NDA/BLA Multi-Disciplinary Review and Evaluation

Application Type	NDA
Application Number(s)	NDA 214154 (IND 110682)
Priority or Standard	Standard
Submit Date(s)	April 15, 2020
Received Date(s)	April 15, 2020
PDUFA Goal Date	April 15, 2021
Division/Office	Division of Urology, Obstetrics, and Gynecology (DUOG) / Office of Rare Diseases, Pediatrics, Urologic and Reproductive Medicine (ORPURM)
Review Completion Date	April 15, 2021
Established/Proper Name	drospirenone and estetrol tablets
(Proposed) Trade Name	Nextstellis
Pharmacologic Class	Combination hormonal contraceptive
Applicant	Mayne Pharma LLC
Dosage form	Tablet
Applicant proposed Dosing Regimen	• Take one tablet by mouth at the same time every day. • Take tablets in the order directed on the blister pack.
Applicant Proposed Indication(s)/Population(s)	For use by females of reproductive potential to prevent pregnancy
Recommendation on Regulatory Action	Approval
Recommended Indication(s)/Population(s) (if applicable)	For use by females of reproductive potential to prevent pregnancy
Recommended Dosing Regimen	• Take one pink tablet (drospirenone 3 mg, estetrol anhydrous 14.2 mg) by mouth at the same time every day for 24 days • Take one white inert tablet (placebo) by mouth at the same time every day for 4 days following the pink tablets • Take tablets in the order directed on the blister pack

Table of Contents

Table of Tables

Table of Figures

Reviewers of Multi-Disciplinary Review and Evaluation

Regulatory Project Manager	Samantha Bell
Chief Project Manager	Margaret Kober
Nonclinical Reviewer	Andrea Benedict
Nonclinical Team Leader	Kimberly Hatfield
Nonclinical Division Director	Mukesh Summan
Office of Clinical Pharmacology Reviewers	Li Li, Fang Li,
Office of Clinical Pharmacology Team Leaders	Yanhui Lu, Jingyu (Jerry) Yu
Office of Clinical Pharmacology Deputy Division Director	Doanh Tran
Clinical Reviewer	Anandi Kotak
Clinical Team Leader	Gerald Willett
Statistical Reviewer	Weiya Zhang
Statistical Team Leader	Guoxing Soon
Cross-Disciplinary Team Leader	Gerald Willett
Deputy Division Director	Audrey Gassman
Deputy Division Director (OB)	Daphne Lin
Deputy Office Director	Janet Maynard

OB = Office of Biostatistics

Additional Reviewers of Application

Interdisciplinary QT review team	Girish Bende, Ferdose Begum, Dalong Huang, Michael Li, Christine Garnett
OPQ	Mark Seggel
Microbiology (clinical)	Not applicable
OPDP	Jina Kwak, Matthew Falter
OSI	Ling Yang, Min Lu, and Kassa Ayalew
OSE/DEPI	Huei-Ting Tsai, Wei Liu, David Money,
OSE/DMEPA – Proprietary Name	Denise Baugh, Briana Rider
OSE/DRISK	Theresa Ng, Laura Zendel, and Cynthia LaCivita
OSE/DMPP	Lonice Carter, Marcia Williams, and LaShawn Griffiths

OPQ = Office of Pharmaceutical Quality
OPDP = Office of Prescription Drug Promotion
OSI = Office of Scientific Investigations
OSE = Office of Surveillance and Epidemiology
DEPI = Division of Epidemiology
DMEPA = Division of Medication Error Prevention and Analysis
DRISK = Division of Risk Management
DMPP = Division of Medical Programs and Policy

Signatures

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Nonclinical Reviewer	Andrea Benedict, PhD	DPT-RPURM	Sections: 5	**Select one:** _X__ Authored ___ Approved
	Signature: Andrea L. Benedict -S Digitally signed by Andrea L. Benedict -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2001432019, cn=Andrea L. Benedict -S Date: 2021.04.13 15:01:24 -04'00'			
Nonclinical Supervisor	Kimberly Hatfield, PhD	DPT-RPURM	Sections: 5, 14	**Select one:** _X__ Authored _X_ Approved
	Signature: Kimberly P. Hatfield -S Digitally signed by Kimberly P. Hatfield -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=1300387215, cn=Kimberly P. Hatfield -S Date: 2021.04.13 15:06:33 -04'00'			
Nonclinical Division Director	Mukesh Summan, PhD, DABT	DPT-RPURM	Sections: 5, 14	**Select one:** ___ Authored _X__ Approved
	Signature: Mukesh Summan -S Digita ly signed by Mukesh Summan S DN: c US o U S Government ou HHS ou FDA ou People cn Mukesh Summan S 0 9 2342 19200300 100 1 1 2000337340 Date: 2021 04 13 15:10:38 04'00'			
Clinical Pharmacology Reviewer	Li Li, PhD	OCP/DCEP	Sections 6	**Select one:** _X__ Authored ___ Approved
	Signature: Li Li -S Dig tally s gned by Li Li S DN c=US o=U S Government ou=HHS ou=FDA ou=People cn=Li Li S 0 9 2342 19200300 100 1 1=2000508577 Date 2021 04 13 15 53 16 04 00'			
Clinical Pharmacology Team Leader	Yanhui Lu, PhD	OCP/DCEP	Sections 6	**Select one:** ___ Authored _X__ Approved
	Signature: Yanhui Lu -S Digitally signed by Yanhui Lu -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Yanhui Lu -S, 0.9.2342.19200300.100.1.1=2001501324 Date: 2021.04.13 16:11:39 -04'00'			
Clinical Pharmacology	Doanh Tran, PhD	OCP/DCEP	Sections 6	**Select one:** ___ Authored _X__ Approved

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Deputy Division Director	**Signature:** Doanh C. Tran -S Digitally signed by Doanh C. Tran -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Doanh C. Tran -S, 0.9.2342.19200300.100.1.1=1300378169 Date: 2021.04.13 16:20:46 -04'00'			
Pharmacometrics Reviewer	Fang Li, PhD	OCP/DOP-	Sections 6	**Select one:** __X_ Authored ___ Approved
	Signature: Fang Li -S Digitally signed by Fang Li -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Fang Li -S, 0.9.2342.19200300.100.1.1=1300430137 Date: 2021.04.13 16:08:40 -04'00'			
Pharmacometrics Team Leader	Jingyu (Jerry) Yu, PhD	OCP/DOP	Sections 6	**Select one:** ___ Authored __X_ Approved
	Signature: Jingyu Yu -S Digitally signed by Jingyu Yu -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Jingyu Yu -S, 0.9.2342.19200300.100.1.1=2000794699 Date: 2021.04.13 15:56:29 -04'00'			
Statistical Reviewer	Weiya Zhang, PhD	OB/DB4	Sections 7, 8	**Select one:** _X__ Authored ___ Approved
	Signature: Weiya Zhang Digitally signed by Weiya Zhang DN: cn=Weiya Zhang, o, ou, email=(b) (6) c=US Date: 2021.04.13 16:48 48 -04'00'			
Statistical Team Leader	Guoxing (Greg) Soon, PhD	OB/DB4	Sections 7, 8	**Select one:** ___ Authored _X__ Approved
	Signature: Guoxing Soon -S Digitally signed by Guoxing Soon -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Guoxing Soon -S, 0.9.2342.19200300.100.1.1=1300124920 Date: 2021.04.14 06:22:51 -04'00'			
Deputy Division Director (OB)	Tsae Yun (Daphne) Lin	OB/DB4	Sections 7, 8	**Select one:** ___ Authored _X__ Approved
	Signature: Tsaeyun D. Lin -S Digitally signed by Tsaeyun D. Lin -S DN: c=US, o=U.S. Government, ou=HHS, ou=FDA, ou=People, cn=Tsaeyun D. Lin -S, 0.9.2342.19200300.100.1.1=1300049055 Date: 2021.04.14 08:38:32 -04'00'			

DISCIPLINE	REVIEWER	OFFICE/DIVISION	SECTIONS AUTHORED/ APPROVED	AUTHORED/ APPROVED
Clinical Reviewer	Anandi Kotak, MD	DUOG	Sections 1.4, 2, 3, 7, 8, 9, 10, 11, 12, 13	**Select one:** _X__ Authored ___ Approved
	Signature: Anandi D. Kotak -A Digitally signed by Anandi D. Kotak -A DN: c=US, o=U S. Government, ou=HHS, ou=FDA, ou=People, 0.9.2342.19200300.100.1.1=2002629744, cn=Anandi D. Kotak -A Date: 2021.04.14 10:23:20 -04'00'			
Clinical Team Leader	Gerald Willett, MD	DUOG	Authored: Sections 1.1, 1.2, 1.3 Approved: All	**Select one:** _X__ Authored _X__ Approved
	Signature: See DARRTS signature			
Deputy Division Director (Clinical)	Audrey Gassman, MD	DUOG	Sections All	**Select one:** ___ Authored _X__ Approved
	Signature: See DARRTS signature			
Office Deputy Director	Janet Maynard, MD	ORPURUM	Sections All	**Select one:** ___ Authored _X__ Approved
	Signature: See DARRTS signature			

Glossary

AC	advisory committee
APC	activated protein C
APTT	activated partial thromboplastin time
ADME	absorption, distribution, metabolism, excretion
AE	adverse event
AR	adverse reaction
AUC	area under the curve
BLA	biologics license application
BMI	body mass index
BPCA	Best Pharmaceuticals for Children Act
BRF	benefit risk framework
BSA	body surface area
CBER	Center for Biologics Evaluation and Research
CDER	Center for Drug Evaluation and Research
CDRH	Center for Devices and Radiological Health
CDTL	cross-discipline team leader
CFR	Code of Federal Regulations
CHC	combined hormonal contraceptive
CHMP	Committee for Medicinal Products for Human Use
CI	confidence interval
Cmax	maximum plasma concentration
CMC	chemistry, manufacturing, and controls
COC	combined oral contraceptive
COSTART	Coding Symbols for Thesaurus of Adverse Reaction Terms
CRF	case report form
CRO	contract research organization
CRT	clinical review template
CSR	clinical study report
CSS	Controlled Substance Staff
DHOT	Division of Hematology Oncology Toxicology
DMC	data monitoring committee
DRSP	drospirenone
E2	estradiol
E4	estetrol
ECG	electrocardiogram
ECAC	Executive Carcinogenicity Assessment Committee
eCTD	electronic common technical document
EE	ethinyl estradiol
EMA	European Medicines Agency

ETASU	elements to assure safe use
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FDASIA	Food and Drug Administration Safety and Innovation Act
GCP	Good Clinical Practice
GRMP	Good Review Management Practice
HCV	hepatitis C virus
HDL	high-density lipoprotein
HED	human equivalent dose
ICH	International Council for Harmonisation
IND	Investigational New Drug
ISE	integrated summary of effectiveness
ISS	integrated summary of safety
ITT	intent to treat
LDL	low-density lipoprotein
MedDRA	Medical Dictionary for Regulatory Activities
mITT	modified intent to treat
MRHD	maximum recommended human dose
NCI-CTCAE	National Cancer Institute-Common Terminology Criteria for Adverse Event
NDA	new drug application
NME	new molecular entity
NOAEL	no-observed-adverse-effect level
OCS	Office of Computational Science
OPQ	Office of Pharmaceutical Quality
OSE	Office of Surveillance and Epidemiology
OSI	Office of Scientific Investigation
PBRER	Periodic Benefit-Risk Evaluation Report
PD	pharmacodynamics
PI	prescribing information
PK	pharmacokinetics
PMC	postmarketing commitment
PMR	postmarketing requirement
PP	per protocol
PPI	patient package insert (also known as Patient Information)
PREA	Pediatric Research Equity Act
PRO	patient reported outcome
PSUR	Periodic Safety Update report
QTc	QT interval corrected for heart rate
REMS	risk evaluation and mitigation strategy
SAE	serious adverse event
SAP	statistical analysis plan
SGE	special government employee

SHBG	sex hormone binding globulin
SOC	system organ class
SPA	special protocol assessment
TEAE	treatment-emergent adverse event
T_{max}	time to reach maximum concentration
VTE	venous thromboembolism

1. Executive Summary

1.1. Product Introduction

Mayne Pharma LLC. seeks approval of Nextstellis under section 505 (b) (2) for use by females of reproductive potential to prevent pregnancy. Nextstellis is a combination hormonal contraceptive (CHC) consisting of oral tablets with a fixed dose of the progestin, drospirenone (DRSP) 3 mg and the novel estrogen, estetrol (E4) 14.2 mg (anhydrous). Estetrol is a new molecular entity not currently approved for marketing in the US. The active tablets are taken for 24 days, followed by 4 days of inactive tablets. A Phase 3 clinical safety and efficacy trial in support of this product was conducted in the US & Canada (MIT-Es0001-C302, also referred to as Study C302 in this review). In addition, a supportive phase 3 trial in Europe & Russia (MIT-Es0001-C301, also referred to as Study C301 in this review) was also submitted. Nextstellis will be referred to in this review either by its proprietary name or by an abbreviation of the combined hormones drosperinone and estetrol (DRSP/E4).

The E4 component in Nextstellis is a synthetic version of estetrol. E4 is an estrogen produced by the fetal liver during human pregnancy. This estrogen was discovered in 1965 in Sweden via isolation from the urine of pregnant women. The physiological function of E4 during pregnancy has not been determined. It has been tested as a marker for fetal well-being, but the large variability in maternal plasma levels has not supported use for diagnostic purposes in pregnancy. In vitro studies have suggested that E4 is a weak estrogen that shows binding to both estrogen receptors alpha and beta with a preference for alpha.

DRSP is a “fourth-generation” progestin derived from the aldosterone antagonist spironolactone - a potassium-sparing diuretic used to treat hypertension. DRSP has been approved for use in the US in both combination and progestin-only hormonal contraceptive products since 2001. The Applicant refers to the Agency’s previous findings of safety for the nonclinical pharmacology, pharmacokinetics, and toxicology of the DRSP component of the approved product YAZ® (NDA 021676, approved March 16, 2006) to support the nonclinical safety of drospirenone in Nextstellis. An adequate scientific bridge has been established to the Listed Drug YAZ®; therefore, the Applicant may rely on the nonclinical sections of the YAZ approved labeling to support Nextstellis labeling.

The primary contraceptive mechanism of action for DRSP/E4, like other CHCs, is ovulation inhibition. As a fixed-combination drug product, NEXTSTELLIS is regulated under 21 CFR Section 300.50. Under the fixed-combination drug rule, a factorial designed study is usually required to demonstrate that the combination is superior to use of either drug alone. For this product, drosperinone is considered the active drug substance that interferes with ovulation while estrogen (estetrol) is supportive (from a safety and tolerability perspective) in maintaining endometrial stability to decrease inter-cycle bleeding and spotting. For that reason, factorial studies to look at the combination were not necessary.

1.2. Conclusions on the Substantial Evidence of Effectiveness

Substantial evidence of effectiveness for DRSP/E4 is provided by one adequate single-arm clinical trial conducted in the target US population with confirmatory evidence from a second supportive study conducted outside of the US target population (in EU and Russia). Study MIT-Es0001-C302 (hereafter Study C302 [US/CA]) – a one-year, multicenter, open-label phase 3 trial conducted in the US and Canada – forms the basis for the Applicant's substantial evidence of effectiveness, with supportive data from Study MIT-Es0001-C301 (hereafter Study C301 [EU/R]), the second phase 3 trial conducted in Europe and Russia.

Open-label single-arm trials are considered an acceptable approach to conducting adequate studies for contraceptives. Ethical considerations preclude the use of a placebo comparator; females desiring contraception should not be intentionally put at risk for unintended pregnancy. Additionally, the absence of a "gold standard" for contraceptives precludes the use of an active comparator. Approval of contraceptives has historically been based on the benefit-risk profile of individual contraceptive products. As a drug class, the benefits of combination hormonal contraceptives outweigh the known risks associated with unintended pregnancy.

Study C302 included 77 sites in the U.S. and 7 sites in Canada. The primary efficacy endpoint was the Pearl Index (PI), defined as the number of on-treatment pregnancies in evaluable (or at-risk) cycles. An evaluable cycle is defined as a menstrual cycle with at least one episode of heterosexual intercourse and no back-up contraception use. The primary efficacy endpoint of on-treatment pregnancies over one year was assessed in US/Canada female participants age 16 to 35 at risk for pregnancy. The safety profile was informed by all females, including those older than 35 years to provide additional safety information.

The U.S./Canadian study (C302) enrolled 1674 participants age 16 to 35 years. The racial demographics for this population included 1,174 (70.1%) White participants, 326 (19.5%) Black or African American participants, 81 (4.8%) Asian participants and 93 (5.5%) "Other" participants. The body mass index (BMI) demographics included 1,298 (77.5%) participants with BMI < 30 kg/m^2 and 376 (22.5%) participants with BMI ≥ 30 to < 35 kg/m^2. A BMI ≥ 35 kg/m^2 was exclusionary, although one participant with BMI 48 kg/m^2 was erroneously enrolled. Of 1,674 participants 1,524 had at least one at-risk cycle. The total number of at-risk cycles was 12,763, which exceeds the Division's recommendation of 10,000 evaluable treatment cycles for a novel CHC. Twenty-six (26) on-treatment pregnancies occurred, resulting in a PI (95% confidence interval) of 2.65 (1.73, 3.88). This PI is consistent with U.S. trials for other recently approved CHCs and below 5 for the upper bound of the 95% confidence interval as outlined in the guidance, "Establishing Effectiveness and Safety for Hormonal Drug Products Intended to Prevent Pregnancy - Guidance for Industry" (July 2019).

Study C301 (EU/R)] was similar to Study C302 (US/CA) with two exceptions: 1) the age range and 2) the definition of on-treatment pregnancies. Enrollees from Study C301 included in the primary efficacy analysis ranged from 18 to 35 years of age (inclusive) compared to 16 to 35 years of age (inclusive) in Study C302. The on-treatment pregnancy definition in Study C301 counted 2 days following last tablet intake compared to 7 days in Study C302.

Study C301 (EU/R) reported 5 on-treatment pregnancies among 1,313 subjects aged 18 to 35 years with at least 1 at-risk cycle. The total number of at-risk cycles was 13,692. The PI was significantly lower than the US study at 0.47 per 100 women-year (95% CI: 0.15, 1.11). The finding of a lower PI in European-based studies is consistent with the Division's experience with trials outside the US over the last twenty years. This difference in pregnancy rate is likely reflective of significant differences between the US population and the EU/R population in body mass index, demographics, compliance, and study site type.

The primary safety population consisted of data from Study C302 with additional supportive data from Study C301 (EU/R) safety population. Data from Study C301 was primarily reviewed for safety purposes and pooled with Study C302 for the integrated analysis (Pooled safety population).

Subgroup analyses of interest conducted by the Review Division related to BMI and race warrant further discussion. Specifically, previous clinical trials for combination hormonal contraceptives have demonstrated trends of decreasing effectiveness and increasing thromboembolic risk with increasing BMI. Therefore, the review team assessed the effectiveness and safety of DRSP/E4 in participants from Study C302 (US/CA) across BMI subgroups. Table 1 shows the PI subgroup analysis by BMI for all participants age 16-35 years (inclusive) from Study C302.

Table 1. Pearl Index by Subgroup for Subjects Aged 16 to 35 Years*, Study MIT-Es0001-C302

Subgroup	N	On-Treatment Pregnancies	At-Risk Cycles	Pearl Index (95% CI)
All participants (age 16–35 yrs.)	1524	26	12,673	2.65 (1.73, 3.88)
BMI (kg/m^2)				
< 30	1,187	20	10,113	2.57 (1.57, 3.97)
≥ 30 to < 35**	337	6	2,650	2.94 (1.08, 6.41)

* Source: Study MIT-Es0001-C302 clinical study report Tables 14.2.1.4 and Appendix 6 in the applicant's responses to the information request submitted on 10/2/2020.
** Includes one subject with BMI 48.4 kg/m^2
Abbreviations: BMI, body mass index; CI, confidence interval

Subgroup analysis by BMI shows a trend of decreasing effectiveness with increasing BMI. However, the small number of at-risk cycles (2,650) in the subgroup of obese females (BMI ≥ 30 kg/m^2) limits interpretation of the data. Study C301 from Europe & Russia does not provide additional supportive safety data as there were only 73 participants with BMIs ≥ 30 kg/m^2 contributing 786 at-risk cycles for the BMI analysis. The paucity of ex-US data limits any additional insight into whether increasing body mass index significantly impacts Nextstellis use.

During development of Nextstellis, a specific criterion for the number of at-risk cycles in females in the higher BMI subgroups was not recommended to the Applicant. At that time, the Agency's recommendations discouraged the use of BMI restriction in contraceptive trials. However, the obesity epidemic has continued to worsen in recent decades. More than 60% of females of reproductive age in the US are now considered overweight (defined as body mass

index ≥ 25 kg/m^2 or < 30 kg/m^2) or obese (defined as body mass index ≥ 30 kg/m^2).[1] Historically, older CHC labels have not restricted use based on BMI and Sponsors limited enrollment of participants with higher BMIs. In recent years, the Agency has labeled newer combined hormonal products with a limitation of use based on BMI and included information on lack of data in females with higher BMI (e.g. exclusion of females with BMI ≥ 35 kg/m^2).

In 2020 the Agency for the first time contraindicated a combination hormonal product for females with BMI ≥ 30 kg/m^2 where the database demonstrated decreased efficacy and a potential for increased thromboembolic risk in the overweight and obese study populations. Recently, the Agency has developed guidance recommending that females of higher BMI (≥ 30 kg/m^2) be routinely included in trials, but development of Nextstellis occurred almost a decade prior to these new recommendations.

Given the limited data in females with BMI ≥ 30 to < 35 kg/m^2, this product will receive a limitation of use in females with obesity. In addition, labeling will reflect the findings in both non-obese and obese females to assist providers and patients in making an informed decision on whether or not to use this product.

Subgroup analysis by race revealed a significant numerical disparity in the PI and 95% CI between White participants (PI 1.77, 95% CI (0.94, 3.02)[see Table 32]) and Black or African American participants (PI 6.80, 95% CI (3.26, 12.51)). The reason for this race disparity is not clear but may have resulted from the limited number of evaluable treatment cycles by race (1,911 evaluable cycles in Black/African American participants vs. 9,570 in White participants). Pharmacokinetic (PK) data did not demonstrate a difference in exposure levels to DRSP and E4 and their metabolites by race. Since these subgroup analyses were exploratory, control for multiplicity was not prespecified. Overall, the limited number of cycles precludes definitive conclusions as to the role of race and BMI regarding differential efficacy among subgroups.

Study C301 lacked supportive data that could be further assessed for race and BMI subgroups, as less than 6% of study participants in Study C301 were of non-White race.

Future drug development efforts may include increasing recruitment within subgroups of interest to allow meaningful analysis and conclusions, and to better inform the population likely to use DRSP/E4 in the US. Additionally, participants with a BMI greater than or equal to 35 kg/m^2 were not studied, highlighting the need to increase recruitment within BMI subgroups to collect sufficient data in this important subset of the population desiring contraception.

In summary, DRSP/E4 demonstrated substantial evidence of effectiveness primarily by the overall PI data from the U.S./Canada study (Study C302) and was supported by the overall data from Europe and Russia (Study C301). The low number of at-risk cycles (2,650) and the upper bound of the PI of 6.41 limits interpretation of the data in the BMI subgroup of ≥ 30 to <

[1] National Institute of Diabetes and Digestive and Kidney Diseases. National Institutes of Health. https://www.niddk.nih.gov/health-information/health-statistics/overweight-obesity

35 kg/m^2. Given that the upper bound of the PI exceeded 5, the review division concludes that this BMI data should be reflected in the product labeling and be presented as a limitation of use. The reviewers conclude that a contraindication for this product for females with a BMI ≥ 30 or < 35 kg/m^2 is not warranted as discussed in the benefit/risk assessment outlined in Section 1.3.

1.3. Benefit-Risk Assessment

Benefit-Risk Summary and Assessment

The benefit-risk analysis for CHCs takes into consideration both the significant risks and consequences of unintended pregnancy in a generally healthy reproductive-age population and balances that against risks of hormonal contraception use that can include serious thromboembolic adverse reactions. The risk of unintended pregnancy can have significant adverse effects on both mother and infant and provides justification for CHC approval in a healthy population of females despite the known risks of hormonal use.

Effectiveness of Nextstellis was demonstrated in a single US/Canada phase 3 trial (MIT-Es0001-C302, hereafter referred to as Study C302) with an acceptable overall Pearl Index (2.65) and upper bound of the 95% confidence interval (3.88). Substantial evidence for contraceptive products is provided by this single adequate clinical trial conducted in the target US/Canada population with supportive evidence from a second supportive trial conducted outside the US. The supportive trial was an additional phase 3 trial conducted in Europe and Russia (MIT-Es0001-C301, hereafter referred to as Study C301). BMI subgroup data in females >30 kg/m^2 was limited, but appeared to have a trend of increasing PI with upper bound of the 95% CI exceeding 5 in females with increasing BMI. This trend will be presented in labeling and will include a limitation of use statement to inform providers and patients. In addition, a higher PI and 95% CI were also seen in the Black/African American subgroup as compared to the White subgroup. However, sub-group analyses of ethnic groups based on race were exploratory, had limited evaluative treatment cycles, and did not adequately control for multiplicity. Future development of these contraceptive products needs to ensure that sufficient numbers of treatment cycles are included for these subgroups to better ascertain precision for labeling.

Safety of Nextstellis was also primarily obtained from Study C302 with supportive data from a European/Russian phase 3 trial (MIT-Es0001-C301, hereafter referred to as Study C301). This trial was conducted in a similar manner to Study C302. The overall safety exposure (>35,000 cycles) and number of 13-cycle (approximately 1 year) completers (>2,200) is acceptable for a drug product containing a new molecular entity (novel estrogen, estetrol). Laboratory findings in regard to DRSP, namely hyperkalemia, were rarely seen and when identified, not associated with any clinical symptomatology. The low rate of hyperkalemia is consistent with other DRSP combination products (e.g., Yasmin, Yaz) and more recently the DRSP progestin-only product, Slynd.

In the US phase 3 study, the safety profile appears to be similar to other oral combined hormonal contraceptive products. Discontinuations due to adverse events and the most common adverse reactions appear clinically similar to other products. In regard to cycle control, which is important for tolerability, the percentage of participants with unscheduled bleeding and the number of unscheduled bleeding days were similar to other CHC products and trended towards improvement over the year-long study. Amenorrhea was seen in approximately 15%% of participants. Amenorrhea is not necessarily desirable by all females who use hormonal contraceptives and will be labeled. No other significant

safety signals emerged for Nextstellis from laboratory findings, vital signs, or endometrial pathology. Several serious adverse events were reported during the development program including depression and ectopic pregnancy and one death that was determined to be unrelated to Nextstellis use.

All combined hormonal contraceptives are associated with a known safety risk of thromboembolic phenomenon. In the Nextstellis database, two cases of deep vein thrombosis occurred in the clinical development of Nextstellis. One occurred in a healthy white female in the EU/R Phase 3 trial (Study C301). The second event occurred in a 54-year-old postmenopausal female receiving five times the therapeutic dose of DRSP/E4 in a Phase 1 safety study evaluating the effects of DRSP/E4 on the QT interval.

Previous epidemiologic studies of CHCs containing DRSP/EE have raised concerns that combination DRSP-containing products may increase the risk for VTE over and above that associated with other CHC progestins (e.g., levonorgestrel, LNG). However, not all epidemiologic studies have clearly identified this increased risk. Of note, a recent FDA-funded study found a hazard ratio of 1.6 when comparing CHC/DRSP users compared to CHC/LNG. Based on the totality of the data on DRSP-containing combined hormonal contraceptives, labeling changes with regard to VTE risk were made to the DRSP-containing products. There is insufficient data to ascertain whether DRSP/E4 will have a similar thromboembolic profile in clinical use to other CHCs. As discussed at a recent Advisory Committee (AC), the higher risk of VTE in females with higher BMI is of significant concern and has resulted in a CONTRAINDICATION in one product based on review of the clinical trial database.

For this product, no safety signal with increasing body mass index was clearly identified. As the risk assessment of VTEs and ATEs require large scale epidemiologic observational data, a postmarketing study will be required to further ascertain the risk of thromboembolism with use in the US population.

An additional safety concern in products containing drospirenone involves the potential for increased serum potassium levels, particularly in users with renal impairment or with concomitant use of medications that can also increase serum potassium levels. The Applicant conducted regular monitoring of serum potassium levels in the Phase 3 clinical trials. A total of 7 participants were listed with “hyperkalemia” or “blood potassium increased.” Upon review, three of these participants had serum potassium elevations greater than 6 mmol/L potentially attributable to study drug, however no adverse events occurred in association with the laboratory findings. The Agency reviewers also evaluated other participants with elevated potassium levels. Many of these lab alterations were 1) identified at screening, 2) very mild elevations or 3) isolated findings. None of the participants in either of the two aforementioned categories showed any symptoms attributable to hyperkalemia. Additional information regarding potassium monitoring can be found in Section 8.2.5.1 of this review.

Review teams from other disciplines including pharmacology/toxicology, clinical pharmacology and chemistry review teams did not identify any approvability concerns.

A whistle blower complaint was received by the FDA prior to review of this application. The Office of Scientific Investigation (OSI) was aware of the details of this complaint and collaborated with other regulatory agencies to assess this application. OSI concluded that the reliability of the efficacy and safety data in support of this application was acceptable.

In summary, based on an acceptable PI, upper bound of the 95% CI and safety profile similar to other oral CHC products, the reviewers find a favorable benefit/risk for Nextstellis and recommend approval of this product for use by females of reproductive potential to prevent pregnancy.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Analysis of Condition	• Unintended pregnancy remains a significant reproductive health problem for females and their families in the United States due in part to compliance, access and affordability. Oral CHC products such as Nextstellis continue to represent a large segment of CHCs but they have been supplanted in the US to some degree by long-acting reversible contraceptives (LARCs) which have less compliance issues.	Additional modifications of contraceptives in regard to ease of use, effectiveness and safety are warranted to continue to reduce the unintended pregnancy rate.
Current Treatment Options	• Contraceptive products that are reversible and utilized by females include progestin/estrogen combinations, progestin-only products and non-hormonal products. Route of administration includes oral, transdermal, dermal implant, injections, vaginal and intrauterine. Ethinyl estradiol is the predominant estrogen used in combination products with estradiol valerate as a component in one product.	Expanding the number of hormonal contraceptive products available improves choices and access for patients.
Benefit	• The primary benefit for Nextstellis is reduction in the rate of pregnancy in reproductive age females who have unprotected intercourse with chronic continuous use.	The overall Pearl Index (PI) 2.65 with an upper bound of 3.88 is acceptable for evidence of Nextstellis effectiveness. Labeling is recommended to provide additional information for BMI and race subgroups.

Dimension	Evidence and Uncertainties	Conclusions and Reasons
Risk and Risk Management	• The safety database during clinical development was acceptable for a product containing a new molecular entity with over 10,000 evaluable cycles. The safety profile for this DRSP-containing product appeared similar to other oral CHCs in discontinuations, common adverse reactions and thromboembolic events. Rare cases of potassium elevation were asymptomatic and were not unexpected with drosperinone use. Uncertainties regarding the incidence of thromboembolic events in females with both normal and increased BMI will be further evaluated with a postmarketing requirement (PMR). One venous thromboembolic case was reported in the clinical development period at the to-be marketed dose.	Compared to other oral CHCs containing DRSP, Nextstellis manifested a similar risk profile although the risk of thromboembolic phenomenon will need further assessment through a postmarketing requirement (PMR) observational study. The totality of evidence from the safety database demonstrates an acceptable risk profile for approval. No other concerns were identified that would require a Risk Evaluation and Mitigation strategy (REMS).

1.4. Patient Experience Data

Patient Experience Data Relevant to this Application (check all that apply)

X	**The patient experience data that were submitted as part of the application include:**			**Section of review where discussed, if applicable**
	☐	Clinical outcome assessment (COA) data, such as		
		☐	Patient reported outcome (PRO)	
		☐	Observer reported outcome (ObsRO)	
		☐	Clinician reported outcome (ClinRO)	
		☐	Performance outcome (PerfO)	
	☐	Qualitative studies (e.g., individual patient/caregiver interviews, focus group interviews, expert interviews, Delphi Panel, etc.)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		
	☐	Natural history studies		
	☐	Patient preference studies (e.g., submitted studies or scientific publications)		
	X	Other: (Please specify): The Applicant submitted patient-reported measures of quality of life, enjoyment, and satisfaction as well as menstrual distress. These questionnaires were not discussed with the Review Division as validated endpoints and are considered exploratory in nature.		Not discussed in this review.
X	**Patient experience data that were not submitted in the application, but were considered in this review:**			
	X	(b) (6)		
	☐	Patient-focused drug development or other stakeholder meeting summary reports		
	☐	Observational survey studies designed to capture patient experience data		

	☐	Other: (Please specify):	
☐	**Patient experience data was not submitted as part of this application.**		

2. Therapeutic Context

2.1. Analysis of Condition

Unintended pregnancy negatively impacts personal, societal, and maternal and infant health in numerous ways. Potential consequences include the following:

Missed opportunities for preconception care
Late initiation of prenatal care
Maternal mental health distress
Relationship stress
Substance abuse
Economic and psychosocial distress
Increased rates of preterm birth and low birth weight
Decreased infant bonding
Decreased breastfeeding
Increased rates of child neglect

Providing females of reproductive potential with contraceptive options to prevent unintended pregnancy will have a significant positive impact on maternal and child health in the US.

2.2. Analysis of Current Treatment Options

Current options for prevention of pregnancy in the US include:

Combination hormonal contraceptives (CHCs), which include:

- Combination oral contraceptives (COC)
- Intravaginal rings
- Transdermal systems

Progestin-only hormonal contraceptives, which include:

- Progestin-only oral contraceptives (POPs)
- Implants
- Injectables
- Hormone releasing intrauterine systems (IUS)

Non-hormone releasing intrauterine systems

Permanent sterilization methods

Barrier methods and vaginal non-hormonal contraceptive agents, including spermicides

Natural-planning methods including phone apps

Abstinence

The effectiveness of these contraceptive methods depends on factors that include the mechanism of action, the tolerability (cycle control) and ease of use/access. For hormonal methods of contraception, effectiveness primarily depends on the consistency of hormone

levels. For non-implantable products, therefore, consistent hormone levels depend on the user. Failure rates range from less than 2 in 1000 users per year with long-acting reversible contraceptive methods – such as the implant or intrauterine systems (non-user dependent) – to nearly 30 in 100 with user-dependent methods – such as barrier or natural-planning methods. Regarding real world combination oral contraceptive effectiveness, failure rates range from 4 to 7 in 100 users per year by current estimates.[2] The goal of including a study in the US/Canada is to provide data on effectiveness and safety that will better inform patients who will use this product in the US.

Table 2 summarizes currently available contraceptive treatment options containing drospirenone, as well as dienogest/estradiol valerate (DNG/E2V) which represents the most recent non-ethinyl estradiol (EE) based estrogen product approved for prevention of pregnancy in the US.

Table 2. Summary of Combination Oral Contraceptives Using Drospirenone and DNG/E2V

Proprietary Name/ NDA Number	Nonproprietary Name	Relevant Indication	Year of Approval	Dosing/ Administration	PI (Rate Per 100 WY)
Yasmin/ 021098	DRSP/EE	Prevention of pregnancy	2001	3/0.03 mg	<1
Yaz/ 021873	DRSP/EE	Prevention of pregnancy PMDD Acne	2006	3/0.02 mg	1.41
Safyral/ 022574	DRSP/EE/ Levomefolate calcium	Prevention of pregnancy Folate supplementation	2010	3/0.03/0.451 mg	Based on Yasmin
Beyaz/ 022532	DRSP/EE/ Levomefolate calcium	Prevention of pregnancy PMDD Acne Folate supplementation	2010	3/0.02/0.451 mg	Based on Yaz
Natazia/ 022252	E2V/DNG	Prevention of pregnancy Heavy menstrual bleeding	2010 2012	3 mg E2V x 2 2 mg DNG/ 2 mg E2V x 5 3 mg DNG/2 mg E2V x 17 1 mg E2V x 5	1.64 (US/Canada) 1.04 (Europe)

Source: Yasmin USPI; Yaz USPI; Safyral USPI; Beyaz USPI; Natazia USPI.
https://www.accessdata.fda.gov/scripts/cder/daf/index.cfm?event = BasicSearch.process
Abbreviations: DNG, dienogest; DRSP, drospirenone; E2V, estradiol valerate; EE, ethinyl estradiol; NDA, new drug application; PMDD, premenstrual dysphoric disorder; PI, Pearl Index; WY, Woman-years

[2] Sundaram A, et al. Contraceptive Failure in the United States: Estimates from the 2006-2010 National Survey of Family Growth. *Persp on Sexual and Repro Health*, 2017, 49(1):7-16.

3. Regulatory Background

3.1. U.S. Regulatory Actions and Marketing History

Mayne Pharma LLC (hereafter referred to as the Applicant) has submitted this application for combination hormonal contraception for a prevention of pregnancy indication. Their product consists of oral tablets containing a fixed dose of drospirenone 3 mg and estetrol 14.2 mg (DRSP/E4) for 24 days, followed by 4 days of inactive (placebo) tablets. The estrogen component of this fixed-dose combination tablet is a new molecular entity not currently approved for marketing in the US. However, this product recently received an approval action from Health Canada (HC) and a positive opinion from the European Medicines Agency (EMA). The preclinical and clinical studies to assess the safety and efficacy of DRSP/E4 were conducted under IND 110682. A number of investigations were conducted in the EU in addition to North America.

The IND application from Mayne Pharma LLC was previously listed under an entity entitled SRA International, Inc.

3.2. Summary of Presubmission/Submission Regulatory Activity

February 28, 2011	PIND meeting: Held to discuss key clinical, non-clinical, and chemistry, manufacturing and control (CMC) issues. FDA advised the Applicant that one or two phase 3 clinical trials would be required, to provide safety and efficacy data in a total of 20,000 28-day treatment cycles. At least 400-500 participants who use the product for one year should be evaluated for safety and at least 200 participants should complete the study. At least 50% of the data should come from North America. The efficacy population should include females age ≤35 years at risk for pregnancy, with exclusion of any cycles in which back-up or emergency contraception was used unless a pregnancy was conceived in such a cycle (see note at end of this section).
August 28, 2012	Executive Committee for Assessment of Carcinogenicity (CAC) meeting held to discuss the carcinogenicity Special Protocol Assessment (SPA) submitted for review. The Committee agreed with the Sponsor's proposed approach to dose selection in rodents.
October 25, 2012	Type B End-of-Phase 2 (EOP-2) face-to-face meeting: FDA advised the following: • Drospirenone remains an acceptable progestin for use in COCs despite recent concerns regarding the potential for increased risk for VTE compared to other progestins used in COCs. This advice is consistent across regulatory agencies globally.

- The Clinical Pharmacology team recommended a food effect studies prior to initiating Phase 3 studies and to consider increasing the sample size for the mass balance study.
- Additional study design considerations communicated to the Applicant included the following:
  - The Pearl Index (PI) should be calculated based on 28-day cycles of exposure, (b) (6)
  - FDA defined "on-drug" pregnancies as all conceptions that occur from Day 1 (the initiation of study drug) to seven days after the final tablet is taken (whether active or inactive).
  - Cycles in which participants used a condom or alternative method of birth control for any reason should not be counted in the PI calculation unless a conception is documented during that cycle. The participant diary should record all use of any back-up contraception (not just condoms) or emergency contraception use.
  - FDA discourages the use of a BMI restriction as an inclusion criterion. If there is a restriction on the basis of BMI in the phase 3 trials, it is likely to be described in labeling.
  - Endometrial biopsies should be conducted in a subset of participants at study entry and after at least 9 months on-treatment and should include 100-200 participants.

