CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-732

Pharmacology Review(s)

DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-732
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	12/12/03
DRUG NAME:	VantasTM (Histrelin acetate subdermal implant)
INDICATION:	Palliative treatment of advanced prostate cancer
SPONSOR:	Valera Pharmaceuticals, Inc. Cranbury, NJ
DOCUMENTS REVIEWED:	Volumes 1.22 – 1.41
REVIEW DIVISION:	Division of Reproductive & Urologic Drug Products) (HFD-580)
PHARM/TOX REVIEWER:	Krishan L. Raheja, D.V.M., Ph.D.
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Date of review submission to Division File System (DFS): 9-13-04

TABLE OF CONTENTS

EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Pharmacology recommends approval of NDA 21-732 for palliative treatment of advanced prostate cancer.

B. Recommendation for nonclinical studies: Based on reference to FDA's previously approved NDA 19-836 for Histrelin subcutaneous injection for the treatment of central precocious puberty and the toxicity studies now submitted under present NDA 21-732, Pharmacology considers that sufficient & adequate P/T information is available to establish safety of the proposed Histrelin implant.

C. Recommendations on labeling: Labeling for Carcingenicity and Teratogenicity and Return to Fertility has been referenced to approved NDA 19-836 for Histrelin subcutaneous injection for precocious puberty. Sponsor has conducted toxicity and a battery of genotoxicity studies for trimethylolpropanetrimethylacrylate (TMPTMA), a NME in the proposed implant formulation according to ICH guidance. All this information will be included in the proposed labeling for Vantas (Histrelin acetate) 50 mg Implant.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: Nonclinical studies reviewed suggest that extracts of HEMA/HPMA copolymer placebo reservoir (Hydrogel implant reservoir) or that of Hydrogel/histrelin implants were not genotoxic. Also long term sub-dermal implant insertion had no significant adverse effects.

B. Pharmacologic activity: Histrelin acetate, a LH-RH agonist, is a potent inhibitor of gonadotropin secretion when given continuously. After an initial stimulatory phase, chronic subcutaneous administration of histrelin acetate desensitizes responsiveness of the pituitary gonadotrophs, which in turn causes a reduction in ovarian and testicular steroidogenesis. In males, testosterone is reduced to castrate levels.

C. Nonclinical safety issues relevant to clinical use: In light of the previous safe use of histrelin acetate under NDA 19-836 at doses higher than proposed under present NDA 21-732 for the palliative treatment of prostate cancer, along with toxicity studies conducted with the proposed histrelin implant, there dose not seem to be any safety concern for its clinical use.

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

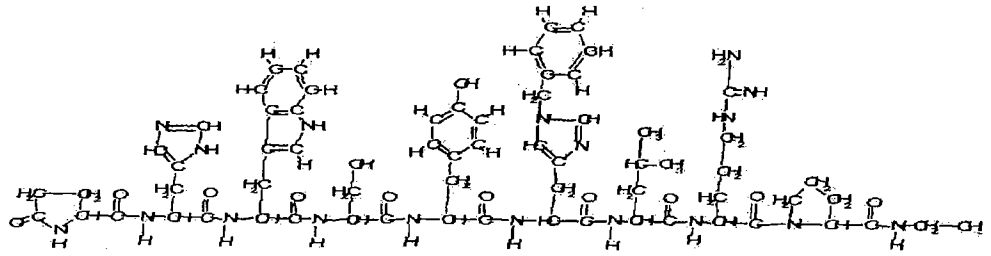
NDA number: 21-732
Review number: 1
Sequence number/date/type of submission: 000/12-12-03/Original submission
Information to sponsor: Yes ( ) No (*)
Sponsor and/or agent: Valera Pharmaceuticals, Inc. Cranbury, NJ
Manufacturer for drug substance: [ ]

Reviewer name: Krishan L. raheja, D.V.M., Ph.D.
Division name: DRUDP
HFD #: 580
Review completion date: 7-20-04

Drug:

Trade name: Vantas (Histrelin subdermal implant)
Generic name: LHRH (luteinizing hormone-releasing hormone)
Code name:
Chemical name: Histrelin acetate: 5-oxo-L-propyl-L-histidyl-L-tryptophyseryl-L -tyrosylN^t-benzyl-D-histidyl-L-leucyl-L-arginyl-N-ethyl-L -prolinamide acetate (salt)

CAS registry number:
Molecular formula/molecular weight: [$C_{66}H_{86}N_{18}O_{12}$. (1.7 –2.8 moles) CH_3COOH, (0.6 – 7.0 moles) H_2O]
MWt: 1323.52
Structural formula for drug substance Histrelin acetate:



Impurities: — synthetic impurities have been isolated and identified in histrelin acetate drug substance. — of the impurities are [ ] and the — is the [ ]

Following are the [ ] impurities:

1. [
2.
3.
4.

5
6

The sponsor has stated that drug substance assay is capable of resolving [ ] impurities with a limited quantitation of [ ] The impurity that is not resolved is [ ] and this impurity along with [ ] have not been detected above [ ] in the histrelin acetate drug substance.

Relevant INDs/NDAs/DMFs: Shire NDA #19-836; [ ] DMF # [ ]

Drug class: LH-RH agonist

Indication: Palliative treatment of advanced prostate cancer

Clinical formulation: The histrelin subdermal implant is a drug/device combination designed to provide a sustained release of a steady amount of histrelin over a period of one year or more when inserted subcutaneously. The system is composed of four hard packed histrelin pallets, which are surrounded by a polymer cartridge. The yearly average rate of histrelin release is 50 ug/day, i.e., approximately 0.7 ug/kg/day for a 70 kg man.

Drug product- Histrelin Subdermal implant is hydrated, autoclaved cylindrical polymeric implant that contains histrelin acetate as the active pharmaceutical ingredient. Each implant contains a total of 50 mg histrelin acetate. The polymeric cartridges (i.e. devices) are packaged in 3.5 ml glass vials containing 2.0 ml of 1.8% Sodium Chloride solution.

The composition of the Implant polymer cartridge is as follows:

[
Purified 2-hydroxyethyl methacrylate (HEMA)
% purified 2-hydroxypropyl methacrylate (HPMA)
Trimethylolpropane Trimethylacrylate (TMPTMA)
Benzoin methyl ether
Perkadox
Triton X-100
]

Note: HPMA, HEMA, and TMPTMA = [ ]
[ ] -sample = remaining weight
Triton, BME, and P-16 are added as % of remaining weight of mixture after sampling.

The implant plug used to seal the polymer cartridge is composed of [ ] HEMA, [ ] TMPTMA, [ ], MBE and [ ], P-16.

The Histrelin pallet is composed of [ ] histrelin acetate and [ ] stearic acid by weight

Route of administration: Subdermal

Proposed use: For the palliative treatment of advanced prostate cancer

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Studies reviewed within this submission:

Under non-clinical pharmacology and toxicology sponsor has provided information on the pharmacological activity of histrelin and toxicology of the implant and its various components. The P/K of histrelin, including administration via implant is also described.

A total of 53 studies are listed. Fifteen of these are referenced to NDA # 19-836 approved in 1991 for the treatment of central precocious puberty.

The safety information for the implant device is based on a number of studies performed over the last 30 years in support of products composed of [ ] polymer.
In addition sponsor has conducted a biocompatibility/toxicology program to test the implant cartridge in its proposed commercial form.

