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APPLICATION NUMBER:

206229Orig1s000

PHARMACOLOGY REVIEW(S)

Memo to the file- addendum to NDA Review

Date: 2/10/2015

NDA #: 206229 SS# 0000 SD# 1

Date of submission: 4/29/2014

Sponsor: Medicines360

Drug Product: (b) (4) an Intrauterine Contraceptive

Trade name: Liletta

Indication: Contraception

Subject: Addendum to the NDA Review dated 2/10/2014 for Section 1.2.3 entitled Additional Non Clinical Recommendations. This section should state that no post-marketing studies are requested or planned.

Reviewer: Krishan L. Raheja, D.V.M., Ph.D.

Through P/T Expert Reviewer: Alex Jordan, Ph.D.

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/s/

KRISHAN L RAHEJA
02/10/2015

ALEXANDER W JORDAN
02/11/2015

Memo to the file-Addendum #2 to NDA Review

Date: 12/10/2014

NDA #: 206229 SS# 0000 SD# 1

Date of submission: 4/29/2014

Sponsor: Medicines360

Drug Product: [REDACTED] ^{(b) (4)}, an intrauterine contraceptive

Trade name: Liletta

Indication: Contraception

Subject: Addendum # 2 to NDA to correct spelling of product Trade name Liletta which was spelled wrong in previous addendum.

Reviewer: Krishan L. Raheja, D.V.M., Ph.D.

Through P/T Supervisor: Alex Jordan, Ph.D.

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/s/

KRISHAN L RAHEJA
12/12/2014

ALEXANDER W JORDAN
12/12/2014

Memo to the file-Addendum to NDA Review

Date: 12/10/2014

NDA #: 206229 SS# 0000 SD# 1

Date of submission: 4/29/2014

Sponsor: Medicines360

Drug Product: [REDACTED]^{(b)(4)}, an intrauterine contraceptive

Trade name: Levlitta

Indication: Contraception

Subject: Addendum to NDA to add Trade name

Reviewer: Krishan L. Raheja, D.V.M., Ph.D.

Through P/T Supervisor: Alex Jordan, Ph.D.

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/s/

KRISHAN L RAHEJA
12/10/2014

ALEXANDER W JORDAN
12/10/2014

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

PHARMACOLOGY/TOXICOLOGY NDA/BLA REVIEW AND EVALUATION

Application number: NDA 206229 eCTD Sequence # 0000 SD# 1
Supporting document/s: e-submission
Applicant's letter date: 4/29/2014
CDER stamp date: 4/29/2014
Product: (b) (4) an intrauterine contraceptive (IUC)
Indication: Prevention of pregnancy
Applicant: Medicines360
Review Division: Division of Bone, Reproductive & Urologic
Products (DBRUP)
Reviewer: Krishan L. Raheja, D.V.M., Ph.D.
Supervisor/Team Leader: Alex Jordan, Ph.D.
Division Director: Hylton V. Joffe, MD, M.MSc
Project Manager: Charlene Z Williamson

Template Version: September 1, 2010

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1 EXECUTIVE SUMMARY

1.1 Introduction

The (b) (4) drug product is a levonorgestrel-releasing intrauterine system. Levonorgestrel is a progestin which has been used in a number of FDA approved hormonal contraceptives. In (b) (4) the progestin is mixed with polydimethylsiloxane to serve as drug reservoir. (b) (4) is indicated as an intrauterine contraceptive to last for a period of 3 years. The safety of the levonorgestrel and all components of the (b) (4) system have been established by preclinical testing and their long clinical use.

1.2 Brief Discussion of Nonclinical Findings

In accordance with discussion between the sponsor and the Division in the Pre-NDA meeting, no preclinical studies were recommended by the Division or conducted by the sponsor. All desired studies to establish the safety of the active ingredient of (b) (4), levonorgestrel and materials used in the manufacture of the drug reservoir and inserter have been supported by either reference to published literature or to studies for which sponsor has right of reference. Based on a review of the referenced material along with the long-term clinical use of the active ingredient, the preclinical requirements for determination of the safety of (b) (4) in the proposed population have been met.

1.3 Recommendations

1.3.1 Approvability

Pharmacology/Toxicology recommends approval of the NDA 206229

1.3.2 Additional Non Clinical Recommendations

No additional nonclinical studies aside from those committed by the sponsor and were agreed by the Division in the Pre-NDA meeting are recommended.

1.3.3 Labeling.

Draft label has been submitted. The sections pertinent to Pharmacology/Toxicology are satisfactory. Under Indications and Usage, (b) (4) and sponsor has been informed to add (b) (4) is a progestogen-containing intrauterine system.”

2 Drug Information

2.1 Drug

CAS Registry Number (Optional): 797-63-7

Generic Name: Levonorgestrel

Code Name: None provided

Chemical Name(s): 18,19-Dinorpregn-4-en-20-yn-3-one, 13-ethyl-17-hydroxy-,(17a)-(-)-(-)-13-ethyl-17-hydroxy-18,19-dinor-17a-pregn-4-en-20-yn-3-one (17a)-(-)-13-ethyl-17-hydroxy-18,19-dinopregna-4-en-20-yn-3-one

Molecular Formula/Molecular Weight:C21H28O2/312.45

Structure or Biochemical Description: Levonorgestrel is a second generation (b) (4) progestogen used as the active ingredient in a number of hormonal contraceptives.

Pharmacologic Class: Progestin

2.2 Relevant INDs, NDAs, BLAs and DMFs

IND 105836, DMF (b) (4) ;

2.3 Drug Formulation:

The (b) (4) drug product is a levonorgestrel-releasing intrauterine system (IUS). The drug product consists of a T-shaped polyethylene frame (T-frame) with a (b) (4) (drug reservoir) around the vertical stem. The drug reservoir consists of a cylinder made of a mixture of levonorgestrel and polydimethylsiloxane (PDMS) formed from silicone base, tetra-n-propyl silicate, and stannous octoate. The drug reservoir is covered with a polydimethylsiloxane (PDMS) membrane. The T-frame has an eyelet at one end of the vertical stem and two horizontal arms at the other end. The low density polyethylene of the T-frame is compounded with barium sulfate, which makes it radio opaque. A blue polypropylene monofilament removal thread is attached to the eyelet at the end of the vertical stem of the T-frame.

Composition

The quantitative composition of the drug product and function of each ingredient are provided in table below:

Component	Function	Quality standard	Quantity
Drug Reservoir	Reservoir of LNG active substance	See below	1 Unit (b) (4)
Levonorgestrel (b) (4)	Drug substance	USP	52.0 mg
Silicone base	(b) (4)	In-house standard	(b) (4)
Tetra-n-propyl silicate		In-house standard	
Stannous octoate		In-house standard	
(b) (4)		In-house standard	

	(b) (4)		
T-frame	T-frame with radio opaque agent	See below	1 unit
Low density polyethylene (LDPE)	(b) (4)	In-house standard	(b) (4)
Barium sulfate	Radio opaque agent	In-house standard	
Polydimethylsiloxane (PDMS) membrane	(b) (4)	In-house standard	1 Unit (b) (4)
	(b) (4)	In-house standard	N/A
Polypropylene thread (b) (4)	Removal thread	In-house standard	1 unit
copper			

2.4 Comments on Novel Excipients

It is stated that there are no compendial excipients used in drug product.

Noncompendial excipients and components with source and quality standard is as follows:

Silicone base, tetra-n-propyl silicate and stannous octoate from (b) (4)

Polydimethylsiloxane membrane from (b) (4)

Polyethylene T-frame (b) (4)

Polypropylene thread (b) (4)

It is stated that there are no ingredients that are derived from human or animal origin- sources. There are no novel excipients.

