002	FE200486DSMUT020	yes/yes	02315601	0	none	none		
				50	none	none		
				160	none	none		
				500	none	none		
				1600	none	none		yes
				5000	none	none		yes
Ames January-2004	FE200486DSMUT0301	yes/yes	03H15-02	0	none	none		
				50	none	none		
				160	none	none		
				500	none	none		yes
				1600	none	none		yes
				5000	none	none		yes
Ames Sep-07	200486-2357	yes/yes	06G18-01	0	none	none		
				16	none	none		
				50	none	none		
				160	none	none		
				500	none	none		
				1600	none	none	minor	yes
5000	none	none	minor	yes				
Ames Sep-07	200486-2368	yes/yes	06G18-02	0	none	none		
				16	none	none		
				50	none	none		
				160	none	none		
				500	none	none		
				1600	none	none		yes
5000	none	none		yes				
Ames Sep-07	200486-2369	yes/yes	06H09-01	0	none	none		
				16	none	none		
				50	none	none		
				160	none	none		
				500	none	none		yes
				1600	none	none		yes
5000	none	none		yes				

- 7) **Mutation assay at the TK locus in L5178Y mouse lymphoma cells using a microtiter cloning technique (trifluorothymidine resistance) with FE200486 - WDM**

Major finding

Degarelix did not cause mutations at the TK locus in L5178Y mouse lymphoma cell culture under the conditions of this assay. The maximum concentration of degarelix tested caused little or no toxicity. The positive controls caused significant numbers of mutations.

Study number: MUT9802
Sponsor reference number: FE200486DSMUT9802
EDR file Name: MUT9802-nonclinical-data.pdf
Conducting laboratory: _____
Date of study initiation: July 1998
GLP compliance: Yes
QA reports: Yes
Drug: Degarelix, Batch 201 1-001-30, and purity 99.8%

b(4)

Method

Cell Strain: L5178Y mouse lymphoma cells
S9: Aroclor induced rat liver microsomes

Toxicity Test

Doses: Without S9, 31.2, 62.5, 125, 250, 500 µg/mL
With S9, 31.2, 62.5, 125, 250, 500 µg/mL
Positive Control: Without S9: Methyl methanesulfonate 10 pg/ml (3 h treatment)
With S9: Cyclophosphamide, 2 pg/ml
Treatment duration: Without S9, short treatment: 3 hours
Without S9, continuous treatment 24 hours
With S9, 3 hours

Mutagenicity Test

Doses without S9: 98.8, 148.1, 222.2, 333.3, 500 (assay 1: 3 hour treatment)
62.5, 125, 250, 500 (assay 2: 24 hour treatment)
Doses with S9: 62.5, 125, 250, 500 (assay 1)
62.5, 125, 250, 500 (assay 2)
Positive Control: Without S9 mix, Methyl methanesulfonate 10 µg/mL (3 h treatment)
Methyl methanesulfonate 2 µg/mL (24 h treatment)
With S9 mix, Cyclophosphamide, µg/mL
No of Replicates: 2 per dose

Results

In the three hour assay without metabolic activation at the maximum concentration tested, 500 µg/mL, the plating efficiency was 78% and the relative survival was 88%. In the 24 hours assay, plating efficiency was 87% and the relative survival was 94%. The highest concentrations tested with metabolic activation were similarly non-toxic. Thus, the highest concentration was only minimally toxic; higher concentrations should have been tested. Precipitation at this highest concentration was only slight. In the study summary, the investigators say that the reason for using 500-µg/mL as the maximum concentration was "quantity limited of the test compound and solubility."

MUTATION ASSAY AT THE TK LOCUS
 IN L5178Y MOUSE LYMPHOMA CELLS
 WITHOUT METABOLIC ACTIVATION
 3 HOUR SHORT TREATMENT

ASSAY 1

TIME 2 days after treatment

Starting date : 14/5/1988

Completion date: 26/5/1988

CULTURE A		MUTATION										VIABILITY at T2				Mutation Frequency x10 ⁻⁶ cells	RATIO		
		Number of positive wells										Number of negative wells							
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells		Plate				PE %	
COMPOUND	DOSE (µg/ml ^(*))	1	2	3	4	1	2	3	4	1	2	3	4	+	-	1	2	per 10 ⁵	%
Solvent Control	0	9	10	8	10	35	13	4	5	9	31	66	318	21	22	43	93.5	100.8	
TEST COMPOUND	98.8	9	12	7	16	44	8	11	5	10	34	78	306	12	13	25	127.4	89.1	0.9
	148.1	5	12	6	8	32	8	8	13	9	38	70	314	15	11	26	125.0	80.5	0.8
	222.2	10	10	10	13	44	5	12	5	13	35	79	305	12	7	19	144.6	79.7	0.8
	333.3	9	5	12	6	32	12	3	8	4	27	59	325	18	21	39	99.6	83.7	0.8
	500	10	11	10	5	36	11	3	8	11	33	69	345	21	17	38	101.2	87.8	1.0
MMS	10	41	33	39	38	151	15	24	14	23	76	227	157	36	25	61	71.7	824	8.2

CULTURE B		MUTATION										VIABILITY at T2				Mutation Frequency x10 ⁻⁶ cells	RATIO		
		Number of positive wells										Number of negative wells							
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells		Plate				PE %	
COMPOUND	DOSE (µg/ml ^(*))	1	2	3	4	1	2	3	4	1	2	3	4	+	-	1	2	per 10 ⁵	%
Solvent Control	0	8	8	10	7	33	11	8	13	10	42	75	309	27	30	57	75.9	143.1	
TEST COMPOUND	98.8	14	16	14	6	50	12	12	13	13	50	100	284	8	9	17	151.5	89.5	0.7
	148.1	11	10	8	15	44	10	7	11	5	33	77	307	18	19	35	108.4	105.2	0.7
	222.2	8	23	14	11	56	9	16	9	11	45	101	283	9	7	16	155.3	96.3	0.7
	333.3	6	11	12	9	38	8	8	4	12	32	70	314	22	21	43	93.5	107.8	0.8
	500	7	8	6	9	28	9	9	8	10	36	64	330	20	22	42	95.0	96	0.7
MMS	10	46	42	45	42	175	17	31	19	21	68	253	121	25	15	40	98.0	589	4.1

PE: Plating efficiency

(*) Doses of the test compound expressed in free base peptide

Appears This Way
 On Original

MUTATION ASSAY AT THE TK LOCUS
 IN L5178Y MOUSE LYMPHOMA CELLS
 WITH METABOLIC ACTIVATION
 3 HOUR TREATMENT

ASSAY 1

Seating date : 12/5/1998

TIME 2 days after treatment

Completion date: 25/5/1998

CULTURE A		MUTATION												VIABILITY at T2				Mutation Frequency $\times 10^{-6}$ cells	RATIO
		Number of positive wells												Number of negative wells		PE %			
		SMALL COLONIES				LARGE COLONIES				TOTAL Wells		Plate							
COMPOUND	DOSE $\mu\text{g/ml}^{(*)}$	Plate				TOTAL per 384	Plate				TOTAL per 384	+	-	TOTAL per 192	TOTAL per 192				
		1	2	3	4		1	2	3	4			1	2					
Solvent Control	0	12	12	8	10	42	6	8	5	5	24	66	318	17	25	43	93.5	100.8	
TEST COMPOUND	62.5	14	15	12	7	48	5	5	3	4	28	75	308	17	20	37	102.9	107.2	1.1
	125	8	8	7	9	32	3	7	3	8	21	53	331	31	28	59	73.7	100.7	1.0
	250	12	7	10	7	36	7	8	9	6	30	66	318	22	14	36	104.6	90.1	0.9
	500	15	9	12	8	45	5	5	2	4	16	61	323	20	25	45	90.7	95.4	0.9
CPA	2	40	50	43	48	189	24	20	15	17	76	285	119	35	23	58	73.7	794.3	7.9

CULTURE B		MUTATION												VIABILITY at T2				Mutation Frequency $\times 10^{-6}$ cells	RATIO
		Number of positive wells												Number of negative wells		PE %			
		SMALL COLONIES				LARGE COLONIES				TOTAL Wells		Plate							
COMPOUND	DOSE $\mu\text{g/ml}^{(*)}$	Plate				TOTAL per 384	Plate				TOTAL per 384	+	-	TOTAL per 192	TOTAL per 192				
		1	2	3	4		1	2	3	4			1	2					
Solvent Control	0	11	8	13	14	46	11	7	7	8	33	79	308	13	22	35	106.4	108.3	
TEST COMPOUND	62.5	12	12	15	12	51	8	7	10	8	29	60	304	13	15	28	120.3	97.1	0.9
	125	12	16	11	10	49	11	7	5	8	31	60	304	11	15	26	125.0	93.5	0.9
	250	6	18	13	10	47	10	8	8	5	29	75	309	15	12	27	122.8	88.6	0.8
	500	17	16	17	12	62	5	8	3	7	23	85	259	17	17	34	108.2	115.6	1.1
CPA	2	35	34	45	34	148	15	20	21	22	78	226	158	22	23	45	90.7	488.7	4.5

PE: Plating efficiency

(*) Doses of the test compound expressed in two base peptide

Appears This Way
 On Original

MUTATION ASSAY AT THE TK LOCUS
 IN L5178Y MOUSE LYMPHOMA CELLS
 WITHOUT METABOLIC ACTIVATION
 24 HOUR CONTINUOUS TREATMENT

