CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-197

PHARMACOLOGY REVIEW(S)

JUL 10 2000

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Cetrorelix, pharmacology, pharmacokinetics, toxicology, toxicokinetics, polyploidy

Reviewer Name: Krishan L. Raheja
Division Name: DRUDP

HFD#: 580
Review Completion Date: 7-3-2000

Review number: 000
IND/NDA number: NDA 21-197
Serial number/date/type of submission: 000/Oct.29, 1999/Original
Information to sponsor: Yes (*) No ( )
Sponsor (or agent): ASTA Medica. Inc. Tewkbury, MA
Manufacturer for drug substance:
(for citrorelix acetate)

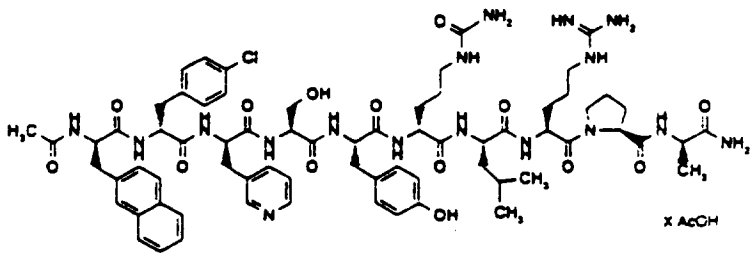
(for protected decapeptide)

Drug:

Code Name: SB-075 acetate, D-20761 (acetate salt)
Generic Name: Cetrorelix acetate
Trade Name: Cetrotide
Chemical Name: Ac-D-Nal-D-p-Cl-Phe-D-Pal-Ser-Tyr-D-Cit-Leu-Arg-Pro-D-Ala-NH_2
IUPAC Name: Acetyl-D-2-naphthylalanyl-D-4-chlorophynylalanyl-D-pyridylalanyl-seryl-tyrosyl-D-citrullyl-leucyl-arginyl-prolyl-D-alaninamide
CAS Registry Number: 130143-01-0
Molecular Formula/ Molecular Weight: $C_{70}H_{92}ClN_{17}O_{14}$ X AcOH/1431.06 (base)

Structure:

Cetrorelix acetate



(Ac-*D*-Nal1-*D*-Cpa2-*D*-Pal3-Ser4-Tyr5-*D*-Cit6-Leu7-Arg8-Pro9-*D*-Ala10-NH_2)

Relevant INDs/NDAs/DMFs:

Drug Class: GnRH antagonist

Indication: The prevention of premature ovulation in patients undergoing controlled ovarian stimulation.

Clinical formulation: Cetrotide is formulated as a sterile powder for injection. There are two dosage strengths containing 0.25 mg and 3 mg cetrorelix. For the reconstitution of the powder, sterile water for injection (1 and 3 ml in ready to use prefilled syringes) is used. The concentration of the final solution is 0.25 mg/ml and 1 mg/ml, respectively. Cetrotide also contains mannitol (USP) in a quantity to obtain an isotonic solution after reconstitution.

Quantitative composition of Cetrotide: For 1 vial Cetrotide 0.25 and 3 mg is as follows:

1 vial Cetrotide	0.25 mg	3 mg
Ingredients	Quantity	
Cetrorelix acetate (active ingredient)	0.25 mg	3.00 mg
Mannitol (inactive ingredient)	[redacted]	[redacted]
Other inactive ingredients:	[redacted]	ı are removed during processing.

Route of administration: Subcutaneous

Proposed clinical protocol or Use: It is to be used for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation. There are two dose regimens i.e., a single dose per treatment cycle or multiple dosing. As a single dose, Cetrotide 3mg is administered for the prevention of premature ovulation with a protective period of 4 days. If hCG has not been administered within 4 days after injection of Cetrotide 3mg, Cetrotide 0.25 mg is administered once daily until and including the day of hCG administration. The multiple dose regimen constitutes administration of Cetrotide 0.25 mg daily until the day of hCG administration to induce ovulation and final maturation of the oocytes.

Previous clinical experience: Included in this NDA are three Phase III studies in adults. These studies were conducted as part of a European clinical program. Two studies were conducted with the 0.25 mg multiple-dosing regimen and one with the 3.0 mg single-dosing regimen. Buserelin and Triptorelin were used as the active controls in these studies.
Seven Phase II efficacy studies were also conducted. These included four Proof of Concept studies, two Dose-finding studies and one Exploratory study using two different gonadotropins (rec FSH or HMG) for ovarian stimulation.

The Division agreed in a meeting on October 30, 1996 that European data would be acceptable as the basis of an NDA submission, if these data were presented together with a suitable historical control consisting of women undergoing ovarian stimulation without concomitant LHRH-agonist or LHRH-antagonist therapy.

The sponsor has obtained the required historical control data from the National In-Vitro Fertilization-Embryo Transfer (IVF-ET) Registry prepared by the American Fertility Society (AFS) and the Society of Assisted Reproductive Technology (SART).
Thus already completed European studies are the basis of this NDA submission.

Disclaimer -- use of sponsor's material

Introduction and drug history: For more than a decade, investigators have been searching for selective potent antagonists of the LHRH decapeptide. The high degree of interest in such antagonists is due to their usefulness in the fields of endocrinology, gynecology, contraception and cancer. The most interesting antagonists to date have been compounds whose structure is a modification of the structure of LHRH.
The first series of antagonists were obtained by replacement of the original amino acids by aromatic acid residues at positions 1, 2, 3 and 6 of the LHRH molecule. These compounds had very poor water solubility.
In order to increase the water solubility of antagonists, basic amino acids, such as D-arginine were introduced into position 6. Although these analogs possessed improved water solubility and showed increased antagonistic activity, they produced transient edema of the face and extremities due to histamine release when administered SC in rats. The edematogenic effects in rats cast doubt on the safety for the use in humans and delayed introduction of these drugs in clinical use.
The third generation antagonists, which include Cetrotide, possess an improved water solubility and high antagonistic potency and are relatively free of edematogenic effects. These compounds are highly potent in inhibiting the release of gonadotropins from the pituitary gland in mammals, including humans.

Studies reviewed within this submission: Studies which have not been reviewed under IND #

Studies <u>not</u> reviewed within this submission: All studies reviewed under [redacted] These however, have been briefly summarized and copy of the original reviews is appended.

PHARMACOLOGY:

<u>Mechanism of Action</u>: Cetrorelix competes with natural LHRH for binding to membrane receptors on pituitary cells and thus controls the release of LH and FSH in a dose-dependent manner. The onset of suppression is immediate and is maintained by continuous treatment. The effect is more pronounced on LH than on FSH.

<u>Drug Activity Related to Proposed Indication</u>: In women who ovulate normally, cetrorelix delays the LH surge, and consequently ovulation, in a dose-dependent manner. This is also the case in women undergoing ovarian stimulation used during controlled ovarian stimulation. In accordance with its specific mode of action, cetrotide does not induce any initial stimulatory effect (flare-up) as with GnRH agonists. The effects of cetrotide on LH and FSH are reversible after discontinuation of treatment.

Ancillary Pharmacology Studies: Studies reviewed under original IND submission were 1) evaluation of the antigonadotropic effects in monkeys and 2) treatment of DMBA-induced mammary carcinomas in rats. Results showed that the treatment lowered testosterone to castrate levels and compound was effective in inhibiting tumor growth.

Summary of pharmacology: Cetrotide competes with natural LHRH for binding to pituitary gonadotrophs and thus controls the release of gonadotropins, LH and FSH. By virtue of its this specific action, it is used during controlled ovarian stimulation to optimize ovulation.

Note: In the rat however, low dose of cetrorelix significantly increased testosterone in males and estradiol in females, an effect similar to that of a GnRH agonist.

SAFETY PHARMACOLOGY: Safety pharmacology studies were reviewed under IND

Neurological effects: Doses of up to 1 mg/kg SC showed no ataxic effects and caused no decrease or increase in spontaneous motor activity in mice.

Cardiovascular effects: An i.v. dose of 100 ug/kg had no effect on CV function in the anesthetized pig. Parameters determined were arterial blood pressure, heart rate and left ventricular contractility.

Pulmonary effects: Administration of 1 mg/kg cetrorelix in saline to anesthetized rats had no effect on blood gas values i.e. pO_2, pCO_2 and pH. High i.v. doses caused bronchospasm in anesthetized guinea pigs.

Renal effects: not investigated

Gastrointestinal effects: Cetrorelix administered to fasted rats as a single SC dose of 0.25, 0.5 and 1 mg/kg had no significant ulcerogenic effect.

Abuse liability: Doses of 0.25, 0.5 and 1.0 mg/kg SC had no effect on hexobarbital-induced or ethanol-induced loss of righting reflex in the mouse.

Other: Using rat peritoneal mast cells, cetrorelix at concentrations below 0.9 ug/ml showed no histamine releasing activity. Concentrations greater than 0.9 ug/ml caused histamine release in a dose-dependent manner with ED_{50} of 9.3 ug/ml (about 20 umol/l). The ED_{50} for LHRH has been reported to be about 30 umol/l. ED_{50} for the first generation LHRH antagonist was reported to be 0.05 ug/ml, which is 180 times stronger in releasing histamine compared to citrorelix.
It was reported that no drug-related allergic reactions were observed in the 10 clinical studies.

Conclusions: Safety pharmacology studies did not demonstrate any adverse effects.

Summary: Cetrorelix did not exhibit any adverse CV, gastric or neurlogical effects. At very high doses, it can cause bronchospasm.

PHARMACOKINETICS/TOXICOKINETICS:

Several ADME studies were conducted in rats and dogs using either [Arg-U-^{14}C] cetrorelix, which is metabolized or [D-Phe-^{14}C] cetrorelix, which is not metabolized. Significant findings of these studies were as follows:

Significantly longer Cetrorelix $T_{1/2}$ observed in both rats and dogs with the use of [Arg-U-^{14}C] cetrorelix was attributed to liberated arginine being involved in protein neosynthesis.

For suppression of testosterone, cetrorelix threshold was 1 ng/ml in rats and 2 ng/ml in dogs.

PK parameters following multiple sc injections were similar to those after a single injection, suggested no enzyme induction.

Only cetrorelix and one water-soluble metabolite (M_1 i.e. urea) was identified in the rat and dog urine after iv and sc cetrorelix administration.

In intact rats, about 70% of the administered dose was excreted in feces and 25% in urine. In dogs, it was 41% in the feces and 47% in the urine.

The metabolite pattern in the rat and dog was similar. Besides cetrorelix, the biliary metabolites were D_1 (tetrapeptide), D_2 (hexapeptide), D_3 (nonapeptide) and D_4 (heptpeptide), suggesting cleavage at positions 4, 6, 9 and 7 respectively.
Heptapeptide was the major metabolite.

Cetrorelix and small amounts of the 4 metabolites found in the rat (15% of the dose) and dog's (8% of the dose) bile were also reported for the human bile. Human urine revealed only unchanged cetrorelix.

No oxidative Phase 1 or conjugative Phase 2 drug metabolism reactions were involved in the in-vitro metabolism of cetrorelix. Cetrorelix was very stable in all incubations except incubation with pancreatin from porcine pancreas.

Toxicokinetics

Six month rat and dog toxicity study results were reviewed under the original IND submission. However, results of TK were not submitted and are now included as study No. D-20761/7096060078 for rat andD-20761/7095260031 for the dog.

TK parameters for the rat study expressed as mean + s.d. for doses of 0.02 and 0.5 mg/kg after week 2 and week 25 is shown in table below. Blood samples were taken up to 24 hours post dose. Blood from 2 animals was pooled.

Table 1

Week 2

Dose mg.kg	Sex	N (Pools/animals)	Cmax ng/ml	Tmax (h)	AUC (ng x h/ml)	T1/2 (h)
0.02	M	3/6	34.7 + 5.7	0.7 + 0.3	64.0 + 5.5	1.1 + 0.2
	F	3/6	32.0 + 4.5	0.7 + 0.3	67.8 + 6.9	1.3 + 0.1
0.5	M	3/6	215.3 + 12.9	1.2 + 0.3	1895.8 +118.2	7.8 + 0.4
	F	3/6	262.0 + 30.6	1.0 + 0.0	1971.1 + 119.4	6.4 + 0.4

Week 25

0.02	m	3/6	23.0 + 1.0	1.3 + 0.8	86.9 + 7.8	1.9 + 0.2
	f	3/6	32.0 + 4.5	0.7 + 0.3	91.5 + 4.3	1.6 + 0.2
0.5	m	3/6	143.3 +32.6	1.2 + 0.8	2012.8 + 430.6	14.3 + 1.8
	f	3/6	166.7 + 9.9	1.3 + 0.8	2038.0 + 29.1	12.7 + 1.7

The results showed a rough dose proportionality with regards to AUC. With the low dose plasma, concentrations were only measurable up to 4-8 hours whereas with the high dose they were >LOQ up to 24 hours. Sponsor suggested that this explains the different half-lives in the 2 dose groups.

Note: It should be pointed out that sponsor has stated that the plasma samples came from study No. 888197, where as in the original study the 26 week toxicity was registered under study No.884564 and was classified as an interim report.

Following are the toxicokinetics data on samples collected in the 26-week dog toxicity study No. 884665. PK parameters were assayed after a single dose and then after 14 weeks. Values are mean + s.d.

