# CENTER FOR DRUG EVALUATION AND RESEARCH

## APPROVAL PACKAGE FOR:

## APPLICATION NUMBER
## 21-343

# Pharmacology Review(s)

# PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA number: 21-343
Review number: 1
Serial number/date/type of submission: 000/3-22-2001/original submission
Information to sponsor: Yes ( ) No (* )
Sponsor and/or agent: ATRIX Laboratories, Fort Cllins, CO
Manufacturer for drug substance: [redacted]

Reviewer name: Krishan L. Raheja, D.V.M., Ph.D.
Division name: Reproductive and Urologic Drug Products
HFD #: 580
Review completion date:

Drug:

Proposed Trade names: One-month [redacted] 7.5 mg; One-month [redacted] 7.5 mg: One-month [redacted] 7.5 mg

Generic name (list alphabetically): Leuprolide acetate

Code name: LA-2500 (7.5 mg)

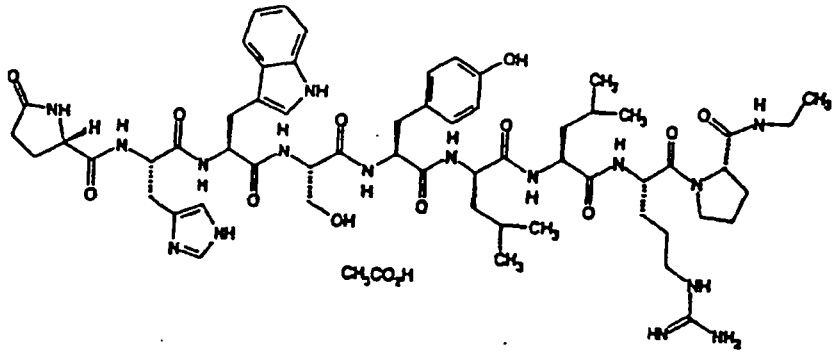
Chemical name: 5-oxo-L-prolyl-L-histidyl-L-tryptophyl-L-seryl-L-tyrosyl-D-leucyl-L-leucyl-L-arginyl-N-ethyl-L-prolinamide acetate

CAS registry number: 74381-53-6

Mole file number: -

Molecular formula/molecular weight: $C_{59}H_{84}N_{16}O_{12} . C_2H_4O_2$/1269.48 Daltons

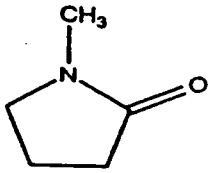
Structure:



Excipient:

Generic name: 1-methyl-2-pyrrolidone
Synonyms/codes: N-methylpyrrolidone
NMP
N-methylpyrrol
H-20417

CAS Registry No.: 872-50-4
Molecular weight: 99.13

Structure: 

Relevant INDs/NDAs/DMFs: INDs [redacted] DMF [redacted]

Drug class: GnRH agonist

Indication: for the palliative treatment of advanced prostate cancer

Clinical formulation: LA-2500 - 7.5 mg is designed as a parenteral drug product that consists of a sterile syringe containing the [redacted] active drug substance, leuprolide acetate, a syringe containing the polymeric ATRIGEL Delivery System and a sterile needle for injection. The delivery system is composed of poly (DL-lactide-co-glycolide)-COO⁻ dissolved in N-methyl-2-pyrrolidone. The drug product is mixed immediately before patient administration and injected subcutaneously. The drug product is designed to deliver a nominal 7.5 mg of leuprolide acetate over a one-month period. The maximum injection mass is approximately 250 mg.

Composition of [redacted] 7.5 mg constituted drug product is as follows:
Table 1

| Component | % w/w | Dose delivered mg/unit |
|---|---|---|
| Leuprolide acetate Ph.Eur. | [redacted] | 7.5 |
| Poly (DL-lactide-co-glycolide) | [redacted] | 82.5 |
| N-methyl-2-pyrrolidone(NMP) | [redacted] | 160.0 |
| Total delivered amount: 250 mg | | |

Based on total absorption of 160 mg NMP, daily exposure will amount to 5.3 mg.

Mechanism of action of the delivery system: The ATRIGEL Delivery System is a polymeric delivery system consisting of a water-insoluble biodegradable polymer (poly DL-lactide-co-glycolide) in a biocompatible water-soluble solvent (N-methyl-2-pyrrolidone). When the polymer system is injected into the body, the water-soluble solvent diffuses from the site and water permeates into the polymer matrix. Because polymer is water-insoluble, it precipitates or coagulates upon contact with aqueous body fluid to form a solid implant.

Route of administration: Subcutaneous

Proposed use: [redacted] 7.5 mg is an injectable extended-release subcutaneous formulation intended for once monthly injection as palliative treatment of advanced prostate cancer.

Disclaimer: Tabular and graphical information is from sponsor's submission unless stated otherwise.

## OVERALL SUMMARY AND EVALUATION:

**Introduction:** Leuprolide acetate is a potent Gn-RH (LH-RH) agonist used clinically for the palliative treatment for advanced prostate cancer. Leuprolide acts by preventing pulsatile hypothalamic stimulation of adenohypophysis, which results in reduced gonadotropic hormone release and suppression of gonadal testosterone to levels associated with surgical castration (< 50 ng/dl in serum). As little as 1 mg leuprolide acetate administered daily or after administration of depot formulations at intervals of one month or longer has been demonstrated to achieve prolonged testosterone suppression.

Atrix Laboratories has developed the [redacted]7.5 mg drug product, a sustained release formulation of leuprolide acetate, for the palliative treatment of advanced prostate cancer. Repeated, monthly treatment with [redacted]7.5 mg provides sustained levels of active drug resulting in continuous suppression of gonadal testosterone synthesis[redacted] is administered using Atrigel Delivery system. As administered, it is biodegradable polymeric formulation consisting of . % 50/50 poly (DL-lactide-co-glycolide) COOH (PLGH)/. % N-methyl-2-pyrrolidone (NMP) combined with leuprolide acetate ( % w/w).

**Safety evaluation:** The safety of leuprolide acetate is well established as it has been approved by the FDA as leuprolide acetate for injection and Lupron Depot as leuprolide acetate depot suspension under various NDAs for the treatment of both malignant and benign conditions. Lupron injection is approved for the palliative treatment of the advanced prostate cancer and for the treatment of precocious puberty. Lupron Depot 3.75 mg is approved for the treatment of endometriosis, Lupron Depot 7.5 mg and Lupron Depot-3 month 22.5 mg for the palliative treatment of prostate cancer, and Lupron Depot-PED 7.5, 11.5 and 15 mg for the treatment of children with central precocious puberty.

The present formulation under consideration differs from Lupron Depot in that its delivery system contains N-methyl-2-pyrrolidone as solvent, which has not been used in any other approved formulation for the proposed indication.

Sponsor however, has an approved product, ATRIDOX (doxycycline hyclate, 8.5%) in the Atrigel Delivery System for controlled release in subgingival application for treatment of chronic adult periodontitis under NDA 50-751. This product is meant for single application which exposes the subject for a period of 7 days. Labeling states that application of Atridox may be repeated 4 months after initial treatment. Because of Atridox short-term treatment, P/T review pertained primarily to doxycycline and a very short summary was provided for NMP.

**Note:** Atridox related IND[redacted] is listed as inactive and as such is not known if any P/T studies were submitted and reviewed.

**Safety issues relevant to clinical use:** Leuprolide acetate is approved for many indications and has excellent safety record. The excipient, NMP used in[redacted] has been approved previously by the FDA for periodontal disease for sponsor's product, Atridox. As such there seems to be no obvious safety concern.

In the ICH guidance document (Q3C) on impurities: residual solvents (Fed. Reg. Vol 62, No. 247, December 24, 1997) NMP is classified as Class 2 solvent and its permitted daily exposure (PDE) is listed as 48.5 mg. The guidance however, also states that the Class 2 solvents use should be limited as these could be nongenotoxic animal carcinogens or possible causative agents of other irreversible toxicity as neurotoxicity or teratogenicity. Also NMP is not listed as GRAS.
As NMP has not been approved for long-term use as for the present proposed indication in any drug product, the review will pertain to PK and toxicity studies conducted with drug product containing the excipient, NMP or excipient alone.

**Other clinically relevant issues:** none

**Conclusions:** Since leuprolide acetate and DL-lactide-co-glycolide polymer are FDA approved articles in many drug products for the treatment of both malignant and benign indications and NMP is approved for the treatment of periodontal disease, Pharmacology considers [redacted] safe for the palliative treatment of advanced prostate cancer.

**Communication review:**

Labeling review: Label for [redacted] is essentially copy of TAP's Lupron label. The fact that no carcinogenicity studies have been conducted with [redacted] is added in the label.