ASSAY 2

TIME 2 days after treatment

Starting date : 27/5/1998

Completion date: 5/8/1998

CULTURE A		MUTATION											VIABILITY at T2			Mutation Frequency $\times 10^{-6}$ cells	RATIO		
COMPOUND	DOSE $\mu\text{g/ml}$	Number of positive wells											Number of negative wells						
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells		Plate				TOTAL per 100	PE %
		Plate				TOTAL per 384	Plate				TOTAL per 384	+	-	1	2				
1	2	3	4	1	2		3	4	+	-						1	2		
Solvent Control	0	5	6	6	8	25	4	6	3	7	20	45	339	14	20	34	108.2	57.6	
TEST COMPOUND	62.5	6	4	4	6	22	6	7	6	6	25	47	337	22	12	34	108.2	60.3	1.0
	125	9	6	6	7	28	1	6	6	4	17	45	339	19	20	48	88.8	71.9	1.2
	250	6	6	6	8	28	6	3	1	5	14	43	341	18	26	44	82.1	84.5	1.1
	500	6	13	4	12	35	2	9	4	6	23	61	323	16	16	32	112.6	77.2	1.3
MMS	2	40	49	40	44	173	25	13	23	13	74	247	137	9	21	30	116.0	444.2	7.7

CULTURE B		MUTATION											VIABILITY at T2			Mutation Frequency $\times 10^{-6}$	RATIO		
COMPOUND	DOSE $\mu\text{g/ml}$	Number of positive wells											Number of negative wells						
		SMALL COLONIES					LARGE COLONIES					TOTAL Wells		Plate				TOTAL per 100	PE %
		Plate				TOTAL per 384	Plate				TOTAL per 384	+	-	1	2				
1	2	3	4	1	2		3	4	+	-						1	2		
Solvent Control	0	6	10	6	6	30	4	4	3	6	17	47	337	25	29	54	79.3	82.3	
TEST COMPOUND	62.5	6	11	15	7	39	9	3	2	5	19	58	326	12	19	31	114.0	71.8	0.9
	125	6	7	6	2	23	2	2	3	3	10	33	351	16	24	40	98.0	45.9	0.8
	250	11	9	4	11	35	2	6	7	5	20	55	329	19	11	30	116.0	66.6	0.8
	500	11	6	9	7	33	5	5	1	2	13	46	338	27	22	49	85.4	74.7	0.9
MMS	2	43	56	36	48	163	20	23	32	19	94	257	127	23	22	45	60.7	610.1	7.4

PE: Plating efficiency

(*) Doses of the test compound expressed in free base peptide

8) **MUT0202-*In vitro* mammalian cell gene mutation test performed with mouse lymphoma L5178Y cells – RTD**

Major findings:

- Degarelix did not significantly increase the mutation frequency in mouse lymphoma (L5178Y) cells under the conditions of this experiment. Higher doses of degarelix were toxic to L5178Y cells, illustrating exposure of the cells to the drug, and all positive controls caused mutations under the experimental conditions.

Study number: FE 200486DSMUT0202
 EDR filename: mut0202-nonclinical-data.pdf
 Conducting laboratory: _____
 Date of study initiation: August 19, 2002
 GLP compliance: Yes

b(4)

QA reports: Yes
Drug: FE200486, Batch No: 02315601, 83.38% purity

Methods

Cell line: L5178Y mouse lymphoma cells
Type of study: TK Locus (Trifluorothymidine Resistance)
Culture medium RPMI 1640 medium supplemented with 10% horse serum, 200 µg/ml sodium pyruvate, and 50 µg/ml gentamycin.

Concentrations used: (for both Toxicity and Mutagenicity tests)

Repeat of First test

without S-9 mix: 313, 625, 1250, 1600, 2000, 2500 µg/ml (4 hrs)
with S-9 mix: 313, 625, 1250, 2000, 2500, 3000, 4000 µg/ml (3 hrs)

Second test

without S-9 mix: 100, 200, 400, 800, 1200 and 1600 µg/ml (24 hrs)
with S-9 mix: 200, 400, 800, 1600, 2400 and 3000 µg/ml (3 hrs)

Calculation of molarity for the doses in this study

Concentration (µg/mL)	Calculated Conc. (µM)
313	191.8
625	382.9
1250	765.8
2000	1225.3
3000	1837.9
4000	2450.5

This study has used concentrations that are sufficiently high and are appropriate to be used in the experiment. Notably, a precipitation occurred dose-dependently in preparations of the peptide leaving some question about full exposure of the cells to degarelix.

Basis of dose selection: Doses were selected to cover a wide range of peptide concentrations, and subsequent experimental doses were altered based on observed toxicity to cells.

Negative controls: Sterile distilled water

Positive controls: Without S-9 mix: N-ethyl-N-nitrosourea (ENU) (50 µg/ml)
With S-9 mix: 7,12-dimethyl-1,2-benzanthracene (DMBA, 3.3 µg/ml)

Incubation and sampling: Test #1 : 4 hours without S9 mix, 3 hours with S9 mix
Test #2 : 24 hours without S9 mix, 3 hours with S9 mix

No of Replicates 2 per dose

Results

The following table from the study report shows the results of this experiment.

FE200486
In Vitro Mammalian Cell Gene Mutation Test
 Performed with Mouse Lymphoma L5178Y Cells

MUTATION DATA - Test 1 (Repeat)

Treatment (µg/ml)	Number of mutant clones		Mutation Frequency (per 10 ⁶ cells)				RTG % of Control
	Large	Small	Large	Small	Total	Mean	
Without S-9 mix							
Untreated	36, 36	23, 28	128, 128	79, 97	227, 250	259	
TA (2500)	3, 3	7, 8	44, 48**	103, 129	149, 179	164ns	1
TA (1600)	14, 12	10, 10	89, 116	63, 96	157, 218	188ns	3
TA (1250)	19, 20	19, 18	98, 89*	98, 80	207, 179	193ns	7
TA (625)	33, 33	25, 21	124, 124	92, 76	237, 217	227ns	83
TA (313)	30, 36	29, 32	138, 128	133, 113	298, 270	284ns	75
ENU (50)	32, 29	23, 28	128, 115	89, 110	236, 247	242ns	70
ENU (50)	112, 109	80, 83	843, 831	519, 561	#, #	#	46
With S-9 mix							
Untreated	35, 37	24, 24	145, 145	96, 91	265, 259	262	
TA (4000)	9, 10	10, 13	133, 143	149, 187*	290, 341	316ns	2
TA (3000)	27, 24	18, 16	150, 248	98, 161	265, 433	349ns	3
TA (2500)	33, 31	27, 26	116, 105	94, 87	231, 210	221ns	17
TA (2000)	35, 36	28, 24	97, 108	76, 69	191, 195	193ns	91
TA (1250)	33, 33	23, 26	132, 132	89, 102	241, 257	249ns	118
TA (625)	31, 32	25, 25	156, 113	123, 86	305, 217	261ns	148
TA (313)	34, 33	27, 23	149, 158	116, 107	293, 288	291ns	126
DMBA (3.3)	116, 113	76, 79	752, 641	409, 382	#, #	#	60

The 3000 and 4000 µg/mL dose of degarelix marginally increased the number of mutant colonies compared to control (<1.8-fold). The slight increase in mutation rate only occurred in Test #1 (Repeat) in presence of S-9 Mix, and was not reproduced in Test #2 where positive controls caused significant mutations. Thus, there is no degarelix-dependent mutation in these cells.

Study validity

The results of the first study were not reported due to high background in negative control samples, leading to a re-test. Subsequent experiments illustrate that degarelix does not cause mutations under these conditions.

Study outcome:

Though high dose (3000 and 4000 µg/mL) degarelix marginally increased the frequency of mutations (<1.8 fold), this was not reproduced in a duplicate experiment. Even moderate doses are orders of magnitude above physiologic levels but did not cause any mutation. Based on the data in this experiment, degarelix is not mutagenic.

9) *In vitro* mammalian cell gene mutation test performed with mouse lymphoma L5178Y cells – WDM

Major finding

Degarelix did not cause mutations at the TK locus in L5178Y mouse lymphoma cell culture under the conditions of this assay with or without S-9. The maximum concentrations of degarelix tested were adequate to demonstrate significant toxicity. The positive controls all caused significant numbers of mutations.

Study number: 53109
 Sponsor reference number: FE200486DSMUT0302
 EDR filename: MUT0302-nonclinical-data.pdf

Conducting laboratory: _____
Date of study initiation: October 2003
GLP compliance: Yes
QA reports: Yes
Drug: Degarelix, Batch 03H 15-02, Peptide content 84.4%

Method

Cell Strain	L5178Y mouse lymphoma cells
S9	Aroclor induced rat liver microsomes
Doses first test	Without S-9: 40, 80, 160, 320, 640 and 960 µg/mL With S-9: 20, 40, 80, 160, 320, 640, 1280, 1920 and 2560 µg/mL
Doses repeat first test	With S-9: 20, 40, 80, 160, 320, 640, 1280, 1920 and 2560 µg/mL
Doses second test	Without S-9: 10, 20, 40, 80, 160 and 320 µg/mL With S-9: 40, 80, 160, 320, 640 and 1280 µg/mL
Repeat of second test	Without S-9: 10, 20, 40, 80, 160, 320, 640 and 1280 µg/mL.
Positive Control	Without S-9, N-ethyl-N-nitrosourea (50 µg/mL) With S-9, 7,12-dimethyl-1,2-benzanthracene (3.3 µg/mL)
Treatment duration	Without S9, short treatment: 3 hours Without S9, continuous treatment 24 hours With S9, 3 hours
No of Replicates	2 per dose

b(4)

Results

The following table from the study demonstrates that the highest doses with S-9 did not cause sufficient toxicity in the first test. Because of this the investigators repeated the first test using higher concentrations and achieved adequate toxicity. Thus, dosing was adequate in this experiment.