Table 2

PK after single dose

Dose mg/kg	Sex	n	Cmax ng/ml	Tmax h	AUC_{0-24} ng x h/ml	AUC ng x h/ml	T1/2 h
0.1	M	3	108.7 + 39.5	1.8 + 0.3	984.2 + 247.3	1086.4 + 279.3	6.8 + 1.1
	F	3	104.3 + 8.9	1.7 + 0.6	934.3 + 65.1	1017.8 + 91.5	6.5 + 0.5
0.5	M	3	160.4 +9.4	2.3 + 1.5	2872.8 + 341.9	5919.6 + 642.5	23.1 + 2.7
	F	3	115.1 + 9.1	1.7 + 0.6	1258.6 + 137.6	1488.4 + 169.9	8.7 + 0.6

PK after multiple dose (in week 14)

Dose mg/kg	Sex	n	Cmax ng/ml	Tmax H	AUC_{0-24}=AUC_t Ng.h/ml	T1/2 H
0.1	M	3	114.5 + 25.6	1.7 + 0.6	1298.2 + 326.5	9.6 + 1.2
	F	3	114.5 + 8.7	1.8 + 0.3	1173.8 + 297.7	9.0 + 1.2
0.5	M	3	252.0 + 48.6	1.3 + 0.3	3875.2 + 585.8	19.6 + 0.8
	F	3	227.0 + 21.6	1.0 + 0.0	3145.8 + 118.3	17.6 + 2.2

In the single dose PK study, females treated with 0.5 mg/kg exhibited low values for Cmax, AUC0-24 and AUC and shorter T1/2 in comparison to male animals. Dose proportionality was not seen with respect to Cmax or AUC in both single and multiple dose studies. A longer half-life was observed with the high dose which may not represent a real elimination half-life as only the 24 h dosing interval was evaluated.

Plasma cetrorelix concentrations determined in a 29-day mouse MTD finding study (Study No. 915726) are shown in table below:

Table 3

Males	Concentration (ng/ml plasma)					
Day	Group 2 (5.0 mg/kg) (1 mg/ml) (5.00 ml/kg)	Group 3 (11.0 mg/kg) (1 mg/ml) (11.0 ml/kg)	Group 4 (24.2 mg/kg) (1 mg/ml) (24.2 ml/kg)	Group 5 (12.5 mg/kg) (2.5 mg/ml) (5.00 ml/kg)	Group 6 (18.8 mg/kg) (2.5 mg/ml) (7.50 ml/kg)	Group 7 (28.1 mg/kg) (2.5 mg/ml) 11.2 ml/kg)
8	65.7	133.0	355.1	117.0	116.2	237.0
15	4.6	14.6	27.6	24.5	32.2	61.0
18	9.2	23.5	69.9	28.7	44.5	70.6
22	6.2	15.5	48.4	23.0	31.2	54.4
24	16.0	34.7	107.0	45.5	73.0	64.0
26	22.2	44.5	110.9	50.7	79.0	154.8
29	14.9	28.7	55.5	89.6	61.2	76.0
Females						
8	32.0	86.0	134.7	42.8	108.9	111.4
15	5.3	10.8	27.0	22.6	20.7	31.4
18	8.4	22.4	42.7	18.1	35.3	42.6
22	6.2	13.8	24.1	19.7	21.8	33.2
24	10.6	27.6	39.0	22.7	39.6	55.2
26	11.5	44.0	76.3	44.6	49.0	85.9
29	6.6	23.2	36.7	28.2	50.3	50.5

Cetrorelix was given on day 1-7 and once on day 8, 15, 18, 22, 24, and 26. Blood samples were taken on day 8, 15, 22. 24 26 just before administration of the compound and on day 29. Samples were pooled from 3 mice for plasma cetrorelix determinatior

Results showed that after 7 days of s.c. administration of cetrorelix acetate there were high cetrorelix concentrations on day 8. The high concentration formulation (2.5 mg/ml) produced lower concentrations compared to lower concentration formulation (1.0 mg/ml). Values for the females were lower compared to those observed in males. Dosage interval of 2 days (day 22-24, day 24-26) led to mean cetrorelix concentrations of > 10 ng/ml at a dose of 5 mg/kg and >85 at a dose of 28.1 mg/kg on day 26. Values were generally dose-proportional and high dose gave a very high multiple of the plasma cetrorelix concentration with human therapeutic dose.

Other studies: For comparative purposes, the PK parameters of cetrorelix following 3 mg single or 0.25 mg single and multiple (daily for 14 days) subcutaneous administration of cetrorelix in adult healthy subjects are summarized in table below.

Table 4

	Single dose 3 mg	Single dose 0.25 mg	Multiple dose 0.25 mg
# of subjects	12	12	12
T_{max} (h)	1.5 (0.5 – 2)	1.0 (0.5 – 1.5)	1.0 (0.5 – 2)
$T_{1/2}$ (h)	62.8(38.2 – 108)	5 (2.4 – 48.8)	20.6 (4.1 – 179.3)
C_{max} (ng/ml)	28.5 (22.5 – 36.2)	4.97 (4.17 – 5.92)	6.42 (5.18 – 7.96)
AUC (ng.h /ml)	536 (451 – 636)	31.4 (23.4 – 42.0)	44.5 (36.7 – 54.2)
Total plasma clearance (ml/min.kg)	1.28[a]		
Volume of distribution (l/kg)	1.16[a]		

a=based on iv administration.

Cmax=maximum plasma concentration; multiple dose Css.max

AUC=area under the curve; single dose AUC0-inf, multiple dose AUC_t

Systemic exposure in the 6 month rat and dog toxicity studies as multiple of human systemic exposure with the proposed therapeutic dose of 0.25 mg/day (5 ug/kg/day) was as follows:

Table 5

Species	sex	Dose ug/kg/day	Treatment duration (weeks)	Cmax Ng/ml	Multiple of clinical Cmax	AUC ng.h/ml	Mulatiple of clinical AUC
Human	F	5	2	6.42	1.0	44.5	1.0
Rat toxicity study	M	20	2	34.7	5.4	64	1.4
	F	20	2	32	5.0	68	1.5
	M	500	2	215	33.4	1896	42.6
	F	500	2	262	40.8	1971	44.3
	M	20	25	23	3.6	87	2.0
	F	20	25	32	5.0	92	2.1
	M	500	25	143	22.1	2013	45.2
	F	500	25	167	26.0	2038	45.8
Dog toxicity study	M	100	14	114	17.8	1298	29.2
	F	100	14	114	17.8	1174	26.4
	M	500	14	252	39.2	3875	87.1
	F	500	14	227	35.4	3146	70.1

Drug administration was by the subcutaneous route. Based on C_{max} values, the multiples of human therapeutic dose observed in mice were 55 in males and 30 in females.

Comments: Although solubility limitation was suggested to be a factor in dose selection, the doses used in these toxicity studies were primarily based on pharmacodynamic rather than toxicokinetic considerations. As such the doses used for the present indication with the proposed dose of 0.25 mg/woman/day gives an adequate safety margin based on systemic exposure. However, the multiples are low if the proposed 3 mg single dose is taken into consideration (maximum of 4 in rats and 7 in dogs).

In vitro protein binding: Under study No. 9444 (report No. D-20761/7094440014) entitled [U-Arg-^{14}C]cetrorelix acetate salt: in vitro binding in dog and rat plasma" it was reported that protein binding in rat and dog plasma is around 87% at concentrations of 40, 500 and 2000 ng/ml. Also binding ex vivo in dog plasma was approximately 90%.

In humans cetrorelix salt was found to be bound up to 83% on human albumin and up to 86% on human plasma proteins at concentrations of 40, 200 and 500 ng/ml plasma or human albumin solution (Study No. 9427).

TOXICOLOGY:

General Comments: Two chronic 6 month toxicity studies, one in rats and the other in dogs were submitted as summaries in the original IND submission and are reviewed here.
Acute toxicity studies in mice and rats, 4-week toxicity in rats and 13-week toxicity studies in rats and dogs were reviewed under the original IND submission, a copy of which is appended.

Study Title: **26-week subcutaneous toxicity study after repeated administration in rats and subsequent 18-week recovery period.**
Study No: 884564
Amendment #, Vol #, and page #: vol 27 of 162/001
Conducting laboratory and location: ASTA Medica AG, Institute of Toxicology, Kantstr. 2, D-33790 Halle/Westfalen
Date of study initiation: 3-17-1992
GLP compliance: yes
QA- Report: Yes (*) No ()
Methods:
Dosing:

- species/strain: Rat/Bor: WISW ($SPFC_{pb}$)
- #/sex/group or time point: 30/s/groups 1 and 4, 20/s/groups 2 and 3
- age: males 6 weeks, females 7 weeks
- weight: males 143-198, females 131-177
- satellite groups used for toxicokinetics or recovery: yes
- dosage groups in administered units: 0, 0.02, 0.1 and 0.5 mg/kg/day (i.e., 0.118, 0.59 and 2.95 mg/m /day)
- route, form, volume, and infusion rate: s.c., solution, I ml/kg in 0.3 molar mannitol solution

Drug, lot#, radiolabel, and % purity: F1 789/ —
Formulation/vehicle: solution in 0.3 molar D-mannitol
Observations and times:

- Clinical signs: clinical signs-daily, mortality-twice daily
- Body weights: once/week
- Food consumption: once/week
- Ophthalmoscopy: predose and then weeks 13 and 26
- EKG: -
- Clinical chemistry: ALTA, albumin, ASTA, blood urea, Ca, Cl, CK, creatinine, Fe, gamma-GT, glucose, GLDH, P, K, Na, total bilirubin, Chol, total protein, & Triglc. On weeks 6, 13, 26 and then weeks 32 and 44 from recovery animals.
- Hematology: RBC, Hct, Hb, WBC, MCH, MCHC, MCV and thrombocytes. Same time points as for hematology.
- Urinalysis: weeks 5, 12 and 25 and then week 31 for recovery animals.
- Organ weights: at necropsy
- Gross pathology: at necropsy
- Organs weighed: yes
- Histopathology: yes

Toxicokinetics: yes

Other: The sponsor stated that the dose selection was based on results of the 13-week toxicity study (D-20761/3000873898) which in turn was based on the results of 4 week toxicity study (D-20761/3000871795). The dose selection for the 4-week toxicity study was based on the results of pharmacological investigations with the trifluoracetate salt of

the test substance, where 0.3 mg/kg was found to be supraoptimal for its efficacy in inhibiting dimethyl benzanthracene (DMBA)-induced mammary carcinomas in SD rats. This coupled with the findings that 0.3 mg total dose in patients gave maximum response to decrease serum levels of LH and FSH significantly, contributed to the dose selection. The dose selection therefore, seems to have been based primarily on pharmacodynamic considerations.

However, it was stated that the high dose was limited by the solubility of the test substance and the maximum amount which could be administered to animals subcutaneously (no data presented). Additionally the high dose produced significant lesions at the site of injection and caused a decrease in body weight gain in mid and high dose males. Taken together, these data suggest the 0.5 mg/kg is near the MTD and/or MFD in rats.

Results:

Clinical signs: Clinical observations revealed no systemic toxic effects. Testes size was decreased in a dose-dependent manner with regards to grade and time of occurrence. Testes were not palpable in the high and mid dose groups during treatment weeks 6 and 10 respectively. The finding was reversed during the recovery period. There was no treatment-related mortality.

Body weights: were significantly decreased in males of groups 3 and 4 starting weeks 8 and 2 of the treatment and persisting up to week 26. At week 26 the mean value + sd (gm) was 407 + 32.5, 406 + 36.5, 357 + 33.4 and 347 + 28.8 respectively for groups 1, 2, 3 and 4. Thus the weight for the low, mid and high dose groups was 100, 88 and 85% compared to control group. Body weights were comparable to control for all groups at the end of recovery period.

Females on the other hand had increased body weight starting week 2 (group 4) and week 11 (group 3) throughout the treatment. Respectively values for low, mid and high dose groups at week 26, were 265 + 21.1, 282 + 21.3 and 286 + 17.7 compared to 246 + 17.3 for the control group. Thus the weight of these groups was 110, 115 and 116% compared to the control group. The high dose group had higher body weight till week 17 of recovery.

Food consumption: was significantly decreased in mid dose males from weeks 3 onwards until the end of treatment as well as in high dose males during the whole treatment period. From weeks 6/7 onwards until the end of treatment, the food consumption of mid and high dose males remained 10-15% below that of control and low dose males. Recovery for the high dose was not complete by the end of recovery period. In contrast in females food intake was increased in a dose-dependent manner up to weeks 17 of treatment. Group 4 females had reduced food intake during the recovery period.

Ophthalmoscopy: There were no ocular changes attributable to treatment with cetrorelix.

Hematology: No drug-related changes in hematology parameters were detected in low dose male and females.

In mid dose males, a decrease in RBCs in weeks 6, 13 and 26, Hb in weeks 6 and 26 and Hct in week 6 was observed. In high dose males, a decrease in RBC in weeks 6, 13 and 26, Hb in weeks 6 and 26 and HCT in week 6 was observed. WBC count was

significantly increased in mid and high dose males in weeks 6, 13 and 26 but was not dose-dependent.
In females changes in hematology parameters were slight though significant and were considered to be incidental and not drug-related. WBC count was increased in mid and high dose females in weeks 6, 13 and 26 but was not dose-dependent in extent. In both males and females an increased WBC count was attributed to increased lymphocytes. All changes in hematology parameters were reversible by the end of recovery period.

Clinical chemistry: TGs were decreased in mid and high dose males in weeks 6, 13 and 26. In both groups alpha-1globulins were decreased and beta and gamma-globulins increased in week 26. All these changes were reversible.
In females, cholesterol was increased in all treated animals and serum iron decreased in mid and high dose groups in weeks 6, 13 and 26. Also alpha-2 globulin were increased in mid and high dose groups.

