Decisional Memorandum

New Drug Application 21998, Supplement 5
Levonorgestrel 1.5 mg Tablet Emergency Contraceptive
Labeling Supplement for Update to Mechanism of Action Information

I. Executive Summary

Levonorgestrel emergency contraception (LNG-EC) is effective in preventing pregnancy when taken within 3 days after unprotected intercourse. LNG-EC does not terminate an established pregnancy and does not affect a continuing pregnancy.

In this supplement, the applicant requests an update to the labeling of nonprescription LNG-EC (NDA 21998), in particular to certain parts of the labeling that concern mechanism of action. The multidisciplinary review team concurs that, based on the totality of both pre-existing and new data, updated labeling is appropriate. Data are strong for a mechanism of action of delay or prevention of ovulation, and data are weak to speculative regarding any postovulatory mechanistic effects, such as on fertilization or implantation.

Key questions answered by the multidisciplinary review include:

A. Does levonorgestrel emergency contraception inhibit ovulation?

Clinical data are strong that LNG-EC, when administered prior to the luteinizing hormone (LH) surge, inhibits or delays ovulation.

B. Does levonorgestrel emergency contraception affect sperm function?

In vitro and human in vivo data do not support a significant effect of LNG on cervical mucus quality, sperm quantity in the genital tract, sperm motility, or acrosomal reaction, when LNG is administered at doses similar to that used in EC.

C. After ovulation occurs, does levonorgestrel emergency contraception affect endometrial receptivity or implantation?

In vitro data and human clinical data show that LNG-EC does not affect exploratory markers of endometrial receptivity after ovulation occurs. It should be noted that such markers are not validated biomarkers, and the multidisciplinary team did not find published data that changes in endometrial receptivity biomarkers translate into a clinical effect on blastocyst implantation. The totality of the evidence indicates that LNG-EC does not alter the endometrium in a clinically meaningful way to prevent implantation. Studies showing no evident reduction in the likelihood of pregnancy when LNG is administered to women postovulation are consistent with histological and biochemical findings and support the conclusion that LNG-EC is ineffective when administered postovulation.

D. Does levonorgestrel emergency contraception affect pregnancy rate after ovulation?

When LNG-EC is administered after ovulation occurs, the rate of pregnancy is what one would expect if LNG-EC had not been administered. This is arguably the most important aspect of the evidence.

The data and citations supporting the above answers are included and discussed later in this memorandum. Given the above findings, it is appropriate to update the Drug Facts label (DFL) and Consumer Information Leaflet (CIL) for LNG-EC to reflect what has been learned about the LNG-EC mechanism of action (MoA) in the years since the original nonprescription LNG-EC approval.

The scientific and clinical concept of the time period of pregnancy is generally consistent with the example found in the Code of Federal Regulations (CFR) under 45 CFR 46.202, which reads: "Pregnancy encompasses the period of time from implantation until delivery." LNG-EC prevents pregnancy by acting on ovulation, which occurs before implantation, and because data do not support that LNG-EC affects implantation, LNG-EC does not terminate pregnancy.

II. Background

Levonorgestrel is a synthetic progestin. Under the brand name Plan B One-Step® (hereafter referred to as Plan B One-Step), the levonorgestrel 1.5 mg tablet is approved as a nonprescription emergency contraceptive, with the Use (nonprescription equivalent to indication) stated on the Drug Facts label (DFL) as "for women to reduce chance of pregnancy after unprotected sex (if a contraceptive failed or you did not use birth control)."

Levonorgestrel was first approved as a prescription emergency contraceptive product in 1999 under the brand name Plan B® (hereafter referred to as Plan B); Plan B was a two-dose product with each tablet 0.75 mg. In 2006, nonprescription Plan B was approved in a partial prescription-to-nonprescription switch. In 2009, Plan B One-Step (the product in this labeling supplement), was approved as a partial prescription-to-nonprescription switch, with a 1.5 mg tablet requiring only one dose. In 2013, the single dose 1.5 mg

product was approved as a full prescription-to-nonprescription switch. The approval history for Plan B and Plan B One-Step can be found at FDA's webpage drugs@FDA available at

https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=021045 and https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event=overview.process&ApplNo=021998 .

Mechanism of action (MoA) information is rarely included in nonprescription labeling. It is not a required element in the regulations that govern nonprescription labeling (21 CFR 201.66, Format and Content Requirements for Over-the-Counter Drug Product Labeling). MoA is often scientifically complex and is usually determined by nonclinical (animal or in vitro) studies, sometimes augmented by human clinical pharmacology or human clinical studies. For prescription products, when MoA has been established, it must be included in the prescription labeling; in that circumstance, the labeling is written for licensed prescribers who have the scientific training to interpret MoA information correctly. By contrast, nonprescription labeling is written for consumers of average US health literacy, generally a reading level of approximately 6th to 8th grade. The US nonprescription label, called the Drug Facts label, is intended to provide consumers with the key information they need to understand how to use a drug safely and effectively without help from a licensed healthcare professional. Mechanism of action information is not needed for the safe or effective use of nonprescription drugs in general, and not for levonorgestrel emergency contraception in particular.

However, MoA-related information was included in the labeling of nonprescription levonorgestrel emergency contraception at the time of its original prescription-to-nonprescription switch in 2006. In 2003, at the public Advisory Committee at which that application (for the LNG-EC 0.75 mg tablet) was discussed, MoA arose as a topic of discussion. In 2004, the FDA requested that the applicant include information relevant to MoA in the nonprescription labeling. In 2005, the applicant agreed, and information relevant to MoA was present in the 2006 labeling for the original approval of the nonprescription LNG-EC 0.75 mg tablet. When the nonprescription LNG-EC 1.5 mg tablet was approved, the same MoA-related information was included in labeling.

In the current approved labeling for Plan B One-Step (NDA 21998, LNG- EC 1.5 mg tablet), the following language relevant to MoA appears:

Drug Facts label:

In the "Warnings" section, under "Do not use":

"Do not use if you are already pregnant (because it will not work)."

In the "Other information" section:

"• this product works mainly by preventing ovulation (egg release). It may also prevent fertilization of a released egg (joining of sperm and egg) or attachment of a fertilized egg to the uterus (implantation)."

Consumer Information Leaflet:

Under the heading "What Plan B One-Step® is not.":

"Plan B One-Step® will not work if you are already pregnant and will not affect an existing pregnancy."

Under the heading "When not to use Plan B One-Step®.":

"Plan B One-Step® should not be used if you are already pregnant, because it will not work."

Under the heading "How does Plan B One-Step® work?":

"Plan B One-Step® is one tablet with levonorgestrel, a hormone that has been used in many birth control pills for several decades. Plan B One-Step® contains a higher dose of levonorgestrel than birth control pills, but works in a similar way to prevent pregnancy. It works mainly by stopping the release of an egg from the ovary. It is possible that Plan B One-Step® may also work by preventing fertilization of an egg (the uniting of sperm with the egg) or by preventing attachment (implantation) to the uterus (womb)."

III. Applicant's Rationale for Requested Labeling Change

In its justification for the requested labeling change, the applicant states that the current labeling of LNG-EC may be misleading to consumers, because it contains information on a possible contribution of postovulatory effects to LNG-EC's mechanism of action, and some consumers are hesitant to use a product that might affect postovulatory events, in particular implantation of the blastocyst.

The science has evolved since the original approval of LNG-EC, and the applicant submitted publications from an updated comprehensive literature search that identified a total of 33 publications concerning LNG-EC MoA. Six of these articles reported on clinical studies. Other types of publications included 4 clinical pharmacology research articles, 2 nonclinical animal research articles, 16 review articles, 1 professional society publication, and 4 opinion/editorial articles. The applicant presented a summary of the findings of these publications, and concluded that data for an effect on ovulation are strong, but data for postovulatory effects are weak or speculative. The applicant asserts that updates to the labeling are needed to make the labeling more accurate, to reduce consumer confusion, and potentially to reduce barriers to use of the legally marketed approved product.