Appears This Way
On Original

FE200486: *In Vitro* Mammalian Cell Gene Mutation Test
 Performed with Mouse Lymphoma L5178Y Cells

TOXICITY DATA - Main Test 1

Treatment ($\mu\text{g/ml}$)	Cloning Efficiency			Suspension Growth	Relative Total Growth	
	Day 0		Day 3		Ind. Values	% of Control
	Ind. Values	% of Control	Ind. Values	Ind. Values		
Without S-9 mix						
Vehicle	0.50, 0.69	-	0.90, 0.87	11.10, 10.08	9.95, 8.73	-
TA (960)	0.41, 0.40	68	1.08, 1.31	2.00, 1.93	2.17, 2.52	25
TA (640)	0.41, 0.43	70	0.96, 0.69	8.32, 11.63	8.01, 8.06	86
TA (320)	0.49, 0.39	74	1.04, 0.84	13.33, 14.99	13.86, 12.54	141
TA (160)	0.48, 0.43	75	0.96, 0.84	16.38, 13.95	15.76, 11.68	147
TA (80)	0.62, 0.67	108	0.78, 0.84	14.24, 14.42	11.17, 12.07	124
TA (40)	0.53, 0.35	74	0.87, 0.76	13.25, 11.07	11.47, 8.41	106
ENU	0.45, 0.52	81	0.60, 0.78	9.64, 8.74	5.77, 6.86	68
With S-9 mix						
Vehicle	0.49, 0.37	-	0.49, 0.60	9.25, 9.74	4.54, 5.83	-
TA (1280)	0.24, 0.28	60	1.04, 1.18	2.11, 1.57	2.19, 1.86	39
TA (640)	0.37, 0.34	82	0.81, 0.84	10.92, 12.71	8.84, 10.64	188
TA (320)	0.29, 0.33	72	1.04, 1.08	9.87, 10.91	10.26, 11.82	213
TA (160)	0.38, 0.41	92	1.00, 0.93	11.90, 12.25	11.90, 11.37	224
TA (80)	0.43, 0.37	92	0.60, 0.84	12.50, 8.99	7.48, 7.52	145
TA (40)	0.52, 0.48	116	1.08, 1.00	8.92, 9.39	9.66, 9.39	184
TA (20)	0.43, 0.55	114	0.84, 0.67	10.98, 10.03	9.19, 6.75	154
DMBA	0.31, 0.31	71	0.71, 0.65	6.38, 6.07	4.56, 3.96	82

Key:

- Ind. Values = Individual values for the duplicate cultures at each test point
- % of Control = Mean value expressed as a percentage of the corresponding negative control value (calculated from individual values expressed to 15 decimal places)
- Vehicle = Sterile distilled water
- TA = Test article: FE200486
- ENU = N-Ethyl-N-nitrosourea (50 $\mu\text{g/ml}$)
- DMBA = 7,12-Dimethyl-1,2-benzanthracene (3.3 $\mu\text{g/ml}$)

Appears This Way
 On Original

FE200486: *In Vitro* Mammalian Cell Gene Mutation Test
 Performed with Mouse Lymphoma L5178Y Cells

Best Possible Copy

TOXICITY DATA - Main Test 1 - Repeat

Treatment (µg/ml)	Cloning Efficiency				Suspension Growth	Relative Total Growth	
	Day 0		Day 3			Ind. Values	% of Control
	Ind. Values	% of Control	Ind. Values	Ind. Values			
With S-9 mix							
Vehicle	0.71, 0.67	-	0.67, 0.74	6.53, 6.14	4.40, 4.52	-	
TA (2560)	0.22, 0.18	29	0.04, 0.44	0.32, 0.57	0.01, 0.25	3	
TA (1920)	0.25, 0.23	35	0.48, 0.09	0.71, 0.36	0.34, 0.03	4	
TA (1280)	0.43, 0.33	54	1.00, 0.78	8.00, 5.67	8.00, 4.44	140	
TA (640)	0.38, 0.43	58	0.76, 0.87	12.26, 12.46	9.31, 10.79	225	
TA (320)	0.39, 0.50	64	1.08, 0.96	10.69, 11.99	11.58, 11.54	259	
TA (160)	0.46, 0.55	73	0.93, 0.84	9.09, 10.46	8.44, 8.75	193	
TA (80)	0.74, 0.63	99	0.84, 0.78	6.92, 6.33	5.79, 4.97	121	
TA (40)	0.58, 0.57	83	0.93, 0.71	5.13, 6.20	4.76, 4.43	103	
TA (20)	0.76, 0.50	91	0.76, 0.65	6.15, 6.25	4.67, 4.08	98	
DMBA	0.31, 0.31	44	0.48, 0.65	5.22, 4.28	2.49, 2.80	59	

Key:

- Ind. Values = Individual values for the duplicate cultures at each test point
- % of Control = Mean value expressed as a percentage of the corresponding negative control value (calculated from individual values expressed to 15 decimal places)
- Vehicle = Sterile distilled water
- TA = Test article: FE200486
- DMBA = 7,12-Dimethyl-1,2-benzanthracene (3.3 µg/ml)

The following tables from this study report demonstrate that degarelix did not cause an increase in mutation frequency with or without S-9. There was some evidence that in the presence of S-9, degarelix was protective against background mutation. In some cases this effect reached statistical significance but there was no clear dose effect.

Appears This Way
 On Original

FE200486: *In Vitro* Mammalian Cell Gene Mutation Test
 Performed with Mouse Lymphoma L5178Y Cells

MUTATION DATA - Main Test 1

Treatment (µg/ml)	Number of mutant clones		Mutation Frequency (per 10 ⁶ cells)				RTG % of Control
	Large	Small	Large	Small	Total	Mean	
Without S-9 mix							
Vehicle	26, 25	28, 30	81, 81	88, 98	184, 195	190	-
TA (960)	31, 33	30, 29	81, 72ns	78, 63ns	176, 149ns	163ns	25
TA (640)	28, 32	31, 35	82, 132ns	91, 145ns	191, 310ns	251ns	86
TA (320)	38, 38	33, 38	106, 132ns	91, 132ns	222, 301ns	262ns	141
TA (160)	32, 31	28, 24	95, 105ns	82, 80ns	195, 202ns	199ns	147
TA (80)	23, 23	25, 25	81, 76ns	89, 83ns	183, 172ns	178ns	124
TA (40)	30, 31	29, 26	98, 116ns	95, 96ns	212, 232ns	222ns	106
ENU	89, 90	90, 91	520, 403nc	528, 410nc	2249, 1823nc	2036nc	68
With S-9 mix							
Vehicle	19, 19	24, 24	106, 87	136, 112	259, 212	236	-
TA (1280)	28, 32	33, 38	76, 77ns	91, 93ns	184, 192ns	188ns	39
TA (640)	12, 15	15, 15	40, 49**	50, 49**	94, 101**	98**	188
TA (320)	20, 18	22, 17	53, 45**	59, 43**	119, 93**	106**	213
TA (160)	23, 17	28, 18	64, 50*	79, 53**	154, 108**	131**	224
TA (80)	23, 33	25, 33	107, 113ns	117, 113ns	240, 252ns	246ns	145
TA (40)	27, 29	31, 33	70, 82ns	81, 94ns	166, 195ns	181ns	184
TA (20)	21, 12	23, 14	69, 48**	76, 56**	155, 108**	132**	154
DMBA	90, 87	89, 92	443, 462nc	436, 499nc	1884, 2061nc	1973nc	82

Key:

Cells in the table containing two values show the individual value for each of the duplicate cultures at that test point.

Number of mutant clones = Number of wells with colonies out of 192 wells examined

RTG % of Control = Mean relative total growth expressed as a percentage of the corresponding negative control value

Large / Small = Large colonies / Small colonies

The following indicators of the statistical significance of the mutation frequencies compared to the corresponding negative control values refer to the data for both duplicate cultures considered together

ns = Not statistically different from negative control value (p>0.05)

nc = Not calculated - the positive controls were not included in the analysis

* = Statistically significant (0.05>p>0.01)

** = Statistically significant (p<0.01)

Vehicle = Sterile distilled water

TA = Test article: FE200486

ENU = N-Ethyl-N-nitrosourea (50 µg/ml)

DMBA = 7,12-Dimethyl-1,2-benzanthracene (3.3 µg/ml)

Best Possible Copy

Best Possible Copy

FE200486: *In Vitro* Mammalian Cell Gene Mutation Test
 Performed with Mouse Lymphoma L5178Y Cells

MUTATION DATA - Main Test 1 - Repeat

Treatment (µg/ml)	Number of mutant clones		Mutation Frequency (per 10 ⁶ cells)				RTG % of Control
	Large	Small	Large	Small	Total	Mean	
With S-9 mix							
Vehicle	14, 9	16, 16	56, 33	65, 59	126, 95	111	-
TA (2560)	0, 3	0, 4	0, 18**	0, 24**	0, 42**	21**	3
TA (1920)	5, 1	6, 1	28, 29ns	33, 29*	62, 57*	60*	4
TA (1280)	16, 15	16, 19	44, 52ns	44, 66ns	91, 124ns	108ns	140
TA (640)	15, 12	18, 19	54, 37ns	65, 60ns	124, 102ns	113ns	225
TA (320)	12, 14	18, 15	30, 39ns	45, 42ns	78, 85ns	82ns	259
TA (160)	8, 8	13, 12	23, 25ns	38, 39ns	62, 66ns	64ns	193
TA (80)	7, 6	7, 8	22, 20ns	22, 27**	45, 48*	47*	121
TA (40)	7, 6	11, 6	20, 22ns	32, 22**	53, 45*	49*	103
TA (20)	14, 12	15, 13	50, 49ns	54, 54ns	108, 107ns	108ns	98
DMBA	58, 62	66, 67	377, 299nc	442, 329nc	1089, 853nc	971nc	59

Key:

Cells in the table containing two values show the individual value for each of the duplicate cultures at that test point.

