

CENTER FOR DRUG EVALUATION AND RESEARCH

Application Number 21-367

PHARMACOLOGY REVIEW(S)

PHARMACOLOGY/TOXICOLOGY COVER SHEET

NDA number: 21-367

Review number: 1

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Information to sponsor: Yes () No (x)

Sponsor and/or agent: Galen Limited
Rockaway 80 Corporate Center
100 Enterprise Drive, Suite 280
Rockaway, NJ 07866
(973) 442-3233

Manufacturer for _____

Reviewer name: Lynnda Reid

Division name: Division of Reproductive and Urologic Drug Products

HFD #: 580

Review completion date: September 13, 2002

Drug:

Trade name: _____ (estradiol acetate vaginal ring)

Generic name (list alphabetically): estradiol acetate

Code name(s): estradiol-3-acetate, E3A, E3A IVR, and estradiol acetate intravaginal ring

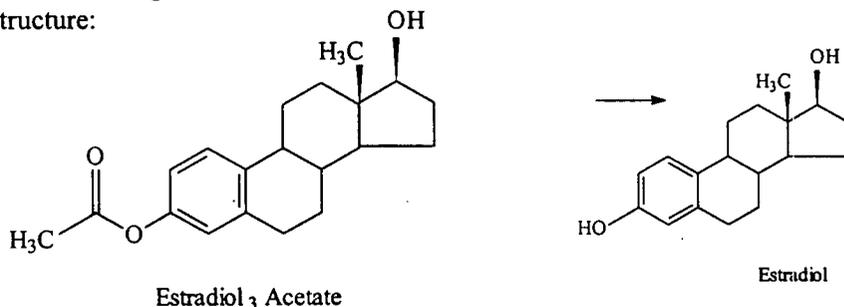
Chemical name (s): 17 β -Estradiol-3-acetate; Estra-1,3,5(10)-triene-3,17 β -diol-3-acetate; and
3-Acetoxy-1,3,5(10)-estratriene-17 β -ol

CAS registry number:

Molecular formula: C₂₀H₂₆O₃

Molecular weight: 314.41

Structure:



Relevant INDs/NDAs/DMFs:

IND _____

MAF _____ for _____ (letter of authorization)

Drug class: estrogen

Indication(s): 1) Treatment of moderate to severe vasomotor symptoms associated with menopause;

2) []

Clinical formulation: Estradiol-3-acetate is incorporated into a silicon polymer intravaginal ring for sustained release of 0.05 or 0.1 mg/day. _____ contains 12.4 mg of estradiol

acetate, released at a rate equivalent to 0.05 mg of estradiol per day for 3 months. _____ contains 24.8 mg of estradiol acetate, released at a rate equivalent to 0.10 mg of estradiol per day for 3 months.

The _____ core of the silicon polymer ring is composed of _____ (_____) _____). _____ In addition to the silicon polymer, the _____ core material of the ring contains the active ingredient estradiol-3-acetate (12.4 or 24.8 mg) _____

Route of administration: insertion into the upper vagina

Proposed use: _____ is a vaginal ring delivering estradiol acetate at rates nominally equivalent to 0.05 and 0.10 mg estradiol per day. Therapy is usually initiated with _____ 0.05 mg/day, inserted vaginally once every 3 months. The dose should be adjusted as necessary to control symptoms at 3- or 6-month intervals.

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

Introduction and drug history: Estradiol-3-acetate (E3A) is a prodrug of estradiol. Estradiol is the principal intracellular human estrogen and is substantially more potent than its metabolites, estrone and estriol at the receptor level. The proposed drug product utilizes an intravaginal silicone polymer ring _____ estradiol-3-acetate for 90 days. The release profile of E3A from the ring is characterized by an initial bolus followed by controlled release for 3 months. Upon release, E3A is converted to estradiol via esterases found in vaginal fluids, tissues and plasma.

An estrogen/intravaginal ring product is approved in the US (ESTRING/Pharmacia NDA 20-472) for the treatment of urogenital symptoms associated with post-menopausal atrophy of the vagina. This product contains 2 mg estradiol and is designed to release approximately 7.5 µg estradiol/24 hours over a period of 90 days.