December 2, 2015 First Special Protocol Assessment (SPA) Request submitted

January 7, 2016 SPA No-Agreement Letter Issued

The Agency issued the letter with a number of no-agreement comments, including sample size, bleeding/spotting definitions, entry criteria, etc. The Agency added that the Applicant confirm that participants had sexual intercourse during evaluable cycles. The following advice regarding endometrial biopsies was communicated to the Applicant:

- The proposed plan to perform endometrial biopsies in 170 participants at screening and after at least 10 completed cycles of treatment was reasonable. However, each biopsy specimen should be read by two independent pathologists, with a third independent pathologist to read the specimen in the event of disagreement. Describe histology results using the descriptions in the 2003 guidance for industry: *Estrogen and Estrogen/Progestin Drug Products to Treat Vasomotor Symptoms and Vulvar and Vaginal Atrophy Symptoms – Recommendations for Clinical*

Evaluation.

February 25, 2016	Applicant submits 2nd SPA request (resubmission)
April 1, 2016	No-Agreement Letter based on lack of nonclinical data on estetrol (E4)
June 20, 2016	IND 110682 opened with phase 3 clinical trial protocol MIT-Es0001-C302 (1st study to be conducted in the US, referred to as Study C302)
April 11, 2019	Agreement reached on initial Pediatric Study Plan (iPSP) with partial waiver for pediatric males and premenarcheal females. The Applicant (b) (4)
April 29, 2019	Type C Teleconference held to discuss Applicant's questions regarding endometrial substudy and discrepancies in readings by the internal pathologists. The Applicant proposed a re-read of the endometrial slides by three independent well-recognized pathologists and submission of the formal re-read as the specific support for the NDA. FDA found the request to be acceptable.
November 22, 2019	Type B Face-to-Face pre-NDA meeting held.
April 15, 2020	NDA 214154 received

A majority of the Agency's recommendations during the drug development for Nextstellis were incorporated into revised clinical and clinical pharmacology protocols for IND 110682.

4. Significant Issues From Other Review Disciplines Pertinent to Clinical Conclusions on Efficacy and Safety

4.1. Office of Scientific Investigations (OSI)

The Office of Scientific Investigations (OSI) conducted a review of inspection reports provided by Health Canada (HC) and the European Medicines Agency (EMA), as well as a remote regulatory assessment (RRA) of the Applicant. Prior to receiving the NDA submission, FDA and other regulatory agencies (EMA and Health Canada) received an anonymous complaint (b) (4) alleging that the sponsor did not adequately assess and report safety information for its E4 containing products in clinical studies. Specifically, the complainant alleged that the sponsor did not have a global safety database for this product that includes cumulative AEs from clinical studies conducted in the US, Europe, and Japan. Safety data were disbursed among several different CROs, and the complainant raised questions about the sponsor's ability

to conduct ongoing evaluation of the product's risk/benefit profile, signal detection, and follow-up of serious AEs (SAEs). The complainant noted that the sponsor's pharmacovigilance (PV) system had recently been outsourced to a vendor.

The ongoing COVID-19 global pandemic significantly limited the Office of Regulatory Affairs (ORA)'s ability to conduct onsite GCP inspections. As a result, onsite inspections or remote data assessment of source records were not conducted due to travel and local restrictions. A remote regulatory assessment (RRA) of the sponsor Estetra SPRL was conducted by FDA. This RRA focused on Studies MIT-Es0001-C301 and MIT-Es0001-C302 and reviewed the sponsor's organizational structure and responsibilities; contractual agreements and oversight of transferred regulatory obligations to the CRO; standard operating procedures (SOPs/guidelines) to assure the integrity of data collection; registration of the studies; financial disclosure and investigator agreements; protocol deviations related to key safety and efficacy endpoints; safety evaluation with AEs and SAEs reporting; electronic trial master file (eTMF) review; record retention; electronic records and electronic signatures; and quality assurance system and audits.

The EMA and Health Canada (HC) also conducted RRA investigations of the applicant and EMA conducted one clinical site inspection remotely. Both EMA and HC were aware of the complaint regarding the sponsor's deficiencies with the PV system during the RRA and shared their findings from remote inspections of the sponsor and sites with the FDA. None of the regulatory agencies identified cGMP violations that would alter the effectiveness or safety findings.

The OSI reviewed inspection reports provided both by HC and the EMA and included the findings into this Clinical Inspection Summary (CIS). Although a few GCP findings were identified by EMA and HC RRA inspections, none were deemed significant to affect the reliability of the effectiveness and safety information in the application. Based on FDA's RRA of the sponsor, the sponsor's study oversight appears adequate and the data generated by the sponsor appear acceptable in support of the NDA.

4.2. Product Quality

NEXTSTELLIS (drospirenone and estetrol) tablets, 3 mg / 14.2 mg, is a fixed-dose combination product containing a previously approved active ingredient, drospirenone (DRSP), and a new molecular entity (NME), estetrol (E4). Each (b) (4) aluminum foil blister pack provides a 28-day 24/4 regimen with 24 active tablets and 4 inert, placebo tablets.

The chemistry, manufacturing and controls (CMC) information for drospirenone is documented in (b) (4) Type II DMF (b) (4). The CMC data and information provided demonstrate that the manufacturing process and controls strategy are adequate to ensure the identity, strength, quality, and purity of the drug substance, and support use of this drug substance in the manufacture of the drug product.

The CMC information for estetrol is documented in (b) (4) Type II DMF (b) (4)

Nextstellis (drospirenone and estetrol tablets)

Estetrol is a synthetic estrogen that is isolated as a white to off-white crystalline monohydrate. Estetrol monohydrate is poorly soluble in aqueous solutions. The CMC data and information provided support use of this drug substance in the manufacture of the drug product.

Each active tablet contains drospirenone, 3 mg, and estetrol monohydrate (15 mg, equivalent to 14.2 mg of anhydrous estetrol), along with lactose monohydrate, sodium starch glycolate, (b) (4) corn starch, povidone, and magnesium stearate. Tablets are coated with a commercially available pink, non-functional film-coating system consisting of hypromellose, hydroxypropylcellulose, talc, hydrogenated cottonseed oil, titanium dioxide, and iron oxide red. The inert, placebo tablets contain lactose monohydrate, (b) (4) corn starch, and a white film-coating system. The film-coating consists of the same components as the pink film-coat except lacks iron oxide red. All inactive ingredients meet compendial requirements and are suitable for the intended use.

In addition to the controls for all materials, manufacturing in-process controls, and the finished product specification ensure the identity, strength, quality, purity, potency, and bioavailability of the drug product. The active tablet specification includes tests for identification of DRSP and E4, assay and content uniformity of DRSP and E4, degradation products, (b) (4) (b) (4) and in vitro dissolution. Based on risk assessments, routine testing for elemental impurities and (b) (4) in the finished product is not required. Testing of the placebo tablets is performed to ensure the absence of the active ingredients. The analytical procedures have been adequately validated. Appropriate acceptance criteria have been established.

A 36-month expiration dating period (shelf-life) for product stored at 20ºC to 25ºC is granted based on the long-term and accelerated stability data provided in the application.

The drug substance and drug product manufacturing, packaging and testing facilities have acceptable Current Good Manufacturing Practice status.

Overall, sufficient information and supporting data have been provided in accordance with 21 CFR 314.50 to ensure the identity, strength, quality, purity, potency and bioavailability of the drug product at release and throughout the shelf-life.

The Applicant's claim for a categorical exclusion from an environmental assessment (EA) in accordance with 21 CFR 25.31(b), which is for substances that increase in use but result in an expected introduction concentration (EIC) of <1 ppb. No extraordinary circumstances are known to the Applicant (21 CFR 25.15). The applicant also submitted EA data to support the exclusion claim based on recent FDA guidance (USFDA, 2016, Environmental Assessment: Questions and Answers Regarding Drugs with Estrogenic, Androgenic, or Thyroid Activity). The FDA EA Team reviewed the data and agreed that approval of this application would not result in a significant environmental impact. Therefore, the claim for an exclusion from an EA is acceptable.

4.3. Clinical Microbiology

Not applicable for an oral tablet.

4.4. Devices and Companion Diagnostic Issues

Not applicable for this combination hormonal contraceptive tablet presentation.

5. Nonclinical Pharmacology/Toxicology

5.1. Executive Summary

NEXTSTELLIS is a proposed fixed-dose combined hormonal contraceptive (CHC) drug product for the prevention of pregnancy in women that contains a novel estrogen, estetrol (E4; 14.2 mg [anhydrous]), and a progestin, drospirenone (DRSP; 3 mg).

The E4 component is a New Molecular Entity (NME), and as such, a full battery of nonclinical pharmacology, pharmacokinetic, and toxicology studies have been completed to support the nonclinical safety of this drug product component.

DRSP is a component of approved progestin contraceptive products, both alone and in combination with estrogens. The Applicant refers to the Agency's previous findings of safety for the nonclinical pharmacology, pharmacokinetics, and toxicology of the DRSP component of the approved product YAZ® (NDA 021676, approved March 16, 2006), as well as information from the published literature, to support the nonclinical safety of drospirenone in NEXTSTELLIS. YAZ® is an approved CHC composed of DRSP (3 mg) and ethinyl estradiol (0.02 mg) and is appropriate for the applicant to reference as a listed drug. The submitted nonclinical literature for DRSP was considered scientifically supportive, but was not deemed necessary for approval or labeling. The nonclinical sections of the labeling for NEXTSTELLIS will align with the DRSP information as represented in the YAZ® approved drug product labeling.

The nonclinical information submitted by the Applicant to support the nonclinical safety and efficacy of NEXTSTELLIS is summarized below.

Estetrol

Primary Pharmacology

E4 is a novel, estrogen class, active pharmaceutical ingredient. E4 is a synthetic version of a naturally occurring estrogen that is produced by fetal liver during human pregnancy. E4 concentration in fetal and maternal plasma increases throughout pregnancy and reaches a maximum at the end of pregnancy (approximately 1 ng/mL in maternal plasma). The physiological function of E4 in pregnancy is unknown.

The in vitro pharmacology data indicate that E4 is a weak and selective estrogen that binds to Estrogen Receptor-α (ERα) and ERβ, with a preference for ERα, and has 60 times less binding affinity to ERα than ethinyl estradiol (EE). E4 inhibits ovulation in animal models and elicits expected estrogenic pharmacological effects in female reproductive tract tissues, but with less potency than established estrogen class compounds, EE and 17β-estradiol (E2).

The major human E4 metabolites, E4-3-glucuronide and E4-16-glucuronide, showed weak estrogenic activity on both ERα and ERβ, with E4-3-glucuronide being less potent than E4-16-glucuronide. The potency of these metabolites was several hundred-fold lower than the potency of E4.

Secondary Pharmacology

E4 did not display significant off-target effects in in vitro studies, and did not bind to the progesterone, androgen, or glucocorticoid receptors at concentrations approximately 170 times higher than the maximal plasma concentration (C_{max}) of E4 at the maximum recommended human dose (MRHD).

Safety Pharmacology

Oral doses of E4 were investigated in the core battery of cardiovascular, respiratory, and central nervous system (CNS) safety pharmacology assays. E4 did not alter the cardiovascular function of telemetered monkeys and did not affect the respiratory functions of rats up to the highest dose tested (100 mg/kg in monkeys and 150 mg/kg in rats), with safety margins of approximately 115 times and 830 times the MRHD on an AUC basis, respectively. In the CNS safety pharmacology study in rats, a minor reduction in body temperature was the only effect observed at the highest dose of 150 mg/kg, with a margin of exposure of 688 times the MRHD on an AUC basis. E4 did not interfere with the hERG channel conduction in in vitro patch-clamp experiments using concentrations of E4 that were 480 times the C_{max} of E4 at the MRHD. As such, these safety margins do not indicate any safety pharmacology-related clinical concerns for E4 at the MRHD.

Pharmacokinetics/ADME

Absorption

E4 is rapidly absorbed by the oral route in mice, rats and monkeys and produces dose-proportional exposure up to 30 mg/kg/day in mice, 50 mg/kg/day in rats and 30 mg/kg/day in monkeys, with limited accumulation upon repeated administration. Plasma half-life of elimination ($T_{1/2}$) was 1.5-3 hours in mice, 2-6 hours in rats, 10-19 hours in monkeys, and is approximately 25 hours in humans. E4 undergoes enterohepatic recirculation in monkeys and humans.

Distribution

E4 displayed moderate protein binding (45-67%) in female mouse, rat, monkey, and human plasma.

E4 and its metabolites were widely distributed in rat tissues after a single oral dose of [^{14}C]-E4 and are rapidly eliminated with no evidence of retention in any organ or tissues, including melanin-containing tissues.

Metabolism

In mouse, rat, rabbit and monkey hepatocytes in vitro, and in mice and rats in vivo, E4 is extensively metabolized by glucuronidation, sulfation, hydroxylation and methylation. Glucuronidation and sulfation are the only metabolic routes observed in humans, and the three major human metabolites are also produced by rats and mice. E4 has no human-specific metabolites. The two main human metabolites, E4-3-glucuronide and E4-16-glucuronide, show weak estrogenic activity, with potency several hundred-fold lower than E4.

Human cytochromes P450 (CYP450) do not appear to play a major role in the in vitro hepatic metabolism of E4 and E4 does not inhibit the major CYP450 isoforms. In vitro, uridine 5'-diphospho-glucuronosyltransferase 2B7 (UGT2B7) is the dominant UGT isoform that catalyzes formation of E4-16-glucuronide, and sulfotransferase 1E1 (SULT1E1) is the dominant SULT isoform that catalyzes formation of a direct sulfate (likely to be E4-3-sulfate).

Excretion

Excretion of E4 in mouse and rat is primarily fecal (67-87%), whereas excretion in humans is primarily urinary (69%) and to a lesser extent fecal (22%).

Pharmacokinetics of Combined E4 and DRSP

In female monkeys treated for 13-weeks with E4 and DRSP alone or in combination, E4 and DRSP co-administration caused a 46% and 42% increase in systemic exposure to E4 at Day 1 and Week 13, respectively; however, E4 co-administration had no impact on exposure to DRSP. After repeated co-administration of E4 and DRSP, the increase in systemic exposure was close to dose-proportional for E4 at doses between 3 and 30 mg/kg and less than dose-proportional for DRSP at doses between 0.6 and 6 mg/kg. For clinical comparison, when E4 was given as single or repeat doses (14-day) alone or in combination with DRSP in healthy female human volunteers, DRSP did not appear to have clinically relevant influence on the PK of E4.

General Toxicology

Repeat-Dose Toxicity Studies With E4 Alone in the Female Rat and Monkey

E4 was examined in chronic repeat-dose toxicology studies for up to 26-months in the female rat and 39-months in the female monkey and was observed to elicit the exaggerated pharmacological effects expected from an estrogen receptor agonist. E4 demonstrated an ability to stimulate mammary and uterine epithelium proliferation, inhibit estrous cyclicity, depress erythropoiesis, and cause cholestasis in both rats and monkeys. There were no toxicities in the rat and monkey that would be considered unexpected for an estrogenic compound. The safety margins for E4 at the NOAEL in the chronic rat and monkey studies were 3.3x and 2.1x the MRHD, respectively, on an AUC basis.

Repeat-Dose Toxicity Study With a Combination of E4 and DRSP in the Female Monkey

E4 given in combination with DRSP in the female cynomolgus monkey for 13-weeks produced treatment-related effects consistent with anticipated hormonal changes of a progestin/estrogen drug combination, specifically histological changes in the female genital tract and mammary glands and inhibitory estrous cycle effects. Adverse effects were considered a result of exaggerated pharmacological effects of supraphysiological concentrations of estrogens and progestins.

Hyperglycemia was observed in female monkeys co-administered E4 and DRSP at the highest dose examined (DRSP/E4 6/30 mg), which resulted in poor condition and early termination of 3 animals in this group, and a subsequent dose-reduction at day 50-53 of the dosing phase. Hyperglycemia was also noted in one mid-dose (MD) female at day 75. At the dose where severe hyperglycemia was observed in female monkeys leading to early termination, the exposure margins for DRSP and E4 were 13x and 97x the MRHD (based on Day 1 toxicokinetic data of DRSP/E4 co-administered at 6/30 mg/kg), respectively, on an AUC basis. NEXTSTELLIS was monitored clinically for associated effects on glucose metabolism and results did not indicate a notable change in glucose or insulin response in treated subjects. However, it should be noted that decreased glucose tolerance, peripheral insulin resistance, and hyperglycemia are known to be associated risks of CHCs.

The combination of DRSP/E4 did not produce any toxicities that were not an anticipated result of estrogen/progestin co-administration at supraphysiological levels, and the concurrent groups treated with E4 or DRSP alone were also representative of estrogenic or progestogenic responses, respectively. The safety margins at the NOAEL in the 13-week study for DRSP and E4 given in combination were 1.2x and 8.2x the MRHD, respectively, on an AUC basis.

Genetic Toxicology

Weight of evidence indicates that E4 is not considered genotoxic in a full battery of in vitro and in vivo assays (Ames assay, in vitro mouse lymphoma assay, an in vivo rat micronucleus assay, and in vivo rat Comet assay). A statement summarizing the nonclinical genotoxicity studies of E4 was proposed by the Division for the Nextstellis labeling that aligns with how mutagenesis information for estrogen components of COC drug products are described (see also the currently approved labeling for the estrogen component of YAZ®).

Carcinogenicity

E4 doses of up to 0.8 mg/kg/day were administered orally to female rats for 104 weeks. At the high-dose of 0.8 mg/kg/day E4 (0.7x the MRHD on an AUC basis), there was an increased incidence of mammary neoplasms (malignant adenocarcinoma and combined malignant adenocarcinoma arising in fibroadenoma, benign adenoma, and malignant adenocarcinoma) in female rats.

E4 doses of up to 1.0 mg/kg/day were administered orally to female mice for 104 weeks. E4 doses ≥0.25 mg/kg/day (≥0.2x the MRHD on an AUC basis), were carcinogenic in female mice,

with an increased incidence of mammary neoplasms (combined benign and malignant adenoacanthoma) at 0.25 and 1.0 mg/kg/day, uterine epithelial, stromal, and glandular neoplasms at ≥0.25 mg/kg, and pituitary gland neoplasms (adenoma and combined adenoma and carcinoma) at 1.0 mg/kg/day.

Long-term continuous administration of natural and synthetic estrogens in animal species, including the rat and mouse, are known to increase the frequency of neoplasms of the breast, uterus, cervix, and vagina. Therefore, E4 carcinogenic potential is aligned with other estrogenic class compounds and there are no unexpected neoplastic signals in the nonclinical carcinogenicity studies of E4.

Reproductive and Developmental Toxicity

Return to Fertility and Early Embryonic Development

In female rats treated for 1-month with E4 up to doses that inhibited estrous cycling (1x the MRHD based on body surface area (BSA) and 2.4x the MRHD based on AUC), there was estrus cycle recovery within one week of treatment cessation and a return to fertility after a 3-week recovery period. There were no E4-related effects on mating, fertility, hysterectomy observations, or on early embryonic development when female rats were mated 3-weeks after the last E4 exposure.

Additionally, in the chronic repeat-dose studies with E4 alone in the female rat and monkey, and in the 13-week repeat-dose study with an DRSP/E4 combination in the female monkey, estrous cycle inhibition was observed in all treated animals. Resumption of normal estrous cycles occurred 20 days into the recovery period in rats treated with E4 alone and 4-weeks after the last dose of combined E4 and DRSP at the highest dose examined in monkeys. In the 39-week monkey study, histopathology examination of the female genital tract indicated that E4-treated animals were beginning new estrous cycles after a 4-week recovery period, though recovery was incomplete at 4 weeks.

Embryo-Fetal Development

In the embryo-fetal development studies of E4 in the rat and rabbit, E4 caused maternal toxicity (reduced body weight and food consumption) and was embryotoxic at 3 mg/kg in the rat and at ≥0.15 mg/kg in the rabbit. E4 caused increased pre- and post-implantation losses and increased early uterine death in both species and abortion in rabbits.

In rats, fetal developmental effects were observed at 1 and 3 mg/kg/day, with increased incidence of skeletal malformations associated with the shortening, thickening and bending of the long bones (humerus, radius) and scapula, and external/visceral malformations associated with malrotated/severely flexed ankle joints. There was also a slight reduction in fetal weight at 3 mg/kg/day, which may be due to the reduced maternal body weight at this dose. The fetal NOAEL was determined to be 0.3 mg/kg/day, with multiples of exposure of <1x the MRHD based on both BSA and AUC.

In rabbits, fetal developmental effects included supernumerary ribs at ≥0.05 mg/kg/day and incomplete/non-ossification of phalanges, several bones, and vertebral centers at 0.15 mg/kg/day. The fetal NOAEL was determined to be 0.05 mg/kg/day, with multiples of exposure <1x the MRHD based on both BSA and AUC.

The observed fetal growth retardation and developmental effects were likely consequences of maternal toxicity in the pregnant female rat and rabbit. There are no indications for intrinsic teratogenic properties of E4 or DRSP in either rat or rabbit.

Pre- and Postnatal Development

In a pre- and post-natal development study in rats, E4 caused maternal toxicity (reduced body weight and food consumption), parturition difficulties or absence of delivery, and enhanced neonatal mortality of the F1-offspring during the first 4 days postpartum, with a NOAEL of 0.17 mg/kg/day (multiples of exposure <1x the MRHD based on both BSA and AUC). There were no adverse E4-related effects on F1 generation offspring outcome after day 4 postpartum through weaning or on F1 generation development, reproductive performance, or fertility, with a NOAEL of 1.5 mg/kg/day (multiples of exposure of approximately 1x the MRHD based on both BSA and AUC).

Reproductive and Developmental Toxicology Summary

NEXTSTELLIS will be used for contraception and pregnant women will not be prescribed this product. However, there is a potential for inadvertent exposure to this drug during pregnancy if the product fails and the woman does not know that she is pregnant. Collectively though, observations in the embryo-fetal and pre- and postnatal development studies with E4 were consistent with pharmacological estrogenic properties and were largely a result of maternal toxicity.

Given natural exposure of the human fetus to E4 during pregnancy and the results of epidemiologic studies and meta-analysis revealing little or no increased risk of birth defects in women who inadvertently use CHCs during early pregnancy, the low exposure multiples at the NOAELs of these studies are not considered a clinically relevant concern.

Drospirenone

No new pharmacology or toxicology studies were conducted to address the nonclinical safety or efficacy of DRSP alone for this 505(b)(2) submission and no additional nonclinical studies with DRSP were required or requested by the Division to support an NDA application, pending an adequate scientific bridge was established to the U.S. comparator YAZ® (listed drug chosen by the Applicant).

The scientific bridge between the DRSP component in NEXTSTELLIS and in YAZ® is provided by a comparative clinical bioavailability (BA) study (MIT-Es001-C112). In this study, the Applicant demonstrated that DRSP exposure following administration of single doses of NEXTSTELLIS (DRSP/E4 3/14.2 mg) was less than or comparable to the reference drug YAZ® (DRSP/EE

3/0.02 mg) in healthy female volunteers. Therefore, an adequate scientific bridge has been established to the Listed Drug YAZ®. It is appropriate for the Applicant to rely on the Agency's previous findings of safety of DRSP in YAZ® to support the nonclinical sections of the NDA, as reflected in the approved Listed Drug labeling. The submitted nonclinical literature for DRSP was considered scientifically supportive, but was not deemed necessary for approval or labeling. The nonclinical sections of the labeling for NEXTSTELLIS will align with the DRSP information as represented in the YAZ® approved drug product labeling.

Drospirenone is a spironolactone analogue with anti-mineralocorticoid activity. In vitro binding affinity and functional activity of DRSP for human steroid receptors have shown that DRSP has no biologically relevant androgenic, estrogenic, glucocorticoid, or anti-glucocorticoid activity.

The contraceptive action of DRSP is attributed to its suppression of gonadotropins.

The toxicity of DRSP has been well established in humans and nonclinical models. In nonclinical repeat-dose toxicity studies with DRSP, findings attributed to DRSP treatment included effects consistent with the pharmacological hormonal effect of progesterone receptor agonism (e.g., ovarian atrophy, uterine and vaginal changes, stimulatory effect on mammary gland development) and anti-mineralocorticoid effects (e.g., diuresis and electrolyte changes).

As described in the approved drug product label for YAZ®: "Mutagenesis studies for DRSP were conducted in vitro and in vivo and no evidence of mutagenic activity was observed."

As reflected for the DRSP component in the current Yasmin® and YAZ® approved drug product labeling: "In a 24-month oral carcinogenicity study in mice with doses up to 10 mg/kg/day DRSP, equating to 2 times the maximum clinical exposure (based on AUC), there was an increase in carcinomas of the harderian gland in the high dose DRSP group. In a similar study in rats given doses up to 10 mg/kg/day DRSP, 10 times the maximum clinical exposure (based on AUC), there was an increased incidence of benign and total (benign and malignant) adrenal gland pheochromocytomas in the high dose DRSP group."

The 13-week repeat-dose toxicity study of combined DRSP/E4 also contained a DRSP alone group for comparison. There were no unexpected toxicities in this study and expected progesterone-related changes were observed in the oviducts (atrophy), uterus (increase in endometrium thickness), and vagina (atrophy).

Nonclinical Integrated Summary

E4 was shown to be a selective estrogen receptor agonist in vitro that has estrogenic activity that is similar, but less potent than, other established estrogenic compounds such as EE and E2. E4 had the anticipated pharmacological effects of inhibiting ovulation and preventing implantation in animal models. The two major active metabolites of E4, E4-3-glucuronide and E4-16-glucuronide, have estrogenic potency several hundred-fold lower than E4. There were no off-target effects or signals of concern in in vitro binding studies or in studies examining E4-related effects on the cardiovascular, respiratory, or central nervous system.

The chronic toxicity studies of E4 alone in female rats and monkeys showed exaggerated pharmacological effects in line with what is expected from an unopposed estrogen at

supraphysiological levels in nonclinical species, including inhibition of estrous cycling, proliferation of mammary and uterine tissues, thymus lymphoid atrophy, adrenal gland hypertrophy, depressed erythropoiesis, and liver dysfunction, which showed full or partial reversibility after a 4- to 8-week recovery period. When E4 was co-administered with DRSP in female monkeys, adverse effects were also attributed to exaggerated pharmacological effects of a progestin/estrogen combination. Adverse effects observed in the nonclinical chronic studies, including cholestasis and hyperglycemia, are known to be associated risks of CHCs.

The findings from the carcinogenicity and reproductive and developmental toxicity studies with E4 alone were also consistent with estrogen class effects and did not indicate any findings that were unanticipated of use of an estrogenic compound.

Overall, the pharmacology and toxicology profile of E4 given alone or in combination with DRSP, a progestin, is consistent with that observed with other estrogenic compounds or CHC combination products and do not raise nonclinical safety concerns for any unexpected effects when used clinically in females of reproductive potential for the prevention of pregnancy.

Pharmacology/Toxicology Recommendation

In conclusion, there are no nonclinical concerns for the safety of this DRSP/E4 CHC product. Pharmacology/Toxicology supports the approval of NEXTSTELLIS for the indication of prevention of pregnancy in females under NDA 214154.

Table 3. Exposure Multiples at the Therapeutic Dose of E4 Based on Nonclinical Studies

Study Description (Study Number)	Daily Dose*	HED (mg/kg)	BSA (mg/m^2)	$AUC_{0\text{-last}}$ (ng·h/mL)	Multiples of Exposure at MRHD	
					BSA	AUC
General Toxicology						
26-week oral toxicity study in female rats (ES-T18)	1.5 mg/kg E4 NOAEL	0.24	9	195.70	1	3.3
39-week oral toxicity study in female cynomolgus monkeys (ES-T20)	1 mg/kg E4 NOAEL	0.32	12	122.56	1.4	2.1
13-week oral toxicity study in cynomolgus monkeys (0030-NC-002)	3/0.6 mg/kg DRSP/E4 NOAEL	0.2/1 (DRSP/E4)	7.2/36 (DRSP/E4)	629.6/482.6 (DRSP/E4)	4.1/4 (DRSP/E4)	1.2/8.2 (DRSP/E4)
Carcinogenicity						
104-week oral carcinogenicity study in female rats (0031-NC-003)	0.27 mg/kg E4; NOAEL	0.04	1.62	18.8	0.18	0.32
104-week oral carcinogenicity study in female mice (0031-NC-004)	0.125 mg/kg E4 NOAEL	0.01	0.375	4.6	0.04	0.08
Return to Fertility						
Oral return to female fertility and early embryonic development (dev.) to implantation (ES-T43)§	1.5 mg/kg E4; NOEL – Return to fertility /Early embryo dev.	0.24	9	142[b]	1	2.4
	0.5 mg/kg E4 Maternal NOAEL	0.08	3	42.8[b]	0.34	0.72
Reproductive and Developmental Toxicity						
Oral embryo-fetal development study in pregnant rats (ES-T03-PR3098)	0.3 mg/kg E4 Maternal/Fetal NOAEL	0.05	1.8	9.33[c]	0.21	0.16
Oral embryo-fetal development study in pregnant rabbits (ES-T19)	0.05 mg/kg E4 Maternal/Fetal NOAEL	0.02	0.6	6.84	0.07	0.12
Oral pre- and postnatal development study in rats (ES0001-NC-003)	0.17 mg/kg E4 Maternal/Fetal NOAEL[a]	0.03	1.02	13.3[d]	0.12	0.23
	1.5 mg/kg E4 F1 Dev./Behavior/Fertility NOAEL	0.24	9	59.8[d]	1	1.01

Estetrol monohydrate (E4) MRHD: 14.2 mg/day*; 0.24 mg/kg/day (based on 60 kg human body weight); BSA =8.76 mg/m^2; $AUC_{0\text{-}24}$=59.1 ng·h/mL
Drospirenone (DRSP) MRHD: 3 mg/day; 0.05 mg/kg/day (based on 60 kg human body weight); BSA =1.85 mg/m^2; $AUC_{0\text{-}24}$=519 ng·h/mL

* E4 doses are presented as nominal estetrol anhydrous doses. For the human dose, 15 mg/day E4 monohydrate is equivalent to 14.2 mg/day E4 anhydrous (i.e., correction factor of 1.06).

§ Female rats were treated for 4 weeks followed by a recovery period of 3 weeks, then followed by a treatment-free mating period of up to 4 weeks. Males were not treated.

[a] NOAEL based on parturition difficulties and neonatal survival only until Day 4 post-partum

[b] Exposure estimate was calculated based on extrapolation from data on Week 13 of a 13-week repeat-dose toxicity study in non-pregnant Wistar rats with E4 doses from 0.2 to 6 mg/kg/day (Study ES-T39 / PR3097).

[c] Exposure data on GD 17 from a dose-finding study in pregnant Wistar rats treated from GD 6-17 with 0.3 mg/kg/day (Study ES-T02 / PR3097).

[d] Estimated PPND study maternal AUC exposure; treatment administered to F0 females on GD 6-18 and lactation day 1-21 post-partum. Exposure estimate was calculated based on extrapolation from data on GD 17 of a dose-finding study in pregnant Wistar rats treated from GD 6-17 (Study ES-T02 / PR3097).

5.2. Referenced NDAs, BLAs, DMFs

NDA 21676: YAZ® (3 mg drospirenone and 0.02 mg ethinyl estradiol), 24/4 regimen; Bayer HealthCare Pharmaceuticals Inc.; Approval Date: March 16, 2006

DMF [redacted]; DMF Holder: [redacted]

DMF [redacted]; DMF Holder: [redacted]

DMF [redacted]; DMF Holder: [redacted]

DMF [redacted]; DMF Holder: [redacted]

5.3. Pharmacology

Primary pharmacology studies were only conducted by the applicant for E4, a novel estrogen. Drosperinone (DRSP) pharmacology information was provided through reference to the YAZ® approved drug product labeling (NDA 21676).

5.3.1. Primary Pharmacology of Estetrol Monohydrate

In Vitro Binding and Functional Activity Assays (Study [redacted] Preclinical Studies of Estetrol, PR3019, PR3046, ES0001-NC-014, Non-GLP)

Estetrol (E4) selectively binds human estrogen receptors ERα (half maximal inhibitory concentration; IC_{50}=17 to 66nM) and ERβ (IC_{50}=91 to 420nM), with an approximate 5-fold preference for ERα in in vitro binding assays. In vitro functional assays indicated that E4 has estrogenic activity and no anti-estrogenic activity.

When compared to other estrogens, E4 has approximately 60 times lower in vitro binding affinity for ERα and ERβ than E2 (17β-estradiol) and has lower transactivating activity at these receptors as compared to E2 (>200 times lower potency for both ERα and ERβ) and EE (ethinyl estradiol) (~700 and 48 times less potent at ERα and ERβ, respectively).

E4 metabolites E4-3-glucuronide and E4-16-glucuronide showed weak estrogenic activity on both ERα and ERβ, with E4-3-glucuronide being less potent than E4-16-glucuronide. The potency of these metabolites was several hundred-fold lower than the potency of E4.

In Vivo Studies Related to the Mechanism of Action of E4

The applicant conducted studies in female rats and rabbits to examine the in vivo estrogenic effects of E4 in relationship to the positive controls EE and E2.

Estrogenic Effects of E4 on the Cornification on the Vaginal Epithelium in Ovariectomized Female Rats (Study PR3014, Non-GLP)

Female ovariectomized Sprague-Dawley rats (n=8/group) were treated with E4 orally (p.o.) or by subcutaneous injection (s.c.) for 7 days at dose levels of 0.1, 0.3, 1 or 3 mg/kg/day. Positive controls included rats treated orally with EE or subcutaneous treatment with E2 at a dose level of 0.05 mg/kg/day. Vaginal smears showed that the onset of vaginal epithelial cornification following oral treatment with E4 was dose-dependent. Vaginal cornification was noted as early as Day 2 at 0.3 (1/8 rats), 1 (4/8 rats) and 3 (6/8 rats) mg/kg/day in E4-treated animals and by Day 7, a vaginal estrogenic response was seen in all E4-treated rats at all dose levels. All rats treated with EE displayed a vaginal estrogenic response by Day 3. Both E4 (at 1 and 3 mg/kg) and EE suppressed terminal body weight and increased uterus weight. The estrogenic potency of E4 was estimated to be 20 times lower than the potency of EE.

Ovulation Inhibitory Effects of E4 in Cycling Sprague-Dawley Rats (Study PR3015 and PR3026, Non-GLP)

Female cycling Sprague-Dawley rats (n=8/dose level) were treated by oral gavage over the 4-day period of the estrus cycle, starting on the day of estrus. Doses of E4 included a range between 0.03 to 3 mg/kg twice daily (BID) or 2 mg/kg once daily (QD). Positive controls included doses of 0.0003 to 0.03 mg/kg BID EE and 0.03 to 3 mg/kg BID E2 or 2 mg/kg QD E2. The number of ova in the oviduct was determined on Day 5 (i.e., one day after the final dose). Animals receiving twice daily E4, EE or E2 showed a dose-related decrease of ovulatory activity. E4 inhibited ovulation with a calculated oral ED_{50} of approximately 0.2 mg/kg BID. EE inhibited ovulation with a calculated oral ED_{50} of 0.01 mg/kg BID. The oral ED_{50} for E2 could not be calculated but was between 0.03 and 0.1 mg/kg BID. At 2 mg/kg QD, E4 blocked ovulation in 4/8 rats, whereas the same dose of E2 blocked ovulation in all animals. E4 displayed an anti-ovulatory potency approximately 20 times lower than that of EE and exhibited lower anti-ovulatory activity than E2.

Effects of E4 on Ovulation and Implantation in Female Rabbits (Study ES-T04, Non-GLP)

Female New Zealand White rabbits (n=3/group) were treated orally with E4 at dose levels of 0 (vehicle control), 0.01, 0.03, 0.1, 0.3 and 1 mg/kg BID from 4 days prior to mating until gestation day 9 inclusive (14 days total treatment). The number of corpora lutea and the number of implants were determined. In rabbits given 1.0 mg/kg BID, one animal ovulated with only 3 corpora lutea noted. Implantation was completely blocked at E4 dose levels of 0.1 mg/kg BID and higher. Ovulation inhibition was observed with an oral ED_{50} of 0.735 mg/kg BID, along with complete block of implantation at dose levels ≥0.1 mg/kg BID.

5.3.2. Secondary Pharmacology of Estetrol Monohydrate

In Vitro Binding Studies With E4 for Potential Off-Target Activities (Study PR3019, Non-GLP)

E4 was evaluated for potential off-target activities in radioligand binding assays with approximately 130 evaluated targets, including neurotransmitter receptors and transporters, neuropeptide receptors, hormone receptors, leukotriene/prostaglandin receptors, cytokine receptors and ion channels. At a concentration of 10 µM (approximately 170 times higher than the C_{max} of E4 at the MRHD), E4 did not display significant inhibition of radioligand binding to any of the targets evaluated with the exception of its primary targets ERα and ERβ. E4 did not bind to the progesterone, androgen or glucocorticoid receptors.

Effects of E4 on Human Breast Cells (Study PR3032, 3101, PR3028, PR3049, PR3066, Non-GLP; Gérard et al., 2015b)

E4 enhanced proliferation of normal human breast cells and MCF-7 breast cancer cells and induced greater migration and invasion of T47-D breast cancer cells. E4 also promoted tumor growth in ovariectomized immunodeficient mice subcutaneously implanted with human MCF-7 breast cancer cells. The potency of E4 towards normal breast cell proliferation in vitro was 1% of the potency of estradiol (E2). However, because of its inhibition of the ERα-dependent extra-nuclear signaling, E4 antagonized the effects of E2 on breast cancer cell proliferation, migration and invasion. Additionally, E4 inhibited tumor development in the DMBA-rat mammary tumor model to an extent similar to ovariectomy and at a dose level similar to the antiestrogen tamoxifen. EE at 20-fold lower (i.e., equipotent) doses had no effect. At high doses, E4 also caused regression of existing tumors in this model. Overall, E4 displays a lower potency for stimulation of breast cancer cell proliferation, migration and invasion than E2, and there is evidence of antagonistic effects compared to E2 both in vitro and in vivo.

Effects of E4 on Markers of Coagulation and the Vascular System (Study PR3060, PR3093, PR3102, Non-GLP; Abot et al., 2014, Hammond et al., 2008, Montt-Guevara et al., 2017)

E4 does not induce or bind to sex hormone binding globulin (SHBG). E4 causes a dose-dependent increase in the relative protein expression of tissue plasminogen activator (tPA), urokinase-type plasminogen activator (uPA) and tissue plasminogen activator inhibitor 1 (PAI-1) in human umbilical vein endothelial cells (HUVEC), but with a potency lower than E2.

E4 induced a rapid nitric oxide (NO) release, endothelial nitric oxide synthase (eNOS) activation and eNOS expression in HUVEC cells and causes vasorelaxation of rat arteries with a lower potency than estradiol. E4 did not cause eNOS activation or NO production in mouse aorta in vitro. E4 had no effect on endothelial healing in ovariectomized C57BL/6J mice following carotid artery electrical injury.

Overall, the data suggest that E4 has some vasoregulation and coagulation effects, but with a lower potency than the endogenous estrogens (e.g., estradiol).

5.3.3. Safety Pharmacology of Estetrol Monohydrate

Cardiovascular System

Effects of Estetrol on Cardiac Ion Channel (Study ES-T30; GLP)

In the in vitro hERG assay, the hERG channel was expressed in HEK-293 cells and E4 was tested at a maximum concentration of 28.17µM (n=4 cells originating from 4 different culture dishes). E4 decreased the hERG tail current amplitude by 7%, a value that is not considered clinically relevant, at a concentration of 28.17 µM (approximately 480 times higher than the C_{max} of E4 at the MRHD). By comparison, E-4031 (selective inhibitor of hERG potassium channel positive control) decreased the hERG tail current amplitude by 84% at 0.1 µM. Therefore, E4 does not have a toxic effect on the hERG channel.