2.5 Comments on Impurities/Degradants of Concern

(b) (4)

Parameter	Acceptance criteria
-----------	---------------------

(b) (4)



Although not strictly applicable to levonorgesrel, USP, all impurities are controlled below the qualification limit, as defined in ICH Guidance: Impurities in New Drug Substance Q3A(R2) and in drug product impurities controlled below the qualification threshold recommended by ICH Guidance: Impurities in New Drug Products Q3B(R2).

(b) (4)



2.6 Proposed Clinical Population and Dosing Regimen

(b) (4) an intrauterine contraceptive (IUC) is intended for prevention of pregnancy for up to 3 years (b) (4). The IUC itself contains 52 mg of levonorgestrel (LNG) that is initially released at a rate of 18.6 ug/day. This rate decreases progressively to 12.6 ug/day (b) (4) 3 years.

2.7 Regulatory Background

At the Pre-IND meeting held in September 2009, the Division agreed that Medicines360 could rely on nonclinical information from the public domain to satisfy the nonclinical requirements for levonorgestrel and the implanted device and that no additional nonclinical studies were necessary at this time. Medicines360 stated that it intends to rely on the following information to satisfy the nonclinical requirements for the LNG20 IUS NDA:

- a. Information on levonorgestrel from the public domain
 1. The nonclinical toxicology information (repeat dose toxicity, carcinogenesis, mutagenesis, and reproductive toxicity) from published studies in the literature.
 2. Nonclinical data from the published literature that evaluates the safety of intrauterine use of levonorgestrel-containing polydimethylsiloxane delivery systems
- b. Nonclinical biocompatibility studies on the components of the delivery system conducted by Medicines360 or to which it has right of reference:
 1. 90-day toxicology study (subdermal implant) conducted to evaluate the safety of the drug reservoir and membrane (Study Tn 020/07-0189)
 2. Biocompatibility studies conducted to evaluate the safety of the thread, drug reservoir, membrane, T-frame, and inserter.

This information is expected to fulfill all nonclinical regulatory requirements for drug product approval: therefore, Medicines360 does not plan to rely upon a listed drug in its 505(b)(2) NDA.

Sponsor's question: Does the Division agree that reliance on these data, which will be provided in the LNG20 IUS NDA, is appropriate and that no additional nonclinical safety data will be required for NDA submission.

FDA response: Yes, the Division agrees, pending review of the submission, and does not anticipate requiring additional nonclinical studies at this time. The sponsor is reminded that reliance on published literature describing a listed drug(s) is considered reliance on FDA's finding of safety and/or effectiveness for the listed drug(s).

3 Studies Submitted

Medicines360 has submitted this NDA for (b) (4) a levonorgestrel-releasing intrauterine system (IUS) via the 505(b)(2) regulatory pathway. The sponsor did not conduct nonclinical toxicology studies for levonorgestrel for the NDA submission and stated that they will rely on data available in the public domain rather than on a listed drug for nonclinical pharmacology and toxicology data. For the components of the intrauterine drug delivery system, sponsor stated that they have conducted one biocompatibility study on the (b) (4) drug reservoir and (b) (4) membrane and 3 biocompatibility studies on the (b) (4) inserter. The remaining toxicology data to support the use of the polymer components of the drug delivery system are from studies in the public domain and studies to which Medicines360 has right of reference. Sponsor's approach was agreed to by the Division in the Pre-NDA meeting on 10/17/2013.

3.1 Studies Reviewed

Sponsor has referred to published literature in the public domain and to studies which sponsor has stated to have right of reference.

3.2 Studies Not Reviewed

No nonclinical toxicity studies have been submitted as agreed by the Division in pre-NDA meeting.

3.3 Previous Reviews Referenced

Sponsor has not conducted any preclinical studies and instead has submitted large list of studies from the published literature available in the public domain and to studies for which sponsor has right of reference to support the safety of the proposed product, (b) (4)

4 Pharmacology

4.1 Primary Pharmacology

The following pharmacological information is from published literature:

Levonorgestrel is a second generation synthetic progestogen used as active ingredient in many hormonal contraceptives. In general, pharmacological effects of LNG are similar to those of the natural progesterone. As a 19-nortestosterone derivative progestin, LNG has potent progestational and anti-estrogenic effects. Use of oral combined (LNG/EE) and emergency contraceptive (which contain high dose of LNG alone) products suppress ovulation; this is the primary mechanism of contraceptive action of these products. In contrast, LNG-releasing IUSs produce high local levels of LNG, and the mechanism of contraceptive action is through local mechanisms such as thickening of the cervical mucus, (which hampers sperm passage), inhibition of sperm motility and function, and alteration of the endometrial morphology rather than through suppression of ovulation.

In contrast to high systemic level attained through oral administration of LNG, clinical studies with LNG releasing IUSs have revealed that they maintain elevated concentrations of API in the endometrium, including myometrium and fallopian tubes, while plasma levels remain low i.e., 4 to 13% of levels observed with daily use of oral contraceptives containing 150 ug LNG. With such regimen women have normal ovulatory cycles, so suppression of ovulation is not a main contraceptive mechanism for LNG-releasing IUS. Animal studies have demonstrated that the local effects of LNG-releasing IUS are thickening of the cervical mucus, alteration of endometrial morphology (endometrial thinning, glandular atrophy, stromal decidualization and inflammation) and a sterile foreign body reaction that impedes sperm and ovum transport, fertilization, embryo development and implantation.

4.2 Secondary Pharmacology

Non-contraceptive effects of LNG include its use in the treatment of a number of gynecological conditions, including menorrhagia, uterine fibroids, endometrial hyperplasia and use in hormone replacement therapy. Rationale for use in these conditions is presumably the endometrial atrophic effect of LNG.

4.3 Safety Pharmacology

Sponsor has mentioned that no studies of the effect of LNG on respiratory or neurological function were identified. However, safety was suggested indirectly because no clinical signs suggesting effect on these organs was reported in the chronic toxicity studies conducted in rats, dogs or monkeys. No effect on monkey ECGs were reported in animals treated every 3 months for a year during a repeat-dose toxicity study with daily oral dosing up to 2.50 mg/kg.

5 Pharmacokinetics/ADME/Toxicokinetics

5.1 PK/ADME

Medicines360 did not conduct nonclinical pharmacokinetics studies on levonorgestrel using (b) (4) and instead decided to rely on data available in the public domain rather than upon a listed drug for nonclinical pharmacokinetics data in support of its NDA submission. The Division agreed to this approach in the Pre-NDA meeting. Sponsor stated that PK of LNG (absorption, distribution, metabolism and excretion) in humans following both oral and intrauterine administration have been well characterized. Although few animal studies on the PK of LNG by either oral or intrauterine administration were identified in the published literature, only one study in rabbits was identified that compared serum levels with intrauterine administration to serum levels attained by another route of administration as shown in Table below:

Serum Concentrations of Levonorgestrel in Rabbits

Study	Route	Dose	Serum levonorgestrel
Nisker, 1988	Intrauterine	25 ug/day (release rate)	➤ 0.12 ng/mL
Zook, 1987	Intravaginal	98 ug/day (release rate)	0.399 +/- 00.083

		rate)	ng/mL
Janne, 2001	Intrauterine	233 ug/day (release rate)	1.3 +/- 0.1 ng/mL
Janne, 2001	Oral	300 ug norgestrel (racemic)	1.3 +/- 0.1 ng/mL

The data suggested that there is correlation between amount of local LNG release by intrauterine or intravaginal administration and serum concentration in rabbits. However, differences in serum LNG concentrations on its contraceptive effect were not identified in nonclinical literature. It was stated that clinical data appears to indicate that there is no relationship between serum LNG and contraceptive effect, ovulation, or side effects. Nonclinical studies of intrauterine administration support a local mechanism of action for the contraceptive effect of LNG-releasing IUSs.