Number of mutant clones = Number of wells with colonies out of 192 wells examined
 RTG % of Control = Mean relative total growth expressed as a percentage of the corresponding negative control value

Large / Small = Large colonies / Small colonies

The following indicators of the statistical significance of the mutation frequencies compared to the corresponding negative control values refer to the data for both duplicate cultures considered together

ns = Not statistically different from negative control value (p>0.05)
 nc = Not calculated – the positive controls were not included in the analysis
 * = Statistically significant (0.05>p>0.01)
 ** = Statistically significant (p<0.01)

Vehicle = Sterile distilled water
 TA = Test article: FE200486
 DMBA = 7,12-Dimethyl-1,2-benzanthracene (3.3 µg/ml)

10) FE200486 Batch No 06G18-01 *In vitro* mammalian cell gene mutation test performed with mouse lymphoma L5178Y cells – WDM

Major findings

Degarelix was not mutagenic under the conditions of this assay.

Study number - 2358
 Conducting laboratory - _____
 Study date - May, 2007
 GLP - Yes
 Audited - Yes
 Drug - Degarelix, Batch No. 06G18-01
 Method
 Cells - Mouse lymphoma L5178Y TK+/- cells

b(4)

The following tables from the sponsor's "Overall Toxicology Summary" show that this test for mutagenicity was negative.

Assay 1

Metabolic Activation	Test Article	Concentration or Dose Level (µg/ml)	Mutation Frequency (per 10 ⁸ cells)		Total	RTG % of Control
			Large	Small		
Without Activation	Control	0	184	163	430	100
	FE200486	5000	0**	0**	0**	0
		2500	192ns	160ns	396ns	11
		1250	169ns	158ns	382ns	44
		625	173ns	141ns	366ns	55
		313	167ns	131ns	351ns	46
		156	140ns	113ns	301ns	96
	N-Ethyl-N-nitrosourea	50	752nc	778.5nc	4432nc	16
With Activation	Control	0	132	126	321	100
	FE200486	5000	114ns	21*	136ns	0
		2500	163	145ns	356ns	8
		1250	173ns	171ns	410ns	45
		625	1ns	152ns	393ns	63
		313	197.5ns	191ns	479ns	66
		156	149.5ns	147ns	362ns	92
	7,12-Dimethyl-1,2-benzanthracene	3.3	1764.5nc	1809.5nc	6953nc	4

RTG % of control = Mean relative total growth (as a percentage of the negative control value)

ns = Not statistically different from negative control value (p>0.05)

nc = Not calculated - the positive controls were not included in the analysis

Analysis of Variance test: * - p<0.05 ** - p<0.01 (The positive controls were not included in the statistical analysis)

Assay 2

Best Possible Copy

Appears This Way
 On Original

Metabolic Activation	Test Article	Concentration or Dose Level (µg/ml)	Mutation Frequency (per 10 ⁶ cells)			RTG % of Control
			Large	Small	Total	
Without Activation	Control	0	141.5	108	293	100
	FE200486	2300	67.5ns	0*	68**	0
		1150	101.5ns	174.5ns	302ns	4
		575	128.5ns	104ns	263ns	40
		288	182ns	87.5ns	317ns	56
		144	142ns	94.5ns	271ns	76
		71.9	117.5ns	86ns	232ns	80
	N-Ethyl-N-nitrosourea	50	6803nc	8609nc	16478nc	0
With Activation	Control	0	175	118	348	100
	FE200486	2300	90*	160.5ns	276ns	9
		1150	186ns	129.5ns	375ns	63
		575	160.5ns	66.5ns	260ns	112
		288	177.5ns	95ns	315ns	93
		144	187.5ns	107.5ns	347ns	94
		71.9	156ns	93.5ns	285ns	88
	7,12-Dimethyl-1,2-benzanthracene	3.3	1191.5nc	1172nc	5127nc	10

RTG % of control = Mean relative total growth (as a percentage of the negative control value)

ns = Not statistically different from negative control value (p>0.05)

nc = Not calculated - the positive controls were not included in the analysis

Analysis of Variance test: * - p<0.05 ** - p<0.01 (The positive controls were not included in the statistical analysis)

Best Possible Copy

11) FE200486 Batch No 06G18-02 *In vitro* mammalian cell gene mutation test performed with mouse lymphoma L5178Y cells – WDM

Major findings

Degarelix was not mutagenic under the conditions of this assay.

Study number -	2370
Conducting laboratory -	_____
Study date -	June, 2007
GLP -	Yes
Audited -	Yes
Drug -	Degarelix, Batch No. 06G18-02
Method	
Cells	Mouse lymphoma L5178Y TK+/- cells

b(4)

The following tables from the sponsor's "Overall Toxicology Summary" show that this test for mutagenicity was negative.

Assay 1

Best Possible Copy

David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Robert Dorsam, Ph. D.

NDA No. 22-201

Metabolic Activation	Test Article	Concentration or Dose Level (µg/ml)	Mutation Frequency (per 10 ⁶ cells)			RTG % of Control
			Large	Small	Total	
Without Activation						
	Control	0	113	124	273	100
	FE200486	5000	78ns	0**	78ns	0
		2500	132ns	95ns	241ns	4
		1250	139ns	145ns	321ns	12
		625	123ns	107ns	262ns	52
		313	105ns	109ns	241ns	75
		156	103ns	88ns	212ns	100
	N-Ethyl-N-nitrosourea	50	1000nc	1201nc	6580nc	10
With Activation						
	Control	0	131	138	311	100
	FE200486	5000	140ns	167ns	334ns	3
		2500	155.5ns	167ns	367ns	11
		1250	134ns	128ns	309ns	55
		625	102ns	106ns	231ns	95
		313	136ns	125ns	301ns	121
		156	79ns	80ns	175ns	121
	7,12-Dimethyl-1,2-benzanthracene	3.3	872nc	1046nc	5543nc	10

= Can not be calculated because cloning efficiency is zero
 RTG % of controls = Mean relative total growth (as a percentage of the negative control value)
 ns = Not statistically different from negative control value (p>0.05)
 nc = Not calculated - the positive controls were not included in the analysis
 Analysis of Variance test: * - p<0.05 ** - p<0.01 (The positive controls were not included in the statistical analysis)

Assay 2

*Appears This Way
On Original*

Best Possible Copy

David McGuinn, Jr., M.S., Ph. D., D.A.B.T.
Robert Dorsam, Ph. D.

NDA No. 22-201

Metabolic Activation	Test Article	Concentration or Dose Level (µg/ml)	Mutation Frequency (per 10 ⁶ cells)			RTG % of Control
			Large	Small	Total	
Without Activation						
	Control	0	142	117	307	100
	FE200486	2500	#	#	#	0
		1250	0ns	521ns	521ns	0
		625	135ns	164ns	353ns	8
		313	186ns	126ns	383ns	42
		156	118ns	95ns	253ns	49
		78.1	105ns	83ns	221ns	117
	N-Ethyl-N-nitrosourea	50	1513nc	1086nc	2655nc	0
With Activation						
	Control	0	151	99	280	100
	FE200486	5000	187ns	135ns	394ns	8
		2500	117ns	78ns	198ns	2
		1250	142ns	97ns	368ns	47
		625	196ns	174ns	445ns	58
		313	174ns	163ns	400ns	85
		156	147ns	126ns	306ns	82
	7,12-Dimethyl-1,2-benzanthracene	3.3	712nc	606nc	2956nc	34

= Can not be calculated because cloning efficiency is zero
 RTG % of control = Mean relative total growth (as a percentage of the negative control value)
 ns = Not statistically different from negative control value (p>0.05)
 nc = Not calculated – the positive controls were not included in the analysis
 Analysis of Variance test: * -p<0.05 ** -p<0.01 (The positive controls were not included in the statistical analysis)

12) FE200486 Batch No 06H09-01 *In vitro* mammalian cell gene mutation test performed with mouse lymphoma L5178Y cells – WDM

Major findings

Degarelix was not mutagenic under the conditions of this assay.

Study number -	2371
Conducting laboratory -	_____
Study date -	June, 2007
GLP -	Yes
Audited -	Yes
Drug -	Degarelix, Batch No. 06H09-01
Method	
Cells	Mouse lymphoma L5178Y TK+/- cells

b(4)

The following tables from the sponsor's "Overall Toxicology Summary" show that this test for mutagenicity was negative.