Cardiovascular Effects of Estetrol in Cynomolgus Monkeys (Study ES-T23; GLP)

Female cynomolgus monkeys (n=6 monkeys; implanted with telemetry devices) were administered single doses of 0 (vehicle: 0.5% carboxymethylcellulose in water), 1, 10, and 100 mg/kg E4 by oral gavage according to a randomized crossover order, with a wash-out period of at least 1 week between two consecutive treatments. Cardiovascular and electrocardiographic (ECG) parameters were monitored before dosing and for 24 h after treatment. E4 had no effect on the cardiovascular function or cardiac electrophysiology in monkeys up to 100 mg/kg. The NOAEL in this study was 100 mg/kg, which provides an exposure margin at the MRHD (15 mg/day) of 115x and 127.5x based on AUC and C_{max}, respectively.

Central Nervous System (CNS)

CNS Safety Pharmacology in Female Rats (Study ES-T25; GLP)

Female Sprague-Dawley rats (n=8 rats/group) were administered single doses of 0 (vehicle: 0.5% carboxymethylcellulose in water), 1.5, 15, and 150 mg/kg E4 by oral gavage and were examined in a functional observation battery (FOB) for up to 4 hours after dosing.

There was no difference in the responses between E4 and vehicle, except for a lower rectal temperature 1 and 2 hours after dosing at 150 mg/kg. The NOAEL was 15 mg/kg, which provides an exposure margin at the MRHD (15 mg/day) of 33x and 58x based on AUC and C_{max}, respectively.

Respiratory System

Pulmonary Safety Pharmacology in Female Rats (Study ES-T24; GLP)

Female Sprague-Dawley rats (n=8 rats/group) were administered single doses of 0 (vehicle: 0.5% carboxymethylcellulose in water), 1.5, 15, and 150 mg/kg E4 by oral gavage (vehicle: 0.5% carboxymethylcellulose in water) and were examined for respiratory parameters by whole body plethysmography for up to 6 hours after dosing. E4 had no effect on the respiratory parameters

at any of the dose-levels tested. The NOAEL in this study was 150 mg/kg, which provides an exposure margin at the MRHD (15 mg/day) of 829x and 804x based on AUC and C_{max}, respectively.

5.3.4. Pharmacology of Drospirenone

No new nonclinical pharmacology studies were conducted with DRSP for this 505(b)(2) submission. The nonclinical pharmacology sections of the labeling for NEXTSTELLIS will align with the DRSP information as represented in the YAZ approved drug product labeling.

DRSP is a progestogen derived from spironolactone with anti-mineralocorticoid activity, but with no biologically relevant androgenic, estrogenic, glucocorticoid, or anti-glucocorticoid activity.

The contraceptive action of DRSP is attributed to its suppression of gonadotropins. DRSP has been shown to have progestogenic (e.g., endometrial transformation, maintenance of pregnancy in ovariectomized rodents, inhibition of ovulation), anti-mineralocorticoid (e.g., increased renal natriuresis via antagonism of aldosterone, decreased blood pressure), and antiandrogenic (e.g., reduced seminal vesicle and prostate size in castrated juvenile rats, feminization of male rat fetuses) activities in animal models.

No new safety pharmacology studies were conducted with DRSP alone. However, in the 13-week oral repeat-dose toxicity study with DRSP and E4 in cynomolgus monkeys, there were no noteworthy clinical signs suggestive of an effect on the central nervous system, cardiovascular, or respiratory system in the group treated with DRSP alone at a dose of 6 (4) mg/kg/day (Study 0030-NC-002, GLP). The systemic exposure at the highest DRSP dose of 6 (4) mg/kg/day provided exposure margins at the MRHD of DRSP of approximately 12x and 15.6x based on AUC and C_{max} after a single dose on Day 1 of treatment, respectively, and 6.1x and 8.2x based on AUC and C_{max} at Week 13, respectively.

5.4. ADME/PK

5.4.1. Pharmacokinetics of Estetrol Monohydrate

Table 4. ADME/PK of Estetrol Monohydrate

Type of Study	Major Findings
Absorption	
[^{14}C]-Estetrol: Absorption, distribution, metabolism and excretion studies following single oral dose administration to female rats (Study 0031-NC-022, non-GLP)	Rat (Females, Sprague Dawley) Dose: 15 mg/kg (3.7 MBq/kg) [^{14}C]-E4 T_{max}: 0.5 h C_{max}: Blood =1.392 µg equivalents/g; plasma =1.561 µg equivalents/g In the female rat, maximal plasma concentrations were obtained at 0.5 h post-dose. Concentrations declined rapidly over the subsequent 24 h.
[^{14}C]-Estetrol: Absorption, distribution, metabolism and excretion studies following single oral dose administration to female mice (Study 0031-NC-023, non-GLP)	Mice (Females, CD-1) Dose: 15 mg/kg (3.7 MBq/kg) [^{14}C]-E4 T_{max}: 0.5 h C_{max}: Blood =2.516 µg equivalents/g; plasma =3.259 µg equivalents/g In the female mouse, maximal plasma concentrations were obtained at 0.5 h post-dose. Concentrations declined rapidly over the subsequent 24 h.
A study to determine the bioavailability of steroid compound Estetrol after subcutaneous and oral administration in female Sprague Dawley rats [single-dose pharmacokinetics] (Study PR3002, non-GLP)	Rat (Females, Sprague Dawley, n=3/group) Dose: Single doses of 0.05, 0.5, 5 mg/kg E4, given p.o. or s.c.

	Dose (mg/kg)		
Parameters	**0.05**	**0.5**	**5**
Oral (p.o.)			
$AUC_{0\text{-last}}$ (ng·h/mL)	33.9	230	1090
C_{max} (ng/mL)	14.6	52.0	204
T_{max} (h)	0.5	0.5	0.5
$T_{1/2}$ (h)	ND	ND	6.4
Subcutaneous (s.c.)			
$AUC_{0\text{-last}}$ (ng·h/mL)	42.2	171	2090
C_{max} (ng/mL)	21.3	86.9	600
T_{max} (h)	0.5	0.5	1.0
$T_{1/2}$ (h)	ND	3.7	2.7

ND; not determined

Oral Bioavailability: Approximately 70% as compared to E4 given by the subcutaneous route of administration.

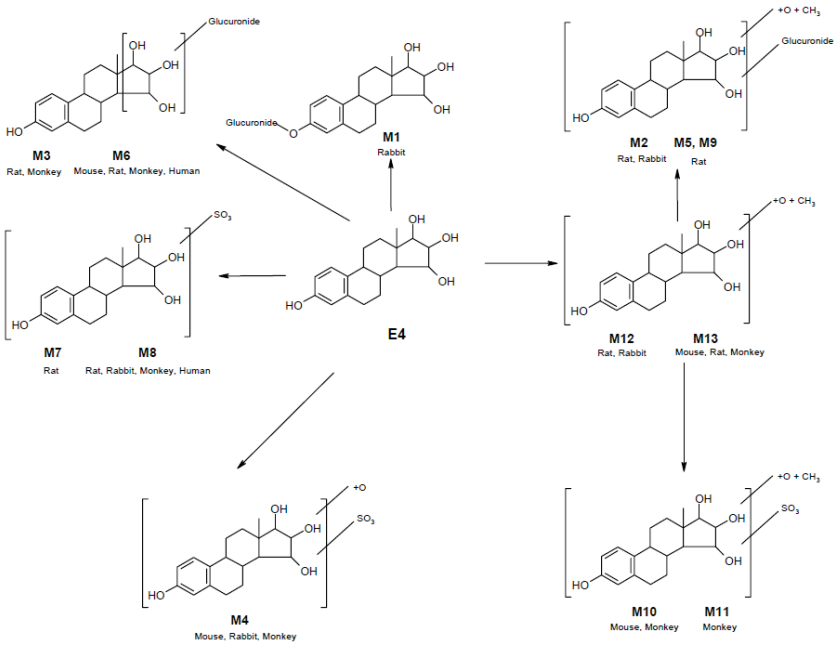
E4 Maximum tolerated dose (MTD) in female cynomolgus monkeys [single-dose pharmacokinetics] (Study ES-T05, GLP)

Monkey (Females, Cynomolgus, n=4)
Dose: Single oral ascending doses of 1, 100, 1000 mg/kg E4

	Dose (mg/kg)		
Parameters	**1**	**100**	**1000**
$AUC_{0\text{-last}}$ (ng·h/mL)	69.3	6816	93303
C_{max} (ng/mL)	30.5	2282	13564
T_{max} (h)	0.5	1.25	4.5
$T_{1/2}$ (h)	9.6	7.4	7.3

MTD; Single-dose in Monkey: 1000 mg/kg

Type of Study	Major Findings
Distribution	
In vitro binding of estetrol to plasma proteins by equilibrium dialysis (Study ES-T33, non-GLP)	E4 displayed moderate protein binding (45-67%) in female mouse (CD-1), rat (Sprague Dawley), monkey (cynomolgus) and human plasma in vitro. There was no indication of species-related differences or concentration dependence up to 1000 ng/mL in animal plasma and up to 50 ng/mL in human plasma.
Estetrol: In vitro plasma/blood cell partitioning in human (Study 0031-104, GLP)	Whole pooled human blood samples (n=3 healthy female volunteers) were spiked with 0.1 or 1 µM [^{14}C]-E4 for up to 120 minutes. In vitro, the blood:plasma ratio of E4 at 0.1 and 1 µM at 120 minutes in human blood was 1.21 and 1.18, respectively. The distribution was not time or concentration dependent.
[^{14}C]-Estetrol: Absorption, distribution, metabolism and excretion studies following single oral dose administration to female rats (Study 0031-NC-022, non-GLP)	Rat Female, Sprague Dawley (non-pigmented, n=7) measured up to 48 h post-dose Lister Hooded (partially pigmented, n=6) measured up to 35 days post-dose Dose: Single dose of 15 mg/kg (3.7 MBq/kg) [^{14}C]-E4 Plasma/blood cell partitioning: At the time of maximal plasma concentration (T_{max} =0.5 h), the amount of radioactivity in the blood was 1.392 µg equivalents/g and 1.561 µg equivalents/g in plasma, indicating a blood:plasma ratio of 0.77 in the rat. Tissue Distribution: [^{14}C]-E4-related radioactivity displayed extensive and rapid tissue distribution in both non-pigmented female rats and in partially pigmented female rats, with maximal radioactivity concentrations at the first time point of 0.5 h post-dose. In non-pigmented rats, tissue levels of radioactivity were below the limit of quantification in all tissues, except liver, thyroid gland, and routes of excretion (i.e., small and large intestine contents and mucosa and bladder contents) at 48 h post-dose (last timepoint examined). The highest radioactivity concentration was found in the liver at all time points examined up to 48 hours post-dose in non-pigmented rats (last time point) and 7 days post-dose in partially pigmented rats. Radioactivity in light-exposed tissues (e.g., skin, eye) was eliminated rapidly and below the limit of quantification within 24 hours in nonpigmented rats and by 7 days in partially pigmented rats. There was no indication for prolonged retention in melanin-containing tissues.
Placental Transfer: Tulchinsky et al., 1975; Coelingh Bennink et al., 2008	Studies on placental transfer of E4 and/or its metabolites have not been performed. Since E4 is produced by the human fetal liver, the fetus is exposed to E4 at physiological levels during pregnancy in the absence of treatment. Transfer of E4 from fetal plasma to maternal plasma occurs during human pregnancy and reaches a maximum at the end of pregnancy (approximately 1 ng/mL in maternal plasma) (Tulchinsky et al., 1975; Coelingh Bennink et al, 2008). Fetal plasma levels have been reported to be over 10 times higher than maternal plasma levels at parturition (Coelingh Bennink et al, 2008).
Metabolism	
Visser et al., 2008	In humans, E4 is a terminal end-product of estrogen metabolism and not converted back into active metabolites like estriol (E3), estradiol (E2) or estrone (E1) in vivo.

Type of Study	Major Findings
E4: Metabolism in cryopreserved hepatocytes from mouse, rat, rabbit, monkey and human (Study ES-T06, non-GLP)	In vitro metabolism study in cryopreserved hepatocytes of 10µM E4 in mice (female, CD-1), rats (female, Sprague Dawley), rabbit (female, New Zealand White), monkey (female, cynomolgus), and 1µM E4 in human (mixed male/female, pool of 10 donors). E4 was metabolized extensively in all species, with unchanged E4 parent compound representing 0%, 10.5%, 3.6%, 0.65% and 4.8% of total peak area after 4 hours of incubation with mouse, rat, rabbit, monkey and human hepatocytes, respectively. E4 was mainly metabolized by phase II metabolic pathways of glucuronidation and sulfation. Only two metabolites were formed by human hepatocytes: M6 (direct glucuronidation of E4; 45% of the total peak area at 4h) and M8 (direct sulfation of E4; approximately 51% of the total peak area at 4 h). These metabolites were also observed after the 4-h incubation with rat (11% of total peak area for both M6 and M8) and monkey (56% and 10% of total peak area for M6 and M8, respectively) hepatocytes. There were no unique E4 metabolites in humans after a 4-h in vitro incubation of E4 in hepatocytes. 

Type of Study	Major Findings
Estetrol: Metabolite comparison between animal and human, and confirmation of glucuronide conjugate position in two major human metabolites (Study Es0001-NC-012, GLP)	Species: Human (healthy females of non-childbearing potential; n=6), Rat (Female, Sprague Dawley; n=3 group plasma/excretion), Mouse (Female CD-1; n=4 group plasma/excretion) Dose (p.o.): Human; 15 mg (2.8 MBq) [^{14}C]-E4; single dose; Rat/Mouse; 15 mg/kg (3.7 MBq/kg) [^{14}C]-E4; single dose In vivo, there were no unique human metabolites. There were 3 metabolites identified as major human metabolites (i.e., >10% of the total drug exposure): E4-3-glucuronide (M1 below), E4-16-glucuronide (M2 below), and an E4-glucuronide-sulfate conjugate, with the C16-position and the C3-position considered the most likely locations for the glucuronide and sulfate substituents, respectively (M3 below). These metabolites were also identified either in the rat and mouse plasma (M1) or in the rat urine and/or bile (M2 and M3).

% Sample Radioactivity in Compound

	Sample (Time [h])									
	Plasma			Urine			Bile	Feces		
Parameter	Human 0-2	Mouse 0.5	Rat 0.5	Human 0-6	Mouse 12-48	Rat 0-24	Rat 0-12	Human 48-72	Mouse 0-48	Rat 0-48
Conc*	231	2549	1595	NA	NA	NA	NA	NA	NA	NA
% of dose	NA	NA	NA	21.74	4.01	9.92	79.17	7.57	74.29	1.62

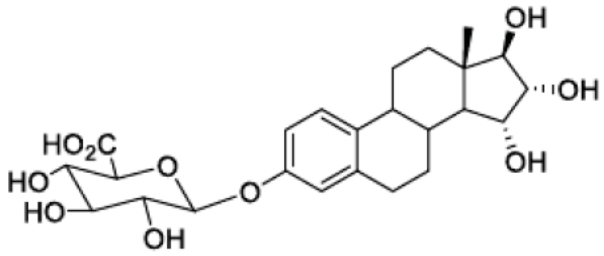
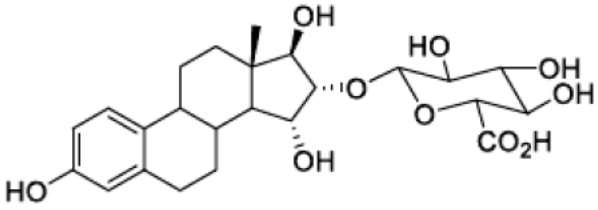
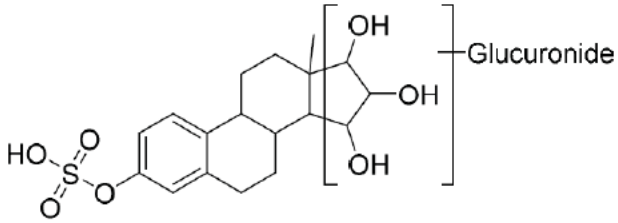
* Concentration in ng·eq/mL

% Sample Radioactivity for Each Compound

	Sample (Time [h])									
	Plasma			Urine			Bile	Feces		
Parameter	Human 0-2	Mouse 0.5	Rat 0.5	Human 0-6	Mouse 12-48	Rat 0-24	Rat 0-12	Human 48-72	Mouse 0-48	Rat 0-48
M1*	15.3	19.9	19.4	16.3	10.0	–	23.7	–	–	–
M2**	61.3	–	–	77.7	–	14.9	16.4	–	–	–
M3†	11.0	–	–	2.1	–	–	15.2	–	–	–
E4	6.9	29.4	49.7	–	13.4	35.9	–	94.6	5.0	11.0
UA	5.5	39.2	24.2	1.0	61.7	33.8	32.2	–	73.7	76.1
Total	100.0	92.6	93.4	97.1	88.6	91.4	89.4	94.6	78.8	87.1

*, **, †See metabolite identity and structure below

Abbreviations: E4, estetrol; UA, unassigned; –, not observed

Type of Study	Major Findings
	Major human metabolites:  E4-3-glucuronide*  E4-16-glucuronide**  E4-glucuronide-sulfate conjugate†
Identification of Drug Metabolizing Enzymes	
Estetrol: Identification of the major drug metabolizing enzymes (CYP450) involved in human hepatic metabolism in vitro (CYP450 Reaction Phenotyping) (Study 0031-102, GLP)	[^{14}C]-E4 (5 µM) was incubated with pooled human liver microsomes (n=150 mixed gender; 0.5 mg protein/mL) for 15 minutes in the presence of NADPH (1mM). [^{14}C]-E4 (5 µM) was also incubated with recombinant cDNA expressed CYPs 1A2, 2B6, 2C8, 2C9, 2C19, 2D6 and 3A4 (125 pmol/mL) in the presence and absence of NADPH (1mM) for 15 minutes. [^{14}C]-E4 showed no evidence of oxidative metabolism when incubated with pooled human liver microsomes or with recombinant human CYP450 enzymes 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, or 3A4.
In vitro study to determine the possible formation of catechol derivatives from estetrol using human recombinant CYP1A1, CYP1B1, and CYP3A4 cDNA (Study PR3100, GLP)	E4 at a concentration of 100µM was incubated with human recombinant CYP1A1, 1B1 and 3A4 supersomes for 30 minutes. After 30 minutes, E4 was metabolized by 10%, 16%, and 2% in the supersomes containing human recombinant CYP1A1, 1B1 and 3A4 cDNA, respectively. Only small amounts of two hydroxylated E4 metabolites were formed by CYP1A1 and CYP1B1.

Type of Study	Major Findings
Estetrol: Identification of the major UGT drug metabolizing enzymes involved in human hepatic metabolism in vitro (UGT Reaction Phenotyping) (Study 0031-106, GLP)	[^{14}C]-E4 (3, 5, and 10 µM) with pooled human liver microsomes (0.1 to 1 mg/mL protein concentrations) for up to 60 minutes in the presence of uridine diphosphoglucuronic acid (UDPGA, 2mM). At all concentrations of [^{14}C]-E4 and microsomal protein used, E4 was metabolized to a single metabolite. This metabolite was likely to be a direct glucuronide of parent E4 as NADPH was not present in the incubations (and hence no CYP-mediated metabolism producing phase I metabolites could occur). Further experiments were conducted with recombinantly expressed UGT enzymes (rUGT). Only UGT2B7, which is primarily present in the liver, catalyzed the formation of the single glucuronide metabolite in vitro. UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B10, 2B15 and 2B17 did not metabolize E4.
Estetrol: Identification of the location of the glucuronide substituent in the glucuronide-estetrol formed after incubation of estetrol with UDP-Glucuronosyltransferase 2B7 (GDP2B7) (Study Es0001-NC-011, non-GLP)	[^{14}C]-E4 at a concentration of 10 µM was incubated with microsomes from cells expressing UGT2B7, with enzyme cofactor UDPGA, for up to 60 min. Incubations of [^{14}C]-E4 with human UGT2B7 produced a single component in the presence of cofactor UDPGA, representing 31.09% of radioactivity after a 60-min incubation. This component was identified as E4-16-glucuronide metabolite, indicating that UGT2B7 is responsible for this biotransformation in vivo.
Sulfation of estetrol by human cytosolic sulfotransferases (Study PR3099, non-GLP)	Seven recombinant human sulfotransferases (rhSULTs) 1A1, 1A3, 1B1, 1E1, 1C2, and 2A1 were expressed in their native forms in E. coli and used in SULT activity assays with E4 concentrations up to 5 µM. E4 undergoes sulfation, mainly by SULT1E1 which showed the lowest K_m (0.54 µM) and highest V_{max} (41 nmol/min/mg) among seven rhSULTs and is likely to be involved in direct E4 sulfation and formation of the E4-3-sulfate metabolite.
Induction and Inhibition of Drug Metabolizing Enzymes	
Estetrol: Assessment of the potential to induce human hepatic cytochrome P450 (CYP) enzymes using human hepatocytes in culture (Study 0031-100, GLP)	E4 concentrations up to 25 µM were used to assess the effects on CYP1A2, 2B6 and 3A4 mRNA expression in cryopreserved human hepatocytes (n=3 individual donors) after up to 72 h. There was no evidence for cytotoxic effects of E4 on human hepatocytes in these experiments. E4 (up to 25 µM for 72 h) did not substantially induce CYP450 enzymes 1A2, 2B6 or 3A4 in human hepatocytes.
Estetrol: Assessment of the potential to inhibit human hepatic CYP450 enzymes in vitro (Study 0031-101, GLP)	The effect of E4 concentrations up to 100 µM (30 µM for 3A4 with testosterone as substrate) on the activities of CYP1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1 and 3A4 was investigated in pooled human liver microsomes (n=150 mixed gender donors). E4 concentrations up to 100 µM were not inhibitory towards any cytochrome P450 enzymes investigated in this study except CYP 3A4. However, the low inhibitory properties of E4 towards CYP3A4 (i.e., IC_{50} of 38.7 µM with testosterone substrate / IC_{50} of >100 µM with midazolam substrate) indicate that clinically relevant systemic drug interactions caused by E4-mediated inhibition of CYP3A4 are unlikely.

Type of Study	Major Findings
Estetrol: Assessment of the potential to inhibit UGT enzymes in vitro (Study 0031-105, GLP)	The effect of E4 concentrations up to 100 µM on the activities of UGT1A1, 1A3, 1A4, 1A6, 1A7, 1A8, 1A9, 1A10, 2B4, 2B7, 2B10, 2B15 and 2B17 in pooled human liver microsomes (n=150 mixed gender donors) and/or cDNA expressed recombinant human UGT (rUGT) enzymes was investigated. E4 inhibits both UGT1A9 and UGT2B7, with IC_{50}s of 95.5 µM and 89.4 µM, respectively in human liver microsomes and IC_{50}s of 25.8 µM and 38.0 µM, respectively, in recombinant cDNA expressed enzymes. E4 was determined to have low inhibitory properties against UGT1A9 and UGT2B7 enzymes.
Drug-Drug Interaction Potential	
Bidirectional transport in human Caco-2 cells: Study of the solubility and the in vitro permeability of Estetrol (0031-NC-017, non-GLP)	Bidirectional transport studies of E4 concentrations up to 200µM using Caco-2 cells indicated that E4 is a medium to high permeability compound and a substrate for an efflux transporter.
Interaction with human efflux ABC transporters: Estetrol: Assessment of efflux-mediated transport (Study 0031-103, GLP) Estetrol: P-Glycoprotein (P-gp) Substrate Interactions (Study Es001-NC-013, GLP)	E4 at concentrations up to 30µM (Study 0031-103) or 190µM (Study Es001-NC-013) was evaluated for its potential interaction with human efflux ABC transporters P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP) as a substrate or inhibitor in bidirectional transport studies in MDCKII cells expressing P-gp or BCRP. E4 acts as a potential substrate for both efflux ABC transporters P-gp and BCRP. The net efflux ratio for E4 in MDCKII cells expressing P-gp or BCRP was >2 and was inhibited by the respective probe inhibitors verapamil and K0143. E4 did not inhibit these transporters. P-gp and BCRP are not expected to impact the oral bioavailability of highly permeable and highly soluble drugs. The intestinal absorption of E4, as a medium to highly permeable drug, is not expected to be significantly affected by BCRP-mediated efflux. Therefore, blocking BCRP or P-gp is unlikely to affect E4 PK.
Estetrol: Assessment of uptake transporter interactions (Study 0031-107, GLP)	E4 concentrations up to 30 µM were assessed as a potential inhibitor of OAT1, OAT3, OATP1B1, OATP1B3, OCT2, MATE1 and MATE2-K in incubations with HEK293 cells transiently transfected with one SLC transporter and wild-type (WT) controls. Uptake or efflux SLC transporters OATP1B1, OATP1B3, OAT1, OCT2, MATE1 and MATE2-K were not inhibited by E4 and these transporters are not involved in the cellular uptake/efflux of E4. E4 was found to be a potential inhibitor of OAT3 with an IC_{50} of 16.5 µM, which is 280x greater than the clinical E4 C_{max} (0.058 µM) suggesting minimal potential for clinically relevant drug interactions at this transporter.

Type of Study | **Major Findings**

Excretion

[^{14}C]-Estetrol: Absorption, distribution, metabolism and excretion studies following single oral dose administration to female rats (Study 0031-NC-022, non-GLP)

Rat (Females, Sprague Dawley); up to 168 h post-dose (n=3) or 48 h post-dose (bile duct cannulated, n=3)

Dose: Single dose of 15 mg/kg (3.7 MBq/kg) [^{14}C]-E4

Recovery (% of Dose)

Sampling Time (h)	Urine	Feces	Carcass	Total
0-6	6.87	–	–	6.87
0-12	8.45	–	–	8.45
0-24	9.85	68.91	–	78.76
0-48	10.69	84.83	–	95.52
0-168	11.11	86.86	0.36	98.62*

* Includes: Air =0.15% and Cage Wash =0.14%; – = not observed

Bile duct cannulated:

Recovery (% of Dose)

Sampling Time (h)	Urine	Feces	Bile	Carcass	Total
0-3	8.12	BLD	44.71	–	52.83
0-6	13.32	BLD	59.84	–	73.16
0-12	17.52	0.29	68.53	–	86.34
0-24	18.42	2.72	71.93	3.61	97.25*

* Includes: Air = Cage Wash =0.56%; BLD = below limit of detection; – = not observed

[^{14}C]-Estetrol: Absorption, distribution, metabolism and excretion studies following single oral dose administration to female mice (Study 0031-NC-023, non-GLP)

Mouse (Females, CD-1) up to 168 h post-dose (n=9)

Dose: Single dose of 15 mg/kg (3.7 MBq/kg) [^{14}C]-E4

Recovery (% of Dose)

Sampling Time (h)	Urine	Feces	Carcass	Total
0-6	0.79	–	–	0.79
0-12	1.43	–	–	1.43
0-24	12.77	62.27	–	75.04
0-48	15.92	65.74	–	81.66
0-168	16.45	66.91	BLD	85.54*

* Includes: Air =0.80% and Cage Wash =1.38%; BLD = below limit of detection; – = not observed

Breast milk excretion

A breast milk excretion study with E4 has not been performed.

Toxicokinetics (TK)

TK Data From General Toxicology Studies

Mouse:

4-week toxicity study by the oral route (gavage) in mice (Study ES-T34, GLP)

Systemic Exposure in Female CD-1 Mice After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 30 mg/kg on Day 28

	Dose (mg/kg)			
Parameter	**0.03**	**0.3**	**3.0**	**30**
$AUC_{0\text{-last}}$ (ng·h/mL)	1.99	21.3	227	2004
C_{max} (ng/mL)	1.04	11.2	159	844
T_{max} (h)	0.5	0.5	0.5	0.5
$T_{1/2}$ (h)	2.68	1.59	3.28	2.21

Accumulation: No evidence of accumulation between Day 1 and Day 28

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 30 mg/kg in female mice

Type of Study

Mouse:

13-week preliminary study by the oral route (gavage) in female mice (Study ES-T40, GLP)

Major Findings

Systemic Exposure in Female CD-1 Mice After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 10 mg/kg on Week 13

	Dose (mg/kg)			
Parameter	**0.3**	**1.0**	**3.0**	**10**
$AUC_{0\text{-last}}$ (ng·h/mL)	21.6	87.2	292	872
C_{max} (ng/mL)	13.2	39.4	136	494
T_{max} (h)	0.5	0.5	0.5	0.5
$T_{1/2}$ (h)	1.59	2.88	3.03	2.68

Accumulation: No evidence of accumulation between Day 1 and Week 13

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 10 mg/kg in female mice.

Rat:

4-week toxicity study by the oral route (gavage) in rats followed by a 2-week treatment-free period (Study ES-T10, GLP)

Systemic Exposure in Female Sprague Dawley Rats After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 150 mg/kg on Day 29

	Dose (mg/kg)			
Parameter	**5**	**15**	**50**	**150**
$AUC_{0\text{-last}}$ (ng·h/mL)	592	1958	7179	40648
C_{max} (ng/mL)	167	1043	2481	9781
T_{max} (h)	0.5	0.5	0.5	0.5
$T_{1/2}$ (h)	2.14	2.59	3.06	2.32

Accumulation: No evidence of accumulation between Day 1 and Day 29.

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 150 mg/kg in female rats.

Rat:

13-week preliminary study by the oral route (gavage) in female Wistar rats (Study ES-T39, GLP)

Systemic Exposure in Female Wistar Rats After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 6 mg/kg on Day 1 and Week 13

Time Point	Dose (mg/kg)			
Parameter	**0.2**	**0.6**	**2**	**6**
Day 1				
$AUC_{0\text{-last}}$ (ng·h/mL)	26.3	89.0	267	1104
C_{max} (ng/mL)	10.2	41.2	98.4	454
T_{max} (h)	0.5	0.5	0.5	0.5
Week 13				
$AUC_{0\text{-last}}$ (ng·h/mL)	21.4	57.6	173	595
C_{max} (ng/mL)	9.22	25.1	87.8	331
T_{max} (h)	0.5	0.5	0.5	0.5

$T_{1/2}$: Approximately 1.8 h at Day 1 and 2.4 h at Week 13

Accumulation: No evidence of accumulation between Day 1 and Week 13. $AUC_{0\text{-last}}$ values were 19% to 46% lower in Week 13 than on Day 1.

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 6 mg/kg in female rats.

Type of Study

Rat:

26-Week toxicity study by the oral rout (gavage) in rats followed by an 8-week treatment-free period (Study ES-T18, GLP)

Major Findings

Systemic Exposure in Female Sprague Dawley Rats After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 15 mg/kg on Day 1, Week 13, and Week 26

Time Point	Dose (mg/kg)		
Parameter	**1.5**	**5**	**15**
Day 1			
$AUC_{0\text{-last}}$ (ng·h/mL)	139	553	1862
C_{max} (ng/mL)	77.4	290	1048
Week 13			
$AUC_{0\text{-last}}$ (ng·h/mL)	158	594	1958
C_{max} (ng/mL)	58.0	189	551
Week 26			
$AUC_{0\text{-last}}$ (ng·h/mL)	196	736	2376
C_{max} (ng/mL)	56.8	238	574

T_{max}: 0.5 h

$T_{1/2}$: Approximately 2.65 h at Week 13 and Week 26

Accumulation: No evidence of accumulation between Day 1 and Week 13. Slight increase (17% to 19%) in systemic exposure was noted between Week 13 and 26.

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 15 mg/kg in female rats. $AUC_{0\text{-last}}$ values were similar on Day 1 and in Week 13 and increased marginally by Week 26.

Monkey:

4-week toxicity study by the oral route (gavage) in adult female cynomolgus monkeys (Study ES-T11, GLP)

Systemic Exposure in Female Cynomolgus Monkeys After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 50 mg/kg on Day 1 and 28

Time Point	Dose (mg/kg)		
Parameter	**5**	**15**	**50**
Day 1			
$AUC_{0\text{-last}}$ (ng·h/mL)	492	1970	6157
C_{max} (ng/mL)	141	834	1250
Day 28			
$AUC_{0\text{-last}}$ (ng·h/mL)	1153	3640	9327
C_{max} (ng/mL)	348	1098	2021

T_{max}: 0.5 to 1.5 h

$T_{1/2}$: 10.8 h

Accumulation: There was slight accumulation between Day 1 and Day 28, with a 34% to 57% increase in AUC and 24% to 61% increase in C_{max} noted across doses.

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 15 mg/kg in female monkeys, and slightly less than dose proportional between 15 and 50 mg/kg. $AUC_{0\text{-last}}$ values after multiple doses were higher than those after the first dose.

Type of Study

Monkey:

13-week toxicity study by the oral route (gavage) in adult female cynomolgus monkeys followed by a 4-week treatment-free period (Study ES-T15, GLP)

Major Findings

Systemic Exposure in Female Cynomolgus Monkeys After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 30 mg/kg on Day 1 and Week 13

Time Point / Parameter	Dose (mg/kg) 3	10	30
Day 1			
$AUC_{0\text{-last}}$ (ng·h/mL)	260	1067	2805
C_{max} (ng/mL)	87.3	471	788
Week 13			
$AUC_{0\text{-last}}$ (ng·h/mL)	363	1215	3497
C_{max} (ng/mL)	122	357	871

T_{max}: 0.5 to 1.0 h

$T_{1/2}$: 10.4 to 18.7 h

Accumulation: There was slight systemic accumulation, with increase in AUC values of 12% to 28% across doses examined between Day 1 and Week 13.

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 50 mg/kg in female monkeys. $AUC_{0\text{-last}}$ values after multiple doses were slightly higher than those after the first dose.

Monkey:

13-week toxicity study by the oral route (gavage) in adult female cynomolgus monkeys followed by a 4-week treatment-free period (Estetrol and Drospirenone) (Study 0030-NC-002, GLP)

Systemic Exposure in Female Cynomolgus Monkeys After Multiple Doses of Combined E4 and DRSP, E4 Alone, or DRSP Alone Given Once Daily by Oral Gavage on Day 1 and Week 13

Parameter / Treatment	Dose (mg/kg) DRSP/E4 0.6/3	2/10	6/30; D1 4/20; W13	E4 30; D1 20; W13	DRSP 6; D1 4; W13
Day 1					
$AUC_{0\text{-last}}$ (ng·h/mL)					
E4	341	1430	5733	3108	–
DRSP	745	2490	6766	–	6186
C_{max} (ng/mL)					
E4	173	658	1965	884	–
DRSP	116	435	930	–	758
Week 13					
$AUC_{0\text{-last}}$ (ng·h/mL)					
E4	483	1608	3733	2181	–
DRSP	630	1867	2769	–	3150
C_{max} (ng/mL)					
E4	173	380	722	687	–
DRSP	105	205	338	–	399

D, day; W, week; **Note: DRSP/E4 HD, E4 and DRSP alone group had a dose reduction on Day 50-53 due to adverse hyperglycemia observed in the DRSP/E4 HD group

T_{max}: 0.5 to 1.0 h for E4 and 1 to 3 h for DRSP given in combination or alone.

$T_{1/2}$: varied between 8.6 to 21.9 h for E4 and 4.5 to 6.1 h DRSP and did not appear to be influenced by dose level or whether given in combination or alone.

Accumulation: No evidence of accumulation for E4 or DRSP.

Type of Study | **Major Findings**

Dose proportionality:

- For E4, increases in C_{max} were close to dose-proportional on Day 1 but less than dose-proportional in Week 13. Increases in $AUC_{0\text{-}last}$ were more than dose-proportional on Day 1 and close to dose-proportional in Week 13.
- For DRSP, increases in C_{max} were less than dose-proportional both on Day 1 and in Week 13. Increases in $AUC_{0\text{-}last}$ were close to dose-proportional on Day 1 and less than dose-proportional in Week 13.

Effects of coadministration of E4 and DRSP: DRSP co-administration caused an increase of E4 AUC exposure (approximately 46% and 42% at Day 1 and Week 13, respectively), while E4 co-administration had no impact on exposure to DRSP.

Monkey:

39-week toxicity study by the oral route (gavage) in adult female cynomolgus monkeys followed by a 6-week treatment-free period (Study ES-T20, GLP)

Systemic Exposure in Female Sprague Dawley Rats After Multiple Doses of E4 Given Once Daily by Oral Gavage Up to 10 mg/kg on Day 1, Week 13, Week 26, and Week 39

Time Point / Parameter	Dose (mg/kg) 1	3	10
Day 1			
$AUC_{0\text{-}last}$ (ng·h/mL)	120	340	1078
C_{max} (ng/mL)	31.7	110	365
Week 13			
$AUC_{0\text{-}last}$ (ng·h/mL)	163	402	1420
C_{max} (ng/mL)	30.4	89.5	433
Week 26			
$AUC_{0\text{-}last}$ (ng*h/mL)	109	312	1024
C_{max} (ng/mL)	19.8	84.9	245
Week 39			
$AUC_{0\text{-}last}$ (ng*h/mL)	123	328	1250
C_{max} (ng/mL)	33.0	97.2	452

T_{max}: 0.5 to 1.0 h

$T_{1/2}$: 11.7 to 16.9 h; Individual plasma-concentration-versus-time curves showed indications of a second peak around 8 to 16 h post-dose, suggesting potential enterohepatic circulation.

Accumulation: No evidence of accumulation between Day 1 and Week 39.

Dose proportionality: Exposure increased with dose in an approximately dose-proportional manner after multiple dosing up to 10 mg/kg in female monkeys.

TK Data from Reproductive and Developmental Toxicology Studies

Rat:

13-week preliminary study by the oral route (gavage) in female Wistar rats (Study ES-T39, GLP):
[Study used to generate data for TK parameters for Return to Fertility and Early Embryo Development in female rats]

Return to Fertility and Early Embryonic Development; In the pivotal study (ES-T43), female rats were treated for 4 weeks followed by a recovery period of 3 weeks, then followed by a treatment-free mating period of up to 4 weeks. Males were not treated. No concurrent TK was performed.

The exposure estimate for female rats was calculated based on extrapolation from data on Week 13 of a 13-week repeat-dose toxicity study in non-pregnant Wistar rats with E4 doses from 0.2 to 6 mg/kg/day (Study ES-T39; see in TK table above).

AUC: Maternal NOAEL (0.5 mg/kg) =42.8 ng*h/mL

Return to fertility/Early embryonic development NOEL (1.5 mg/kg) =142 ng·h/mL

Type of Study | **Major Findings**

Rat:

Estetrol (E4): Oral (Gavage) Range-Finding Study of Embryo-Foetal Development in the Rat (Study ES-T02, GLP) [Study used to generate data for TK parameters in pregnant female rats]

Study ES-T02: Exposure Data on GD17 After Multiple Doses of E4 in Pregnant Female Rats

	Dose (mg/kg)				
Parameter	**0.1**	**0.3**	**1**	**3**	**10**
$AUC_{0\text{-last}}$ (ng·h/mL)	4.58	9.33	37.7	139	350
C_{max} (ng/mL)	3.03	6.83	20.9	67.8	141
T_{max} (h)	0.5	0.5	0.5	0.5	1

Embryo-fetal development (EFD); No concurrent TK was performed in the pivotal study (ES-T03-PR3098). Exposure data on GD 17 from a dose-finding study in pregnant Wistar rats treated from GD 6-17 with 0.3 mg/kg/day (Study ES-T02).

AUC: Maternal/Fetal NOAEL (0.3 mg/kg) =9.33 ng·h/mL

Pre- and postnatal development (PPND); Estimated PPND study maternal AUC exposure; treatment administered to F0 females on GD 6-18 and lactation day 1-21 post-partum. Exposure estimate was calculated based on extrapolation from data on GD 17 of a dose-finding study in pregnant Wistar rats treated from GD 6-17 (Study ES-T02).