5.2 Toxicokinetics

(If not included in toxicity studies)

None identified in the referenced published toxicity studies.

6 General Toxicology

Sponsor has not conducted any toxicology studies with levonorgestrel, the active pharmaceutical ingredient in (b) (4) (levonorgestrel releasing intrauterine system). Instead all toxicology studies required to support the safety of levonogestrel have been referred to published literature. As such review given below does not give details of the studies referred by the sponsor. Instead the studies are summarized describing only significant finding which could impact drug safety. This approach is taken since LNG has been used over 40 years as contraceptive agent without any significant adverse effects.

6.1 Single-Dose Toxicity

Reference to two single dose toxicity studies, one in mice and one in rabbits (Hillesheim, et al 1988) is provided. In mice (10/s) LNG was administered by ip route at doses levels of 1000, 2000, 2500, 3000 and 4000 mg/kg. It resulted in observed maximum lethal dose of 1000 mg/kg. Doses > 2000 mg/kg caused central depressant symptoms and animals generally died in 1 – 3 days.

In 2 rabbits dose levels of 2000 and 3000 mg/kg were used via ip administration. No lethality was reported.

6.2 Repeat-Dose Toxicity

In a one year oral gavage study (Hite, 1991) in female rats having 10 rats each from the control, 0.5 and 25 mg/kg/day and 30 animals in the control and 0.5 and 25.0 mg/kg/day groups. Animals were dosed seven days per week up to 52 weeks followed by a 3-month recovery period. At the dose of 25 mg/kg/day body weight was increased

significantly but at recovery no clear difference was noted. Increased triglyceride and decreased cholesterol concentrations at 12 months were noted at 25 mg/kg/day. The mean clotting time was increased at 0.5 mg/kg/day in the recovery period. Drug related significant decreases in mean absolute and mean relative weights of the uterus at the terminal and recovery sacrifices, and for the pituitary at the terminal sacrifice at 25.0 mg/kg/day were noted. Increased liver weights and ratios at 25 mg/kg/day were associated with increases in body weight and correlated microscopically with increased glycogen and fat deposition. No macroscopic or microscopic correlation with decreased pituitary and uterine weights was found. NOAEL was 0.01 mg LNG/kg/day in female rats.

In a 6 month oral study (Hoffman et al.1988) in dogs (4 groups of 4/s/g) given 1.0 mg/kg/day, 100% survival was observed. Decreased organ weights for ovary, uterus, prostate and testes were observed in LNG-treated dogs.

A study by Wadsworth et al, 1979 entitled "Treatment of rhesus monkeys (Macaca Mulatta with intrauterine device loaded with levonorgestrel)" was designed to assess local toxicity and determine the effects of levonorgestrel-loaded plastic intrauterine devices on endometrial morphology in 15 rhesus monkeys with evidence of cyclic activity for 14 weeks. The devices were designed to release 25 ug of the hormone/day and were inserted in the uterus by hysterectomy. Control animals were sham-operated or received inert placebo devices. Hematological and biochemical investigations were conducted during the pre-treatment period and then again after 6 and 12 weeks treatment. After 14 weeks treatment, animals were killed and various organs were weighed. Representative samples of ovary, fallopian tubes, uterus, cervix, and vagina were processed for microscopic examination.

Results: Data showed no treatment-related changes in any hematological or biochemical parameters and results of urinalysis were within normal range. The group mean weights of ovaries were significantly reduced. Changes observed in endometrial morphology included atrophy of the endometrial mucosal and glandular epithelium and decidualization of the endometrial stroma. With inert placebo control devices only minor changes in endometrial morphology were observed.

In a 1 year oral gavage study (Hite, 1991) in female Cynomolgus monkeys (5- 8 animals/g) administered LNG at doses of 0, 0.00025, 0.025, 0.25 and 2.50 mg/kg/day, a NOAEL of 0.00025 was reported. There was 100% survival in all groups. Dose-related increase in body weight was observed at doses > 0.025 mg/kg/day. Prothrombin time was increased and cholesterol concentration was decreased at 2.5 mg/kg/day. All changes were within normal range at the end of 3-month recovery period. Occasional increases in fibrinogen levels were detected at 0.25 and 2.50 mg/kg/day. All values for PTs and fibrinogen levels remained within normal range of historical control values. There was a LNG-related increase in the incidence of thickening of the uterine wall in monkeys in the 0.25 and 2.50 mg/kg/day treatment groups, which was noted macroscopically at the terminal sacrifice. Decidual transformation of uterine endometrium, increased glandular secretion in cervix, decreased mucosal keratinization

in vagina, follicular atresia and absence of corpora lutea in the ovaries was observed in LNG treated monkeys.

In a 2-year SC implant study in female cynomolgus monkeys at doses of 0.014 – 0.050 mg/monkey/day, there was no effect on survival. Uteri and ovaries in high dose group weighed less compared to that of the controls. No adverse tissue reactions were observed at implantation site nor were any tumors found. Increased hematocrit and hemoglobin were noted as were decreased cholesterol and triglycerides.

A 90-day study entitled "Toxicity study 90 days after implantation in the subcutaneous tissues of a rat: test element- Levosert Placebo" was conducted in 2007 by EVIC France, Blanquefort for Mithra Pharmaceuticals, Liege-Belgium. The study was conducted in accordance with GLP regulations of the OECD.

The objective of the study was to evaluate the toxicity of Levosert Placebo- Batch 060512, consisting of reservoir and membrane.

Test system: Twenty 8 week old SD female rats were used. Animals were divided in 2 batches of 10 females. On the day of implantation, 2 subcutaneous pockets (0.5 cm x 1.5 cm) were made on both sides of the vertebral axis of each animal. A sample of test element (sterile cylinder 10mm x 3.5 mm, weight 100mg) was placed in each SC pocket of the treated rats. The mass of test element with tissues represented 800 mg/kg b.w. The control batch received in each pocket 0.1 ml of control element (NaCl 0.9%)

During the 13 week observation period, animals were examined for clinical signs, body weight and weekly food intake, ophthalmoscopic examination before implantation and then at the end of experiment, hematology and serum chemistry at the experiment in all animals. All animals were autopsied, organ weights were taken and processed for histopathology.

Results: There was no mortality during the experiment, and no clinical signs of toxicity were noted. Body weight and food consumption was comparable to control group.

Compared with control group, there was significant decrease in average cell volume and increase in Hb ($p < 0.05$). No significant effect on serum chemistry.

Autopsy examination: No lesions related to implantation of Levosert were observed. There was significant ($p < 0.05$) increase in the absolute and relative weight of the adrenal glands compared to animals in the control group.

Histopathological examination: No lesions were noted related to implant. No cutaneous tissue reaction in contact with implant was reported.

Conclusion: Under the experimental conditions used, Levosert Placebo implant (reservoir and membrane) implanted in the SC tissue had no systemic toxic effect or local tissue reaction.

7 Genetic Toxicology

7.1 An *In Vitro* Reverse Mutation Assay in Bacterial Cells (Ames) was conducted (Lang and Reimann, 1993) to test the genotoxicity of levonorgestrel using *Salmonella typhimurium* strains TA1535; TA100; TA1537; TA 1538 and TA98 with and without S9 mix. Dose levels used included 5, 25, 125, 500 and 2500 ug/plate. Appropriate positive and negative controls were used. Revertant colony count was based on mean of 3 plates.

There were no cytotoxic effects. At the highest concentration precipitation was noted for all strains. Negative and positive controls exhibited their expected effects. Based on the finding of this study, levonorgestrel was considered not genotoxic.