Studies reviewed within this submission:

Studies conducted with estradiol-3-acetate:

- 1) Determination of the Rate of Conversion of Estradiol-3-Acetate to Estradiol *In Vitro* in Human Serum and Human Whole blood using _____
- 2) A Study to Determine the Conversion of Estradiol-3-Acetate Following the Insertion of an IVR Releasing Estradiol-3-Acetate in Healthy Postmenopausal Women
- 3) Reverse Mutation in four Histidine-Requiring Strains of *Salmonella typhimurium* and Two Tryptophan-Requiring Strains of *Escherichia coli*

Studies conducted with elastomer extracts:

- 1) Cytotoxicity Test of a Sample Extract using the MEM Elution Method in the L-929 Mouse Fibroblast Cell Line
- 2) *In Vitro* Hemolysis Test by Direct Contact (Rabbit Whole Blood)
- 3) Elastomer Extract Intracutaneous Study in the Rabbit
- 4) Elastomer Extract Acute Systemic Toxicity Study in Mice
- 5) Elastomer Muscle Implantation Study in Rabbits with Histopathology (90 Days)
- 6) Elastomer Extract Ames Salmonella Mutagenicity Assay

Studies not reviewed within this submission: none

Executive Summary

I. Recommendations

- A. Recommendation on Approvability: Approval
- B. Recommendation for Nonclinical Studies: None
- C. Recommendations on Labeling: Labeling for reproductive and carcinogenic effects should be consistent with the most current standard language recommended by the Division of Reproductive and Urologic Drug Products for all estrogen drug products.

II. Summary of Nonclinical Findings

A. Brief Overview of Nonclinical Findings:

Estradiol Acetate: E3A is quickly hydrolyzed to estradiol *in situ* and was undetectable systemically following use of the IVR in clinical studies. Estradiol is the principal intracellular human estrogen and is currently available in numerous approved drug products at concentrations comparable or higher than those anticipated following clinical use of _____

_____ Elastomer _____ has been widely used in medical implant materials, drug delivery devices, prostheses, and in reconstructive surgery. There has been no evidence of chronic toxicity or carcinogenic effects in studies in rats, dogs and mice following administration of oral doses of _____ or in studies in mice, rats, rabbits and baboons following implantation of _____ cured silicone elastomers. _____ has not been reported to be cytotoxic, genotoxic or antigenic. Implantation of the _____ cured _____ material for 90 days in the paravertebral muscle of rabbits resulted in a mild local inflammatory reaction characterized by formation of a fibrous capsule, similar to the reactions observed with the previously approved Dow product, Silastic® 382.

The following potential leachables have been identified from the _____ intravaginal ring material: _____ were within the permissible exposure limits, while potential exposure levels of _____ were below the no observable adverse effect level in rodents. There was no significant evidence of local or systemic toxicity following intracutaneous injection of elastomer extracts in rabbits or following i.v. injections of elastomer extracts in mice. Extracts were also negative in the Ames bacterial mutagenicity assay, cytotoxicity assay in mouse fibroblasts, and the hemolysis assay in rabbit whole blood.

- B. Pharmacologic Activity: Estradiol-3-acetate is a prodrug of estradiol.

C. Nonclinical Safety Issues Relevant to Clinical Use: none

III. Administrative

A. Reviewer signature: _____

B. Supervisor signature: Concurrence - _____

C. cc: list:

NDA 21-367
HFD-580
HFD-580/Pharm/Reid
HFD-580/Pharm/Jordan
HFD-580/CSO/Spell-LeSane
HFD-580/MO/Van der Vlugt

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PHARMACOLOGY/TOXICOLOGY REVIEW

I. PHARMACOLOGY:

Primary pharmacodynamics: Estradiol acetate is a prodrug of estradiol. Estradiol is the principal endogenous form of estrogen. Estrogens are largely responsible for the development and maintenance of the female reproductive system and secondary sexual characteristics. Following menopause, ovarian estrogen production ceases and most endogenous estrogen is produced by conversion of androstenedione, secreted by the adrenal cortex, to estrone by peripheral tissues, resulting in a state of relative estrogen deficiency associated with symptoms including 'hot flashes' and vulvovaginal atrophy.

Drug activity related to proposed indication: exogenous estrogen replacement in post-menopausal women

II. SAFETY PHARMACOLOGY:

Safety pharmacology studies were not performed with estradiol acetate. The safety pharmacology of relies on studies performed and published with estradiol.

III. PHARMACOKINETICS/TOXICOKINETICS:

With respect to the use of estradiol acetate, the non-clinical development program focused on demonstrating that the rate of hydrolysis of estradiol acetate to estradiol was very rapid and that there would be no significant systemic exposure to estradiol acetate.

PK parameters: Sponsor's summary of mean pharmacokinetic data following administration of to postmenopausal women:

Study (Duration)	E3A Dose (mg/day)	n	Analyte	T _{max} (h)	C _{max} (pg/ml)	C _{min} (pg/ml)	C _{ave} (pg/ml)	AUC _{0-t} (pg.day/ml)
IVR 1006 (13 wks)	0.05	12	estradiol	0.9	1120	22.8	40.6	3696
			estrone	6.2	141	25.8	35.9	3270
			estrone sulfate	9.3	2365	137.4	494.6	45,007
IVR 1001 (12 wks)	0.10	25	estradiol	4.3	102.6	69.96	76.0	6394
			estrone	4.2	74.5	44.0	47.5	3837

In clinical studies, drug release from the intravaginal ring was characterized by an initially rapid delivery rate followed by a relatively constant delivery rate for the remainder of the dosing interval. The duration of the initial faster delivery rate is about 1 hour, with steady state release achieved by 24-36 hours after instillation.