Urinalysis: No treatment-related effects were reported

Gross pathology: All male rats in groups 3 and 4 had small testes, prostate and seminal vesicles. In low dose 1/20 had these conditions. In recovery animals, 1/10 high dose animal had small testis with flaccid consistency.
In females of groups 3 and 4, small ovaries and uterus were seen in all animals and were fully reversible.
Injection site: were discolored and had slight to severe hemorrhages in animals of both sexes including the controls. All changes were reversible.
Organ Weights: While the relative organ weights of mid and high dose males were reduced for liver, kidney, testes, prostate and seminal vesicles, they were increased for brain and thymus gland. In group 4 males, relative weight was for heart, liver, kidneys, testes and prostate were still below the control values at the end of the recovery period. The relative weight of brain, spleen and ovaries was decreased in all cetrorelix treated groups. Also mid and high dose females had reduced relative weight of pituitary, heart, liver, kidneys, adrenals, ovaries and genital tract. At the end of recovery period, the relative weights of pituitary, liver, adrenals and that of the genital tract were still reduced in group 4 recovery females.

Histopathology: Treatment-related microscopic findings were observed in the genital system, mammary gland, injection site, pituitary gland, adrenals, bone, spleen and kidneys.
Testes- Treatment-related changes consisted of diffuse atrophy of seminiferous tubules and interstitial cells. Seminiferous tubules were markedly reduced in diameter and the germinal epithelium was characterized by maturation arrest. In cases of massive atrophy it consisted of Sertoli cells and spermatogonia. In cases of less severe atrophy early stages of spermatocytes were also present. Late stages of spermatocytes, spermatids and spermatozoa were absent. The incidence of diffuse atrophy was 1/20 in low dose and in all animals in mid and high dose groups. The increase in severity was dose-related. It was 4.2 for group 3 and 4.6 for group 4 on a scale of 1-5 (4=marked and 5=massive). Diffuse atrophy of interstitial cells was observed in all group 3 and 4 males with

moderate to marked severity. Atrophy was shown by a reduction in size, small-condensed nuclei and increased nucleus to cytoplasm ratio. All changes were completely reversible.

Epididymides- showed atrophic changes in epididymal epithelium. Atrophic cells have small condensed nuclei and a reduced amount of cytoplasm. The spermatozoa were reduced in 1/20 low dose and in all mid and high dose animals. These changes showed complete reversibility.
Prostate and seminal vesicles- The seminal vesicles and the acini of prostate were markedly reduced in size and atrophic cells had same appearance as in the epididymides. Diffuse atrophy of both organs was seen in all mid and high dose animals with severity of 4.7 in group 3 and 4.9 in group 4 for prostate and 4.7 in group 3 and 4.9 in group 4 for s.v.
Ovaries- treatment was associated with reduced follicular activity i.e. secondary, tertiary and Graafian follicles were absent. The incidence and number of atretic follicles was increased and c.l were absent in low, mid and high dose animals. Instead atypical c.l occurred. Atretic follicles were seen in 3/20 low dose and in all mid and high dose animals with a mean severity of 1 in low dose and 3.5 in high dose group. Secondary follicles and later stages of the follicular maturation were absent in 13/20 group 3 and 14/20 group 4 rats. It was not observed in group 1 and 2 animals. Only atypical corpora lutea were seen in cetrorelix treated animals. These consisted of elongated cells which were morphologically intermediate between theca cells and luteinized cells, and center having degenerated cells with deposits of brown pigment. Atypical c.l were observed in 1/20, 12/20 and 14/20 group 2, 3, and 4 rats, respectively. The changes of the ovary showed complete reversibility.

Uterus and vagina- Uterus of mid and high dose animals showed diffuse atrophy with moderate to massive severity. According to the cycle of the vaginal epithelium and uterine endometrium, the animals in these groups were in anestrus. Majority of the animals in the control and low dose groups were in proestrus/estrus. Diffuse uterine atrophy did not occur in low dose group.

Mammary gland region- showed dose-related diffuse atrophy in mid and high dose groups.

Pituitary gland- showed reduction of basophilic cells and focal vacuolization of pituicytes in pars distalis. In males the incidence was 7/20 in low and 20/20 in mid and high dose groups. In female 9/20 were affected in low dose and all animals in mid and high dose groups. The severity was dose-related ranging from 1 to 3.6 in both sexes. The incidence of focal vacuolation was dose- related but exhibited minimal severity. All changes showed complete reversibility.

Adrenals- Diffuse atrophy of the zona reticularis occurred in 0/20, 4/20, 19/20 and 20/20 males and in 5/20, 3/20, 20/20 and 20/20 in females in control and 3 treated groups. The severity was dose-related.
Vacuolation of the adrenal cortex, which occurred only in males, showed drug-related reduction in incidence and severity. This finding was not completely reversed during the recovery period.

Bone- 13/20 and 17/20 of groups 3 and 4 males and all 20/20 group 3 and 4 females had reduction of the spongiosa i.e. decreased bone density. Complete reversibility was seen in males but not in females during the recovery period.

Injection sites- histopathological changes at the injection site consisted of macrophage infiltrates, focal necrosis, focal mononuclear infiltrates and focal fibrosis/fibroplasia. The incidence and severity of these changes was dose-related. These findings showed strong tendency towards reversibility during the recovery period.
Focal hemorrhage occurred in control and drug treated animals and was attributed to the traumatic character of the subcutaneous injections.

Spleen- Reduced extramedullary hematopoiesis was observed in all mid and high dose females only. Minimal follicular hyperplasia of the white pulp occurred in an increased incidence and severity in both sexes treated with high dose and also in mid dose females. These findings were completely reversible.

Kidneys- Mid and high dose rats showed less severe changes in chronic-progressive nephropathy than in control rats and those treated with low dose.

Toxicokinetics: Is reviewed under toxicokinetics as table # 1

Key Study Findings: Aside from the expected effects from the suppression of sex hormones, significant observations consisted of 1) drug-related atrophy of the adrenal's zona reticularis, reduction of the bone spongiosa, reduced spleen extramedullary hematopoisesis and dose-related incidence and severity of inflammatory changes at the injection site. No significant unexpected toxicity was observed at the dose levels used.

Overall Toxicology Summary: Mostly pharmacodynamic effects of treatment were observed. These were attributed to suppressive effects of cetrorelix on pituitary gonadotropins and sex steroid (testosterone and estradiol) secretion. At the application site treatment-related changes consisted of macrophage infiltrates, focal fibrosis/fibroplasia, focal edema and focal necrosis.

These changes showed a dose-related increase in incidence and severity ranging from slight to marked. The adrenal and the bone changes were not completely reversed during the recovery period.

Comments: In contrast to results of this 26 week toxicity study, similar doses used in the 4 week and 13 week toxicity studies using same strain of rats produced some significant systemic adverse findings.

In the 4 week toxicity study, high dose males had significantly elevated ASAT at week 4 compared to ccntrols (37 vs 34 U/l) and groups 3 and 4 males had significantly lower alkaline phosphatase (431 and 457 vs 523 U/l).

In the 13-week toxicity study, significant findings consisted of the following:

In males alkaline phosphatase was decreased at all determinations. At week 13, increase in ASAT was observed with values being 34, 37, 66 and 67 U/l respectively for the controls and 3 treated groups. Cholesterol was increased in the mid and high dose groups.

In females alkaline phosphatase was increased at all determinations. At week 13 changes included increases in ASAT (35, 38, 60 and 84 IU/l), in ALAT (29,39, 34 and 40 IU/l), in AlkPase (198, 173, 247 and 234 U/l), and in Gamma glutamyl transferase (0.19, 0.15, 0.33 and 0.53 U/l). Cholesterol was increased in all treated groups. TG, iron and albumin were decreased in mid and high dose groups.
No morphological correlates were reported for increase in liver enzymes.

Study title: **26-week subcutaneous toxicity study after repeated administration in Beagle dogs and subsequent 49-week recovery period.**

Study No.: 884665
Amendement #, Vol #, and page #: 30 of 162, page 001
Conducting laboratory: same as for the 26-week rat toxicity study reviewed above.
Date of study initiation: 8-5-1991
GLP Compliance: yes
QA-Report: Yes

Methods:

Dosing:
Species/strain: Beagle/Harlan CP
#/sex/group or time point: 6/s in groups 1 and 4 and 4/s in groups 2 and 3.
age: males 7-8 months; females 8 to 9 months
weight: males 9.2-14.0 kg; females 7.6 – 12.4 kg
satellite groups used for TK or recovery: 2/s in groups 1 and 4 used for recovery
dosage groups in administered units: 0, 0.02, 0.10 and 0.50 mg/kg/day (i.e., 0.4, 2 and 10 mg/m^2 /day)
route, form, volume, and infusion rate: s.c. test solution in 0.3 molar D-mannitol solution, 0.5 ml/kg
Drug lot #, radiolabel, and % purity: FI 701, ——.
Formulation/vehicle: 0.3 molar mannitol solution

Observations and times:
Clinical signs: twice daily
Body weights: once a week
Food consumption: daily
Ophthalmology: prior to first dose, week 13 for groups 1 and 4, week 26 for all groups.
EKG: -
Hematology: RBC, Hct, Hb, WBC, MCH, MCHC, MCV, thrombocytes on weeks 6, 13, and 26 and then at the end of recovery period (wk 75)
Clinical chemistry: ALTA, albumin, ASTA, blood urea, Ca, Cl, CK, creatinine, Fe, gamma-GT, glucose, glutamate dehydrogenase (GLDH), P, K, Na, total bilirubin, Cholesterol, total protein, triglyceride. Same time points as for hematology
Urinalysis: in test weeks 27 and 75
Organ weights: weeks 27 or 75
Gross pathology: at necropsy
Organ weighed: yes
Histopathology: yes
Toxicokinetics: yes

Results:

Clinical signs: No treatment-related effect on behavior and general condition was noted in any group. Reduction of testicular size was observed in treated males and onset was dose-dependent. It was reversible during the recovery period. In high dose animals of both sexes, swollen sites of administration were noted from week 2 of treatment until week 4 of recovery. Diarrhea and vomiting were frequently observed in treated and control animals but was more frequent in cetrorelix treated animals.

Body weights: At the end of 26-week treatment body weights were slightly higher in all treated males and females. These were 14750, 15375, 16600 and 15567 g for the male groups 1, 2, 3 and 4 respectively. Values for the females were 10950, 11275, 12225 and 12317 g respectively. Pretest values were similar among groups.

Food consumption: No significant effect on food consumption related to treatment with cetrorelix.

Ophthalmology: There were no ocular changes attributable to treatment.

EKG: No changes in heart rate were noted.

Andrological examination: No difference was observed between control vs group 4 recovery male animals for total volume of ejaculate (4.35 vs 7.10 ml), total number of sperm cells x 10^6 (770 vs 825), the active movement of sperm cells (82 vs 84%), and the incidence of morphological changes of sperm cells (12.6 vs 6.9%). No malformations were observed in either group.

Hematology: No changes in hematology were reported in relation to treatment.

Clinical chemistry: At various time determinations, significant changes were observed which did not occur at all dose levels and at all determination points.
These changes in males involved increases blood urea (weeks 6 & 13), gamma glutamyltransferase (weeks 6 & 13), and inorganic phosphate (weeks 6 and 13). Plasma glucose decreased on weeks 13 and 26.
In females changes were seen only at week 6. These included increases were seen in ALAT, blood urea, inorganic phosphate, and glutamate dehydrogenase (GLDH) and decrease in total bilirubin.

None of these changes were observed at the 26 week determinations except plasma glucose.

Urinalysis: Revealed no changes that could be attributed to treatment with cetrorelix.

Gross pathology: Treatment-related changes were observed at the site of injection and in the genital systems of both sexes. Injection site changes consisted of subcutaneous hemorrhages and thickening and/or brown discoloration. SC thickening was observed in all high dose males and females and was not observed in groups 1-3. These changes were reversible.
The male genital system was characterized by a marked reduction in the size of the testes and prostate in all treated animals compared to controls. In treated females, ovaries and uterus were reduced in size. These changes were reversible.

Organ weights: There was a marked reduction in the absolute and relative weights of testes and prostate in treated males and of the ovaries and genital tract in treated females. These changes were normalized in females but in males testes weight was higher and prostate weight did not reach control level at the end of recovery period. Relative wt of left kidney was decreased in all treated groups but in the absence of no change in the right kidney it was not considered treatment-related.

Histopathology: Treatment-related atrophic changes were seen in testes, epididymides and prostate in all treated males and in ovaries, uterus, vagina and mammary glands in treated females.

Changes in testes consisted of diffuse atrophy graded as moderate to marked. The seminiferous tubules were markedly reduced in diameter. The germinal epithelium was characterized by maturation arrest. It consisted of spermatogonia and early stages of spermatocytes while spermatocytes, spermatides and spermatozoa were absent. Interstitial cells also showed atrophy. Tubular atrophy occurred in 4/4 dogs in each group and severity was dose-related. Epididymal atrophy was associated with reduction of spermatozoa. The atrophy was dose-related in severity. The severity score was 2.3, 2.8 and 3.3 for groups 2, 3 and 4 respectively (2=slight, 3=moderate and 4=marked). Spermatozoa were completely absent in all treated dogs.

Prostate was atrophied in all treated dogs. All changes showed reversibility during the recovery period.

The female genital system was in an inactive stage. Ovaries had markedly reduced follicular activity i.e. tertiary and Graafian follicles were absent and the incidence and number of atretic follicles was increased in these animals. Reduced follicular activity occurred in 1/4 control and all treated females. The severity was dose-dependent (minimal to marked). The ovaries of animals treated with cetrorelix were devoid of corpora lutea.

Uterus and vagina of all treated females reflected the inactive stage of the ovaries and were morphologically in atrophy/anestrus. Mammary gland was also atrophied in treated females.

Pituitary gland of all animals treated with cetrorelix showed reduction of basophilic cells in the dorsocephalic region of the pars distalis. These changes were completely reversed during the recovery period.

At the injection site treatment related changes consisted of macrophage infiltrates, focal fibrosis/fibroplasia, focal edema and focal necrosis. These changes showed a dose-related increase in incidence and severity ranging from slight to marked.