7.2 *In Vitro* Assays in Mammalian Cells

In a review article (Jordan, 2002) entitled "Toxicology of progestogens of implantable contraceptives for women". Levonorgestrel was examined for genotoxicity in a number of systems. It was reported that LNG did not increase point mutations in the Ames *Salmonella*/microsome test with or without a metabolic activating system. The steroid was also negative in a mammalian mutagenesis assay in the mouse lymphoma cells, and in-vitro chromosomal aberration assay in Chinese hamster ovary cells, as well as in an in vivo mouse micronucleus test. No data was presented in this review article.

7.3 *In Vivo* Clastogenicity Assay in Rodent (Micronucleus Assay)

Mouse micronucleus test was negative when tested with LNG as stated in review article by Jordan described under 7.2.

7.4 Other Genetic Toxicity Studies

None given

8 Carcinogenicity.

In a literature review article entitled "Predictability of the safety of hormonal contraceptives from canine toxicological studies" by Larsson & Machin (1989), data was collected for a great number of studies through courtesy of the pharmaceutical industry and by FOIA, USA. Special analyses have been made on the data on nodule rate and appearance of malignancies in the mammary glands. Authors concluded that strong support was obtained for the view that the long-term beagle studies could not predict toxicological effects of contraceptive steroids in humans. It was further stated that there is reason to believe that all progestogens if they reach a certain concentration can cause and increased nodule rate and also induce malignant tumors in the mammary gland of the beagle bitch. Tumors in other organs were not found to be induced by contraceptive steroid, which included levonorgestrel and d, l-norgestrel with and without ethinyl estradiol. Studies with d,l-norgestrel given at a dose 25 times the human dose, the mammary nodules incidence by third year was already 37% and increased to about

75% in the fourth, fifth and sixth years with dose response relationship. No malignant neoplastic changes in mammary glands were reported.

With LNG administered at 2, 10 and 25 times the human dose, there was low mortality during the study. The incidence of mammary nodules was low in the low and mid dose groups compared to both control and high dose groups. The incidence of malignant tumors was reported in all groups except the mid dose. The rate of malignant tumors developed in 9/32 of the control group, 6/16 in the highest dose group and 1/16 in the low dose group.

In another review article entitled "Toxicology of progestogens of implantable contraceptives for women" by Jordan (2002), use of four progestogens used in implantable contraceptives i.e., LNG in Norplant and Jadelle; etongesterol in Implanon; nesterone in Elcometrine, and nomegesterol acetate in Uniplant and Surplant were discussed. It was stated that all four progestogens underwent preclinical testing following the format for animal testing of steroid contraceptives published by the WHO and the US FDA. Most of the progestogens have been tested for genotoxicity in bacterial and mammalian cultured cells and in rodents. All were tested in short and long-term toxicology studies in rodents, dogs and monkeys, all were tested for their effects on reproduction and fetal development. Most of these were tested for carcinogenic effect in two rodent species, rats and mice. The author concluded that the published data and data submitted to the FDA demonstrate that the implantable progestogens have no significant or unusual toxicities and have similar safety profile to the progestogens found in the approved oral contraceptives.

In a study entitled "Reduced incidence of rabbit endometrial neoplasia with levonorgestrel implant" by Nisker et al (1988), authors tested the hypothesis that progestogens protect against the development of neoplasia. To test their hypothesis, they placed PDMS implants (levonorgestrel or inert) in to the right uterine horn at random in 114 old female rabbits. Cross-sectional uterine biopsy specimens were taken from both horns at the time of implantation and at 6, 12 and 24 months thereafter. Twenty-nine LNG-treated and 33 controls survived to the completion of the study.

The incidence of endometrial neoplasia was 17.2% in the LNG-treated group which was significantly less than the 42.4% incidence observed in the control does. One treated doe which died before completion of the study had endometrial tumor. No endometrial neoplasia was found in the 8 does with serum LNG concentrations >0.12 ng/ml. Only one of the 5 tumors in the LNG-treated group occurred in the horn containing LNG implant, which authors suggested that a dose effect is likely. The data shows that the protective effect of LNG was not complete. Furthermore, it is pointed out that it is difficult to assess the importance of serum and intrauterine LNG levels in this study. Also although no tumors were found in rabbits with serum LNG >0.12 ng/mL, the small numbers make it difficult to assign importance to the possibility of an absolute serum level above which no neoplasia occurs. All does with tumors had lower LNG levels. However, a significant correlation between the serum LNG level and tumor incidence was not observed.

Although a short study entitled "Tissue concentrations of levonorgestrel in women using a levonorgestrel-releasing IUD" was not planned to determine the effect of plasma LNG concentration and its relation on the incidence of uterine tumor occurrence, it does provide information on the quantitative levels of LNG in various reproductive organs with LNG-IUD which could suggest a protective effect of LNG IUD on uterine tumors. The LNG levels after 36 to 49 days of implant insertion releasing 30 ug LNG daily and oral daily contraceptive tablet (Cyclabil) containing 2 mg estradiol valerate and 250 ug of LNG given for 7 days is shown in the table below on levonorgestrel concentrations (mean +/-SD) in blood plasma, fat, myometrial and fallopian tube tissues:

Tissue	IUD	Cyclabil
Plasma (pg/mL)	202+/-102* (7)	559+/-209* (4)
Fat tissue (ng/g/wet wt)	1.23+/-0.46** (7)	4.41+/-1.06** (4)
Myometrium (ng/g wet wt)	2.43+/-1.86 (6)	1.42+/-0.46 (4)
(pg/mg protein)	34+/-23 (4)	25+/-15 (4)
Endometrium (ng/g wet wt)	808+/-511 (4)	3.5 (2)
(pg/mg protein)	6937+/-3126 (4)	44 (2)
Fallopian tube (ng/g wet wt)	1.8 (3)	1.7 (2)
(pg/mg protein)	17 (3)	19 (2)

* P<0.05. ** p <0.01 Values in parenthesis indicate number of samples.

The results showed that the concentration of levonorgestrel in myometrium, fallopian tube and fat tissue were between 1 and 5 ng/g wet weight of tissue in both the intrauterine device group and orally treated group. In the endometrium the LNG concentrations were many-fold higher in the IUD group.

It was reported that in in vitro experiments there was rapid uptake of LNG and an unsaturability of fat tissue by the steroid at the concentrations used. Fat tissue concentration of LNG correlated with the plasma concentrations suggesting possible implications in obese patients using steroid contraception.

9 Reproductive and Developmental Toxicology

9.1 Fertility and Early Embryonic Development

In a study by Shirley and Bundren (Contraception 51:209, 1995) entitled "Effects of levonorgestrel on capacity of mouse oocytes for fertilization and development" authors examined whether LNG implants may exert their contraceptive effects in part by adversely affecting oocyte quality. Time-release pellets and silastic capsules containing LNG were implanted subdermally in mice and left in situ for 60 or 90 days. Mice were then injected with gonadotropins to stimulate ovulation and oocytes, inseminated and

subsequently cultured for 96 hours, some in medium supplemented with 1.5 or 3.0 mg LNG for day 60 examination or with 5 mg LNG for day 90 examination. Appropriate controls were used for both 60 and 90 day experiments. The number of oocytes that were fertilized and the number that developed to at least to the morula stage were determined

Results for the 60 day groups showed that total number of oocytes were significantly more for the treated groups than the respective control groups. However, the percent fertilized was greater for the LNG treated compared to controls as was the percentage to the morula stage.

In the 90 day groups, number of total oocytes was statistically greater than the controls while percent to morula stage was similar.

Results therefore, suggested that contraceptive effect of LNG should be attributed to effects other than diminished oocyte quality. LNG thus will not adversely affect early embryonic development in the event that pregnancy be initiated during its use.