Pertinent studies related to present device formulation have been reviewed under the appropriate sections.

Studies <u>not</u> reviewed within this submission: Studies which have been previously reviewed under NDA 19-836 for histrelin for the treatment of central precocious puberty.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary: No pharmacology studies were submitted. However, reference is made to NDA 18-836 for pharmacological studies conducted in rats on the effects of histrelin on reproductive organs, pituitary desensitization, inhibition of prostatic carcinoma growth, stimulation of the reproductive endocrine system, and inhibition of the reproductive endocrine system with histrelin in combination with flutamide in male rats.

2.6.2.2 Primary pharmacodynamics

<u>Mechanism of action</u>: The continuous administration of histrelin via the implant device results in pituitary gonadotrophs insensitization to GnRH with subsequent suppression of gonadotropin secretion & ovarian and testicular steroidogenesis. Since prostate cancer

growth is testosterone-dependent, with suppression of testosterone tumor growth is inhibited.

Drug activity related to proposed indication: Suppression of testosterone and inhibition of tumor growth.

2.6.2.3 Secondary pharmacodynamics: Suppression of PSA & LH

2.6.2.4 Safety pharmacology

There were no safety pharmacology studies submitted under the present application. The non-clinical safety assessment of the drug itself is largely based on the non-clinical program performed in support of Supprelin injection (active ingredient: histrelin, U.S.-NDA Number 19-836, approved in 1991 for treatment of central precocious puberty (CPP)).

2.6.2.5 Pharmacodynamic drug interactions: none described

2.6.3 PHARMACOLOGY TABULATED SUMMARY

None submitted

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

No new data submitted

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

None submitted

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

As pointed out earlier, the non-clinical safety assessment of the drug itself is largely based on the non-clinical program performed in support of histrelin injection under NDA 19-836 approved in 1991 for the treatment of central precocious puberty.
However, sponsor has conducted studies and submitted results under the present submission to establish the safety of the histrelin subdermal implant, which is a drug/device combination.
The hydrogel (hydroxyethyl methacrylate) polymer of the cartridge has been suggested to be safe when used in ocular devices such as soft contact lenses in humans.
However, for the polymer implant cartridge Hydro Med Sciences has submitted studies which are reviewed under specific headings.

General toxicology: Nonclinical studies reviewed suggest that extract of HEMA/HPMA copolymer placebo reservoir (Hydrogel implant reservoir) or that of Hydrogel/histrelin implants had no significant adverse effects.

Genetic toxicology: Extracts of HEMA/HPMA copolymer placebo reservoir (Hydrogel implant reservoir) or that of Hydrogel/histrelin implants were not genotoxic in a battery of genotoxicity assays.

Carcinogenicity: The rat and mouse carcinogenicity studies with histrelin are referred to Shire's approved NDA 19-836 for precocious puberty.

Reproductive toxicology: All reproductive toxicity studies are referred to Shire's approved NDA 19-836 for precocious puberty.

Special toxicology: Primary subdermal irritation study of histrelin implants in rats and administration of HEMA/HPMA copolymer placebo extracts prepared in either saline of cottonseed oil in various toxicity studies i.e., intracutaneous injection test in rabbits, Kligman Maximization test in guinea pigs, systemic injection test in mice, and pyrogen test in rabbits did not exhibit any significant adverse effects.

2.6.6.2 Single-dose toxicity

No single dose toxicity studies were submitted

2.6.6.3 Repeat-dose toxicity

The following studies were submitted:

Study title: A 6-month study with two formulations of histrelin hydrogel implants in male rats. Volume # 38.

This study was conducted by [redacted] in 1989. No study project number was given. It was not stated if study was conducted in accordance with GLP regulations. The 2 formulations used were formulation 132 ([redacted] Equilibrium Water Content, EWC) and formulation 136 ([redacted] EWC) having in vitro histrelin release of 80 ug and 60 ug histrelin/day, respectively.
The purpose of this study was to ascertain if sufficient amounts of histrelin could be released from these implants, in vivo, to cause a biological effect in rats. The implants were 2 cm in length and 2.8 mm in diameter, and each contained 25 mg of histrelin. The hydrogel polymer used in the implants was made from monomers of HEMA and HPMA and was cross-linked with trimethylpropane trimethylacrylate. The EWC of the hydrogel polymer is changed by varying the proportion of each monomer in the formulation.

Experimental design: Sixty male SD rats weighing 200-210 g were divided in 3 groups of 20/g. Group 1 served as the control group. Group 2 animals were each implanted with a single histrelin hydrogel implant of formulation 132 and animals in group 3 were each implanted with a single implant of formulation 136. Five animals from each group were sacrificed at intervals of 30, 60 120 and 210 days. Their body weights were recorded. Paired testes and epididymides, ventral prostate ad seminal vesicles were removed and weighed.

Results: Testes and epididymides weights as well as ventral prostate and seminal vesicle weights were significantly suppressed at each time interval by histrelin compared to the controls. There was no difference between treatment with formulation 132 and 136. Body weight increased in all treatment groups and was not significantly different from each other.
Severe mineral deposits were seen on the surface of the implants that were removed at 210 days with adhesion between the implants and their fibrous capsules. The degree of mineralization was less at 60 and 120 days.

Based on these findings it was concluded that sufficient histrelin was released from the implants to have biological effect. It was suggested that increased mineralization with prolonged used could alter histrelin release.

Study title: A 6-month study with two formulations of histrelin hydrogel implants in male dogs. Study 1. Volume # 38

This study was conducted by [redacted] in 1990. No study number was assigned.

In this study as in the above reviewed rat study, implants of formulation 132 and 136 with peptide content of 25 mg were used. However, histrelin hydrogel implants of formulation 132 were in preconditioned hydrated state and measured 2 cm long and 3.1 mm in diameter. Formulation 136 implants were non-hydrated as well as in hydrated state. The dry implants measured 2 cm in length and 2.8 mm in diameter.

Thirty-seven adult Beagle dogs were used and histrelin implants were removed at various time intervals after insertion as shown in table below:

Treatment formulation	Intervals following implant insertion			
	30 days	60 days	120 days	180 days
Prehydrated 132	4	4	4	4
Prehydrated 136	4	4	4	4
Nonhydrated 136				5

Blood samples were collected before implant insertion and periodically throughout the course of the study for plasma histrelin and testosterone determination. Testes of each dog treated with implant of formulation 136 were measured and testis volume calculated.

Results: Treatment with preconditioned, prehydrated implants resulted in immediate detection of histrelin in the blood soon after implant insertion. There was an initial burst of 750 pg/ml followed by a decline to about 400 pg/ml. The level was fairly constant throughout the course of the study. Hydrogel formulation with higher water content (formulation 132) released greater amounts of histrelin than formulation with lower EWC (formulation 136), which showed relatively constant level between 100-200 pg/ml. Dogs treated with dry or non-hydrated implants of formulation 136 had a lag time of 30 days before the peptide could be detected. Similar lag time of about 28 –30 days was reported

for in vitro release rate studies. Histrelin levels fell below the level of detection following removal of implants on day 180.
Prehydrated implant treatment resulted in an earlier detection of plasma testosterone. In the non-hydrated implant treatment, the delay in reaching maximum plasma concentration of testosterone was attributed to lag-time encountered before the hydrogel became hydrated in vivo. Suppression of plasma testosterone to undetectable levels was noted at 60 days in animals treated with the preconditioned implants compared to 75 days for the non-hydrated implant treatment group.
The prehydrated implants resulted in immediate suppression of testis volume at 1 month. At 4 month, the testis volume was approximately 40% of the original. No measurements were taken at 5 or 6 months. Testicular volume in dogs treated with non-hydrated implants showed a net increase of about 9% at day 30 and then steadily declined, resulting in a 70% loss at 6 months.
The intensity of mineralization increased with length of implant use.