IV. Summary of Multidisciplinary Review

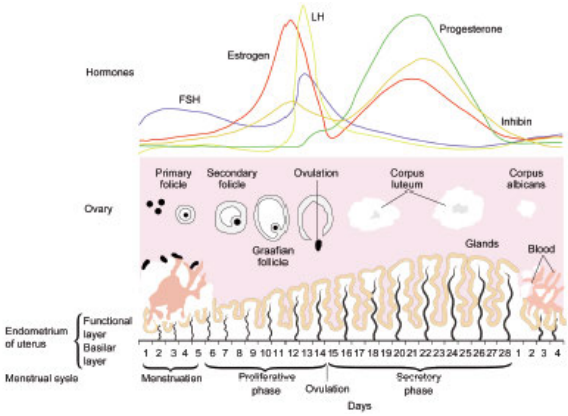
A. The Typical Events of the Early Reproductive Process

In order to understand the points in the reproductive process at which an emergency contraceptive might have its effect(s) to prevent pregnancy, it is useful to understand the typical sequence of events that occur early in the reproductive process. The process is highly complex, but some major events of interest include ovulation, fertilization, and implantation.

Figure IV.A.1 below illustrates the hormonal and follicular changes that occur in a typical menstrual cycle. Day 1 of the cycle (the first day of menstruation) starts the follicular phase (referred to in the figure below as the proliferative phase), in which the pituitary hormone follicle-stimulating hormone (FSH) stimulates

the development of follicles in the ovary. As follicles mature, systemic estrogen levels rise and hypothalamic feedback occurs. In the final stages of follicular maturation, near the middle of the cycle, a surge in systemic levels of the pituitary hormone luteinizing hormone (LH) occurs. Approximately 24-48 hours later, a dominant follicle ruptures and an ovum is released; this is referred to as ovulation. The phase after ovulation is called the luteal phase (referred to in the figure below as the secretory phase); during this time, there is a rise in systemic progesterone and estrogen, with accompanying changes in the uterine endometrium.

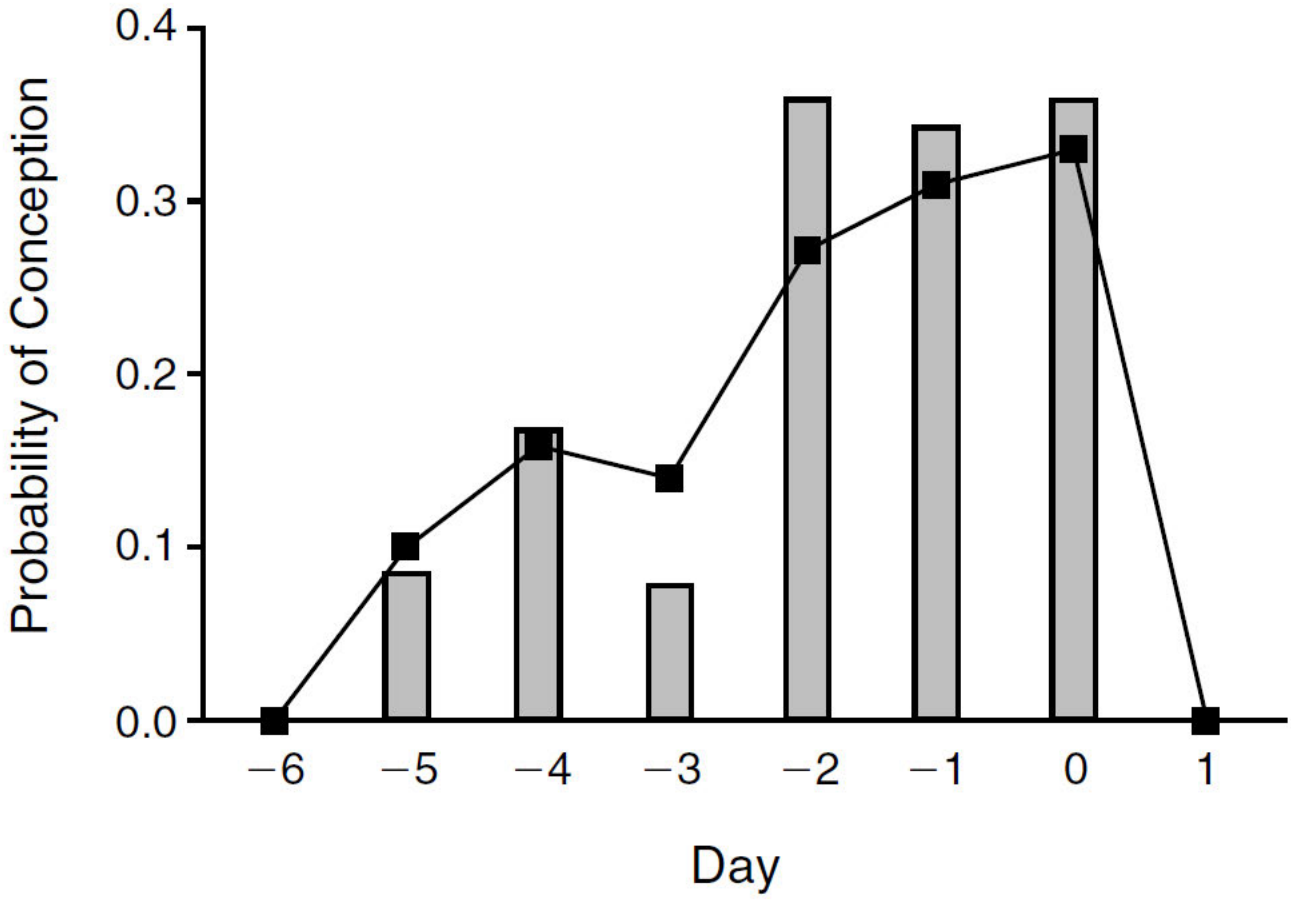
Figure IV.A.1: Hormonal Changes and Ovarian Follicular Development in a Typical 28-Day Menstrual Cycle



Source: Leung 2010, pg 159.
Abbreviations: FSH = follicle-stimulating hormone; LH = luteinizing hormone
Note: In many sources, the "proliferative phase" is often referred to as the "follicular phase", and the "secretory phase" is often referred to as the "luteal phase".

The days in which unprotected intercourse is most likely to result in fertilization are approximately the five days prior to ovulation, and the day of ovulation (Wilcox 1995).

Figure IV.A.2: Probability of Conception Related to Timing of Intercourse During Menstrual Cycle*



Source: Adapted from Wilcox 1995, pg 1519
***Day 0 is the day of ovulation**
"Probability of Conception" for a given day was calculated by dividing the number of pregnancies observed by the number of cycles with intercourse only on that specific day (and no intercourse occurring on other days within Days -6 to +1).

After unprotected intercourse, sperm that reach the fallopian tube generally survive for 3-4 days, although this is variable. The released ovum enters the fallopian tube, and generally must encounter sperm within 12-24 hours in order for fertilization to occur. If fertilization happens, the fertilized egg develops into a blastocyst, which generally moves through the fallopian tube in about 3 days and enters the uterus on about Day 17 or 18 of the menstrual cycle. The blastocyst begins implantation about 3 days later. In order for implantation to occur, the endometrium needs to be in a receptive state. Numerous biochemical markers of a receptive endometrium have been explored. While none of these is qualified as a validated biomarker, they include measurements of estrogen and progesterone levels, appropriately timed with ovulation, followed by assessment of endometrial downregulation of receptors for estrogen and progesterone; and may also include expression of such factors as glycodelin, prostaglandins, interleukins, integrins, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), leukemia inhibitory factor (LIF), tumor necrosis factor (TNF), and cell adhesion molecules (Achache 2006, Lindhard 2002). Note that if intercourse occurs prior to ovulation, administration of a hypothetical drug to prevent pregnancy *after* ovulation could still prevent pregnancy (based upon the number of days between intercourse, fertilization, and implantation) if postovulation events (fertilization or implantation) were

directly inhibited. However, as discussed in detail below, LNG-EC administered prior to ovulation is effective to prevent pregnancy, but administered after ovulation is ineffective to prevent pregnancy, providing evidence that LNG-EC does *not* directly alter events after ovulation.