9.2 Embryonic Fetal Development

In a study by Kwarta et.al. 1991 entitled “Levonorgestrel/ethinyl estradiol study of developmental toxicity in rabbits” LNG/EE oral contraceptive was administered orally by gavage to 20 mated NZW rabbits/g during organogenesis to evaluate the potential for producing developmental toxicity (embryo/fetal toxicity). Dosage levels of 1.6, 8.0 and 40.0 ug/kg/day were administered based on a pilot study where doses up to 1000 ug/kg/day were used. Untreated controls and lactose vehicle controls were used for comparison. Body weights were recorded on GD 0, 6, 12, 19, 24 and prior to sacrifice on GD 29 and fetuses were examined.

There was no drug-related effect on survival, fertility and abortion rates, postmortem examination findings or hysterectomy findings.

In does, drug-related effects included significant decreases in body weight gains and food consumption on GD 6 – 11 in the 40 ug/kg/day group during the first half of the treatment period, GD 6 -11 in the 8 and 40 ug/kg/day groups. The decreased food consumption persisted during the second half of the treatment period, GD 12 – 18. Water consumption was also significantly reduced on GD 6 – 11 in the 40 ug/kg/day group.

With respect to fetuses, LNG/EE had no adverse effect on body weight, placental weight, sex distribution or external, visceral and skeletal morphological development.

NOEL for maternal effects was 1.6 ug/kg/day and NOAEL for developmental effects was 40.0 ug/kg/day.

In a publication entitled “Levonorgestrel/ethinyl estradiol developmental toxicity with behavioral and reproductive assessment of offspring (Seg II-rat) by Kwarta et al. (1991), LNG/EE combination at dose levels of 3.2(2.0/1.2 LNG/EE), 16 (10.0/6.0 LNG/EE) and 80 (50.0/30.0 LNG/EE) ug/kg/day was administered to 40 mated females/g from GD 7 to 17.

The effect of treatment was evaluated for its effect on potential development toxicity (embryo/fetal toxicity and teratogenicity), post-natal survival, growth, reflex and morphological development, learning and memory capability, and reproductive performance of the offspring.

The selected doses were based on a pilot study in which doses of 0.32, 8.0, 20.0, 800.0 and 40,000.0 ug/kg/day were tested in comparison with a vehicle control group. Doses of 800.0 and 40000.0 ug/kg/day were considered inappropriate for a teratology study.

One rat in the 800 ug/kg group and all five rats in the 40,000 ug/kg group had complete litter resorption. Retarded fetal development was reported at 800 ug/kg dose group with reduction of ossification of the sternbrae and skull bones.

On GD 29, the first 20 pregnant females from each group were sacrificed. Thoracic and abdominal viscera were examined. Uterus and ovaries were examined and weighed. Corpora lutea in each ovary were counted. Uteruses were examined for number and relative position of implantations.

Conceptuses were identified as early resorptions, late resorptions, dead fetuses or live fetuses. Live fetuses were weighed and examined palatal closure and gross external malformations. Placenta weights were recorded.

One third of the live fetuses from each litter were processed for visceral and skeletal malformations. The remaining female rats of each group were allowed to deliver and raise their progeny for 21 days.

F1 pups were examined for reflex and morphological development parameters and other development and behavioral parameters. Beginning PD 22 to PD 25, rats were tested for learning and memory in a passive avoidance paradigm. At 11-12 weeks of age F1 rats were mated for reproductive performance. F2 pups were examined and sacrificed on PD 14.

Results: In dams, drug-related effects occurred in the 16 and 80 ug/kg/day dose groups including significant decrease in gestation body weight gains and food consumption during the treatment period. Increased number of rats with alopecia was reported in all drug-treated groups compared to lactose/vehicle control and untreated control groups with no dose-response relationship.

In pups, no adverse drug-related effects were observed for f1 or f2 generation, including no effects on F1 external, visceral and skeletal morphological development.

Based on the data presented, the maternal no observable effect level (NOEL) was 3.2 ug/kg, and the developmental NOEL for the F1 and F2 generation was 80.0 ug/kg.

9.3 Prenatal and Postnatal Development

AS described under section 9.2 above.

10 Special Toxicology Studies

The following three toxicity studies for test article, Levosert-20 inserter (graduated tube, flange, pusher, EO sterilized) Lot Batch # 110726 were conducted by (b) (4) for Odyssea Pharma sa, Belgium in 2011 for which sponsor has right of reference..

Study 1: (b) (4) Final GLP Report: 11-4048-G1 entitled "L929 neutral red uptake cytotoxicity test (1 concentration) – ISO".

This study was conducted in compliance with U.S. Food and Drug Administration regulations set forth in 21 CFR, Part 58. This study was based upon ISO 10993-5-2009, Biological Evaluation of Medical Devices- Part 5: Tests for In Vitro Cytotoxicity.

The purpose of the study was to determine the biological reactivity of the mammalian cell culture (mouse fibroblast L929 cells) in response to test article extract. The study was based on the measurement of the viability of cells via metabolic activity. Neutral red (NR) was actively internalized in the lysosomes of viable cells. The number of viable cells correlated to the color density determined by photometric measurements after extraction of the incorporated NR.

Test article was (b) (4) inserter, negative control article was high density polyethylene (negative control plastic), positive control article was Natural rubber, untreated control was (extraction medium) serum supplemental MEM.

Preparation of test and control articles:

The test article was combined with vehicle at ratio of 6 cm²/ mL MEM per ISO 10993-12 guidelines and extracted at 37+/- 1 C for 24+/-2 hours. The positive and negative controls were extracted at a ratio of 3 cm²/mL in complete MEM at 37 +/- C for 24+/-2 hours. The extracts were not filter sterilized prior to cell monolayer. All cultures were incubated for 24 -26 hours at 37 C in humidified atmosphere containing 5% CO₂.

The cell viability was expressed as $Viability \% = 100 \times OD_{540e} / OD_{540a}$
Where OD_{450e} is mean value of optical density of the 100% extract of test article;
OD_{540a} is the mean value of the measured optical density of blanks (cells exposed to extraction medium = untreated).

The lower the viability % value, higher the cytotoxic potential of the test article is. If the viability is reduced to < 70% of the blank, the test article is considered to have a cytotoxic potential.

Results: Table below provides optical densities and calculated viability:

Without blank (no cell) subtraction (per ISO 10993-5, 2009)

	Untreated	Negative control	Positive control	Test article
Average of 12 replicates	0.393	0.400	0.132	0.421
Viability%	100 %	102%	34%	107%
With blank (no cell) subtraction. Blank OD=0.088				

Average	0.305	0.312	0.044	0.333
Viability	100%	102%	15%	109%

Conclusion: Based on the criteria of the protocol and the ISO 10993-5, 2009 standard, the test article is not considered to have cytotoxic potential.

Study 2: (b) (4) Final Report: 111-4048-G2 entitled "Intracutaneous Injection Test – ISO". for (b) (4)-20 inserter.

This study was conducted following reference:

ISO 10993-10, 2010, Biological Evaluation of Medical Devices – Part 10; Tests for Irritation and Skin Sensitization.

The test article for this study was same as used in the above cytotoxicity study, and conducted by the same laboratory and for the same sponsor using the same article Lot/Batch : 110726.

The negative controls were 0.9% sodium chloride for injection and cottonseed oil (CSO).

Three NZW rabbits (1 male and 2 female) were used after 5 days of acclimation. They were examined that their skin was free from irritation, trauma and disease. Animals were clipped free of fur on the dorsal side prior to injection.

The test article was separately extracted in NaCl and CSO at 70 C for 24 hours. Each control article was prepared in the same manner as the test article.