The results showed that histrelin can be delivered in dogs from hydrated implants in a controlled sustained manner for a period of at least 6 months and that the dosage can be regulated by changing the EWC of the hydrogel polymer used.

Study title: Correlation of in vivo and in vitro release rates of histrelin from hydrogel implants in the dog: Study 2

This study was conducted by [ ] in 1995.

The goals of this study were to determine the in vivo release rate of histrelin from hydrogel implants in dogs and to ascertain whether they differ from the in vitro release rate of the peptide from similar implants.

The release rates were obtained using 3 different approaches, namely, by determining (a) the in vitro release rate of the peptide in an incubation medium of physiological saline, maintained at 37 C in a water bath, with agitation; (b) the average daily release rate in vivo, obtained by dividing the amount of peptide lost from implants inserted into dogs, by dividing the number of days of implant use and; (c) by calculating the in vivo release rate from the measured metabolic clearance rate (MCR) of the peptide and serum levels of histrelin at various time points during implant use in the dog.

Histrelin hydrogel implants were made from hydrogel formulation 142, containing 27.5% equilibrium water content. They were preconditioned, prehydrated and were 20 cm in height and 3.1 mm in diameter. Each implant manufactured by Hydro Med Sciences, contained 51.76+/- 1.36 mg (X+/-SD) histrelin.

[ ] Osmotic Pumps were used in the determination of the MCR. The pumps were loaded with a solution of known concentration of histrelin.

In vitro release rate of histrelin:

In vitro release rate assessment from hydrogel Implants was determined on 4 separate implants. The implants were not prehydrated before initiation of the first incubation. The incubation medium was changed every 7 days. Histrelin was determined by HPLC method and quantification of histrelin done by both peak area and peak height.

In vivo release rate of histrelin (A): Average daily release rate:

Each of 6 adult male beagle dogs were implanted with 4 histrelin hydrogel implants (2 on each side of the ventral midline of the abdomen). The peptide content of each implant was predetermined. The implants were removed at predetermined intervals of 8, 16, 24, 32, 40 and 48 weeks, following initiation of the study. One implant was removed from each of 4 dogs at each time point and assayed for their peptide content by HPLC. The amount of peptide lost was divided by the number of days of implant use in the dog, to obtain the average daily release rate of histrelin from the hydrogel implants.

In vivo release rate of histreliln (B): MCR and serum levels of histrelin:

The in vivo release rate of histrelin from the hydrogel implant was determined using the following equation:

RR=MCR x SL
Where RR= rate of release of histrelin from implant or rate of infusion of histrelin from [ ] pumps
MCR= metabolic clearance rate
SL = serum level of histrelin

One can calculate MCR by rearranging the equation as
MCR = RRi/Sli

Where MCR= metabolic clearance rate
Rri = rate of infusion of histrelin from [ ] pump
Sli = serum level of histrelin during pump use.

Each of 6 dogs was implanted with [ ] osmotic pump calibrated to deliver a predetermined amount of histrelin for at least 2 weeks. Blood was collected on 4 consecutive days following one week of pump use. Histrelin was assayed by RIA methodology.

Dose-response curve:

The same 6 dogs were later implanted with 1, 2, 3 and 4 histrelin implants and blood samples were collected at 8 weeks of implant use for histrelin determination by RIA.

Results:

In vitro release of histrelin: The maximum release rate was 45 ug/day at week 12. The release rate declined to about 20 ug/day at 48 weeks.

In vivo release rate of histrelin (A) average daily percentage rate: At 8, 16, 24, 32, 40 and 48 weeks, the mean +/- SE average daily release rate was estimated to be 39.6+/-3.2, 41.8+/-9.0, 33.7+/-5.0, 26.4+/-5.4, 33.7+/- 1.6, and 26.3+/-3.8 ug/day respectively. These results were considered in close agreement with in vitro release data except for the data obtained at 32 weeks.

In vivo release rate of histrelin (B) metabolic clearance rate and serum levels of histrelin:

The estimated MCR ranged from 255 L/day to 430 L/day. The in vivo release rate in 6 dogs over a period of 48 weeks was estimated to be 55 ug histrelin at 8 weeks, slowly declining thereafter to about 22 ug/day at week 48.

Dose-response curve: Data showed an excellent correlation between serum levels of histrelin and the number of implants used.

Study title: "Morphological assessment of testicular function in the dog following treatment for one year with a single histrelin hydrogel implant and after recovery period of either 45 or 90 days" Study No.3 This study was conducted by [ ] in 1995. Volume # 41

In this study 9 adult Beagle dogs were used. Six dogs were implanted with a single preconditioned (hydrated) implant, formulation 136 (28.5% EWC) releasing approximately 50 ug histrelin/day. Three dogs received no implants and served as controls.

The implants remained in situ for one year. At the end of treatment period, the implants were removed, and one testis from each dog was removed. The dogs were allowed to recover for 45 or 90 days. At 45 days the remaining testes from 3 of the treated dogs were removed and one testis from each of the 3 of the control dogs was also removed. At 90 days post-treatment the remaining testes from other 3 treated dogs and the 3 remaining control dogs were removed. All testes were processed for examination with [ ] microscope. Images were recorded on [ ] film.

Results: Following one year of treatment, it was reported that the plasma testosterone was suppressed significantly to below the assay lower limits of the detection. Testicular volume was reduced to 70% of the original. Spermatogenesis was arrested.

Following removal of the histrelin implants, at Day 45, the morphology of the testis was not restored to normal. However, complete recovery of the architecture of the testis was evident at Day 90 following cessation of histrelin implant treatment.

Based on the results of this study sponsor stated that "it may be concluded that a single histrelin hydrogel implant, releasing 50 ug histrelin/day, markedly altered testis function. Plasma testosterone, testicular volume and morphology of the testes were all altered significantly by histrelin treatment. Spermatogenesis was interrupted as evidence by morphological evaluation. Full recovery of testicular function was attained at 90 days after termination of treatment".

Study title: A one year study of mineral deposit on histrelin hydrogel implants (formulation 136) in Cynomolgus monkey. Study 2. Volume # 38

The study was conducted by [redacted] in 1995 to determine if and when mineralization occur in histrelin hydrogel implants placed in monkeys for one year and to determine the ease of implant removal from the capsules.
Six monkeys were used in this study. Histrelin implants (formulation 136) were prehydrated.
Implant insertions: Monkeys # 1 and 2 were implanted with 2 implants which were removed 12 months post-implantation. Monkeys # 3 and 4 were implanted with 2 implants which were removed at 2 months post-treatment, and then implanted with a second set of 2 implants each which were removed at 8 months post-treatment. Monkeys # 5 and 6 were each implanted with 2 implants, which were removed after 4 months. They received a second set of implants, which were removed 6 months later. With this schedule implants were in situ for 2, 4, 6, 8, and 12 months in the monkeys. The fibrous capsules after implant removal were processed for histological examination to determine cellular response and whether duration of use of implants would result in differences in fibrous capsule morphology and wall thickness.