In vitro experiments revealed that estradiol acetate was rapidly hydrolyzed in serum with a half-life of approximately 28 seconds.

Study title: Determination of the Rate of Conversion of Estradiol-3-Acetate to Estradiol *In Vitro* in Human Serum and Human Whole blood using

Key study findings: At concentrations up to 5000 pg/ml, the half-life of estradiol-3-acetate *in vitro* is <1 minute in human serum and whole blood samples.

Study no: IVR/SP/011

Volume #, and page #: Volume 1.15, Section 5.6.3.1, Page 671

Conducting laboratory and location: _____
(_____ Report Nos. 1450/011-D1145 and RR 06801.1)

Date of study initiation: May 4, 2001

GLP compliance: yes

QA reports: yes

Drug, lot #, radiolabel, and % purity: estradiol-3-acetate, batch #38052685; 99.7%

Formulation/vehicle: none

Methods: The objective of this study was to determine the conversion rate of estradiol-3-acetate to estradiol *in vitro* in human serum and whole blood. Human serum and whole blood samples (pooled from 75 male subjects) were incubated at 37°C ($\pm 2^\circ$) and spiked with 500, 2000 or 5000 pg/ml estradiol-3-acetate. At defined time intervals over a period of 10 minutes, aliquots were taken and mixed with ethyl acetate (containing internal standards) to stop the conversion to estradiol. Studies were performed in triplicate with two aliquots/sample analyzed by _____ one for estradiol-3-acetate and the other for estradiol. Regression analysis was performed by PROC REG using SAS for Windows.

Observations and times: 0.25, 0.5, 1.0, 1.5, 2, 3, 5 and 10-minutes post/estradiol acetate addition

Results: Hydrolysis of estradiol-3-acetate to estradiol was rapid in both human serum and whole blood. A plot of logarithm of estradiol acetate concentration versus time was linear indicating that estradiol acetate hydrolysis followed first order kinetics over the range of concentrations studied. In human serum, the mean $-k_1$ value was 1.48 min^{-1} ; the corresponding harmonic mean half-life value was 28 seconds. At 3 minutes, estradiol acetate was undetectable in serum sample (limit of detection _____). The hydrolysis rate was slower in whole blood where the mean $-k_1$ value was 0.71 min^{-1} and a corresponding harmonic mean half-life value of 59 seconds. Estradiol acetate became undetectable in human whole blood samples between 5 and 10 minutes. The Sponsor has hypothesized that the slower hydrolysis time in whole blood samples may be due to inhibition of esterases by the anti-coagulant lithium heparin.

Summary of individual study findings: Results indicate that estradiol acetate is rapidly hydrolyzed *in vitro* in human serum and whole blood samples. By approximately five half-lives (~2.5 minutes in serum and ~5 minutes in whole blood) concentrations of estradiol acetate in human serum were below the limit of detection _____

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Study title: A Study to Determine the Conversion of Estradiol-3-Acetate Following the Insertion of an IVR Releasing Estradiol-3-Acetate in Healthy Postmenopausal Women

Key study findings:

Study no: IVR 1005 (Report No. RR00801.1)

Volume #, and page #: Volume 1.16, Section 5.6.3.2, Page 829

Bioanalytical laboratory and location: []

Date of study initiation: February 1, 2000

Drug, lot #, radiolabel, and % purity: Batch no. 960902

Formulation/vehicle: Estradiol Acetate Intravaginal Ring (IVR) delivering estradiol acetate equivalent to approximately 0.1 mg/day estradiol

Methods: The objective of this study was to investigate the conversion of E3A to estradiol in blood up to 90 minutes and at 27 and 72 hours after insertion of the drug product. This study was conducted in 14 healthy, postmenopausal women, each wearing an IVR for 72 hours. Serial blood samples were collected for determination of estradiol-3-acetate concentrations in blood and estradiol concentrations in blood and serum. Blood estradiol and estradiol-3-acetate concentrations were determined by _____ at _____ Serum estradiol concentrations were determined by an _____ with _____ method at _____

Observations and times: Blood samples were collected predose and at 5, 15, 30 and 45 minutes, 1, 1.5, 24, 48 and 72 hours post IVR administration

Results: Estradiol-3-acetate was not found in any of the blood samples (LOQ = _____ evaluated. The highest observed individual serum concentration of estradiol was _____ pg/ml at 30 minutes after IVR insertion. Mean serum estradiol PK parameters were as follows: C_{max} = 1501.9 ± 450.8 pg/ml, T_{max} = 0.98 ± 0.5 hr, and AUC₍₀₋₇₂₎ = 27,029.9 ± 5520.2 pg.h/ml. In the majority of samples, whole blood estradiol concentrations were below the limit of quantitation (_____). Mean serum estradiol levels (SD) are presented in the following table.