AUC: Maternal/Fetal NOAEL based on parturition difficulties and neonatal survival until Day 4 post-partum (0.17 mg/kg) =13.3 ng·h/mL

F1 Development/Behavior/Fertility NOAEL (1.5 mg/kg) =59.8 ng·h/mL

Rabbit:

Study for effects on embryo-fetal development by oral route (gavage) in rabbit (Test Article: Estetrol E4) (Study ES-T19, GLP)

Study ES-T19: Exposure Data on GD18 (or GD15 at 0.45 mg/kg) After Multiple Doses of E4 in Pregnant Female Rabbits

	Dose (mg/kg)		
Parameter	**0.05**	**0.15**	**0.45**
$AUC_{0\text{-last}}$ (ng·h/mL)	6.84	28.5	57.8
C_{max} (ng/mL)	0.925	4.14	4.17
T_{max} (h)	2.0	2.0	2.0
$T_{1/2}$ (h)	13.4	ND	18.0

Embryo-fetal development (EFD); concurrent TK was performed (Study ES-T19)

AUC: Maternal/Fetal NOAEL (0.05 mg/kg) =6.84 mg/kg

TK Data from Carcinogenicity Studies

Rat:

Carcinogenicity study by the oral route (gavage) in female Wistar rats (Study 0031-NC-003, GLP)

Exposure Data in Female Rats on Week 25 of the 104-Week Carcinogenicity Study

	Dose (mg/kg)		
Parameter	**0.08**	**0.27**	**0.8**
$AUC_{0\text{-last}}$ (ng·h/mL)	6.73	18.8	40.7
C_{max} (ng/mL)	3.39	9.99	25.5
T_{max} (h)	0.5	0.5	0.5

Type of Study	Major Findings				
Mouse: Carcinogenicity study by the oral route (gavage) in female mice (Study 0031-NC-004, GLP)	**Exposure Data in Female Mice on Week 25 of the 104-Week Carcinogenicity Study**				
		Dose (mg/kg)			
	Parameter	**0.125**	**0.25**	**0.5**	**1.0**
	$AUC_{0\text{-last}}$ (ng·h/mL)	4.6	12.4	23.6	54.6
	C_{max} (ng/mL)	3.75	8.91	14.9	29.1
	T_{max} (h)	0.5	0.5	0.5	0.5

Abbreviations: $AUC_{0\text{-last}}$, area under the plasma concentration-time curve from time zero to time of last measurable concentration; C_{max}, maximum (peak) plasma drug concentration; DRSP, drospirenone; E4, estetrol; GD, gestation day; GLP, good laboratory practice; NOAEL, no-observed-adverse-effect level; $T_{1/2}$, elimination half-life; T_{mas}, time to reach maximum (peak) plasma concentration following drug administration

5.4.2. Pharmacokinetics of Drospirenone

No new nonclinical pharmacokinetic/ADME/toxicokinetic studies were conducted with DRSP for this 505(b)(2) submission. The nonclinical pharmacokinetic sections of the labeling for NEXTSTELLIS will align with the DRSP information as represented in the YAZ approved drug product labeling. The following summary for drospirenone PK/ADME is based on the current approved YAZ® label (NDA 21-676).

Metabolism

The two main metabolites of DRSP found in human plasma were identified as the acid form of DRSP generated by opening of the lactone ring and the 4,5-dihydrodrospirenone-3-sulfate, formed by reduction and subsequent sulfation. These metabolites were also identified in the plasma of rats, mice, and rabbits. These metabolites were shown not to be pharmacologically active. Drospirenone is also subject to oxidative metabolism catalyzed by CYP3A4.

Excretion

Breast Milk Excretion: Excretion of DRSP or its metabolites into milk has not been studied in animals.

The current YAZ® labeling states:

> After oral administration of 3 mg DRSP/0.03 mg EE (Yasmin) tablets, about 0.02% of the DRSP dose was excreted into the breast milk of postpartum women within 24 hours. This results in a maximal daily dose of about 0.003 mg DRSP in an infant.

5.5. Toxicology

5.5.1. General Toxicology of Estetrol Monohydrate

A 26-Week Toxicity Study by the Oral Route (Gavage) in Rats Followed by an 8-Week Treatment-Free Period (Study ES-T18)

Key study findings:

Daily oral administration of E4 up to 15 mg/kg for 26 weeks in female rats was associated with dose-dependent decreases in body weight, clinical signs of poor health and alopecia, minor anemia and disturbances in clotting parameters, and slight changes in cholesterol and triglycerides that correlated with liver hypertrophy and increased liver weight at doses ≥5 mg/kg/day.

Pathologic changes in the female genital tract and mammary gland, and inhibitory effects on estrous cyclicity, were considered anticipated pharmacological effects of an unopposed estrogen, and were primarily observed at ≥5 mg/kg/day.

Normal estrous cycling was observed following a 20-day recovery period after high dose (15 mg/kg/day) administration.

E4-related changes in clinical signs, body weight gains, food consumption, and pathologic changes in the thymus, spleen, kidneys and liver suggest that oral administration at ≥5 mg/kg/day creates stress and induces hepatic metabolism in female rats.

The NOAEL in this study was determined to be 1.5 mg/kg/day, with a corresponding safety margin of 3.3x the MRHD on an AUC basis.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 5. Methods, Study ES-T18

Method	Details
Dose:	0 (vehicle), 1.5 (LD), 5 (MD), 15 (HD) mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in drinking water
Species/Strain:	Rat / Sprague Dawley
Number/Sex/Group:	Main study: 20 females/group; all groups Recovery: 6 females/group; vehicle and HD only
Age:	8 weeks at study initiation
Satellite groups/ unique design:	Recovery: 8-week recovery period for 6 females/group for vehicle and HD only TK analysis: 3 in vehicle and 9 in each treatment group were allocated for the determination of test article plasma levels
Deviation from study protocol affecting interpretation of results:	No

Abbreviations: HD, high dose; LD, low dose; MD, mid dose; TK, toxicokinetic

Table 6. Observations and Results, Changes From Control, Study ES-T18

Parameters	Major Findings
Mortality	No premature deaths or unscheduled sacrifices attributed to treatment.
Clinical signs	Signs of poor clinical health (thin appearance, hunched posture, piloerection and soiled body parts) associated with cutaneous manifestations (thinning of hair and generalized alopecia) were reported in all E4 groups (only thinning of hair at LD), with increased frequency at MD and HD. These signs were observed for several weeks or until sacrifice, and persisted through recovery.
Body weights	Dose-dependent decreases in mean body weight (BW) in all E4-treated groups (up to -22% at HD) at the end of the 6-month treatment period as compared to controls. This correlated with reduced food consumption during weeks 1-5 of the dosing phase. This effect reversed during the recovery period with HD animals gaining weight 13% faster than the controls.
Ophthalmoscopy	No E4 treatment-related ophthalmological findings were observed
Hematology	E4-treatment produced dose-dependent and statistically significant: • Reduced red blood cell counts (up to -10% HD), • Reduced hemoglobin content (-10 to -12% all doses), • Reduced hematocrit (-10% all doses), • Increased neutrophil count (up to +51% HD) • Clotting parameters: prolonged prothrombin time (up to +12% HD), prolonged activated prothrombin time (up to +24%) and higher fibrinogen concentration (up to +17% HD). All effects were modest and showed full reversibility.
Clinical chemistry	E4 caused a dose-dependent decrease in cholesterol (-42% HD) and albumin/globulin ratio (-8% HD), and a dose-dependent increase in triglycerides (+14% HD), total proteins (+4% HD), and inorganic phosphorus (+27% HD) at the end of the 26-week dosing phase compared to controls. The changes were minor, but clearly dose-dependent, with complete reversibility.
Urinalysis	No E4 treatment-related changes were observed
Gross pathology	No gross lesions were attributed to the test article at the end of the treatment period.
Organ weights	Comparison of relative organ weights was more meaningful in this experiment due to the significant effect of dosing on body weight gain. Statistically significant changes in organ weights relative-to-body weight in all E4-treated groups: • Adrenal glands (LD: +66%, MD: +60%, HD: +55%) • Kidneys (LD: +21%, MD: +25%, HD: +33%) • Liver (LD: +31%, MD: +51%, HD: +68%) • Thymus (LD: -8.2%, MD: -11%, HD: -20%) *Effects attributed to the disruption of estrus cycles by E4:* • Relative full uterus weight decreased in all E4 groups (LD: -5.3%, MD: -10%, HD: -3.8%), but the weight of the empty uterus increased (LD: +8.5%, MD: +3.3%, HD: +9%), indicating a loss of uterus content and thickening of the uterine membranes • Relative vagina weight decreased (LD: -5%, MD: -7.5%, HD: +9%) • Relative ovary weight increased (LD: +10%, MD: +15%, HD: +0.8%)

Parameters	Major Findings
Histopathology Adequate battery: Yes	***E4-related microscopic findings in the female rat observed at 26-weeks:*** Female genital tract (ovaries, uterus, vagina): Cellular changes were characteristic of estrous cycle suppression by treatment, and were dose-dependent: • *Ovaries* - interstitial cell atrophy in all treatment groups, follicular cysts at higher incidence in MD/HD groups, and inhibition of corpora lutea development in the HD group. • *Uterus* - minimal to slight endometrial hypertrophy with squamous metaplasia in all E4 groups. • *Vagina* - epithelium was mucified in all E4 groups, and hypertrophy and eosinophilic infiltration were observed at the HD. Mammary gland: Lobuloalveolar hyperplasia in MD/HD Thymus: Lymphoid atrophy in MD/HD Spleen: Brown pigmentation [hemosiderin] in the red pulp, higher severity in MD/HD Liver: Centrilobular hepatocellular hypertrophy in all treated groups, correlating with increased liver weights, suggestive of CYP450 induction Adrenal glands: Cortical hypertrophy in the reticular layer in all E4 groups Kidney: Brown pigments were observed in the cortical tubular cells at higher incidence in E4 groups and minimal dilation of the cortical tubules in MD/HD Femur: Increased trabecular bone in all E4 groups ***Reversibility (8-week recovery):*** The effects on the thymus, spleen, ovaries, vagina and liver completely recovered. The kidney, uterus, adrenal, spleen and mammary gland findings did not fully recover.
Estrous cycle	Estrous cycling was disrupted in HD females after 26 weeks of treatment, as evidenced by the decrease or absence of corpora lutea development at this dose. Histology of the female genital tract in control animals at the end of the treatment phase indicated normal cycling. ***Reversibility (8-week recovery)*:** Normal estrous cycles resumed in HD females around 20 days after the start of recovery. The number of normal cycles during the first 37 days of recovery was lower in HD (10/35) when compared to controls (24/39). Mean cycle length in HD recovery females was also slightly higher when compared to controls (5.62 vs. 5.02).

-: indicates reduction in parameters compared to control.
+: indicates increase in parameters compared to control.
Abbreviations: E4, estetrol; HD, high dose; LD, low dose; MD, mid dose

A 39-Week Toxicity Study by the Oral Route (Gavage) in Adult Female Cynomolgus Monkeys Followed by a 6-Week Treatment-Free Period (Study ES-T20)

Key study findings:

Daily oral administration of E4 up to 10 mg/kg for 39 weeks in female monkeys was associated with clinical signs of thinness and alopecia, minor dose-dependent anemia, and evidence of mild dose-dependent hepatocellular injury and altered hepatic function at doses ≥3 mg/kg/day.

Pathologic changes in the female genital tract, mammary gland, and pituitary gland, and inhibitory effects on estrous cyclicity, primarily observed at ≥3 mg/kg/day, were anticipated exaggerated pharmacological effects of an unopposed estrogen.

In the female monkey, dose-dependent cholestasis with corresponding increased liver weight at ≥3 mg/kg/day is considered an adverse E4-related effect. Additionally, the severity of the pathologic changes in the thymus, pituitary gland, mammary glands, and bone marrow were more pronounced at ≥3 mg/kg/day. The NOAEL in this study was determined to be 1 mg/kg/day, with a corresponding safety margin of 2.1x the MRHD on an AUC basis.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 7. Methods, Study ES-T20

Method	Details
Dose:	0 (vehicle), 1 (LD), 3 (MD), 10 (HD) mg/kg
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water
Species/Strain:	Monkey / Cynomolgus
Number/Sex/Group:	Main study: 8 females/group; all groups Recovery: 4 females/group; vehicle and HD only
Age:	44 to 61 months old (sexually mature) at study initiation
Satellite groups:	Recovery: 6-week recovery period for 4 females/group for vehicle and HD only
Deviation from study protocol affecting interpretation of results:	No

Abbreviations: HD, high dose; LD, low dose; MD, mid dose

Table 8. Observations and Results, Changes From Control, Study ES-T20

Parameters	Major Findings
Mortality	No premature deaths or unscheduled sacrifices occurred.
Clinical signs	Thin appearance (LD: 25%, MD: 13%, HD: 42%) as compared to controls and generalized alopecia, for which the incidence and severity was not dose-dependent and was related to microscopic changes in the hair follicles.
Body weights	Vehicle control females body weight gain over the dosing phase was 12%, at LD and MD no weight was gained over the dosing phase, and the HD group lost 9% of their body weight at the end of the dosing phase. Both controls and HD females gained weight in a similar manner over the recovery period.
Ophthalmoscopy	No ophthalmological findings were observed in any group.
Hematology	E4 caused a dose-dependent decrease in red blood cells (RBC; up to -18% HD), hemoglobin (up to -15% HD), and hematocrit (up to -15% HD). These changes were significant at all dose levels on week 25 and 38. These changes were fully reversible.
Clinical chemistry	E4-treatment produced dose-dependent and statistically significant increases in cholesterol (+52% HD), ALT (+170% HD) and total bilirubin (+200% HD) and decreases in total protein (-11% HD), albumin (-17% HD), ALP (-64% HD), and GGT (-30% HD). The changes were minor and reversible.
Urinalysis	No E4 treatment-related changes were observed.

Parameters	Major Findings
ECG	No E4 treatment-related abnormalities in ECG parameters or blood pressure were observed that were considered to have toxicological significance.
Gross pathology	No gross lesions were attributed to the test article at the end of the treatment period.
Organ weights	Statistically significant changes in organ weights relative-to-body weight in all E4-treated groups: • Adrenal glands (LD: +20%, MD: +36%, HD: +28%) • Kidneys (LD: +0.7%, MD: +6%, HD: +6%) • Liver (LD: +0%, MD: +4.5%, HD: +9%) • Thymus (LD: -19%, MD: -41%, HD: -58%) • Spleen (LD: -22%, MD: -22%, HD: -23%) • Ovaries (LD: -13%, MD: -28%, HD: -22%)
Histopathology Adequate battery: Yes	**E4-related microscopic findings in the female monkey observed at 39-weeks:** Female genital tract (ovaries, oviducts, uterus including cervix, vagina): • *Ovaries* - no corpora lutea in all E4 groups • *Oviducts* - hypertrophy/hyperplasia of epithelium and myometrium at MD/HD • *Uterus* - decreased thickness of endometrium, increased thickness of myometrium at HD • *Cervix* - hypertrophy/hyperplasia of epithelium with squamous metaplasia in all E4 groups • *Vagina* - atrophy of epithelium at MD/HD Mammary glands: Proliferation of the acini characterized by the presence of irregular size cells with enlarged clear cytoplasm and irregular/enlarged nuclei in MD and HD groups. Minimal lymphoid cell infiltrate at HD. Pituitary gland: Enlarged pituitary cells filled with eosinophilic small granules seen in the pars distalis in MD and HD groups. Thymus: Lymphoid atrophy in MD and HD animals. Adrenal glands: Hypertrophy and vacuolation in zona fasciculata in all E4 groups and atrophy of zona reticularis at MD and HD. Skin: Diffuse alopecia was reported in 1/8 controls, 4/8 LD and MD, and 6/8 HD females. Histologically, atrophy of hair follicles was observed with a dose-related trend. Bone marrow: Increase in marrow adipose tissue at the MD and HD. Decreased cellularity at HD. Liver: Reduction in hepatocyte glycogen at the HD. ***Reversibility (6-week recovery):*** All the histologic changes were fully recovered except for alopecia which appeared to be in the process of recovery.

Parameters	Major Findings
Estrous cycle	Prior to E4 administration, bleeding periods indicative of estrous cycles were observed consecutively and at regular intervals in all the animals. Four LD females and 3 MD females had an estrus period during the first month of treatment. After the first four weeks of treatment, estrous cycles were no longer observed in these two groups. Two HD females showed bleeding during the first week of treatment and no further estrus period was observed in any animal of this group during treatment. Control animals continued to cycle normally. ***Reversibility (6-week recovery)*:** During the recovery period, 2 of 4 HD animals showed signs of returning to cycling (spotting and minor bleeding) on Day 315-316. Additionally, histopathological findings of the presence of corpora lutea at the end of the 6-week recovery in these 2 HD animals suggested that ovulation occurred, indicating a return to estrous cycles.

-: indicates reduction in parameters compared to control.
+: indicates increase in parameters compared to control.
Abbreviations: E4, estetrol; ECG, electrocardiogram; HD, high dose; LD, low dose; MD, mid dose

5.5.2. General Toxicology of Combined Estetrol Monohydrate and Drospirenone

13-Week Toxicity Study by the Oral Route (Gavage) in Adult Female Cynomolgus Monkeys Followed by a 4-Week Treatment-Free Period (Estetrol and Drospirenone) (Study 0030-NC-002)

Key study findings:

A combination of DRSP/E4 administered to female cynomolgus monkeys for 13-weeks resulted in treatment-related effects consistent with anticipated hormonal changes of a progestin/estrogen drug combination. Adverse effects were considered a result of exaggerated pharmacological effects of supraphysiological concentrations of progestins and estrogens.

Hyperglycemia was observed in HD females (DRSP/E4 6/30 mg), which resulted in poor condition and early termination of 3 animals in this group, along with a subsequent dose-reduction at day 50-53 of the dosing phase. Hyperglycemia was also noted in one MD female at day 75. At the dose where severe hyperglycemia led to early termination, the exposure margins for DRSP and E4 were 13x and 97x (based on Day 1 toxicokinetic data of DRSP/E4 co-administered at 6/30 mg/kg), respectively, at the MRHD on an AUC basis.

The NOAEL for the DRSP/E4 combination was determined to be 0.6/3 mg/kg/day DRSP/E4, with exposure margins for DRSP and E4 in combination of 1.2x and 8.2x, respectively, at the MRHD on an AUC basis.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 9. Methods, Study 0030-NC-002

Method	Details
Dose:	DRSP/E4: 0 (vehicle), 0.6/3 (DRSP/E4 LD), 2/10 (DRSP/E4 MD), 6/30 (4/20) (DRSP/E4 HD) mg/kg DRSP only: 6 (4) mg/kg (DRSP HD) E4 only: 30 (20) mg/kg (E4 HD)
Frequency of dosing:	Once daily
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water
Species/Strain:	Monkey / Cynomolgus
Number/Sex/Group:	Main study: 9 females/group; vehicle and DRSP/E4 HD; 5 females/group; DRSP/E4 LD, DRSP/E4 MD, DRSP HD, E4 HD Recovery: 4 females/group; vehicle and 2 females/group DRSP/E4 HD
Age:	44 to 61 months old (sexually mature) at study initiation
Satellite groups:	Recovery: 6-week recovery period for 4 females/group for only vehicle and DRSP/E4 HD
Deviation from study protocol affecting interpretation of results:	No; Poor clinical condition in DRSP/E4 HD led to a 2-day dosing gap (D48-49) and dose reduction from 6/30 to 4/20 (DRSP/E4) on D50. HD DSRP and E4 alone were also reduced to 4 mg/kg and 20 mg/kg, respectively, starting on Day 53. The Agency was consulted on the dose reduction and for allocation of surviving animals in the HD group and sponsor followed the Agency recommendations. The dose reduction at the DRSP/E4 HD, DRSP HD, and E4 HD groups did not affect the interpretation of the study results.

Abbreviations: DRSP, drospirenone; E4, estetrol; HD, high dose; LD, low dose; MD, mid dose

Table 10. Observations and Results, Changes From Control, Study 0030-NC-002

Parameters	Major Findings
Mortality	3 DRSP/E4 HD females sacrificed on D45 or D47 of the dosing phase due to poor condition attributed to adverse effects caused by hyperglycemia.
Clinical signs	Transient episodes of hypothermia and pallor of the gum were observed in 2 DRSP/E4 HD and 1 E4 HD animals.
Body weights	No significant differences in treatment groups.
Ophthalmoscopy	No ophthalmological findings were observed in any group.
ECG	No treatment-related difference in ECG parameters.
Hematology	Decrease in RBC in the DRSP/E4 MD and HD (-7%) and E4 HD (-11%) groups. Decrease in hemoglobin (HB) in all groups (up to -10% in E4 HD). Decrease in prothrombin time (PT) in DRSP/E4 MD and HD (-9%). All 3 parameters recovered at the end of the recovery period.

Parameters	Major Findings
Clinical chemistry	Most clinical chemistry changes were dose dependent and were reversed by the end of recovery period. All changes are an expected and pharmacologically exaggerated response to E4, and not seen with DRSP alone. Statistically significant changes included: • Decreased calcium in all groups (up to -11% in E4 HD) • Decreased total proteins in all groups (up to -13% in E4 HD) • Increased glucose in DRSP/E4 MD (+34%) and HD (+47%) • Increased triglycerides in all groups except DRSP HD (+115 to +510%). • Decreased ALP in all groups (up to +74% in DRSP/E4 HD) • Changes in ALAT (+250% in E4 alone), GGT (-40 to -48% all DRSP/E4)
Urinalysis	No E4 treatment-related changes were observed
Gross pathology	Thymus size reduced in all treatment groups except DRSP HD. Uterus enlarged in 4 DRSP/E4 MD and 2 DRSP/E4 HD animals.
Organ weights	Thymus size reduced in all treatment groups except DRSP HD with a correlating decreased thymus weight (all E4-treated groups, ~ -55%), that was partially reversible. Uterus enlarged in several DRSP/E4 MD and HD animals, with increased uterus weight in all DRSP/E4 groups (+51% to +271%) that was reversible. Ovary weight reduced (~ -50%) in all treatment groups and decreased oviduct weight in all treatment groups except E4 HD (~ -54%), with reduction in ovary weight not reversible. Pituitary weight increased (DRSP/E4 MD/HD and E4 HD, ~ +35%), which was reversible.
Histopathology Adequate battery: Yes	Histopathology at the end of 13-week treatment period in female monkeys: Female genital tract (ovaries, oviducts, uterus, cervix, vagina): Anticipated hormonal changes associated with combined administration of E4 and DRSP in the female genital tract were observed.: • *Ovaries* - no corpora lutea in all DRSP/E4 groups and E4 HD and hyalinization of zona pellucida in all treated groups • *Oviducts* - minimal to slight atrophy of epithelium in all treated groups except E4 HD; minimal to moderate hypertrophy/hyperplasia of the epithelium and lumen dilation in E4 HD, • *Uterus/Cervix* - myometrium hypertrophy, decidual endometrial stroma, basal glands, enlarged cervix in all DRSP/E4 groups; endometrial stroma hyalinization, epithelia hyperplasia, gland dilation, and squamous metaplasia in E4 HD; decreased epithelial thickness in DRSP HD • *Vagina* - atrophy of epithelium in all treated groups Mammary gland: Increased acinar development (moderate to marked) in all DRSP/E4 groups and E4 HD; acinar vacuolation in DRSP/E4 MD/HD, tubule-alveolar dilation in all treated groups, anisokaryosis/anisocytosis in all DRSP/E4 groups and E4 HD, and lymphoid infiltrate in all treated groups. Pituitary gland: Presence of eosinophilic cells in all treated groups; reflected an estrogenic effect.

Parameters	Major Findings
	Thymus: Increased lymphoid atrophy in all treated groups and less severe in DRSP HD.
	Adrenal gland: Atrophy in zona reticularis in all treated groups except DRSP/E4 LD, and hypertrophy in zona glomerulosa in all treated groups.
	Pancreas: Minimal vacuolation was observed in islet cells at DRSP/E4 HD.
	Sternal bone marrow: Minimally decreased cellularity in DRSP/E4 MD/HD.
	Reversibility (4-week recovery):
	Recovery was partial but incomplete in female genital tract tissues, which was consistent with the beginning of new estrous cycles (return to fertility). Pituitary gland findings were not reversible.
	Histopathology in moribund animals:
	In early terminated animals from DRSP/E4 HD group on D45/47, changes in endocrine pancreas (single cell necrosis/apoptosis of islet cells), along with pronounced kidney and liver changes, and decreased cellularity of the bone marrow, mainly involving erythroid cells, were present. Findings in the pancreas, kidney, and liver were possibility related to hyperglycemia that was a major contributing factor to the poor status leading to premature sacrifice in combination DRSP/E4 HD animals.
Hormone levels	Cortisol levels increased (+40-60%) in all treatment groups except DRSP HD.
	Aldosterone levels increased (up to +473%) in all treatment groups except E4 HD, which is an expected progestogenic effect of DRSP.
Estrous cycles	Estrous cycle bleeding was absent in all drug-treated groups during the dosing period, whereas vehicle control females had regular estrous cycles during the entire study.
	Isolated incidences of bleeding occurred in all treatment groups: 1 episode in 3 DRSP/E4 LD females, 1 episode in 4 DRSP/E4 MD females, 1 episode in 7 DRSP/E4 HD females, 1 episode in 1 E4 alone female, and 3 incidences in 1 DRSP alone female.
	Estrous cycles returned in both recovery females of DRSP/E4 HD.

"D": indicates the study day that a finding was observed.
-: indicates reduction in parameters compared to control.
+: indicates increase in parameters compared to control.
Abbreviations: DRSP, drospirenone; E4, estetrol; HD, high dose; LD, low dose; MD, mid dose

5.5.3. Genetic Toxicology of Estetrol Monohydrate

E4 was not considered genotoxic in a full battery of in vitro and in vivo assays (Ames assay, in vitro mouse lymphoma assay, an in vivo rat micronucleus assay, and in vivo rat Comet assay) described below. Bacterial Reverse Mutation assays showed positive results only in the *Salmonella typhimurium* strain TA102. Due to the sensitivity of the TA 102 strain to oxidative mutagens, the positive results observed only in this strain may involve the generation of free radical species without any direct DNA damaging effect. Subsequent follow-up studies in the *Escherichia coli* WP2 uvrA strain and in vivo genetic toxicology studies failed to substantiate the ability of E4 to cause gene mutations or induce chromosomal damage. Therefore, the weight-of-evidence indicates that E4 is not genotoxic.

In Vitro Reverse Mutation Assay in Bacterial Cells (Ames)

Bacterial Reverse Mutation Test (Study ES-T21)

Key study findings:

E4 was found negative for mutagenicity in tester strains TA 1535, TA 1537, and TA 98 up to the limit dose of 5000 µg/plate and in strain TA 100 up to 2500 µg/plate, in the presence or absence of metabolic activation.

Increases in the number of revertant colonies were noted in strain TA 102, both in the absence and presence of metabolic activation, starting at 1250 µg/plate and exceeding the threshold of two-fold the vehicle control value at 2500 and/or 5000 µg/plate (maximum of 3.3-fold at 5000 µg/plate). These increases were observed in both assays. Therefore, the data met the criteria for a positive response for strain TA 102.

GLP compliance: Yes

Test system:

Salmonella typhimurium: TA 1535, TA 1537, TA 98, TA 100 and TA 102; up to 2500 µg/plate for the TA 100 strain and 5000 µg/plate for all other strains

Assay 1: ±S9 mix; direct plate incorporation

Assay 2: -S9 mix; direct plate incorporation / +S9 mix; direct plate incorporation followed by plate incorporation

Study is valid: Yes

Bacterial Reverse Mutation Test (Study ES-T29)

Key study findings:

No precipitate and no toxicities were observed.

A dose-related increase in the number of revertants was noted in both experiments, with and without S9 mix. These increases exceeded the threshold of 2-fold the vehicle control value only at the dose-levels of 2500 and 5000 µg/plate, with a maximum of 4-fold at 5000 µg/plate.

GLP compliance: No

Test system:

Salmonella typhimurium: TA 102; up to 5000 µg/plate

Assay 1: ±S9 mix; direct plate incorporation

Assay 2: -S9 mix; direct plate incorporation / +S9 mix; pre-incubation followed by plate incorporation

Study is valid: Yes

Evaluation of the Mutagenic Activity of E4 in the Salmonella typhimurium Reverse Mutation Assay With the Tester Strain TA 102 and the Escherichia coli Reverse Mutation Assay With the Tester Strain WP2 uvrA (Study ES-T48)

Key study findings:

The *S. typhimurium* TA102 and *E. coli* WP2 uvrA strains are interchangeably used to detect mutagens that cause base pair substitutions (AT base pair at the primary reversion site) and are also used to detect cross-linking mutagens in the Ames assay system. This study concurrently compared the mutagenic potential of E4 in these two strains.

Under the experimental conditions of this study, E4 up to the limit dose of 5000 µg/plate was concluded to be not mutagenic in the Ames test with *S. typhimurium* strain TA102 and *E. coli* strain WP2 uvrA, either in the absence or in the presence of metabolic activation.

GLP compliance: Yes

Test system:

Salmonella typhimurium: TA 102 and *Escherichia coli* strain WP2 uvrA; up to 5000 µg/plate; *Assay 1*: ±S9 mix; direct plate incorporation

Assay 2: ±S9 mix; pre-incubation followed by plate incorporation

Study is valid: Yes; for Assay 1 WP2 uvrA only and Assay 2 both strains. In Assay 1, positive controls for strain TA 102 did not meet the criteria for a valid test.

<u>In Vitro Assays in Mammalian Cells</u>

In Vitro Mammalian Cell Gene Mutation Test in L5178Y$^{+/-}$ Mouse Lymphoma Cells (Study ES-T45)

Key study findings:

E4 did not display mutagenic activity in the mouse lymphoma assay, either in the presence or in the absence of a rat metabolizing system.

GLP compliance: Yes

Test system: L5178Y TK$^{+/-}$ cells

Assay 1: -S9 mix; Doses: 0.031, 0.063, 0.13, 0.25, 0.5 and 1mM, 3-h incubation / +S9 mix; Doses: 0.031, 0.063, 0.13, 0.25, 0.5, 0.75, and 1mM, 3-h incubation

Assay 2: ±S9 mix; Doses: 0.031, 0.063, 0.13, 0.25, 0.5, 0.75 and 1mM, 24-h incubation (-S9 mix) / 3-h incubation (+S9 mix)

Study is valid: Yes

In Vivo Clastogenicity Assay in Rodent (Micronucleus Assay)

Bone Marrow Micronucleus Test by Oral Route in Rats (Study ES-T37)

Key study findings:

E4 did not induce damage to the chromosomes or the mitotic apparatus of rat bone marrow cells after a single oral administration up to 2000 mg/kg.

E4 plasma concentrations at 2000 mg/kg were between 39.2 and 48.6 µg/mL at 0.5 h post-dose and between 19.5 and 65.6 µg/mL at 3 h post dose.

GLP compliance: Yes

Test system: Female Sprague Dawley Rat (n=5 per group and time point)

Doses (single-dose; oral gavage): E4; 0 (vehicle), 500, 1000, 2000 mg/kg; Cyclophosphamide (positive control); 15 mg/kg

Sampling Time: 24 h post-dose (all treatment groups) and 48 h post-dose (vehicle and 2000 mg/kg only)

Study is valid: Yes

Other Genetic Toxicity Studies

In Vivo Comet Assay in Rodent

In Vivo Comet Assay in Rat (Study ES-T46)

Key study findings:

E4 did not induce primary DNA damage in the liver, whereas findings in the duodenum were considered equivocal for this study.

E4 induced a statistically and biologically significant increase in DNA strand breaks only at the lowest dose tested of 500 mg/kg/day (x2) in female rat duodenum cells after oral administration.

E4 plasma concentrations at 2000 mg/kg averaged 39.8 µg/mL at 0.5 h and 28.5 µg/mL at 3 h after the final dose.

GLP compliance: Yes

Test system: Female Sprague Dawley Rat (n=4 per group and time point)

Doses (oral gavage): E4 (2 doses at 24-h interval); 0 (vehicle), 500, 1000, 2000 mg/kg; Dimethylhydrazine (positive control; single-dose); 10 mg/kg (liver), 20 mg/kg (duodenum)

Sampling Time / Tissues: 3 and 6 h after last dose (all treatment groups) / Liver and Duodenum

Study is valid: Yes

Detection of DNA damage in the liver and duodenum of treated rats using the Comet assay (Study 0031-NC-001)

Key study findings:

E4 did not induce DNA damage in the liver or duodenum of female rats treated orally by gavage at doses up to and including 2000 mg/kg/day.

E4 plasma concentrations at 2000 mg/kg were 22.8 µg/mL at 0.5 h and 14.5 µg/mL at 3 h after the final dose.

GLP compliance: Yes

Test system: Female Sprague Dawley Rat (n=5 per group and time point)

Doses (oral gavage):

E4 (2 doses at 0 and 21 h); 0 (vehicle), 62.5, 125, 250, 500, 1000, 2000 mg/kg;
Ethylmethanesulfonate (positive control; single-dose at 21 h); 250 mg/kg

Sampling Time / Tissues: 3 and 4 h after last dose (all treatment groups) / Liver and Duodenum

Study is valid: Yes

5.5.4. Carcinogenicity of Estetrol Monohydrate

Standard 2-year carcinogenicity studies for E4 in the female rat and mouse were conducted by the oral route of administration. The Executive Carcinogenicity Assessment Committee (ECAC) concurred with the doses used for both studies through special protocol assessment (SPA).

Rat:

Carcinogenicity Study by the Oral Route (Gavage) in Female Wistar Rats (Study 0031-NC-003)

Female Wistar rats (n=100/group) were treated with 0 (vehicle), 0 (vehicle), 0.08, 0.27, and 0.8 mg/kg/day E4 for 104 weeks. E4 was not found to affect overall survival, clinical signs, or estrous cycling up to 0.8 mg/kg/day. At 0.27 and 0.8 mg/kg/day, E4 decreased body weight by 4% to 6% and body weight gain by 6% to 10% as compared to vehicle control groups, which correlated with a decrease in food consumption. E4 caused non-neoplastic proliferative changes at almost all the dose levels in the mammary gland and uterus, accompanied by uterine inflammation. E4-related non-neoplastic histopathology changes were consistent with estrogenic effects or were considered secondary effects to these estrogen-related changes.

E4 was found to be carcinogenic at the high-dose of 0.8 mg/kg/day (0.7x the MRHD on an AUC basis) increasing the incidence of mammary neoplasms (malignant adenocarcinoma and combined malignant adenocarcinoma arising in fibroadenoma, benign adenoma, and malignant adenocarcinoma) in female rats. E4 did not induce tumors in female rat at the mid-dose of 0.27 mg/kg/day, with an exposure margin of approximately 0.32x the MRHD on an AUC basis.

Mouse:

Carcinogenicity Study by the Oral Route (Gavage) in Female Mice (Study 0031-NC-004)

Female CD-1 mice (n=100/group) were treated with 0 (vehicle), 0 (vehicle), 0.125, 0.25, 0.5, and 1.0 mg/kg/day E4 for 104 weeks. E4 was not found to affect overall survival. At ≥0.5 mg/kg, body weight and body weight gain were increased, with body weight increases of +3% at 1.0 mg/kg/day and body weight gain increases of +15% and +33% for the 0.5 and 1.0 mg/kg-treated groups, respectively, at Week 104. The increase in body weight and body weight gain at ≥0.5 mg/kg correlated with an increase in food consumption at ≥0.25 mg/kg. E4-related non-neoplastic histopathology findings were generally consistent with anticipated exaggerated pharmacological effects of supraphysiological levels of estrogenic compounds or were considered secondary effects to these estrogen-related changes.

In female mice, E4 was carcinogenic at doses of 0.25 mg/kg/day (0.2x the MRHD on an AUC basis) and above, including mammary neoplasms (combined benign and malignant adenoacanthoma) at 0.25 and 1.0 mg/kg/day, uterine epithelial, stromal, and glandular neoplasms at ≥0.25 mg/kg/day, and pituitary neoplasms (adenoma and combined adenoma and carcinoma) at 1.0 mg/kg/day. E4 did not induce tumors in female mice at the lowest-dose of 0.125 mg/kg/day, with an exposure margin of approximately 0.08x the MRHD on an AUC basis.

Adequacy of Carcinogenicity Studies of E4: The tumor findings from the rat and mouse final carcinogenicity studies were reviewed and agreed upon by the Division and ECAC on November 24, 2020. As the carcinogenic potential of E4 was aligned with other estrogenic class compounds and there are no unexpected neoplastic signals in the nonclinical carcinogenicity studies of E4, it is proposed that estrogen class labeling be used for the Nextstellis label and that the carcinogenicity study data for E4 not be included in the labeling.

5.5.5. Reproductive and Developmental Toxicology of Estetrol Monohydrate

Return to Fertility and Early Embryonic Development

Study of Return to Female Fertility and Early Embryonic Development to Implantation by Oral Route (Gavage) in Rats (Test Article: Estetrol E4) (Study ES-T27)

Key study findings:

The NOAEL for estrous cyclicity was determined to be 0.17 mg/kg/day.

E4 was effective in reducing estrous cycling, particularly at the high-dose of 1.5 mg/kg/day.

Following recovery, no treatment-related effects on fertility or hysterectomy data were observed up to 1.5 mg/kg/day; however, due to the low number of pregnant females in all groups because of the use of immature males, the study results do not present the true effect of E4 on fertility and preclude a definitive conclusion on these parameters.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 11. Methods, Study ES-T27

Method	Details
Dose and frequency of dosing:	0 (vehicle), 0.17 (LD), 0.5 (MD), 1.5 (HD) mg/kg/day; once daily for 4 weeks
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water (b) (4)
Species/Strain:	Rat / Wistar Han; WI (Glx/BRL/Han) IGS BR
Number/Sex/Group:	24 females/group
Study design:	Study design: Daily dosing of non-pregnant female rats for 4 weeks (Days 1-29) followed by a recovery period of 3 weeks, then followed by a treatment-free mating period of up to 4 weeks. Males were not treated. Day of C-section was Day 15 post-coitum.
Deviation from study protocol affecting interpretation of results:	Yes; Males used in this study were determined to be sexually immature (i.e., 8 weeks at time of mating). Therefore, effects of E4 on fertility and reproductive parameters were not reliable. However, results for female estrous cyclicity are still relevant to E4 mechanism of action and are interpretable.

Abbreviations: E4, estetrol; HD, high dose; LD, low dose; MD, mid dose

Table 12. Observations and Results, Study ES-T27

Parameters	Major Findings
Mortality	No animals were found dead or prematurely sacrificed.
Clinical signs	No treatment-related clinical signs were observed in female animals.
Body weights	LD/MD/HD: The dose-related decrease in BW (-7% HD) and BWG (-25-52% all doses) throughout the treatment and pregnancy phases correlated with a decrease in food consumption (-5-10%) in these groups.
Estrous cycle	MD/HD: Increased numbers of non-cycling females at MD (4/24) and HD (9/24) compared to control (1/24). Mean estrous cycle duration was not significantly affected. Estrous cycles returned to control within first week of recovery.
Necropsy findings	Following recovery, no clear treatment-related effects on reproductive parameters and indices or effects on hysterectomy data were observed. **However, the number of pregnant females was overall low, especially control (i.e., 9/24 (Ctrl), 11/24 (LD), 15/24 (MD), 16/24(HD)). The decrease in pregnant females in all groups including control was attributed to incomplete sexual maturity of male animals used for mating in this study (i.e., 8 weeks old). *Therefore, the study results do not present the true effect of E4 on fertility*.

Abbreviations: BW, body weight; BWG, body weight gain; HD, high dose; LD, low dose; MD, mid dose;

Study of Return to Female Fertility and Early Embryonic Development to Implantation by Oral Route (Gavage) in Rats (Test Article: Estetrol E4) (Study ES-T43)

Key study findings:

There were no treatment-related changes in fertility parameters in female rats treated with up to 1.5 mg/kg/day E4 prior to recovery and mating (i.e., total of 3 recovery and up to 4 weeks treatment-free mating period).

There were no treatment-related effects on hysterectomy data and early embryonic development (i.e., number of corpora lutea, number of implantation sites, pre- or post-implantation loss, number of live concepti) up to 1.5 mg/kg/day, the highest dose tested. The NOEL for return to female fertility and early embryonic development was determined to be 1.5 mg/kg/day.