Assay 1

Best Possible Copy

Metabolic Activation	Test Article	Concentration or Dose Level (µg/ml)	Mutation Frequency (per 10 ⁸ cells)			RTG % of Control
			Large	Small	Total	
Without Activation						
	Control	0	102	80	200	100
	FE200486	5000	#	#	#	0
		2500	82ns	0**	82ns	0
		1250	91ns	73ns	184ns	26
		625	104ns	98ns	227ns	27
		313	87ns	75ns	175ns	64
		156	105ns	79ns	204ns	94
	N-Ethyl-N-nitrosourea	50	861nc	915nc	4643nc	9
With Activation						
	Control	0	114	101	253	100
	FE200486	5000	0**	0ns	0*	0
		2500	181*	177ns	389ns	6
		1250	118ns	111ns	270ns	32
		625	139ns	103ns	278ns	59
		313	134ns	83ns	244ns	95
		156	115ns	90ns	230ns	103
	7,12-Dimethyl-1,2-benzanthracene	3.3	733nc	685nc	4218nc	17

RTG % of control = Mean relative total growth (as a percentage of the negative control value)

ns = Not statistically different from negative control value (p>0.05)

nc = Not calculated - the positive controls were not included in the analysis

Analysis of Variance test: * - p<0.05 ** - p<0.01 (The positive controls were not included in the statistical analysis)

Assay 2

Appears This Way
 On Original

Best Possible Copy

Metabolic Activation	Test Article	Concentration or Dose Level (µg/ml)	Mutation Frequency (per 10 ⁶ cells)			RTG % of Control
			Large	Small	Total	
Without Activation						
	Control	0	205	145	429	100
	FE200486	2500	***	0ns	0**	0
		1250	176ns	157ns	336ns	0
		625	265ns	222ns	583*	7
		313	219ns	196ns	513ns	26
		156	158ns	135ns	358ns	61
		78.1	206ns	168ns	464ns	70
	N-Ethyl-N-nitrosourea	50	2981nc	3113nc	8879nc	1
With Activation						
	Control	0	229	163	491	100
	FE200486	2500	115ns	99ns	214ns	0
		1250	211ns	259ns	604ns	25
		625	215ns	187ns	509ns	73
		313	237ns	172ns	508ns	93
		156	196ns	186ns	460ns	105
		78.1	198ns	175ns	469ns	120
	7,12-Dimethyl-1,2-benzanthracene	3.3	1013nc	1026nc	4594nc	21

RTG = Mean relative total growth (as a percentage of the negative control value)
 ns = Not statistically different from negative control value (p>0.05)
 nc = Not calculated - the positive controls were not included in the analysis
 Analysis of Variance test: * - p<0.05 ** - p<0.01 (The positive controls were not included in the statistical analysis)

13) Rat Micronucleus Test – WDM

Major findings:

Degarelix did not cause any statistically significant increases in the number of micronucleated immature erythrocytes at either sampling time. Mitomycin C caused large, highly significant increases in the frequency of micronucleated immature erythrocytes.

Study number: ADR0050
 Sponsor reference number: 200486-2373
 Conducting laboratory: _____
 Date of study initiation: July 2007
 GLP compliance: Yes
 QA reports: Yes
 Drug: Degarelix, Batch 06A23-01, and purity 99.8%
 Methods: _____
 Animal: WI(Han)

b(4)

Doses and N used in definitive study (for dose ranging see table below)

Group	Treatment	Dose mg/kg	Dose mg/m ²	N males
1	Vehicle Control	-	-	7
2	Degarelix	7	54	7
3	Degarelix	12	72	7
4	Degarelix	22	132	7
5	Mitomycin C	3	18	5

*Additional animals, dosed concurrently, to replace any that might die

Schedule 2 doses 24 hours apart
 Route IV tail vein
 Formulation Mannitol_{aq} 5% w/v
 Positive controls Mitomycin C

Observations

Mortality Daily
 Clinical signs Daily
 body weight Before dosing and end of study.
 Bone marrow 24 hours
 Number of cells analyzed/ animal 2000

Results

Mortality None

Clinical signs The investigators constructed the following table to demonstrate the clinical signs observed in the dose ranging study. Because there were no sex-related differences the investigators did the main study with males only. Doses of 0, 2.2, 3.9 or 12.5 mg/kg/day caused no clinically observable toxicity.

Range Finder Phase Dose level	RF5 22 mg/kg/day	RF5 22 mg/kg/day	RF6 40 mg/kg/day ¹	RF6 40 mg/kg/day ¹
Clinical sign	Male	Female	Male	Male
Reduced Activity	3/3	3/3	3/3	3/3
Piloerection	2/3	3/3	3/3	3/3
Slow Breathing			3/3	3/3
Eyes Part Closed			3/3	3/3
Cold to Touch			3/3	3/3

Note: x/y = number of animals exhibiting clinical sign / number of animals in group 1: tails took approximately 10 minutes to stop bleeding from the injection site following the 2nd dose administration

There was no significant difference in the PCE/NCE ratio between males and females at any dose of degarelix. The investigators considered the clinical signs observed and their persistence following a dose of 40 mg/kg/day degarelix to be too severe for this dose to be used on animals in the main study.

The investigators constructed the following table to demonstrate the results of the main study.

	Group 1 Negative Control	Group 2 7 mg/kg/day FE200486	Group 3 12 mg/kg/day FE200486	Group 4 22 mg/kg/day FE200486	Group 5 Positive Control
Mean MN-PCE	1.57	1.29	1.57	2.00	42.43**H
Mean PCE/NCE Ratio	0.93	0.93	0.74	0.94	0.68

H= Within group heterogeneity detected, comparisons made using Wilcoxon test
 **= Statistically significant p<0.01

The incidence of MN-PCE and the PCE/NCE ratio in the vehicle control group fell within the historical vehicle control range. Males dosed with MMC, the positive control article, had statistically significantly more micronuclei than the negative control animals. There was no evidence of a statistically significant degarelix treatment-related increase in the numbers of MN-PCE compared to control. There was no evidence of a statistically significant reduction in the PCE/NCE ratio of any of the male degarelix groups or the positive control group compared to the negative control group.

14) Rat Micronucleus Test – WDM

Major findings:

Degarelix did not cause any statistically significant increases in the number of micronucleated immature erythrocytes at either sampling time (p > 0.01). Cyclophosphamide caused large, highly significant increases (p < 0.001) in the frequency of micronucleated immature erythrocytes.

Study number FRG 032/994546
 Conducting laboratory _____
 Date of study initiation April 1999
 GLP compliance Yes
 QA reports Yes
 Drug Degarelix, Batch 2011-032-30, and purity 97.58%
 Methods

Animal Sprague-Dawley Rats
 Doses and N used in definitive study (dose ranging study not reviewed)

Group	Treatment	Concentration mg/mL	Dose mg/kg	Dose mg/m ²	N females	N males
1	Vehicle Control	-	-	-	10	
2	Degarelix	5	10	60	5	
3	Degarelix	10	20	120	5	
4	Degarelix	20	40	240	10+2*	10+2*
5	Cyclophosphamide	2	20	120	5	5

*Additional animals, dosed concurrently, to replace any that might die

Schedule single dose
 Route PO gavage
 Formulation Mannitol_{aq} 5% w/v
 Positive controls Cyclophosphamide

b(4)

Observations

Mortality Daily
 Clinical signs Daily
 body weight Before dosing and end of study.
 Bone marrow 24 hr in the main study, 48 hr in the dose ranging study
 Number of cells analyzed/ animal 2000

Results

Mortality Three HD males died within one hour of treatment. The investigators considered these deaths due to toxicity and not gavage error. These animals were replaced
 Clinical signs None
 Body weight Dose dependent weight loss (as much as 9%).

Micronucleated mature erythrocytes (mme)

Degarelix did not cause any significant increases in the incidence of micronucleated mature erythrocytes at either sampling time.

Proportion of immature erythrocytes (% ie/ie + me)

Degarelix did not cause any significant decreases in the proportion of immature erythrocytes [p > 0.01]. Cyclophosphamide caused statistically significant decreases in the proportion [p < 0.001].

The investigators constructed the following table to present the results of this experiment.

Sample Time	Treatment	Dose mg/kg	Proportion of immature erythrocytes	Incidence of mie (mean)	Incidence of mme (total)
24 hours	Vehicle Control	-	45	1.2	-3
	Degarelix	10	41	1.7	0.0
	Degarelix	20	41	1.9	0.0
	Degarelix	40	39	1.4	0.6
	Cyclophosphamide	20	20 **	51.7 **	0.9
48 hours	Vehicle Control	-	39	1.5	0.3
	Degarelix (dose rangeing)	40	35	1.4	0.3

% ie/(ie+me) Proportion of immature erythrocytes
 mie Number of micronucleated cells observed per 2000 immature erythrocytes examined
 mme Number of micronucleated cells observed per 2000 immature erythrocytes examined

Results of statistical analysis using the appropriate nonparametric method of analysis based on permutation (one-sided probabilities):

** P < 0.001 (highly significant), * P < 0.01 (significant), otherwise P > 0.01 (not significant)

2.6.6.5 Carcinogenicity

1) 104 Week Carcinogenicity Study in Rats with Subcutaneous Administration (WDM)

Major Findings:

After 24 months (52 fortnightly subcutaneous injections) overall mortality was less in treated rats than in controls. This difference reached statistical significance in low and mid dose females. The high dose in this study was about the same as the proposed clinical loading dose and about 3 times greater than the proposed monthly maintenance dose on a mg/m² basis. The high dose males weighed 24% less than controls at the end of the study. Treated females weighed significantly more than controls throughout the study depending on the dose. Toxicokinetic analysis established that exposure was roughly dose proportional but highly variable since subcutaneous absorption is rate limiting at the high dose. Steady-state was reached after about the twelfth dose (week 24).