Serum estradiol levels following IVR/E3A exposures in postmenopausal women:

Pre-dose	0.5 min	15 min	40 min	45 min	1 hr	1.5 hr	24 hrs	72 hrs
20.4	182.6	896.4	1211.2	1264.1	1280.3	1267.9	244.6	104.8
(7.6)	(177.1)	673.9)	(630.8)	(580.6)	(455.7)	(295.8)	(54.0)	(24.6)

Summary of individual study findings: Estradiol-3-acetate was not detectable in any of the whole blood samples evaluated following intravaginal insertion of _____ for a period of up to 72 hours.

PK/TK summary: Estradiol acetate is quickly hydrolyzed to estradiol *in situ*, with no detectable concentrations observed in the systemic circulation.

IV. GENERAL TOXICOLOGY:

Estradiol Acetate: Local and systemic nonclinical toxicity studies were not conducted with estradiol acetate. Evaluation of local toxicity and *in situ* conversion of estradiol acetate to estradiol in the human vagina were conducted in Phase 1 clinical study IVR 1005 (See Clinical Review).

Intravaginal Ring (IVR): Silicones represent a very broad family of synthetic polymers containing a repeating silicon-oxygen backbone with organic side groups and are classified into 3 major types (fluids, resins and elastomers), depending on the degree of elastomerization. The IVR is a _____ cured silicone _____ elastomer.

Chronic toxicity studies in rats, dogs and mice have shown no evidence of chronic or carcinogenic effects following administration of oral doses of _____ Implantation studies in mice, rats, rabbits and baboons with _____ cured silicone elastomers _____ stannous octoate _____) demonstrate the induction of a typical foreign body reaction characterized by an inflammatory response and fibrous capsule formation. There were no indications of adverse systemic effects or any increase in tumorigenesis in these studies. _____ was not found to be genotoxic or antigenic.

To establish equivalency of the _____ elastomer with the previously approved Dow Corning elastomer, Silastic® 382, the Sponsor performed the following CDRH recommended comparability studies:

- identification and quantification of leachables using the following solvents and conditions:
 - pH 4.5 phosphate buffer, saline, methylene chloride, ethanol, and hexane
 - room temperature; after 12 hours at 200°C, and after _____ for 2 hours
- USP Class VI *in vitro* and *in vivo* toxicity studies:
 - acute systemic toxicity
 - 90-day gross and histological implantation study
 - intracutaneous irritation tests
 - bacterial and mammalian mutagenicity
 - *in vivo* cytogenetic damage
 - skin sensitization tests

Leachables: The following table gives the quantities of leachables found following dissolution in pH 4.5 phosphate buffer for three months. _____

Leachables	Maximum Quantity per IVR	Amount Leached (mg/IVR)	
		2 weeks*	40 months*
_____	_____	_____	_____
_____	_____	_____	_____
_____	_____	_____	_____

*Age of IVR (n=6) when dissolution started.

Assays for leachable _____ were also conducted, but there were no detectable levels (LOD = _____) in any of the various extracts tested.

The amount of leachables from an aged IVR is substantially less than that leached from a newer IVR. The Sponsor has attributed this to evaporation of _____ and continuation of the curing process during storage.

_____ is a volatile _____ commonly found in antiseptics used in preparations for disinfecting the hands, skin surfaces and instruments. Potential symptoms of over-exposure to _____ vapors include mild irritation to the eyes, nose, throat and upper respiratory tract. Severe intoxication by inhalation, swallowing or skin absorption may result in central nervous system depression (headache, nausea, vomiting and dizziness), hypotension, anemia and respiratory depression. _____ was not found to be genotoxic or antigenic. Exposures of 50 mg/day is generally considered safe for pharmaceutical products.

_____ has been evaluated in 13-week dietary studies in mice and rats. The main effects were on the liver with an increased relative liver weight and hepatic peroxisomes. Triglyceride levels were decreased and cholesterol levels increased at doses greater than 71 mg/kg/day. All effects were reversible following a 28-day recovery period. The NOAELs for maternal and developmental toxicity were 250 and 100 mg/kg/day, respectively, in the rat, and 25 and 250 mg/kg/day, respectively, in the rabbit. When applied dermally to rabbits, _____ is rapidly absorbed and causes focal erythema and necrosis of underlying dermis and hypodermis at high concentrations (1 g/kg). There was no evidence of induction of mutation in the Ames Assay. However, a low positive response rate (1.8 x the control) was reported in the *in vitro* human lymphocyte sister chromatid exchange test at concentrations ranging from 0.63 to 2.5 mM.