Results: Examination of the implantation sites showed no erythema or edema. The microscopic findings are detailed in table below:

Group	2 months				4 months				8 months					12 months		
Capsule #	1	2	3	4	1	2	3	4	1	2	3	4	5	1	2	3
Implant capsule thickness	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1
Cellular response Polymorphonuclear	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Lymphocytes	1	1	1	1	1	-	-	-	-	-	1	-	-	1	-	-
Plasma cells	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-
Mononuclear macrophages	1	1	1	2	2	1	1	1	1	1	1	1	1	1	1	1
Foreign body giant cells	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Fibrosis (capsules)	2	2	2	2	2	2	2	2	2	1	1	1	1	1	1	1

1, 2, 3, 4= degree of severity of indicated change
1= minimal, 2= mild, 3= moderate, 4= marked
- = Indicated change is not present

In the early 1970s, [redacted] conducted studies (study # 16, 17, and 18 volume 38) to determine the toxicity of Hydron monomer in rats. Rats received Hydron monomer polyvinyl alcohol implants SC for a period of 14 or 28 days. The effects of

Hydron monomer upon fibroplasia and repair were studied by examining implants histologically and for collagen content.
It was reported that no systemic toxic effects were observed in animals receiving 2 polyvinyl alcohol sponges containing in excess of 100 mg Hydron monomer each (dose about 0.2% of body weight). Hydron monomer was locally toxic at this dose at 14 days but inhibition of fibroplasia and intense inflammation was largely resolved by 28 days.

In a repeat study with a lower dose of Hydron monomer (0.06% of body weight), no systemic effects and inflammatory response was observed.

Subcutaneous injection of saline extractables obtained from boiled Hydron polymers in rats for 5 days had no effect on rat body weight, organ weights and exhibited no microscopic evidence of systemic toxic effects on examination of heart, lung, liver, kidney, spleen, adrenal, nerve, fibroconnective tissue or muscle.

Study title: 14 day repeat dose intravenous toxicity study (subchronic) –ISO- For: Hydrogel implant reservoir. [ ]. Final Report: 99-2949-G10. Volume # 39

In this study the test article was HEMA/HPMA copolymer placebo reservoir and control article was 0.9% USP sodium chloride for injection. Twenty Swiss Albino mice (10/s) were used.
The test article was extracted at a ratio of 60 cm^2 per 20 ml of NaCl vehicle at 37C for 72 hours. The control article (NaCl) was prepared in the same manner as the test article for parallel treatment and comparison.
The test and control article extracts were administered i.v. to 10 animals (5/s), 5 days a week for 14 days at a dose level of 25 ml/kg body weight.

Results:

There were no significant differences in body weight for the test and control groups. None of the test or control animals exhibited any signs of toxicity over the course of the study. No signs of toxicity were reported at gross necropsy in either test or control animals.
Hematologic examination revealed female test animals had significantly greater WBCs counts than control animals but were reported to be within normal range for this species. Compared to controls in treated animals the absolute weight of kidneys was increased in males (0.75 vs 0.56 g) and females (0.53 vs 0.40 g) and that of liver in females (1.69 vs 1.26 g). Relative weight of kidneys in males and liver and kidneys in females were also significantly increased.
On histopathological examination, one treated female had mild focal mononuclear cell infiltration in lung and unilateral focal mononuclear cell infiltration in the renal pelvis. One control female had moderate epicardial vascular calcification.

Study title: Long term subcutaneous implantation test with histopathology (4 week timepoint) –ISO For: hydrogel implant reservoir. [ ] Final Report: 99-2949-G12.

Volume # 39

This study was conducted in 2000 to determine the potential for test article, HEMA/HPMA copolymer placebo reservoir (solid material, batch No. 019-01), to induce local toxic effects in the SC tissues of NZW rabbits was tested. Control article was negative control plastic.
Three rabbits were implanted with 5 strips of test and control article, maintained for a period of 4 weeks, and then sacrificed.

Following gross observations, the implanted sites were excised from the rabbits and examined macroscopically and histologically. Inflammation, encapsulation, hemorrhage, necrosis and discoloration were recorded.

Results:
All animals gained weight; none exhibited any signs of toxicity. Macroscopic examination indicated no signs of inflammation, encapsulation, hemorrhage, necrosis or discoloration.
Microscopic examination of test implant site revealed no significant signs of inflammation, fibrosis, hemorrhage, necrosis or degeneration as compared to control article sites.
The average toxicity rating was 0.29 on a scale of 1-4 indicating no toxicity.

Study title: Long term subcutaneous implantation test with histopathology (12 week timepoint)- ISO For: hydrogel implant reservoir. [ ] Final Report: 99-2949-G13.
Volume # 39

In this study 3 rabbits were used as in the 4-week study. The results were similar i.e., the test article was non toxic when implanted for 12 weeks. The average toxicity rating was 0.48 when compared to control article implant sites.

Study title: Long term subcutaneous implantation test with histopatholgy – ISO 4, 12 and 26 week timepoints For: hydrogel implant reservoir. [ ] Final Reports: 99-2949-G12, 99-2949-G13, 99-2949-G14.

Sponsor has stated that the study was conducted for 26 weeks and according to [ ] current policy, each time point was reported as an independent report.

The overall results indicated that the test article was non toxic when implanted for 4, 12, and 26 weeks with Toxicity ratings of 0.29, 0.48 and 0.20, respectively, when compared to control article implant sites. It was stated that biological reaction to the test article implantation did not significantly change over the course of the 26-week exposure.

2.6.6.4 Genetic toxicology

The following 3 genotoxicity studies for Hydrogel Implant Reservoir were sponsored by Roberts Pharmaceuticals Corporation and conducted by [ ] in 2000.

The test article (HEMA/HPMA copolymer placebo reservoir) was prepared by extraction in NaCl at a ratio of 60 cm^2 per 20 ml and extracted was conducted at 37 +/-2 C for 72 hours according to ISO 10993.

Study title: Rodent bone marrow micronucleus assay – ISO For: hydrogel implant reservoir.

Key findings: Test article was negative in the MMN assay

Study no.: [ ] final report # 99-2949-G1

Volume #, and page #: 41 of 344, page 008
Conducting laboratory and location: [ ]
Date of study initiation: 11-15-99
GLP compliance: yes
QA reports: yes (*) no ( )
Drug, lot #, and % purity: Lot/Batch # 507

Methods

Strains/species/cell line: mice/Swiss albino

Doses used in definitive study: The test extract was administered intravenously as a single dose (50 ml/kg).

Basis of dose selection: neat concentration as per sponsor's specifications, which were not described.

Negative controls: The negative control (NaCl) was administered intravenously as a single injection (50 ml/kg)

Positive controls: Positive control was Mitomycin C administered at a dose of 200 ug/kg (dissolved in NaCl) as a single dose intravenously.