**Recommendations:** Based on the safety of leuprolide acetate (active ingredient), which has been approved under several NDAs for many malignant and benign indications and that of N-methyl-2-pyrrolidone (excipient) as established via review of literature and that of DMF [redacted] Pharmacology recommends approval of NDA 21-343 for the palliative treatment of advanced prostate cancer.

**Internal comments:** none

External **recommendations (to sponsor)**: none

**Draft letter content for sponsor (if not same as above):**

**NDA issues:** none

**Reviewer signature:**

**Team leader signature** [concurrence/non-concurrence]:

cc: list:
Original NDA 21-343
HFD-580
HFD-580/A.Jordan/A.Batra/J.Best
N 21343.000/11-14-01

**Memorandum of non-concurrence** (if appropriate, attached):

**Addendum to review** (if necessary):

**Studies reviewed within this submission:** All pre-clinical studies, which have not been reviewed under sponsor's INDs.

**Studies not reviewed within this submission:** All studies previously reviewed under various INDs. Copies of the IND reviews are appended with the present NDA review.

**Introduction and dug history:** see under Overall Summary and evaluation.



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## PHARMACOLOGY:

**Primary pharmacodynamics:**

Mechanism of action: Leuprolide acetate is an agonist analog of leutinizing hormone releasing hormone (LH-RH, Gn-RH). Following the administration of leuprolide acetate, there is an initial increase in circulating levels of leuteinizing hormone (LH) and follicle stimulating hormone (FSH). This causes a transient increase in levels of the gonadal steroids (testosterone and dihydrotestosterone in males, and estrone and estradiol in females). However, continuous administration of leuprolide acetate at therapeutic doses inhibits pituitary gonadotropin secretion and suppresses testicular and ovarian steroidogenesis. As a result serum testosterone is reduced in males to castrate levels i.e. equal or below 50 ng/dl and estrogen in females is reduced to postmenopausal levels. The efficacy of leuprolide acetate in prostate cancer treatment is based on its ability to decrease plasma testosterone levels to castrate levels.

The ATRIGEL Delivery System, by virtue of the polymer being insoluble in water, precipitates or coagulates after solvent, NMP diffuses from the injection site and polymer comes in contact with aqueous body fluid to form a solid implant for sustained release of leuprolide acetate.

Drug activity related to proposed indication: Since the growth of hormone sensitive prostate cancer is dependent on circulating testosterone levels, reduced testosterone levels by the administration of [redacted] results in regression of tumor tissue.

**Secondary pharmacodynamics: -**

**Pharmacology summary:** Continuous administration of the drug substance, leuprolide acetate desensitizes the pituitary GnRH receptors, resulting in decreased gonadotropin secretions and subsequent suppression of testosterone levels associated with regression of hormone sensitive prostate cancer.

**Pharmacology conclusions:** The drug product, [redacted] works by inhibiting pituitary gonadotropin secretion and suppression of testicular steroidogenesis.

## SAFETY PHARMACOLOGY:

Safety pharmacology of the drug substance, leuprolide acetate is well established. However, the safety pharmacology of the excipient, NMP has not been directly evaluated. Some information on the various parameters of safety pharmacology of NMP have been are studied in some of the toxicity studies referred to literature citations, which is described below.

**Neurological effects:** Neurobehavioral battery of tests conducted pre-dose and then 4, 8 and 13 weeks of treatment in rats with NMP at oral doses of 90, 225 and 540 mg/kg/day had no adverse effects except increased foot splay at the mid and high doses and high incidence of low arousal and slight palpebral closure, the later suggestive of sedative effect in high dose.

**Cardiovascular effects**: Intravenous administration of NMP at doses of 100 and 200 mg/kg in rats produced a slight transient hypotensive effect without alterations in the ECG and a dose of

50 mg/kg had no effect. A high dose of 500 mg/kg produced marked hypotension and ECG alterations of bradycardia and A/V block, attributed to a reflex vagal action.

**Pulmonary effects:** none mentioned

**Renal effects:** none mentioned

**Gastrointestinal effects:** none mentioned

**Abuse liability:** none mentioned

**Other:** Intravenous doses of 200 or 500 mg/kg produced hyperglycemia via catecholamine release.

**Safety pharmacology summary:** Safety pharmacology of NMP is not adequately investigated. However information available from literature suggests that it had no significant neurological/behavioral effects. It causes slight transient hypotensive effects at very high dose levels.

**Safety pharmacology conclusions:** NMP as used in the proposed formulation, [redacted] seems quite safe for the proposed indication. The daily total dose of NMP from the monthly dose of [redacted] will amount to 5.3 mg, which is very low compared to doses used in the PK/TK and toxicity studies and permitted daily exposure (PDE) of 48.5 mg as a Class 2 solvent. Moreover, NMP is an approved excipient in Atridox used as a single application of 450 mg for the treatment of chronic periodontal disease.

## PHARMACOKINETICS/TOXICOKINETICS:

PK/TK of leuprolide acetate is not reviewed as it has been extensively studied and published.

The following PK studies were conducted with Atrigel formulation.

**Study title: ATLS-81: Evaluation of the pharmacokinetics of an Atrigel formulation containing leuprolide acetate following multiple subcutaneous injections in dogs". volume 1.16 0f 1.55. page 1.**

This study was conducted by [redacted] in accordance with the FDA Good Laboratory Practices Regulations.

The primary objective of this study was to determine the PK of Atrigel formulation containing leuprolide acetate by measuring serum testosterone levels after 3 sc injections given once every 30 days at implant sites A, B and C. The secondary objective was to compare effects of sterile filtration vs gamma-irradiation of Atrigel formulation on serum testosterone levels. A supplementary objective was to retrieve all SC test sites at termination for histological analysis.

Eight mature, male beagle dogs were used in each of the 2 groups. Group 1 received the irradiated while group 2 received the sterile filtration product. A dose of 0.25 cc was used to deliver 7.5 mg of leuprolide acetate, an amount that is delivered by the 30-day Lupron Depot formulation. Blood was collected on Study Day 0 and on Days 1, 3, 7, 15, 21 and 30 or 31 following each injection. On the 31$^{st}$ day after the final injection all dogs were euthanized and test sites were retrieved. Macroscopic observations were made of the SC tissue and area around each implant. All identifiable test sites recovered at necropsy were evaluated histologically. No gross necropsies were performed.

**Results:**

The average amount of formulation injected was 238 and 242 mg on Day 0, 242 and 231 mg on Day 30 and 246 and 248 mg on Day 69 for groups 1 and 2, respectively.

Serum testosterone and leuprolide levels: As shown in table 2 below, testosterone levels were decreased to castration levels and no increase was observed on repeat administration.

Table 2

| Day | Serum testosterone (ng/nl) group 1 | group 2 | Serum leuprolide (ng/ml) group 1 | group 2 |
|---|---|---|---|---|
| 0 | 0.61 | 2.42 | 0.10 | 0.11 |
| 1 | 2.95 | 4.86 | 4.50 | 6.79 |
| 3 | 4.84 | 3.01 | 1.05 | 1.16 |
| 7 | 0.22 | 2.23 | 0.38 | 3.53 |
| 15 | 0.80 | 0.18 | 0,30 | 0.44 |
| 21 | 0.50 | 0.09 | 0.61 | 0.61 |
| 30 | 0.19 | 0.13 | 0.31 | 0.33 |
| 31 | 0.08 | 0.05 | 4.40 | 9.06 |
| 33 | 0.09 | 0.05 | 1.18 | 2.01 |
| 37 | 0.22 | 0.06 | o.30 | 1.03 |
| 45 | 0.58 | 0.06 | 0.29 | 0.40 |
| 51 | 0.38 | 0.05 | 0.48 | 0.41 |
| 60 | 0.33 | 0.05 | 0.43 | 0.39 |
| 61 | 0.15 | 0.05 | 4.20 | 7.64 |
| 63 | 0.12 | 0.05 | 1.00 | 0.89 |
| 67 | 0.12 | 0.05 | 0.41 | 1.78 |
| 75 | 0.07 | 0.05 | 0.28 | 0.43 |
| 81 | 0.10 | 0.05 | 0.38 | 0.30 |
| 91 | 0.09 | 0.05 | 0.64 | 0.52 |

Macroscopic observations: At site A no implants were found in 4 dogs in group 1 and in 2 dogs in group 2. When found implants were small in size and soft in consistency. Only 4 implants were found in group 1 and 5 in group 2 at site B. All C implants were identifiable in both groups. The implants were larger than those seen at previous time points. The consistency of implants ranged from hard to soft and all were fragmenting and showing signs of degradation. Tissue around implants appeared normal. Most implants were scored none to moderate for irritation.