Toxicokinetics: See Table 2

Overall toxicology summary: Most significant treatment-related findings were restricted to sex hormone dependent organs i.e., testes in males and ovaries in females. At the injection site, treatment related changes consisted of macrophage infiltrates, focal fibrosis, edema and necrosis. These changes showed a dose-related increase in incidence and severity. These changes were reversible on cessation of treatment. Following the recovery period, an andrological examination of drug treated dogs revealed no differences when compared to controls for total volume of ejaculate, total number of sperm cells, active movement of sperms and the incidence of morphological changes of sperm cells. No sperm malformations were reported.

Histopathology Inventory for NDA #21-197 for rat and dog 26-wk tox. studies

Study				
Species	Rat	Dog		
Adrenals	•	•		
Aorta	•	•		
Bone Marrow smear	•	•		
Bone (femur)	•	•		

Brain	•	•		
Cecum	•	•		
Cervix	•	•		
Colon	•	•		
Duodenum	•	•		
Epididymis	•	•		
Esophagus	•	•		
Eye	•	•		
Fallopian tube				
Gall bladder		•		
Gross lesions	•	•		
Harderian gland	•			
Heart	•	•		
Hyphophysis	•	•		
Ileum	•	•		
Injection site	•	•		
Jejunum	•	•		
Kidneys	•	•		
Lachrymal gland				
Larynx	•			
Liver	•	•		
Lungs	•	•		
Lymph nodes, cervical	•	•		
Lymph nodes mandibular	•	•		
Lymph nodes, mesenteric	•	•		
Mammary Gland	•	•		
Nasal cavity	•			
Optic nerves	•	•		
Ovaries		•		
Pancreas	•	•		
Parathyroid	•	•		
Peripheral nerve	•	•		
Pharynx				
Pituitary	•	•		
Prostate	•	•		
Rectum	•	•		
Salivary gland	•	•		
Sciatic nerve				
Seminal vesicles	•			
Skeletal muscle	•	•		
Skin	•	•		
Spinal cord	•	•		
Spleen	•	•		
Sternum	•	•		
Stomach	•	•		
Testes	•	•		
Thymus	•	•		
Thyroid	•	•		
Tongue	•	•		
Trachea	•	•		
Urinary bladder	•	•		
Uterus	•	•		
Vagina	•	•		
Zymbal gland				

Addendum to toxicology: About 8 months after the original submission, sponsor discovered that a number of preclinical studies performed with citrorelix were not included in the initial NDA. These studies listed below constitute 5 volumes, which were received on 5-19-2000.

1. A single subcutaneous toxicity study of NSD75A in rats and associated toxicokinetic study.
2. A single subcutaneous toxicity study of NS75A in dogs.

3. Antigenicity study of NS75A in mice.
4. Anatigenicity study of NS75A in guinea pigs.
5. Orientating maximum tolerated dose (MTD)-finding study and plasma levels determination after repeated subcutaneous administration in mice.
6. Orientating MTD-finding study and plasma level detrmination after repeated subcutaneous administration in rats.
7. MTD-finding study by s.c. administration (twice weekly; 26 weeks) in mice with an associated toxicokinetic report.
8. MTD-finding study by s.c. administration (daily;26 weeks) in mice with an associated toxicokinetic report.
9. MTD-finding study by s.c. administration (twice weekly; 26 weeks) in rats with an associated toxicokinetic report.
10. MTD-finding study by s.c. administration (daily; 26 weeks; 6 week recovery) in rats with an associated toxicokinetic report.
11. Acute toxicity after single intravenous administration in mice.
12. Acute toxicity after single intravenous administration in rats.
13. Toxicological examination after single intravenous administration in rats (pretreated with orally administered Azelastine HCl).
14. Toxicological examination after single intravenous administration in rats (pretreated with orally administered Cyproheptadine (Peritol)).
15. A local tolerability study after single intramuscular and subcutaneous injection in rabbits.
16. A local tolerability study after single intramuscular and subcutaneous injection in beagles.
17. A local tolerability study after repeated intramuscular and subcutaneous injection in beagles.

The above studies are summarized as follow:

Studies 1 and 2 were conducted by [redacted] in accordance with GLP Regulations.
In the single s.c. toxicity study in rats (Report No. 918731), dose levels of 1, 10, 25 and 100 mg/kg (5/s/g) were used.
No deaths were reported in any treatment group. Drug effect was observed in reproductive organs and body weight over 10 mg/kg treatment groups.

Gross pathology findings were atrophy of testes, prostate gland and seminal vesicles and atrophy of the uterus.
In histopathology, hypospermatogenesis in testes, absence of spermatozoa in epididymides, atrophy of epithelium in prostate and seminal vesicles, atrophy of the ovaries with the decrease of
secondary follicles and Graafian follicles, atrophy of the uterus with thinning of endometrium and myometrium and thinning of epithelium in vaginal was observed.
Body weight gain was decreased in males and increased in females.
There was swelling at the injection site. In histopathology, granuloma in the 10 mg/kg and higher dose groups and cysts in the 100 mg/kg group were seen in s.c. tissue.
The highest plasma cetrorelix (NS75A) concentration was reported to be in the range of 523 – 1091 ng/ml compared to Cmax of 31.5 + 15.7 ng/ml at a dose of 3 mg/body in clinical trial.

In the single dose s.c. toxicity study in dogs, dose levels of 2 and 20 mg/kg (2 females/g) were evaluated.
No treatment-related changes were reported for general signs, body weight, food and water consumption, hematology and biochemistry in either group.
Ovaries were atrophied with decreased secondary follicle and Graafian follicles. Uterus and vagina was in anestrus state. Hemorrhage and edema at injection site in gross pathology and granuloma with hemorrhage in histopathology were noted suggesting irritation.
Plasma drug concentration was reported to be similar in both groups and ranged from 618 to 2825 ng/ml.

Study 3 on the antigenicity of cetrorelix in mice (report No. 918707) and **study 4** on antigenicity of cetrorelix in guinea pigs (Report No. 918718) was conducted by
Both these studies were conducted in compliance with GLP Regulations.
In mice the antigenicity potential of cetrorelix was evaluated by enzyme-linked immunosorbent assay (ELISA). In guinea pigs aside from these 2 assays, immunogenicity was also examined by

Results showed that cetrorelix was not immunogenic in either mice or guinea pigs.

Studies 7-10 were conducted to identify the optimum doses for planned carcinogenicity studies. Sponsor stated that since the carcinogenicity studies were cancelled, no final reports were written and submitted for review.

The sponsor stated that no treatment-related systemic toxicological findings were observed in these MTD-finding studies. It was however, reported that a granuloma-like reaction occurred at the injection site and subsequent injections of cetrorelix into the vascularized granuloma tissue effectively resulted in a quasi-intravenous injection. This quasi-intravenous injection led to mast cell degranulation with subsequent clinical symptoms of hypotensive shock and eventually death.

Because of the above observations in the MTD-finding studies, acute single dose intravenous toxicity in mice (report No. 917886) and in rats (Report No. 916525) were conducted to reconfirm the assessment of the MTD-finding studies.
In the mouse toxicity **study 5,** treatment groups were used as shown in table below:
Table 6

Study group	1		2		3		4	
Dosage mg/kg	21.5		31.6		31.6*		46.4*	
Sex	M	F	M	F	M	F	M	F
Animals/dosage	5	-	5	5	5	-	5	-
Deaths	1	-	0	0	5	-	5	-

* higher concentration i.e. 2 mg/ml instead of 1 mg/ml. All males in groups 3 and 4 died within 2 minutes of injection. One mouse in group 1 died during days 2-7.

Adverse effect was characterized by hypokinesia and staggered gait. Mice in groups 3 and 4 also showed loss of muscle tone and righting reflexes. Histological examination revealed vascular thrombi in the lungs of all dead mice in groups 3 and 4. Thrombi were also recorded in the hearts of animals treated with 31.6 mg/kg and the diffuse vascular thrombosis was suggested as the cause of sudden deaths. Since no deaths occurred in animals dosed with 31.6 mg/kg employing concentration of 1 mg/kg concentration, findings were attributed to dosing solution concentration and not to the toxicity of the drug substance?.

Intravenous injection of cetrorelix in rats was also lethal as it was in mice. Five treatment groups were used as shown in table below:

Table 7

Study group	1		2		3		4		5	
Dosage mg/kg	**0.681**		**1.00**		**1.47**		**2.15**		**3.16**	
Sex	m	F	M	f	M	F	m	F	m	F
Animal/dose	5	5	5	5	5	5	-	5	-	5
Deaths	0	1	2	1	5	1	-	2	-	5

Concentration of test substance in the solution was 1 mg peptide base/ml.

Main signs of toxicity were hypokinesia, lack of coordination, decreased muscle tone, loss of reflexes and cyanosis. Incidence and severity increased dose-dependently. Males were more sensitive than females.

Necropsy findings consisted of reddened mucosa of the stomach and the intestinal tract in both sexes. Microscopic examination revealed degranulation of mast cells of various organs, more pronounced in mesenchymal than in epithelial tissues.

A diffuse subcutaneous and gastric mucosal hyperemia/congestion was recorded in all animals. This finding was also present in the tongue as well as the skeletal muscle and perineural tissue suggesting that citrorelix causes the release of vasoactive amines preferentially out of connective tissue mast cells.

Since mast cells in rat and mouse contain both histamine and serotonin, studies were conducted in rats to determine their involvement in the sudden deaths observed in mice and rats after intravenous administration of cetrorelix.

Under study No. 918450, 3 groups of male rats were administered an i.v. dose of 1.47 mg/kg and one group of female rats was administered 3.16 mg/kg. Two hours prior to cetrorelix injection the male rats were treated orally with histamine H_1 antagonist Azelastine at a dose of 1.00, 2.15 and 4.64 mg/kg and female rats with 4.64 mg/kg respectively.

Results of this study showed that 4/5 females died, suggesting that histamine was not involved in the lethal effects of cetrorelix.

For male rats, one of 5 rats died in the groups pretreated with 1 and 2.15 mg/kg Azelastine while none died in the group pretreated with 4.64 mg/kg. It was pointed out that all male rats were in very critical condition for about 4-5 hours showing signs of a marked status of cardiovascular insufficiency.

Comments: Based on the results in female rats, the sponsor concluded that histamine release does not play a role when cetrorelix is administered intravenously. Sponsor however, ignored the protective effect of Azelastine in male rats by simply stating that they were partially protected in spite of the fact that at least 4/5 rats survived a dose, which was LD_{100} in the absence of histamine H_1 antagonist. Results also showed that male rats are more sensitive than female rats to cetrorelix toxicity.

Under study No. 917831, six treatment groups (5/s/g) were used. In all groups males were given an iv injection of cetrorelix at a dose of 1.47 mg/kg and females were given a dose of 3.16 mg/kg, doses which have been shown to cause 100% mortality. Both male and female rats were pretreated with Cyproheptadine, a serotonin and histamine antagonist at doses of 0.313, 0.625. 1.25, 2.5, 5.0 and 10.0 mg/kg, respectively for the 6 treatment groups, two hours before cetrorelix injection.

Mortality: Only 1/5 male rats died in group 1 where the lowest dose of cyproheptadine was used. In females, 1/5, 4/5 and 1/5 died in groups treated with 0.313, 0.625 and 1.25 mg/kg cyproheptadine, respectively. The mortality rate was similar to previous experiment where histamine antagonist Azelastine was used. This showed that the protective effect of treatment was not dose-related. No deaths occurred with higher doses of cyproheptadine.

Sponsor's conclusion: Based on the results of the above studies, sponsor stated that serotonin plays an important role in the pathogenesis of the lethality in rats, when cetrorelix is administered intravenously. This was supported by the observation that Azelastine could not prevent deaths induced by i.v. cetrorelix administration in female rats.

Sponsor assumption is based on protective effect in female rats only because males were equally protected by both Azelastine and cyproheptadine pretreatment. This could suggest mechanistic sex differences.

Comments: Based on the results of the two studies, this reviewer doubts sponsor's conclusion that the cause of death in rats after intravenous administration of cetrorelix is unequivocally due to release of serotonin from mast cells. Sponsor has ignored the fact that both Azelastine (antagonist of histamine release) and Cyprohepadine (antagonist of histamine and serotonin release) were equally effective in protecting male rats. It is quite likely that Cyproheptadine is more potent antagonist of histamine release than Azelastine or the sensitivity of male and female rats is different.

Sponsor has also argued "that observed findings in rats are not relevant for the intended use of cetrorelix in humans because the safety factor of 10 between the toxicological no effect level (0.5 mg/kg b.w.) and the human therapeutic dose (approximately 0.05 mg/kg b.w.) is sufficiently wide".
This may not be correct safety factor since if values are expressed on body surface area, the safety factor will be below 2.

However, if one accepts sponsor's assumption that lethality in mice and rats after iv injection of cetrorelix is due to serotonin rather than histamine, the rodent findings have little significance for humans since mast cells in human have been demonstrated to contain no serotonin. There were no serious adverse effects reported when cetrorelix was administered intravenously at a dose of 3 mg to 6 female and 6 male human subjects. Also the risk if any is further reduced by the subcutaneous clinical route of administration and the concentration of peptide being 1 mg/ml or less (0.25 mg/ml) in the proposed clinical formulation.

Summary: Toxicity of cetrorelix administered intravenous in mice and rats was characterized by hypokinesia, staggered gait, loss of muscle tone and righting reflexes, cynosis and death. Males were more sensitive than females. Sponsor attributed mortality to release of serotonin from mast cells, although review of the data submitted does not seem to support sponsor's assumption.

The following two toxicology studies conducted recently have not been reviewed before:

For all toxicity studies submitted under [redacted] or under the present NDA originally submitted on 10-28-1999, maximum dose level used in any repeat dose-toxicity study in any species was 0.5 mg/kg. The dose selection for these studies was based on pharmacodynamic rather than toxicokintic parameters and was not an MTD or MFD.