Based on the magnitude of the body weight gain decrements during the dosing and recovery phase, the maternal NOAEL was determined to be the mid-dose of 0.5 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 13. Methods, Study ES-T43

Method	Details
Dose and frequency of dosing:	0 (vehicle), 0.17 (LD), 0.5 (MD), 1.5 (HD) mg/kg/day; once daily for 4 weeks
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water
Species/Strain:	Rat / Wistar Han; (b) (4) WI (Glx/BRL/Han) IGS BR
Number/Sex/Group:	24 females/group
Study design:	Study design: Daily dosing of non-pregnant female rats for 4 weeks (Days 1-29) followed by a recovery period of 3 weeks, then followed by a treatment-free mating period of up to 4 weeks. Males were not treated. Day of C-section was Day 15 post-coitum. *Males used in this study were sexually mature.
Deviation from study protocol affecting interpretation of results:	No

Abbreviations: HD, high dose; LD, low dose; MD, mid dose

Table 14. Observations and Results, Study ES-T43

Parameters	Major Findings
Mortality	No animals were found dead or prematurely sacrificed.
Clinical signs	No treatment-related clinical signs were observed in female animals.
Body weights	MD/HD: BWG was significantly decreased on Days 1-8 only at MD and HD (-45-73%) but partially recovered by Day 29 to decreases of -19-38%. During the pre-mating recovery (up to Day 50), BWG was still decreased -18-25% for the MD and HD groups. The dose-related decrease in BWG correlated with a decrease in food consumption in these groups from Day 1-8 for MD (-5%) and from Day 1-15 for HD (-5 to -10%).
Necropsy findings	Following recovery, no treatment-related effects on mating, fertility, or hysterectomy data were observed in any E4 group.

Abbreviations: BWG, body weight gain; HD, high dose; LD, low dose; MD, mid dose

Embryo-Fetal Development

Oral (Gavage) Study of Embryo-Fetal Development in the Rat (Test Article: Estetrol E4) (Study ES-T03-PR3098)

Key study findings:

Body weight decreases at the MD and HD levels were considered to be adverse. At the LD of 0.3 mg/kg/day, only minimal body weight loss was noted, and no other signs of maternal toxicity were observed. The maternal NOAEL was determined to be 0.3 mg/kg/day.

Embryotoxicity was observed at the HD of 3 mg/kg/day, with increased post-implantation loss resulting in 7 females with total embryo-fetal loss.

Fetal developmental effects were observed at 1 and 3 mg/kg/day, with the increased incidence of skeletal malformations associated with the shortening, thickening and bending of the long bones (humerus, radius) and scapula, and external/visceral malformations associated with malrotated/severely flexed ankle joints. There was also a slight reduction in fetal weight at 3 mg/kg/day, which may be due to the reduced maternal body weight at this dose. The fetal NOAEL was determined to be 0.3 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 15. Methods, Study ES-T03-PR3098

Method	Details
Dose and frequency of dosing:	0 (vehicle), 0.3 (LD), 1 (MD) and 3 (HD) mg/kg/day; once daily from gestation day (GD) 6 to GD 17
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water
Species/Strain:	Rat / HsdHan™:WIST
Number/Sex/Group:	24 females/group
Study design:	Study design: The test and control articles were administered to mated female rats daily from GD 6 to GD 17, inclusive. The females were maintained to GD 20, sacrificed, and examined macroscopically. The fetuses were removed, sacrificed, and examined.
Deviation from study protocol affecting interpretation of results:	No

Abbreviations: HD, high dose; LD, low dose; MD, mid dose

Table 16. Observations and Results, Study ES-T03-PR3098

Parameters	Major Findings
Mortality	HD: Four HD female, were sent to necropsy on GD 14, 18, and 20 due to aborting/signs of abortion and poor clinical condition.
Clinical Signs	HD: Clinical signs were observed in HD females sent to necropsy early as noted above, and appear to be treatment related: sluggish, red-discharge in uro-genital area, raised body hair, pale body, and hunched.

Parameters	Major Findings
Body Weights	MD/HD: Excluding females with total embryo-fetal loss, there were no significant BW changes, with only a slight BW reduction (-4% to -5%) at the MD and HD from GD 15-20. BWG was reduced on GD 7-12, with overall reduction in BWG of -27% compared to controls. Decreased BW/BWG correlated with reduced food consumption in MD (-13%) / HD (-28%) during the treatment phase (GD 6-17) and continued for HD until study termination on GD 20.
Necropsy findings Cesarean section data	Surviving females: 20, 16, 23, and 15 in the control, LD, MD, and HD, respectively. Females with live fetuses: 19, 15, 22, and 8 in the control, LD, MD, and HD groups, respectively. Total embryo-fetal loss: Increased total embryofetal loss at the HD (7/15 (47%)); all other groups had only one female with total embryo-fetal loss. Necropsy/Histopathology: In females with live fetuses, large/thick placentas were observed in several MD (3/22, slight to moderate severity) and HD (5/8, slight to marked severity) females Cesarean section data: LD and MD: No effects on pre- or post-implantation loss, total resorptions, or number of fetuses/female. HD: In all surviving HD females, there was increased pre-implantation loss (HD: 36.5% vs. Control: 21.0%), increased early resorptions, and increased post-implantation loss (HD: 55.4% vs. Control: 14.8%). When females with total embryo-fetal loss were excluded, the only treatment-related effect observed was an increase in post-implantation loss at the HD (HD: 16.4% vs. Control: 10.3%).
Necropsy findings Offspring	Litter weight and sex ratio: No E4 treatment-related effects. Fetal BW: slight, non-statistically significant decrease in fetal weight at HD as compared to control, which may be correlated to the reduced maternal BWG for HD dams. Fetal malformations/Variations: LD: No external, visceral, or skeletal malformations observed MD/HD: Dose-related increases in % fetuses and % litters with external/visceral/skeletal malformations, which was statistically significant at the HD. • Skeletal malformations observed at the MD and HD consisted of shortening/thickening/bending of long bones (humerus, radius) and scapula, which were above the background incidence in laboratory historical controls. • External/visceral malformations in the MD and HD groups consisted of malrotated/severely flexed ankle joints. • Skeletal variations at the HD included incomplete/non-ossification of sternebrae and nodulated and kinked ribs, which are likely related to the reduction in maternal BW and BWG.

Abbreviations: BW, body weight; BWG, body weight gain; E4, estetrol; HD, high dose; LD, low dose; MD, mid dose

Study for Effects on Embryo-Fetal Development by Oral Route (Gavage) in Rabbit (Test Article: Estetrol E4) (Study ES-T19)

Key study findings:

Findings of maternal toxicity at ≥0.15 mg/kg/day included clinical signs of abortion and marked reductions of body weight and body weight gain, corresponding with significant reductions in food consumption during the dosing phase, as well as additional clinical signs of emaciated appearance and absence of feces in HD females. The maternal NOAEL was determined to be 0.05 mg/kg/day.

The maternal toxicity at ≥0.15 mg/kg/day contributed to the observed marked, dose-related effects on pregnancy at the MD and HD level (e.g., increases in total embryofetal loss, late litter resorptions, and post-implantation loss).

Findings associated with fetal developmental delays in rabbit fetuses, which were likely associated with the observed maternal toxicity at ≥0.15 mg/kg/day, included supernumerary 13th ribs at ≥0.05 mg/kg/day and incomplete/non-ossification of phalanges, several bones, and vertebral centers at 0.15 mg/kg/day. Fetal malformations and variations were related to maternal toxicity. The fetal NOAEL was determined to be 0.05 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 17. Methods, Study ES-T19

Method	Details
Dose and frequency of dosing:	0 (vehicle), 0.05 (LD), 0.15 (MD), and 0.45 (HD) mg/kg/day; once daily from GD 6 to GD 18 inclusive [**Dosing duration of HD dose group was reduced to GD 6 to GD 15 only (see deviations below)]
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water
Species/Strain:	Rabbit / KBL New Zealand White
Number/Sex/Group:	20 females/group
Satellite groups:	4 females/group for toxicokinetic analysis
Study design:	Study Design: The test and control articles were administered to mated females daily from GD 6 to GD 18, inclusive. The females were maintained to GD 29, sacrificed and examined macroscopically. The fetuses were removed, sacrificed and examined.
Deviation from study protocol affecting interpretation of results:	No; Due to clinical signs of blood in the bedding in the HD group from GD 16 in the first subset of HD females (7/9 dams) treated from GD 6 to 18, the last 11 HD main study females and all HD satellite females were treated from GD 6 to 15 only. The reduction in dosing period did not improve pregnancy outcome. Therefore, data from all HD females have been assessed as one group instead of 2 subsets. This reduction in dosing phase at the HD and combination of HD subsets did not affect the interpretation of the study results.

Table 18. Observations and Results, Study ES-T19

Parameters	Major Findings
Mortality	MD/HD: Due to evidence of abortion or excessive blood loss, 6 MD, 1 HD (main group), and 1 HD (satellite group) were sacrificed prematurely.
Clinical signs	MD/HD: Signs of abortion (blood, fetal/placental remnants in bedding) for 6 MD dams on GD21-22 and 7 HD dams starting on GD 16. Areas of hair loss on the thorax, abdomen and/or limbs were noted in some MD and HD animals from as early as GD 11. At HD, emaciated appearance (8/19) and absence of feces (5/19) were observed, corresponding with the marked decrease in food consumption and consequent weight loss.
Body weights	MD/HD: BW was statistically decreased at GD 19 in MD (-7%) and HD (-13%) groups and at GD 29 for HD (-9%) animals as compared to controls. BWG was statistically and markedly decreased during the dosing phase on GD 6-19 (MD: -113% and HD: -213%). BWG increased in the MD and HD females after termination of E4 treatment on GD 19 to GD 29. Reduced BW and BWG correlated with a dose-related reduction in food intake from GD 6-19 (MD: -19-44% and HD: -41-74%).
Necropsy findings Cesarean section data	Females with live fetuses: 19, 19, 9, and 2 in the control, LD, MD, and HD groups, respectively. • Non-pregnant animals; 4 MD and 1 HD. • 17 HD dams had implantation site scars at necropsy and/or total early resorption and there were 7 MD dams with evidence of abortion. • Total embryofetal loss was increased at MD (7/16, 46%) and HD (17/19, 89%) • Gravid uterus weight was reduced at the HD (-24%). Necropsy/Histopathology: Uterine endometrial stroma was slightly more prominent (increased size and more compact appearance) in 1/8 MD and 4/8 HD examined. Minimal atrophy of uterine endometrial epithelium was observed in 5/8 HD. Cesarean section data: No treatment-related effects on the mean number of corpora lutea, implantations, and pre-implantation loss were observed when compared with control values. MD/HD: Increased post-implantation loss at MD (10.6%) and HD (37.4%) were observed compared to controls (7.0%), with statistical significance at the HD. Increases in late resorptions at the HD (3.5/group vs. 0/group controls) were observed. Mean number of fetuses per female was reduced at the HD due to increased post-implantation loss in 1 of the 2 surviving litters.

Parameters	Major Findings
Necropsy findings Offspring	**There were only 2 surviving litters with a total of 12 surviving fetuses at the HD. This reduced number of surviving fetuses was largely attributed to post-implantation loss. Due to the limited number of live fetuses in the HD, the statistical assessment of fetal findings was based on LD and MD only. Fetal weight: No E4-related effects Fetal malformations/Variations: No treatment-related increases in external/visceral malformations or variations considered to be unrelated to maternal toxicity: LD/MD: Skeletal variations related to developmental delay included an increased incidence of fetuses per litter with supernumerary 13th ribs. MD: Minor skeletal variations attributed to slight developmental delay included incomplete ossification and/or unossified frontal, supraoccipital and parietal bones of the skull, sternebrae (1st to 4th), cervical and caudal vertebral centra, phalanges of the forepaws and/or hindpaws. HD: skeletal variation of fused sternebrae.

Abbreviations: BW, body weight; BWG, body weight gain; GD, gestation day; HD, high dose; LD, low dose; MD, mid dose

Prenatal and Postnatal Development

Study for Effects on Pre- and Postnatal Development by Oral Route (Gavage) in Rats (Study ES0001-NC-003)

Key study findings:

Due to dose-related body weight and weight gain decreases with correlating reduction of food consumption in F0 females at ≥0.5 mg/kg/day, the maternal NOAEL was determined to be 0.17 mg/kg/day.

Parturition difficulties or absence of delivery was associated with premature sacrifice at 1.5 mg/kg/day (7/17) and one dam at 0.5 mg/kg/day being found dead during or soon after delivery. These deaths contributed to a decrease in gestation index at 0.5 and 1.5 mg/kg/day. There were no significant effects on the duration of gestation in females that survived parturition. The NOAEL for parturition was 0.17 mg/kg/day.

At 0.5 mg/kg/day, one entire litter died on Day 2 postpartum. At 1.5 mg/kg/day, viability index on Day 4 postpartum was reduced due to increased incidences of cannibalized pups and pups found dead. The reduced viability index on Day 4 postpartum at 0.5 and 1.5 mg/kg/day was considered adverse and treatment-related. The NOAEL for Day 4 postpartum viability is 0.17 mg/kg/day.

There were no treatment-related effects on survival of pups after Day 4 postpartum or on litter observations during the lactation phase. The NOEL for survival after Day 4 postpartum to weaning is 1.5 mg/kg/day.

There were no adverse E4-related effects on F1 generation development, reproductive performance, or fertility, and the F1 generation NOAEL was determined to be 1.5 mg/kg/day.

Conducting laboratory and location: (b) (4)

GLP compliance: Yes

Table 19. Methods, Study ES0001-NC-003

Method	Details
Dose and frequency of dosing:	0 (vehicle), 0.17 (LD), 0.5 (MD), and 1.5 (HD) mg/kg/day; once daily from GD 6 to GD 18 inclusive and then from Day 1 postpartum (p.p.) to at least Day 21 p.p. inclusive
Route of administration:	Oral gavage
Formulation/Vehicle:	0.5% (w/v) carboxymethylcellulose aqueous solution in purified water
Species/Strain:	Rat / Wistar Han (Rj Han:WI)
Number/Sex/Group:	24 females/group
Study design:	Study Design: The test and control articles were administered to pregnant female rats daily from GD 6 to GD 18, inclusive, and then again from Day 1 p.p. to at least Day 21 p.p. There was a treatment-free period of approximately 3 days on GD 19 to 21. On Day 22 p.p., 20 female and 20 male pups per group were selected to form the F1 generation. F1 animals were monitored for mortality and clinical signs and tested for auditory startle reflex, pupil constriction, learning and memory, spontaneous locomotor activity and the onset of sexual maturation (preputial separation or vaginal opening). At 12 to 15 weeks of age, F1 animals were paired for mating. The F1 females were sacrificed on Day 15 post coital (p.c.). Males were sacrificed after most female hysterectomies had been completed. A macroscopic post-mortem examination was performed for all animals and, in females, the numbers of corpora lutea and implantations (classified as dead and live concepti, early and late resorptions or implantation scars) were recorded. No microscopic examination was performed.
Deviation from study protocol affecting interpretation of results:	No

Table 20. Observations and Results, Study ES0001-NC-003

Generation	Major Findings
F0 dams	Mortality: MD: 1 female found dead on Day 1 p.p. (death occurred soon after or during parturition) and 1 female was prematurely sacrificed as litter was found dead on Day 2 p.p. HD: 8/17 females were prematurely sacrificed due to littering difficulties (2/8), found dead litters (1/8), and/or the absence of pup delivery (5/8). Clinical signs: MD: Reddish vaginal discharge was observed in 1 lactating female. HD: Multiple clinical signs (e.g., pallor of extremities, cold to the touch, piloerection, hypoactivity, prostration, eyes half-closed/pallor and/or blood in the bedding) were noted in females sacrificed prematurely. Reddish vaginal discharge was observed in 4 HD females during the later portion of the gestation period (i.e., GD 14 to GD 20). Body weight:

Generation	Major Findings
	MD/HD: dose-related, statistically reduced BWG during pregnancy between Day 6 and 20 p.c. and reduced BW and BWG (HD only) during lactation between Day 1 and 21 p.p., which correlated with a reduction in food consumption. Parturition: MD/HD: Decreased gestation index (MD: 94.7% and HD: 64.7%) due to a reduced number of females surviving delivery in these groups due to adverse treatment-related effects on parturition, which was also associated with a low number of delivered pups at the HD (6.7 HD vs. 10.6 controls).
F1 generation	Survival indexes prior to weaning: *Viability Index at Day 4 p.p.:* MD: One entire litter died on Day 2 p.p.; however, viability index was not substantially affected by this loss. HD: Increased cannibalized pups and found dead pups resulted in a statistically reduced viability index. *Lactation Index (Day 4 p.p. to Day 21 p.p.):* No treatment-related effects on survival from Day 4 p.p. to Day 21 p.p. *Clinical and necropsy observations prior to weaning*: There were no treatment-related clinical signs, external abnormalities or abnormal behavior in pups, and there were no effects on pup body weight or body weight gain from Day 1 p.p. to Day 21 p.p. There were no treatment-related macroscopic post-mortem findings in found-dead pups nor at necropsy for pups that were not selected at weaning and were euthanized. F1 Generation Development, Fertility, and Reproductive Parameters: There were no treatment-related observations in F1 generation animals for mortality, clinical signs, sexual development, neurobehavioral assessments, mating and fertility, hysterectomy data, or macroscopic necropsy findings. MD/HD: F1 generation males had a slight non-adverse reduction of BW and BWG (-6% to -12% compared to controls) from F1 generation Day 1 through the termination of the study (Day 92), correlating with decreased food consumption in F1 males on Days 29-50.
F2 generation	F2 generation was not assessed.

5.5.6. Other Toxicology Studies of Estetrol Monohydrate

None.

5.5.7. Summary of Toxicity of Drospirenone

The toxicity of DRSP has been well established in humans and nonclinical models. In nonclinical repeat-dose toxicity studies with DRSP, findings attributed to DRSP treatment included effects consistent with the pharmacological hormonal effect of progesterone receptor agonism (e.g., ovarian atrophy, uterine and vaginal changes, stimulatory effect on mammary gland development) and anti-mineralocorticoid effects (e.g., diuresis and electrolyte changes).

The Applicant conducted a 13-week bridging repeat-dose toxicology study that incorporated treatment groups of combined DRSP and E4 at fixed doses compared to DRSP and E4 alone. The observations noted in the group administered 6(4) mg/kg/day DRSP alone reflected exaggerated pharmacological (progestogenic and anti-mineralocorticoid) properties of DRSP, such as a minimal increase of insulin levels, cessation of estrous cycling, changes in female genital tissues and mammary glands, enhanced serum aldosterone, increased adrenal weights associated with minimal to slight hypertrophy of the zona glomerulosa, and marginally lower glycogen content in liver.

A complete battery of genetic toxicology studies has been conducted with DRSP in vitro and in vivo and no evidence of mutagenic activity was observed (YAZ® label, 08/2017).

In a 24-month oral carcinogenicity study in mice with doses up to 10 mg/kg/day DRSP, equating to 2 times the maximum clinical exposure (based on AUC), there was an increase in carcinomas of the harderian gland in the high dose DRSP group. In a similar study in rats given doses up to 10 mg/kg/day DRSP, 10 times the maximum clinical exposure (based on AUC), there was an increased incidence of benign and total (benign and malignant) adrenal gland pheochromocytomas in the high dose DRSP group (YAZ® label, 08/2017).

The nonclinical toxicology sections of the labeling for NEXTSTELLIS will align with the DRSP information as represented in the YAZ approved drug product labeling.

6. Clinical Pharmacology

6.1. Executive Summary

Nextstellis (DRSP/E4) is a new monophasic combined oral contraceptive (COC) containing 3 mg of drospirenone (DRSP) and 15 mg of estetrol (E4) monohydrate (equivalent to 14.2 mg E4). The proposed dosing regimen is one tablet daily for 24 days followed by a 4-day drug free (placebo) interval for each treatment cycle. E4, the estrogen component of the COC, is considered as a new molecular entity (NME) in the U.S. E4 is a synthetic version of a natural hormone produced by the human fetal liver and reaches the maternal circulation through the placenta. DRSP, the progestin component of the COC, has been approved for contraception as a fixed dose combination with ethinyl estradiol (EE) under NDA 021676 (Yaz®, EE/DRSP 0.02 mg/3 mg). The applicant submitted this NDA under 505(b)(2) pathway relying on the Agency's finding of safety and/or effectiveness of DRSP from Yaz®.

The Office of Clinical Pharmacology, Divisions of Cardiometabolic and Endocrine Pharmacology and Pharmacometrics, have reviewed the information contained in NDA 214154 and recommend approval of this NDA. The key review issues with specific recommendations/ comments are summarized in Table 21.

Table 21. Key Review Issues for NDA 214154 With Recommendations and Comments

Review Issues	Recommendations and Comments
Pivotal and supportive evidence of effectiveness and safety	One pivotal phase 3 studies (Study MIT-Es0001-C302, referred to as Study C302) demonstrated the safety and efficacy of DRSP/E4 for the proposed indication of prevention of pregnancy and supportive data was provided by Study C301 (Study MIT-Es0001-C301) phase II dose-finding studies showed adequate ovulation suppression and bleeding control at the selected dose of 14.2 mg E4 combined with 3 mg DRSP. A PK bridging study (Study MIT-Es001-C112) indicated that DRSP/E4 and Yaz® are bioequivalent on systemic exposure of DRSP. At a dose 5 times the maximum recommended dose (i.e., supertherapeutic dose of DRSP/E4 15 mg/71 mg), DRSP/E4 did not prolong the QT interval to any clinically relevant extent.
General dosing instructions	The recommended dosing regimen is one tablet daily for 24 days followed by a 4-day drug free (placebo) interval for each treatment cycle.
Dosing in patient subgroups (intrinsic and extrinsic factors)	DRSP/E4 is contraindicated in patients with renal or hepatic impairment due to potential for increased risk of hyperkalemia. CYP3A inducers: Strong and weak CYP3A induction reduced DRSP concentrations by 86% and 30%, respectively, leading to a potential risk of contraception failure. Therefore, an alternative method of contraception or a back-up method is recommended when CYP3A inducers are used concomitantly with DRSP/E4. Strong CYP3A inhibition: Strong CYP3A inhibitor ketoconazole increased DRSP exposure by 2.6-fold. Consider monitoring serum potassium concentration in women who take a strong CYP3A4 inhibitor long-term and concomitantly with DRSP/E4.
Labeling	Refer to Section 11.1 for the review team's recommendations.
Bridge between the to-be-marketed (TBM) and clinical trial formulations	Not applicable. The TBM formulation was used in the pivotal clinical trials.
Other (specify)	None.

6.2. Summary of Clinical Pharmacology Assessment

6.2.1. Pharmacology and Clinical Pharmacokinetics

Mechanism of Action and Pharmacodynamics (PD)

DRSP/E4 is a fixed-dose combined hormonal contraceptive, which prevents pregnancy primarily by suppressing ovulation.

Absorption

E4 is rapidly absorbed upon oral administration with a median (range) T_{max} value of 0.5 (0.5 to 2) hours. After this initial absorption phase, there are lower secondary reabsorption peaks due to enterohepatic recycling (Figure 1 and Table 22). The PK of E4 is dose-proportional over the dose range of 14.2-71 mg. The absolute bioavailability of E4 has not been determined but is

expected to be ≥69% based on the recovery of administered radioactivity in urine in a mass balance study. Following daily dosing of DRSP/E4, steady-state E4 concentrations were observed after 4 days. Steady-state maximum plasma concentrations ($C_{max,ss}$) was 17.9 ng/mL. $AUC_{(0–24h)}$ of E4 accumulated by a factor of about 1.6 at steady-state compared to that following a single dose administration.

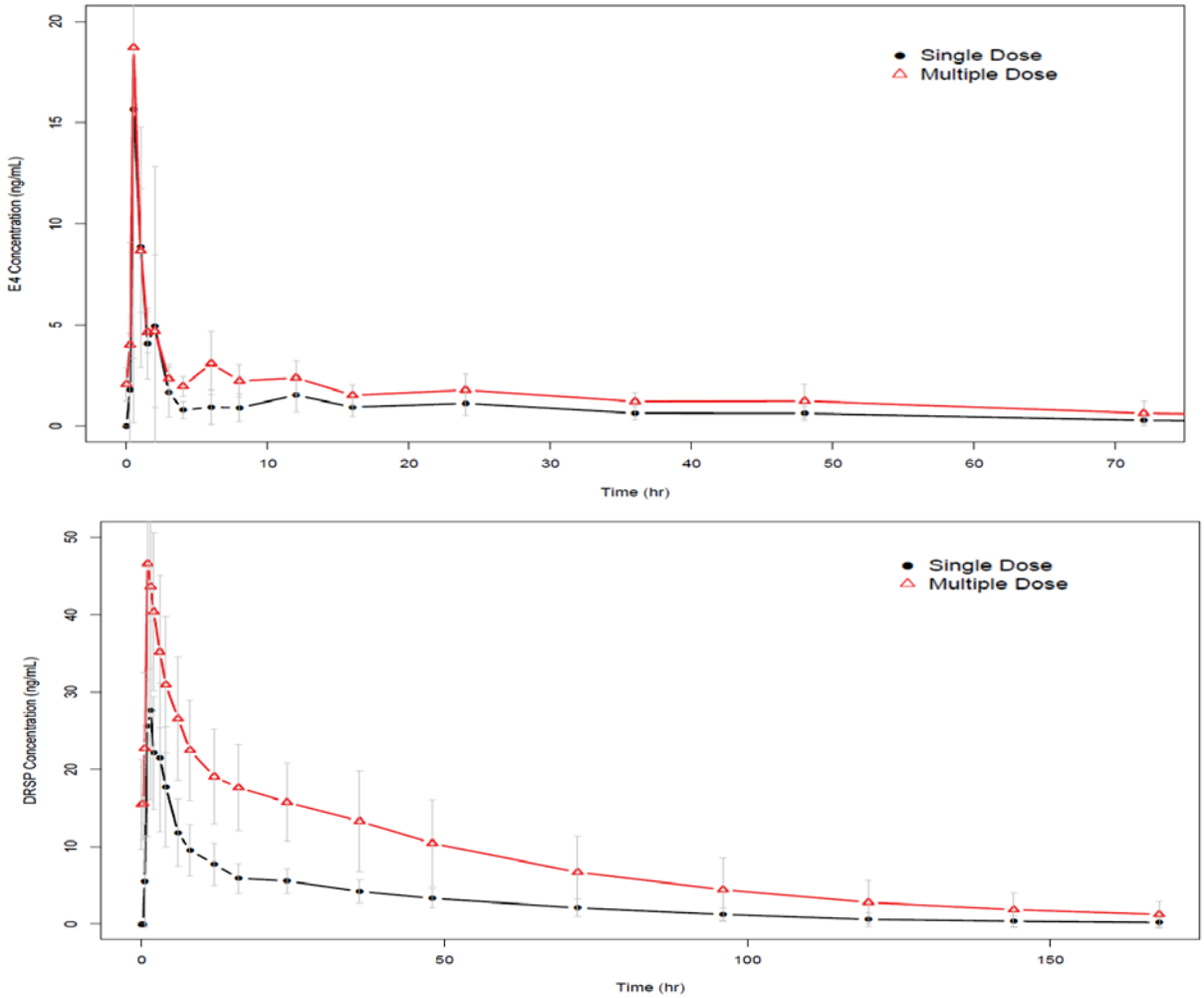
DRSP: Mean $C_{max,ss}$ of DRSP was 48.7 ng/mL and median T_{max} was 1 hour following multiple doses of DRSP/E4 (Figure 1 and Table 22). The PK of DRSP is dose-proportional over the dose range of 1-10 mg. The absolute bioavailability of DRSP was reported to be about 76%. Following daily dosing of DRSP/E4, steady-state DRSP concentrations were observed after 10 days with approximately 2.3-fold accumulation in $AUC_{(0–24h)}$.

Table 22. PK Parameters of E4 and DRSP Following Single and Multiple Oral Administration of DRSP/E4, Study MIT-Es0001-C103

	E4		DRSP	
Parameter	**Day 1 (N=10)**	**Day 14 (N=10)**	**Day 1 (N=10)**	**Day 14 (N=10)**
C_{max} (ng/mL) GM (CV%)	18.0 (61.3)	17.9 (68.1)	32.4 (30.9)	48.7 (24.6)
T_{max} (hr) median (min–max)	0.5 (0.5–2.0)	0.5 (0.5–2.0)	1.5 (1.0–4.0)	1.0 (1.0–3.0)
AUC_{0-24} (ng·hr/mL) GM (CV%)	36.4 (29.9)	59.1 (24.3)	224.0 (31.9)	519.0 (27.7)
R_{AC} GM (min–max)	n/a	1.6 (1.3–2.0)	n/a	2.3 (1.8–3.2)
$t_{1/2}$ (hr) GM (CV%)	24 (39)	24 (27)	33 (30)	34 (32)

Abbreviations: AUC_{0-24}, area under the concentration versus time curve from zero to hour 24; C_{max}, maximum (peak) plasma drug concentration; CV, coefficient of variation; DRSP, drospirenone; E4, estetrol; GM, geometric mean; PK, pharmacokinetic; R_{AC}, accumulation ratio; $t_{1/2}$, elimination half-life; T_{max}, time to reach maximum (peak) plasma concentration following drug administration

Figure 1. Arithmetic Mean (SD) Plasma Concentration Vs. Time Profile for E4 and DRSP Following Single and Multiple Oral Administration of DRSP/E4 (N=10/Group), Study MIT-Es0001-C103



Abbreviations: DRSP, drospirenone; E4, estetrol; SD, standard deviation

Distribution

E4: E4 is 46% - 50% bound to human plasma proteins and does not bind to sex hormone-binding globulin (SHBG). There is limited distribution of E4 into red blood cells.

DRSP: Per Yaz® label, DRSP is 95% to 97% bound to serum albumin and does not bind to SHBG. The apparent volume of distribution of DRSP is approximately 4 L/kg.

Metabolism

E4: E4 undergoes extensive phase II metabolism to form glucuronide and sulfate conjugates. Main metabolites are E4-3-glucuronide and E4-16-glucuronide, both of which are not pharmacologically active. In vitro studies show that UGT2B7 is the dominant UGT isoform that catalyzes the formation of E4-16-glucuronide.

DRSP: DRSP is extensively metabolized after oral administration. Two main metabolites of DRSP are the acid form of DRSP generated by opening of the lactone ring and 4,5-dihydrodrospirenone-3-sulfate via reduction and subsequent sulfation. These metabolites are not pharmacologically active. DRSP is also subject to oxidative metabolism catalyzed by CYP3A4.

Excretion

E4: Following a single oral administration of 14.2 mg [^{14}C]-E4, 69% of the administered radioactivity was detected in urine (all as glucuronide and sulfate conjugates) and 22% in feces (all as unchanged E4). E4 undergoes enterohepatic circulation. The terminal phase half-life was about 24 hours following oral administration of DRSP/E4.

DRSP: DRSP is extensively metabolized and only trace amounts of unchanged DRSP are excreted in urine and feces. The terminal phase half-life of DRSP is about 34 hours following oral administration of DRSP/E4.

6.2.2. General Dosing and Therapeutic Individualization

General Dosing

The proposed dose is 3 mg DRSP combined with 15 mg E4 monohydrate (equivalent to 14.2 mg of E4) as an oral tablet to be administered once daily for 24 days followed by a 4-day drug free (placebo) interval during each treatment cycle which is roughly equivalent to the normal menstrual cycle.

Therapeutic Individualization

Renal Impairment and Hepatic Impairment

The effect of renal or hepatic impairment on the PK of DRSP/E4 has not been studied. As the listed drug Yaz® is contraindicated in patients with renal or hepatic impairment due to increased risk of hyperkalemia, the review team agrees with the applicant to contraindicate the use of DRSP/E4 in these patients.

Obesity

Higher body weight is associated with decreased systemic exposures of E4 and DRSP. Considering the limitation of use in women with BMI ≥30 kg/m^2 in labeling, no further PK information in women with BMI >35 kg/m^2 is required at this time.

Race

Study MIT-Es0001-C109 shows that systemic exposures of E4 and DRSP are comparable between Japanese and Caucasian women following a single dose of DRSP/E4. Other ethnic groups have not been specifically studied.

Pediatric Use

The applicant requests a waiver for pre-menarche children as they are not at risk of becoming pregnant and use of this product before menarche is not indicated. This is clinically acceptable.

The DRSP/E4 clinical pro/gram did not include female subjects below the age of 16 years and there were only 3 subjects aged ≥16 and ≤18 years in the Phase 3 Study. Although PK may be comparable between post-menarcheal adolescents and adults, reduced compliance among adolescents may affect contraceptive efficacy. In addition, safety and tolerability findings observed from adults need to be validated in adolescents. Therefore, (b) (4)

Drug-Drug Interaction (DDI)

Effects of Other Drugs on DRSP/E4

Although E4 is not metabolized by CYP3A, DRSP is a substrate of CYP3A. Strong and weak CYP3A induction decreased the systemic exposure of DRSP by about 86% and 30%, respectively. Therefore, co-administration with drugs or herbal products that induce CYP3A4 may decrease the effectiveness of DRSP/E4. As a result, an alternative method of contraception or a back-up method is recommended when CYP3A inducers are used concomitantly with DRSP/E4. In addition, back-up contraception should be continued for 28 days after discontinuation of CYP3A inducers to ensure contraceptive reliability.

Per Yaz® label, once daily co-administration of DRSP/EE 3 mg/0.02 mg with the strong CYP3A4 inhibitor ketoconazole (200 mg twice daily) for 10 days increased the $AUC_{(0\text{-}24h)}$ of DRSP by 2.68-fold (90% CI: 2.44, 2.95). Due to potentially increased serum potassium levels with elevated DRSP systemic exposures, serum potassium levels may be monitored in high-risk patients who take DRSP/E4 with a strong CYP3A4 inhibitor long-term and concomitantly.

Study MIT-Es0001-C110 showed that co-administration of the strong UGT2B7 inhibitor valproic acid (500 mg BID for 12 days) with a single dose of DRSP/E4 increased the $AUC_{(0\text{-}\infty)}$ and C_{max} of E4 by 1.13- and 1.36- fold, respectively, but did not impact DRSP PK. The exposure changes of E4 are not considered clinically relevant.

Effects of DRSP/E4 on Other Drugs

Currently available data, including in vitro studies of E4 and DRSP and clinical DDI studies of DRSP, do not indicate an inhibitory or induction potential towards human CYP enzymes at clinically relevant concentrations of DRSP/E4.

Outstanding Issues

None.

6.3. Comprehensive Clinical Pharmacology Review

6.3.1. General Pharmacology and Pharmacokinetic Characteristics

Table 23. General Pharmacology and Pharmacokinetic Characteristics

Pharmacology	
Mechanism of action	DRSP/E4 prevents pregnancy primarily by suppressing ovulation.
Active moieties	DRSP and E4
QT prolongation	At a dose 5 times the maximum recommended dose (i.e., supertherapeutic dose of 15 mg DRSP/ 71 mg E4), DRSP/E4 does not prolong the QT interval to any clinically relevant extent.
General Information	
Bioanalysis	LC-MS/MS methods were used to measure DRSP and E4 concentrations in plasma.
Healthy vs. patients	N/A; the target population of DRSP/E4 are healthy pre-menopausal women
Drug exposure at steady state	Following oral administration of DRSP/E4 once per day for 14 days, geometric mean (CV%) values of steady-state C_{max} and AUC_{0-24h} were 17.9 (68%) ng/mL and 59.1 (24%) hr*ng/mL, respectively, for E4, and 48.7 (25%) ng/mL and 519.0 (28%) hr*ng/mL, respectively, for DRSP.
Range of effective dose or exposure	Phase 3 studies assessed the efficacy (Pearl Index) of DRSP/E4 at one dose level, i.e., 3 mg DRSP combined with 14.2 mg E4. See Pharmacodynamics section below.
Maximally tolerated dose or exposure	Maximally tolerated dose was not established. A combination dose of 3 mg DRSP and 18.9 mg E4 were studied in healthy women for 6 months from which no particular safety concerns were identified.
Pharmacodynamics	DRSP/E4 lowers the risk of pregnancy primarily by suppressing ovulation. PD data in Study MIT-Es0001-C202 showed that luteal activity remained adequately suppressed at the selected daily dose of 3 mg DRSP combined with 14.2 mg E4.
Dose proportionality	Following oral administration, E4 and DRSP PK was dose-proportional over the dose range of 14.2-71 mg and 1-10 mg, respectively.
Accumulation	The accumulation ratios of E4 and DRSP upon once daily dosing of DRSP/E4 were about 1.6 and 2.3, respectively.
Variability	At steady-state, CV% values of E4 C_{max} and AUC_{0-24h} were 68% and 24%, respectively. CV% values of DRSP C_{max} and AUC_{0-24h} were 25% and 28%, respectively.
Absorption	
Bioavailability	The absolute bioavailability of E4 has not been determined but is expected to be ≥69% based on data from the mass balance study.
$T_{max,}$ median (min - max)	E4: 0.5 (0.5–2.0) hours; DRSP: 1.0 (1.0–3.0) hours
Food effect	There was no significant food effect on the PK of DRSP/E4.
Distribution	
Volume of distribution (mean CV%)	Apparent volume of distribution (Vz/F) was determined to be 5940 (40%) L for E4 and 339 (23%) L for DRSP.
Plasma protein binding	E4 is 46%–50.4% bound to human plasma proteins and does not bind to SHBG. DRSP is 95% to 97% bound to serum albumin and does not bind to SHBG.
Elimination	
Terminal elimination Half-life (mean CV%)	24 (27%) hrs for E4 and 34 (32%) hrs for DRSP following daily dosing of DRSP/E4
CL/F (mean CV%)	196 (32%) L/hr for E4 and 6.8 (31%) L/hr for DRSP

Metabolism	
Fraction metabolized (% dose)	E4: 69% of total radioactivity was recovered in urine with all as metabolites; DRSP was extensively metabolized and only trace amounts of unchanged DRSP were excreted in urine and feces.
Primary metabolic pathway(s)	E4 undergoes extensive phase 2 metabolism to form glucuronide and sulphate conjugates which have negligible estrogenic activity. UGT2B7 is the dominant UGT isoform that catalyzes formation of E4-16-glucuronide. DRSP is subject to oxidative metabolism catalyzed by CYP3A4 and reduction followed by sulfation.
Excretion	
Primary excretion pathways (% dose)	E4: 69% and 22% of total radioactivity was excreted in urine and feces, respectively. DRSP: 38% and 44% of the total administered dose of radioactivity was detected in urine and feces, respectively.
In vitro interaction liability (Drug as a perpetrator)	
Inhibition/Induction of metabolism	E4 is unlikely to inhibit CYP3A4, CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, UGT1A9 and UGT2B7 or induce CYP1A2, CYP2B6, CYP3A4 at clinically relevant dose. The major metabolites of E4 (E4-3-glucuronide and E4-16-glucuronide) are unlikely to inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A at clinically relevant concentrations. DRSP inhibits human CYP450 enzymes 1A1, 2C9, 2C19 and 3A4 in vitro, with CYP2C19 being the most sensitive enzyme. DRSP does not inhibit CYP2C19 and CYP3A4 in vivo.
Inhibition/Induction of transporter systems	E4 and its major metabolites are unlikely to inhibit transporters P-gp, BCRP, OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K at clinically relevant dose.
In vitro interaction liability (Drug as a victim)	
Metabolism and transporter systems	E4 is not a substrate of CYP1A1, CYP2B6, CYP2C8, CYP2C9, CYP2D6, or CYP3A4. E4 is a substrate of P-gp and BCRP. However, P-gp or BCRP is unlikely to affect E4 PK considering its high cellular permeability. Uptake or efflux solute carrier transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K did not play a role in the cellular transport of E4.