Incubation and sampling times: The test article extract and negative control were each administered to 2 groups of 5 mice/s and were scarified at 24 and 48 hours after treatment. The positive control article was administered to a single treatment group that was sacrificed at 24 hours.
At each sacrifice bone marrow slides were prepared. A total of at least 2000 polychromatic erythrocytes per animal were scored for the presence of micronuclei. The ratio of polychromatic to normochromatic erythrocytes was determined for each animal.

Results: The micronucleus scoring for negative control, test article and positive control, 24 and 48 hours after extract administration is shown in table below Average for 5 mice/s):

Negative control (24 hours)					
Sex	#PCE	#NCE	PCE/NCE	#MNC	MNC/PCE
Male	2000	751	2.68	2.20	0.0011
Female	2000	698	2.88	2.60	0.0013
Negative control (48 hours)					
Male	2000	746	2.70	4.00	0.0011
Female	2000	705	2.86	2.40	0.00122
Test article (24 hours)					
Male	2000	676	2.99	2.60	0.0013
Female	2000	708	2.83	1.80	0.0009
Test article (48 hours)					
Male	2000	777	2.59	1.80	0.0009
Female	2000	737	2.73	2.00	0.0010

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Study deemed valid as conducted

Study outcome: The HEMA/HPMA copolymer placebo reservoir extract was negative in the mouse micronucleus assay as conducted.

Study title: Salmonella typhimurium and escherichia coli reverse mutation assay- ISO-For: hydrogel implant reservoir

Key findings: Extracts of hydrogel implant reservoir were negative in the Reverse Mutation assay.

Study no.: [ ] final report # 99-2949-G3

Volume #, and page #: 41 of 344, page 025
Conducting laboratory and location: [ ]
Date of study initiation: 11-12-99
GLP compliance: yes
QA reports: yes (*) no ( )
Drug, lot #, and % purity: Lot/Batch # 507

Methods

Strains/species/cell line: Salmonella typhimurium strains TA98, TA100, TA1535, TA 1537 and Eschirichia coli WP2.

Doses used in definitive study: 100 ul/plate at neat concentration.

Basis of dose selection: neat concentration as per sponsor's specifications, which were not described

Negative controls: NaCl and DMSO 100 ul/plate

Positive controls: 2-nitrofluorene, sodium azide, 9-aminoacridine, N-ethyl-N-nitro-N-nitroguanidine

Incubation and sampling times: All strains were treated with the neat concentration of each of the test article extracts. All controls and test groups were plated in triplicate. For the non-activated assay top agar supplemented with 0.5mM histidine and 0.5mM biotin per 1.0 ml of agar was used as overlay. Tryptophan was used in place of histidine for WP2.

The overlay consisted of tubes containing the following:

2 ml of molten top agar
0.1 ml of the appropriate tester strain
0.1 ml of the appropriate concentration of the test article or control article
0.5 ml of phosphate buffer pH 7.4

For the metabolic activation assay 0.5 ml of S9 mix was substituted for phosphate buffer.

The tubes were vortexed and poured onto minimal glucose agar plates. All plates were incubated at 37 c for 72 hours. Plates were checked for uniform background lawns and the number of revertant colonies counted.

Results:
The results of the reverse mutation assay with NaCl extract without and with microsomal activation are given in table below. Values are revertant/plate

NaCl extract without S9 mix

Strain	Controls		Test article
	Positive control	Negative control	Dose level: neat extract
TA98	137	31.3	28.3
TA100	366	103.3	99.7
TA1535	124	21.7	17.3
TA1537	155	10.0	9.7
WP2	414	138.3	112
NaCl extract with S9 mix			
TA98	168	40.3	47.3
TA100	416	136.7	129.7
TA1535	163	21.0	21.3
TA1537	187	13.0	12.3
WP2	491	134.0	116.7

Results for the confirmatory assay with and without S9 activation with NaCl extract and with DMSO as negative control with and without metabolic activation were similar to results shown in table above.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Study deemed valid as conducted

Study outcome: The HEMA/HPMA copolymer placebo reservoir extracts in NaCl and DMSO were negative in the Reverse Mutation assay

Study title: CHO/HGPRT forward mutation assay – ISO For: hydrogel implant reservoir.

Key findings: The HEMA/HPMA copolymer placebo reservoir test extract was negative in the CHO/HGPRT forward mutation assay.

Study no. : [ ] final report # 99-2949-G7

Volume #, and page #: 41 of 344, page 047
Conducting laboratory and location: [ ]
Date of study initiation: 11-16-99
GLP compliance: yes
QA reports: yes (*) no ( )
Drug, lot #, and % purity: Lot/Batch # 507

Methods

Strains/species/cell line: Chinese Hamster Ovary (CHO-KI) cells
Doses used in definitive study: Test article tested at neat concentration of the extract.

Basis of dose selection: The extract of the test article was directly assayed.
Negative controls: Ham's F-12 cell medium served as negative control article for the non-activated and activated assays.

Positive controls: The positive control for the non-activated system was ethylmethanesulfoxide (EMS) and for the activated system dimethylbenzanthracene (DMBA)

Incubation and sampling times: The cells were exposed to the test article for 16 hours in the non-activated assay and 5 hours in the activated assay. After exposure cells were washed twice with medium or PBS and supplemented with complete medium.
Approximately 28 hours following termination of non-activated exposure period and approximately 43 hours following termination of activated exposure period, cells were trypsinized, counted, and plated at 1 x 10^6 cell per 100 mm dish. The cells were passed every 48-72 hours to maintain exponential growth during phenotypic expression for 6 days.
Following the phenotypic expression period, cells were grown in selective medium with 6-thioguanine to select mutant cells. The cultures were incubated for 7 days to allow colonies to develop. Concurrently cloning efficiency was determined by plating cells on 6 dishes (3 from each duplicate seeded at 200 cells/60 mm dish) in selective medium without 6-thioguanine.
At the end of the incubation period, plates were rinsed with PBS, fixed in methanol and stained with Giemsa. Only colonies with 50 or more cells were counted.
The results of the CHO/HGPRT locus mutation assay were evaluated based on the number of TG-resistant mutants per 1 x 10^6 surviving cells.

The results of the CHO/HGPRT locus mutation assay were evaluated based on the number of TG-resistant mutants per 1 x 10^6 surviving cells.

Results:

In the cytotoxicity assay positive control article exhibited a cell survival of 91 – 100%, the test article 98 – 104% and negative control article 95 – 100% in the non-activated and activated assays.
As shown in table below, the neat test article extract did not show a statistically significant increase in the number of mutants per 1 x 10^6 surviving cells as compared to corresponding negative controls.

Non-activated assay

Treatment	Total # of plates	Total # of foci	Av. foci/plate	Av. foci/plate normalized*
Negative control	10	4	0.4	0.75
Positive control	10	1279	127.9	285.8
Test article extract	10	3	0.3	0.53
Activated assay				
Negative control	10	4	0.4	1.04
Positive control	10	544	54.4	171.8
Test article extract	10	4	0.4	1.01

* Normalized to 1 x 10^6 cells per plate based upon the corresponding survival fraction which ranged 0.45 to 0.57 for the non-activated and 0.32 to 0.39 for the activated systems.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Study deemed valid as conducted

Study outcome: The HEMA/HPMA copolymer placebo reservoir extract was negative in the CHO/HGPRT forward mutation assay

In 2002 [ ] conducted 3 genotoxicity assays sponsored by Hydro Med Sciences, Inc. Cranbury, NJ. In these assays a saline extract of Hydron/Histrelin implants was used in place of extracts of Hydrogel implant reservoir used in the genotoxicity studies described above. All studies were conducted in accordance with FDA's GLP regulations following OECD Guideline 471 dated July 21,1997.