_____ is not readily absorbed. In the US, drinking water guidelines limits the presence of _____ in the region of 4 mg/l and the recommended acceptable intake from foods is 14 mg/kg/week. A mean normal urine level of 16.6 µg/L or 23.4 µg/day has been reported.

The following toxicology studies were conducted to support the safety of the intravaginal silicone polymer ring.

Study title: Elastomer Extract Intracutaneous Study in the Rabbit

Key study findings: no findings of significant irritation in rabbits following intracutaneous injection of elastomer extracts

Study no: TU013-800

Volume #, and page #: Volume 1.15, Section 5.6.2.3, page 536

Conducting laboratory and location: _____

Date of study initiation: 9/25/95

GLP compliance: yes

QA report: yes () no (x)

Drug, lot #, radiolabel, and % purity:

Formulation/vehicle: 1) 0.9% sodium chloride (USP)
2) Cottonseed Oil (NF)

Methods: Test samples were extracted in saline and cottonseed oil at 50°C for 72 hours per USP requirements. 0.2 ml of extract was injected intracutaneously into 5 separate sites approximately 2 cm apart on the right side of the back of each rabbit and the corresponding vehicle was injected on the left side.

Dosing:

Species/strain: New Zealand White Rabbits
 #/sex/group or time point (main study): 2 animals/extract (no specific gender specified)
 Doses in administered units: 0.2 ml extract
 Route, form, volume, and infusion rate: intracutaneous

Observations and times: Observations for erythema and edema were conducted at 24, 48 and 72 hours after injection.

Results: Cottonseed oil with or without elastomer caused significant signs of irritation, e.g., slight to moderate erythema and edema, within 24 hours of injection.

Test Article	Mean Irritation Score	
	0.9% sodium chloride	Cottonseed Oil
Negative Control	0.0	1.7
— Elastomer	0.0	1.7
Dow Corning Elastomer	0.0	1.5

Summary of individual study findings: Under the conditions of this study, there was no evidence of significant irritation from the elastomer extracts injected intracutaneously in rabbits.

Study title: Elastomer Extract Acute Systemic Toxicity Study in Mice

Key study findings: none

Study no: TU012-500

Volume #, and page #: Volume 1.15, Section 5.6.2.4, page 566

Conducting laboratory and location: _____

Date of study initiation: 9/25/95

GLP compliance: yes

QA report: yes () no (x)

Drug, lot #, radiolabel, and % purity: ?

Formulation/vehicle: 0.9% sodium chloride (USP)

Methods: _____ and Dow Corning elastomers were extracted in saline at 50°C for 72 hours per USP requirements. An acute i.v. dose was administered and animals monitored for 72 hours.

Dosing:

Species/strain: Non-Swiss Albino CF1 mice: _____
 #/sex/group or time point (main study): 5 males/test material
 Age: ?
 Weight: 19-22 g

Doses in administered units: 50 ml/kg extract
Route, form, volume, and infusion rate: i.v.

Observations and times:

Clinical observations: immediately after dosing, and at 4, 24, 48 and 72 hours
Body weights: at dosing and at 72 hours

Results:

Mortality: none
Clinical observations: unremarkable
Body weights: all animals gained or maintained weight

Summary of individual study findings: Under the conditions of this study, there was no evidence of systemic toxicity following i.v. injections of elastomer extracts.

Study title: Elastomer Muscle Implantation Study in Rabbits with Histopathology (90 Days)

Key study findings: muscle implants were slightly irritating

Study no: TU014-890 (TH035-800)

Volume #, and page #: Volume 1.15, Section 5.6.2.5, page 595

Conducting laboratory and location: _____

Date of study initiation: 9/19/95

GLP compliance: yes

QA report: yes

Drug, lot #, radiolabel, and % purity: ?

Formulation/vehicle: none

Methods: — and Dow Corning elastomers and USP negative control strips were loaded into 16 gauge needles and sterilized — then implanted in the muscle tissue of the rabbit for a period of 90 days. Six test article sections were implanted in the right paravertebral muscle of each rabbit. In the opposite muscle, four USP negative control strips were similarly implanted.

Dosing:

Species/strain: New Zealand White Rabbit

#/sex/group or time point (main study): 3 animals/test material (no gender specified)

Age: ?

Weight: 3.0 to 3.4 kg

Route, form, volume, and infusion rate: solid material, i.m.