Microscopic observations: The cellular reaction was mild to moderate with some granulation tissue observed. Most sites were discrete nodules with a central area of amorphous material or a clear space representing the site of the implanted material surrounded by a layer of epitheliod macrophages. Just beyond this layer was a cellular layer, which was surrounded by a layer of collagenous connective tissue that was well demarcated from surrounding fat. No lesions were found in the epidermis, dermis or skeletal muscle.

Clinical observations: No irritation or adverse events were noted.
Overt toxicity: No signs of overt toxicity were reported.
Body weight: was not affected by treatment.

**Note:** It was reported that the sterile filled formulation was more difficult to inject and was painful upon injection in some of the dogs.

**Conclusion:** Results showed that testosterone was suppressed to castrate level and remained suppressed following second and third injections. Repeat administration did not result in increased serum leuprolide levels.
Also under study ATRS-277, it was observed that circulating leuprolide acetate levels of > 100 – 200 pg/ml were sufficient to suppress testosterone following SC administration in dogs.

**ATLS-82: Evaluation of the extended release of leuprolide acetate from an Atrigel formulation following subcutaneous injection in dogs. volume 1.16 of 1.55, page 167**

The objective of this study was similar to those described under ATLS-81, except that irradiated and sterile filtration Atrigel products were compared with Lupron Depot.
There were 8 mature, male beagle dogs in each of the 3 treatment groups, i.e., gamma-irradiation group 1, sterile filtration group 2 and Lupron Depot group 3. Preparations were injected SC once and serum testosterone determined at various time intervals up to Day 63. Average injection weights for the test article were 240.4 mg for group 1 and 239.6 mg for groups 2. The injection weight for Lupron Depot was 1.0287 mg.

**Results:**

Serum testosterone levels: As shown in table 3 below, over a period of 30 days, mean testosterone levels (ng/ml) were suppressed to near 0.5 ng/ml a few times in group 1. Suppression below 0.5 ng/ml was seen in groups 2 and 3. The effect of Lupron Depot lasted up to 56 days while that of Sterile Atrigel preparation up to 30 days.

Table 3

| Day | Group 1 Gamma irradiated | Group 2 Sterile Filtration | Lupron Depot 30 day |
|---|---|---|---|
| -3 | 2.31 | 2.06 | 5.17 |
| 6hr | 6.06 | 4.49 | 5.23 |
| 12 hr | 3.03 | 2.59 | 2.66 |
| 1 | 4.56 | 4.04 | 5.09 |
| 2 | 5.81 | 4.41 | 6.19 |
| 3 | 6.69 | 5.77 | 4.99 |
| 7 | 0.55 | 0.98 | 1.90 |
| 14 | 0.66 | 0.46 | 0.24 |
| 22 | 0.96 | 0.49 | 0.15 |
| 28 | 0.49 | 0.31 | 0.11 |
| 30 | 1.01 | 0.35 | 0.17 |
| 32 | 0.90 | 0.22 | 0.25 |
| 34 | 1.53 | 0.47 | 0.35 |
| 36 | 0.98 | 0.38 | 0.27 |
| 38 | 1.93 | 0.37 | 0.41 |
| 40 | 2.54 | 0.58 | 0.75 |
| 42 | 2.02 | 0.67 | 0.44 |
| 49 | 2.73 | 0.91 | 0.62 |
| 56 | 2.21 | 1.41 | 0.43 |
| 63 | 2.37 | 1.58 | 1.58 |
| | | | |

As under ATLS-81 clinically no irritation or adverse events or any signs of overt toxicity were reported. Body weight was not affected by treatment.

**Note:** Although the sponsor stated that sterile filled product is comparable to Lupron Depot in efficiency, results clearly demonstrate that Lupron effect last much longer. The gamma irradiated product was inferior compared to other 2. Also it was reported that the sterile filled formulation was more difficult to inject and that the injections were painful in some of the dogs.

**Comments:** Based on this data it would seem that Atrigel may not keep testosterone suppressed for 30 days in humans with irradiated formulation. The product seems less effective compared to Lupron.

**Conclusion:** Although sponsor concluded that the sterile filled Atrigel formulation and Lupron Depot are comparable, results clearly suggest that Lupron Depot has longer duration of action. Also it was stated that the Atrigel formulation was difficult to inject and injections were painful.

However, in an other study, SC administration of gamma-irradiated formulation containing % PLGH in % NMP and Lupron Depot were considered equally effective in suppressing serum testosterone in dogs as shown in table 4 below (ATRS –219)

Table 4

| Day | Av. serum testosterone (ng/ml) | |
|---|---|---|
| | Group 1 | Group 2 |
| 1 | 2.23 | 3.52 |
| 3 | 5.50 | 4.85 |
| 7 | 0.47 | 1.05 |
| 14 | 0.60 | 0.39 |
| 21 | 0.36 | 0.07 |
| 28 | 0.24 | 0.08 |
| 35 | 0.15 | 0.07 |
| 42 | 0.51 | 0.31 |
| 49 | 2.09 | 0.30 |
| 55 | 2.85 | 1.08 |
| 63 | 4.95 | 0.77 |
| 77 | 4.82 | 1.22 |
| 91 | 2.23 | 2.84 |
| 135 | a/a | n/a |

Group 1 is Atrigel formulation and group 2 is Lupron depot

Also gamma-irradiated and non-irradiated Atrigel had similar testosterone suppression profile (ATRS 180 and 226) and leuprolide release profile following SC Vs IM administration in male rats (ATRS-186).
Under study ATRS-170: Evaluation of the 28-day release kinetics of leuprolide acetate from various Atrigel formulations made in NMP and DMSO following subcutaneous injection in rats (volume 1.18 of 1.55, page 98), it was reported that the solvent had no effect either on release of leuprolide acetate or in affecting the suppression of testosterone.

**Pharmacokinetic studies with the excipient, NMP:**

Only PK data for the excipient, N-methyl-2-pyrrolidone as submitted by the under DMF for (NMP) entitled as **"Oral, dermal and inhalation pharmacokinetics and disposition of [2-$^{14}$C] NMP in the rat" Report # 630-95** was reviewed under IND submission dated 3-17-2000. Copy of this review is appended.

**Results** of this study demonstrated that NMP is absorbed after all routes of administration. Once absorbed it is distributed in the organs of elimination i.e. the kidney, liver, lungs, G.I. tract, skin and carcass. It was metabolized and three radioactive components were separated in the urine. The major component (peak 3) in urine was identified as 1-methyl-5-hydroxy-2-pyrrolidone. Peaks 1 and 2 were not identified. Excretion was mostly by the urinary route.

In a study by Well and Digenis (ASPET 16: 243-249, 1988) entitled "Disposition and metabolism of double-labeled [$^{3}H$ and $^{14}C$] N-methyl-2-pyrrolidone in the rat" following PK information was provided after a single iv dose of 45 mg/kg of [methyl-$^{14}C$]NMP, [4-$^{3}H$]NMP, or [ring-$^{14}C$]NMP.

**PK parameters:**

**Absorption:** Absorption was rapid. Plasma levels of intact NMP were relatively unchanged at about 50 ug/ml from 30 minutes to 2 hours. At 6 hours post-dose, plasma levels declined to about 35 ug/ml. $T_{1/2}$ of elimination of radioactive intact NMP plasma were 6.6, 6.9 and 9.9 hours for 3 labeled NMP, respectively. The apparent Vd was 0.33, 0.25 and 0.47 litters/kg, respectively. Metabolites were not detected in plasma until 6 hours.

**Distribution:** Tissues containing the greatest amount of radioactivity at 6 hours (expressed as % of the dose) for all 3 radiolabled isomers of NMP were liver, small and large intestine, testes, stomach and kidneys. Total average recovery of radioactivity from combined excised tissues at 6 hours was 9.3%, 9.6% and 10.2% respectively for the 3 isomers of NMP. Values decreased to 0.9%, 0.8% and 2.9% of the dose at 24 hours.

**Metabolism:** Urine contained one major and 2 minor metabolites. The major metabolite, representing 70-75% of the administered dose of radioactivity, was found to retain all 3 radiolabeled positions. Acid hydrolysis of the major metabolite yielded 4-(methylamino)-butenoic acid. The major metabolite was identified by the same authors as 5-hydroxy-NMP (Wells et al. Isolation and identification of the major urinary metabolite of N-methylpyrrolidone in the rats. ASPET 20: 124-126, 1992).

**Excretion:** The major route of excretion of radioactivity was urine. Within 6 hours after dosing, 17-26% was eliminated for all 3 radiolabled isomers. The rate of excretion increased from 6-12 hours, in which time about 50% of the dose was eliminated. By 12 hours accumulated excretion was 70%, 75% and 70%, respectively. At 72 hours the respectively values were 85%, 82% and 93%.
Cumulative excretion of radioactivity in to feces was only 2.4% of the administered dose recovered after 72 hours for the $^{14}C$ isomers and 1.4% for the tritium labeled NMP. The majority of $^{14}C$ exhaled occurred between 9-12 hours and amounted from 0.84% to 1.1%.
Cumulative biliary excretion in radiolabeled isomers after 3.5 hours in bile cannulated rats was 1.9%.