B. Approach to the Review

Broadly speaking, publications related to the question of MoA of LNG-EC fall into three categories: original research articles (meaning publications of original scientific or clinical research studies), review articles, and commentary/opinion articles. The latter two categories include writings of a wide range of scientific rigor, varying from careful systematic reviews to commentaries containing hypothetical or speculative statements. The review for this labeling supplement focuses only on original research articles, both those submitted by the applicant, and additional articles identified in systematic literature searches conducted by members of the multidisciplinary review team. Although review and commentary/opinion articles were not the focus of this review, all articles selected by the applicant (which included both supportive and dissenting points of view), as well as additional review articles from separate systematic literature searches by members of the multidisciplinary review team, were reviewed for identification of possible additional sources of original research, and to consider all viewpoints. The following articles were identified as original research articles by the applicant: do Nascimento 2007; Lalitkumar 2007; Marions 2002; Meng 2009 and 2010; Muller 2002; Noé 2011; Novikova 2007; Ortiz 2004; Palomino 2010; Ugocsai 2002, and Vargas 2012. Full citations for these and all articles mentioned in this document are in the References section.

The following additional original research articles were identified by the multidisciplinary review team in a systematic literature search and were reviewed. (A few of these articles were also submitted by the applicant, but were not identified as original research articles in the applicant's Table of Studies on page 10 of the applicant's justification document.): Durand 2001, 2005, and 2010; Hapangama 2001; Landgren 1989; Ling 1979 and 1983; Marions 2004; Matsuo 2020; Okewole 2007; Tirelli 2008; and Yeung 2002.

Of the aforementioned original research articles, the review team identified the following articles as the strongest clinical evidence of LNG-EC's effects on ovulation: Durand 2001; Marions 2002; Croxatto 2004; Okewole 2007; Novikova 2007; Tirelli 2008; and Noé 2011. The strengths and limitations of each of these studies are discussed further below.

Other review articles and commentary/opinion articles that were identified by the applicant, and additional such articles identified by the multidisciplinary review team, are also cited in the References section. As noted above, the multidisciplinary team reviewed all articles and materials submitted by the applicant, as well as additional articles from the FDA team's literature search.

The review below begins with a description of the information (from research studies) that was known at the time of FDA's review of the literature in preparation for the 2003 Nonprescription Drugs Advisory Committee meeting, then proceeds through the data published since then on new original research. The review then proceeds to an integrated summary of what the current state of knowledge is about the mechanism of action of LNG-EC.

C. Information on Mechanism of Action that was Available at the Time of the Review Division's Analysis in Preparation for 2003 Nonprescription Drugs Advisory Committee Meeting

At the time of FDA's review in preparation for the 2003 public Advisory Committee meeting at which the application for nonprescription LNG-EC was discussed, limited published data were available from original research regarding MoA for LNG-EC.

Some work had been published in 1979 and 1983 by Ling and colleagues, but because the treatment given in these studies was a combination of levonorgestrel and ethinyl estradiol, and not LNG alone, these studies were not informative as to the individual effect of LNG.

1. Clinical Studies Evaluating Effect of Levonorgestrel on Ovulation and the Endometrium

a. Landgren and colleagues (1989) published a human pharmacodynamic study that showed that suppression of ovarian function by LNG was seen only when the drug was administered in the late follicular phase and around the time of ovulation.

On pathological assessment of endometrial biopsy specimens, a decrease in the number and diameter of endometrial glands was observed only when LNG was administered during the follicular phase, and not when it was administered in the luteal phase. A limitation of this study was that the total amount of LNG administered per subject (3 mg, administered as 0.75 mg every 2 days for 4 doses) was higher than that of the approved LNG-EC product in this application (1.5 mg given as a single dose). However, given this, it seems unlikely that LNG-EC at the current (lower) approved dose would have exhibited greater effects than that seen at higher doses in this study.

b. Durand and colleagues (2001) published a clinical study that showed that LNG prevented ovulation when administered on Day 10 of the menstrual cycle (pre-ovulatory) and did not find impairment of corpus luteum function or endometrial morphology when LNG was administered in the peri-ovulatory or postovulatory period.

The study included 45 healthy surgically sterilized women who ranged in age from 29 to 35 years, and who had regular menstrual cycles. Subjects were randomized into one of three groups. All participants were studied for two menstrual cycles; a control cycle without administration of LNG, and an active treatment cycle during which LNG was administered. In the second cycle, each group received two doses of LNG 0.75 mg, with 12 hours between doses. The difference in regimen between groups was in when in the menstrual cycle each group received the LNG. Group A received it on Day 10 of the menstrual cycle (a time expected to be pre-ovulatory); Group B received it immediately after detection of luteinizing hormone (LH) in the urine (a time expected to be peri-ovulatory); and Group C received it 48 hours after detection of LH in the urine (a time expected to be postovulatory). A Group D was also formed after it was noted that there were inconsistencies between serum LH and urinary LH in 12 of the 90 studied cycles. Because of this observation, the investigators decided to use serum LH, estrogen, and progesterone levels for cycle dating, rather than using urinary LH. Thus, Group D was formed from four participants from Group B and four from Group C. Group D received LNG 3 +/- 1 day prior to the serum LH surge.

During both the control cycle and the treatment cycle, from the time of urinary LH detection, the investigators performed daily transvaginal ultrasound until the investigators observed follicular rupture (FR). Also, during both the control and treatment cycles, endometrial biopsies were performed on all participants 9 days after urinary LH detection. This day was chosen for biopsy because it occurs during the

time when the endometrium was felt to be optimally receptive to implantation, based on other published data (Harper 1992, Yaron 1994).

In Group A (the pre-ovulatory group), after receiving LNG, 12 of 15 participants did not ovulate; follicular rupture was not detected on ultrasound, and urinary LH was not detected. For the 3 of 15 Group A participants who did ovulate, there was a delay in urinary LH detection, serum LH surge, and follicular rupture. In Groups B (presumed peri-ovulatory), C (presumed postovulatory), and D (use of serum LH for ovulation detection, with LNG dosing 3 +/- 1 day prior to LH surge), all participants ovulated, and LNG-EC did not alter the day of the cycle on which ovulation occurred.

Regarding the endometrial biopsy results, which were all read in blinded fashion, morphology did not differ between control and treated samples. Of note, there was no difference between groups in the presence of spiral arteries, which are important for implantation (Pijnenborg 2006, Craven 1998, Blankenship 1997). Similarly, stromal edema was similar between treated and control groups, also suggesting a lack of effect on endometrial morphology important for preparation for implantation (Okada 2001).

Table IV.C.1.b: Endometrial Morphology in Control and Treated Groups

	Control (n = 41)	Group B (n = 10)	Group C (n = 11)	Group D (n = 3)
Postovulatory day	8.6 ± 1.3	8.7 ± 0.6	9.0 ± 0.8	9.0 ± 0
Total area of tissue (mm^2)	1,988 ± 55	2,003 ± 45	1,984 ± 62	2,015 ± 26
# of glands/visual field	59 ± 12	58 ± 7	55 ± 8	58 ± 1
# of glands/mm^2	30 ± 6	29 ± 4	28 ± 4	29 ± 0.8
Stromal edema (mm^2)	1,049 ± 308	1,225 ± 261	1,011 ± 209	1,142 ± 40
% of tissue with stromal edema	53 ± 15	61 ± 14	51 ± 10	57 ± 1.4
Spiral arteries per visual field	6 ± 3	4 ± 1	5 ± 2	4 ± 0.7

Source: Durand 2001, pg 231

Results expressed as mean +/- SD

24/33 biopsies from treated ovulatory cycles included. Reasons for exclusion: Insufficient tissue (4 control, 1 Grp A, 1 Grp D); sampling not correlated with cycle day (3 Grp A, 4 Grp D)

Overall, the data reported by Durand supported prevention of ovulation by LNG. In addition, peri- and postovulatory administration did not affect endometrial morphology, making an effect on implantation unlikely.

c. Marions and colleagues (2002) published a clinical study in healthy fertile women; this study examined the effect of LNG on ovarian function and endometrial development. With regard to ovarian function, the authors observed inhibition of the LH surge and ovulation when LNG was given pre-ovulation. However, the authors did not measure the timing of the LH surge relative to LNG dosing, precluding definitive conclusions.