Red and white cell parameters increased in females and low and mid dose males; these changes were more pronounced in females. These parameters decreased in high dose males. Changes in clinical chemistry parameters were minor. Dosing caused profound atrophy of the sex organs in both males and females. In males there were significant dose related decreases in the weights of the thymus and adrenals; in females the weights of the pituitary and adrenals were significantly less than controls. There was some evidence of mild kidney damage particularly in females. Other changes in organ weight were consistent with changes in total body weight.

The incidence of benign adenoma of the pituitary gland decreased in all groups of treated females (p < 0.02). The incidence of benign fibroadenoma of the breast decreased in all groups of treated females (p < 0.024). The incidence of eosinophilic cell foci in the liver increased in low dose females (p < 0.001). This preneoplastic finding is consistent with mild hepatic damage seen in males and females. Lastly, there was an increase in metastatic hemangiosarcoma of the mesenteric lymph node in HD females (p < 0.04, with a positive trend by Peto analysis p = 0.015). The incidence of this tumor was 8% which is within the range seen in historical controls. There was no similar finding in males. The combined incidence of all benign and malignant hemangiomas and hemangiosarcomas (16%) was significantly different from controls by pairwise comparison (p = 0.0013, Exact test) in the high dose group. This difference remained significant when analyzed by the asymptotic trend test (p = 0.0008).

Study number FE200486DSCAR0101
 EDR filename car0101-nonclinical-data.pdf
 Conducting laboratory _____
 Date of study initiation November 2002
 GLP compliance Yes
 QA report Yes
 Drug Degarelix, Batches listed in the table below from the study report

b(4)

Batch Number	Weight (g)	Date Received	Peptide Content (%)	Correction Factor	Expiry Date
0048262#PPL-FE4860001	22.73	03 July 2002	91.77		02 July 2004
PPL-FE4860002*	13.74	28 January 2003	89.50		31 May 2004
PPL-FE4860002*	7.14	03 February 2003	89.50		31 May 2004
0051981#PPL-FE4860002*	25.00	05 March 2003	89.50		25 May 2004
PPL-FE4860201A	84.39	10 July 2003	91.40		31 March 2005

b(4)

*The same batch, although the complete batch number was not given on the first two batches.

Methods

Animal Male and female Han Wistar rats — WI(Glx/BRL/Han)IGSBR
 6 weeks old

b(4)

Males 142 to 205 g
 Females 111 to 167 g
 Doses 0, 2, 10 or 25 mg/kg, (0, 12, 60, 150 mg/m²)
 Dose levels are expressed as 100% peptide content
 Based on 26 week study (FRG 067/014270 / FE200486DSTOX010)
 N 50 per sex per dose level, ten per sex per dose level for toxicokinetics
 Schedule Once fortnightly
 Route Subcutaneous injection rotated over four different sites
 Dose volume 5 mL/kg
 Formulation Mannitol_{aq} 5% w/v

Observations

Mortality Twice daily
 Clinical signs Twice daily
 body weight weekly
 Ophthalmoscopy every 6 months (20 animals per sex only)
 Hematology End of study
 Urinalysis End of study (10 animals per sex only)
 Toxicokinetics in relation to doses 1, 8, 13, 20, 26, 33, 39 and 46 as follows (0.75 mL)
 Each animal was sampled at two time points according to the following table from the study report

Dose No.	Timepoints Relative to Dosing									
	Predose	+2 h	+4 h	+8 h	+12 h	+24 h [Day 2]	+48 h [Day 3]	+96 h [Day 5]	+192 h [Day 9]	+336 h [Day 15]*
1	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
8	(10)	-	-	-	-	-	-	-	-	-
13	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
20	(10)	-	-	-	-	-	-	-	-	-
26	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
33	(10)	-	-	-	-	-	-	-	-	-
39	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)	(2)
46	(10)	-	-	-	-	-	-	-	-	-

() = Number of animals per sex per treatment group to be sampled
 * = On Day 15, the sample was taken prior to administration of the next dose

In relation to dose 52, the number of animals had decreased so the sampling schedule was as shown in the following table from the study report.

Dose No. 52	Timepoints Relative to Dosing																			
	Predose		+2 h		+4 h		+8 h		+12 h		+24 h [Day 2]		+48 h [Day 3]		+96 h [Day 5]		+192 h [Day 9]		+336 h [Day 15]	
Group	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F	M	F
1	0	1	2	1	2	2	2	1	2	2	0	1	2	2	1	2	2	1	1	2
2	1	2	1	1	2	2	1	1	2	2	1	2	2	2	2	2	2	2	2	2
3	2	2	2	2	2	2	2	2	2	2	2	2	2	2	1	2	2	2	1	2
4	1	2	2	1	1	1	2	1	1	1	1	2	2	2	2	2	2	2	2	2

Necropsy Week 104 or 105
 Histopathology Adequate battery
 Statistical evaluation Pairwise comparison of tumor incidence was done using Fisher's Exact test (two-tailed). Males and females were analyzed separately. Tumor data was also analyzed using SAS (v8.2) PROC MULTTEST. All significance tests were one-tailed (testing for an increase) with a significance level of 5%. Since this test was one-tailed it did not detect decreases in tumor incidence and the p-value reported in the Peto tables is 1.00.

Results

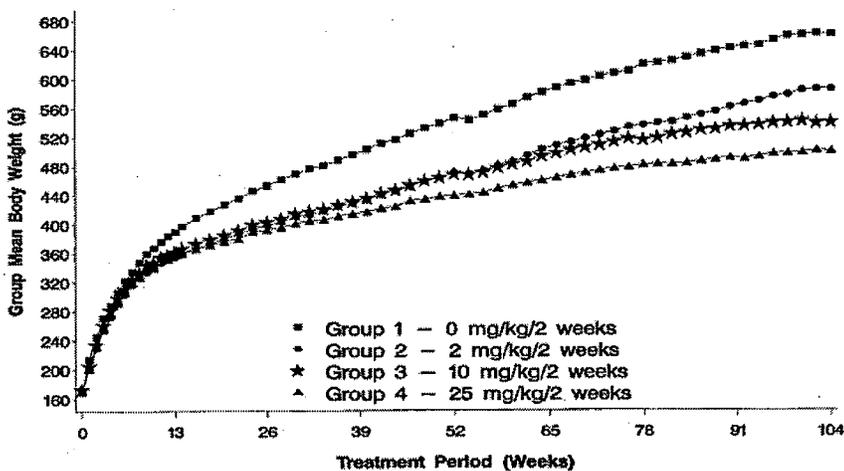
Mortality 49 animals died or were killed humanely due to poor health before scheduled necropsy. The following table from the study report shows that mortality was lower (increased survival time) in males and females in the low and mid dose groups compared to control. This difference reached significance in low and mid dose females ($p < 0.03$ and 0.017 respectively). The investigators did not attribute any of these deaths to degarelix treatment.

Dose Group (mg/kg/2weeks)	Number of Premature Decedents	
	Males	Females
1 (0)	9	11
2 (2)	5	3
3 (10)	4	3
4 (25)	6	8

Clinical signs Injection site damage was most common in the high dose group and to a lesser extent in the low and mid dose groups

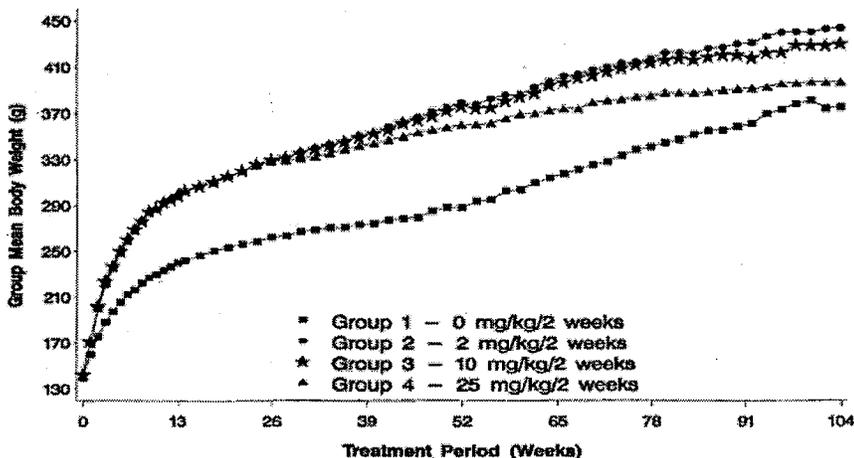
Body weight

The following graph from the study report demonstrates that male treated rats gained less weight than controls. This decreased weight gain was dose proportional. In week 104 the males in the control group had a mean weight of 661 g (N = 41, sd = 95) and those in the high dose group had an average mean weight of 500 g (N = 44, sd = 74). The HD animals weighed 24% less than the controls and the difference was highly significant ($p < 0.001$). Low dose males weighed 11% less than controls ($p < 0.001$) and mid dose males weighed 18% less than controls ($p < 0.001$).



The following graph from the study report demonstrates that female treated rats gained more weight than controls. In week 104, the females in the control group had a mean weight of 376 g (N = 39, sd = 63) and those in the high dose group had an average mean weight of 397 g (N = 42, sd = 44). Thus, the high dose females weighed 6% more than the controls. This difference was did not reach

significance. Nevertheless, the low dose females weighed 18% more than controls ($p < 0.001$) and mid dose females weighed 14% more than controls ($p < 0.001$). This pattern (bell shaped dose response) suggests that the high dose was causing a toxicity that the other two doses were not. Dosed females all gained significantly more weight than controls in the first 20 weeks of the experiment in a pattern that is statistically indistinguishable by dose. At week 20 all three dosed groups weighed 16% more than controls ($p < 0.001$). At this point the high dose group begins to gain less weight presumably due to some toxicity. At week 104, the high dose group weighs 12% less than the low dose group. This unusual pattern of weight gain is likely related to the primary pharmacology of the drug and sufficient to demonstrate adequate dosing in the high dose group.