**Other studies:** none

**PK/TK summary:** NMP had a rapid distribution phase followed by a slow elimination phase. The route of excretion of radioactivity was mainly via the urine. Liver and intestine accumulated the highest radioactivity. Urine revealed the presence of one major and 2 minor metabolites.

**PK/TK conclusions:** Only PK and no TK data was submitted for NMP.

## GENETIC TOXICOLOGY:

**Study title: Genotoxicity: Salmonella typhimurium reverse mutation study report**

**Key findings:** Drug product showed mutagenic potential only when tested with S.typhimurium strain TA1538

**Study no**: ATLS-85
**Study type** (if not reflected in title):
**Volume #, and page #:** 1.18 of 1.55
**Conducting laboratory and location:**

**Date of study initiation:** 10-13-1998
**GLP compliance:** yes
**QA reports:** yes ( * ) no ( )
**Drug, lot #, radiolabel, and % purity:** Lot: 271077-A (irradiated polymer formulation) 50090-LA-1 ( leuprolide acetate drug), purity not given
**Formulation/vehicle: solution/DMSO**

**Methods:**

Strains/species/cell line: salmonella typhimurium strains TA98, TA100, TA1538, TA1537 and TA1535
Dose selection criteria:
Basis of dose selection: not given. Only preliminary toxicity screen using 0.1 ml of test article in DMSO used (concentration of solution used not mentioned)
Range finding studies: none
Test agent stability: dissolved in DMSO. Not tested in other solvent, especially NMP which is used in clinical formulation
Metabolic activation system: S-9 mix
Controls:
Vehicle: DMSO
Negative controls: DMSO
Positive controls: Dexon with and without S9 activation with strains TA98, TA100 and TA1537; nitrofluorene with and without S9 activation with strain 1538; 2-aminofluorene with and without S9 activation with strains TA100 and TA1538; and sodium azide with and without S9 activation with strains TA100 and TA1538.
Comments:
Exposure conditions for standard plate incorporation assay: Separate tubes containing 2 ml of molten top agar with histidine-biotin solution were inoculated with 0.1 ml of culture for each of the 5 tester strains and 0.1 ml of the DMSO test article solution. A 0.5 ml aliquot of the S9 activation system was added when necessary. The mixture was poured across triplicate minimal E plates. Parallel testing was conducted with a negative control and 4 positive controls.
Incubation and sampling times: Plates were incubated at 37 C for 48-72 hours. Following incubation spontaneous revertant, number of revertants from triplicate testing for the negative and positive control was determined.

Doses used in definitive study: O.1 ml used with no concentration given
Study design: The study design used only a single 0.1 ml dose, it did not describe the drug concentration. Also in the toxicity screen sponsor did not use dose higher that used in the definitive assay.

Analysis:
No. of replicates: 3
Counting method: not mentioned
Criteria for positive results: A two-fold or greater increase in the number of mean revertants over the mean values obtained from the negative control

**Results:** standard plate incorporation assay results for the 5 S. typhimurium strains used are shown in table 9 below. Values for revertants are mean of 3 plates.
Table 9

| Treatment | Salmonella typhimurium tester strains | | | | |
|---|---|---|---|---|---|
| | TA98 | TA100 | TA1535 | TA1537 | TA1538 |
| DMSO(-control) | 28 | 142 | 16 | 6 | 9 |
| DMSO test solution | 33 | 128 | 16 | 8 | 8 |
| DMSO w/S9(-control) | 35 | 131 | 16 | 7 | 11 |
| DMSO test solutionw/S9 | 29 | 139 | 17 | 6 | 42* |
| Dexon | 661 | 779 | NA | 517 | NA |
| Dexon w/S9 | 560 | 821 | NA | 437 | NA |
| Sod. Azide | NA | NA | 1472 | NA | NA |
| Sod. Azide w/S( | NA | NA | 1461 | NA | NA |
| 2-nirtofluorenw | NA | NA | NA | NA | 1195 |
| 2-nitrofluorene w/S9 | NA | NA | NA | NA | 352 |
| 2-aminofluorene | NA | NA | NA | NA | 23 |
| 2-aminofluoren w/S9 | NA | NA | NA | NA | 523 |

NA=not applicable

Thus according to criteria set for a positive result, the drug product was mutagenic when tested with TA1538.

To confirm the positive response, sponsor conducted a dose-response assay using 3 lower non-toxic doses. Since there was no linear dose response relationship, it was concluded that compound is not mutagenic. Sponsor however, ignored the fact that the original dose itself was non-toxic in the toxicity screen.

**Summary of individual study findings:**

Study validity: Study as conducted without a dose range finding study and only single dose of 0.1 ml/plate was used.
Study outcome: The product had positive response only with S.typhimurium TA1538 and was attributed to the test article being composed of peptide having 11% by weight histidine.

**Study title: Mutagenicity and cytotoxicity of N-methyl-2-pyrrolidone and 4-(methylamino)butanoic acid in the salmonella/microsome assay.**

This study was conducted by David A. Wells, Harvey F. Thomas and George A. Digenis and published in Journal of Applied Toxicology 8(2): 135-139, 1988. Volume 1.22 of 1.25 page 274

In this study NMP and its hydrolytic product N-MeGABA were examined for mutagenicity and cytotoxicity in the Ames test. Salmonella strains TA100, TA102, and TA104 (base pair substitution strains); TA97 and TA'98 (frameshift strains); and TA2638, UTH8413 and UTH8414 (repair proficient strains) were used. The dose ranged from 0.01 to 1000 umol/plate for NMP and 0.01 to 316 umol/plate for N-MeGABA.
It was summarized that neither compound was detectably mutagenic when tested in the presence and absence of metabolic activation. NMP did show significant responses with strains TA102 and TA104 that were less than 2 fold over background, but no clear dose-response relationship was evident. With pre-incubation modification of the assay, using strains TA98 and TA104, mutagenic activity was not observed for NMP, while N-MeGABA showed a significant responses with TA104 but dose-related mutagenicity was not established. Using pre-incubation method both compounds were cytotoxic at the highest treatment doses.
**Note:** The authors concluded that additional short-term studies and those employing mammalian cell systems might further elucidate any possible genetic activity with either NMP or N-MeGABA and provide data for risk assessments.

**Note:** It was not stated if the study was conducted in accordance with GLP regulations.

A mutagenicity test on NMP using the salmonella/mammalian microsome reverse mutation assay was also conducted by the [redacted] in 1991 and is described as HLA study No. 12596-0-401R under DMF [redacted] volume 3.1.

The study was entitled **"Salmonella/mammalian-microsime reverse mutation assay (Ames test) with a confirmatory assay"**
It was stated that the study was conducted in accordance with general requirements of appropriate GLP regulations.
The doses were selected based on results of a dose-range finding study. Doses used in final assay ranged from 100 to 5000 ug/plate both in the presence and absence of S9. The of S. typahimurium consisted of TA98, TA100, TA1535, TA1537 and TA1538.

Results: At all dose levels appearance of background lawn was normal. In both the initial and confirmatory assay, NMP did not cause a positive increase in the number of histidine revertants/plate of any of the tester strains either in the presence or absence of S9.

**Study title: Aneuploidy induction in Saccharomyces cerevisiae by two solvents compounds, 1-methyl-2-pyrrolidone and 2-pyrrolidone.**

This study was conducted by Vernon W.Meyer, Carol J. Goin, and Rhoda E. Taylor-Meyer and published in Environmental and Molecular Mutagenesis 11: 31-40, 1988.

No statement was provided if the study was conducted in accordance with GLP regulations.

The study was conducted because a number of solvent compounds that were tested in S. cerevisiae were potent inducers of aneuploidy, although they did not induce any other genetic effects. The authors tested 1-methyl-2-pyrrolidone and found it to induce aneuploidy. When several other structurally related compounds were tested 2-pyrrolidone induced aneuploidy but succiniamide, pyrrolidine, 1-methylpyrrolidine, 1-methyl-3-pyrrolidol, and 2-pyrrolidineethanol did not. Maleimide and its N-hydroxy, N-methyl, and N-ethyl derivatives were also negative for aneuploidy induction.

conducted mutagenicity test on NMP in the CHO/HGPRT (Chinese hamster ovary cells/hypoxanthine-guanine phosphoribosyl transferase) forward mutation assay (HLA study No. 10194.0-435. DMF volume 1.1).