Dose administration: A volume of 0.2 mL per site of one extract was injected intracutaneously on one side of each of 3 rabbits, 5 sites for the test article extract and 5 posterior sites for the control. Similarly, on the other side of each rabbit, the other extract was injected.

Post-dose procedure: The injection sites on each animal were observed for erythema and edema immediately following injection and at 24, 48 and 72 hours after injection of test extract. Skin reactions were scored for erythema and eschar formation on a scale of 0 (no erythema) to 4 (severe erythema to eschar formation). Similarly edema was scaled as 0 (no edema) to 4 (severe edema). Observation also included clinical and toxicological signs.

Evaluation of animal data: After 72 hour grading, all erythema grades plus edema grades for 24, 48 and 72 hours were totaled separately for each test article and vehicle control for each animal. For calculating score for each individual animal, each of the totals is divided by 15 (3 scoring points x 5 injection sites). For overall mean, scores are added for the 3 animals and divided by 3. The final test article score is obtained by subtracting the control score from the test article score.

Results: All test animals had increase in body weight. No animal exhibited signs of toxicity. The biological reaction at the test injection sites was similar to that observed for the control sites. The difference of the overall mean score between the test article and the control was 0.0.

Conclusion: The test article did not show a significant greater biological reaction than sites injected with control article.

Study 3. (b) (4) Final Report: 11-4048-G3 entitled "Kligman Maximization Test – ISO" for (b) (4)-20 inserter.

This study was conducted based on the following reference:

ISO 10993-10,2010, Biological Evaluation of Medical Devices – Part 10: Tests for irritation and Skin sensitization.

ISO 10993-12, 2007, Biological Evaluation of Medical Devices – Part 12: Sample preparation and reference materials.

Magnusson, B. and A.M.Kligman "The identification of contact allergens by animal assay. The guinea pig maximization test." J Invest.Dermatol. 52 (1969): 268-276.

Study was conducted in accordance with FDA's GLP regulations.

The test material (b) (4)-20 inserter Lot/Batch # 110728 was as in previous 2 studies. The control articles were NaCl and CSO. The positive control article was Dinitrochlorobenzene (DNCB).

Thirty-five Hartley guinea pigs (16 males and 19 females were used). Distribution of animals was as follows:

1. Experimental (10 animals/extract)
2. Negative controls (5 animals/extract)
3. Positive control (5 animals/study)

Preparation of test and control articles were prepared as described under Study 1. The positive control was dissolved in 95% ethanol. Extracts were used at 100% concentration.

Induction/intradermal application (Day 0):

Three pairs of intradermal injections were made so that on each side of the midline three was one row of 3 injections each. Injection sites (6) were within the boundaries of a 2 x 4 cm patch, which was applied one week following the injections. Dosing solutions were prepared for experimental, negative control and positive control groups and extracts were used neat.

Topical application: On Day 6, animals that showed no signs of irritation or corrosion after induction application were pretreated with 10% sodium dodecyl sulfate in petrolatum 24 hours before topical application.

On Day 7, the test article extract was spread over a 2 x 4 cm piece of filter paper to saturation and it was secured with an occlusive wrapping wound around the torso of the animal. The dressing was left in place for 48 hours. Negative control animals were exposed to vehicle without test article and positive control group exposed to 0.1% DNCB solution in the same manner as the experimental group.

Challenge application (Day 23): Pieces of the extract saturated filter paper, measuring 2 x 2 cm, were secured to the previous unexposed area of the animal for 24 hours with the same occlusive bandage used for topical application. Negative and positive controls were applied same way.

Post-dose procedure: Skin reading: Immediately after removing the patches, the challenge sites were cleaned and shaved and readings were taken at 24, 48 and 72 hours after challenge exposure period. For evaluation of skin reactions a four point scale was used (0 as no visible change and 3 as intense erythema and swelling). Any animal showing skin reaction of 1 or greater was considered positive.

The sensitization classification was based on sensitization rate (%). A sensitization rate of 0 – 8 was classified as weak and 81 – 100 was termed as extreme.

Results: All animals gained in body weight. No systemic signs of toxicity were observed in treated or control animals.

Sensitization: None of the treated (NaCl or CSO extracts) or negative control animals elicited any reaction to the challenge (0% sensitized). Positive control article elicited moderate to intense reactions in all animals (10% sensitized).

Conclusion: Based on scoring system of Kligman, the test article was as having weak allergenic potential.

P/T Comment: Treatment's allergic and sensitizing potential for humans will be best evaluated in the clinical trials.

The following studies for testing the toxicity of polypropylene, monofil EP 2, CU blue were conducted by [REDACTED] (b) (4) [REDACTED] in 2007 – 08 to which sponsor has right of reference

Study 1. Investigation of acute systemic toxicity according to DIN EN ISO 10993-11: 2006. Code No. AT.27120278

The test material was extracted in polar (0.9% NaCl) and apolar (CSO) extraction medium. A 6 cm² of the test material/mL of extraction medium was kept at 37 C for 72 hours in an orbital shaker and was used within 4 hours.

Healthy 6 – 12 month old mice were used in two groups of 5 animals to test the extracts and two groups of 5 animals to test the solvents (control). The polar extract (50 mL/kg) was injected in the tail vein at a rate of 0.1 mL/s. The apolar extract was injected intraperitoneally. Mice were checked for toxic symptoms and weighed 24, 48 and 72 hours after injection.

Acute toxicity was scored on a scale of 0 – 5.

0 (normal) - Mouse exhibits no adverse physical symptoms and

1 (Slight) – mouse exhibits slight but noticeable symptoms of hypokinesia, dyspnea, or abnormal irritation.

2 (moderate) – mouse exhibits definite evidence of abdominal irritation, hypokinesia, prosis or diarrhea

3 (marked) – mouse exhibits prostration, cyanosis, tremors, or severe symptoms of abdominal irritation, diarrhea, ptosis, or dyspnea ot extreme weight loss.

4 (dead, expired) – mouse dies after injection

Test criteria was as follows:

No acute toxic reactivity if during the 72 hours observation period all animals are without any biological reaction when compared to controls.

Toxic reactivity was considered if 2 or more animals show either marked symptoms (3) of toxicity or die i.e. samples do not meet the requirement of the test.

Symptoms observed: No symptoms were observed in either test or control animals in either test or control groups. Treatment had no effect on body weight.

Conclusion: The test material, Polypropylene, monofil EP 2, CU blue did not cause acute toxicity under the described test conditions.

Study 2. Sensitization test according to DIN EN ISO 10993-10: 2007 “Closed patch sensitization test.” Code No. ST 27120278

Preparation of extracts: Both polar and apolar extract were prepared as described under Study 1 above.

Animals: Healthy adult albino guinea pigs (strain Dunkin Hartley) were used.

Test procedure: Two groups of 10 animals were selected for the extracts, and two groups of 5 animals were selected to test the solvents.

Induction Phase: An area of 3 x 3 cm on the left flanks was clipped and following day 0.5 mL of polar and apolar extracts on 2.5 x 2.5 cm 4-ply gauze patch were applied. The gauze patches were attached to the skin covered with non-occlusive gauze patch and wrapped with an occlusive bandage for 6 hours. The wrapping was then removed and residual substances washed off with warm water. This sensitization procedure was performed 3 times at weekly interval.

Challenge phase: Two weeks after the last application, the challenge test was performed. A day before the test procedure, the fur of guinea pigs right flanks was clipped. Next day they underwent the same procedure with polar and apolar extracts for 6 hours as described under induction phase. 24 hours after the challenge, both polar and apolar treated animals were closely clipped at the test area and 2 hour after that, the skin reaction was graded. The grading was repeated after 24, and 48 hours.