Study title: Cetrorelix acetate lyophilisate (D-20761) orientating MTD finding study inclusive plasma level determination after repeated subcutaneous administration in mice.

Study No.: 915726
Amendment #, vol # and page#: Amendment dated 5-18-2000/2 of 5/001
Conducting laboratory and location: ASTA Medica AG, [redacted]

Date of study initiation: 11-24-1997
GLP compliance: yes
QA report: yes

Methods:
Dosing: once daily for 7 consecutive days during week 1, then one injection on days 8, 15, 18, 22, 24 and 26.
Species/strain: mouse/Hds/Win: NMRI
#/sex/group: 21/s/g except controls 6/s
age: 6 weeks
weight: males 28-36 g; females 21 – 28 g
Satellite groups usde for TK or recovery:
Dosage groups in administered units: as given in expt design table below

Table 8

Group	1 (control)	2	3	4	5	6	7
Dose mg/kg	0	5.00	11.0	24.2	12.5	18.8	28.1
Concentration mg/ml	0	1.00	1.00	1.00	2.50	2.50	2.50
Injection volume ml/kg	24.2	5.00	11.0	24.2	5.00	7.50	11.2

Dose selection was based on limited injection volume for repeated injections and local tolerability of the test solution.

Route, form, volume and infusion rate: subcutaneous
Drug lot#, radiolabel and purity: 9602-001/03 & 9610-001/09/ [redacted]
Formulation/vehicle: Drug substance prepared in water/water
Observations and times:
Clinical sign: twice daily
Body weight: once pretest and then weekly
Food consumption: once pretest and then weekly
Ophthalmology:-
EKG:-
Hematology:no
Clinical chemistry: no
Urinalysis: no
Organ weights:no

Gross pathology: yes
Organ weighed:-
Histopathology: yes
Toxicokinetics: Blood samples were taken on day 8, 15, 18, 22, 24, 26 just before administration of the compound and on day 29. Plasma cetrorelix concentrations were determined by RIA on pooled samples from 3 mice.
Other: none

Results:

Clinical signs: No drug-related clinical symptoms of systemic toxicity were observed. Changes at injection site occurred except in controls and low dose group 2 females with drug concentration of 1.0 mg/ml. Groups 2-4 had slight swelling at the injection sites while groups 5-7 had slight to moderate swelling. No mortality occurred in any group.
Body weights: No significant change in weight gain in males or females was observed during the 4 week study.
Food consumption: mean food consumption was decreased up to 13% in males and 11% in treated female.
Hematology: not done
Clinical chemistry: not done
Urinalysis: not done
Organ weights: not weighed
Gross pathology: No changes were noted in control animals. Drug treated animals showed dose and concentration related increased incidence and severity of discoloration and thickening of the injection sites. Higher concentration had more pronounced effect when compared to low concentration formulations. All treated males had small testes, seminal vesicles and prostates. Small ovaries and uteri were reported in all treated female groups.
Histopathology: There was a dose and concentration-dependent increased incidence and severity of central focal necrosis and infiltrates of macrophages around the deposited test material at the injection sites (granuloma formation). The reaction was more pronounced with higher the concentration formulation and was suggested poor bioavailability of the higher concentration material with poor local tolerability.
Toxicokinetics: Blood was collected on day 8, 15, 18, 22, 24, and 26 immediately before drug administration and then on test day 29. See Table 3

Key study findings: The treatment resulted in expected findings of GnRH analog's physiological effect on sex hormones and on hormone dependent tissues. Concentrations greater than 1 mg/ml showed more pronounced irritation at the injection sites. Some of the parameters like organ weights, hematology and clinical chemistry, which are routinely included in these toxicity studies, were not determined. Plasma cetrorelix concentrations reported under PK/TK section showed that high dose produced 30 and 50 times higher Cmax in female and male mice respectively, when compared to human exposure with a therapeutic dose.

Study title: Cetrorelix acetate lyophilisate (D-20761) orientating MTD-finding study inclusive plasma levels determination after repeated subcutaneous administration in rats.

Study No: 913498
Amendment #/vol# and Page #.: Amendment dated 5-18-2000/3 of 5/001
Conducting laboratory and location: ASTA Medica AG, [redacted]
[redacted]
Date of study initiation: 8-26-1997
GLP compliance: yes
QA report: yes

Methods:
Species/strain: Rat/HsdCpb: WU
#/sex/group: 5/s/g
age: males 6 weeks; females 8 weeks
Weight: males 175 –192 g; females 150-168 g
Satellite groups for TK or recovery: no
Dosage groups in administered units: as shown in table below:
Table 9

	Control	Cetrorelix acetate lyophilisate dissolved in water						Cetrorelic acetate in gluconic acid	
Group	1	2	3	4	5	6	7	8	9
Dose mg/kg	0	0.50	1.00	2.00	1.25	2.50	5.00	1.25	5.00
Conc mg/ml	1.00	1.00	1.00	2.50	2.50	2.50	2.50	2.50	2.50
Inj volume ml	2.00	0.50	1.00	2.00	0.50	1.00	2.00	0.50	2.00

Dosed for 7 days once daily, thereafter once weekly up to week 9 and twice weekly to test week 10 i.e., on day 8, 15, 22, 29, 36, 42, 50, 57, 60, 64 and 67.

Route, form, volume and infusion rate: Subcutaneous

Drug lot #, radiolabel/% purity: 9602-001/03; 9601-001/06 & 9610-001/09/[redacted]
Formulation/vehicle: As shown in table above

Observations and times:
Clinical signs: clinical symptom and local toxicity daily, mortality twice daily
Body weights: Once pretest and then weekly
Food consumption: once pretest and then weekly
Ophthalmology: no
EKG: no
Hematology: no
Clinical chemistry: no
Urinalysis: no
Organ weights: no
Gross pathology: yes
Organs weighed: -
Histopathology: yes
Toxicokinetics: Blood samples were taken on day 8, 15, 22, 29, 42, 43, 44, 45, 46, 49, 60, 64, 67 and 71 just before the compound administration.
Other: none

Results:

Clinical signs: No systemic toxicity was observed in any treatment group. It was reported that some pain reactions occurred during s.c. injection procedure in group 8 and 9 animals starting in week 9. In treated males testes were small or unpalpable. Swelling at injection site was observed in both sexes. No mortality occurred during the course of the study.
Body weights: not recorded
Food consumption: Changes were not consistent but on the whole food consumption was decreased in males and increased in females.
Ophthalmology: -
EKG: -
Hamtology: -
Clinical chemistry: -
Organ weights: -
Gross pathology: macroscopic treatment-related changes in males consisted of small testes, seminal vesicles and prostates. Females showed dose-related increased incidence of small ovaries and uteri. At the injection sites focal thickening occurred. The incidence of this was greater in animals of dose groups 6-9, which received cetrorelix in the higher concentration of 2.5 mg/ml.
Histopathology: There was a dose-related increase in incidence and severity of focal inflammatory lesions at the s.c. injection sites. These lesions consisted predominantly of focal infiltrates of macrophages. Animals treated with higher concentrated formulation had central core of necrotic and fibrillar material and it was more prevalent in animals treated with the gluconic acid reconstituted formulation.
Toxicokinetics: Plasma cetrorelix concentrations on day 8 i.e. 24 hours after drug administration were almost similar to those observed in study No. 7096060078 (Table 1).

Key study findings: As in the 4-week mouse toxicity study, treatment-related findings in the present 10-week rat toxicity study consisted of expected pharmacodynamic effects of a Gn-RH antagonist on sex organs in both treated male and female rats. Treatment caused irritation at the injection site, which was greater with concentrated formulations and was slightly more prevalent with the gluconic acid reconstituted formulation.

Overall toxicology summary: Treatment had no systemic adverse effect up to the doses used. It exerted its expected pharmacodynamic effects. There was local skin reaction at the injection site but no mortality occurred at any dose level. The skin reaction was greater for formulations with concentration greater than 1 mg/ml. As in the mouse toxicity study, toxicology parameters like organ weights, hematology and clinical chemistry, which are routinely included in these studies, were not determined. Plasma cetrorelix concentrations determined 24 hour after the last of 7 daily administration (i.e. on day 8) was not different from that reported in the 6 month repeat dose toxicity study with a cetrorelix dose of 0.5 mg/kg.

Comment: The maximum dose volume used in this study was 2 ml/kg compared to 24 ml/kg in the mouse study.

Under studies 15, 16 and 17, local tolerability after single i.m. and s.c. injections in rabbits, and after single and repeated i.m. and s.c. administration in Beagle dogs was determined.

The concentration of dosing solution in these studies was 2.5 mg/ml of peptide base. The volume of administration was 0.5 ml in rabbits and maximum of 0.2 ml/kg for the dog studies.
In rabbits, macroscopic and microscopic examination of injection site revealed no sign of severe toxicity. Common finding after both routes of administration was macrophage accumulation representing a common phagocytic reaction to foreign material.

In dogs acute inflammation seen 3 days after a single injection was of greater severity after subcutaneous than after intramuscular administration. This reaction was not seen 27 days after the injection. Once a day adminstration for 7 days was well tolerated. Both macroscopic and microscopic examination revealed no irritational changes after a post-treatment period of 8 to 9 weeks.

Summary: Both the rabbit and dog studies demonstrated that cetrorelix at concentration of 2.5 mg/ml was well tolerated at the dose levels used in contrast to the rat studies, where it was recommended that concentration higher than 1 mg/ml should not be used.

CARCINOGENICITY: Studies have not been conducted and are not required for the proposed indication.
IMMUNOTOXICOLOGY: None submitted. Cetrorelix had no sensitizing potential in the guinea pig delayed sensitization test conducted as study No. 873641 and reviewed in the original IND submission dated 10-10-1994.
REPRODUCTIVE TOXICOLOGY: Rat and rabbit segment II was reviewed under original [redacted] 3 submission dated 10-10-1994. The following studies were conducted after the original IND submission and have not been reviewed before.

Study title: **Examination of the influence on the fertility and general reproductive performance of female Sprague-Dawley rats after subcutaneous administration.**

Study No: 908100. Report No. D-20761/3000908100
Site and testing facility: ASTA Medica AG. [redacted]
GRP compliance: Yes
QA- Reports: Yes (*) No ( ):
Lot and batch numbers: Ber x 443
Protocol reviewed by Division: Yes ( ) No (*):

Methods:
- Species/strain: Rat/SD/Crl: CD.BR
Doses employed: 0, 0.01, 0.0681 and 0.464 mg/kg/day as 0.3 molar mannitol solution (i.e., 0.059, 0.402 and 2.738 ~~mg/m~~ ^{2}/day).
Administration volume: 1.0 ml/kg.
Route of Administration: S.C.
Study Design: is shown in table below:

Table 11

Group	1	2	3	4
Dose mg/kg	0	0.01	0.0681	0.464
Test substance conc (mg/ml)	0	0.01	0.0681	0.464

Fertility				
1st.Mating period (week 5)	20f	20f	20f	20f
2nd mating period (week 12)	20f	20f	20f	20f
Estrus cycle/histology	5f	5f	5f	5f

40 males used for mating remained untreated. First and second matings were one and 8 weeks after the cessation of treatment. Doses were selected based on the results of preliminary studies. In the first study (No. 903993) dose of 0.01 and 0.10 mg/kg were used for 3 weeks. There was some inhibition of ovulation and arrested estrus cycle but the animals were still fertile. In the second study (No 906118), treatment was extended to 4 weeks and dose was increased to 0.5 mg/kg b.w.. With this study design an infertile state was achieved in female rats. The ovaries and genital tract were atrophic and inactive (anestrus). In both studies the changes were reversible Based on these results the doses used in definitive study were selected.

Results:

Clinical signs: No treatment-related clinical symptoms and no influence of the treatment on behavior and external appearance were observed.

Mortality: None of the animals died prematurely.

Body weight: Body weight was increased in all dose groups during the 4 week treatment and in group 4 remained sig. higher compared to controls until second mating period.

Food consumption: There was dose-dependent increase in mid and high dose groups during the 4 week premating treatment period.

Toxicokinetics: none

(- Fertility in Males): males were untreated

In-life observations: Mating results after 5 and 12 weeks i.e. one and 8 weeks after cessation of 4 week treatment are shown in table below

Table 12

Test week 5 (first mating period/gestation phase 1)

Dose group	Animals mated	Mating Index %	Animals pregnant	Fertility Index %
1 (control)	20/20	100	20/20	100
2(0.01 mg/kg)	20/20	100	18/20	90
3(0.0681 mg/kg)	20/20	100	19/20	95
4(0.464 mg/kg)	0/20	0	-	-
Test week 11 (2nd mating period)				
1(control)	19/20	95	16/19	84
2(0.01 mg/kg)	17/20	85	14/17	82
3(0.0681 mg/kg)	19/20	95	19/19	100
4(0.464 mg/kg)	20/20	100	19/20	95

It was reported that during the second mating period, in the control group 1 animal did not mate and 3 animals did not become pregnant, though the sperm was found in the vaginal smear. In the low dose 3 animals did not mate and another 3 did not become pregnant although sperm was found in the vaginal smear. Sponsor stated that these findings may still be within the normal range for this species and not treatment-related.

Reproductive performance: In the low and mid dose groups the number of implantations and number of conceptuses/dam were reduced and these correlated with an increased incidence of resorptions and hence pre- and post-implantation loss. Reproductive performance could not be determined in high dose group since all females were infertile.

During test week 12 all findings were fully reversed. No macroscopically abnormal conceptus was reported on laprotomy on gestation day 15.

Histopathology of reproductive organs in satellite animals on necropsy: In low and mid dose satellite groups, changes in ovaries and genital tract were indicative of an inhibition of ovulation. This resulted in the persistence of tertiary and/or Graafian follicles with a shift in vaginal cycle towards the estrus period. This pattern was suggested to be indicative of suppression of LH. The absence of corpora lutea (c.l) in the low and mid dose was due to permanent estrus in these animals, which serves as a trigger for resolution of c.l. The estrogenic trigger was lacking in the anestrus high dose animals, which resulted in persistence of c.l. for several weeks. All changes were reversible by week 12.