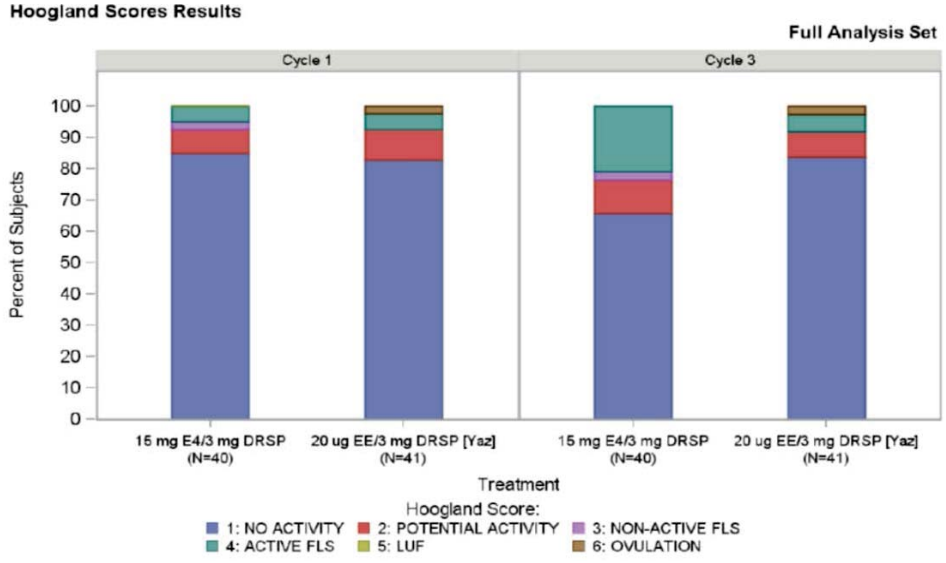
6.3.2. Clinical Pharmacology Questions

Does the clinical pharmacology program provide supportive evidence of effectiveness?

DRSP/E4 lowers the risk of pregnancy primarily by suppressing ovulation. PD data in Study MIT-Es0001-C202 show that luteal activity remains adequately suppressed at the selected dose of 3 mg DRSP in combination with 14.2 mg E4.

Study MIT-Es0001-C202 evaluated the ovarian function inhibition in 82 pre-menopausal women. During the study, eligible studies were randomized in a 1:1 ratio to DRSP/E4 or DRSP/ EE 3 mg /0.02 mg (Yaz®) treatment groups. The designated contraceptives were administered orally once daily in a 24/4-day regimen for three consecutive cycles. Hypothalamic-ovarian activity was monitored by measuring hormone levels and ultrasound assessments of ovarian follicular growth during Cycle 1 and Cycle 3. As shown in Figure 2, no Hoogland scores were above 4, and hence ovulation was adequately suppressed in DRSP/E4 treatment group.

Figure 2. Distribution of Hoogland Scores Following DRSP/E4 and Yaz® Treatments, Study MIT-Es0001-C202



Abbreviations: DRSP, drospirenone; E4, estetrol; FLS, follicle-l ke structure; LUF, luteinized unruptured follicle; N, number of subjects

Is the proposed dosing regimen appropriate for the general patient population for which the indication is being sought?

Yes. The safety and efficacy of DRSP/E4 for prevention of pregnancy have been demonstrated in the Phase 3 studies. The selected daily dose of 3 mg DRSP combined with 14.2 mg E4 for the Phase 3 studies was based on adequate ovulation inhibition and optimal cycle control.

Pilot study: Study PR3081 showed that E4 suppressed ovarian activity in a dose-dependent manner. However, E4 alone in daily doses of up to 18.9 mg was not capable of consistently inhibiting ovulation. Therefore, the applicant conducted 2 additional PD studies (Studies ES-C01 and ES-C02) using different doses of E4 in combination with approved progestins, i.e., DRSP at 3 mg or LNG at 0.15 mg.

Ovulation inhibition: Study ES-C01 showed that 4.7 and 9.5 mg E4 combined with 3 mg DRSP, and 4.7, 9.5 and 18.9 mg E4 combined with 0.15 mg LNG inhibited ovulation in all cycles and the most significant effect on ovarian suppression was achieved with LNG/E4 0.15 mg/18.9 mg.

Cycle control: Study ES-C02 assessed the cycle control of DRSP/E4 3 mg/14.2 mg and LNG/E4 0.15 mg/ 18.9 mg in a 24/4 regimen for 6 cycles compared to marketed COC containing estradiol valerate and DNG (Qlaira®). Of the studied groups, DRSP/E4 3 mg/14.2 mg showed the lowest incidence of unscheduled bleeding (16.9% subjects in Cycle 6) and the lowest incidence (3.5% subjects in Cycle 6) of an absence of withdrawal bleeding.

Dose confirmation study: Study MIT-Es0001-C202 confirmed the robust ovulation inhibition from the selected dose combination of DRSP/E4 3 mg/14.2 mg using Yaz®, a marketed COC, as a comparator.

The following clinical pharmacology studies supported the safety of DRSP/E4.

PK bridging of DRSP/E4 to the listed drug Yaz®

The Applicant proposed to rely on the safety findings of DRSP from Yaz®, an approved COC containing 3 mg DRSP and 0.02 mg EE. To this end, a PK bridging study (Study MIT-Es001-C112) was conducted to compare the rate and extent of DRSP absorption from DRSP/E4 3 mg/14.2 mg (test) and the listed drug Yaz® (reference). The results showed that the 90% CIs on the test/reference geometric mean ratio of C_{max} and AUC are contained within the bioequivalence (BE) acceptance criteria of 80.00% to 125.00% (Table 24). Although single dose was used in this PK bridging study, considering the accumulation ratios of DRSP upon multiple doses are about 2.3 and 2.8 for DRSP/E4 and Yaz®, respectively, it is expected that steady-state DRSP exposure from DRSP/E4 is similar or lower than that from Yaz®.

Table 24. PK Parameters of DRSP Following Single Oral Doses of DRSP/E4 and Yaz® to Healthy Female Subjects, Study MIT-Es001-C112

	DRSP/E4 3 mg/14.2 mg (Test)		DRSP/EE 3 mg/0.02 mg (Reference)			
PK Parameter	**N**	**GM**	**N**	**GM**	**Ratio**	**90% CI (%)**
C_{max} (ng/mL)	27	35.0	27	36.0	97.26	88.63, 106.72
$AUC_{0\text{-tlast}}$ (ng·hr/mL)	27	454	27	493	92.11	88.68, 95.68
$AUC_{0\text{-inf}}$ (ng·hr/mL)	26	504	26	550	91.65	87.89, 95.56

Abbreviations: $AUC_{0\text{-tlast}}$, area under the concentration versus time curve from time 0 to time t (time of last measurable concentration); $AUC_{0\text{-inf}}$, area under the concentration versus time curve from zero to infinity; CI, confidence interval; C_{max}, maximum (peak) plasma drug concentration; DRSP, drospirenone; E4, estetrol; GM, geometric mean; PK, pharmacokinetic

Thorough QT Study

Study Es0001-C106 was performed to evaluate the effect of once daily multiple therapeutic and supratherapeutic doses of DRSP/E4 at steady-state on the QTc interval as compared to placebo. Single-dose oral administration of moxifloxacin was used as a positive control. Based on the review by Dr. Girish Bende (DARRTS on August 31, 2020), at a dose 5 times the maximum approved recommended dose (i.e., supratherapeutic dose of DRSP/E4 15 mg/71 mg), DRSP/E4 does not prolong the QT interval to any clinically relevant extent.

Is an alternative dosing regimen or management strategy required for subpopulations based on intrinsic patient factors?

Yes. DRSP/E4 should not be used in patients with renal or hepatic impairment.

Higher body weight is associated with lower systemic exposures of E4 and DRSP. The impact of observed magnitude of exposure reduction on the efficacy of DRSP/E4 is not known. Based on increased pearl indices, use of DRSP/E4 should be limited in women with BMI ≥30 kg/m^2 (see Section 1.2).Providers and patients will need to use this information to decide whether to use this product based on their individual risk/benefit.

No significant difference was observed between the PK of E4 or DRSP in Japanese versus Caucasian women. Therefore, similar efficacy is expected between the two patient populations and data from the pharmacokinetic and pharmacodynamic studies performed in Japanese participants can be considered applicable to the broader US population.

Renal Impairment

There is no dedicated renal impairment study for DRSP/E4. According to the label of Yaz®, after administration of DRSP 3 mg daily for 14 days, serum DRSP concentrations were on average 37% higher in subjects with creatinine clearance (CLcr) of 30–49 mL/min compared to those in a control group with CLcr ≥80 mL/min. DRSP treatment did not show any clinically significant effect on serum potassium concentration. Although hyperkalemia was not observed in the study, in five of the seven subjects who continued use of potassium-sparing drugs during the study, mean serum potassium concentrations increased by up to 0.33 mEq/L. Therefore, Yaz® is contraindicated in patients with renal impairment due to a potential to develop hyperkalemia. Considering the current product contains the same dose of DRSP as Yaz®, the review team agrees with the applicant to contraindicate the use of DRSP/E4 in women with renal impairment.

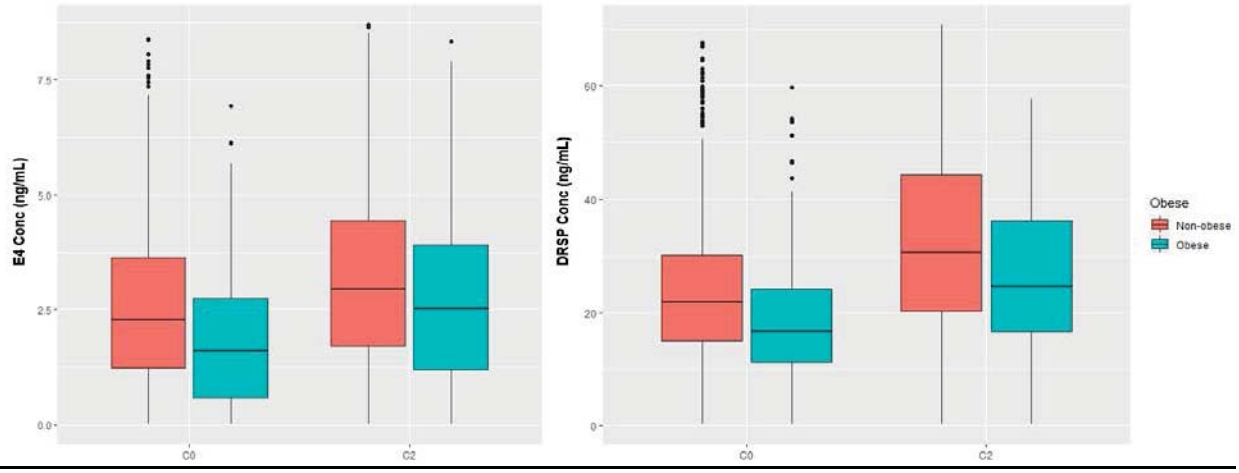
Hepatic Impairment

There is no hepatic impairment study for DRSP/E4. Yaz® is contraindicated in patients with hepatic disease based on three times higher exposure to DRSP in women with moderate liver impairment compared to women with normal liver function. Therefore, the review team agrees with the applicant to contraindicate the use of DRSP/E4 in women with hepatic impairment.

Obesity

Higher body weight is associated with decreased systemic exposures of E4 and DRSP. In the pivotal Phase 3 Study (Study MIT-Es0001-C302), 23% of subjects had a BMI ≥30 to <35 kg/m^2 and steady state concentrations of E4 and DRSP at pre-dose (C_0) and at 2 hours post-dose (C_2) were determined in a subset of 460 subjects. As shown in Figure 3, despite large variability, median values of C_0 and C_2 for both E4 and DRSP were lower in obese women (BMI ≥30 to <35 kg/m^2) compared to non-obese women (BMI <30 kg/m^2). Pearl Indices (95% confidence interval) were 2.57 (1.57, 3.97) and 2.94 (1.08, 6.41) for women with BMI <30 kg/m^2 and BMI ≥30 to <35 kg/m^2, respectively. Per recommendations under Section 1.2, use of DRSP/E4 should be limited in women with BMI ≥30 kg/m^2.

Figure 3. Boxplot of Steady State E4 and DRSP Concentrations at Predose and 2 Hours Postdose, Study MIT-Es0001-C302 (N=334 for Non-Obese Subjects and N=104 for Obese Subjects)



Source: reviewer's analysis based on data from Study MIT-Es0001-C302
Abbreviations: DRSP, drospirenone; E4, estetrol; N, number of subjects in each body weight group

Race

Study MIT-Es0001-C109 showed that systemic exposures of E4 are comparable between Japanese and Caucasian women (Table 25). This finding is consistent with that from a study of Yaz® where comparable DRSP exposures were observed in these 2 ethnic groups.

Table 25. PK Parameters of E4 Following Single Administration of DRSP/E4 in Caucasian and Japanese Women, Study MIT-Es0001-C109

DRSP/E4 Dose	Caucasian/ Japanese	C_{max} (ng/mL) Mean (SD) (N=10)	AUC_{0-72h} (ng·hr/mL) Mean (SD) (N=10)	AUC_{0-inf} (ng·hr/mL) Mean (SD) (N=10)
DRSP/E4 3 mg/4.7 mg	Caucasian	3.19 (1.76)	18.90 (7.85)	22.93 (9.79)
	Japanese	6.14 (3.96)	24.16 (8.92)	28.03 (12.58)
DRSP/E4 3 mg/14.2 mg	Caucasian	12.72 (9.70)	73.97 (26.50)	87.42 (32.65)
	Japanese	13.34 (6.04)	55.46 (24.36)	78.26 (33.56)
DRSP/E4 3 mg/18.9 mg	Caucasian	23.88 (11.85)	87.10 (40.73)	96.54 (44.97)
	Japanese	22.84 (11.55)	98.66 (39.10)	116.80 (46.02)
E4 14.2 mg	Caucasian	19.76 (7.126)	64.10 (27.68)	81.35 (41.56)
	Japanese	17.64 (9.425)	75.93 (19.65)	83.88 (22.15)

Abbreviations: AUC_{0-72h}, area under the concentration versus time curve from time 0 to 72 hours; AUC_{0-inf}, area under the concentration versus time curve from zero to infinity; C_{max}, maximum (peak) plasma drug concentration; DRSP, drospirenone; E4, estetrol; PK, pharmacokinetic; SD, standard deviation

Are there clinically relevant food-drug or drug-drug interactions, and what is the appropriate management strategy?

There is no clinically significant food-drug interaction with DRSP/E4. The applicant conducted several in vitro DDI studies and a clinical DDI study with a strong UGT2B7 inhibitor to address the DDI potential of E4 and proposed to rely on the information from Yaz® for drug interactions with DRSP. Based on the study results, there is no significant drug interaction with E4 at the clinically relevant dose. Per Yaz® Label, drug interactions of DRSP with strong CYP3A inhibitors

and CYP3A inducers are considered as clinically significant due to increased risks of hyperkalemia and contraception failure, respectively.

No Significant Food Effect on DRSP/E4

Study Es0001-C101 showed that high fat meal decreased the rate, but not the extent of absorption of E4 and DRSP following a single dose of DRSP/E4. Geometric mean ratios (90% confidence intervals) of C_{max} were 0.75 (0.66, 0.84) for DRSP and 0.51 (0.37, 0.70) for E4. The peak concentrations of E4 and DRSP were also reached sooner in fasted conditions than in fed conditions. No food restrictions were applied in the Phase 3 pivotal efficacy and safety studies. Overall, DRSP/E4 can be taken with or without food.

Effects of Other Drugs on DRSP/E4

CYP3A Induction:

DRSP is a CYP3A substrate. A recent literature[3] showed that DRSP exposure decreased by 86% (geometric mean ratio of AUC and 90% confidence interval: 0.14; 0.13-0.15) in the presence of a strong CYP3A inducer. In addition, weak CYP3A induction, as confirmed by a 46% decrease in midazolam exposure, resulted in 30% reduction in DRSP exposure (geometric mean ratio of AUC and 90% confidence interval: 0.70; 0.66-0.75). The effect of CYP3A induction on E4 was not studied. Nonetheless, considering that the contraceptive efficacy is mainly driven by progestin, i.e., DRSP, in this case, the effectiveness of DRSP/E4 may be compromised when taken with CYP3A inducers. As a result, an alternative method of contraception or a back-up method is recommended when CYP3A inducers are used concomitantly with DRSP/E4. In addition, back-up contraception should be continued for 28 days after discontinuation of the CYP3A inducer to ensure contraceptive reliability.

CYP3A Inhibition:

Per Yaz® label, co-administration of Yaz® with a strong CYP3A4 inhibitor ketoconazole (200 mg twice daily) for 10 days increased the AUC_{0-24h} and C_{max} of DRSP by 2.68-fold (90% CI: 2.44, 2.95) and 1.97-fold (90% CI: 1.79, 2.17), respectively. Although E4 is not expected to be affected by CYP3A inhibition, serum potassium concentration may be monitored in high-risk patients who take a strong CYP3A4 inhibitor long-term and concomitantly with DRSP/E4.

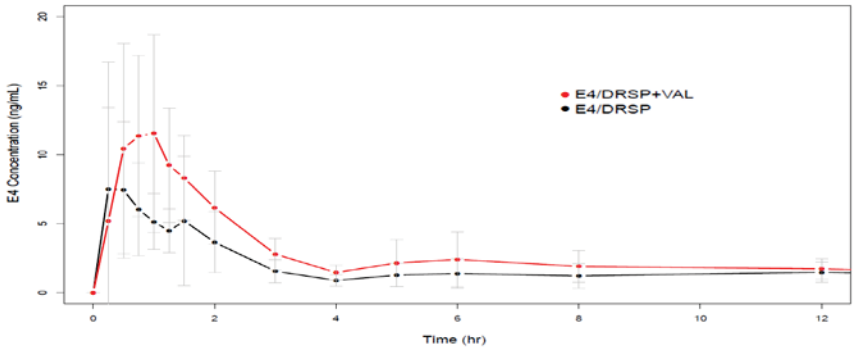
UGT2B7 Inhibition:

In vitro studies showed that the metabolism of E4 is mainly mediated by phase 2 enzymes (UGT and SULT). UGT2B7 is the dominant UGT isoform that catalyzes formation

[3] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7540325/pdf/CPT-108-798.pdf

of E4-16-glucuronide, the major E4 metabolite. A clinical DDI study (Study MIT-Es0001-C110) was performed to assess the effect of valproic acid, a UGT2B7 inhibitor, on the systemic exposure of E4, DRSP, and E4-glucuronide metabolites. The results showed that co-administration of the UGT2B7 inhibitor valproic acid with DRSP/E4 increased $AUC_{0\text{-}168h}$ and C_{max} of E4 by 24% (90% CI 9% - 42%) and 36% (90% CI 11% - 65%), respectively. The metabolite to parent ratios decreased from 2.45 to 2.0 for E4-3-glucuronide and from 4.8 to 2.3 for E4-16-glucuronide in the presence of valproic acid (Figure 4 and Table 26). There was no impact on DRSP PK. These changes are considered of no clinical relevance.

Figure 4. Mean (±SD) Plasma E4 Concentration up to 12 Hours Following Single Oral Doses of DRSP/E4 Administered Alone or in Combination With Valproic Acid, N=21/Group, Study MIT-Es0001-C110



Abbreviations: DRSP, drospirenone; E4, estetrol; N, number of subjects in the treatment group; SD, standard deviation; VAL, valproic acid

Table 26. Assessment of Relative Bioavailability of E4 After Treatment With DRSP/E4 Alone or in Combination With Valproic Acid, Study MIT-Es0001-C110

	Adjusted Geometric Mean		
Parameter	**Treatment A (N=21) (DRSP/E4 + VAL)**	**Treatment B (N=21) (DRSP/E4)**	**Ratio Treatment A/ Treatment B (90% CI)**
C_{max} (ng/mL)	15.6	11.5	135.51% (111.16, 165.19)
$AUC_{0\text{-}tlast}$ (ng·hr/mL)	81.1	65.1	124.51% (109.19, 141.98)
$AUC_{0\text{-}inf}$ (ng·hr/mL)	87.7[a]	77.6[a]	113.06% (102.97, 124.14)

[a] N=16

Abbreviations: $AUC_{0\text{-}tlast}$, area under the concentration versus time curve from time 0 to time t (time of last measurable concentration); $AUC_{0\text{-}inf}$, area under the concentration versus time curve from zero to infinity; CI, confidence interval; C_{max}, maximum (peak) plasma drug concentration; DRSP, drospirenone; E4, estetrol; N, number of subjects in the treatment group; VAL, valproic acid

In vitro studies show that E4 is a substrate of P-gp and BCRP. However, P-gp or BCRP is unlikely to affect E4 PK considering its high cellular permeability. Uptake or efflux solute carrier transporters OATP1B1, OATP1B3, OAT1, OAT3, OCT2, MATE1 and MATE2-K did not play a role in the cellular transport of E4.

Effects of DRSP/E4 on Other Drugs

E4: In vitro studies showed that E4 did not induce CYP1A2, CYP2B6 or CYP3A4 nor inhibit the major CYP isoforms (CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1) and drug transporters (P-pg, BCRP, OATP1B1, OATP1B3, OAT1, OCT2, MATE1, and MATE2-K). E4 inhibited CYP3A4, UGT1A9, UGT2B7 and OAT3 with an IC_{50} of 38.7 µM, 95.5 µM, 89.4 µM, and 16.5 µM, respectively. Given that the steady-state C_{max} of E4 is about 59nM, it is unlikely that E4 will affect the PK of other drugs via inhibition of CYP3A, UGT1A9, UGT2B7 and OAT3.

E4-3-glucuronide: In vitro studies showed that E4-3-glucuronide did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, CYP3A, P-gp, BCRP, MATE1, MATE2-K, OAT1, OAT3, OATP1B3 and OCT2 and is unlikely to inhibit OATP1B1 at clinically relevant concentration (C_{max} of 0.09 µM).

E4-16-glucuronide: In vitro studies showed that E4-16-glucuronide did not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, and CYP2E1, CYP3A, P-gp, MATE1, MATE2-K, OAT1, OAT3, OATP1B3, OCT2 and is unlikely to inhibit BCRP, OAT1, OATP1B3, OCT2, OAT3 at clinically relevant concentration (C_{max} of 0.45 µM).

DRSP: Per Yaz® label, in vitro studies showed that DRSP inhibited CYP1A1, CYP2C9, CYP2C19, and CYP3A4, with CYP2C19 being the most sensitive enzyme. Clinical DDI studies using omeprazole, simvastatin, or midazolam as marker substrates for CYP2C19 and/or CYP3A4 demonstrated that DRSP at dose of 3 mg did not inhibit CYP2C19 or CYP3A4.

7. Sources of Clinical Data and Review Strategy

7.1. Table of Clinical Studies

Table 27 summarizes the clinical studies conducted in support of this application.

Table 27. Listing of Clinical Studies Conducted in Support of DRSP/E4[*]

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
Uncontrolled Clinical Studies to Support Efficacy and Safety							
MIT-Es0001-C301 Efficacy/Safety NCT02817828	Open-label Single-arm Evaluate the contraceptive efficacy, cycle control, impact on psychological and social functioning and well-being, and endometrial safety	DRSP/E4 combined tablet DRSP/E4 3/14.2 mg	Primary: Number of on-treatment pregnancies by Pearl Index in ITT population	Multiple dose 13 cycles[***]	1553	Healthy premenopausal females aged 18-50 years	Multicenter (69) Belgium Czech Republic Finland Germany Hungary Norway Poland Sweden Russia
MIT-Es0001-C302 Efficacy/Safety NCT02817841	Open-label Single-arm Evaluate the contraceptive efficacy, cycle control, impact on psychological, and social functioning and well-being, and the effect of various individual characteristics/covariates (e.g., body weight, race, smoking, and fed/fasted condition) on the PK of E4 and DRSP	DRSP/E4 combined tablet DRSP/E4 14.2/3 mg	Primary: Number of on-treatment pregnancies by Pearl Index in ITT population	Multiple dose 13 cycles[*]	1864	Healthy premenopausal females aged 16-50 years	Multicenter (77) USA Canada

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
Controlled Clinical Studies							
ES-C01 Efficacy/Safety (dose-finding)-PD N/A	Randomized Open-label Active-control Dose-finding Investigate the ovulation inhibition, PD effects on liver function	E4 and DRSP separate tablets E4 and LNG separate tablets DRSP/EE combined tablet (Yaz®) DRSP/E4 3/5 mg DRSP/E4 3/10 mg LNG/E4 0.15/5 mg LNG/E4 0.15/10 mg LNG/E4 0.15/20 mg DRSP/EE 3/0.02 mg	Primary: Follicle development Liver parameters Serum LH, FSH, E2, P, and T Return to fertility by follicular growth on TVUS Vaginal bleeding patterns Endometrial thickness by TVUS PK parameters of E4 Metabolism and excretion of E4 in urine Safety and tolerability Participant well-being by questionnaire	Multiple dose 3 cycles*	109	Healthy premenopausal females aged 18-35 years	Single center Netherlands

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
ES-C02 Efficacy/Safety (dose-finding)-PD N/A	Randomized Open-label Active-control Dose-finding Evaluate vaginal bleeding patterns (cycle control)	E4 and DRSP separate tablets E4 and LNG separate tablets DRSP/EE combined tablet (Yaz®) DNG/E2V combined tablet (Qlaira)	Primary: Cycle control Ovulation inhibition Serum SHBG Pregnancy rate Participant satisfaction and health-related effects Assessment of acne Body weight Return of menstruation after treatment discontinuation Acceptability AEs, VS, PE, pap smear, Labs	Multiple dose 6 cycles*	389	Healthy premenopausal females aged 18 to 35 years	Multicenter Finland
Comparative BA and BE Studies							
0030CA001 BA/BE Safety N/A	Two-way crossover BE between a combined tablet vs. two separate tablets	DRSP/E4 combined tablet E4 and DRSP separate tablet E4 15 mg DRSP 3 mg	Primary: PK parameters for E4 and DRSP AEs, VS, ECG, PE, Labs	Single dose	Part 1: 12 Part 2: 36	Healthy premenopausal females aged 18 to 45 years	Single center Belgium
0031CA001 BA/BE Safety N/A	Two-way crossover BA between a combined tablet vs. two separate tablets	LNG/E4 combined tablet E4 and LNG separate tablet E4 20 mg LNG 0.15 mg	Primary: PK parameters for E4 and LNG AEs, VS, ECG, PE, Labs	Single dose	28	Healthy premenopausal females aged 18 to 45 years	Single center Belgium

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
0031CA002 BA/FE Safety N/A	Two-way crossover Food-effect	LNG/E4 combined tablet E4 20 mg LNG 0.15 mg	Primary: PK parameters for E4 and LNG AEs, VS, ECG, PE, Labs	Single dose	28	Healthy premenopausal females aged 18 to 45 years	Single center Belgium
Es0001-C101 BA/FE Safety NCT02852681	Two-way crossover Food-effect	DRSP/E4 combined tablet E4 15 mg DRSP 3 mg	Primary: PK parameters for E4 and DRSP AEs, VS, ECG, PE, Labs	Single dose	28	Healthy premenopausal females aged 18 to 45 years	Single center Bulgaria
MIT-Es001-C112 BA Safety NCT02817841	Open-label Randomized Two-period Two-treatment Two-way crossover Compare DRSP BA	DRSP/E4 15/3 mg DRSP/EE 3/0.02 mg (Yaz®)	Primary: PK parameters for DRSP/E4 and DRSP/EE AEs, VS, ECG, PE, Labs	Single dose	28	Healthy female participants aged 18 to 50 years	Single center USA
Healthy Participant PK and Initial Tolerability Studies							
PR3050 Safety PK/PD N/A	Randomized Double-blind Placebo-controlled Safety Tolerability PK/PD	E4 solution/ E4 0.1 mg E4 1 mg E4 10 mg E4 100 mg Placebo	Primary: Safety and tolerability of E4 PK and PD parameters of E4	Single dose	32	Healthy post-menopausal females	Single center Netherlands
PR3054 Safety PK/PD N/A	Partly randomized Open-label Safety Tolerability PK/PD	E4 solution-E2V tablet E4 2 mg E4 10 mg E4 20 mg E4 40 mg E2V 2 mg/	Primary: Safety and tolerability of E4 PK and PD parameters of E4 and E2	Multiple dose 28 days	49	Healthy post-menopausal females not older than 70 years of age	Single center Netherlands

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
PR3077 Safety PK/PD N/A	Randomized Double-blind Placebo-controlled Safety Tolerability PK/PD	E4 solution E4 2 mg E4 10 mg Placebo	Primary: Safety and tolerability of E4 PK and PD parameters of E4	28 days	20	Healthy men aged 40 to 70 years	Single center Netherlands
MIT-Es0001-C102 PK Safety NCT03075956	Randomized Open-label PK	E4 tablet E4 5 mg E4 15 mg E4 45 mg	Primary: PK parameters of E4 Safety and tolerability of E4	Single dose and Multiple dose for 14 days	31	Healthy females aged 18-55 years	Single center Bulgaria
MIT-Es0001-C103 PK Safety NCT02874248	Randomized Double-blind Placebo-controlled PK	DRSP/E4 combined tablet Placebo tablet DRSP/E4 3/15 mg DRSP/E4 6/30 mg DRSP/E4 12/60 mg DRSP/E4 15/75 mg Placebo	Primary: PK parameters of E4 and DRSP Safety and tolerability of E4 and DRSP Effect of E4 on QTc Effect of E4 on HR, PR, and QRS	Single dose and Multiple dose for 14 days	39	Healthy pre-menopausal females aged 18-50 years	Single center Netherlands
MIT-Es0001-C105 Mass-balance PK Safety NCT02720224	Open-label Mass balance recovery PK E4 metabolite profile E4 metabolite identification	E4 solution [^{14}C]-E4 15 mg	Primary: PK parameters for absorption, metabolism, and excretion of E4 Safety and tolerability of E4	Single dose	6	Healthy female volunteers of non-childbearing potential	Single center United Kingdom

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
MIT-Es0001-C106 PK Safety NCT03512860	Randomized Double-blind Parallel group with nested crossover PK Investigate the effects of therapeutic and supratherapeutic concentrations of DRSP/E4 on heart rate corrected QT interval (QTc)	DRSP/E4 combined tablet Placebo tablet Moxifloxacin tablet DRSP/E4 3/15 mg DRSP/E4 15/75 mg Placebo Moxifloxacin 400 mg	Primary: Plasma concentrations and PK parameters of E4, DRSP, and moxifloxacin AEs, VS, PE, labs, ECG, Holter monitoring	Multiple dose for 10 days with therapeutic dose followed by multiple dose for 10 days with supra-therapeutic dose	64	Healthy premenopausal females aged 18-55 years	Multicenter USA
Intrinsic Factor PK Study							
MIT-Es0001-C109 PK Safety N/A	Randomized Double-blind Placebo-controlled Ethno-bridging study Characterize and compare the PK of E4 and DRSP in Japanese and Caucasian participants	E4 tablet DRSP/E4 combined tablet Placebo tablet E4 15 mg DRSP/E4 3/5 mg DRSP/E4 3/15 mg DRSP/E4 3/20 mg	Primary: PK parameters of E4 and DRSP Safety and tolerability of DRSP/E4 combination and E4 alone	Single dose	96	Healthy premenopausal females aged 18 to 50 years of Japanese and Caucasian origin	Multicenter USA

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
Extrinsic Factor PK Study							
MIT-Es0001-C110 DDI NCT03512860	Two-way crossover Evaluate the effect of VAL on the PK of E4 and DRSP	DRSP/E4 combined tablet VAL tablet DRSP/E4 3/15 mg VAL 500 mg	Primary: PK parameters of DRSP/E4 Safety of DRSP/E4 alone or in presence of VAL Plasma concentrations of E4, DRSP, and E4-glucuronide metabolites	DRSP/E4 Single dose on two occasions VAL: 500 mg BID for 11 days and a final dose on day 12	48	Healthy premenopausal females aged 18-45 years	Multicenter USA United Kingdom
Human Pharmacodynamic Studies							
PR3081 PD-PK/ PD-Safety N/A	Randomized Open-label Feasibility Investigate: PD (ovarian suppression and HPO axis inhibition) Vaginal bleeding pattern PK of E4 Safety Tolerability	E4 solution DSG tablet P4 capsule E4 10 mg E4 20 mg E4 20 mg + DSG 0.15 mg E4 20 mg + P4 200 mg	Primary: Follicle development and endometrial thickness by TVUS Serum levels of LH, FSH, E2, P, and SHBG Vaginal bleeding pattern PK of E4 Safety and tolerability	Multiple dose 28 days	52	Healthy premenopausal females aged 18 to 40 years	Single center Netherlands

Trial Identity Purpose of Trial NCT no.	Trial Design/ Objective	Regimen/ Schedule/ Route**	Study Endpoint(s)	Treatment Duration/ Follow Up	No. of Participants Enrolled	Study Population	No. of Centers and Countries
MIT-Es0001-C201 Safety NCT02957630	Randomized Open-label Active-control Evaluate the effects on hemostasis, endocrine function, and lipid and carbohydrate metabolism parameters	DRSP/E4 combined tablet LNG/EE combined tablet DRSP/EE combined tablet DRSP/E4 3/15 mg LNG/EE 0.15/0.03 mg DRSP/EE 3/0.02 mg	Primary: Hemostatic and endocrine parameters Liver proteins Lipid profile AEs, VS, PE, Labs, ECG	Multiple dose 6 cycles*	98	Healthy premenopausal females aged 18 to 50 years	Single center Netherlands
MIT-Es0001-C202 Efficacy/Safety NCT03091595	Randomized Open-label Active-control Evaluate the effects on ovarian inhibition	DRSP/E4 combined tablet DRSP/EE combined tablet DRSP/E4 3/15 mg DRSP/EE 3/0.02 mg	Primary: Hoogland score at Treatment Cycle 1 and Treatment Cycle 3 Serum LH, FSH, E2, and P Double layer endometrial thickness by TVUS Return to fertility by follicular growth on TVUS Safety and tolerability by AEs, VS, PE, Labs, ECG, and echocardiogram	Multiple dose 3 cycles*	82	Healthy premenopausal females aged 18 to 35 years	Single center Netherlands

Source: NDA 214154, Tabular Listing of Clinical Studies (m5.2, Table 5.2, eCTD SN:0001)
* Dose of E4 is expressed for the estetrol monohydrate form
** All routes were oral unless specified otherwise.
*** 1 cycle =28 days
Abbreviations: AEs, adverse events; BA, bioavailability; BE, bioequivalence; DDI, drug-drug Interaction; DRSP, drospirenone; DSG, desogestrel; E2, estradiol; E2V, estradiol valerate; E4, estetrol monohydrate; ECG, electrocardiogram; FE, food-effect; FSH, follicle-stimulating hormone; HR, heart rate; ITT, intent-to-treat; Labs, clinical laboratory tests; LH, luteinizing hormone; LNG, levonorgestrel; P, progesterone; P4, progesterone; PD, pharmacodynamic; PE, physical examination; PK, pharmacokinetic; PR, P-R interval; QRS, QRS interval; SHBG, sex-hormone binding globulin; T, testosterone; TVUS, transvaginal ultrasound; VAL, valproic acid; VS, vital signs

7.2. Review Strategy

The overall strategy for review of this marketing application for DRSP/E4 includes a primary focus on a single Phase 3 trial – MIT-Es0001-C302 (Study C302; NCT02817841) conducted in the target US population for the efficacy assessment, which is adequate to provide substantial evidence of effectiveness for a contraceptive product. Additional analyses from MIT-Es0001-C301 (Study C301, NCT02817828) provide supportive evidence for effectiveness. The safety assessment, however, includes analysis of adverse events across all studies in which participants received the to-be-marketed dose of DRSP/E4 (3/14.2 mg) for at least three 28-day treatment cycles.

Study C302 forms the primary focus of the efficacy review because the majority of study sites were located in the US with a small component in Canada, and therefore more accurately reflects the target US population. Key differences between the two Phase 3 trials – Study C302 versus Study C301 – include: 1) differences inherent to the target populations (e.g., higher rates of smoking in Europe, higher BMI in the US), and 2) differences in calculation of the Pearl Index between FDA and other regulatory agencies (e.g., definition of evaluable cycles; definition of "on-treatment" pregnancy; etc.). See details in Section 8.1.

The review of the safety profile for DRSP/E4 includes assessments of serious adverse events across all studies in which subjects received DRSP 3 mg and E4 14.2 mg. In addition, both Phase 3 trials – Study C302 and Study C301 – as well as Phase 2 studies – ES-C02, MIT-Es0001-C201 and MIT-Es0001-C202, are included in the integrated safety assessment. However, Studies Study C301 and Study C302 only determined the safety profile for labeling purposes. See details in Section 8.2.

8. Statistical and Clinical and Evaluation

8.1. Review of Relevant Individual Trials Used to Support Efficacy

8.1.1. Study MIT-Es0001-C302 (Study C302)

Trial Design

This was a Phase 3, multicenter, open-label, single-arm trial to evaluate the contraceptive efficacy and safety of DRSP/E4 – a combined oral contraceptive containing drospirenone 3 mg and estetrol 14.2 mg. The design and conduct of this trial was generally consistent with previous phase 3 trials for contraceptive products. The trial was performed in 77 sites in North America: 70 sites in the US and 7 sites in Canada.

The primary objective was to evaluate the contraceptive efficacy of DRSP/E4 using the Pearl Index in participants aged 16 to 35 years, inclusive, at the time of screening. The secondary objectives of this study (as stated by the Applicant) were:

1. To evaluate the contraceptive efficacy of DRSP/E4 using the "method failure" Pearl Index and life-table analysis in subjects aged 16 to 35 years, inclusive.
2. To evaluate the contraceptive efficacy of DRSP/E4 using the Pearl Index, the "method failure" Pearl Index and life-table analysis in the overall study population.
3. To evaluate cycle control and bleeding pattern associated with DRSP/E4.
4. To evaluate the general safety of DRSP/E4.
5. To evaluate the impact of DRSP/E4 on physical, psychological, and social functioning and well-being.
6. To assess the effect of various individual characteristics/covariates (e.g., body weight, race, smoking, and fed/fasted condition) on the pharmacokinetics (PK) of DRSP/E4 (Population PK Sub-study).

Eligible subjects were to be treated with DRSP/E4 in a 24/4-day regimen (i.e., 24 active tablets followed by 4 inactive tablets) for up to a maximum of 13 consecutive cycles. Participating subjects were asked to record their daily pill intake, use of other contraceptive methods, the occurrence of sexual intercourse, and their daily bleeding/spotting occurrences in a subject diary. Those subjects who discontinued and desired a pregnancy were to be followed for a maximum of one year after study discontinuation for return of post-treatment spontaneous menstruation and until pregnancy or initiation of a contraceptive method, whichever occurred first.

Study Population

The study enrolled healthy female subjects at risk for pregnancy, between ages 16 and 50 (inclusive at the time of screening) and requesting contraception.

Key inclusion criteria consisted of the following:

1. Heterosexually active female at risk for pregnancy and requesting contraception.
2. Negative serum pregnancy test at enrollment.
3. Aged 16 to 50 years inclusive at the time of signing of informed consent.
4. Willing to use the investigational product as the primary method of contraception for 13 consecutive cycles.
5. Body mass index ≤35.0 kg/m^2.

Key exclusion criteria consisted of the following:

1. Menstrual cycle length shorter than 21 days or longer than 35 days.
2. Smoking nicotine-containing products if ≥35 years old.

3. Increased risk for cardiovascular events including: dyslipoproteinemia requiring active treatment with antilipidemic agent; diabetes mellitus with vascular involvement; hypertension with vascular involvement, etc.
4. Increased risk for venous thromboembolism including personal history of deep vein thrombosis (DVT) or pulmonary embolism (PE); known hypercoagulopathy, etc.
5. Undiagnosed abnormal vaginal bleeding
6. Hyperkalemia, or condition that predisposes to hyperkalemia
7. Presence or history of hormone-related malignancy
8. Renal or hepatic impairment
9. Concomitant use of medications with known or potential for drug-drug interaction with study product

The inclusion/exclusion criteria were consistent with other trials for combined hormonal contraceptive products.

Study Endpoints

The primary efficacy endpoint of the study was the number of on-treatment pregnancies assessed by the Pearl Index based on at-risk cycles in subjects aged 16 to 35 years in the Intent-to-Treat (ITT) Population.

The secondary endpoint of interest to the clinical review team included:

The cumulative pregnancy rate estimated by the life-table method based on all cycles for subjects aged 16 to 35 years in the ITT Population, respectively.