The study was entitled **"Mutagenicity test on N-methyl-2-pyrrolidone Lot number 9094-126A in the CHO/HGPRT forward mutation assay: Final report**
This study was conducted in 1988 in accordance with general requirements of appropriate GLP regulations.
In the dose range finding study, it was observed that NMP was non-toxic at all dose levels tested from 0.005 mg/ml to 5.0 mg/ml under both non-activation and S9 activation test conditions. The relative survival was 100% and cloning efficiency was 90 -108%. For each test condition, 6 dose levels that ranged from 0.5 mg/ml to 5.0 mg/ml were used in the mutation assays.

Under non-activation test conditions, the 6 cultures treated with NMP showed no dose-related toxicity as measured by either relative clonal survival, which ranged from 99 to 127% of negative control or relative population growth, which ranged from 69 to 95% of control.
No cultures had mutant frequencies (= total mutant colonies/No of dishes x 2 x $10^5$ x absolute cloning efficiency) that were statistically higher than mutant frequencies (MF) of the concurrent controls. All treated cultures had MF less than threshold of 15.0 X $10^{-6}$ for acceptable background mutant frequency values. Mutant frequency (in $10^{-6}$ units) was 2.1-8.4 for the negative controls; 0.8 to 4.2 for the test article and 145.7 for the positive control (150 ug/ml 5-bromo-2-deoxyuridine).

Under metabolic activation conditions, all 6 treated cultures survived treatment and were analyzed. Under these conditions, relative clonal survival ranged from 75 to 97% of negative control and relative population growth ranged from 94 to 109% of control.
The mutation frequencies for the negative control ranged from 0.8 to 2.8, that for the test article from 0.8 to 6.5 and for the positive control (3-methylcholanthrene) 192.0. The 6.5 value for the high dose test article (5 mg/ml) was statistically significantly higher compared to negative control values of 0.8 and 2.8 x $10^{-6}$ but well within the accepted background mutant frequency of <15 x $10^{-6}$.

Also negative result was obtained in a rat primary hepatocyte UDS assay conducted by Results of this GLP study entitled **"Mutagenicity test on N-methylpyrrolidone in the rat primary hepatocyte unscheduled DNA synthesis assay" Final report. This report is included in DMF volume 1.1.**

This study as the above CHO/HGPRT forward mutation assay was conducted in accordance with general requirement of appropriate GLP regulations.

In this assay freshly prepared hepatocytes were exposed for 18 hours to NMP at concentrations up to 5000 ug/ml to 0.500 ug/ml. Treatments from 5000 ug/ml to 500 ug/ml were selected for UDS analysis and covered a range of toxicity (53.2% to 98.6% survival).

The results of the study are given in table 10 below:

Table 10

| Test condition | Concentration Ug/ml | UDS [1] grains/nuclelus | Avg [2] % nuclei with > 6grains | Avg [2] % nuclei with>20 grains | % Survival at 21 hours |
|---|---|---|---|---|---|
| Negative control | -- | -1.14 | 3.3 | 0.0 | 100.0 |
| Positive control | 0.1 | 13.58 | 80.0 | 18.7 | 98.2 |
| NMP | 5000 | -0.59 | 2.7 | 0.0 | 53.2 |
| | 4000 | -0.45 | 1.3 | 0.0 | 64.6 |
| | 3000 | -0.12 | 0.7 | 0.0 | 72.2 |
| | 2000 | -0.07 | 0.0 | 0.0 | 81.7 |
| | 1000 | -0.87 | 0.7 | 0.0 | 97.7 |
| | 500 | -0.06 | 0.7 | 0.0 | 98.6 |

1 UDS= average of net nuclear grains counts on triplicate coverslips (150 total cells)
2 = average values for triplicate coverslips
positive control was 2-acetylaminofluorene

Based on the above results, NMP was considered as inactive in the rat primary hepatocyte UDS assay.

Literature search via Toxnet, revealed in a study conducted by G. Engelhardt and H. Fleig (Mutat Res 298(3): 149-55, 1993) that NMP at single oral doses up to 3800 mg/kg body weight (80% of LD50) in male and female mice or in male and female hamsters did not lead to an increase in micronucleated erythrocytes in the **mouse micronucelus assay** or in structural or numerical chromosomal aberrations in the **Chinese bone marrow test** when bone marrow was sampled 16, 24 and 48 hours post-treatment in the micronucleus test or after 24 and 48 hours after for karyotype analysis.
Cyclophosphamide (40 mg/kg p.o.) and vincristine (0.15 mg/kg i.p) in the mouse micronucleus assay and cyclophosphamide (40 mg/kg p.o.), vincristine (2 or 3 mg/kg i.p) and benomyl (2500 and 5000 mg/kg o.p) in the Chinese hamster bone marrow had the expected clastogenic effects. Cyclophosphamide significantly ($p<0.01$) increased the number of small micronuclei/1000 polychromatic erythrocytes while vincristine increased both the number of small and large micronuclei in the mouse micronucleus assay. In the Chinese hamster bone marrow assay, vehicle and NMP had no effect on the % aberrant cells, both including or excluding gaps, while cyclophosphamide significantly increase the % of aberrant cells both excluding and including gaps. Both CP and NMP had no effect on polyploid cells. Vincristine increased significantly both the % of aberrant cells as well as hyperploid and polyploid cells. Benomyl did not affect % of aberrant cells but increased both hyperploid and polyploid cells.
The results of this study showed that NMP did not lead to clastogenic and especially not to aneugenic effects in intact animals. It was suggested that the findings using Sacchromyces cerevisiae can not be extrapolated to the in vivo situation.

**Note:** Sponsor referred for mouse lymphoma assay (vol 1.21, page 276) to a publication entitled **"Toxicity of N-methyl-2-pyrrolidone (NMP): Teratogenic, subchronic, and two-year**

**inhalation studies**" published in Fundamental and Applied Toxicology 9: 222-235, 1987. However, review of this publication showed no reference to mouse lymphoma test.

In response to Pharmacology inquiry, sponsor under SS# 006 dated 10-5-2001 has provided summary data for a study entitled "Mutagenicity in the mouse lymphoma L5178Y cell line, NMP" [redacted] Project 677.76, September 10, 1976.

Although in summary it was stated that maximum doses of up to 10,000 ppm were used, results table showed that maximum dose of 1000 ppm was used in non-activated system and 10,000 ppm in system with S-9 mix. Percent relative growth was not effected with any dose level (100 – 1000 ppm) in the non-activate system and was 49.3% at a dose of 10,000 ppm in the activated system, suggesting that MT dose was not used since maximum dose should reduce the % RG to 10 – 20% accodring to OECD guidance document.

From the results of this study however, sponsor concluded that 2-pyrrolidone,N-methyl tested at concentrations up to 10,000 ppm, produced no significant increase in the spontaneous mutation frequency in either the presence or absence of a liver microsomal preparation, indicating that the compound is not mutagenic.
Pharmacology however, considers that since the doses used were not based on cytotoxicity results in any dose range-finding study, and as study was conducted long before present GLP regulations, the study does not meet the present ICH recommendations.

**Key findings:**

**Summary of individual study findings:**

Study validity:

Study outcome:

**Genetic toxicology summary:** As regards Ames test, results were variable. A study conducted by the [redacted] showed that NMP gave positive results with salmonella typhimurium strain TA1538. Wells et al (1988) reported that NMP gave positive response with strains TA 102 and TA 104 and its acid hydrolytic product, 4-(methylamino)butanoid acid gave positive response with strain TA 104. However, study conducted by [redacted] reported that NMP did not give positive response with any tester strains either in the presence and absence of S9.
NMP was reported to induce aneuploidy in Sacchromyces cerevisiae. NMP was positive in the CHO/HGPRT forward mutation assay at the highest concentration (5 mg/ml) compared to negative control values but within the sponsor's accepted background mutant frequency.
NMP was negative in the rat primary unscheduled DNA synthesis assay.
Literature review indicates that NMP was negative in the mouse micronucleus assay and did not increase structural or chromosomal aberrations in the Chinese hamster bone marrow test suggesting that the findings using Sacchromyces cerevisiae can not be extrapolated to in vivo situation.
In a study conducted by the [redacted] as project # 677.76 in 1976 and submitted under SS# 006 dated 10-5-2001, it was reported that NMP was negative in the mouse lymphoma test under the assay conditions used.

**Genetic toxicology conclusions:** Taking into consideration findings of all mutagenicity tests conducted, it seems that NMP does not possess significant mutagenic potential. It is however, suggested that in vitro mouse lymphoma assay and in vivo mouse micronucleus test should be conducted according to present ICH guidance if the product in the future is submitted for any benign indication.

**Labeling recommendations:** NMP has no significant mutagenic potential

## CARCINOGENICITY:

Carcinogenicity studies with leuprolide acetate have been referred to literature. Also leuprolide acetate is an approved drug substance.