Observations and times:

Clinical observations: daily

Body weights: at dosing, monthly and at termination

Gross Pathology: Representative tissue implant sites were excised. At least 4 of the test article sites and 2 of the control sites were examined histologically and capsule formation or other signs of irritation were scored on a 0-4 scale.

Histopathology: representative implant site tissues were examined microscopically

Results:

Mortality: none

Clinical observations: unremarkable

Body weights: unremarkable

Gross Pathology: unremarkable

Histopathology: There was microscopic evidence of formation of a fibrous capsule and some inflammatory reaction around the elastomer fragments. Macrophages, giant cells, fibrosis and fatty infiltrates were observed at the site of implantation with all three articles.

However, the average score for _____ and the Dow Corning Elastomers was slightly higher (9.3) than with the USP control material (5.0-7.0), resulting in a classification of slightly irritating for both elastomers. There were no signs of necrosis or fibroplasia.

Summary of individual study findings: Under the conditions of this study, _____ was classified as a slight irritant as compared to the USP negative control material.

Toxicology summary: The following potential leachables have been identified from the _____ intravaginal ring material: _____, and _____ Both _____ and _____ were within the permissible exposure limits, while potential exposure levels of _____ were below the no observable adverse effect level in rodents. Furthermore, there was no significant evidence of local or systemic toxicity following intracutaneous injection of elastomer extracts in rabbits or following i.v. injections of elastomer extracts in mice.

Implantation of the _____ cured _____ material for 90 days in the paravertebral muscle of rabbits resulted in a mild local inflammatory reaction characterized by formation of a fibrous capsule, similar to the reactions observed with the previously approved Dow product, Silastic® 382.

Toxicology conclusions: It is considered that the Sponsor has submitted sufficient data to show the comparability and safety of the _____ elastomer with the previously approved Dow Corning Silastic® 382 elastomer based on the following:

- 1) acute and sub-chronic studies conducted with the _____ elastomer and extractables
- 2) similarity between the _____ and Dow Corning _____
- 3) availability in the public domain on the safety (clinical and nonclinical) of _____ cured silicone elastomers

Further chronic testing of the _____ product will not be recommended.

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V. GENETIC TOXICOLOGY:**Study title: Reverse Mutation in four Histidine-Requiring Strains of *Salmonella typhimurium* and Two Tryptophan-Requiring Strains of *Escherichia coli*****Key findings:** negative**Study no:** 1450/2-1052**Volume #, and page #:** Volume 1.14, Section 5.6.1, Page 334**Conducting laboratory and location:** _____**Date of study initiation:** October 19, 1995**GLP compliance:** yes**QA reports:** yes**Drug, lot #, radiolabel, and % purity:** estradiol-3-acetate lot M500002, 99%**Formulation/vehicle:** DMSO**Methods:**

Strains/species/cell line: *S. typhimurium* strains TA98, TA100, TA1535 and TA1537
E. coli strains WP2pM101 and WP *uvrAp*KM101

Dose selection criteria:

Basis of dose selection: solubility

Range finding studies: 8-5000 µg/plate

Test agent stability: stable**Metabolic activation system:** Aroclor induced S-9 from rat hepatocytes**Controls:**

Vehicle: DMSO

Negative controls: DMSO

Positive Controls:

Sodium Azide

9-Aminoacridine

2-Nitrofluorene

4-Nitroquinolone

2-Aminoanthracene

Strains:

TA 1535 & TA100 w/o S9

TA1537 w/o S9

TA98 w/o S9

E. coli WP2 strains

All Strains w/S9

Concentration:

2 µg/plate

50 µg/plate

50 µg/plate

2-10 µg/plate

5 µg/plate

Comments: Estradiol hemihydrate was also tested in all strains as a reference compound**Exposure conditions:**

Incubation and sampling times: 37°C for 3 days, with second phase studies including a preincubation step of 60 minutes

Doses used in definitive study: 6.25 - 4000 µg/plate

Study design: The 1st assay was performed using the standard plate incorporation procedures in both the presence and absence of an Aroclor 1254-induced rat-liver metabolic activation system. The 2nd assay incorporated a 60 minute preincubation both the presence and absence of an Aroclor 1254-induced rat-liver metabolic activation system prior to plating.

Analysis:

No. of replicates: duplicate assays; 3 replicate plates/assay

Counting method: electronic automatic counter _____ or manually for plates treated with ≥ 2000 $\mu\text{g}/\text{plate}$ estradiol-3-acetate due to precipitation of test compound

Criteria for positive results: A test is considered positive if the assay was valid and when a reproducible increase in mean revertant was significant at $p \leq 0.01$ in Dunnett's test and the data set showed a significant dose correlation.

Summary of individual study findings:

Study validity: This assay was considered valid: controls performed as expected.