Statistical Analysis Plan

Analysis Populations (Definitions)

Screened Population included all subjects who signed an informed consent form. The Screened Population was used for disposition data.

Enrolled Population included enrolled subjects (i.e., subjects who had a non-missing enrollment date on the enrollment page of the CRF).

Safety Population included all enrolled subjects who receive at least one dose of study medication.

Intent-to-Treat (ITT) Population was the same as the Safety Population. The ITT Population were used for baseline, protocol deviations, bleeding, quality of life data, and contraceptive efficacy analysis.

Sample Size Consideration

The applicant proposed a study sample size to have a sufficient number of cycles such that the difference between the Pearl Index and the upper limit of the two-sided 95% confidence interval (CI) for the Pearl Index did not exceed 1. Assuming that the true Pearl Index was 1.0 and that a Poisson model was used to derive the CIs, then at least 12,337 at-risk cycles were required for a power of 90% in the 16 to 35-year-old population (Gerlinger et al., 2003[4]).

Assuming 80% of the study cycles were at-risk cycles and a dropout rate of approximately 45% (assuming that an average of 4 cycles was for subjects that discontinued) were assumed, approximately 1800 subjects aged 16 to 35 years old needed to be enrolled. Additionally, it was planned to enroll a maximum of 200 subjects 36-50 years. Therefore, in total, approximately 2000 subjects were to be enrolled in the study. Additionally, a subset of approximately 500 subjects were to be enrolled in the PK Substudy.

Analysis for Primary Endpoint

The Pearl Index was summarized in subjects aged 16 to 35 years in the ITT Population using the at-risk cycles. The Pearl Index was presented with corresponding 95% CI.

The Pearl Index, defined as the number of pregnancies per 100 women-years of treatment, were calculated as:

$$Pearl\ Index = \frac{Number\ of\ on-treatment\ pregnancies}{number\ of\ 28\ day\ equivalent\ cycles\ of\ treatment} \times 1300$$

Only at-risk cycles were included in the denominator of the Pearl Index calculation. At-risk cycles were defined as cycles in which the following criteria were met:

- No other methods of birth control (including condoms and emergency contraception) are used by the subject as confirmed in the subject diary
- The subject confirmed that sexual intercourse occurred during the cycle in the study diary

If conception occurred in a treatment cycle, then that cycle was included as an at-risk cycle in the denominator even if other methods of birth control were used during that cycle or the subject did not confirm that they had sexual intercourse. Cycles after the cycle of conception were not included as at-risk cycles and were excluded from the denominator to calculate the Pearl Index.

All on-treatment pregnancies were included in the numerator for the Pearl Index. The on-treatment pregnancies were defined as pregnancies with an estimated date of conception during the on-Treatment Period for contraceptive efficacy. On-Treatment Period was defined as

[4] Gerlinger, C., Endrikat, J., van der Meulen, E. A., Dieben, T. O. & Dusterberg, B. Recommendation for confidence interval and sample size calculation for the Pearl Index. Eur.J.Contracept.Reprod.Health Care 8, 87-92 (2003).

the period of time between the date of first dose of study medication and 7 days after the last dose of study medication, inclusive.

The number of 28-day equivalent cycles of treatment was the sum of the number of at-risk cycles across subjects.

The 95% CI for the Pearl Index was calculated using a Poisson distribution. The confidence interval limits were calculated by the following equations:

$$CL_{\text{lower}} = \frac{\frac{1}{2}\chi^2\,(0.025, 2x)}{number\ of\ 28\ day\ equivalent\ cycles\ of\ treatment} \times 1300$$

$$CL_{\text{upper}} = \frac{\frac{1}{2}\chi^2\,(0.025, 2(x+1))}{number\ of\ 28\ day\ equivalent\ cycles\ of\ treatment} \times 1300$$

Where, x was the number of on-treatment pregnancies, $\chi^2\ (alpha, df)$ is the alpha quantile from chi-square distribution with df degrees of freedom.

The Pearl Index and the corresponding 95% confidence interval based on at-risk cycles for subjects aged 16 to 35 years were presented for the following subgroup categories:

- Body Mass Index (BMI) categories (<30 kg/m^2, ≥30 kg/m^2) at Baseline
- Race category (White, Black or African American, Asian, American Indian or Alaska Native, Native Hawaiian or other Pacific Islander, Other Races). Other races group included subjects who selected more than one race or whose race was recorded as other race on the CRF.
- Starters (including true new users)/Switchers. Starters were defined as subjects who had not used hormonal contraceptives within the 3 months prior to the date of first dose of study medication. Switchers were defined as subjects who had used hormonal contraceptives within the 3 months prior to the date of first dose of study medication.
- Smokers/Non-smokers at Baseline. The non-smokers group included subjects who are former smokers and never smokers. The smokers group included subjects who are current smokers.
- Age (≥16 to ≤25; >25 to ≤35 years) at Screening

Analysis for Secondary Endpoints

The cumulative pregnancy rate and the corresponding 95% CI, as determined by the life table method, were presented based on on-treatment pregnancies and all cycles for subjects aged 16 to 35 years in the ITT Population. Life Table methodology was analyzed as a sensitivity analysis for the PI.

Protocol Amendments

There were no major protocol amendments that significantly altered the methodology or analyses of primary efficacy and safety outcomes in Study C302. However, several minor protocol amendments occurred during the study. None of these amendments contained changes that were objectionable to the clinical or statistical review team. See Section 17.8 for a summary of all protocol amendments.

8.1.2. Study Results

Compliance With Good Clinical Practices

The Applicant states that the study was conducted in accordance with Good Clinical Practice (GCP) as contained in ICH Guidelines and US CFR governing the protection of human subjects (Title 21, Part 50) and the obligations of clinical investigators (Title 21, Parts 312.60 through 312.69). The Applicant also states the study was conducted in accordance with World Medical Association Declaration of Helsinki and all amendments.

As previously mentioned, there was a complaint received by the FDA and other regulatory agencies regarding GCMP violations by the applicant. After investigation, none of the issues identified during inspection by the three agencies (see review Section 4) affect the approvability of this application.

Financial Disclosure

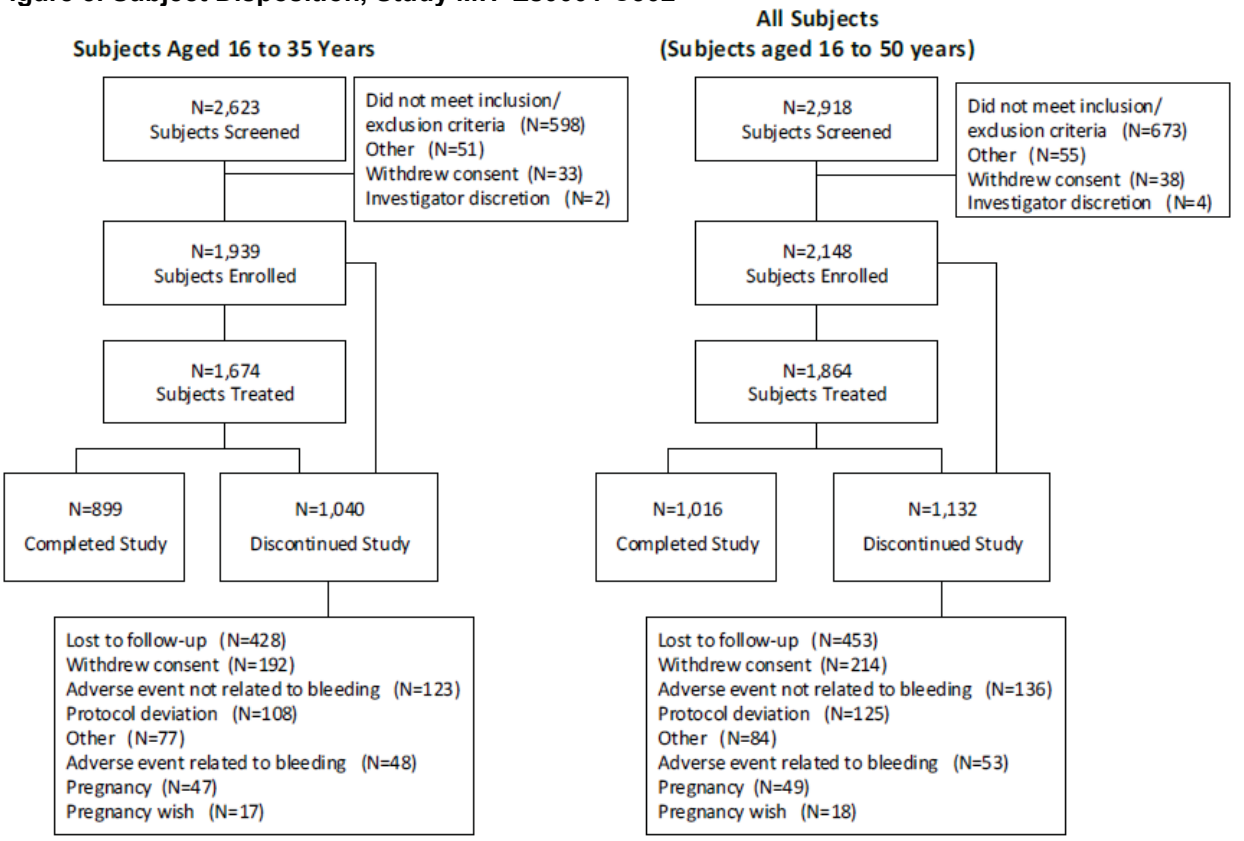
The Applicant has submitted the names of all clinical investigators who conducted covered clinical studies in accordance with 21 CFR part 54. The Applicant certifies that all financial interests and arrangements have been assessed and monitored from the initiation to the end of the study and one year after study completion. A form 3455 was not necessary as there were no clinical investigators (or spouses or dependent children) that had disclosable financial interests in and/or arrangements with the sponsor of the covered clinical study.

Refer to Section 17.2 for detailed review of the financial disclosures.

Subject Disposition

A total of 2,918 subjects were screened for the study, of whom 2,148 were enrolled: 1,939 subjects aged 16 to 35 years and 209 subjects aged 36 to 50 years. Overall, 1,864 of 2,148 (86.7%) enrolled subjects took study medication (i.e., subjects in the Safety and ITT Population), including 1,674 of 1,939 (86.3%) subjects aged 16 to 35 years in the ITT Population. See Figure 5 below.

Figure 5. Subject Disposition, Study MIT-Es0001-C302



Source: Study MIT-Es0001-C302 clinical study report Figure 1 and confirmed by the statistical reviewer.

For all subjects enrolled in the study, 1,016 of 2,148 (47.3%) subjects completed the study. The most common reasons for study discontinuation were lost to follow-up (453 subjects, 21.1%), consent withdrawal (214 subjects, 10.0%), AEs not related to bleeding (136 subjects, 6.3%), protocol deviations (125 subjects, 5.8%), and AEs related to bleeding (53 subjects, 2.5%). The cumulative discontinuation rate from the Kaplan-Meier estimate for the enrolled population and the ITT population are presented in Figure 9 and Figure 10 in the Appendix.

In response to the Division's information request submitted on October 2, 2020, the applicant explained the major reasons for 13.2% (N=284) of the enrolled subjects to discontinue the study prior to treatment start were due to lost to follow-up (7.7%, N=166), withdrawal of consent (1.4%, N=31) and protocol deviations (1.4%, N=31). See Figure 11 in the Appendix.

This preceding paragraph highlights the fact that lost to follow-up is fairly prevalent even prior to treatment. Unfortunately lost to follow-up throughout trials seems to plague many U.S. contraceptive trials. It is more commonly seen in the U.S. compared to Europe. The Agency discussed the issue with the Applicant during this review cycle. One difference between the two regions is that there are more research centers in the U.S. running these trials compared to individual offices in Europe. It is possible that study participants may be more comfortable with their own health care providers who they have seen for a long time and be more likely to follow up with care.

In response to the Division's information request submitted on December 8, 2020, the applicant explained that the cumulative discontinuation rate was higher in Black or African American than White, or other races (Figure 12 in the Appendix), and higher in subjects with BMI ≥30 kg/m^2 than subjects with BMI <25 kg/m^2 or BMI between 25 kg/m^2 and 30 kg/m^2 (Figure 13 in the Appendix).

Protocol Violations/Deviations

The Applicant defined important protocol deviations as those that might significantly affect the completeness, accuracy, and/or reliability of the study data or that might significantly affect a subject's rights, safety, or well-being. Protocol deviations determined to be "important" by the applicant were reported in 1026 subjects (55.0%). Protocol deviations determined by the Review Division to be "important" were those related to the timing of missed pregnancy tests, as this could have implications for the efficacy analysis.

Study drug-related protocol deviations identified by the Applicant constituted the most common type of important protocol deviations (e.g., the subject did not return study drug blisters, did not follow the instructions for missing pills, was not compliant with the instructions for use, etc.). The Applicant reported study drug-related protocol deviations in 477 subjects (25.6%); these were the most common type of protocol deviation leading to discontinuation.

Other types of protocol deviations reported in >5% of subjects were:

1. Laboratory/endpoint data (e.g., laboratory samples taken without fasting, the subject did not return the diaries for some cycles or did not return questionnaires, etc.), reported in 430 subjects (23.1%);
2. Safety assessment (e.g., urine pregnancy test not performed despite the subject not having menses, physical or gynecological examinations not performed, etc.), reported in 208 subjects (11.2%);
3. Visit window, reported in 188 subjects (10.1%);
4. IC (e.g., subject did not re-consent and updated IC, re-consent process not correctly documented, etc.), reported in 95 subjects (5.1.%); and
5. Prohibited co-medication (e.g., fluconazole), reported in 94 subjects (5.0%).

Clinical review identified significant protocol deviations related to missed pregnancy tests in six subjects in Study C302. In response to an Information Request sent by the Review Division on March 16, 2021, the Applicant provided clarification regarding the missed pregnancy testing in these six subjects. One subject discontinued due to pregnancy wish, and one subject discontinued due to withdrawal of consent. Two subjects were discontinued from study participation due to protocol deviations unrelated to the missed pregnancy test. Two subjects were lost to follow-up, with documentation of appropriate steps taken to contact the subject. The pregnancy status of the two lost-to-follow-up subjects is unknown.

After review, the clinical and statistical review teams concurred that none of these deviations had a significant clinical impact on the primary effectiveness results. The deviations likely

represent the types of issues that will occur in the overall population that will use these products in the US. However, assessment by specific demographic and baseline characteristics is descriptive only.

Demographics and Baseline Characteristics

Subjects' demographics and baseline characteristics in the ITT Population are shown in Table 28. 69.7% of the subjects were white (70.1% in subjects aged 16 to 35 years), and 19.8% were black or African American (19.5% in subjects aged 16 to 35 years). 26.2% of subjects (25.6% in subjects aged 16 to 35 years of age) were Hispanic or Latino origin. The subjects' mean age was 27.3 years old (25.8 in subjects aged 16 to 35 years). Mean body mass index was 25.9 kg/m^2 (25.8 in subjects 16 to 35 years of age).

Table 28. Demographics and Baseline Characteristics, Study MIT-Es0001-C302

	DRSP/E4	
Characteristic	**Subjects Aged 16 to 35 Years (N=1,674)**	**All Subjects (N=1,864)**
Race, n (%)		
White	1,174 (70.1)	1,300 (69.7)
Black or African American	326 (19.5)	369 (19.8)
Asian	81 (4.8)	87 (4.7)
American Indian or Alaska native	15 (0.9)	15 (0.8)
Native Hawaiian or other Pacific Islander	7 (0.4)	9 (0.5)
Other	71 (4.2)	84 (4.5)
Ethnicity, n (%)		
Hispanic or Latino	429 (25.6)	488 (26.2)
Not Hispanic or Latino	1,245 (74.4)	1,376 (73.8)
Age (years) at screening		
Mean (SD)	25.8 (4.7)	27.3 (6.5)
Median	25	26
Range	16, 35	16, 50
Age (years) group at screening, n (%)		
≥16–≤25	839 (50.1)	839 (45.0)
>25–≤35	835 (49.9)	835 (44.8)
>35–≤50		190 (10.2)
Baseline weight (kg)		
Mean (SD)	69.0 (14.2)	69.4 (14.2)
Median	67.1	67.6
Range	36.7, 148.8	36.7, 148.8
Baseline height (cm)		
Mean (SD)	163.5 (6.9)	163.6 (6.9)
Median	162.6	162.6
Range	144.8, 195.6	144.8, 195.6
Baseline BMI (kg/m^2)		
Mean (SD)	25.8 (4.7)	25.9 (4.7)
Median	25.1	25.3
Range	14.3, 48.4	14.3, 48.4
BMI (kg/m^2) categories, n (%)		
<30	1,298 (77.5)	1,432 (76.8)
≥30	376 (22.5)	432 (23.2)

Characteristic	DRSP/E4 Subjects Aged 16 to 35 Years (N=1,674)	DRSP/E4 All Subjects (N=1,864)
Highest level of education, n (%)		
Less than Upper Secondary School	89 (5.3)	95 (5.1)
Completed Upper Secondary School	771 (46.1)	827 (44.4)
Completed Vocational School	151 (9.0)	173 (9.3)
Completed College	530 (31.7)	611 (32.8)
Completed Graduate School	133 (7.9)	158 (8.5)

Source: Study MIT-Es0001-C302 clinical study report Table 3 and confirmed by the statistical reviewer.
Abbreviations: BMI, body mass index; DRSP, drospirenone; E4, estetrol; SD, standard deviation

Overall, the demographic characteristics reported in Study C302 approximates the population in the US that is expected to use DRSP/E4, although non-White participants and overweight and obese females were underrepresented.

Treatment Compliance, Concomitant Medications, and Use of Back-up Contraception

Treatment Compliance

Treatment compliance was assessed based on the response values entered by the subject in the diary on a daily basis. Comparison was made between the number of tablets expected to be used, and the number of tablets taken according to the subject's diary. The data were also used to identify when a pill had been missed.

At each study visit, diary entries were compared to the returned tablets to ensure consistency, however compliance assessment was based on diary data. In the case of discrepancy between the diary data and the returned tablets, the Investigator discussed with the subject and documented this in the source data.

Concomitant Medications

The following medications are defined as concomitant medications (WHO Drug Dictionary PT):

1. Medications with a start date during the on-treatment period for the investigational product.
2. Medications with a start date prior to the date of first dose of investigational product and an end date on or after the date of first dose of investigational product.

Prior medications are defined as medications with a start date and end date prior to the start date of the investigational product. Post-study medications are defined as medications with a start date after the date of the last dose of investigational product.

More than half of subjects in the safety population (54%, n=999, N=1864) had taken at least 1 prior medication. The most commonly used prior medications were oral contraceptives (36.6%, n=683). The most commonly used prior medication after contraceptives were azithromycin (3.4%, n=63), metronidazole (3.1%, n=57), and fluconazole (1.0%, n=19).

Nearly two-thirds of subjects (62.5%, n=1165) took concomitant medications during the study.

The most commonly used concomitant medications (by >5% of subjects) were ibuprofen (18.9%, n=352), multivitamins (9.4%, n=176), and acetaminophen (6.5%, n=121).

None of the commonly used concomitant medications would be expected to interfere with the effectiveness or safety analyses for DRSP/E4.

Use of Back-up Contraception

For the purposes of this review the use of back-up contraception is defined as the use of a barrier method such as condoms, or emergency contraception in addition to use of the study product. A cycle in which back-up contraception was used was excluded from the efficacy analysis unless pregnancy is observed in that cycle. Table 29 shows the percentage of subjects per cycle of study product use who reported use of back-up contraception during that cycle.

Table 29. Proportion of Subjects Using Back-up Contraception, Study C302

Cycle	Subjects Reporting Use of Back-Up Contraception (n)	Subjects Reporting Use of DRSP/E4 (N)	Proportion of Subjects Using Back-Up Method as Percent of Total No. of Subjects Using DRSP/E4 ([n/N]*100)
1	436	1852	23.5%
2	186	1686	11.0%
3	149	1611	9.2%
4	121	1468	8.2%
5	99	1414	7.0%
6	92	1370	6.7%
7	75	1273	5.9%
8	59	1231	4.8%
9	68	1198	5.7%
10	47	1088	4.3%
11	49	1060	4.6%
12	56	1040	5.4%
13	57	1012	5.6%

Source: NDA 214154, Study C302, Dataset ADFA, Clinical Reviewer Analysis.
Abbreviations: DRSP, drospirenone; E4, estetrol; N, number of subjects using study product during the cycle; n, number of subjects using back-up contraception

The proportion of participants using back-up contraception decreased markedly from Cycle 1 through Cycle 6, and then remained constant at approximately 6% through Cycle 12. This trend and plateau appear to correlate with discontinuation rates. From a clinical perspective, the information on the use of back-up contraception suggests that participants at increased risk for pregnancy may be more likely to discontinue early from study participation. Cycles in which back-up contraception use occurred were excluded from the efficacy analysis. However, use of back-up contraception is not unexpected and this likely mirrors real-world use.

Data Quality and Integrity

A complaint was received by the FDA and other regulatory agencies (EMA, HC) and investigated by OSI as described in Section 4.1. After remote inspections were conducted by these agencies, the sponsor's study oversight appears adequate and the data generated by the sponsor from Study C302 appeared acceptable in support of the NDA.

Efficacy Results – Primary Endpoint

In Study C302, 28 on-treatment pregnancies were reported. Of those pregnancies, 26 of 28 occurred during the treatment period in subjects between 16 to 35 years of age, inclusive, at initial screening (ITT population). 1,524 subjects aged 16 to 35 years with at least 1 at-risk cycle in the study reported 12,763 at-risk cycles. The Pearl Index was 2.65 per 100 women-year (95% CI: 1.73, 3.88). See Table 30 below.

Table 30. Pearl Index in Subjects Age 16 to 35 Years, Study MIT-Es0001-C302

Variable	DRSP/E4 (N=1,674)
Number of subjects with at least 1 at-risk cycle	1,524
Number of cycles	12,763
Number of on-treatment pregnancies	26
Pearl Index (95% CI)	2.65 (1.73, 3.88)

Source: Study MIT-Es0001-C302 clinical study report Table 11 and confirmed by the statistical reviewer.
Abbreviation: CI, confidence interval

Efficacy Results – Secondary and Other Clinically Relevant Endpoints

Life-table estimates of the cumulative pregnancy rates based on the on-treatment pregnancies and all cycles in subjects aged 16 to 35 years from the ITT Population were conducted as sensitivity analyses. The estimates are presented in Table 31. The cumulative pregnancy rate after 13 cycles of use was 2.07% (95% CI: 1.40%, 3.05%) for subjects aged 16 to 35 years in the ITT Population.

Table 31. Life-Table Estimate of Cumulative Pregnancy Rate, Study MIT-Es0001-C302

Variable	Subjects Aged 16–35 Years of Age With All Cycles (N=1,674)
Number of subjects with at least 1 at-risk cycle	1,673
Number of cycles	15,467
Number of on-treatment pregnancies	26
Cumulative pregnancy rate (95% CI)	2.07% (1.40%, 3.05%)

Source: Study MIT-Es0001-C302 clinical study report Tables 14.2.2 and statistical reviewer's analysis.
Note: Subject (b) (6) had a post-treatment pregnancy but did not have any diary data from which the cycle of censoring could be derived. This subject was included in the ITT Population but not included in the analysis.
Abbreviation: CI, confidence interval

The Life-table estimates from the table above were reviewed and further support the PI findings.

Efficacy Results – Subgroup Analysis

The Pearl index by subgroups of race, age, BMI, hormonal contraceptives use, and smoking status at baseline for subjects aged 16 to 35 years is presented in Table 32. The Pearl Index was numerically higher in Black or African American subjects, and slightly higher in subjects aged 25 years or younger, BMI ≥30 kg/m^2, hormonal contraceptive starters, and smokers.

The reviewers explored the potential reasons for the numerically higher Pearl Index observed in Black or African American participants. An additional subgroup analysis by race and BMI was presented in Table 33. The proportion of White participants (n=867, 56.9%) with BMI <30 kg/m^2 provides a reasonable number of at-risk cycles (7,710) for analysis. The Pearl Index was 1.35 (95% CI: 0.58, 2.66) for this subgroup which was in line with the overall Pearl Index in subjects aged 16 to 35 years of age. The Pearl Index in Black or African American participants (n=193, 12.7%) was 9.59 (95% CI: 4.6, 16.63). These subgroup analyses were exploratory and not controlled for multiplicity resulting from many analyses. The finding should be interpreted with caution.

Table 32. Pearl Index by Subgroup for Subjects Aged 16 to 35 Years, Study MIT-Es0001-C302

Subgroup	N	On-Treatment Pregnancies	At-Risk Cycles	Pearl Index (95% CI)
Race				
White	1,090	13	9,570	1.77 (0.94, 3.02)
Black or African American	278	10	1,911	6.80 (3.26, 12.51)
Asian	73	2	608	4.28 (0.52, 15.45)
American Indian or Alaska native	12	0	82	0
Native Hawaiian or other Pacific Islander	5	0	53	0
Other	66	1	539	2.41 (0.06, 13.44)
Age (years)				
≥18 to ≤25	748	14	5,936	3.07 (1.68, 5.14)
>25 to ≤35	776	12	6,827	2.29 (1.18, 3.99)
BMI (kg/m^2)				
<30	1,187	20	10,113	2.57 (1.57, 3.97)
≥30	337	6	2,650	2.94 (1.08, 6.41)
BMI (kg/m^2)				
<25	742	12	6,416	2.43 (1.26, 4.25)
≥25 to <30	445	8	3,697	2.81 (1.21, 5.54)
≥30	337	6	2,650	2.94 (1.08, 6.41)
Hormonal contraceptives use				
Starters	868	16	6670	3.12 (1.78, 5.06)
Switchers	656	10	6,093	2.13 (1.02, 3.92)
Smoking status at baseline				
Smoker	198	5	1,552	4.19 (1.36, 9.77)
Non-smoker[s]	1,326	21	11,211	2.43 (1.51, 3.72)

Source: Study MIT-Es0001-C302 clinical study report Tables 14.2.1.4 and confirmed by the statistical reviewer, and the applicant's submission on 10/2/2020. a "Non-smoker" included previous smoker and never smoker.
Abbreviations: BMI, body mass index; CI, confidence interval; N, number of subjects in each subgroup

Table 33. Pearl Index by Race & BMI for Subjects Aged 16 to 35 Years, Study MIT-Es0001-C302

BMI (kg/m^2) at Baseline	Racial Subgroup	N	On-Treatment Pregnancies	At-Risk Cycles	Pearl Index (95% CI)
<30	White	867	8	7,710	1.35 (0.58, 2.66)
	Black or African American	193	10	1,356	9.59 (4.6, 16.63)
	Other races*	127	2	1,047	2.48 (0.30, 8.97)
≥30	White	223	5	1,860	3.49 (1.13, 8.15)
	Black or African American	85	0	555	0
	Other races*	29	1	235	5.53 (0.14, 30.82)

Source: The applicant's submission on 10/2/2020 and statistical reviewer's analyses.
* "Other Races" included Asian, American Indian, or Alaska Native, Native Hawaiian or other Pacific Islander, and Other.
Abbreviations: BMI, body mass index; CI, confidence interval; N, number of subjects in each racial subgroup

As seen in Table 33 above, the small proportion of non-White participants with BMI ≥30 kg/m^2 disallows conclusions regarding the effect of BMI by racial subgroups on efficacy of DRSP/E4. Future efforts to obtain additional data during development may include increasing recruitment within subgroups of interest to allow meaningful analysis and conclusions to better inform the population likely to use CHCs in the US.

Dose/Dose Response

Dose and dose response were not evaluated in this study.

Durability of Response

Durability of response was not evaluated in this study and is not expected beyond immediate use.

Persistence of Effect

Historically, oral CHC products have not demonstrated persistence of effectiveness beyond intake of the last tablet. There is limited information in this application on this. The mean time to return of spontaneous menses was 42.8 days, with median 39 days. The range was -15 to 135 days post-treatment. Data on timing of return to spontaneous menses was only available for 10 subjects.

A total of 4 subjects became pregnant out of 17 subjects who discontinued the study due to desiring pregnancy. The mean time from last dose of DRSP/E4 to estimated date of conception was 170.7 days based on data from 3 subjects who became pregnant. Pregnancy dates were not available for the fourth subject.

After review, it appears that most patients have a return to spontaneous menses within 2 menstrual cycles and that is clinically reassuring. The limited information on return to menses and the few patients that become pregnant after dosing with DRSP/E4 does not suggest that DRSP/E4 has a different profile when treatment is discontinued compared to other products.

Secondary or Exploratory COA (PRO) Endpoints

Not applicable.

Additional Analyses Conducted on the Individual Trial

Refer to discussion above regarding sub-group analyses on BMI and race considerations.

Integrated Review of Effectiveness

No integrated effectiveness review was performed because the population and results of the EU/R efficacy database was not considered sufficiently similar to the population that will use this in the US.

8.1.3. Supportive Effectiveness Information From Study C301

An additional study MIT-Es0001-C301 (or Study C301) conducted in the Europe and Russia was submitted in this application. The efficacy of DRSP/E4 for prevention of pregnancy is based on the Study C302 conducted in the US and Canada. For a complete review, the efficacy results from study C301 are summarized in this section. More details on Study C301 results, considered supportive of the DRSP/E4 application for the prevention of pregnancy, are listed in Appendix (Section 16.6).

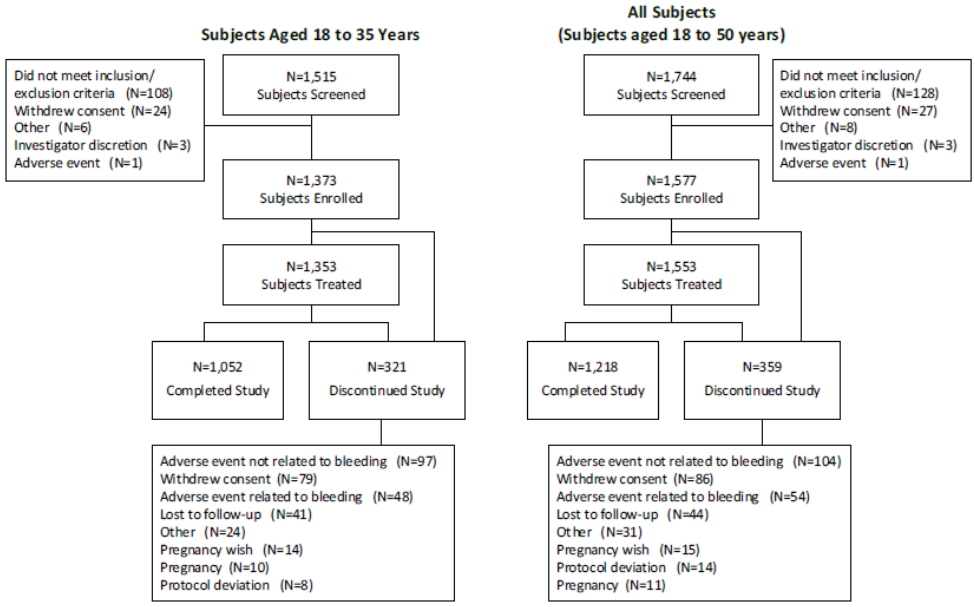
The study design, study endpoints, and statistical analysis plan of Study C301 was similar to the Study C302, except Study C301 enrolled subjects 18 to 35 years of age, and the On-Treatment Period was defined as the period of time between the date of first dose of study medication and 2 days after the last dose of study medication (regardless of whether this is an active or inactive tablet), inclusive. For details of study design, study endpoints, and statistical analysis plan, please refer to Section 8.1.1.

Subject Disposition

A total of 1,744 subjects were screened. Of whom 1,577 were enrolled in the study: 1,373 subjects aged 18 to 35 years and 204 subjects aged >35 years. Overall, 1,553 of 1,577 (98.5%) enrolled subjects took study medication (i.e., subjects in the Safety and ITT Populations), including 1,353 of 1,553 (87.1%) in the ITT Population of subjects aged 18 to 35 years. See details in Figure 6.

For all subjects enrolled in the study, 1,218 of 1,577 (77.2%) subjects completed the study. The most common reasons for study discontinuation were AEs not related to bleeding (104 subjects, 6.6%), consent withdrawal (86 subjects, 5.5%), AEs related to bleeding (54 subjects, 3.4%) and loss to follow-up (41 subjects, 2.6%). The loss to follow-up difference between study regions is evident with this low percentage. The Kaplan-Meier estimates of cumulative discontinuation rates based on the Enrolled Population and ITT population are displayed in Figure 7 and Figure 8 and In the Appendix.

Figure 6. Subject Disposition, Study MIT-Es0001-C301



Source: Study MIT-Es0001-C301 clinical study report Figure 1 and confirmed by the statistical reviewer.

Demographics and Baseline Characteristics

Subjects' demographics and baseline characteristics in the ITT Population are shown in Table 34. 98.6% of the subjects were white (98.6% in subjects aged 18 to 35 years), and 0.5% were black or African American (0.6% in subjects aged 18 to 35 years). The subjects' mean age was 27.1 years old (25.0 in subjects aged 18 to 35 years). Mean body mass index was 23.0 kg/m^2 (22.9 in subjects 18 to 35 years of age).

Table 34. Demographic and Baseline Characteristics, Study MIT-Es0001-C301

	DRSP/E4	
Characteristic	**Subjects Aged 18 to 35 Years (N=1,353)**	**All Subjects (N=1,553)**
Race, n (%)		
White	1,334 (98.6)	1,532 (98.6)
Black or African American	8 (0.6)	8 (0.5)
Asian	9 (0.7)	10 (0.6)
Other	2 (0.1)	3 (0.2)
Age (years) at screening		
Mean (SD)	25.0 (4.5)	27.1 (6.9)
Median	24	25
Range	18, 35	18, 49
Age (years) group at screening, n (%)		
≥18–≤25	793 (58.6)	793 (51.1)
>25–≤35	560 (41.4)	560 (36.1)
>35–≤50		200 (12.9)
Baseline weight (kg)		
Mean (SD)	63.7 (10.7)	63.8 (10.5)
Median	61.6	62.0
Range	35.0, 111.5	35.0, 111.5
Baseline height (cm)		
Mean (SD)	166.8 (6.3)	166.5 (6.2)
Median	167.0	166.0
Range	145.0, 189.0	145.0, 189.0
Baseline BMI (kg/m^2)		
Mean (SD)	22.9 (3.5)	23.0 (3.5)
Median	22.3	22.4
Range	15.0, 35.0	15.0, 35.0
BMI (kg/m^2) categories, n (%)		
<30	1,278 (94.5)	1,464 (94.3)
≥30	75 (5.5)	89 (5.7)
Highest level of education, n (%)		
Less than upper secondary school	113 (8.4)	130 (8.4)
Completed upper secondary school	518 (38.3)	584 (37.6)
Completed vocational school	130 (9.6)	150 (9.7)
Completed college	413 (30.5)	481 (31.0)
Completed graduate school	179 (13.2)	208 (13.4)

Source: Study MIT-Es0001-C301 clinical study report Table 3 and confirmed by the statistical reviewer.
Abbreviations: BMI, body mass index; DRSP, drospirenone; E4, estetrol; SD, standard deviation

It is clear that the demographics and baseline characteristics of the population in Study C301 are significantly different from the population in Study C302 and also from the population that will likely use this product in the US. Major differences between Study 301 and Study C302 included: 1) race: White/Caucasian participants comprised over 98% of the Study C301 (EU/R) study population, compared to 70% of the Study C302 (US/CA) population; and 2) BMI: obese individuals, defined as BMI ≥30 kg/m^2 comprised only 5.7% of the study population in Study C301 (EU/R) compared to approximately 23% of the study population in Study C302 (US/CA).

Efficacy Results – Primary Endpoint

The applicant reported 5 on-treatment pregnancies in the study, all of which occurred in subjects aged 18 to 35 years at screening. 1,313 subjects aged 18 to 35 years with at least 1 at-risk cycle reported 13,692 at-risk cycles. The Pearl Index was 0.47 per 100 women-year (95% CI: 0.15, 1.11). See Table 35 below.

Table 35. Pearl Index in Subjects Age 18 to 35 Years, Study MIT-Es0001-C301

Variable	DRSP/E4 (N=1,353)
Number of subjects with at least 1 at-risk cycle	1,313
Number of cycles	13,692
Number of on-treatment pregnancies	5
Pearl Index (95% confidence interval)	0.47 (0.15, 1.11)

Source: Study MIT-Es0001-C301 clinical study report Table 9 and confirmed by the statistical reviewer.
Abbreviations: DRSP, drospirenone; E4, estetrol

It is clear that the PI in Study C301 is significantly lower than Study C302. As demonstrated by the demographic and baseline data, this study was not conducted in a population that is sufficiently similar to that of the expected population that will use this product in the US. It is also possible that differences in compliance with regular use of CHCs and investigator site type may also play a role in the differences reported in PI between these phase 3 studies. After review, the team concluded that no further analyses or sub-analyses of Study C301 were needed. Efficacy findings from this study will not be included in labeling.

8.1.4. Integrated Assessment of Effectiveness

Given the significant differences between the effectiveness seen in the two studies – Study C301 and Study C302, an integrated assessment of effectiveness was not requested. Effectiveness data from Study C301 was not considered informative for labeling for the US population.

8.2. Review of Safety

8.2.1. Safety Review Approach

The safety review for DRSP/E4 focused primarily on the safety population in Study C302 database from US and Canadian sites. The reason for this focus is that the demographic composition and baseline characteristics of the population in Study C302 most closely approximates the population in the US likely to use this product. The most important demographic characteristic impacting the safety profile of DRSP/E4, as for all CHC products, is baseline overweight (defined as BMI ≥ 25 kg/m^2 and < 30 kg/m^2) and obesity (defined as BMI ≥ 30 kg/m^2).

All participants who received at least one dose of DRSP/E4 in Study 302 (US/CA) comprised the primary safety population. Other analyses included an integrated analysis of common adverse events encompassing all studies conducted in the indicated population (healthy premenopausal

females 16-50 years old), where the duration of treatment was for at least three 28-day cycles, and where the dosage and regimen of DRSP/E4 was 3/14.2 mg for 24/4-days (Integrated Safety Population [or ISP]). The ISP includes data from two Phase 3 trials - MIT-Es0001-C301 (Study C301) and MIT-Es0001-C302 (Study C302) – and three Phase 2 trials - Es-C02, MIT-Es0001-C201 (Study C201), and MIT-Es0001 (Study C202). Adverse event analysis also includes a comparison of frequencies in the target US population (Study C302) versus the overall ISP population. Serious adverse events or other adverse events of special interest from all studies, regardless of duration, are described in Section 8.2.4.

8.2.2. Review of the Safety Database

Overall Exposure

Evaluation of overall exposure to DRSP/E4 includes all participants that received at least one dose of the proposed strength in this marketing application: drospirenone 3 mg and estetrol (anhydrous) 14.2 mg. A total of 3727 participants received at least one dose of DRSP 3 mg/E4 14.2 mg across the clinical development program (from two phase 3 trials [MIT-Es0001-C301 and MIT-Es0001-C302]), three phase 2 trials [Es-C201, MIT-Es0001-C201, and MIT-Es0001-C202] and six phase 1 trials [Es0001-C101, MIT-Es0001-C103, MIT-Es0001-C109, MIT-Es0001-C106, MIT-Es0001-C110, and MIT-Es001-C112]) with 1863 participants from the US/Canada trial (C302). Table 36 summarizes total clinical exposure to DRSP/E4.