The carcinogenic potential of NMP has been referred to 18 month carcinogenicity study in mice ( Project No. 76C0225/93065. July 7, 1999) and 24 months carcinogenicity study in rats ( Project No. 9738-001. Dec 18, 1997). Both these studies were stated to have been conducted according to GLP regulations.

In both the mouse and rat studies, NMP was administered via the diet. Dose levels were selected on the basis of findings in repeat dose toxicity studies of up to 3-month duration.

**In the mouse carcinogenicity study**, mice of B6C3F1 strain received doses of 89, 173 and 1089 mg/kg/day in males and 115, 221 and 1399 mg/kg/day in females.
Treatment had no effect on survival, which was in excess of 96% in all groups. Body weight and food consumption was not affected. Liver weight was increased in both sexes at high dose and in males at mid dose. As shown in table below, the incidences of males with liver carcinoma and of males and females with adenoma and foci of cellular alteration were increased at the high dose. Hepatocellular hypertrophy was also seen in the high dose males.

Table 11

| Incidence of liver lesions in mice (50/sex/group) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Males | | | | Females | | | |
| Mg/kg/day | 0 | 89 | 173 | 1089 | 0 | 115 | 221 | 1399 |
| Carcinoma | 4 | 1 | 3 | 13 | 0 | 0 | 0 | 3 |
| Adenoma | 5 | 2 | 4 | 12 | 2 | 2 | 1 | 7 |
| Foci or cellular alteration | 5 | 5 | 6 | 25 | 3 | 2 | 1 | 17 |
| Hepatocellular hypertrophy | 0 | 0 | 3 | 43 | 0 | 1 | 0 | 0 |

Sponsor suggested that hepatic changes observed are typical of compounds which induce adaptive changes in the liver such as non-genotoxic microsomal enzyme inducers, phenobarbital and dichloroacetic acid.
Sponsor concluded that based on lack of hepatocarcinogenic effect in the rat and the negative results for genotoxicity, the effect on the mouse liver was epigenetic in origin.

**In the rat carcinogenicity study**, SD rats were administered in diet doses of 66.4, 207 and 678 mg/kg/day to males and 87.8, 283 and 939 mg/kg/day in females for 104 weeks.

The survival was reduced in the high dose group compared to controls (24% vs 32%) and was attributed to increased incidence of severe chronic progressive nephropathy in this group, which was considered related to treatment with NMP.
The only significant treatment related finding was the occurrence of stromal sarcoma in the uterus. The incidence was 0/62, 2/62, 3/62 and 3/62 in control, and low, mid and high dose NMP treated rats, respectively. Sponsor concluded that since there was no dose-response and the incidence was within historical laboratory control values, relationship to treatment is unlikely and NMP is considered not carcinogenic in the rat.
Note: Complete data base for both the mouse and the rat carcingenicity studies will be requested for review if the drug produced is proposed to be used for benign indications.

A 2-year inhalation carcinogenicity study in rats was referred to a publication by Lee et al (Fundamental and Applied Toxicology 9:222-235, 1987). Rats were exposed to vapor of NMP at concentrations of 0.04 mg/liter (10 ppm) and 0.4 mg/liter (100 ppm) for 6 hr/day, 5 days/week for 2 years. There were 120 rats/s in each group. Another groups with 120 rats/sex were exposed to air only and served as controls. 10/s/g were sacrificed after 1, 2, 6, 12 and 18 months and all surviving at the end of 2 year exposure. Routine clinical chemistry and hematology and serum enzymes chemistries was evaluated at 1, 3, 6, 12 and 18 months. Tissues were saved at necropsy for histological examination.
It was reported that both male and female rats discharged dark yellow urine and the males had greater urine volume. After 2-years of exposure, the high dose male rats gained 6% less body weight than the control males. There were no significant differences in either morbidity or mortality between exposed and control rats. There was no difference in myeloid/erythroid ratios of exposed rats and controls after 24 months of exposure to NMP. There were no differences in hematology and clinical chemistry and urinalysis between controls and exposed rats. The incidence and severity of neoplastic and non-neoplastic lesions was reported similar in exposed and control rats. In rats that died or were killed in extremis before 18 months, chronic progressive nephropathy was found in 8/23 high dose rats and 4/19 control rats. High dose female rats had decreased incidence of mammary gland tumors but had increased incidence of mammary gland hyperplasia.

**Summary of individual study findings:**

Adequacy of the carcinogenicity study and appropriateness of the test model: Complete data was not available and as such adequacy of the study can not be judged.

Evaluation of tumor findings: The incidence of liver carcinoma and adenoma as well as foci or cellular alteration was increased in both male and female high-dosed mice although it was greater in males. Also the incidence of hepatocellular hypertrophy was increased in mid and high dose males but no hypertrophy was observed in females.
Only treatment-related effect in rats was increased incidence of stromal carcinoma in the uterus in NMP treated females.
In the inhalation carcinogenicity study it was reported that treatment-related findings was a decreased incidence of mammary gland tumors but increased incidence of mammary gland hyperplasia in high dose females.

**Carcinogenicity summary:** Only significant NMP treatment-related tumorigenic findings involved hepatic effects in mice and uterine and mammary gland effects in rats.

**Carcinogenicity conclusions:** Based on the data provided, NMP has carcinogenic effect only at very high dose levels (1089 – 1399 mg/kg/day) compared to maximum human exposure of 75 ug/kg/day. As such there is no carcinogenic risk for NMP.

Recommendations for further analysis: none at this time

**Labeling Recommendations**: Literature review suggests that NMP is not carcinogenic at recommended dose levels.

## REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:

Reproductive toxicity for leuprolide acetate in not reviewed as it has been approved for many malignant and benign indications.

References have been provided for number of studies conducted in rats, rabbits and mice by various routes of administration to study the embryotoxic and teratogenic potential of NMP. A statement was made that these studies were conducted in accordance with GLP regulations.

In an oral study in rats using doses up to 400 mg/kg, maternal and fetal body weights were reduced. There was an increased incidence of stunted fetuses but no effect on the type and incidence of malformation or developmental variations were noted. The NOAEL was 125 mg/kg. ( June 5, 1992)

**Note:** The days and duration of drug administration was not described. The sponsor referred the study to DMF( ) However, the study could not be located.

**A development toxicity study was conducted in New Zealand White rabbits by** ( ) in 1991. DMF( ) volume2.1.

This study was conducted in accordance with GLP regulations.
Inseminated female rabbits were assigned to control and 3 treatment groups (20/g) and an oral of 55, 175 and 540 mg/kg of NMP were administered by gavage as a single daily dose on gestation days 6 through 18 at a volume of 3 ml/kg. Controls got only the vehicle, deionized water. Caesarian section examination was performed on all females on gestation day 29. Fetuses were examined for tertogenicity.

**Results:**
Maternal toxicity was reported with mid and high doses.
Between day 6 and 12 the controls gained 77 g while high dose lost 36 g. Food consumption was reduced by 24% in high dose and there was marginal effect on body weight with mid dose, which was significant during gestation days 6-12. One rabbit on high dose aborted.

At the high dose there was increased post implantation loss as shown in table 12 below. Embryolethality occurred at the high dose with statistically significant increase in overall fetal

malformations, mainly of the heart and associated vessels, and the skull bones. Developmental variations included misshapen skull bones. The maternal NOAEL was 55 mg/kg, while 175 mg/kg was the developmental toxicity NOAEL.