The authors also observed no effects on markers of endometrial receptivity, whether LNG was given before or after ovulation. However, there are no validated markers of endometrial receptivity modulating blastocyst implantation. Therefore, the biomarkers evaluated in this study would be considered as exploratory. That being said, the consistent lack of alteration of any biomarker in this study provides support for the conclusion that there were no meaningful changes in the endometrium with LNG administration.

d. Ugocsai and colleagues (2002) published a report on three women who, "due to enormous fear of unwanted pregnancy", had intentionally taken excess LNG (3-3.75 mg as a single dose, followed by at least one other dose of 0.75 mg). The women then sought care "as anxiety emerged regarding health consequences and possible sequelae." Approximately 96 hours after the second dose, each woman underwent endometrial biopsy. Their specimens were compared to two ovulating women, one in each half of the menstrual cycle. The investigators performed scanning electron microscopy on the specimens and reported that the endometria of the women who intentionally overdosed on LNG differed from those of the control women, notably in a lack of ciliated cells. This study has the limitation that it evaluated ingestion of doses well above the clinical dose; the relevance of the observations to the approved LNG-EC product, which is a much lower dose, is uncertain.

e. Hapangama and colleagues (2001) conducted a study in 12 healthy women with regular cycles. This study intended to examine the effect of LNG at different times during the menstrual cycle. The study utilized urinary monitoring of estrone-3-glucuronide to detect ovulation with no transvaginal ultrasounds obtained. The method used in this study does not accurately determine the specific time during the cycle; that is, the method cannot accurately determine if the women were pre-, peri-, or post-ovulatory. Thus, the results of this study are confounded, and the study is not clinically interpretable.

2. Effects of Levonorgestrel on Sperm Function

After unprotected intercourse, sperm can be found in the endocervix within 90 seconds. Based on literature review conducted by the multidisciplinary review team, it is unlikely that LNG, when used for emergency contraception, directly affects sperm function.

Yeung and colleagues (2002) reported data from an in vitro study that evaluated the effects of LNG on sperm function. The authors obtained semen samples from men attending infertility clinics and incubated the samples with either control or LNG in concentrations of 1, 10, or 100 ng/mL. The authors chose their doses based on prior studies of pharmacokinetic data on serum concentrations of LNG, which reached a Tmax of 5-10 ng/mL at 2 hours post-dose. The LNG concentrations to which sperm were exposed in the Yeung study appear to be considerably higher than those that would occur with use of an emergency contraceptive. The authors noted a decrease in some parameters of sperm motility (curvilinear velocity and straight-line velocity) at the 10 and 100 ng/mL LNG concentrations, and a decrease in average path velocity at the 100 ng/mL concentration. As noted above, these observations were at concentrations likely to be many-fold above pharmacologically relevant concentrations for the LNG-EC. Several other sperm motility measures (linearity, head beat cross frequency, and amplitude of lateral head displacement) were the same for control and LNG at all concentrations. Limitations of the study include the high concentration of LNG used; the unknown correlation between serum and uterine/fallopian LNG concentration; and the fact that specimens were obtained from men seeking infertility care, who may not have had normal sperm function at baseline.

To summarize the information on LNG-EC MoA that was available at the time of the 2003 review, the studies by Landgren, Durand, and Marions, and their colleagues, showed that LNG suppressed the midcycle LH surge and suppressed ovulation. In addition, all three studies evaluated endometrial changes in either morphology and/or endometrial biomarkers and reported no meaningful changes. Finally, the study by Yeung and colleagues showed no evident effect on sperm function. This evidence suggests that

the mechanism by which LNG prevents pregnancy is through suppression of ovulation, and not through changes in endometrial function/receptivity or changes in sperm function leading to impaired fertilization. However, the limited number of studies available at the time of the 2003 review of the data precluded a firm conclusion regarding whether other mechanisms were unlikely to contribute.

D. Information on Mechanism of Action that Became Available After the 2003 Review

As noted in the Background (Section II) above, the decision to take the unusual step of inclusion of MoA in the nonprescription labeling for LNG followed discussion of MoA at the 2003 Advisory Committee (AC) meeting (note that the labeling for MoA was based upon the assessment of the literature leading up to the AC meeting). Subsequent to that meeting, additional research (nonclinical, clinical pharmacology, and clinical) has been published relevant to LNG-EC MoA. Some of these publications became available during the time period between the 2003 review and the 2006 nonprescription approval of LNG-EC; others were published later.

1. Nonclinical Studies on Suppression of Ovulation with Levonorgestrel

There are two nonclinical studies, one in rats (Muller 2003) and one in monkeys (Ortiz 2004), that address LNG effects on ovulation. Full study reports were not available for either publication, but a review was conducted on peer-reviewed publications of summary information.

a. In the rat study, Muller and colleagues dosed animals to determine if LNG had an effect on ovulation, fertilization, or implantation. None of the animals were dosed after implantation. First, rats were dosed subcutaneously (SC) with LNG (50 µg/kg), with Group 1 dosed twice per day, for 2 days early in the menstrual cycle (at diestrus and proestrus); Group 2 dosed once during diestrus; and Group 3 dosed once at proestrus. These animals were terminated at estrus to determine the percentage of animals ovulating, and the mean number of released eggs per ovulating animal. Two SC LNG administrations early in the menstrual cycle (at diestrus and proestrus) completely prevented ovulation in the rat, but this effect decreased with single doses, and as dosing approached estrus.

   Following the initial rat study, which evaluated Groups 1-3 (as described above), several additional groups of rats (groups 4-11) were mated and dosed with single doses of LNG during proestrus or estrus to determine if LNG affected fertilization or implantation. If mating occurred, the day of estrus was designated Day 1 of pregnancy (P1). The following eight different groups were tested on P2 to assess the number of fertilized eggs, or P12 to assess the number of implantations:

   - Dosing before mating and ovulation: females were dosed during proestrus and then mated the same day (Group 4), or overnight (Group 5)
   - Dosing after mating and before ovulation: females were mated during proestrus, then dosed with LNG once that day (Group 6), or twice (the day of mating and 12 hours later; Group 7)
   - Dosing after ovulation and before mating: females were injected with LNG on the day of estrus and immediately mated (Group 8)
   - Dosing after ovulation and mating: females were mated on the day of estrus, and injected immediately with LNG (Group 9)

- Dosing after fertilization upon implantation: proestrus rats were mated overnight, then injected with LNG once on P1 and again on P2 (Group 10), or on P3 and again on P4 (Group 11)

All groups had matching vehicle-injected controls, and data from LNG-injected females were compared to controls. In all groups, there were no notable observed differences in implantation rates between the control and LNG-treated animals. The primary mode of action of LNG appeared to be ovulation suppression, based on the absence of ovulation in rats treated with LNG at proestrus and diestrus (Groups 1-3), and there was no notable effect in the LNG-treated animals that were mated (but dosed after proestrus), compared to concurrent control animals. LNG does not appear to exert any postimplantation effects on the rat, nor did it appear to affect implantation of fertilized eggs. It is not clear how systemic exposure in the rat studies compares to those expected in humans who take LNG-EC, because systemic exposure was not measured in rats.

b. The studies reported by Ortiz in monkeys cannot directly inform whether LNG affects implantation of fertilized eggs or causes loss of fertilized eggs. In the first study, monkeys were mated to determine if LNG affected pregnancy rates. In the second study, monkeys were dosed with LNG to determine if LNG suppressed ovulation in the absence of mating. Limitations to both studies are described below:

i. In a monkey mating study, animals were treated with either one dose of 0.75 mg LNG or two doses of 0.75 LNG (1.5 mg LNG total), subcutaneously or orally after confirmed mating. The authors conclude that overall, the pregnancy rate was identical between LNG-treated and vehicle-treated controls. The authors state, "In 41 [out of] 48 [animals], mating took place between 2 days before ovulation and the day of ovulation." In only seven cycles did mating precede ovulation by greater than 2 days (3 – 5 days). Therefore, the number of monkeys mated early relative to ovulation is limited making it difficult to interpret how timing of LNG treatment affects pregnancy rate. Seven animals appear to have been treated with LNG after fertilization likely occurred; however, the pregnancy rate in these animals was not different from those treated prior to fertilization.