Summary: Cetrorelix induced the expected pharmacodynamic effects on the functional and morphological state of female gonads. At high dose level of 0.464 mg/kg, complete infertility of female rats was induced. All cetrorelix-induced effects were reversible by week 12 of the experiment.

Fertility and Early Embryonic Development in Females

Study title: Examination of the influence on the early embryonic development after subcutaneous administration to Sprague-Dawley rat on days 0-7 of Pregnancy. Study No. 909573.

This study like the above study was conducted in accordance with GLP Regulation by the same laboratory and using the same batch of cetrorelix acetate.

This study was performed to gather information on the influence of cetrorelix acetate on early embryonic development after repeated s.c. administration on days 0-7, which corresponds to early pregnancy and implantation phase of the gestation period. On day 15 of pregnancy the dams were laprotomized and examined for implantation sites, resorptions n the uterus, and for condition of the conceptuses.

Dosing and dose groups were as follows:

Table 13

Group	1	2	3	4
Nominal dose (ug/kg	0	16.3	49.0	147
Nominal consc, ug/ml	0	16.3	49.0	147
Effective conc ug/ml	0	11	38	139
Effective dose(ug/kg)	0	11	38	139
Total number of pregnant females	20	20	20	20

Dose volume was 1ml/kg. 24 females were mated for each treatment group to have 20 pregnant females. Laprotomy was performed on day 15 of gestation.
Dose selection was based on the results of 2 preliminary studies. In the first study, no influence on early embryonic development was noted in the dose range between 4.64 and 46.4 ug/kg b.w. (Study No. 908256), dose levels used in rat segment 2 study (Study No. 861287). In the second preliminary study (No. 909077), dose of 147 and 464 ug/kg b.w. were used which resulted in resorption rate and thus post-implantation loss up to 100%. On this basis, nominal concentrations of 16.3, 49 and 147 ug/kg were used. Because of the adsorption of cetrorelix to glass vessels used effective dose was lower i.e. 11,38 and 139 ug/kg b.w. respectively. The mid dose is equal to maximum dose for clinical trials for the indication of in-vitro fertilization.

Results:

Clinical observations: no treatment-related clinical symptoms and no mortality was reported
Food consumption: there was no treatment effect
Body weight: not affected by treatment
Mating: 1/20, 2/20, 1/20 and 4/20 animals in groups 1-4 respectively were reported not pregnant, though sperm was found in the vaginal smear.

Reproductive/early embryonic development: data is shown in table below:

Table 14

Study group	1	2	3	4
Dosage (ug/kg	0	11	38	139
Parents				
Females with sperm	20	20	20	20
Pregnant females	19	18	19	16
Evaluated pregnant females				
Litters (values as mean/litter				
Corpora lutea	18.2	17.8	19.1	19.2
Implantation	16.5	15.8	17.3	17.6
Live fetuses	15.9	14.5	15.3	0.0
Resorptions	0.6	1.3	2.1	17.6
Weight of fetuses (g)	0.229	0.279	0.283	-

* These were effective dose. Nominal doses were 16.3, 49.0 and 147 ug/kg b.w.

The number of c.l., implantations, and conceptuses as well as uterine weights were within the normal range for animals in the low and mid dose groups. Also resorption rate and thus post-implantation loss as well as embryonic body weight was not sig influenced by treatment.

Summary: results showed that cetrorelix affected the development of the implanted conceptus in the high dose group, but did not influence the low and the mid dose groups. NOAEL was determined at an effective dose of 38 ug/kg b.w.

Embryo-fetal Development: Rat and rabbit reproductive toxicity segment II was reviewed under original [redacted] submission dated 10-10-1994.

Overall summary: In both rats and rabbits, treatment during period of organogenesis resulted in early resorptions and total implantation losses but no increase in the incidence of fetal abnormalities.

In clinical studies overall, 235 pregnancies have been reported. From these 235 pregnancies, 46 miscarriages and 9 ectopic pregnancies occurred. In addition two stillbirths occurred. There was one neonate (of twins) with major congenital anomaly, the newborn died after 4 days due to

anencephaly. The other twin was born healthy. One minor congenital abnormality was reported in a new born (pyelocaliceal junction syndrome). No other malformations were reported.

Prenatal and postnatal development, including maternal function: not submitted

Study title: Examination of the influence on fertility and general reproductive performance of male Sprague Dawley rats after subcutaneous administration.

Study No: 913320
Site and testing facility: Same as for the above segment 1 study
GLP compliance: Yes
QA-reports: Yes
Lot and batch Nos.: Same as used in segment 1 study
Protocol reviewed by Division: No

Methods:

Species/strain: Rat/Sprague-Dawley
Doses employed: 0, 0.01, 0.0681 and 0.464 mg/kg/day (i.e., 0.059, 0.402 and 2.738 mg/m^2/day)
Route of administration: S.C.
Study design: Dosing and dose groups were as given in table below:

Table 15

Group	1	2	3	4
Dose ug/kg	0	10.0	68.1	464
Concentration ug/ml	0	10.0	68.1	464
Animal #s Males (for fertility)	20	20	20	20
For sperm evaluation & histopatholgy	10	10	10	10
Females (untreated)	40	40	40	40

Dose volume was 1ml/kg. Males were treated for 4 weeks before mating with untreated females. Study was conducted in 2 mating trials (recovery period was 1 and 14 weeks i.e. test weeks 5 and 19 respectively).
Doses were selected based on results of preliminary studies. In the first study (No. 903982) dose of 10 and 100 ug/kg were used for 4 weeks while in the second study (No.910091) dose was increased to 464 ug/kg for 4 weeks. At test week 5, a dose-dependent depression of gonadal function and sexual behavior was observed at dose levels of approximately 50 ug/kg (see table 16 below). Recovery was also dose-dependent. Drug influence was no longer present in the high dose group after 14 weeks of recovery.

Parameters and endpoint evaluated: Sperm evaluation and reproductive organs weight & histopathology
Statistical evaluations: Means + s.d. were calculated. Dunnett-Test, Fisher –Exact-Test as well as Trend-Test were used.

Results:
Clinical signs: No treatment-related clinical symptoms or behavioral changes were observed.
Mortality: No rat died prematurely
Body weight: No influence of cetrorelix on body weight was noted.

Food consumption: Treatment did not affect food consumption.
Treatment effect on fertility: Results of first mating trial (test week 5) and second mating trial (test week 19) is shown in table below:

Table 16

First mating trial

Group	1 (control)	2 (10 ug/kg)	3 (68.1 ug/kg)	464 ug/kg
Animals paired	20	20	20	20
Copulation index	19/20 (95%)	19/20 (95%)	18/20 (90%)	3/20 (15%)
Fertility index	19/19 (100%)	18/19 (95%)	10/18 (56%)	0/3 (0%)

Second mating trial

Animals paired	20	20	20	20
Copulation index	19/20 (95%)	18/20 (90%)	20/20 (100%)	18/20 (90%)
Fertility index	17/17 (100%)	18/18 (100%)	19/20 (95%0	15/18 (83%)

Sponsor stated that fertility index of high dose males was on the lower limit of physiological range and therefore represented almost full recovery of fertility.

Reproductive performance:

First mating trial, test week 5: None of the reproductive parameters determinable in untreated females was influenced by the treatment of males with the low dose. A slight decrease in the number of implantations and an increase in pre-implantation loss was reported for mid dose group. There were no pregnancies in females mated to HD males.

Second mating trial, test week 19: After recovery time of 14 weeks, all reproductive parameters at all dose levels had recovered. One female fetus in group 3 had a malformation compatible with spina bifida.

Sperm evaluation:

Test week 5: No changes in sperm parameters in the low dose group. There was a decrease in viable sperm as well as a decrease in epididymal sperm head count in mid dose males. High dose caused a marked decrease of viable sperm as well as of spermatid and sperm head count.
No significant morphological findings were reported during sperm evaluation in any of the dose groups.

Test week 19: All parameters were recovered after 14 weeks recovery period. Epididymal sperm head count was slightly below the control value.

Necropsy and histopathology of reproductive organs:
Necropsy- Test week 5: Testes and epididymides in all high dose and one mid dose animals were small. Prostate and seminal vesicles were reduced in mid and high dose groups in a dose-dependent manner.

Test week 19: No treatment-related necropsy findings were reported after 14 weeks recovery.

Male reproductive organ weights:

Test week 5: Although absolute weight of gonads and prostate/seminal vesicles was reduced with dose-dependent severity in the mid and high dose groups, relative gonad weights was reduced only in high dose group males.
Test week 19: All changes had returned to normal.

Histopathology:

Test week 5: There was a marked atrophy of seminiferous tubules in high dose animals characterized by a virtual block of spermatogenesis after the spermatocyte stages. Spermatides were virtually absent leading to a total absence of spermatozoa in the epididymides. There was moderate to marked atrophy of the Leydig cells. Only one mid dose animal showed these lesions.

The prostate and seminal vesicles had a mild to moderate atrophy in the mid dose and marked to severe atrophy in the high dose group.

Test week 19: There was complete reversibility of reproductive effects.

Toxicokinetics: None submitted

Summary and Evaluation: Expected effect of a GnRH antagonist were observed on male fertility. All observed effects on fertility were fully reversible during the recovery period.

GENETIC TOXICOLOGY: The following genotoxicity studies were reviewed under sponsor's original [redacted] submission dated 10-10-1994.

1. Salmonella typhimurium reverse mutation assay on D-20761. Study No. 876960

2. In vitro assessment of the clastogenic activity of D-20761, cetrorelix in cultured human peripheral lymphocytes. Study No. 876971.

3. Mammalian cell (V79) gene mutation test on D-20761. Study No. 876993

4. D-20761- Mouse micronucleus test (Single intraperitoneal administration). Study No. 876982

All these studies were conducted in accordance with GLP Regulations following OECD guidelines. All studies were negative.

Results of the following studies have not been submitted or reviewed previously.

Study title: Addendum No.1 to the report: In vitro assessment of the clastogenic activity of D-20761, cetrorelix in cultured human peripheral lymphocytes (date of report; April 22, 1993)- re-evaluation on polyploidy. Study No 876971

The coded slides of the study conducted in 1992 (study No. 876971) were re-examined for polyploidy in 1996. Sponsor however, did not provide any information as to why the slides were re-evaluated.

For each treatment and culture 1000 metaphases were evaluated, which was only possible for doses up to 150 ug/ml with metabolic activation and for 37.5 and 75 ug/ml without metabolic activation.
The re-evaluation of all slides revealed no increase in the incidence of polyploidy for cetrorelix.

Study title: D-20761- In vitro mammalian cytogenetic test in CHL Chinese hamster fibroblasts ( ) to get information on the polyploidy-inducing potency of the test substance.

Study No.: 3000914545
Study type: induction of polyploidy
Volume and page No: 30/027
Conducting laboratory: ASTA Medica AG, Halle/Westfalen
Date of study initiation/completion: 3-10-1997/3-13-1997
GLP compliance: yes
QA repots: yes
Drug lot No.:Ber x 836
Study endpoint: Induction of numerical chromosomal aberrations (polyploidy)

Methodology:

Strains/cell line: CHL/IU No,: CRL 1935, batch F-9374 (Chinese hamster lung fibroblasts)

Basis of dose selection: doses were based on the results of the in vitro cytogenetic tests in human lymphocytes (ASTA report No. 3000876971), in V79 Chinese hamster fibroblasts (ASTA Report No. 3000912014) and in CHL cells (ASTA Report NO. 3000912778).

Vehicle: DMSO
Negative controls: DMSO
Positive controls:Ethyl methane sulphonate & cyclophosphamide.
Note: it was stated that a standard positive control substance in respect to numerical chromosomal aberrations is not available.

Exposure conditions:

Incubation and sampling time: First experiment: 18 hours (without S-9 mix)
3 hours (with S-9 mix)
Sampling time 18 hours for both
Second experiment: 6 hours each (without and with S-9 mix)

Doses used in definitive study: First experiment: 6.25 – 400 ug/ml without S-9 mix and
25 –400 ug/ml with S-9 mix
Second experiment: same concentrations as in the first experiment for both 24 and 48 hours sampling times

Study design:
No of slides/plates/replicates: two parallel cultures

Counting method: 400 metaphases per group
Cytotoxic endpoints: effect on mitotic index (% of cells in mitosis)
Genetic toxicity endpoints: hyperploid cells
Statistical methods:
Criteria for positive results: If the test substance produced neither a statistically significant, biologically relevant, and reproducible positive response at any test point nor a concentration related statistically significant and biologically relevant increase in numerical chromosomal aberrations compared to the respective negative control group, it is considered non-suspicious to have a polyploidy-inducing potency in this system.

Results: Values for pH and osmolality were considered acceptable for in vitro tests.