Food consumption Decreased in all dosed males throughout the experiment
 Decreased in dosed females week 54 through 104
 Water consumption No toxicologically significant changes
 Ophthalmoscopy No toxicologically significant changes

Hematology

The following table from the study report shows that in males, changes in red and white cell parameters were less severe and reached significance only in the low dose group in most cases. They did not show a clear pattern of change. Again prothrombin time increased in dosed animals.

FE200486
 104 Week Carcinogenicity Study in Rats with Subcutaneous Administration
 Haematology: Main Study: Termination
 Group Mean Values: Males

Best Possible Copy

Group/Dose Level (mg/kg/2weeks)		Hb	RBC	Hct	MCH	MCV	MCHC	RetH	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat	PT	APTT
1 (0)	Number	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	36	28
	Mean	15.6	8.61	0.463	18.2	53.9	33.7	2.3	8.03	1.80	4.02	0.25	0.11	0.02	0.03	717	16	20
	SD	1.0	0.67	0.027	1.1	2.8	0.8	1.3	2.79	1.29	1.54	0.23	0.06	0.01	0.02	218	1	3
2 (2)	Number	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	29
	Mean	15.7	8.41	0.465	18.7	55.4	33.9	2.3	5.24	1.32	3.63	0.15	0.09	0.01	0.03	625	16	19
	SD	0.7	0.42	0.018	0.8	2.0	0.7	0.4	2.99	2.61	1.00	0.07	0.04	0.03	0.03	196	1	2
3 (10)	Number	45	45	45	45	45	45	45	45	45	45	45	45	45	45	45	48	41
	Mean	15.6	8.42	0.460	18.5	54.7	33.8	2.4	5.74	1.80	3.46	0.22	0.12	0.01	0.03	703	17	20
	SD	1.0	0.52	0.027	0.9	2.0	0.8	0.9	1.97	1.30	1.19	0.11	0.06	0.01	0.02	200	1	2
4 (25)	Number	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	40	33
	Mean	15.4	8.50	0.459	18.2	54.0	33.6	2.0	6.34	1.87	3.31	0.21	0.11	0.01	0.03	580	16	20
	SD	0.7	0.39	0.023	0.6	1.7	0.7	0.3	1.31	0.89	0.82	0.12	0.05	0.01	0.02	155	1	5

Significantly different from control: * $p < 0.01$, ** $p < 0.01$, *** $p < 0.001$

The following table from the study report shows that RBC, Hb, and Hct increased in all dosed female groups. MCH and MCV decreased. Changes in red cell parameters are all less than 10% but reached significance in many cases because of the large sample size. Increases in white cell parameters were somewhat greater. WBC was 31, 29 and 48 percent higher than controls in the low dose, mid dose and high dose groups respectively. Other white cell parameters followed this pattern. Prothrombin time increased in all dosed groups but platelets were unchanged.

Best Possible Copy

FE200486
104 Week Carcinogenicity Study in Rats with Subcutaneous Administration
Haematology: Main Study: Termination
Group Mean Values: Females

Group/Dose Level (mg/kg/2weeks)		Hb	RBC	Hct	MCH	MCV	MCHC	Retl	WBC	Neut	Lymp	Mono	Eos	Baso	LUC	Plat	PT	APTT
1 (0)	Number	37	37	37	37	37	37	37	37	37	37	37	37	37	37	37	33	28
	Mean	15.1	7.78	0.436	19.4	58.1	34.5	2.4	3.62	1.68	2.19	0.15	0.08	0.01	0.02	848	15	20
	SD	0.8	0.38	0.023	0.7	2.0	0.6	1.03	0.62	0.59	0.07	0.04	0.04	0.01	0.01	185	1	3
2 (2)	Number	46	46	46	46	46	46	46	46	46	46	46	46	46	46	44	44	27
	Mean	15.9	8.28	0.465	19.2	56.3	34.1	2.3	4.61	0.89	3.29	0.19	0.11	0.01	0.03	808	16	20
	SD	0.5	0.42	0.019	0.9	1.9	0.8	0.3	1.21	0.37	0.93	0.10	0.05	0.02	0.02	131	1	5
3 (10)	Number	46	46	46	46	46	46	46	46	46	46	46	46	46	46	39	39	30
	Mean	15.8	8.25	0.460	19.0	55.8	34.0	2.3	4.54	1.27	2.95	0.18	0.10	0.01	0.03	577	17	20
	SD	0.6	0.51	0.022	0.9	1.8	0.8	0.6	1.49	0.89	1.15	0.08	0.04	0.01	0.03	184	1	2
4 (25)	Number	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	29
	Mean	15.4	8.35	0.456	18.5	54.6	33.8	2.1	5.22	1.77	3.07	0.21	0.13	0.01	0.03	810	17	20
	SD	0.8	0.53	0.024	0.9	1.7	0.7	0.4	1.43	1.10	0.78	0.09	0.08	0.06	0.02	180	1	5

Significantly different from control: * p < 0.01, ** p < 0.01, *** p < 0.001

Clinical Chemistry

The following table from the study report shows that changes in clinical chemistry in dosed males were relatively minor (< 10% change) and not consistently dose dependant except for the increase in triglycerides (about 30 % decrease). The changes in urea are small but probably dose related. The changes in transaminases are equivocal and do not correlate with microscopic damage to the liver. There is an increase in plasma creatine phosphokinase in treated males but these animals actually showed a decreased incidence of cardiomyopathy (below). This result is possibly due to the lower total muscle mass of the chemically castrated males. The decreases in alkaline phosphatase are consistent with the effect of chemical castration on bone metabolism.

Group/Dose Level (mg/kg/2weeks)		Urea	Glu	AST	ALT	AP	LDHIL	Na	K	Cl	TP	Alb	Glob	AG-R	Chol	Crea	Ca	Phos	T.Bi	Trig	CPK
1 (0)	Number	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41
	Mean	4.9	7.30	78	58	230	431	148	4.1	104	72	42	29	1.5	3.4	54	2.82	1.20	1.3	3.04	234
	SD	0.6	1.08	29	24	75	303	1	0.5	2	3	4	4	0.3	1.1	4	0.09	0.15	0.9	1.20	207
2 (2)	Number	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44	44
	Mean	5.4	7.79	152	181	191	369	148	3.7	105	71	44	27	1.8	3.5	69	2.81	1.12	1.6	2.51	160
	SD	1.5	1.03	353	728	61	342	2	0.4	3	4	3	3	0.2	0.9	5	0.14	0.18	0.9	0.90	148
3 (10)	Number	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47	47
	Mean	5.5	7.46	88	57	199	333	148	3.8	105	70	42	28	1.6	3.3	58	2.84	1.15	2.1	2.05	150
	SD	1.8	0.98	31	18	72	171	2	0.4	2	3	4	3	0.3	0.7	8	0.12	0.14	1.0	0.70	111
4 (25)	Number	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43	43
	Mean	5.5	7.44	136	88	184	321	148	3.9	105	70	42	27	1.6	3.4	57	2.81	1.16	1.9	2.15	139
	SD	0.8	0.95	89	46	57	147	1	0.3	2	3	3	4	0.3	0.6	5	0.13	0.13	1.0	0.82	77

Significantly different from control: * p < 0.01, ** p < 0.01, *** p < 0.001

Similar changes were seen in females.

Best Possible Copy

Best Possible Copy

Group/Dose Level (mg/kg/2weeks)		Urea	Glu	AST	ALT	AP	LDHIL	Na	K	Cl	TP	Alb	Glob	AG-R	Chol	Crea	Ca	Phos	T.Bi	Trig	CPK
1 (0)	Number	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	39	33	39	39
	Mean	5.1	7.35	80	61	137	269	145	3.7	102	74	49	25	2.0	2.7	55	2.83	1.15	0.9	3.94	114
	SD	0.9	1.33	36	22	52	148	2	0.5	3	4	3	3	0.3	0.8	5	0.12	0.20	0.8	2.19	75
2 (2)	Number	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46
	Mean	5.5	7.70	85	63	193	243	148	3.7	105	70	46	24	1.9	3.2	56	2.76	1.08	1.1	3.06	135
	SD	0.7	0.99	24	24	64	94	2	0.3	2	4	3	3	0.3	0.8	4	0.12	0.14	0.9	1.42	79
3 (10)	Number	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46	46
	Mean	5.4	7.80	95	67	152	284	148	3.7	105	69	44	25	1.8	3.1	56	2.80	1.12	1.4	2.49	136
	SD	0.5	1.03	40	26	56	128	2	0.3	2	4	4	4	0.4	0.5	4	0.14	0.14	0.7	1.28	86
4 (25)	Number	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	40	39	40	40
	Mean	5.7	7.53	99	84	181	312	149	3.9	108	69	43	26	1.7	3.1	56	2.79	1.13	1.6	2.18	159
	SD	0.8	0.97	46	28	60	171	2	0.4	3	4	4	4	0.4	0.6	4	0.12	0.15	0.8	1.03	125
	Prob.	***		**	**	**	***	**	***	***	***	***	***	***	**	4	0.12	0.15	***	***	***

Significantly different from control: * p < 0.01, ** p < 0.01, *** p < 0.001

Urinalysis

In all male groups receiving degarelix, there was a decrease in urinary volume (-48, -62 and -58% in LD, MD and HD respectively) with a corresponding increase in specific gravity. These findings were statistically significant for males treated at 2, 10 and 25 mg/kg with the exception of the specific gravity for the 2 mg/kg animals. In females, the decrease in urine volume was somewhat less (about 30% in all groups) and did not reach significance due to sample size (N = 10). This change is probably related to the primary pharmacology of the drug. In treated males and females there was a decrease in protein concentration most which was notable at 2 and 10 mg/kg. This finding is particularly striking in light of the fact that the vehicle 5% mannitol_{aq} increases urine volume. It is probably related to the adrenal atrophy (manifest as decreased relative organ weight) seen in both males and females.