Results: For skin reaction, erythema and eschar formation and edema formation were graded on a scale of 0 – 4

Grades of 1 or larger in the test group indicate sensitization, provided that grades given for control animals are less than 1. If grades of 1 or larger are given for control animals, then reactions of test animals which exceed the most severe control reaction are presumed to be due to sensitization.

On the basis of sensitization rate, the allergic rate of the test material was classified as weak (0 – 8), mild (9 – 28), moderate (29 – 64) strong (65 – 80) and extreme (81 – 100).

The skin reaction grading was 0 for all animals in both groups after 2, 24 and 48 hours.

Conclusion: Results suggested that during polar and apolar extractions no substances were derived that cause sensitization. As a result of the observed sensitization rate, the test material was considered to cause no sensitization under the described conditions.

Study 3: Tests for interaction with blood.

This study was conducted in 2008 to test hemocompatibility according to DIN EN ISO 10993-4: 2007. Code No. HK 27120278.

Summary: The test material “polypropylene, monofil EP 2, CU blue” did not cause interactions with blood and is haemocompatible under the test conditions selected.

Study 4: Reverse mutation assay according to DIN EN ISO 10993-3 (2004). Code No. GT 27120278

This study was conducted in 2008. The test method used Salmonella typhimurium reverse mutation assay following OECD guideline for testing of chemicals No. 471. Moltex "Salmonella Mutagenicity Assay Kit" with S. typhimurium strains TA97a, TA98, TA100 and TA102.

Summary: The test material "Polypropylene, monofil EP 2, CU blue" did not cause any genotoxic activity under the experimental conditions used.

Study 5. Test for local effects after implantation (3 months).

This study was conducted in 2008 in accordance with DIN EN ISO 10993-6: 2007.

Control specimens DACON USA 3/10 EP 2 has been used as reference substance for comparative tissue reaction.

The biological reactivity of the testing material was checked by implanting the test article in to subcutaneous tissue of the neck of rabbits for 90 days. The local effects were assessed by a comparison of the tissue reactions of the test substance with the control test material.

In a preliminary implantation test 4 monofil polypropylene filaments were implanted as a pooled. In comparison to control sites local reaction was detected on histological examination and was assumed to be due to mechanical effects of implants because no other pathologic systemic or clinical findings were observed. To confirm this assumption every specimen was implanted in one separate rabbit at 3 test sites. The local reactions of the implants were compared to control implants.

Conclusion: The test material caused under the described test conditions of implantation test for 90 days a very slight tissue reaction with an irritating quotient of 0.8 and is therefore assessed as biocompatible.

The following 3 studies on Silicone Material were conducted in 1996 by (b) (4)

. for which sponsor has right of reference

Study # 1 Study title: Cytotoxicity test using the ISO elution method in the L-929 mouse fibroblast cell line.

The test article, Silicone material (ID# CH-037) was extracted and the extracts were subjected to an in vitro cytotoxicity study for biocompatibility in accordance with ISO 10993-5 guidelines. The test was performed in order to determine whether leachables extracted from the material would cause cytotoxicity.

Test article preparation: Three 19.5 cm² portions were each covered with 7 ml of MEM and were extracted at 37C for 24 hours.

Negative control material, reagent control and positive control (sodium chloride granules, 1.2 g portions in 6 mL of MEM) were subjected to the extraction conditions as described fir test article.

Each extract was placed onto separate confluent monolayers of L-929 mouse fibroblast cells. Separate triplicate samples were prepared for a negative control, reagent control and for a positive control. The monolayer in the test, negative, and reagent control were examined microscopically at 48 hours to determine any change in cell morphology. The changes were grade on a 0 – 4 basis.

Results: The MEM test extracts showed no evidence of causing cell lysis or toxicity while negative control, reagent controls and positive controls performed as expected.

Conclusion: Under the conditions of this study, the MEM test extracts were not cytotoxic.

Study # 2: Study title “In vitro hemolysis test by direct contact”.

An in- vitro biocompatibility study was conducted on the test article, silicone material ID-021 sample A to determine whether test article will cause hemolysis. The test represent a measure of blood compatability. The study was conducted in accordance with the provisions of the FDA GLP.

Preparation: In each of two tubes, 10 mL of 0.9% NaCl solution was added to 29.2 cm² sample of test article. The combination was evaluate to determine whether direct contact or leachables from the test material will cause hemolysis in vitro. NaCl solution, the vehicle was the negative control while purified water was the positive control. :

Experimental procedure: A 0.2 mL sample of the rabbit clot-free blood was added to each of the following tubes:

A negative control containing 10 mL of the NaCl solution (SC), a positive control containing 10 mL of purified water (PW) and two tubes each containing 0.2 mL of test article and 10 mL of saline. Contents were mixed and placed in water bath at 37C for one hour. Following incubation, samples were again inverted and decanted into separate centrifuge tubes. These tubes were centrifuged for 10 minutes at 1000xg. Absorbance of each test solution as well as of negative and positive controls was determined spectrophotometrically at 545 nm. % hemolysis was calculated as follows:

Test – SC negative control/PW positive control x 100 = % hemolysis

Results: Absorption for negative control was 0.00, that for PW positive control was 1.97 and hemolysis for duplicate test article was 0.5%.

Conclusion: Under the condition of this study, the combined SC and blood in direct contact with test article was not considered hemolytic as mean hemolysis value of 1% was acceptable.

Study # 3: Study entitled “Ames Salmonella/mammalian microsome mutagenicity assay”

An Ames mutagenicity standard plate incorporation assay was conducted to determine whether a saline extract of silicone material, ID No. CH-037 would cause mutagenic changes in histidine-dependent *S. typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 in the absence and presence of S9 metabolic activation. The methodology of Ames et al (Mutation Research 1975) was followed using saline extract.

Extract preparation: A 39 cm² of test article was covered with 13 mL of saline and extracted at 50°C for 72 hours. Vehicle without test material was similarly subjected to extraction as a negative control.

Test procedure: The saline article extract was found to be non-inhibitory to growth of 5 tester strains used. Separate tubes containing 2 mL of molten top agar supplemented with histidine-biotin solution were incubated with 0.1 mL of culture for each of the tester strains, and 0.1 mL of the saline extract. A 0.5 mL aliquot of S9 homogenate simulating metabolic activation was added when necessary. The mixture was poured across triplicate Minimal E plates. Parallel testing was run with negative control and 4 positive controls i.e. Dexon, 2-nitrofluorene, 2-aminofluorene and sodium azide). The mean number of revertants of the triplicate test plates were compared to mean number of revertants of the triplicate negative control plates for each of 5 tester strains.

Conclusion: Using mean values obtained for positive controls as point of reference, it was considered that under the conditions of this assay, the saline test extract was not mutagenic to *S. typhimurium* tester strains used.

Aside from the studies described above, sponsor has provided literature references for polymer toxicity. The following some are described below:

A review article by P.V. Shastri published in *Contraception* 65 (2002) 9-13 titled “Toxicology of polymers for implant contraceptives for women” stated that two main classes of synthetic, nondegradable polymers used in the delivery of female contraceptives are silicone elastomers (i.e., Silastic) and ethylene co-vinyl acetate. The epidemiological data obtained so far have overwhelmingly concluded that no correlation exists between certain chronic symptoms and silicone breast implants, a conclusion supported by Institutes of Medicine. The author concluded that the prognosis for Silastic and EVA is excellent. However, it cautioned that any future implant development using these polymers should place an emphasis on processing parameters to minimize potential small molecule leachants and establish their safety.

The PDMS manufacturer has provided safety of leachables and degradants for the polymer used in the sponsor's clinical studies.