Study outcome:

- Precipitation of test article was observed at ≥ 2000 $\mu\text{g}/\text{plate}$ w/o S9 and at ≥ 4000 $\mu\text{g}/\text{plate}$ w/S9.
- Slight toxicity was observed in all strains at 5000 $\mu\text{g}/\text{plate}$ with and without S9 and in strains TA98, TA100, and TA1537 at 1000 $\mu\text{g}/\text{plate}$ without S9 or preincubation. In the preincubation assay, slight to moderate cytotoxicity was observed at much lower concentrations: strains TA 98 and TA100 at ≥ 100 $\mu\text{g}/\text{plate}$, strains TA1535, TA2537 and WP2pKM101 at ≥ 200 $\mu\text{g}/\text{plate}$, and strain WP2uvrApKM101 at 400 $\mu\text{g}/\text{plate}$. Significant toxicity was only observed in the preincubation study in strains TA1537 and WP2pKM101 in the presence of S9.
- There was no significant, consistent, or dose-dependent increase in the number of revert colonies which met the criteria for a positive response.
- The reference compound, estradiol hemihydrate, did not induce any increase in the number of revertants at equimolar concentrations to those of estradiol-3-acetate.

Study title: Elastomer Extract Ames Salmonella Mutagenicity Assay

Key findings: negative

Study no: MG019-211

Volume #, and page #: Volume 1.15, Section 5.6.2.6, Page 632

Conducting laboratory and location: _____

Date of study initiation: October 10, 1995

GLP compliance: Yes

QA reports: yes

Drug, lot #, radiolabel, and % purity: CH-021 Sample A - — Silicon Material

CH-021 Sample D - Dow Corning Silicone polymers

Formulation/vehicle: 0.9% USP sodium Chloride for Injection

Methods:

Strains/species/cell line: *S. typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538

Dose selection criteria:

Basis of dose selection: Extracts were evaluated by a spot plate technique, modified after the antimicrobial zone of inhibition test. This screen was used to evaluate the extract's toxicity to determine whether dilution of the extract was required to provide an extract non-inhibitory to *Salmonella typhimurium*. No significant inhibition was observed with either extract.

Range finding studies: none reported

Test agent stability: not reported

Metabolic activation system: Aroclor induced S-9 from rat hepatocytes

Controls:

Vehicle: Saline

Negative controls: Saline

Positive Controls:

Sodium Azide

Dexon (paradimethylaminobenzene
diazosulfonic acid sodium salt)

2-Nitrofluorene in DMSO

2-Aminofluorene in DMSO

Strains:

TA 1535

TA98, TA100 & TA1537

TA1538

TA100 & TA1538;

All Strains w/S9

Concentration:

100 µg/plate

100 µg/plate

100 µg/plate

10 µg/plate

Exposure conditions:

Incubation and sampling times: Plates were incubated at 37°C for 48-72 hours, with or without S9. Following incubation, spontaneous revertants from each plate were recorded.

Doses used in definitive study: 0.1 ml extract

Study design: The assay was performed using standard plate incorporation procedures in the presence and absence of an Aroclor 1254-induced rat-liver metabolic activation system.

Extracts were performed based on the recommended UPS ratio of surface area of test article to volume of vehicle (i.e., 60 cm²/20 ml). 35.6 cm² of _____ material and 59.8 cm² of the Dow Corning silicone polymers were covered with 12 ml of saline and extracted at 121°C for 1 hour. This resulted in almost complete dissolution of the silicone polymers. Test extracts were clear with some particulate matter present.

Analysis:

No. of replicates: 3 replicate plates/assay

Counting method: not specified

Criteria for positive results: A test is considered positive if the assay is valid, i.e., controls perform as expected, and when a reproducible, two fold increase over the negative control in the mean number of revertant for any tester strain. Positive results will be confirmed by demonstrating reproducibility and a dose-related increase in the number of revertants over a minimum of two increasing dose increments.

Summary of individual study findings:

Study validity: valid

Study outcome: Under the conditions of this study, a saline extracts of Silicone material, CH-021, did not induce a significant increase in the number of revertant colonies when compared to the vehicle control.

Genetic toxicology summary: *In vitro* bacterial and mammalian genotoxicity assays performed with _____ extracts did not show any evidence of potential genotoxicity.

Labeling recommendations: none

VI. CARCINOGENICITY:

Carcinogenicity summary: Chronic toxicity studies in rats, dogs and mice have shown no evidence of chronic or carcinogenic effects following administration of oral doses of _____.

_____ Implantation studies in mice, rats, rabbits and baboons with _____ cured silicone elastomers _____, stannous octoate _____ have shown no evidence of systemic toxicity or tumorigenesis.