Table 12

Summary of maternal and fetal observation at caesarian section

| Parameter | Dosage mg/kg/day | | | |
|---|---|---|---|---|
| | 0 | 55 | 175 | 540 |
| Animals on study | 20 | 20 | 20 | 20 |
| Pregnant | 20 | 18 | 18 | 17 |
| Aborted | 0 | 0 | 0 | 1 |
| Animals with viable fetuses | 20 | 17 | 18 | 15 |
| Animals with all resorptions | 0 | 1 | 0 | 1 |
| Mean corpora lutea | 13.6 | 14.1 | 13.5 | 13.5 |
| Total corpora lutea | 259 | 239 | 243 | 203 |
| Mean implantation sites | 9.1 | 7.6 | 6.7 | 7.0 |
| Total implantation sites | 182 | 137 | 120 | 112 |
| Mean post-implantation loss | 0.4 | 0.8 | 0.7 | 1.8* |
| Total postimplantation loss | 7 | 15 | 12 | 29 |
| Mean early resorptions | 0.3 | 0.4 | 0.5 | 1.4* |
| Total early resorptions | 6 | 8 | 9 | 22 |
| Mean late resorptions | 0.0 | 0.1 | 0.2 | 0.4** |
| Total late resorptions | 0.0 | 1 | 3 | 7 |
| Mean % pre-implantation loss | 35.5 | 44.4 | 50.6 | 45.3 |
| Mean % post-implantation loss | 5.5 | 10.9 | 10.0 | 25.9 |
| **Incidence of fetal malformations.** Values as No.of fetuses (No. of liters) | | | | |
| Number of litters examined | 19 | 17 | 18 | 15 |
| Number of fetuses examined externally | 161 | 128 | 108 | 83 |
| Number of fetuses examined viscerally | 161 | 128 | 108 | 83 |
| Number of fetuses examined skeletally | 161 | 128 | 108 | 83 |
| Aortic arch stenosis | 1(1) | | 1(1) | 20(8) |
| Bulbus aortic arch | 1(1) | | 1 (1) | 17(6) |
| Ductus arteriosus stenosis | 2(2) | | 1(1) | 14(6) |
| Interventricular septal defect | 2 (2) | | 2(2) | 24(8) |
| Malformed skull bones | | | | 6 (4) |
| Fused sternebrae | 3(2) | 2(2) | 5(4) | 7(6) |
| Forked scapula | | | 2 (1) | 1(1) |
| Total fetuses with malformation | 9 (7) | 5 (5) | 10(7) | 36(12) |
| Total fetuses with variations | 126(19) | 101(17) | 102(17) | 82(15) |

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

A **dermal developmental toxicity study was conducted in rats** (Beci el al. Fundam Appl Toxicol 2:73-76, 1982. Dose levels of 75 mg; 237 mg and 750 mg/kg were used. There were no adverse effects in the dams or fetuses at 75 and 237 mg/kg. At 750 mg/kg, maternal weight during gestation and fetal body weights were significantly reduced; embryolethality occurred and developmental skeletal anomalies were observed including missing sternebrae, incomplete ossification of vertebrae, incomplete skull closure and reduced hyoid. Skeletal malformations included fused/split ribs, supernumerary ribs and fusion of the atlas and exoccipital bones. There were no visceral malformations.

**Note:** The days and duration of drug administration was described.

In another study in rats, oral dosage of 1000 mg/kg on days 6-15 of pregnancy was markedly embryotoxic and induced fetal malformations; maternal body weight was reduced. There were no adverse effects at 330 mg/kg dose level (no reference provided).

Exposure to 0.1 and 0.36 mg/l, 6 hours/day from days 6 to 15 of gestation in rats, caused initial sporadic lethargy and irregular respiration in the dams at both dose levels. No other adverse effects were seen (Lee et al. Fundam Appl Toxicol 9:222-235, 1987).

The neurobehavioral effects of NMP in the offspring of rats exposed to NMP for 6 hours/day on gestation days 7- 20 at 150 ppm was investigated (Hass, U; Lund, S.P. and Elsner J. Neurotox Teratol 16(3) 241-249, 1994). This dose was calculated to be maternally toxic or leading to decrease viability of the offspring. The occupational exposure level is 100 ppm.

Minor effects in pups during the pre-weaning period were lower body weight and delayed physical development. Neurobehavioral evaluation of the male pups revealed no effects on basal functions of central nervous system. The animals appeared normal and motor function (rotarod), activity level (open filed). It was stated that the performance in learning tasks with a low grade of complexity in two groups was similar, however, in more difficult tasks such as the reversal procedure in Morris water maze and operant spatial alteration (Skinner boxes) performance was impaired in exposed offspring.

In an article entitled " **Experimental investigations about the embryotoxic and terstogenic effect of N-methyl-pyrrolidone (NMP)"** by Von Reiner Schmidt and published in a German journal, it was stated that in experiments with 2 defined strains of mouse, NMP proved to be an effective embryotoxic compound. The embryo damaging effects depended on the doses and took place both after single and repeated application. Moreover, NMP induced malformations of different severity in both strains (volume 1:22 of 1:55, page 157).

**Note**: with the limited English translation provided, although it is not possible to adequately review the data, it seems that NMP is likely teratogenic.

Sponsor summarized the NOAEL for maternal and developmental toxicity in the rat and rabbit studies as given in the following table:

Table 13

| Species/route | NOAEL for maternal Toxicity | NOAEL for developmental Toxicity |
|---|---|---|
| **Rat** | | |
| Oral<br>Deraml<br>Inhalation | 330 mg/kg<br>237 mg/kg<br>0.62 mg/L | 330 mg/kg<br>237 mg/kg<br>0.36 mg/L |
| **Rabbit** | | |
| Oral | 55 mg/k | 175 mg/kg |

**Conclusion:** It was concluded that in terms of embryotoxicity and teratogenicity, effects have been confined to dose levels inducing maternal toxicity

The potential effects of NMP on gonadal function, estrous cycles, mating behavior, conception, parturition, lactation, weaning, and the growth and development of the offspring was investigated in a study conducted by [redacted] in 1991. This study was entitled **"Multi-generation rat reproduction study with N-methylpyrrolidone"** and was conducted in accordance with FDA Good Laboratory Practices regulations. Three groups of male and females rats were treated with NMP in diet to achieve dose levels of 50, 160 and 500 mg/kg/day. The fourth group was an untreated control and fed standard rat chow.
P1 males and females were treated for at least 10 weeks prior to mating and during mating the mating period. P2 litters were dosed beginning on post-natal day 21 for 10 weeks before mating and continued during mating gestation and lactation.

In the parental generations (P1 and P2), no overt clinical signs of toxicity or mortality were reported up to the high dose of 500 mg/kg. Body weight and food consumption was not affected in males but was decreased in females. P2 male and female body weight and food consumption for the high dose group was decreased.

There were no significant differences in reproductive data for P1 generation. However, high dose P2 male mating, fertility and fecundity indices significantly lower than controls. Survival and growth rate of offspring of the high dose group was decreased.

No treatment-related microscopic changes were observed in the reproductive organs or any tissues with gross changes from male and female P1 generation rats given 500 mg/kg/day NMP. Also no treatment-related microscopic changes were reported in P2 males at any dose level.

Based on these results, sponsor stated that a dose of 160 mg/kg/day was established as the parental, reproductive and developmental NOAEL.

**Comments:** The study findings suggested that male fertility is not adversely affected by prior 10 weeks treatment with NMP at a dose of 160 mg/kg/day before mating.

**Summary of individual study findings:**

**Reproductive and developmental toxicology summary:** NMP did not adversely effect male or female fertility up to a dose of 160 mg/kg/day. Also a dose of 175 mg/kg was the NOAEL for developmental toxicity in rabbits.

**Reproductive and developmental toxicology conclusions:** There is no concern about reproductive and developmental toxicity at dose of NMP used in the proposed formulation.

**Labeling recommendations:** NMP is not teratogenic

## SPECIAL TOXICOLOGY STUDIES:

**Study title: ATLS-83: Subcutaneous implantation study in the rabbit with histopathology Volume 1.16 of 1.55, page 298**

This study was conducted at the [redacted] in accordance with FDA's GLP Regulations.

The objective of the study was to evaluate compatibility of polymer formulation + leuprolide acetate with the SC tissue. The test article was previously exposed to 2 different sterilization processes and comparison of both was made to a reference material (USP Negative control Plastic RS)

The experimental procedure was as follow:

Table 14

| Polymer formulation & leuprolide acetate | Number of female rabbits | Termination (days) 7 | 21 | 28 | 56 |
|---|---|---|---|---|---|
| Sterile filled polymer | 8 | 2 | 2 | 2 | 2 |
| Gamma irradiated polymer | 8 | 2 | 2 | 2 | 2 |

US negative control Plastic strips were used as location markers.

A 0.25 ml dose of the test article was implanted into 2 separate sites in the SC tissue on the right side of the back. One 0.25 ml dose of test article was similarly implanted on the left side of the back. One USP negative control article was also implanted on the left side of the back of each rabbit. At termination, capsule formation or other signs of irritation were scored at a scale of 0 to 4 dependent on the extent of reaction area. Cellular response on microscopic evaluation was graded on a 0-4 scale.

As shown in table below both formulations was considered slight irritant following the 56-day implantation period.

Table 15

| | Macroscopic classification | Microscopic Observation |
|---|---|---|
| 7 days | | |
| group 1 | Not significant | Slight irritant |
| group 2 | Not significant | Slight irritant |
| 21 days | | |
| group 1 | Not significant | Slight irritant |
| group 2 | Not significant | Moderate irritant |
| 28 days | | |
| group 1 | Not significant | Moderate irritant |
| group 2 | Not significant | Slight irritant |
| 56 days | | |
| group 1 | Not significant | Slight irritant |
| group 2 | Not significant | Slight irritant |

**Conclusion:** Macroscopic reaction of both formulations of the test article was not significantly different as compared to the USP negative control implant material. Microscopically, both formulations were classified as slight to moderate irritants.