The 3 studies were as follows:

1. Salmonella-Escherichia coli/mammalian microsome reverse mutation assay with a saline extract of Hydron/Histrelin implant. [ ] study # 24-97-0-409
2. L5178Y $TK^{+/-}$ mouse lymphoma forward mutation assay. [ ] study # 24097-0-4311CHI
3. In vivo mouse micronucleus assay with a saline extract of hydron/histrelin implant. [ ] study # 24097-0-455OECD.

Results of these studies are summarized below:

1. Ames test

Mutagenicity assay reults - Summary

	Mean revertants/plate					
Dose/plate	TA98	TA100	TA1535	TA1537	WP2uvA	Background lawn[a]
Microsomes: rat liver Vehicle/extract control	32	92	8	11	15	N
Test article 25.0 ul	24	103	13	8	14	N
50.0 ul	29	84	12	5	15	N
100 ul	20	83	16	10	11	N
150 ul	22	78	13	8	11	N
200 ul	22	105	8	7	17	N
Positive control	238	382	107	62	627	N
Microsomes: none Vehicle/extract control	14	3	88	9	7	N
Test article 25.0 ul	12	2	71	11	6	N
50.0 ul	12	4	77	11	5	N
100 ul	11	4	81	10	8	N
150 ul	14	1	75	8	7	N
200 ul	11	5	85	15	6	N
Positive control	121	17	728	885	1269	N

*Background lawn N=normal

2. L5178Y TK$^{+/-}$ mouse lymphoma forward mutation assay, without activation-4 hour treatment

Test condition	Daily cell count (cell/ml, x 10^5 units		Cumulative RSG[a]	Total mutant colonies	Total viable colonies	Cloning efficiency[b]	Relative growth (%)[c]	Mutant freqency (x 10^{-6} units)[d]
	Day 1	Day 2						
Nonactivated controls[e]								
Extract/vehicle control	17.4	14.6	28.2	155	465	77.5	104.1	66.7
Extract/vehicle control	16.8	13.0	24.3	148	483	80.6	93.1	61.4
Extract/vehicle control	15.8	13.5	23.7 **AVG=25.4**	170	540	90.0 **AVG=82.7**	101.6	63.0
Untreated/negative control	16.3	15.4	27.9	194	489	81.5	108.2	79.5
Negative vehicle control	19.9	12.7	28.1	160	598	99.6	133.3	53.6
MMS 13 ug/ml	8.7	9.2	8.9	554	283	47.1	19.9	393.3[f]
MMS 13 ug/ml	9.9	8.0	8.8	575	312	52.0	21.8	368.5[f]
Test compound ul/ml			Relative to vehicle cont (%)					
0.785	18.0	16.5	129.9	133	461	93.0	120.9	57.7
1.57	20.1	14.9	131.0	145	517	104.3	136.6	56.1
3.13	15.5	13.7	92.9	178	480	96.8	89.9	74.1
6.25	19.2	13.9	116.8	155	525	105.8	123.5	59.0
12.5	16.1	14.4	101.4	165	551	111.1	112.7	59.8
25.0	18.1	14.1	111.7	146	497	100.3	112.0	58.8
50.0	16.4	14.8	106.2	175	527	106.2	122.8	66.3
100	19.0	12.2	101.4	176	659	132.8	134.7	53.3

[a] RSG= (day 1 count/3) x (day 2 count/3
[b]cloning efficiency = total viable colony count/number of cells seeded x 100
[c] relative growth =(relative suspension growth x relative cloning efficiency)/100
[d] mutant frequency = (total mutant colonies/total viable colonies) x (2 x 10^{-4})
[e] extract/vehicle control=10% mock extracted saline
untreated negative control = untreated control (5% Fisher medium), negative/vehicle control=10% saline
positive control = methyl methanesulfonate
[f]= mutagenic = exceeds minimum criterion of 127.4 x 10^{-6}

The ratio of small: large colonies was 1:1 for extract/vehicle control. Untreated negative control, negative vehicle control and test article. However for the positive control number of small colonies was about 2 times higher compared to large colonies.

Test article used was a saline extract of Hydron/histrelin implant. Vehicle was saline. Selective agent was TFT 3.0 ug/ml. Treatment period was 4 hours and expression period was 2 days.

Similar results were reported 24-hour treatment without activation and 4 hour treatment with activation.

3. Mouse micronucleus summary results

Treatment	Dose	Harvest time	% micronucleated PCEs Mean of 2000 per animal+/-SE	Ratio of PCE;NCE mean +/- SE
Control Vehicle extract	50 ml/kg i.v	24 hour 48 hour	0.08 +/- 0.02 0.03 +/- 0.01	0.55 +/- 0.15 0.52 =/- 0.06
Positive	CP80 mg/kg (10 ml/kg) oral gavage	24 hour	3.49 +/- 0.33*	0.51 +/- 0.06
Test article extract	50 ml/kg i.v.	24 hour 48 hour	0.08 +/- 0.02[a] 0.03 +/- 0.02	0.26 +/- 0.04 0.40 +/- 0.04**

*Compared to vehicle control $p<0.05$
**Compared to vehicle control $p<0.01$
[a] 4000 PCE counted for one animal
CP = cyclophosphamide, PCE = polychromatic erythrocyte, NCE = normochromatic erythrocyte, vehicle extract = 0.9% mock extracted saline, test article = saline extract of hydron/histrelin implant

Results of the above 3 studies showed that saline extracts of Hydron/Histrelin implants were not mutagenic under the experimental conditions used.

2.6.6.5 Carcinogenicity

The rat and mouse carcinogenicity studies with histrelin are referred to Shire's approved NDA 19-836 for precocious puberty.

2.6.6.6 Reproductive and developmental toxicology: All reproductive toxicity studies are referred to those conducted under NDA 19-836 for histrelin. See label for Histrelin.

2.6.6.7 Local tolerance

Primary subdermal irritation study of histrelin implant components in rats.

This study was conducted by [ ] as project # — SA-01-KS/93 in accordance wit GLP regulations.

Seven groups of [ ] CD BR COBS male and female rats with body weight range of 175 – 200 g (5/x/g) were used as shown in table below:

Group #	Group identification	Treatment level
1	Sham Control	0
2	Stearic acid	1 pallet
3	Histrelin	1 pallet
4	Strearic acid + histrelin	1 pallet
5	Hydrogel placebo implnat	1 Implant
6	Hydrogel histrelin implant	1 implant
7	Hydrogel stearic acid + histrelin implant	1 implant

Stearic acid pallets were made from 100% stearic acid. Histrelin pallets were made from histrelin. Histrelin pallets with stearic acid contained 2% stearic acid. These 3 pallets were 5 mm x 1.75 mm, and weighed about 13 – 14 mg.
Hydrogel placebo implants were prepared from implant polymer in the form of rods 12 mm x 3.2 mm. The samples were leached in sterile saline solution for 7 days, changing

saline daily. The placebo was packaged individually in 2 ml of sterile saline and sterilized.
Hydrogel histrelin implants are sealed polymer cartridges containing non-pulverized histrelin powder. These implants were leached as the placebo implants. The hydrated implants are 31 mm long and 3.2 mm in diameter and contained approximately 50 mg of 100% histrelin. Hyrogel implants containing 98% histrelin and 2% stearic acid were prepared as above with the same dimensions. However, instead of 100% histrelin the implants were loaded with 4 pallets made of 98% histrelin and 2% stearic acid. The implant weighed approximately 55 mg. In the implant, stearic acid is added at 2% level to facilitate the fabrication of histrelin pallets.