Table 17
Effect of treatment on polyploidy is shown in table below:

Treatment		**# of metaphases analyzed**		**Mitotic index Mean of 2 cultures (%)**		**Number of polyploidy**		**% polyploidy**	
First experiment 18 hr sampling		-	+	-	+	-	+	-	+
Culture medium		400	400	38	65	7	12	1.8	3.0
Culture medium with 1 % DMSO		400	400	38	34	11	8	2.8	2.0
Cetrorelix (ug/ml)									
-	+								
6.25	25	400	400	47	31	9	9	2.3	2.3
12.5	50	400	400	51	32	12	4	3.0	1.0
25	100	400	400	38	33	15	20*	3.8	5.0
50	200	400	287	34	26	13	4	3.3	1.4
100	400	+++	249	-	12	-	13*	-	5.2
Second experiment 24 hour sampling									
Culture medium		400	400	36	69	4	4	1.0	1.0
Culture medium with 1% DMSO		400	400	48	47	4	9	1.0	2.3
-	+								
12.5	25	400	400	62	70	4	12	1.0	3.0
25	50	400	400	54	56	6	14	1.5	3.5
50	100	400	400	35	32	6	24	1.5	6.0
100	200	400	272	11	6	38 **	15	9.8	5.5
200	400	400	+++	12	-	56 **	-	14.0	-
Second experiment 48 hour sampling									
Culture medium		400	400	19	48	6	6	1.5	1.5
Culture medium with 1% DMSO		400	400	21	45	4	5	1.0	1.3

-	+								
12.5	25	400	400	38	40	9	15	2.3	3.8
25	50	400	400	22	35	4	8	1.0	2.0
50	100	400	400	21	35	10	30**	2.5	7.5
100	200	200	400	26	31	47**	23**	11.8	5.8
200	400	400	400	32	26	54**	20**	13.5	5.0

- and + = without and with metabolic activation

Ethyl methane sulphonate (EMS) and cyclophosphamide (CP) were used at concentrations of 400 ug/ml and 2 ug/ml respectively. Mitotic index for EMS ranged from 24 to 42; number of polyploidy from 6 to 8; and % of polyploidy from 1.5 to 2.0. Respective values for CP were 49-60; 5; and 1.3. The mean number of chromosomes in normal cells of the cell line used is 25.

Results: The mitotic index was reduced by more than 50% at cetrorelix concentrations of 100 and 200 ug/ml without metabolic activation and 200 and 400 ug/ml in the presence of metabolic activation. Precipitation was observed at 400 ug/ml.

A treatment-related increase in polyploidy was seen without metabolic activation at 100 and 200 ug/ml. In experiments with exogenous metabolic activation an increase in polypoidy was present at 100, 200 and 400 ug/ml. The standard positive control substances in respect to structural chromosomal aberrations, ethyl methane sulphonate and cyclophosphamide, did not increase the number of polyploid cells.

Study validity: Study as conducted seems valid.
Study outcome: Cetrorelix induced polyploidy both without and with exogenous metabolic activation.

Summary: Under the conditions of the test, solutions of cetrorelix with and without a metabolic activation system induced numerical chromosomal aberrations (polyploidy) in Chinese hamster lung fibroblasts (from ATCC) in vitro.

Study Title: D-20761: In vitro mammalian cytogenetic test in V79 Chinese hamster fibroblasts to get information on the polyploidy-inducing potency of the test substance.
Study No: 912014

Study Type: Assessment of the potential to induce numerical chromosomal aberrations in V79 cells
Amendment #, Volume # and Page #: volume 35 of 162/ p.090
Conducting Laboratory: ASTA Medica AG, Institute of Technology, KantstraBe 2, D-33790 Halle/Westfalen
Date of Study Initiation/completion: 4-17-1996/4-26-1996
GLP Compliance: Yes
QA- Reports: Yes (*) No ( ):
Drug Lot Number: Ber x 443
Study Endpoint: Numerical chromosomal aberration

Methodology:

- Strains/Species/Cell line: V79 A2/ Chinese hamster lung fibroblasts
- Dose Selection Criteria: According to results of preliminary trial with evaluation of pH value, osmolality, solubility, cell number, plating efficiency, mitotic index.

- Basis of dose selection: The highest dose was chosen to get at least 50% reduction of the mitotic index compared to negative controls or to get other signs of distinct cytotoxicity.
- Range finding studies: yes
- Test Agent Stability: Was reported to be stable up to 12-31-1996
- Metabolic Activation System: yes

- **Controls:**
  - Vehicle: DMSO
  - Negative Controls: DMSO batch No. 02781
- Positive Controls: Ethyl methane sulphonate (EMS batch No. 074 H 1107
- Cyclophosphamide batch No. 065038

- **Exposure Conditions:** First experiment
- 18 and 28 hours (without exogenous metabolic activation)
- 3 hours (with exogenous metabolic activation)
- Second experiment
- 6 hours without or with exogenous metabolic activation
-
- Doses used in definitive study:
- 6.25 – 200 ug/ml without metabolic activation and
- 25 – 400 ug/ml with metabolic activation

Study design: Preparation of chromosomes done 18 and 28 hours after the start of treatment in the first experiment and 24 and 48 hours in the second experiment

- Analysis:
- No. slides/plates/replicates/animals analyzed: 2 for each sampling time and treatment
- Counting method: 400 metaphases per group counted
- Cytotoxic endpoints: Mitotic index reduction
- Genetic toxicity endpoints/results: Increase in polyploidy
- Statistical methods: Chi-square test
- Other:
-
- Criteria for Positive Results: If a test substance produced neither a statistically significant, biologically relevant and reproducible positive response at any test point nor a concentration related statistically significant and biologically relevant increase in numerical chromosomal aberrations compared to the respective negative controls, it is considered to have no polypoidy-inducing potency in this system.

Results:

Preliminary trial:

pH and osmolality of solutions for chromosomal preparations and for the determination of cell number and plating efficiency ranged from 7.97 to 8.20 for pH and 0.324 to 0.518 for osmolality were considered acceptable.
Solubility: Cetrorelix was soluble up to 100 ug/ml. A beginning ppt was noted at 200 ug/ml.
Cell number: Cell number was significantly decreased above 50 ug/ml. Morphological changes were observed at 50 ug/ml and above with severity ranging from slight at 50 ug/ml to severe at 400 ug/ml.

Plating efficiency: was significantly decreased above 100 ug/ml.
Mitotic index: was significantly decreased above 50 ug/ml.
Note: All the above criteria were determined in cultures without S-9 mix.

Main trials:
Numerical chromosomal aberrations: No increase in polyploidy was observed in drug treated or in negative and positive controls except for 3/40 cetrorelix treated groups (group Nos. 15, 104 and 126), which had statistically significant increases in the number of polyploidy cells but these were considered incidental because the increases were small, not concentration related and noted only in one of the two parallel cultures.

Cytotoxicity: At the 18 hour sampling time a reduction of mitotic index of 50% or higher at concentrations of 12.5, 25 and 50 ug/ml cetrorelix without S-9 compared to cells treated with culture medium containing 1% DMSO was observed. At 100 ug/ml, evaluation was not possible due to high cytotoxicity. At 28 hours evaluation was not possible due to high cytotoxicity at 50 and 100 ug/ml.

No decreases in the mitotic index were observed in the second experiment in tests without S-9 activation as well as in both expts in tests with exogenous metabolic activation. However, a clear precipitation of the test substance was observed at concentration of 400 ug/ml.

Note: Sponsor has not explained the discrepancies with regards to mitotic index in 2 experiments.

- Study Validity: seems valid
- Study Outcome: Based on the criteria for a positive response, data suggested that cetrorelix did not induce polyploidy.

Summary: Sponsor concluded that under the conditions of the study, cetrorelix without as well as with a metabolic activation did not induce numerical chromosomal aberrations in V79 Chinese hamster fibroblast in vitro.
However, statistically significant increase in the number of polyploidy cells occurred in 3/40 cetrorelix treated groups.

Study title: D-20761: In vitro mammalian cytogenetic test in CHL Chinese hamster fibroblasts to get information on the polyploidy-inducing potency of the test substance.

Study No.: 912778
Study type:

Volume and page #: 35 of 162/145
Conducting laboratory: same as for the above study
Date of study initiation/completion:GLP Compliance:8-20-1996/9-4-1996
QA-report: yes
Drug lot #: Ber x 443
Study endpoint: numerical chromosomal aberrations

Methodology:

Strain/species/cell line: CHL/IU Chinese hamster lung fibroblasts
Dose selection criteria, metabolic activation system, negative and positive controls, exposure conditions, incubation and sampling times, cytotoxic and genotoxic endpoints and criteria for positive results were exactly the same as in the above study using V79 A2 cell strain. Thus the only difference between study No. 912014 and this study was the cell strain used.

Results:

Preliminary trials: Determination of pH and osmolality, cell number, plating efficiency and mitotic index determined in culture medium without S9- mix gave essentially similar results as in study No. 912014 conducted under similar experimental conditions. Solubility was clear up to 100 ug/ml. Precipitate began to appear at 200 ug/ml.

Main trials: As shown in table below, in contrast to previous study (study NO. 912014) treatment-related polyploidy was reported in this study:
Expt. 1

Table 18

			18 hour sampling time						28 hour sampling time			
Treatment	Number of metaphases analyzed		Mitotic index (X of 2 cultures		# of polyploidy		% of polyploidy		Number of metaphases analyzed		Mitotic index (x of 2 cultures)		# of polyploidy		% of polyploidy	
	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+
Culture medium	400	400	12.5	21	5	4	1.3	1.0	400	400	32	40	6	7	1.5	1.8
Culture medium C 1% DMSO	400	200	38.5	29	6	4	1.5	2.0	400	346	31.5	13	8	11	2.0	3.2
6.25 ug/ml 25 ug/ml	200	400	32	37.5	2	9	1.0	2.3	400	400	21.5	39.5	6	13	1.5	3.3
12.5 ug/ml 50 ug/ml	400	400	17.5	32.0	3	15	0.8	3.8	365	400	20.0	29.5	6	6	1.6	1.5
25 ug/ml 100 ug/ml	400	400	25	35.5	3	25*	0.8	6.3	400	400	26	35.0	6	7	1.5	1.8
50 ug/ml 200 ug/ml[P]	400	400	28.5	29.0	19*	39**	4.8	9.8	400	400	4.5*	46.5	33*	12	8.3	3.0
100 ug/ml 400 ug/ml[P]	+++	400	-	25.0	-	41**	-	10.3	+++	400	-	31.5	-	25	-	6.3

P=precipitation *= p< 0.05 **=p<0.01 +++= no evaluation possible

Results showed that there was a significant increase in polyploidy at 18 and 28 hours sampling without metabolic activation at a dose of 50 ug/ml. Because of toxicity metaphases at higher dose of 100 ug/ml were not evaluable. With exogenous metabolic activation, there was a statistically significant increase in polyploidy at 100, 200 and 400 ug/ml at the 18 hour sampling time. Cytotoxicity was observed at the 28 hour sampling

In the second experiment (6 hour pulse treatment) essentially similar results were obtained. Polyploidy was increased without S9-mix at 100 ug/ml at 24 hour sampling time (200 ug/ml was not evaluable because of toxicity) and at 100 and 200 ug/ml at the 48 hour sampling time. With S9-mix activation, increases were present at 100, 200 and 400 ug/ml (24 hour sampling time) and at 100 and 200 ug/ml at 48 hour sampling time. 400ug/ml were not evaluable because of toxicity.
Positive control did not increase polyploidy.
Significant toxicity was observed at a concentration of 50 ug/ml at 28-hour sampling time without S9-mix activation. There was no significant decreased in mitotic index at any concentration in the presence of exogenous metabolic activation.

Study validity: Study seems valid
Study outcome: Cetrorelix increased polyploidy in CHL fibroblasts.

Micronucleus assay in cytokinesis-blocked Chinese hamster lung (CHL) cells with D-20761 (Cetrorelix)

The objective of the study was to evaluate the ability of cetrorelix to induce micronuclei in cytokinesis-blocked CHL cells under conditions where a significant increase in polyploidy is induced. It was stated that since cetrorelix induced polyploidy in CHL cells both in the absence and in the presence of metabolic activation, the present study was performed only in the absence of metabolic activation.

Study title: **Micronucleus assay in cytokinesis-blocked Chinese hamster lung (CHL) cells with D-20761 (Cetrorelix).**

Study No.: 901833
Amendment #, volume # and page #: 36 of 162/076
Conducting laboratory: [redacted]
Date of study initiation/completion: 9-9-1997/4-28-1998
GLP compliance: yes
QA-report: yes
Drug lot #: not given
Study endpoint: a. induction of polyploidy; b. micronuclei and c. binucleate cells

Methodology:

Strains/species/cell line: Chinese hamster lung cells

Dose selection criteria:
Basis of dose selection: Toxicity assays. Whenever possible, the high dose was selected to result in at least 50% depression of cell density, mitotic index or binucleated cell index as compared to that recorded for the concurrent set of controls cultures.
Range finding studies: yes
Test agent stability: stated to be stable under the condition used
Metabolic activation system: none

Controls:

Vehicle: DMSO
Negative controls: DMSO

Positive controls: a. 0.1 ug/ml colchicine in distilled water
b. 1 and 2 ug/ml colchicine in distilled water
c. 3 ug/ml cytochlasin B in distilled water

Exposure conditions:

Incubation and sampling time: a. 4 hr treatment + 22 hr recovery
b. 4 hr treatment + 26 hr recovery
c. 4 hr treatment + 22 hr recovery

Doses used in definitive studies: 12.5 – 100 ug/ml, dissolved in DMSO

Study design: Five concentrations of cetrorelix plus concurrent negative and positive controls were used in the polyploidy, micronucleus and binucleated cell assays. Treatment started at 16 – 24 hr of culture. Culture termination for polyploidy assay and assay for the induction of binucleated cells was at 1.5 cell cycles after treatment initiation, and for micronuleus assay at 1.7 cell cycles.

Analysis:
No. slides/plates/replicates/animals analyzed: Two independent experiments for a and b and one for c. Two replicate cultures per dose group for a, b, and c.
Counting method: a. 200 metaphase cells/culture (400/dose group)
b. 1000 binucleated cells/culture (2000/dose group)
c. 1000 cells/culture (2000/dose group)
Cytotoxic endpoints: depression of cell density
Genetic toxicity endpoints/results: induction of polyploidy, micronucleated binucleated cells and binucleated cells.
Statistical methods: For polyploid metaphase cells and micronucleated binucleated cells One-tailed Cochran-Armitage trend test and one-tailed Fisher's exact test comparison for each treatment group against concurrent negative control group. Cell density, the MI and BCI analyzed by ANOVA.