Pharmacokinetic data were not collected in this study to allow for comparison to human exposures. There were limited pharmacokinetic data collected for one of the doses (0.75 mg oral or subcutaneous) employed in monkeys in a separate bioavailability assessment (see item iii below).

ii. A monkey study was also conducted to determine if LNG affected ovulation, but monkeys in the study were not mated. Because animals were not mated, this study cannot inform how LNG affects fertilization or implantation. In the monkey ovulation study, six female monkeys were dosed with two subcutaneous injections of LNG (0.75 mg), 12 hours apart. Follicle development and ovulation were inhibited in monkeys dosed when the leading follicle was less than 5 mm in size. Ovulation occurred when the leading follicle was greater than 5 mm at the time of LNG treatment.

iii. It is not clear how systemic exposure compares between monkeys and humans in either the mating or ovulation studies (described directly above). In a bioavailability monkey study, three monkeys received 0.75 mg LNG (oral or subcutaneous), and the total systemic exposure (area under the curve) of LNG was not quantified.

In conclusion, the rat data from Muller (2003) show that subcutaneous administration with LNG (50 µg/kg, twice per day, for 2 days) early in the menstrual cycle inhibits ovulation and does not affect implantation. The monkey studies from Ortiz (2004) also show LNG inhibits ovulation when administered early in the cycle but the data did not show that LNG decreased pregnancy rates when administered close to fertilization. These papers did not address the impact of LNG when dosed after implantation. The data, however, are hard to extrapolate to consumer use of LNG-EC because it is not clear how the dosing regimen in the animals compares to clinically relevant exposures of LNG, due to the lack of adequate pharmacokinetic data in either the rat or monkey study.

2. Clinical Studies Evaluating the Effect of Levonorgestrel Emergency Contraception on Ovulation

a. Marions and colleagues (2004) published another human clinical study further examining the postovulatory effects of LNG-EC; LNG again delayed or stopped ovulation, and was associated with a delay in rise of pregnanediol in the time following the expected LH peak, and a lower estrone peak at the time of expected LH peak. In this study, after a control cycle to determine the expected day of LH peak, 7 women were administered 2 doses of LNG 0.75 mg given 12 hours apart, 2 days prior to their expected LH peak. Daily transvaginal ultrasounds were performed, as were daily urine assays for LH, pregnanediol glucuronate, and estrone glucuronate. Follicle rupture did not occur in any participants. Similar to findings in other studies, the LH peak did not occur or was attenuated and delayed in LNG cycles vs control cycles. Pregnanediol levels rose more slowly in the LNG-exposed cycle than in the control cycle, reaching peak on Day +9 (with Day 0 having been the expected LH peak day, 2 days after administration of LNG) as opposed to Day +7 for the control cycle, but had a similar area under the concentration curve (AUC). Estrone levels peaked on Day 0 for both the LNG and control cycles, with a lower Cmax for the LNG cycle. The authors state that the latter two observations suggest that LNG-EC may have some effect in the luteal phase. However, because ovulation did not occur, this postulation is moot, because pregnancy could not have occurred anyway. It is unclear whether one can attribute an effect of LNG, or whether the observations may be the natural findings of reduced luteal activity in an anovulatory cycle. Mean cycle length shortened by 4 days with LNG vs control. Also, the study examined similar parameters for mifepristone, with each participant receiving mifepristone in one cycle, then having a washout cycle with no drug given, then receiving LNG in a third cycle. This presents a small possibility of confounding from mifepristone's effects in the prior cycle in the same participant.

b. Tirelli and colleagues (2008) published a study in which women with regular menstrual cycles were enrolled within 72 hours of unprotected intercourse and received LNG 0.75 mg x 2 doses 12 hours apart. The study included a subgroup of eight women who received LNG during Days 11-13 of the cycle, and who underwent detailed evaluation with transvaginal ultrasound every 3 days starting on the day of recruitment before LNG intake until follicular rupture was documented. This subgroup of eight women also underwent endocrine blood sampling (LH, FSH, estradiol, and progesterone).

In seven of eight women, an anovulatory cycle was documented with no LH or FSH peak. In addition, menstrual cycle length was significantly shortened. One of the eight women, who had received LNG on Day 13 of the cycle, had an ovulatory cycle, and an LH surge. This study demonstrates that follicular phase

LNG administration generally (in seven of eight women in this study) results in suppression of ovulation and consequent shortening of the menstrual cycle. This supports the mechanism of LNG as suppressing ovulation, which thereby prevents pregnancy.

c. Croxatto and colleagues (2004) published a study evaluating the effect of LNG-EC on ovulation and ovulatory function. Each subject was studied during a placebo- and a drug-treated (LNG 0.75 mg x 2 doses at 12-hour interval) cycle. Three groups were studied: one group received drug when the leading follicle was 12-14 mm (18 women), one group when the leading follicle was 15-17 mm (22 women), and one group when the leading follicle was ≥ 18 mm (18 women). All women were evaluated with repeated transvaginal ultrasounds, and venous blood sampling for endocrine measurements (LH, FSH, estradiol, and progesterone).

In women with the leading follicle 12-14 mm, no rupture of the follicle was documented within 5 days of dose administration in 83% of women with LNG administration vs 56% of women in the placebo cycle. In comparison, ovulation was not suppressed relative to placebo in women with the leading follicle ≥ 18 mm or when the follicle was 15-17 mm. However, with LNG administration, LNG markedly suppressed the LH peak and/or the rise in progesterone in 94% of women with leading follicle of 12-14 mm (vs 61% with placebo), 91% of women with their leading follicle 15-17 mm (vs 45% with placebo), and in 47% of women with leading follicle of ≥18 mm (vs 13% with placebo).

This study shows that LNG suppresses or delays ovulation (beyond 5 days) and/or suppresses ovulatory function in a high proportion of women with leading follicle sizes under 18 mm, and less consistently in women with their leading follicle ≥18 mm. In women with a smaller leading follicle size, the primary mechanism is prevention or delay (beyond 5 days) of follicular rupture, while when the leading follicle is larger, suppression of ovulatory function (i.e., prevention of the midcycle LH surge) is more prominent as a mechanism. Both prevention of follicular rupture and suppression of ovulatory function may contribute to prevention of pregnancy.

d. Okewole and colleagues (2007) studied 14 women with regular menstrual cycles; all were advised to use condoms during the study to prevent pregnancy. Women were followed from 5 days prior to expected ovulation, and daily blood samples for estradiol, FSH, and LH were collected. Women were followed through 2 cycles: a pretreatment cycle and a cycle in which all women received LNG 1.5 mg (8 women randomly assigned to receive drug 3 days prior to expected ovulation and 6 women to receive drug 1 day prior to expected ovulation). In the pretreatment cycle, all women had ovulatory cycles as shown by the LH peak. In women receiving LNG 3 days prior to expected ovulation, mean cycle length was prolonged and LH and FSH peaks were delayed by 4-5 days relative to the control cycle; in comparison, in 6 women who received LNG 1 day prior to expected ovulation, mean cycle length was shortened and LH/FSH peak was only delayed by 1 day relative to the control cycle. The authors concluded that the primary mechanism is to delay the events triggering ovulation when administered sufficiently in advance of ovulation.

3. Studies Evaluating the Effectiveness of Levonorgestrel Based Upon Timing in Cycle

a. Novikova and colleagues (2007) published a clinical study showing that LNG 1.5 mg was effective in preventing pregnancy when taken before ovulation, but when LNG was taken after ovulation, the pregnancy rate was consistent with expected rates if LNG had not been administered.

The study was done in 99 women who presented requesting emergency contraception. While in clinic for their initial consultation, they had a blood sample taken for LH, estradiol, and progesterone; then they took 1.5 mg LNG. The serum hormone samples were used to estimate the day of cycle on which the woman took LNG. Follow-up occurred 4-6 weeks later by phone to determine pregnancy status; those who became pregnant underwent ultrasound for confirmation.

Of the 99 women studied, 51 had their unprotected intercourse during the "fertile window", i.e., between days -5 to 0 of the cycle, with Day 0 being the day of ovulation. Using data from Wilcox (1998), Novikova and colleagues estimated that eight pregnancies should have occurred among these women if no EC was used. Three pregnancies occurred: one in a woman who had intercourse on Day -1 of her cycle, and two in women who had intercourse on Day 0 of their cycle. Notably, these three women took LNG approximately 2 days after ovulation.