A paper by Isquith et al entitled "Genotoxicity studies on selected organosilicon compounds: In vitro assays" published in Fd. Chem. Toxic 26: 255 – 261, 1988 tested a series of 12 organosilicon compounds representing potential intermediates in the synthesis and degradation of polydimethylsiloxanes were evaluated with a battery of in vitro assays. Microbial assays included the Ames bacterial reverse mutation in Salmonella, mitotic gene conversion in Saccharomyces cerevisiae D4 and in E.coli. These assays were conducted with and without S9. Also forward gene mutation, sister-chromatid exchange, DNA alkaline elution and chromosome aberration potential were evaluated in mouse lymphoma L1578Y tissue culture cells. The tissue culture assays were performed with and without mouse liver S9 metabolic activating system.

No evidence of gene mutation was observed. However, following 6 of the 12 compounds evaluated with S9 demonstrated potential in vitro clastogenic (chromosomal damaging) activity:

Trimethylchlorosilane, dimethyldichlorosilane, methyltrichlorosilane, methyltriethoxysilane, hexamethyldisiloxane, hexamethylcyclotrisiloxane, decamethylcyclopentasiloxane

Cutler et al in a study published in Fd.Cem Toxici 12:443, 1974 entitled "A lifespan study of a polydimethylsiloxane in the mouse" tested the toxicity of silicone antifoam agent containing 94% PDMS silicone oil and 6% finely divided silicone dioxide. The product was given in diet at levels of 0.25 and 2.5% to groups of mice for 76 weeks from weaning. Another group was given a SC injection of 0.2 ml of the antifoam at weaning and a control group was given a single sc injection of 0.2 ml liquid paraffin BP. All surviving mice were sacrificed when 80 weeks old.

No silicone was detected in the bodies of the mice given diet containing 2.5% of the antifoam. Similarly, none was detected in the liver, kidneys, spleen, or perirenal fat of mice given the injection. There was no increase in the incidence of malignant or benign tumors in the group of mice receiving the antifoam either in the diet or by injection and there were no toxic effects that could be ascribed to administration of silicone. However, sc injection of liquid paraffin caused an increased incidence and earlier appearance of sc fibromas at the injection site in male mice.

11 Integrated Summary and Safety Evaluation

Sponsor has not conducted any pharmacology/toxicology studies for API and instead has supported all pharmacology and toxicology (single dose and repeat-dose toxicity, genetic toxicity, carcinogenicity, reproductive & development toxicity) of drug substance by reference to published studies available in the public domain or to studies where the Sponsor has right of reference. The toxicity of Levosert-20 inserter was conducted by (b) (4). The toxicity of polypropylene, monofil EP 2,CU blue

was conducted by [REDACTED]^{(b) (4)}. The toxicity of Silicone material was conducted by [REDACTED]^{(b) (4)}

[REDACTED] Based on a review of all the submitted studies for the API and the intrauterine device, no toxicity was detected which would pose a risk to users of [REDACTED]^{(b) (4)}. Pharmacology/Toxicology considers [REDACTED]^{(b) (4)} use safe as planned.

12 Appendix/Attachments/References

Cutler M.G., Collings A.J., Kiss I.S & Sharratt M. (1974). A lifespan study of a polydimethylsiloxane in the mouse. *Fd Chem. Toxic* 12, 443 – 450.

Hoffman H. Hillesheim H.G., Gutner J., Stade K., Merbt E.M., & Holle K. (1988). Long term toxicological studies on progestin STS 557. *Exper. Clin. Endocrinol.* 81(22), 179 – 196.

Hillesheim H.G., Hofman H. (1988). Data on acute toxicity of progestin STS 557. *Exper. Clin. Endocrinol.* 81 (2), 175 – 178.

Hite M., Blair M., Nakayma T., Suzuki M., & Yago N. (1991). One year toxicity study of levonorgestrel in female *Cynomolgus* monkeys. *Oyo Yakuri/Pharmacometrics* 42 (3), 301-312.

Hite M., Blair M., Nakayma T., Sazuki M. and Yago, N. (1991). One year rat toxicity study of levonorgestrel I female rats. *Oyo Yakuri/Pharmacometrics* 42 (3), 291-299.

Isqith A., Matheson D., & Slesinski R. (1988). Genotoxicity studies on selected organosilicon compounds in vitro assays. *Fd. Chem. Toxic.* 26, 255 – 261.

Janne O.A., Zook H.A. (2001). The role of estrogen and progestin in producing deciduosarcoma and other lesions in rabbit. *Toxicol. Pathol.* 29 (4), 417 – 421.

Jordan A. (2002). Toxicology of progestogens of implantable contraceptives for women. *Contraception* 65, 3 – 8.

Kawarta R. F., Hemm R. D., Pollock J.J., Chritain M.S., Usui T., Sazuki M. & Yago N. (1991). Levonorgestrel/ethinyl estradiol developmental toxicity study with behavioral and reproductive assessment of the offspring (Seg II – rat). *Oyo Yakuri/Pharmacometrics* 42 (4), 327 – 340.

Kawata R.F., Hemm R.D., Pollock .J.J., Chritian M.S., Usui T., Sazuki M., & Yago N. (1991). Levonorgestrel/ethinyl estradiol study of developmental toxicity in rabbits. *Oyo Yakuri/Pharmacometrics* 42(4),341-349.

Lang R., & Reimann R. (1993). Studies on the genotoxic potential of some endogenous and exogenous sex steroids. 1 Communication: Examination for the induction of gene

mutation using the Ames Salmonella/microsome test and HGPRT test in V79 cells. *Environmental and Molecular Mutagenesis*. 21, 272 – 304.

Larsson K.S., & Machin D. (1989). Predictability of the safety of hormonal contraceptives from canine toxicological studies. A review PP 230 – 269. Journal source not provided.

Nakamura A., Kawasaki Y., Takada K., Aida Y., Kurokama Y., Kojima S., Shintani H., Matsui M., Nohmi T., Matsuoka A., Sofuni T., Kurihara M., & Miyata N. (1992). Difference in tumor incidence and other tissue responses to polyetherurethanes and polydimethylsiloxane in long-term subcutaneous implantation into rats. *Journal of Biomedical Materials research*. 26, 631 – 650.

Nilsson C. G., Hukkamaa M., Vierola H. & Laukkamaa M., Vierola H., & Luukkainen (1982). Tissue concentrations of levonorgestrel in women using a levonorgestrel-releasing IUD. *Clinical Endocrinology* 17, 529 – 536.

Nisker J. A., Kirk M. e., & Nunez-Troconis J. T. (1988). Reduced incidence of rabbit endometrial neoplasia with levonorgestrel implant. *Am. J. Obstet. Gynecol.* 158, 300 – 303

Rodriguez M.I., Darney P. D. (2010). Non-contraceptive applications of the levonorgestrel intrauterine system. *International Journal of Women's Health*. 2, 63 – 68.

Shastri P. V. (2002). Toxicology of polymers for implant contraceptives for women. *Contraception* 65, 9 – 13.

Shirley B., and Bundren J.C. (1995). Effects of levonorgestrel on capacity of mouse oocytes for fertilization and development. *Contraception* 51, 209 – 214.

Wadsworth P.E., Heywood R., Allen D.G., Sortwell R. J. and Walton R. M. (1979). Treatment of rhesus monkey (*Macaca mulatta*) with intrauterine devices loaded with levonorgestrel. *Contraception* 20(2), 177 -179.

Zook B. C., Spiro I., & Hertz R. (1987). Malignant neoplasms of decidual origin (deciduomas) induced by estrogen-progestin-releasing intravaginal devices in rabbits. *American Journal of Pathology* 128 (2), 315 - 327.

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/s/

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12/10/2014

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