Carcinogenicity conclusions: It is considered that the Sponsor has submitted sufficient data to show the comparability of the _____ elastomer with the previously approved Dow Corning elastomer. Therefore, the need for carcinogenicity studies using the _____ elastomer are not deemed necessary at this time.

Labeling Recommendations: none

VII. REPRODUCTIVE AND DEVELOPMENTAL TOXICOLOGY:**Reproductive and developmental toxicology summary:**

Estradiol Acetate: There is no indication for estrogen therapy during pregnancy. Estrogen therapy during pregnancy is associated with an increased risk of congenital defects in reproductive organs of the fetus.

_____ Elastomer _____ Potential adverse reproductive and developmental effects were not evaluated with _____ Elastomer.

Reproductive and developmental toxicology conclusions: Products containing estrogens should not be used during pregnancy or by women actively trying to conceive.

Labeling recommendations: current standard labeling for estrogenic compounds

VIII. SPECIAL TOXICOLOGY STUDIES:

Study title: Cytotoxicity Test of a Sample Extract using the MEM Elution Method in the L-929 Mouse Fibroblast Cell Line

Key study findings: Extracts of test elastomer in MEM were not considered toxic to L-929 cells.

Study no: MG023-100

Volume #, and page #: Volume 1.14, Section 5.6.2, page 432

Conducting laboratory and location: _____

Date of study initiation: September 26, 1995

GLP compliance: yes

QA report: yes

Drug, lot #, radiolabel, and % purity:

Sample	Maker	Curing Procedure
A	— Elastomer	J
B	— Elastomer	
C	— Elastomer	
D	Dow Corning Elastomer	
E	Dow Corning Elastomer	
F	Dow Corning Elastomer	

Formulation/vehicle: MEM

Methods: Based on the recommended USP extraction ratio of 60 cm²:20 ml, the following extractions were performed at 37°C for 24 hours:

Sample	Elastomer (cm ²)	MEM (ml)
A	18	6
B	38.1	13
C	29.8	10
D	26.5	9
E	38.1	13
F	38.1	13

Test extracts (5 ml/flask) were placed onto a confluent monolayer (≥80%) of L-929 mouse fibroblast cells and incubated at 37°C in MEM supplemented with 5% calf serum and 2% antibiotics. MEM and sodium chloride granules were used at the negative and positive controls, respectively.

Observations and times: Monolayers were examined microscopically at 24, 48 and 72 hours to determine any change in cell morphology.

Results: Under the conditions of this study, MEM test extracts showed no evidence of causing cell lysis or toxicity (vacuolization, swelling or crenation).

Summary of individual study findings: The MEM test extract was not considered toxic to L-929 mouse fibroblast cells.

Study title: *In Vitro* Hemolysis Test by Direct Contact (Rabbit Whole Blood)**Key study findings:**

Study no: CB037-100-C

Volume #, and page #: Volume 1.15, Section 5.6.2.2, page 510

Conducting laboratory and location: _____

Date of study initiation: 9/15/95

GLP compliance: yes

QA reports: yes () no (x):

Drug, lot #, radiolabel, and % purity: ?

Formulation/vehicle: 0.9% sodium chloride (USP)

Methods: Test articles were added to 10 ml of 0.9% sodium chloride. Whole rabbit blood (0.2 ml) was added to the test solution and each tube was inverted gently to mix the contents then incubated for 1 hour at 37°C. After incubation, solutions were centrifuged and the resulting supernatant examined _____

Results:

Test Article	Absorbance	% Hemolysis
Negative Control - 0.9% sodium chloride (USP)	0.00	0.0
Positive Control - whole blood in purified water	1.97	-
29.2 cm ² _____ Elastomer	0.01	0.5
29.7 cm ² Dow Corning Elastomer	0.01	0.5

Summary of individual study findings: Under the conditions of this study, there was no evidence of hemolytic activity associated with _____ Elastomer following incubation in 0.9% sodium chloride at 37°C for 1 hour.

IX. DETAILED CONCLUSIONS AND RECOMMENDATIONS:

Conclusions: Nonclinical studies performed with _____ cured _____ Elastomer _____ or extractables did not result in any safety concerns regarding its use for sustained intravaginal delivery of estradiol acetate. It was found to be comparable to the previously approved Dow Corning elastomer, Silastic® 382.

General Toxicology Issues: none

Recommendations: approval

Labeling with basis for findings: Labeling for reproductive and carcinogenic effects should be consistent with the most current standard language recommended by the Division of Reproductive and Urologic Drug Products for all estrogen drug products.

X. APPENDIX/ATTACHMENTS:

Addendum to review: none

Other relevant materials (Studies not reviewed, appended consults, etc.): none

Any compliance issues: none