Organ weights

Best Possible Copy

Organ weights	Absolute Change				Relative Change *		
	Control value	Low Dose	Mid Dose	High Dose	Low Dose	Mid Dose	High Dose
male body weight	698	560	538	508			
Heart male	1.57	-19.7%	-14.0%	-20.4%	1.000	1.116	1.094
Kidney male	3.24	-33.6%	-25.9%	-28.4%	0.827	0.961	0.984
Liver male	19.79	-34.9%	-32.5%	-35.6%	0.812	0.875	0.885
Lung male	2.05	-10.7%	1.0%	-9.3%	1.113	1.310	1.247
Epididymides	1.258	-91.2%	-88.8%	-91.2%	0.109	0.146	0.121
Prostate	0.351	-93.7%	-94.0%	-93.2%	0.078	0.078	0.094
Seminal Vesicles	1.614	-96.1%	-95.4%	-96.3%	0.048	0.060	0.052
Testes	4.57	-94.1%	-93.4%	-93.9%	0.074	0.085	0.084
Spleen male	1.19	-28.6%	-16.0%	-20.2%	0.890	1.090	1.097
Thymus male	0.889	-89.8%	-86.6%	-90.1%	0.128	0.174	0.136
Adrenals male	0.1987	-69.8%	-72.2%	-67.2%	0.377	0.361	0.451
Salivary gland male	0.5871	-17.4%	-13.3%	-18.0%	1.030	1.125	1.127
Female body weight	362	445	425	391			
Ovaries	0.102	-77.5%	-67.6%	-76.5%	0.183	0.276	0.218
Uterus	1.23	-88.6%	-88.6%	-89.4%	0.093	0.097	0.098
Pituitary female	0.05	-82.0%	-86.0%	-84.0%	0.146	0.119	0.148
Adrenals female	0.0648	-20.5%	-17.6%	-13.1%	0.647	0.702	0.804
kidney female	2.15	-18.6%	-12.6%	1.9%	0.662	0.745	0.943

= (organ weight in control ÷ body weight of control) ÷ (organ weight in treated group ÷ (body weight in treated group))
Values close to 1 show no change in organ weight relative to body weight

The difference in male heart, kidney, liver, spleen and lung weight may be related to the fact that these animals did not grow as much as controls, but the major changes in sex organs are atrophic and directly related to the drug mechanism. The decrease in male adrenal and salivary gland weight may also

be atrophic. These values did not reach significance because the investigators only weighed the organs of 10 animals per sex and because there was considerable variability. In females the decreased weight in sex organs is atrophic and due to the mechanism of the drug. The changes in female pituitary and kidney weight are also probably atrophic since these animals weighed more than controls. This is an unusual toxicity. It may be directly related to the primary pharmacology of the drug and its effect on the adrenal glands but it could also be due to a secondary pharmacology.

Gross Pathology

Most males from all groups receiving degarelix had small testes, epididymides, prostate and seminal vesicles. Similarly the females from the same groups had small ovaries and uterus. Females treated at 2, 10 and 25mg/kg/2-weeks also exhibited fewer necropsy findings in the pituitary gland, such as enlarged, dark focus or raised focus than controls.

Treated animals showed increased numbers of injection site findings, such as thickening, reddening or swelling, but the incidence of subcutaneous masses was reduced in treated animals compared with controls.

Microscopic Pathology

The following tables from the study report summarize the histopathological findings in this study. The table does not include all tumor data (see below). In many cases, treatment with degarelix decreased the incidence of certain pathologies, particularly in organ that were atrophic. The table is divided only for convenient display. In all cases, the number of animals examined between 48 and 50, thus the table includes the results for both animals that died at scheduled necropsy and before. The table does not include injection site damage which was significant and dose related.

Tissue and lesion	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
Lung vascular mineralization	15	13	15	12	5	15*	18**	16**
Mandibular lymph node plasmacytosis	6	5	11	8	12	5	7	3*
Mesenteric lymph node no abnormality found	42	34	40	38	46	41	34**	40
Spleen no abnormality found	13	11	13	18	0	7*	2	4
Spleen pigment increased	4	3	4	5	28	9***	13**	16**
Thymus no abnormality found	31	39	46	42	23	36**	33	34
Thymus cystic hyperplasia	7	5	2	5	21	5***	3***	7**
Thymus Agonal congestion or hemorrhage	5	16*	16*	14	7	17*	14	10
Heart no abnormality found	22	33*	29	37**	40	35	43	36
Heart progressive cardiomyopathy	27	15*	21	12**	7	14	6	11
Thyroid no abnormality found	14	17	13	20	15	19	17	26*
Thyroid focal C-cell hyperplasia	12	3*	11	3*	7	10	9	4
Thyroid diffuse C-cell hyperplasia	27	27	24	26	28	17*	28	19
Adrenal gland pigmentation increased	12	19	24*	34**	17	32**	29*	32**
Adrenal cystic degeneration	0	0	1	1	15	4**	0***	1***
Adrenal gland cortical vacuolated cell focus	10	5	4	5	1	1	3	8*
Pituitary gland no abnormality detected	26	29	29	28	7	23***	24***	27***
Pituitary gland Adenoma anterior lobe	9	5	5	4	27	9***	10***	9***
Pancreas no abnormality detected	42	49*	49*	47	49	49	50	47
Pancreas islet cell hyperplasia	6	0*	1	2	0	0	0	0
Testes no abnormality found	33	0***	0***	0***				

Significantly different from the Control: P < 0.05, ** P < 0.01, *** P < 0.001

**Appears This Way
 On Original**

Tissue and lesion	Male				Female			
	Control	Low Dose	Mid Dose	High Dose	Control	Low Dose	Mid Dose	High Dose
Kidney transitional cell hyperplasia diffuse	18	19	11	19	22	9**	13	14
Kidney increased tubular pigment	1	4	7	7	23	4***	10*	10*
Kidney progressive nephropathy	28	13**	20	41**	8	10	19*	42***
Kidney tubular mineralisation	1	3	6	1	13	21	25*	15
Kidney pelvic mineralisation	14	24*	22	24	36	18***	21**	27
Urinary bladder no abnormality found	41	49**	50**	48**	49	47	48	49
Stomach no abnormality found	26	35	38*	40**	23	36*	32*	42***
Stomach dilated glands	5	6	4	2	15	11	5*	2***
Liver no abnormality found	1	4	4	5	2	10*	11*	13*
Liver basophilic cell focus, tigroid	24	16	12*	22	42	13***	11***	10***
Liver eosinophilic cell focus	43	35	30**	31*	8	25***	15	12
Liver bile duct hyperplasia	3	6	6	11*	4	7	3	4
Liver hepatocyte vacuolization	6	10	5	2	9	3	1*	1*
Liver glycogen vacuolization	11	8	7	3*	2	7	6	6
Salivary gland no abnormality found	44	46	44	41	38	46	43	48**
Salivary gland acinar hypertrophy	1	2	3	1	11	2*	4	2*
Pancreas (exocrine) no abnormalities found	21	36**	31	35**	39	24**	26*	33
Pancreas (exocrine) inflammatory cell foci	1	0	1	1	1	8*	3	4
Pancreas (exocrine) lobular atrophy	19	8*	10*	8*	5	14*	13	5
Skin no abnormality found	29	45***	34	27	42	47	41	40
Skin macrophage infiltration	0	1	7*	16***	0	0	3	4
Mammary gland no abnormality found	20	6***	8**	5**	15	10	10	8
Mammary gland fibroadenoma benign	0	0	0	0	10	1*	0**	0**
Mammary gland atrophy								
minimal	1	1	2	1	1	3	5	4
mild	5	4	7	4	0	7**	8**	10***
moderate	1	12***	4	4	1	5	8**	11**
marked	1	3	7	6*	0	12***	8**	5*
severe	0	0	0	0	0	1	1	0
total incidence	8	20***	20***	15**	2	28***	30***	30***
Mammary gland cystic hyperplasia	0	0	0	0	22	2***	0***	0***
Lacrimal gland no abnormality found	19	45***	40***	40***	41	45	47	44
Harderian gland alteration	27	1***	2***	5***	2	0	1	2
Brain no abnormality found	45	50	48	48	37	47*	46*	47*
Brain compression by pituitary tumor	2	0	0	0	11	1**	1**	1**
Sternum no abnormality detected	36	45*	44	44	31	39	41*	35
Sternum mucinoid degeneration of cartilage	8	4	4	5	17	7*	6*	10

Significantly different from the Control: P < 0.05, ** P < 0.01, *** P < 0.001

Best Possible Copy

Appears This Way
 On Original