**ATLS-86: Comparison of the effects of a gamma-irradiated Atrigel formulation manufactured by two different processes containing leuprolide acetate or vehicle control alone on tissue irritation when injected subcutaneously in rats. Final report. Volume 1.18 of 1.55, page 32**

This study was conducted by the [redacted] in accordance with FDA's GLP Regulations.

Four Atrigel formulations, 2 with and 2 without leuprolide acetate were injected SC into mature rats (20/g). One formulation with and one without was irradiated at 20 kGy while the other 2 were irradiated at 25 kGy. All formulations contained [redacted]% 50/50 PLGH (IV 0.48)/[redacted]% NMP with [redacted]% w/w leuprolide acetate. Injection volume was 100 ul. Animals showing any signs of irritation were immediately terminated and tissue from test sites and any other remaining test article were retrieved for histologic examination. All remaining animals were terminated on Day 7. Test sites were examined macroscopically in all animals and test sites taken from 4 to 5 animals from each group for histologic examination. Macroscopic and microscopic scoring was similar to that described for rabbit study.

**Results:** Minimal to mild irritation was observed at all test sites at the time of necropsy on Day 7. Microscopically all sites were similar in cellular reaction with only mild localized reaction to the implanted material. It was concluded that the formulation with or without leuprolide acetate is a slight irritant at 7 days post-implantation causing a mild foreign body reaction.

**ATLS-78: Subcutaneous implantation study in rabbits with histopathy- Final report. Volume 1.20 of 1.55, page 2.**

This study was conducted in accordance with FDA's GLP Regulations at the [redacted]

The purpose of the study was to evaluate the local effects of Atrisite (PLGH in NMP, [redacted]% polymer/[redacted]% NMP, lot #1042) in direct contact with SC tissue of the rabbit. A total of 42 rabbits were used as shown in table below. The test article was injected on right side of the back, while negative control (USP polyethylene strips) were used on the left side of each rabbit. USP plastic was implanted at each test site to serve as location marker. When multiple doses were used, the same site was selected for all injection. Rabbits were anesthetized for the implant insertion procedure.

Table 16

| Group | # of animals | Implantation schedule | Termination intervals (3 rabbits/interval) |
|---|---|---|---|
| 1 | 12 | Day 0 | Days 7, 21, 28 and 56 |
| 2 | 9 | Day 0, 29 | Days 36, 50, and 57 |
| 3 | 12 | Day 0, 29, 58 | Days 65, 79, 86, and 114 |
| 4 | 9 | Day 0, 58 | Days 65, 79, and 86 |

At each termination, rabbits were weighed and anesthetized. SC tissue was examined and capsule formation or other signs of irritation were scored on a scale of 0-4. The parameters in macroscopic evaluation included inflammation, polymorphonuclear, lymphocytes, plasma cells, macrophages, giant cells, and necrosis. The score of these was added and multiplied by 2 and to it was added score for fibroplasia, fibrosis and fatty infiltrate to get total score. Reaction index was test minus control and was classified as not significant, trace, slight, moderate and marked when the difference was 0.0 to 0.5, 0.6 to 1.0, 1.1 to 2.0, 2.1 to 3.0 and over 3.0 respectively. Microscopic evaluation was based on cellular changes scored on scale of 0-4 as for macroscopic changes.

**Results:**

Clinical observations: sporadic episodes of ocular discharge, reduced feces and animals off feed.
Body weight: no significant treatment-related effects
Macroscopic observations: It was stated that at multiple termination intervals, the tissue surrounding the test material at several sites was obaque and edematous and the test article appeared white and firm. At day 28, most of the test sites were surrounded by area of white colored tissue, which was not present at the earlier termination intervals. The USP control sites were said to be unremarkable at each termination interval. The test article was considered to be a trace irritant after 7, 21, 28 and 50 days of implantation as compared to USP control. On day 56 reaction was slight and was not significant compared to the control at the remaining termination intervals.
Microscopic observations: Following a single injection, implantation of test article stimulated a slight foreign body cellular response, but had little effect on adjacent tissue. By day 21, there was subacute inflammatory response and showed macrophage/foreign body giant cell reaction in clearing the test article from the sites. Material seemed to be biodegraded over time and the cellular reaction subsided by days 28 and 56.
Upon reinjection after day 29, the secondary sites were characterized by subacute inflammation with macrophage and foreign body giant cell activity; there was necrosis of the dermal collagen and cutaneous muscle tissue. Moderate irritation was observed on days 57 and 65 at secondary injection sites. Reaction at the original injection sites was minimal.
Treatment sites receiving 3 injections of test material were characterized similar to 2 injections (group 2). The degree of response was time-dependent. By day 114, there was minimal to no reaction present.
On day 65, at sites injected on day 58 (group 4), no evidence of irritation was present. The secondary sites were slightly reactive. In animals terminated on day 79, the secondary sites were characterized as moderate and the original injection sites were slightly reactive. Similar observations were made on day 86.

**Conclusion:** It was concluded that under the conditions of this study, the macroscopic observations did not vary considerably following single or multiple injections of test article. Reactions were characterized as nonirritating to trace signs of irritation. Microscopically test article stimulated a slight foreign body cellular response but had little effect on adjacent tissues. By day 28, the material appeared to biodegrade and the cellular reaction subsided. Following multiple injections, primary sites showed little response while secondary sites showed subacute inflammatory responses along with macrophage/foreign body giant cell activity. These observations were time dependent and appeared to subside over time.

**ATRS-189: A 28-day study to determine the irritation potential of an Atrigel formulation containing NMP when injected subcutaneously in the rat- final report.**

This non GLP study was conducted by [redacted]

The study was designed to assess by histology and macroscopic tissue evaluation, the 28 day local tissue response to NMP when used as the solvent in a gamma irradiated Atrigel formulation.

The experimental design was as shown in a table 17 below:

Table 17

| Group | Number/sex | Test article/site | Dosage |
|---|---|---|---|
| 1 | 15 M, 15 F | Atrigel SC | 0.10 cc |
| 2 | 15 M, 15 F | USP marker SC | 0.10 cc |
| 3 | 15 M, 15 F | Polymer particles SC | 0.50 cc |

The average amount of formulation injected was about 115 mg

Six animals from each group (3male and 3 female) were terminated at days 3, 7, 14, 21, and 28. The implants and surrounding tissues were then evaluated macroscopically and microscopically.

**Results:**

Both male and female rats gained in body weight during the course of the study.
As shown in table 18 below macroscopic examination revealed that there was no macroscopic tissue irritation in group 3, some minimal irritation at Day 3 in group 2 and minimal to mild irritation in group 1 at the start of the study but appeared to dissipate by Day 14. Values are for males and females combined on a scale of 0-4 (0=none, 1=minimal, 2=mild, 3=moderate and 4=marked)

Table 18

| group | Termination Time point(day) | Vasodilation | erythema | edema | Capsule Formation |
|---|---|---|---|---|---|
| 1 | 3 | 1.0 | 0.3 | 0.3 | 0.0 |
| | 7 | 1.5 | 0.0 | 1.5 | 0.0 |
| | 14 | 0.8 | 0.0 | 1.0 | 0.0 |
| | 21 | 0.5 | 0.0 | 0.0 | 0.0 |
| | 28 | 0.2 | 0.0 | 0.0 | 0.0 |
| 2 | 3 | 0.8 | 0.3 | 0.3 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 14 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 21 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 28 | 0.0 | 0.0 | 0.0 | 0.5 |
| 3 | 3 | 0.0 | 0.2 | 0.0 | 0.0 |
| | 7 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 14 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 21 | 0.0 | 0.0 | 0.0 | 0.0 |
| | 28 | 0.0 | 0.0 | 0.0 | 0.0 |

Summary of the histological analysis is provided in a table 19 below. Values are average score on a scale of 1-3 (1=mild, 2=moderate and 3=severe):

Table 19

| Treatment Group | Day of sampling of test sites | | | | |
|---|---|---|---|---|---|
| | 3 | 7 | 14 | 21 | 28 |
| 1 | 1.5 | 1.0 | 1.3 | 0.7 | 0.7 |
| 2 | 1.0 | 1.0 | 0.2 | 0.4 | 0.8 |
| 3 | 0.8 | 0.3 | 0.5 | 0.0 | 0.0 |

It was reported that no necrosis or suppuration was present and no dramatic changes were found in the dermis or epidermis of any animal.

**Conclusion:** There was some increased macroscopic irritation in group 1 at the start of the study, which disappeared by day 14.

**ADDENDUM TO REVIEW:**
(if necessary)

**APPENDIX/ATTACHMENTS:**

IND [redacted] original submission dated 3-17-2000 P/T review



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