The authors conclude that these results support the conclusion that LNG-EC has little or no effect on postovulation events but acknowledge the limitation of a study in which only three pregnancies occurred. It is noted that this study was conducted outside the US, while the Wilcox data upon which expected pregnancy rates were based came from a US study. The number of expected pregnancies would not, however, have been expected to differ meaningfully.

b. Noé and colleagues (2011) published a clinical trial on the contraceptive efficacy of LNG given before or after ovulation; the trial showed that LNG-EC given before ovulation was highly effective, but when given after ovulation, was ineffective and had no effect on the expected occurrence of pregnancy.

This study enrolled 450 women who presented to a clinic and requested emergency contraception. All were given 1.5 mg LNG-EC, and had blood drawn for LH, progesterone, and estradiol (in order to determine the day of the cycle relative to ovulation). Transvaginal ultrasound was also performed to assess the presence and size of a leading follicle or corpus luteum. Women who had a follicle ≥12 mm or a recent corpus luteum had follow-up ultrasounds. Women were later contacted to determine if menstruation occurred; if it had not, women were followed weekly for urinary human chorionic gonadotropin until pregnancy or menstruation was confirmed. Of most interest were 393 women who had data sufficient to determine whether they had intercourse on 1 of the 6 fertile days of the cycle (the day of ovulation and the 5 days prior), as described by Wilcox (1998). Women who had intercourse during those days (total N = 148) were divided into two groups; those who took LNG before (days -5 to -1, n=103) or after (day 0 or after, n=45) ovulation.

Among those who received LNG in the fertile window but were preovulatory, there were no observed pregnancies, while 16 were expected. Among those who took LNG at ovulation or after in the fertile window, 8.7 pregnancies were expected and 8 were observed. All pregnancies were intrauterine (i.e., none were ectopic); five women delivered healthy term babies vaginally. Three pregnancies ended in abortion (spontaneous versus elective not stated).

The authors conclude that reproductive processes subsequent to ovulation were not affected by LNG-EC. As with the Novikova study, it is noted that this study was conducted outside the US, while the Wilcox data upon which expected pregnancy rates were based came from a US study. The number of expected pregnancies would not, however, have been likely to differ meaningfully.

In summary, the studies by Noé and by Novikova both show that LNG is effective when given pre-ovulatory and not when administered after ovulation. These results support the conclusion that the mechanism of LNG is to suppress ovulation and that LNG either does not alter postovulation processes (i.e., fertilization or implantation) or that any effect of LNG on postovulation processes is insufficient to contribute to the mechanism of drug action to prevent pregnancy.

4. Studies Assessing Levonorgestrel Effect on Endometrial Receptivity or Sperm Function

a. Durand and colleagues (2005) published a clinical study using specimens collected in their previously described 2001 study; this new study examined one additional exploratory endometrial marker (serum glycodelin-A). In the study, serum glycodelin-A concentrations rose earlier and endometrial expression of glycodelin was lower among women who took LNG prior to the LH surge than in control cycles with no LNG. This effect was not seen when women took LNG on the day of the LH surge. Glycodelin-A is an endometrial glycoprotein that is postulated to have many possible effects. In an immunologic sense, the blastocyst and subsequent embryo are partial allografts, and thus at risk of natural immune rejection. One of glycodelin-A's roles may be as a suppressor of Natural Killer cells that would otherwise naturally attack and destroy the blastocyst; another role may be in adhesion of the blastocyst to endometrial epithelial cells (Uchida 2013). Thus, glycodelin-A may play a role in postovulatory events, although it has not been validated as a biomarker. Glycodelin-A is not usually expressed in the endometrium until the last week of the luteal phase (Brown 2000). This timing is potentially important, because in vitro, glycodelin-A may inhibit binding of human spermatozoa to the zona pellucida of the oocyte (an event relevant to fertilization), but if glycodelin-A is not normally expressed until later in the luteal phase (after fertilization would be expected to occur), this potential antifertilization effect would not generally be present naturally. As noted above, serum glycodelin-A concentrations rose earlier among women who took LNG prior to the LH surge than among women with control cycles with no LNG. This effect was not seen when women took LNG on the day of the LH surge. In the endometrial biopsies done on Day +9, staining intensity for glycodelin-A was less intense for women who took LNG prior to the LH surge than it was in control cycles and when women took LNG at the time of the LH surge. The observation of low expression of glycodelin-A in the biopsy specimens while at the same time seeing an early increase in serum glycodelin concentration is unexplained; the authors postulate that it may be due to variability in glycodelin-A expression in different parts of the endometrium.

The authors conclude that LNG may affect glycodelin-A in two phases of the cycle: the phase when fertilization occurs, and the phase of endometrial receptivity. Possible limitations of this study include that it is unclear how long specimens were stored prior to testing; because these specimens came from a study published years earlier, storage may have been for years, and the stability of glycodelin-A under the storage conditions of the study is unknown. Also, as mentioned earlier, glycodelin-A has not been validated as a biomarker. The practical relevance of a glycodelin-A effect seen only in the absence of ovulation is unclear, as pregnancy could not have occurred in this circumstance anyway. It is not clear what the normal expected profile of glycodelin-A would be in an anovulatory cycle with consequent lack of luteal activity.

b. Lalitkumar and colleagues (2007) published in vitro data showing that levonorgestrel, compared to control, did not inhibit human blastocyst attachment to an endometrial three-dimensional cell culture model. However, mifepristone (a drug approved for medical termination of early pregnancy) did inhibit

blastocyst attachment. Thus, in an in vitro model with both a positive and negative control, LNG did not affect blastocyst attachment.

c. do Nascimento and colleagues (2007) published a clinical study conducted in surgically sterilized normal women; the study showed little to no effect of LNG on sperm function or endometrial glycodelin-A levels.

Note that prior to this work, the same group had published the results of several in vitro studies that showed no effect of LNG on the fertilizing capacity of spermatozoa (Bahamondes 2003, Brito 2005, Munuce 2005).

In this clinical study, they recruited fertile, surgically sterilized women (ages 30-41 years). Participants were divided into four groups:

Group I: LNG 1.5 mg or placebo (pbo) taken 12 hours after coitus; uterine flushing performed 24 hours after LNG/pbo intake

Group II: LNG 1.5 mg or pbo taken 12 hours after coitus; uterine flushing performed 48 hours after LNG/pbo intake

Group III: LNG 1.5 mg or pbo taken 36 hours after coitus; uterine flushing performed 24 hours after LNG/pbo intake

Group IV: LNG 1.5 mg or pbo taken 24 hours after vaginal artificial insemination; uterine flushing performed 24 hours after LNG/pbo intake

The women underwent daily intravaginal ultrasound and daily serum progesterone measurements. Couples for groups I-III were asked to abstain from intercourse for 5 days, then have intercourse on the evening of the day when the greatest ultrasound-detected follicular diameter showed positive correlation with cervical mucus characteristics (spinnbarkheit >10 cm, crystallization >2+). Group IV participants had artificial insemination the next morning. In addition to the administration of LNG/pbo and uterine flushing for sperm retrieval as described above, cervical mucus was also obtained, and endometrial biopsies were obtained after uterine flushing. Endometrial tissue was assayed for glycodelin-A. Uterine flushings were examined for quantification of spermatozoa present. Spermatozoa from uterine flushings and cervical mucus were assessed microscopically for evidence of the acrosomal reaction (the attachment and dissolution of the acrosome, a structure at the tip of a spermatozoan; this reaction is necessary for penetration of the ovum and thus fertilization).

Measures of cervical mucus quality, as measured by the World Health Organization Laboratory Manual (World Health Organization 1999) were not affected by LNG treatment. This observation is of note, because previous authors had hypothesized that LNG-EC might affect cervical mucus, because progestin-only routine daily oral contraceptive tablets using a different progestin, such as norethindrone, do appear to affect cervical mucus (Moghissi 1973).