Th pallets and implants were inserted subdermally. Rats were checked every day for behavioral or clinical changes and study was terminated after 14 days. For necropsy, all rats were killed by asphyxiation and the pallets/implants with implantation sites were removed. Tissues surrounding the test articles were subjected to histopathological evaluation.

Results:

Mortality: There were no treatment-related deaths
Body weight: There were no treatment-related effects on the body weight of males at week one or at necropsy at week 2. In female rats, treatment with histrelin in groups 3, 4, 6 and 7, led to significant increase in body weight at necropsy compared to control group due to loss of estradiol synthesis as a result of gonadotropin suppression by histrelin.

Necropsy: No skin lesions were observed in any of the rats treated with pallets/implants. It was stated that stearic acid pallets appeared unchanged and retained their original shape. They elicited no observable adverse reaction locally and slipped off their fibrous tissue capsule easily. In group 3, histrelin pallets had shrunk and lost shape and seemed undergoing biodegradation. They were enclosed in a yellowish mass of thicker tissue than seen in group 2 (stearic acid pallets). Stearic acid + histrelin had findings similar to those seen in group 3. Hydrogel placebo elicited no local reaction. Implants slipped out of their fibrous capsule easily and implant surface was smooth. The findings for hydrogel implant and hydrogel stearic acid + histrelin implant were similar to those for hydrogel placebo.
Microscopic examination: Cellular response and irritation response of each implantation site compared to sham control is shown in table below:

Group #	Group identificaiton	Male	Female
1	Sham Control	2.0 non irritant	0.6 non irritant
2	Stearic acid	6.4 slight irritant	3.2 slight iritant
3	Histrelin	8.4 slight irritant	12.0 slight irritant
4	Stearic acid + histrelin	12.4 slight irritant	13.2 slight irritant
5	Hydrogel placebo implnat	7.0 slight irritant	9.6 slight irritant
6	Hydrogel histrelin implant	7.0 slight irritant	6.6 slight irritant
7	Hydrogel stearic acid + histrelin implant	5.4 slight irritant	6.8 slight irritant

0 –3 = non irritant, 4 – 15 = slight irritant, 16 – 30 = moderate irritant, >30 = severe irritant

Sponsor concluded that all of the single or multiple material test articles caused irritation, however, the degree of irritation for each of the test articles was considered to be within the safe range for subdermal use.

2.6.6.8 Special toxicology studies:

To support the safety of the polymer implant cartridge Hydro Med Sciences has performed the following biocompatibility tests (volume # 38) of the hydrogel implants according to ISO10993 and GLP guidelines:

The following 5 extraction studies were conducted by [ ] for Roberts Pharmaceutical Corporation, Eatontown, NJ in accordance with GLP regulations with some exemptions. All these studies were conducted during 1999-2000.

The HEMA/HPMA Copolymer placebo reservoir extracts were prepared in either saline (NaCl) or cottonseed oil (CSO) as per ISO 10993-12 guidelines. The test article was extracted in 0.9% USP Sodium chloride for injection and cottonseed oil at a ratio of 60 cm^2 per 20 ml of vehicle at 37+/-2 C^o for 72 hours.

1. Intracutaneous injection test – ISO for hydrogel implant reservoir. [ ] Final Report: 99-2949-G8.

In this study 2 male and one female NZW rabbits 10-12 weeks old were used. A volume of 0.2 ml of each test article extract was injected intracutaneously at five sites on one side of each of three rabbits. At five other sites on the other side per rabbit, 0.2 ml of the corresponding control was injected. The injection sites of each animal were observed for signs of erythema and edema immediately, 24, 48 and 72 hours after injection of the test article. Animals weighed at the end of observation period and returned to the colony.

Scoring method: Scores for each animal for erythema and edema at 24, 48, and 72 hours were recorded and total was divided by 15 (5 sites x 3 observation periods). Similar assessment was made for the controls. The control score was subtracted from the test article score and the calculated value for each animal is added and total divided by 3 to obtain Primary Irritation Index (PII).

Results: All rabbits gained weight. None of the animals exhibited overt signs of toxicity. The biological reaction at sites injected with the test article was no greater than that at the control injection sites. The PII for both NaCl and CSO extracts of test article was 0.0. Based on this test article is considered a negligible irritant.

2. Kligman Maximization test (17 animals) – ISO for hydrogel implant reservoir (saline extract). [ ] Final Report: 99-2949-G4.

Seven male and 10 female adult guinea pigs, 26 –67 days old and weighing 381 – 429 g were used. The animals were distributed in 1) experimental NaCl extract (5/s), 2)

negative control NaCl (2 males and 3 female), 3) primary irritation intradermal (1 female) topical NaCl extract (1 female) and 4) historical positive control using DNCB.

Preparation of test animals: On day 0 and on day 7, an approximately 5x7 cm area over the shoulder was prepared by clipping the hair. On day 23, a 4x4 cm area of flanks was prepared.
Before Induction Phase, 2 primary irritation test were performed. The animals were injected 24 hours prior to the start of Primary irritation test with 0.1 ml of FCA mixed 1:1 with the vehicle.
The highest test article concentration that did not cause extensive destruction of the skin after 24 hours was used in the Intradermal Induction Phase. For the Topical irritation study, skin observations were scored at 24 hours to determine whether test article was or was not a skin irritant.
Based on results of topical primary irritation study, the test article extract was found to be non irritating and was applied in full strength at both induction and challenge.

Induction/intradermal application: Three pairs of intradermal injections were made simultaneously as one row of 3 injections on each side of midline. The injection sites were within 2x4 cm patch, which was applied one week following the injection. The dosing solution were as follows:

Experimental group:

1) 0.1 ml FCA 1:1 with NaCl
2) 0.1 ml test article extract
3) 0.1 ml test article extract 1:1 with FCA

Negative control group:

1) 0.1 m FCA 1:1 with NaCl
2) 0.1 ml vehicle alone
3) 0.1 ml vehicle 1:1 with FCA

Positive control group (historical response to DNCB)

1) 0.1 ml FCA 1:1 with NaCl
2) 0.1 ml 0.1% DNCB in 95% EtOH
3) 0.1 ml 0.1% DNCB in 95% EtOH 1:1 with FCA

Topical application (Day 7). Since the test article was not an irritant, the area was pretreated with 10% sodium lauryl sulfate in petrolatum 24 hours before the topical application. For the experimental group, the test article was spread over a 2x4 cm piece of filter paper to saturation, control group animals were exposed to vehicle and positive control animals were exposed to 0.1% DNCB in 95% EtOH. The patch was covered by an impermeable sheet and secured with non-adhesive bandage for 48 hours.