Results:

Cytotoxic effects:
a. 28 % depression in cell density at 100 ug/ml
b. 28% depression in cell density at 100 ug/ml
c. 41% depression in cell density at 100 ug/ml

Doses above 100 ug/ml could not be used because of toxicity as it destroyed cell morphology.

Genotoxic effects:
a. Induced a dose–dependent increase in polyploidy index at 50, 75 and 100 ug/ml. Treatment caused a significant dose-dependent decrease in mitotic index.
b. negative for induction of micronucleated binucleate cells at 12.5, 25, 50, 75 and 100 ug/ml.

c. Positive for induction of binucleated cells at 50, 75 and 100 ug/ml. All doses were equally effective. There was no change in replicative index.

Effects of positive controls:

a. Statistically significant increase in cells with polyploidy
b. Statistically significant increase in micronucleated binucleate cells
c. Statistically significant increase in binucleated cells.

Study validity: study as conducted seems valid.
Study outcome: Cetrorelix induced polyploidy.

Summary: Under conditions where cetrorelix induced an increase in polyploidy, it did not increase micronuclei in CHL cells when tested in the absence of metabolic activation for 4 hours. Since cetrorelix induced a statistically significant increase in the frequency of binuleated cells, sponsor suggested that this process was responsible for the increase in polyploidy.

In vivo chromosomal aberration test on NS75A with bone marrow cells of mice. Toxicokinetic analysis in an in vivo chromosomal aberration test on NS75A with bone marrow cells in mice

Since induction of numerical aberration (polyploidy) by cetrorelix was observed in vitro chromosomal aberration test with CHL/IU cells but not with human lymphocytes nor with V79 cells, this in vivo chromosomal aberration test was conducted to elucidate if cetrorelix induces chromosomal aberrations, especially numerical one, with bone marrow cells of mice.

Study No. 914128
Study type: In vivo chromosomal aberration test with bone marrow cells of mice
Volume/page #: 35 of 162/200
Conducting laboratory: [redacted]

Date of study initiation/completion: 10-30-1997
GLP Compliance: yes
QA-report. yes
Drug lot #: Ber x 922, Positive (mitomycin) lot # 051AEG, Vehicle (distilled water and physiological saline)

Study endpoint: Chromosomal damage

Methodology:

Species/strain: Jcl:ICR mice
Sex: Male and females
Age: 7-weeks old
Body weight: Males 36- 43 g; females 27-35 g

Dose selection criteria: The high dose was selected based on physical limit of preparing a cetrorelix solution. At concentrations of more than 1 mg/ml cetrorelix partially coagulates in solution. For this high viscosity solution it was difficult to prepare concentrations more than 10 mg/ml.

Controls:

Vehicle: 0.3 M mannitol solution

Positive control: Mitomycin C

Exposure conditions: Single administration of 20 ml/kg for the vehicle and test substance groups and a single administration of 10 ml/kg for the positive control groups (2 mg/kg).

Doses used: 20, 63 and 200 mg/kg of cetrorelix

Study design: as shown in table below:

Table 19

Group	Substance	Dose mg/kg	Concentration mg/ml	Volume ml/kg	No. of mice	Sampling time
Chromosomal aberation test						
A	Vehicle control	0	0	20	5	18 hr
B	Cetrorelix	20	1	20	5	18 hr
C	Cetrorelix	63	3.15	20	5	18 hr
D	Cetrorelix	200	10	20	5	18 hr
E	Mitomycin C	2	0.2	10	5	18 hr
F	Vehicle control	0	0	20	5	42 hr
G	Cetrorelix	20	1	20	5	42 hr
H	Cetrorelix	63	3.15	20	5	42 hr
I	Cetrorelix	200	10	20	5	42 hr
	Exposure assessment					
J	Vehicle control	0	0	20	4	1 hr
K	Cetrorelix	20	1	20	4	1 hr
L	Cetrrorelix	63	3.15	20	4	1 hr
M	Cetrorelix	200	10	20	4	1 hr
N	Vehicle control	0	0	20	4	4 hr
O	Cetrorelix	20	1	20	4	4 hr
P	Cetrorelix	63	3.15	20	4	4 hr
O	Cetrorelix	200	10	20	4	4 hr

Vehicle was 0.3M mannitol Route of administration was ip.

Preparation of specimen: Prior to sacrifice mice were administered intraperitonially with spindle inhibitor (1mg/kg colchicine) for one hour to accumulate cells in metaphase. Bone marrow was flushed into MEM containing inactivated fetal bovine serum. Tubes were centrifuged and supernatant discarded. Cells were suspended in hypotonic KCl, slides were air dried and stained. Chromosomal damages were recorded.

For chromosomal aberration, 50 metaphases/slide (100/animal, 500/group) were analyzed.
Polyploidy was assessed by scoring 100 metaphases/slide i.e 1000/group.
Mitotic index (MI) was determined as a measure of bone marrow cytotoxicity by counting 500 cells/slide i.e. 5000 cells/group.

Results: Results of chromosomal aberration test are shown in table below:

Table 20

Male

Substance	Dose Mg/kg	Sampling Time(h)	MI [a](%)	TAG (%)	TA (%)	Polyploidy[b] (%)
Vehicle[c]	0	18	1.7	7(1.4)	1 (0.2)	1 (0.1)
Cetrorelix	20	18	2.2	7 (1.4)	3 (0.6)	1 (0.1)
	63	18	2.3	7 (1.4)	1 (0.2)	1 (0.1)
	200	18	2.1	7 (1.4)	4 (0.8)	2 (0.2)
Mitomycin C	2	18	1.4	187 (37.4)	162 (32.4)**	1 (0.1)
Vehicle	0	42	1.7	5 (1.0)	2 (0.4)	1 (0.1)
Cetrorelix	20	42	2.1	5 (1.0)	2 (0.4)	1 (0.1)
	63	42	2.7	6 (1.2)	4 (0.8)	1 (0.1)
	200	42	2.8	3 (0.6)	1 (0.2)	1 (0.1)
Female						
Vehicle	0	18	2.4	1 (0.2)	1 (0.2)	2 (0.2)
Cetrorelix	20	18	2.8	3 (0.6)	1 (0.2)	3 (0.3)
	63	18	2.9	3 (0.6)	2 (0.4)	3 (0.3)
	200	18	3.0	3 (0.6)	2 (0.4)	1 (0.1)
Mitomycin C	2	18	1.2	147 (29.4)	122 (24.4)**	5 (0.5)
Vehicle	0	42	2.4	3 (0.6)	2 (0.4)	2 (0.2)
Cetrorelix	20	42	2.6	3 (0.6)	2 (0.4)	2 (0.2)
	63	42	2.7	1 (0.2)	1 (0.2)	2 (0.2)
	200	42	3.6	3 (0.6)	2 (0.4)	2 (0.2)
Hostorical control[d]				135 (2.3)	20 (0.3)	13 (0.1)[e]

** Significantly different $p<0.01$ a=mitotic index b=polyploid cells/1000 metaphase cells/dose
c=0.3 M mannitol d=Historical control data in male mice collected during 15 year before this study (total cells 5750)
e=polyploid cells/12500 metaphase cells. TAG and TA= total number of aberrant cells including and excluding gaps, respectively.

Plasma drug concentrations given in table below shows that all treatment groups had systemic exposure to cetrorelix

Table 21

Dose (mg/kg)	Sex	Plasma concentration (ng/ml)	
		1 h after administration	4 h after administration
20	Male	9961 + 1192	8511 +1210
	Female	7295 + 636	3035 + 2002
63	Male	3349 + 3009	6685 + 1237
	Female	2275 + 1123	3228 + 848
200	Male	5491 + 266	10005 + 2276
	Female	3691 + 1066	5915 + 921

Each value is mean + s.d. of 3 mice. Considering C_{max} of 6.42 ng/ml in humans with the proposed therapeutic dose of 0.25 and 28.5 ng/ml with a dose of 3 mg/person/day, the systemic exposure levels observed in this study represent a very high multiples of human exposure with a therapeutic dose.

Conclusion: The in-vivo chromosomal aberration test was negative.

Summary: Cetrorelix did not show mutagenicity in the in-vitro Ames, HPRT and chromosomal aberration tests or in vivo mouse chromosomal and micronucleus tests. Cetrorelix however, induced polyploidy in CHL fibroblasts in a dose-related and reproducible manner, both in the absence and presence of S-9 activation system. Such findings have not been reported with any other GnRH analog.

SPECIAL TOXICOLOGY STUDIES: None

OVERALL SUMMARY AND EVALUATION: This summary covers studies conducted under ——— as well as studies reviewed under this NDA.

Introduction: Cetrorelix, a LH-RH antagonist has a highly modified LH-RH sequence. It is comprised of 10 amino acids, 5 out of them in non-natural D-configuration.

Cetrorelix is indicated for the prevention of premature ovulation in patients undergoing controlled ovarian stimulation.

Cetrorelic competes with natural LHRH for binding to membrane receptors on pituitary cells and thus controls release of LH and FSH in a dose-dependent manner. The onset of suppression in humans is immediate and is maintained by continuous treatment. In the rat cetrorelix administration at low doses increased testosterone in male and estradiol in female rats.

Safety pharmacology studies demonstrated that cetrorelix has no neurological, cardiovascular, pulmonary or gastrointestinal adverse effects.

Cetrorelix threshold level for testosterone suppression was 1 ng/ml for the rat and 2 ng/ml for the dog.

TK parameters determined in the 6-month rat and dog studies with a maximum dose of 0.5 mg/kg/day showed AUC values at least 40 times in rats and 70 times in the dog as compared to human systemic exposure with the proposed therapeutic dose of 0.25 mg/day i.e. 5 ug/kg/day. However, the multiples of human systemic exposure will be below 4 for the rat and below 7 for the dog when compared with single 3 mg dose administered in humans.

In the toxicity studies in rats and dogs, pharmacodynamic effects of treatment were observed but very little systemic toxicity. At the injection site, treatment-related changes consisted of macrophage infiltrates, focal fibrosis, focal edema and focal necrosis. These changes showed a dose-related increase in incidence and severity.

In both the 4-week and 13-week rat toxicity studies the low dose of cetrorelix increased testosterone in male and estradiol in female rats. Hepatic enzymes were increased in both studies. In the 26-week rat toxicity study, there was atrophy of the adrenal gland zona reticularis in all treated groups. Bone density was decreased in both the 13 and 26 week toxicity studies. Bone density however, was not affected in treated dogs.

In the MTD-finding studies in rats, it was reported that an agranuloma-like reaction occurred at the injection site and subsequent injections of cetrorelix into the vascularized granuloma tissue effectively resulted in a quasi-intravenous injection. This quasi-intravenous injection led to mast cell degranulation with subsequent clinical symptoms of hypotensive shock and eventually death. Sponsor attributed the mortality to release of serotonin and suggested that it is irrelevant to humans because human mast cells do not contain serotonin. However, the submitted data do not unequivocally demonstrate this assumption, although iv injections into 12 human subjects produced no adverse effects.

Cetrorelix had the expected effects in reproductive toxicity studies and these were reversible on cessation of treatment. No teratogenic effects were reported.

Cetrorelix was reported negative in the Ames test, showed no clastogenic activity in cultured human peripheral lymphocytes, was negative in the mammalian cell (V79) gene mutation test and in the mouse micronucleus test. However, cetrorelix was shown to have polyploidy-inducing potency in the in-vitro cytogenetic test using Chinese hamster lung fibroblasts.
The in-vivo chromosomal aberration test using bone marrow cells in mice was negative even when cetrorelix plasma concentrations in mice were at least 200 times higher compared to plasma concentration in humans with a single 3 mg dose of cetrorelix.

The significance of polyploidy in humans seems to be unknown as apparently it is for the induction of gaps in the chromosomal aberration assay. Thus the consideration of polyploidy in the assessment of a drug's genotoxic potential and human risk assessment is uncertain (consultation with Dr. D.Benz, HFD 901).

Safety Evaluation: Preclinical safety evaluation suggests that cetrorelix is safe for the short-term use for in vitro controlled ovarian stimulation.

Clinical Relevance of Safety Issues: Few if any for short term use. Care should be taken to minimize accidental iv administration. Longer use may reduce bone density, increase serum transaminases and cause adrenal atrophy. The relevance of cetrorelix-induced polyploidy is unknown.

Other Clinically Relevant Issues: none in relation to present indication. Cetrorelix in rats at low sc dose acted as a GnRH agonist as it increased testosterone in male and estradiol in female rats. This will have bearing for a future prostate carcinoma indication and should be carefully examined in future clinical trials with early blood sampling.

Conclusions: Pharmacology considers that Cetrorelix is safe for the proposed indication.

Communication Review:

- Labeling Review (NDA): Sponsor was sent specific labeling comments.
- Investigator's Brochure/Informed consent review (IND):

RECOMMENDATIONS: Pharmacology recommends approved of NDA 21-197 for the proposed indication.

Internal comments:
External Recommendations (to sponsor):
Draft letter Content for Sponsor:

Future development or NDA issues:
Reviewer signature/team leader signature [Concurrence/Non-concurrence]

/S/ /S/ 7/7

cc:
HFD-580
HFD-580/A.Jordan/J. Willett/K.L.Raheja/J.Best
Draft date (# of drafts):
Memorandum of Non-concurrence (if appropriate, attached):
Addendum to review (if necessary): none
Appendix/attachments: Copy of the review of ——— original submission dated 10-10-1994. review

APPEARS THIS WAY
ON ORIGINAL

NDA 21-197
Cetrotide™ (cetrorelix acetate for injection)
ASTA Medica, Inc.

There was no Carcininogenicity review done for this Drug product.

NDA 21-197
Cetrotide™ (cetrorelix acetate for injection)
ASTA Medica, Inc.

This Drug Product was not presented to the CAC/Executive Committee for Review.