Number of recovered spermatozoa did not differ between LNG and pbo groups, either at 24 or 48 hours after coitus. Viable, motile spermatozoa were obtained from the cervical and uterine cavity at Hours 36, 48, and 60 after coitus. There were no differences between LNG and pbo groups in numbers of observed acrosomal reactions, whether observed in spermatozoa from uterine washings or cervical mucus, regardless of whether specimens were obtained at 24 or 48 hours.

Treatment with LNG was not associated with changes in expression (immunostaining intensity) of glycodelin-A from endometrial biopsy specimens. This differed from observations in the 2005 Durand study discussed above.

Potential limitations of the do Nascimento study included the fact that although a total of 33 women enrolled with a plan for 2 cycles each, 18 women were discontinued due to various problems, resulting in a total of only 48 experiments being completed. Problems included inadequate cervical mucus (n = 11 cycles), inadequate postcoital mucus test by World Health Organization (WHO 1999) criteria (12 cycles), premature ovulation (9 cycles), and "problems with the couple" (6 cycles).

The authors of the do Nascimento study concluded that LNG did not affect acrosomal reaction status, endometrial expression of glycodelin-A, quality of cervical mucus, or penetration of spermatozoa into the uterine cavity. This points away from postovulatory MoAs.

d. Palomino and colleagues (2010) published a clinical study that showed that LNG 1.5 mg administered on the day of the LH surge did not alter multiple putative markers of endometrial receptivity in endometrial biopsies from 12 women, when compared to untreated women. Unchanged markers included progesterone, L-selectin ligand, integrin αvβ3, and glycodelin-A. In 3 of 12 biopsies from LNG-treated women, areas of glandular atrophy and stromal decidualization were noted, which are common after progestin administration (Deligdisch 2000). The authors' overall conclusion was that the MoA of LNG-EC given at the time of LH surge does not involve impairment of expression of progesterone receptors or experimental biomarkers of endometrial receptivity.

e. Meng and colleagues (2009) published in vitro data using a three-dimensional human endometrial cell culture model similar to that used by Lalitkumar as described above. This in vitro model expressed numerous putative markers of endometrial receptivity, including alpha and beta receptors for estrogen and progesterone; VEGF; LIF; interleukin-1β; cyclooxygenase-2 (COX-2); integrin αvβ3; and surface molecule MUC1. LNG exposure, compared to control, did not affect expression of these markers. Mifepristone exposure, however, was associated with upregulation of epithelial estrogen receptor-β and progesterone receptor-β; and with downregulation of stromal VEGF, MUC1, and integrin αvβ3. Thus, an in vitro experiment with both a positive and negative control did not show an effect of LNG on multiple putative markers of endometrial receptivity to blastocyst implantation. A limitation of this study is that these markers of endometrial receptivity, while extensively studied, are not validated biomarkers. The in vitro model, while robust in that it shows expression of many receptors and markers associated with the state of endometrial receptivity, may not be fully translatable to the human in vivo state.

f. Vargas and colleagues (2012) studied the direct effects of LNG-EC on the endometrium, focused on changes in gene expression. Eight women were administered LNG 1.5 mg or placebo after ovulation, so as to avoid indirect effects of the suppression of ovulation on the endometrium. The results showed, as expected, no effects on cycle length or bleeding, nor on endometrial morphology or progesterone levels. A wide range of gene expression was assessed and only small changes were observed in four genes. The authors concluded that LNG caused minimal changes in transcript levels and that the changes observed were unlikely to interfere with endometrial receptivity.

g. Brito and colleagues (2005), in an in vitro sperm study, evaluated the acrosome reaction in semen samples from fertile men. Concentrations relevant to systemic exposure with LNG-EC were used, and no effects on the acrosome reaction were observed.

h. Hermanny and colleagues (2012) comprehensively evaluated sperm function in the fallopian tube after LNG administration. In this study, fallopian tube segments removed from women undergoing surgical sterilization were removed and perfused with a suspension containing motile spermatozoa, with or without LNG. The number of motile spermatozoa recovered was similar in the tubal isthmus and in the ampulla in the LNG and control groups. In addition, there were no statistically significant differences in the number of spermatozoa recovered after flushing, the number of sperm adhering to the oviductal epithelium, or the acrosome reaction rate using a fluorescent probe.

E. Integrated Summary of Current State of Knowledge Regarding Mechanism of Action of Levonorgestrel Emergency Contraception

The events of the reproductive cycle are highly complex. Since unprotected intercourse may occur at various stages of the cycle, LNG postcoital dosing may occur either in the follicular phase, right at the time of the expected midcycle surge in LH, or after ovulation. The totality of the evidence indicates that LNG-EC is effective when administered during the follicular phase of the menstrual cycle, before the LH surge. Available clinical evidence suggests that the constellation of events leading to the LH surge and follicular rupture is interrupted when LNG is administered during the follicular phase, and that this interruption of pre-ovulatory events is the main mechanism by which LNG prevents pregnancy.

As discussed above, studies available prior to the 2003 review, including clinical studies by Landgren (1989), Durand (2001), and Marions (2002), showed that LNG suppressed the midcycle LH surge and suppressed ovulation. Studies subsequently available provide additional support for this mechanism. Nonclinical studies, by Muller (2003) in rats and by Ortiz (2004) in monkeys, both support the conclusion that LNG inhibits ovulation, although in monkeys, inhibition was noted predominantly when LNG was administered before follicle growth exceeded 5 mm in size. Clinical studies published after the 2003 review, by Marions (2004), Okewole (2007), and Tirelli (2008), also demonstrated that LNG suppresses the LH surge and ovulation.

Studies by Novikova (2007) and Noé (2011) provide the strongest clinical data that suppression of the midcycle LH surge and interference with ovulation are the main mechanisms by which LNG-EC prevents pregnancy. These studies showed that after intercourse occurred during the “fertile window”, LNG administered during the follicular phase, prior to ovulation (through Day -1 prior to ovulation), was effective in preventing the occurrence of pregnancy, but when administered from Day 0 (ovulation) and beyond, the expected pregnancy rate was not reduced. In other words, LNG-EC is shown to be effective only if administered in the follicular phase of the menstrual cycle *prior* to ovulation when it is possible to suppress or delay follicle release, impair follicle development, and/or suppress the midcycle LH surge. These observations also strongly support the conclusion that direct effects on postovulatory processes are not relevant to the mechanism by which LNG-EC prevents pregnancy since LNG-EC is not effective when given after ovulation.

Further support for these conclusions comes from the studies that investigated the direct effects of LNG-EC on the endometrium, implantation, or sperm function/fertilization. As discussed above, the earlier (before the 2003 review) studies by Durand (2001) and Marions (2002) did not find meaningful changes in endometrial morphology or endometrial exploratory biomarkers. Landgren (1989) did report a decreased number of endometrial glands when LNG is administered in the follicular phase, but not when

it was administered in the luteal phase, suggesting the possibility of an indirect effect through suppression of ovulation rather than a direct effect on the endometrium. In studies reported subsequent to the 2003 review, Durand (2005) found reduction in an endometrial biomarker, glycodelin-A, when LNG was administered during the follicular phase of the cycle, and not when LNG was administered at the time of ovulation, consistent with the report by Landgren, suggesting that the changes may reflect an indirect effect of a reduced LH surge. In contrast to the findings of Durand, the study by do Nascimento (2007) did not find any effects on endometrial glycodelin-A, nor on cervical mucus quality. Similarly, the study by Palomino (2010) also did not find changes in exploratory endometrial biomarkers (including glycodelin-A) in women administered LNG, although some morphological changes (e.g., glandular atrophy) were observed. The study by Meng (2009), using an in vitro system, also found no changes in any of a range of exploratory endometrial biomarkers assessed. The study by Vargas (2012) found no meaningful changes in endometrial morphology or gene expression when LNG was administered after ovulation, supporting the conclusion that there are no direct effects of LNG on the endometrium and hence endometrial receptivity is unlikely to be reduced. Finally, the study in rats by Muller (2003) showed that LNG did not alter implantation rates but suppressed ovulation. These studies show that endometrial morphological changes with LNG administration are variably seen and such changes, if they occur, likely reflect suppression of ovulation rather than a direct effect on the endometrium. Studies of exploratory endometrial biomarkers and gene expression generally show no notable effects of LNG, particularly when administered after ovulation. Overall, these results support the conclusion that changes in the endometrium are variable, limited, and if seen, may reflect the effects of LNG on ovulation, and are unlikely to contribute to the mechanism of action of LNG-EC. The observation that postfollicular-period administration of LNG is not shown to be effective in preventing pregnancy strongly supports the lack of the drug's direct effects on postovulation processes involved in conception.