Challenge application: (Day 23). On the left flanks a patch as for induction phase was secured for 24 hours. Patch was saturated with neat test article extract and in positive control animals DNCB in 0.1% in ethanol.

Skin reaction was evaluated at 24, 48 and 72 hours after the challenge exposure period and reaction of 1 or greater for erythema and edema was considered positive.

The scoring system of Kligman was as follows:

Sensitization rate (%) i.e.,Showing skin reaction	Grade	Class
0.-9	1	Weak
9-28	2	Mild
29-64	3	Moderate
65-80	4	Strong
81-100	5	Extreme

Results: all animals showed an increase in body weight. No signs of systemic toxicity were observed. None of the treated or negative control animals exhibited any reaction to the challenge (0% sensitized). Positive controls exhibited skin reaction of 1, 2, or 3 at each observation point (100% sensitized).

Based on these results it was concluded that sodium chloride extract of test article(HEMA/HPMA copolymer placebo reservoir), elicited weak reaction at the challenge following an induction phase, and with a grade 1, it was classified as having weak allergenic potential.

3. Kligman maximization test (17 animals) – ISO for hydrogel implant reservoir (cottonseed oil extract). [ ] Final Report: 99-2949-G5.

This study was repeat of the above study except that a CSO extract of the test article i.e, HEMA/HPMA placebo reservoir was used instead of the saline extract used in the above study.

Results: Cottonseed oil extract elicited no reaction at the challenge (i.e., 0% sensitization), following the induction phase. As such it was classified as a grade 1 reaction and test article was classified as having weak allergenic potential.

4. L929 MEM elution test – ISO for hydrogel implant reservoir. [ ] Final Report: 99-2949-G11.

The purpose of this test is to determine the biological reactivity of a mammalian cell culture in response to test article. The test is designed for the evaluation of test article extracts e.g., polymeric materials and high-density materials.
The test article (HEMA/HPMA copolymer placebo reservoir), positive control article (natural rubber) and negative control article (silicone) extracts were tested. Extracts were adjusted to pH 7.21 and sterilized by passage through 0.22 um pore filter before being applied to cell monolayer.

The cultures were incubated for 48 hours, at 37 +/-1 C, in a humidified atmosphere containing 5% carbon dioxide. The response of the cell monolayer is evaluated under a microscope and the biological activity (cellular degeneration and malformation) is rated on a scale of 0 –4. The test article meets the requirement of the test if none of the cultures treated with the test article show greater than a mild reactivity (grade 2).

Results: Slight biological reactivity (grade 1) was observed in the L929 mammalian cells at 24 and 48 hours which was not considered significant according to ISO. The score for positive control and negative control were 4 and 0, respectively.

Based on these findings, sponsor stated the HEMA/HPMA placebo reservoir to be non-cytotoxic and met requirements of the elution test, ISO 10993-5.

5. Systemic injection test – ISO for hydrogel implant reservoir. [ ] Final Report: 99-2949-G2.

In this study, 20 adult male Albino Swiss mice weighing 17.0 – 22.9 g were used in 4 treatment groups (5/g) as follows:

NaCl test 50 ml/kg, IV 0.1 ml/second
NaCl control 50 ml/kg, IV 0.1 ml/second
CSO test 50 ml/kg, IP
CSO control 50 ml/kg, IP

Animals were observed immediately after injection and then 4, 24, 48, and 72 hours after injection for clinical and toxicological signs. Animals were weighed prior to injection and then at the end of observation period.

Evaluation criteria: The test was considered negative if none of the animals injected with test article show a significantly greater biological reaction than the animals treated with the control article.
If 2 or more mice die, or show signs of toxicity such as convulsions or prostration, or if 3 or more mice lose more than 2 g of body weight, the test article does not meet the requirements of the test.

Results: All test and control animals gained weight. None of the test animals exhibited overt signs of toxicity at any of the observation points.
The test was considered negative for extract of test article (HEMA/HPMA copolymer placebo reservoir) because none of the animals injected with the extracts of the test article showed significantly greater biological reaction than the animals treated with the control articles.

6. Rabbit pyrogen test (material mediated)- ISO for hydrogel implant reservoir. [ ] Final Report: 99-2949-G9.

The pyrogen test involves measuring the rise in temperature of NZW rabbits following intravenous injection of a test article. It is designed for products that can be tolerated by the test rabbit in a dose not to exceed 10 ml/kg, within a period of not more than 10 minutes.
Four adult NZW rabbits (2/s, 3 treated ad one control) were used. The test article extract in NaCl was injected into the marginal ear vein of each rabbit at a dose (10 ml/kg) based on the body weight of each animal. Each injection was completed within 10 minutes. Five temperature readings were taken over a 3-hour period subsequent to recording a baseline temperature using a digital thermometer with thermistor probes.

Evaluation criteria: If no rabbit shows an individual rise in temperature of 0.5 C or more above the baseline temperature, the test article meets the requirements for the absence of pyrogens.

Results: The temperature increases were 0.3, 0.4, and 0.4 C for the 3 treated animals and 0.1 C for the control. The NaCl extract of HEMA/HPMA copolymer placebo reservoir was therefore considered non-pyrogenic.

7. Hemolysis – rabbit blood – ISO for hydrogel implant reservoir. [ ] Final Report: 99-2949-G6.

Experimental design: Whole rabbit blood was collected in . [ ] vacutainer tubes and was utilized within one hour of testing. It was diluted sufficiently in NaCl until 0.2 ml was hemolyzed in 10 ml of USP water for injection and the spectrophotometric absorbance reading at 545 nm was approximately 1.0 absorbance unit. The test article, positive and negative controls were tested in triplicate.
Positive control article solution was obtained by adding 10 ml of USP water for injection per vial. Negative control article solution was obtained by adding 10 ml of 0.9% USP sodium chloride for injection per vial. All vials were incubated in water bath for 30 minutes at 37 C. 0.2 ml of diluted blood was added to all vials and incubated for 60 minutes. After incubation, vials were centrifuged and absorbance of supernatant was determined against NaCl blank at 545 nm.

% hemolysis = Ave. Abs of test article – Ave. Abs of negative control divided by Ave. Abs. of positive control – Ave. Abs. of negative control multiplied by 100.
If the % hemolysis was 5% or less, the test article is considered non-hemolytic.

Results: % hemolysis for the test article was 0.41% and was considered non-hemolytic.

2.6.7 TOXICOLOGY TABULATED SUMMARY

None submitted

OVERALL conclusions and recommendations

Conclusions: Based on review of the nonclinical in-vitro and in-vivo toxicology studies conducted with Hydrogel/Histrelin implant, P/T concludes that the drug/device is safe for the proposed indication.

Unresolved toxicology issues (if any): None

Recommendations: Pharmacology recommends approval of NDA 21-732 for Hydrogel/Histrelin Implant for the palliative treatment of advanced prostate cancer.

Suggested labeling: The mutagenicity studies performed with extracts of the implants with and without histrelin should be included in the label. Also the multiples of the human dose used in animals described under Carcinogenesis, Mutagenesis, Impairment of Fertility and under Overdosage sections should be expressed in relation to Vantas, the histrelin subdermal implant.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

APPENDIX/ATTACHMENTS- NONE

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/s/

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