Several studies evaluated sperm function (Yeung 2002, Brito 2005, do Nascimento 2007, Hermanny 2007) and found no evidence of reduced spermatozoa number recovered from the cervical or uterine cavity; reduced sperm function; alterations in the acrosomal reaction; or inhibition of fertilization. These studies support the conclusion that alterations in sperm number or function, or fertilization, are unlikely to be a relevant mechanism of LNG-EC prevention of pregnancy.

The conclusions from this review are that there is strong evidence that LNG-EC acts by actions on ovulation, including inhibiting or delaying follicle rupture and suppressing the LH surge; there is no substantive evidence that other mechanisms such as direct alteration of endometrial receptivity leading to reduced implantation or alterations in sperm function or fertilization contribute to the mechanism of action.

It is of note that the evidence prior to the 2003 review already pointed to a predominant mechanism of suppression of ovulation; however, at that time, the evidence was as yet insufficient to conclude firmly that other mechanisms were unlikely to be contributory. Evidence subsequent to the 2003 review provides strong evidence that mechanisms beyond changes in ovulation are noncontributory to the mechanism of LNG-EC. Novikova (2007) and Noé (2011) provided especially persuasive evidence showing that dosing after ovulation does not reduce the occurrence of pregnancy. Additional studies provided evidence that LNG-EC suppresses ovulation and/or ovulatory function, and that there is no substantive evidence of direct changes in the endometrium or reductions in sperm numbers or function with LNG-EC. In summary, LNG-EC is shown to act primarily by interfering with ovulation (delay or inhibition of follicle

rupture and/or the midcycle LH and related hormonal changes) and not to act through direct effects on fertilization or on the endometrium to impede implantation.

In the Executive Summary in Section I above, summaries of answers to the following key questions were provided. These answers are expanded upon in the following paragraphs, with supporting citations:

1. Does levonorgestrel emergency contraception inhibit ovulation?

Clinical data are strong that LNG-EC, when administered prior to the LH surge, inhibits ovulation (Durand 2001; Marions 2002; Croxatto 2004; Okewole 2007; Novikova 2007; Tirelli 2008; and Noé 2011).

2. Does levonorgestrel emergency contraception affect sperm function?

In vitro and human in vivo data do not support a significant effect of LNG on cervical mucus quality; sperm quantity in the genital tract; sperm motility; or acrosomal reaction when administered at doses similar to that used in EC (Yeung 2002, Bahamondes 2003, Brito 2005, Munuce 2005, do Nascimento 2007).

3. After ovulation occurs, does levonorgestrel emergency contraception affect endometrial receptivity or implantation?

In vitro data, and human clinical data, show that LNG-EC does not affect exploratory markers of endometrial receptivity after ovulation occurs (Lalitkumar 2007; Meng 2009; do Nascimento 2007; Palomino 2010; Durand 2005). In these research studies, a wide range of endometrial characteristics and markers was evaluated, and overall, essentially no changes were consistently observed. It should be noted that such markers are not validated biomarkers, and the multidisciplinary review team did not find published data showing that changes in endometrial receptivity biomarkers translate into a clinical effect on blastocyst implantation. The totality of the evidence indicates that LNG-EC does not alter the endometrium in a clinically meaningful way to prevent implantation. The findings of Noé that there is no evident reduction in the likelihood of pregnancy when LNG is administered to women postovulation are consistent with the histological and biochemical findings of the aforementioned studies and support the conclusion that LNG-EC is ineffective when administered postovulation.

4. Does levonorgestrel emergency contraception affect pregnancy rate after ovulation?

When LNG-EC is administered after ovulation occurs, the rate of pregnancy is what one would expect if LNG-EC had not been administered (Noé 2011, Novikova 2007). This is arguably the most important aspect of the evidence.

As noted earlier, the scientific and clinical concept of the time period of pregnancy is generally consistent with the example found in the Code of Federal Regulations (CFR) under 45 CFR 46.202, which reads: “Pregnancy encompasses the period of time from implantation until delivery.” LNG-EC prevents pregnancy by acting on ovulation, which occurs before implantation, and because data do not support that LNG-EC affects implantation, LNG-EC does not terminate pregnancy.

V. Decision on Action to be Taken

In response to the applicant's submission of this labeling supplement, FDA reviewed currently available scientific evidence regarding the mechanism of action for LNG-EC as described above. Based on that review, the multidisciplinary review team agrees that it is appropriate to update nonprescription LNG-EC labeling information relevant to mechanism of action.

As explained in this review, LNG-EC prevents pregnancy - it does not terminate pregnancy. For people who are capable of becoming pregnant, LNG-EC is effective in preventing pregnancy when taken within 3 days of unprotected intercourse and before ovulation. LNG-EC is not effective if pregnancy has already occurred.

The current science supports an effect on inhibiting or delaying ovulation and the midcycle hormonal changes, and the evidence also supports the conclusion that there is no direct effect on postovulatory processes, such as fertilization or implantation.

Therefore, based on careful consideration of the applicant's labeling supplement, as amended, and additional scientific evidence, the labeling of LNG-EC will be updated. In the Drug Facts label, some (but not all) information relevant to mechanism of action will be edited. In the Consumer Information Leaflet, information relevant to mechanism of action will be updated to reflect the current state of knowledge. The following section details the changes to be made.

VI. Labeling

The multidisciplinary review team proposes the following labeling changes.

In the Drug Facts label for Plan B One-Step, in the "Other Information" section, remove the following statement:

> " * this product works mainly by preventing ovulation (egg release). It may also prevent fertilization of a released egg (joining of sperm and egg) or attachment of a fertilized egg to the uterus (implantation)."

In the Consumer Information Leaflet (CIL), under the heading "How does Plan B One-Step® work?", make the following edits; additions are underlined, and removed statements are struck through:

"Plan B One-Step® works before release of an egg from the ovary. As a result, Plan B One-Step® usually stops or delays release of the egg from the ovary. Plan B One-Step® is one tablet ~~with levonorgestrel, a hormone that has been used in many birth control pills for several decades. Plan B One-Step®~~ that contains a higher dose of levonorgestrel than birth control pills ~~but~~ and works in a similar way to prevent pregnancy. ~~It works mainly by stopping the release of an egg from the ovary. It is possible that Plan B One-Step may also work by preventing fertilization of an egg (the uniting of sperm with the egg) or by preventing attachment (implantation) to the uterus (womb).~~"

Without the above editorial markings, that CIL section will read as follows:

> "Plan B One-Step® works before release of an egg from the ovary. As a result, Plan B One-Step® usually stops or delays the release of an egg from the ovary. Plan B One-Step® is one tablet that contains a higher dose of levonorgestrel than birth control pills and works in a similar way to prevent pregnancy."

Of note, there are several other sections of the DFL and CIL that are relevant to mechanism of action, and these sections will remain unchanged.

The DFL will retain the following language that is relevant to mechanism of action:

In the "Warnings" section, under "Do not use", the following statement will remain:

> "Do not use if you are already pregnant (because it will not work)."

In the Consumer Information Leaflet, the following statements relevant to mechanism of action will be retained:

Under the heading "What Plan B One-Step® is not.", the following statement will remain:

> "Plan B One-Step® will not work if you are already pregnant and will not affect an existing pregnancy."

Under the heading "When not to use Plan B One-Step®.", the following statement will remain:

> "Plan B One-Step® should not be used if you are already pregnant, because it will not work."

These retained statements are relevant to mechanism of action because they continue to inform the consumer that levonorgestrel emergency contraception will only work prior to establishment of a pregnancy. Thus, when considering where in the reproductive cycle that LNG-EC might exert its mechanism of action, the consumer can still know that LNG-EC works very early in the reproductive process, before pregnancy has actually begun.

VII. References

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