Table 36. Exposure to DRSP/E4

Clinical Trial Groups	Number of Participants(n)	Duration of Exposure	Total Number of Cycles of Exposure
Phase 1 trial			
Single dose	47	Once	
Single dose – repeated	64	2 times	0
Single, then multiple dose	10	Day 1, then Day 15 to 28	
Multiple dose	32	20 days	
Subtotal	153		0
Phase 2 trial			
Multiple dose	158	28-day cycles	
Subtotal	158		
Phase 3 trial			
Single-arm, open- label, multiple dose		28-day cycles	
Europe/Russia	1553		17680
US/Canada	1863*		17413
Subtotal	3416		35093
Grand total	3727		

* Excludes one participant (b) (6) who reported study drug intake and had a post-treatment pregnancy determination; however, study drug intake could not be confirmed by participant diary or returned study drug accountability (participant lost study materials).Source: NDA 214154, Summary of Clinical Safety (m2.7.4) - Table 10 and Clinical Reviewer analysis of Applicant's datasets.
Abbreviations: DRSP, drospirenone 3 mg; E4, estetrol 14.2 mg; N, total number of participants; n, number of participants in each subgroup

For ease of review, the "Integrated Safety Population" was defined as including all participants who were treated with at least one dose of DRSP/E4 as recorded in the participant diary, contributing a total of more than 35,000 cycles of exposure for the safety analysis with the

US/Canada contributing 17,412 cycles. Additionally, participants who were dispensed pills, but for whom there was no pill intake record showing treatment in the participant diary nor return of the dispensed pills documented in the drug accountability, were also included in the Integrated Safety Population. One participant enrolled and received study product in two Phase 2 studies and is therefore counted twice for exposure data, but once in the safety population. One participant enrolled in Study C302 and had a post-treatment pregnancy determination but misplaced her diary and unused study product. This participant is included in both the Integrated Safety population and the exposed population.

In total:

- The Integrated Safety Population includes 3790 participants: 3575 participants with confirmed exposure to DRSP/E4 and 215 participants for whom neither exposure nor non-exposure to DRSP/E4 could be confirmed.
- The US/Canada population (primary safety database, Study C302) is a subgroup of the Integrated Safety Population and includes a total of 1864 participants with confirmed exposure and 209 with unconfirmed exposure (a total of 2073 participants), contributing 17,413 treatment cycles of exposure in the US/Canada population.

The Review Division requested additional details on the 215 enrollees with unconfirmed exposure to study product (6 in Study C301-EU/R; 209 in C302-US/CA) in an Information Request to the Applicant on March 10, 2021. The Applicant clarified that these enrollees contributed to the overall Integrated Safety Population but are not included in the safety and efficacy populations for Studies C301 and C302. After review of the adverse event data and disposition of these individuals, the Review Division concludes that the inclusion of these subjects who had unconfirmed use of the study product does not adversely affect the safety analysis or dilute the safety populations. However, the Review Division has included the 209 individuals with unconfirmed exposure in the safety population of Study C302 as the Applicant confirmed that these participants did contribute adverse reactions to the safety database.

In summary, a total of 2,212 participants completed 13 cycles of treatment in the two Phase 3 trials, contributing a total of 35,677 cycles of exposure for the safety analysis. In assessing the safety from the US/Canada population a total of 1864 participants contributed 17,413 cycles of confirmed exposure. As with previously approved CHC products, the safety profile contained sufficient numbers of subjects and treatment cycles from the US/Canada population to inform the safety of the product with support from the totality of the ISP safety database information.

Table 37 lists the Integrated Safety Population by study conducted in support of NDA 214154.

Table 37. Safety Populations for DRSP/E4 by Study

	Integrated Safety Population (N=3790)	
Study	**No. of Participants With Confirmed Exposure to DRSP/E4***	**No. of Participants for Whom Neither Exposure Nor Nonexposure to DRSP/E4 Could be Confirmed**
MIT-Es0001-C302 (pivotal phase 3 – US/Canada)	**1864****	**209**
MIT-Es0001-C301 (pivotal phase 3 – Europe/Russia)	1553	6
ES-C02 (dose finding active control)	79	0
MIT-Es0001-C201	38	0
MIT-Es0001-C202	41	0
Total (Integrated Safety Population)	3575	215

Source: NDA 214154, Summary of Clinical Safety (m2.7.4) - Table 9 and Dataset ADSL Clinical Reviewer Analysis
* Includes all participants to whom study drug was dispensed and for whom study drug intake was confirmed either by participant diary entry and/or returned study drug accountability except as described for participant (b) (6) below.
** Includes one participant (b) (6) who reported study drug intake and had a post-treatment pregnancy determination; however, study drug intake could not be confirmed by participant diary or returned study drug accountability (participant lost study materials).
Abbreviations: DRSP, drospirenone 3 mg; E4, estetrol 14.2 mg

The majority of clinical investigations for DRSP/E4 were conducted in the EU. However, the pivotal Phase 3 clinical trial MIS-Es0001-C302 (Study C302) was conducted solely in the US and Canada as requested by the Agency. Several Phase 1 studies (including C106 – the thorough QT [TQT] study) were also conducted in the US, contributing a total of 113 exposures in US participants.

Safety data included information on drug exposure, demographic and baseline characteristics, concomitant medications including contraceptive use, medical/gynecologic history, adverse events/reactions, vital signs and laboratory data and pregnancy outcome information. Additional information on physical & gynecological exams; electrocardiogram and echocardiogram findings; and endometrial biopsy data, when available, were also reviewed using the US/Canada and ISP databases.

Relevant Characteristics of the Safety Population

The safety population in the US consisted of 2073 participants with the following racial composition: 64.1% (n=1736) Caucasian; 25.4% (n=687) Black or African-American; and 4.3% (n=117) Asian. Participants of Hispanic or Latino ethnicity represented 27.0% (n=731) of the total study population in the US. These participants contributed a total of 17,413 cycles of exposure.

The proportion of the US study population that was either overweight (defined as a BMI ≥25 kg/m^2 and <30 kg/m^2) (n=549, 20.3%), obese (defined as a BMI ≥30 kg/m^2)(n=480, 17.7%), or overweight and obese (n=1029, 38.0%) significantly under-represents the target population. Most recent estimates indicate that more than 65% of females over the age of 20 in the United

States are overweight and obese.[5] Likewise, the proportion of participants in the total study population (EU plus US/Canada) that was overweight (n=898, 23.7%), obese (n=585, 15.4%), or overweight and obese (n=1483, 39.1%) is unlikely to keep pace with the rapidly rising rates of overweight and obesity worldwide.

In light of this, there is a significant limitation of data on overweight and obese participants in the study population for DRSP/E4. For most of the adverse reactions seen with CHCs including DRSP/E4, the safety in overweight and obese females of reproductive potential is expected to be similar to those of normal weight females of reproductive potential. However, one concern is the rate of thromboembolism in overweight and obese patients as these patients are at an increased risk for these events. Therefore, a postmarketing requirement will be necessary to capture sufficient numbers of normal, overweight and obese patients to determine if there is an increased risk of thromboembolism when compared to other oral CHC containing products.

In contrast, participants in the Integrated Safety Population (N=3790) were primarily Caucasian (n=3094, 81.6%) and non-Hispanic (n=3231, 85.3%), with a mean age of 27.1 (±6.6) and mean baseline BMI of 24.6 (±4.5). Overall, 14.4% of participants (n=544) were current smokers in the age group of 16-35 only. The majority of participants had completed upper secondary school or higher (n=3111, 82%); 354 (9.5%) participants had completed vocational school; and 246 (6.6%) participants had completed less than upper secondary school.

Table 38 summarizes the demographics and baseline characteristics of the US/Canadian safety population compared to the Integrated Safety population.

Table 38. Demographics, Integrated Safety Population[1] for DRSP/E4

Demographic Parameters	Primary Safety Population C302 (N=2073)*	Integrated Safety Population (Ex-US) (N=3790)
Age		
Mean years (SD)	27.3 (6.4)	27.1 (6.6)
Median (years)	26	26
Min, max (years)	(16, 50)	16, 50
Race, n (%)		
White	1406 (67.8)	3094 (81.6)
Black or African American	457 (22.0)	467 (12.3)
Asian	89 (4.3)	100 (2.6)
American Indian or Alaska Native	19 (0.9)	19 (0.5)
Native Hawaiian or Other Pacific Islander	9 (0.4)	9 (0.2)
Other	93 (4.5)	101 (2.7)
Ethnicity[2]		
Hispanic or Latino	532 (25.7)	545 (14.4)
Not Hispanic or Latino	1541 (74.3)	3231 (85.6)

[5] National Health and Nutrition Examination Survey (NHANES): Overweight and Obesity Statistics. National Institute of Diabetes and Digestive and Kidney Diseases. https://www.niddk.nih.gov/health-information/health-statistics/overweight-obesity. August 2017.

Demographic Parameters	Primary Safety Population C302 (N=2073)*	Integrated Safety Population (Ex-US) (N=3790)
Body mass index (BMI)		
Mean kg/m^2 (SD)	26.0 (4.8)	24.6 (4.5)
Median (kg/m^2)	25.4	23.7
Min, max (kg/m^2)	14.3, 48.4	14.3, 48.4
Body mass index category, n (%)		
Underweight (BMI <18.5 kg/m^2)	60 (2.9)	126 (3.3)
Normal weight (18 kg/m^2≤ BMI <25 kg/m^2)	924 (44.6)	2181 (57.5)
Overweight (25≤ BMI <30 kg/m^2)	595 (28.7)	898 (23.7)
Obese (BMI ≥30 kg/m^2)	494 (23.8)	585 (15.4)
Region		
United States/Canada	n/a	2073 (54.7)
Europe	n/a	1717 (45.3)

Source: NDA 214154, ISS, ADSL Dataset, Clinical Reviewer Analysis.
* The Safety population for C302 includes 1864 participants with confirmed exposure and 209 participants with unconfirmed exposure but contributed adverse reactions to the safety database.
[1] Integrated Safety Population includes participants from studies: ES-C02 (n=79), C201 (n=38), C202 (n=41), C301 (n=1559), and C302 (n=2073).
[2] Missing data =14 participants
[3] Missing data =79 participants
Abbreviations: DRSP, drosperinone; E4, estetrol; SD, standard deviation; US, United States

Differences in US/Canadian versus European study populations by race and BMI have been noted in previous trials. Therefore, the US/Canada database is the primary focus of the review as this is the most likely to provide a safety profile that is sufficiently similar to the US for labeling purposes.

Adequacy of the Safety Database

The primary safety database for DRSP/E4 from the US/Canadian Study C302 is clinically adequate and provides sufficient information to draw general conclusions regarding the safety of DRSP/E4 in the target US population from 2073 participants with 17,413 treatment cycles. The integrated safety database for DRSP/E4 includes confirmed exposure for a minimum of three 28-day cycles in 3575 participants. A total of 2,212 participants completed 13 cycles of treatment in the two Phase 3 trials, contributing a total of 35,677 cycles of exposure for the safety analysis and provides supportive information to complement the safety profile identified in the phase 3 US/Canada study.

8.2.3. Adequacy of Applicant's Clinical Safety Assessments

Issues Regarding Data Integrity and Submission Quality

Refer to Section 4 for additional details.

Categorization of Adverse Events

Adverse events (AEs) and treatment-emergent adverse events (TEAEs) were classified using standardized terminology according to the Medical Dictionary of Regulatory Activities

(MedDRA) system organ class (SOC) and preferred term (PT). The latest version of MedDRA at the time of study completion was employed for AE classification, and updated to the most current version of MedDRA prior to database lock. Each participant was only counted once for a given SOC or PT where a SOC or PT was reported more than once by that participant.

For the pooled analysis, which included Phase 2 and Phase 3 studies, AE terms, medical history terms, and concomitant medication data were re-coded/up-versioned using MedDRA v20.0, ATC 2019, and WHO-DD version 2017. For supportive studies, if 2 or more studies used different MedDRA versions, the SOC and PT from each version was retained (no pooling or reclassification).

The Applicant retrospectively identified the following TEAEs of Special Interest (TEAESIs):

Venous thromboembolism (VTE; known or suspected) including deep vein thrombosis (DVT) and pulmonary embolism (PE)

Arterial thromboembolism (ATE) including myocardial infarction (MI) and cerebrovascular accident (CVA or stroke)

Mood changes

Bleeding irregularities

Acne, seborrhea, oily skin

Sexual interest (increase/loss of libido)

Breast complaints (pain, tenderness, masses, galactorrhea)

Headache/migraine

Potassium-related AEs (hyperkalemia, hypokalemia)

Weight gain/loss

These TEAEs of Special Interest are consistent with other CHC applications. AEs related to changes in serum potassium are reviewed for every contraceptive product containing drosperinone as it is an analog of spironolactone.

Routine Clinical Tests

Blood hematology, biochemistry, and lipids were assessed at Visits 1 and 7. Visit 5 assessments included: serum glucose levels, glycated hemoglobin (HgbA1C), total cholesterol, HDL- and LDL-cholesterol, triglycerides, lactate dehydrogenase (LDH) 1 and 2, renal function (urea, creatinine, and glomerular filtration rate [GFR]), and serum ion concentrations of sodium, potassium, chloride, calcium, phosphorus, and bicarbonate.

The Sponsor had investigators record all out-of-range laboratory values as AEs as considered to be clinically significant.

8.2.4. Safety Results

Deaths

One death occurred in the clinical development program due to accidental overdose of alprazolam and fentanyl. The Review Division agreed with the applicant that this case was unrelated to study drug. See Section 17.9 for a full narrative.

Serious Adverse Events/Reactions

A total of 45 serious adverse events (SAEs) were reported in 41 participants in the clinical development program for DRSP/E4. The Review Division assessed six of these as related to study drug: 2 cases of venous thrombosis, and 1 case each of depression, suicidal ideation, ectopic pregnancy, and complicated migraine, respectively. Refer to Section 17.9 for detailed narratives of these events.

Further review was conducted of the narratives of the serious adverse events that were considered related to DRSP/E4 use. These included:

Thromboembolism

- No thromboembolic events were reported in Study C302
- One venous thromboembolism event (VTE) occurred in Study C301 (EU/R) in an otherwise healthy 32-year-old female with normal baseline BMI and no significant medical history.
- Another VTE occurred in an otherwise healthy 54-year-old postmenopausal female with normal baseline BMI enrolled in Study MIT-Es0001-C106 (TQT study). She received five times the therapeutic dose of DRSP/E4 for 10 days per protocol in the Phase 1 cardiac conduction study and developed a deep vein thrombosis (DVT) of the fibular vein after prolonged motor vehicle travel.

As previously stated, venous thromboembolism associated with CHC use is a rare event. A one-year clinical trial database is insufficient to adequately assess the incidence of VTE. Therefore, a larger epidemiologic study will be necessary to further assess the risk of VTE with this new combination oral contraceptive product.

Mood disturbances (including depression and suicidal ideation).

- One participant (Study C302) experienced worsening of pre-existing depression four months after study drug initiation, requiring addition of a new concomitant medication for management of depression and cessation of study drug.
- One participant (Study C302) experienced a recurrence of intermittent suicidal ideation. The event occurred on Study Day 14 and study drug was withdrawn. A causal relationship between the study drug and the event cannot be definitively ruled out based on the available information.

Mood disorders and depression are known adverse reactions that are associated with CHC use. Review of these specific serious adverse events does not reveal a specific new safety signal or

trend. Therefore, the review team determined that existing class-labeling for a possible association between CHC use and depression adequately reflects the potential risks.

Migraine headache, particularly when aura is present, represents an adverse reaction of special interest due to the increased risk of stroke in females with migraine with aura using CHCs.

- One participant experienced complicated migraine after receiving five times the therapeutic dose of DRSP/E4 for 10 days in Study C106 (TQT study). No specific neurologic deficits were identified in the report. The subject had completed the treatment and therefore no action was taken with the study drug. Review of the details of this case did not identify a specific signal or pre-existing contributing factor that would increase the participant's risk of migraine. Migraine with aura is class-labeled as a contraindication for estrogen-containing products and will be included in labeling for DRSP/E4. Therefore, additional labeling regarding migraine with aura is not warranted.

Ectopic pregnancy occurred in the Phase 3 trials

- Two participants in Study C302 experienced ectopic pregnancy.

These events are of interest due to the potential for ectopic pregnancy to occur in any CHC user experiencing contraceptive failure. Ectopic pregnancy is not described in class-labeling for CHCs as available evidence does not suggest an increased risk of ectopic pregnancy with CHC use and are more likely related to previous STI infection. Therefore, these events will not be described in labeling for DRSP/E4.

See Section 17.9 for full narratives of these cases.

Overall, these serious adverse events from the DRSP/E4 safety database appear to be similar to those previously reported with other combination hormonal contraceptives. None of these serious adverse events demonstrated new safety signals or trends that warranted additional labeling language at this time.

Dropouts and/or Discontinuations Due to Adverse Effects

One hundred fifty-three of 2073 (7.4%) participants in Study C302 discontinued due to an adverse event, accounting for nearly half of all participants in the ISP. In comparison, 346 of 3790 (9.1%) participants in the Integrated Safety Population discontinued due to an adverse event. The most frequently reported reasons for discontinuation due to an adverse event are listed in Table 39.

Table 39. Discontinuations Due to Adverse Reactions

Preferred Term	Participants Discontinued Due to AE in C302 (US/CA) (n [%]) (N=153)	Participants Discontinued Due to AE in Overall ISP (n [%]) (N=346)
Metrorrhagia	18 (11.8)	42 (12.1)
Menorrhagia	16 (10.5)	21 (6.1)
Acne	11 (7.2)	36 (10.4)
Weight increased	12 (7.8)	16 (4.6)
Vaginal hemorrhage	11 (7.2)	28 (8.1)
Headache	9 (5.9)	14 (4.0)
Mood swings	7 (4.6)	18 (5.2)
Depression	7 (4.6)	9 (2.6)
Mood altered	5 (3.3)	15 (4.3)
Abdominal pain	6 (3.9)	7 (2.0)
Libido decreased	4 (2.6)	20 (5.8)
Irritability	4 (2.6)	9 (2.6)
Anxiety	4 (2.6)	8 (2.3)
Dysmenorrhea	3 (2.0)	7 (2.0)
Breast pain	2 (1.3)	8 (2.3)
Menstruation irregular	2 (1.3)	7 (2.0)

Source: NDA 214154 ADAE, Clinical Reviewer Analysis, Analysis variable "Drug Withdrawn"
Abbreviations: AE, adverse event; CA, Canada; ISP, integrated safety population; US, United States

Overall, the most frequently reported preferred terms leading to discontinuation included mood disturbances and bleeding irregularities.) When grouped by related preferred terms, more participants in Study C302 appear to have discontinued due to bleeding irregularities and mood disturbances (22.8% and 17.0%, respectively) compared to the ISP (17.6% and 9.%, respectively).

Significant Adverse Events

Significant adverse events attributable to DRSP/E4 are discussed elsewhere in this review: hyperkalemia and venous thromboembolism. Hyperkalemia (see Section 8.2.5.1) is likely related to drosperinone use (as it is chemically similar to spironolactone). Venous thromboembolism (see Section 8.2.4) is a known risk associated with use of combined hormonal contraceptives.

Treatment Emergent Adverse Reactions

The three most frequent adverse reactions (by preferred term) reported by participants in the target US population in Study C302 included: headache (n=79 [3.8%]), metrorrhagia (n=74 [3.6%]), and acne (n=52 [2.5%]). Adverse reaction frequencies appear low when examined by individual preferred terms. Due to demographic and baseline differences discussed previously, adverse reaction frequencies in the US were assessed and then compared to those reported in the Integrated Study Population.

Table 40 lists the most frequent adverse reactions occurring in Study C302 compared to the ISP listed by individual preferred terms.

Table 40. Frequency of Adverse Reactions in Participants Who Received DRSP/E4, Integrated Safety Population

Preferred Term (PT)	Study C302 n (%) [N=2073]	ISP (Pooled) n (%) [N=3790]
Headache	79 (3.8)	256 (6.8)
Metrorrhagia	74 (3.6)	175 (4.6)
Nausea	65 (3.1)	105 (2.8)
Dysmenorrhea	60 (2.9)	131 (3.5)
Weight increased	56 (2.7)	108 (2.7)
Acne	52 (2.5)	147 (3.9)
Breast pain	48 (2.3)	89 (2.3)
Vaginal hemorrhage	37 (1.8)	117 (3.1)
Abdominal pain	37 (1.8)	82 (2.2)

Source: NDA 214154, ISS ADAE, Clinical Reviewer Analysis
Abbreviations: DRSP, drosperinone; E4, estetrol; ISP, integrated safety population

After assessment of the safety databases, the clinical review team grouped multiple related preferred terms to estimate adverse reactions of interest for DRSP/E4. Since the two phase 3 studies (Studies C302 and C301) were similar in many aspects, it was decided that it might be helpful for providers to show both the adverse reaction profile from the US/Canada study (alone) and the pooled phase 3 population data from both phase 3 studies.

Table 41 lists the frequency of adverse reactions grouped by related preferred terms, comparing the target US population to the pooled phase 3 population (Studies C302/C301 only) and the overall Integrated Safety Population (ISP as described in Section 8.2.1 above).

Table 41. Frequency of Adverse Reactions for DRSP/E4 Grouped by Related Preferred Term

Preferred Term (PT)	Study C302 (US/CA) (n [%]) (N=2073)*	Pooled C301/C302 (n [%]) (N=3632)†	ISP (Pooled) (n [%]) (N=3790)‡
Any adverse reaction	1205 (58.1)	2126 (58.5)	2286 (60.3)
Mood disturbance[1]	226 (10.9)	329 (9.1)	347 (9.6)
Bleeding irregularities[2]	201 (9.7))	393 (10.8)	393 (10.4)
Breast symptoms[3]	110 (5.3)	197 (5.4)	230 (6.1)
Headache[4]	100 (4.8)	227 (6.3)	262 (6.9)
Dysmenorrhea[5]	84 (4.1)	133 (3.7)	150 (4.0)
Weight increased[6]	68 (3.3)	108 (3.0)	109 (2.9)
Acne[7]	66 (3.2)	136 (3.7)	148 (3.9)
Libido decreased/lost[8]	27 (1.3)	72 (2.0)	73 (1.9)

Source: NDA 214154, ISS ADAE, Clinical Reviewer Analysis
* Represents the safety population of C302 only (US/Canada).
† Represents the safety population of C301/C302 described in the USPI for DRSP/E4.
‡ Represents the Integrated Safety Population (3 Phase 2 trials [Es-C02, C201, C202] and 2 Phase 3 trials [C301, C302])
[1] Includes PTs: adjustment disorder, affective disorder, agitation, anger, anxiety, depressed mood, depression, depressive symptom, disorientation, emotional disorder, emotional distress, euphoric mood, generalized anxiety disorder, insomnia, irritability, mood altered, mood swings, nervousness, panic attack, panic disorder, performance fear, restlessness, sleep disorder, stress, suicidal ideation, tearfulness.
[2] Includes PTs: abnormal withdrawal bleeding, amenorrhea, cervix hemorrhage uterine, coital bleeding, dysfunctional uterine bleeding, menometrorrhagia, menorrhagia, menstrual disorder, menstruation irregular, metrorrhagia, oligomenorrhea, polymenorrhea, uterine hemorrhage, vaginal hemorrhage.
[3] Includes PTs: anisomastia, breast cyst, breast discoloration, breast discomfort, breast disorder, breast engorgement, breast enlargement, breast mass, breast edema, breast pain, breast swelling, breast tenderness, fibrocystic breast disease, galactorrhea, gynecomastia, mastoptosis, nipple disorder, nipple pain.
[4] Includes PTs headache, premenstrual headache, and tension headache.
[5] Includes PTs adnexa uteri pain, dysmenorrhea, premenstrual cramps, pelvic discomfort, pelvic pain, uterine spasm.
[6] Includes PTs: weight increased, weight fluctuation, body mass index increased, weight loss poor, and obesity.
[7] Includes PTs acne and cystic acne.
[8] Includes PTs: libido decreased and loss of libido.

This table demonstrates that participants in Study C302 more frequently reported mood disturbances, while the overall safety population more frequently reported bleeding irregularities. However, the two study populations demonstrate similar overall adverse reaction profiles.

Adverse Reactions of Special Interest

Adverse reactions of particular interest in the clinical development of DRSP/E4 included adverse reactions known to be associated with the two hormonal components: Estetrol/E4 – the estrogen drug product, and drospirenone – the progestin drug product. The adverse reaction of greatest known clinical significance with DRSP is hyperkalemia and is discussed in Section 8.2.5.1. This section reviews adverse reactions of interest primarily associated with the E4 (estrogen) component including hypertension, migraine (with and without aura), mood disturbances, and weight changes.

Hypertension

The association between combination hormonal contraceptives and hypertension is well-recognized. The mechanisms by which CHCs cause hypertension are incompletely understood,

but are believed to be related to oxidative stress.[6,7] The relationship appears to be dependent on the dose of both the estrogen and progestin components.[8] However, drospirenone may be protective against hypertension due to its anti-mineralocorticoid diuretic effects.[9]

In the clinical development program for DRSP/E4, five participants in the Integrated Safety Population (0.13%) experienced adverse reactions related to elevated blood pressure (preferred term "hypertension"): four participants in Study C302 (US/CA) and one participant in Study C301 (EU/R). Of note, two participants (b) (6) (Study C302) and (b) (6) (Study C302) were over 35 years of age; each had normal blood pressures at baseline and developed diastolic blood pressure >90 mm Hg. Participant (b) (6) discontinued the study. All five of the adverse events of hypertension were likely related to study drug product. In total, three participants discontinued due to hypertension (one from Study C301 and two from Study C302).

Hypertension is an expected adverse reaction with CHC use. The label will include a warning to monitor blood pressure and discontinue if blood pressure cannot be adequately controlled, which is consistent with class-labeling for estrogen-containing CHCs.

Migraine With and Without Aura

Twenty-three (0.6%) participants reported migraine headache. Eight (34.8%) participants categorized the event of migraine headache as mild intensity; 13 (56.5%) participants categorized the event as moderate intensity; and two (8.7%) participants reported severe symptoms. Six (26%) participants discontinued study participation due to the event.

Of specific interest to the clinical review team were cases of migraine with aura due to an increased risk of stroke in CHC users with current or previous history of migraine with aura. A total of six (0.2%) participants in the two Phase 3 clinical trials reported migraine with aura. All 6 participants discontinued study participation due to the adverse event and these cases were individually assessed:

Participant (b) (6) was a 34-year-old G3P2 white female with baseline BMI of 20.6 kg/m^2 who enrolled in Study C301 on (b) (6) and started treatment with DRSP/E4 on (b) (6) Past medical history was significant for headache that was ongoing at the time of enrollment. At the time of the event, the participant was taking ibuprofen. On (b) (6) Study Day 142, the participant experienced migraine with aura that resolved

[6] Goldhaber SZ, et al. Plasma renin substrate, renin activity, and aldosterone levels in a sample of oral contraceptive users from a community survey. *Am Heart J.* 1984; 107(1): 119.

[7] Harvey RE, et al. Oral Contraceptive Use, Muscle Sympathetic Nerve Activity, and Systemic Hemodynamics in Young Women. *Hypertension.* 2015 Sep; 66(3): 590-7.

[8] Woods JW. Oral contraceptives and hypertension. *Hypertension.* 1988; 11(3 Pt 2):II11.

[9] De Morais TL, et al. Effects of a contraceptive containing drospirenone and ethinyl estradiol on blood pressure, metabolic profile, and neurohumoral axis in hypertensive women at reproductive age. *Eur J Obstet Gynecol Reprod Biol.* 2014 Nov; 182:113-7.

spontaneously on the day of the event. The participant was not treated with medication for the event. Study drug was withdrawn due to the adverse event of migraine with aura. Per the participant's diary, the last dose of study drug was on (b) (6), however, blister accountability indicated that last dose was taken on (b) (6)

Participant (b) (6) was a 25-year-old G0P0 white female with baseline BMI 22.9 kg/m2 who enrolled in Study C301 on (b) (6) and started treatment with DRSP/E4 on (b) (6) Past medical history was significant for allergy to pollen and animals (ongoing) and stress (ongoing). Concomitant medications at enrollment included sertraline. On (b) (6) Study Day 17, the participant experienced migraine with aura [PT: migraine with aura]. The participant received treatment with codeine phosphate, ibuprofen, and paracetamol for the event. Study drug was withdrawn due to the event; last dose of study drug was taken on (b) (6)

Participant (b) (6) was a 23-year-old G0P0 white female with baseline BMI 20.1 kg/m^2 who enrolled in Study C301 on (b) (6) and started treatment with DRSP/E4 on (b) (6) No relevant medical history or concomitant medications were reported. On (b) (6) Study Day 22, the participant experienced migraine with aura [PT: migraine with aura], treated with paracetamol and rizatriptan benzoate. The event was considered recovered/resolved on (b) (6)

Participant (b) (6) was a 25-year-old G0P0 white female with baseline BMI 24.7 kg/m^2 who enrolled in Study C301 on (b) (6) and started treatment with DRSP/E4 on (b) (6) Past medical history was significant for migraine with aura (ongoing). The participant did not report concomitant medications at the time of enrollment. On (b) (6) Study Day 221, the participant experienced worsening migraine with aura [PT: migraine with aura]. The participant was not treated with any medication for the event. The study drug was withdrawn due to the event; the last dose of study drug was taken on (b) (6).

Participant (b) (6) was a 21-year-old G0P0 white female with baseline BMI 18.7 kg/m^2 who enrolled in Study C302 on (b) (6) and started treatment with DRSP/E4 on (b) (6) Past medical history was significant for bipolar disorder, depression, dysmenorrhea (ongoing), and migraines without aura (ongoing). At the time of the event, the participant was taking ibuprofen. On (b) (6) Study Day 21, the participant experienced worsening of migraines with possible aura [PT: migraine with aura]. The participant was not treated with any medication for the event. The study drug was withdrawn due to the event; the last dose of study drug was taken on (b) (6). The event was considered recovered/resolved on (b) (6)

Participant (b) (6) was a 25-year-old G1P0 white female with baseline BMI 28.0 kg/m^2 who enrolled in Study C302 on (b) (6) and started treatment with DRSP/E4 on (b) (6) No relevant medical history or concomitant medications were reported. On (b) (6) (b) (6) Study Day 170, the participant experienced migraine with aura [PT: migraine with aura]. The participant was not treated with any medication for the event. The study drug was withdrawn due to the event; the last dose of study drug was taken on (b) (6) The event was considered recovered/resolved on (b) (6)

All six of these cases of migraine with aura are related to study drug and resulted in patients being discontinued. A total of 4 cases occurred in Study C301 (Europe/Russia), while 2 cases occurred in Study C302 (US/CA) with no significant difference in presentation of the migraine with aura. None of these events resulted in stroke. Of note, one participant who had a history of migraine with aura should probably not have been enrolled in a CHC study given the risk of stroke.

A prior history of migraine with aura is a contraindication to CHC use and is currently class-labeled. The six cases of migraine with aura occurring in the clinical development program for DRSP/E4 will be described in the Adverse Reactions – Clinical Trials Experience (Section 6.1) of the product label. Participants with a prior history of migraine may be at increased risk for worsening of migraine. This risk is described in current class-labeling for CHCs and will be included in product labeling for DRSP/E4.

Mood Changes and Depression

Whether CHCs may cause new onset depression or exacerbation of existing depression remains unclear. Of participants receiving DRSP/E4, 19 experienced “depression,” “depressed mood,” or “depressive symptom” requiring management with an anti-depressant medication. Of these, five participants reported no prior history of depression or other mood disorder. Five participants reported adverse events indicating worsening of their previously existing condition (n=2 with anxiety, n=3 with depression). Nine participants had “drug withdrawn” as a result of the adverse event related to depression.

Current class-labeling warns providers of the potential for CHCs to cause new onset of depression or exacerbation of pre-existing depression and will be included in product labeling for DRSP/E4.

Changes in Body Weight

The majority of individuals reporting changes in body weight participated in Study C302. Among participants in Study C302, 57 (2.1%) reported the adverse event “weight increased.” Of these, 14 (24.6%) had baseline BMI in the overweight range (BMI ≥25 kg/m^2 or <30 kg/m^2), while 22 (38.6%) had baseline BMI in the obese range (BMI ≥30 kg/m^2). In the overall Safety Population, 104 participants (2.7%) reported the adverse event “weight increased.” Among these participants, nearly one-third (n=29, 27.9%) had a baseline BMI less than 25 kg/m2. The majority of reports of increased weight occurred in individuals with baseline BMI in the overweight (n=19, 18.3%) or obese (n=24, 23.1%) category. Eleven participants reported decreased weight.

While individual participants may have subjectively experienced changes in weight, as a group, there were no overall changes in weight in the safety populations for Study C302 or the ISP as discussed later in this section. In summary, there was no evidence that use of DRSP/E4 resulted in reduction or increase in weight or BMI. This is consistent with data from other CHC trials.

Bleeding Patterns

For the purposes of this review, scheduled bleeding was defined as withdrawal bleeding that occurred during the hormone-free interval from Day 25 of the current cycle through Day 3 of the next cycle as defined by Mishell et al.[10] Unscheduled bleeding was defined as any episode of bleeding and/or spotting that occurred during Days 4 through 24 of the current cycle. Amenorrhea was defined as the *absence* of scheduled bleeding from Day 25 of the current cycle through Day 3 of the next cycle. Bleeding patterns in the US/CA Phase 3 clinical trial - MIT-Es0001-C302 (US/CA) – are considered most relevant to the indicated population and are the focus of this review. Because of differences in definitions of bleeding patterns, other phase 2 studies conducted to support the application are not included in this analysis.

In Study C302, approximately 63% of participants reported unscheduled bleeding in the first cycle of use, followed by 53% in Cycle 2, as is typical for CHC users beginning product use on the first day of menses. Rates of unscheduled bleeding gradually decreased with increasing duration of use of DRSP/E4. Participants reported approximately 2.6 mean bleeding days in Cycle 1, followed by 1.9 mean bleeding days in Cycle 2, which also gradually decreased with increasing duration of use. Amenorrhea rates also began with a higher rate of 58% in Cycle 1, followed by 54% in Cycle 2, and gradually decreased with increasing duration of use.

Table 42 illustrates the proportion of participants reporting unscheduled bleeding and amenorrhea across cycles in Study C302 (US/CA).

[10] Mishell DR Jr, Guillebaud J, Westhoff C, Nelson AL, Kaunitz AM, Trussell J, Davis AJ. Recommendations for standardization of data collection and analysis of bleeding in combine hormone contraceptive trials. *Contraception.* 2007 Jan; 75(1):11-5.

Table 42. Rates of Unscheduled Bleeding and Amenorrhea in Participants Receiving DRSP/E4, Study MIT-Es0001-C302[*,†]

	No. of	Unscheduled Bleeding[‡]		Amenorrhea[**]
Cycle	Evaluable Cycles (m)	No. of Participants [n] (Rate [n/m])	No. of Bleeding Days (Mean [SD])	No. of Participants [n] (Rate [n/m])
1[¥]	1758	1121 (63.8)	2.6 (4.45)	1026 (58.4)
2	1579	841 (53.3)	1.9 (2.96)	851 (53.9)
3	1526	784 (51.4)	2.0 (3.62)	819 (53.7)
4	1420	705 (49.6)	1.8 (3.79)	717 (50.5)
5	1352	628 (46.4)	1.6 (2.74)	664 (49.1)
6	1319	651 (49.4)	1.6 (2.45)	688 (52.2)
7	1235	597 (48.3)	1.6 (2.61)	602 (48.7)
8	1208	558 (46.2)	1.5 (2.43)	593 (49.1)
9	1162	559 (48.1)	1.6 (4.10)	566 (48.7)
10	1053	474 (45.0)	1.4 (2.28)	482 (45.8)
11	1028	457 (44.5)	1.3 (2.32)	471 (45.8)
12	1006	453 (45.0)	1.4 (2.48)	459 (45.6)

Source: NDA 214154, m2.7.3 Summary of Clinical Efficacy
* Represents Intention-to-Treat (ITT) Safety Population i.e., all subjects who enrolled and received at least 1 dose of study drug in Study C302
† Scheduled bleeding is defined as bleeding or spotting that occurred on Cycle Day 25 of the current cycle through Day 3 of the next cycle.
‡ Unscheduled bleeding is defined as bleeding or spotting that occurred on Days 4 through 24 of the current cycle.
** Amenorrhea is defined as the absence of bleeding or spotting on Cycle Day 25 of the current cycle through Cycle Day 3 of the next cycle.
¥ Cycle 1 data may represent a larger proportion of participants due to Day 1 start in the first cycle of DRSP/E4 use.
Abbreviations: m, number of participants with cycle data; n, number of participants with bleeding or spotting; SD, standard deviation

Unscheduled bleeding and amenorrhea are not serious adverse events, but can result in tolerability issues with some deciding to discontinue the product. Although amenorrhea can be a benefit in some females, others will worry about possibly being pregnant and discontinue their product. Rates of unscheduled bleeding and amenorrhea decreased over time. Unscheduled bleeding and amenorrhea rates did not differ significantly between the US/Canadian (Study C302) and European/Russian (Study C301) populations (data not shown). Overall, rates of unscheduled bleeding and amenorrhea appear consistent with bleeding profiles of other approved CHCs. Unscheduled bleeding and amenorrhea rates appear in the [redacted] (b) (4) section of Adverse Reactions [redacted] (b) (4)

Laboratory Findings

Data from Phase 2 and Phase 3 studies were not pooled due to dissimilar time points for laboratory assessments. Laboratory assessments from the Phase 3 studies were pooled for review as it was unlikely that there would be differences in these routine laboratory findings between the two trials. Additional results from Phase 2 studies are discussed where relevant.

Hematology Parameters

Hematology parameters of interest include hemoglobin and hematocrit due to the indirect effects of both decreased blood loss with menses in some participants and increased loss due to inter-cycle bleeding in others associated with CHC use. The Applicant also measured multiple

hematology parameters at baseline and at end of study, but are not the focus of this review due to lack of clinical significance.

Table 43 summarizes the proportions and direction of change for shifts in hemoglobin.

Table 43. Hematology Parameters Shift in Pooled Phase 3 Studies of DRSP/E4 (14.2/3) mg (N=3632)

	Baseline Value		
End-of-Study Value	**Low**	**Normal**	**High**
Hemoglobin			
Low	133 (4.6)	167 (5.7)	0 (0.0)
Normal	109 (3.7)	2491 (85.2)	10 (0.3)
High	0 (0.0)	9 (0.3)	3 (0.1)

All lab parameters are presented as n (%).
Abbreviations: DRSP, drospirenone; E4, estetrol

Overall, hemoglobin parameters demonstrated the highest proportion of shifts from one category (low, normal, or high) at baseline to another category at end of study, but there was no specific trend of concern. However, of clinical importance, mean values of hemoglobin (in g/L), did not differ between baseline and end of study. Significant shifts in other hematology parameters did not occur.

COCs have not been demonstrated to have clinically concerning effects on hematologic parameters. After review, no clinical adverse events were reported in association with shifts in other hematology parameters (including erythrocytes, leukocytes and lymphocytes). The effect of DRSP/E4 on hematology parameters lacks clinical significance and is therefore not represented in labeling.

Biochemistry Parameters

The Applicant measured multiple biochemistry parameters at baseline and at end of study. Significant shifts in biochemistry parameters did not occur, did not result in reported adverse events, and are therefore not represented in labeling. The effects of DRSP/E4 on serum potassium levels are of particular interest due to the drospirenone component of DRSP/E4, are represented in labeling, and are discussed separately in Section 8.2.5.1.

Special Safety Parameters

Combination hormonal contraceptives are known to have specific effects on androgens, liver proteins and lipids. Details of the results of the laboratory findings are discussed in Section 8.2.8. None of the results from these studies raised concerns that required labeling clinically different from CHC class labeling.

Vital Signs

The majority of participants demonstrated no clinically significant differences in blood pressure from baseline to end-of-study, although some participants did experience hypertension. The adverse event of “hypertension” is discussed separately in Section 8.2.4. Significant changes from baseline to end-of-study in group heart rate or body weight did not occur, as seen in Table

44 below.

Table 44. Vital Signs at Baseline and End-of-Study in Participants Using DRSP/E4

	Baseline		End-of-Study	
Vital Signs	**Mean (SD)**	**Median (Min, Max)**	**Mean (SD)**	**Median (Min, Max)**
Systolic blood pressure	113.8 (10.5)	113 (83, 142)	113.8 (10.5)	114 (73, 164)
Diastolic blood pressure	73.1 (7.9)	73 (49, 96)	72.8 (7.7)	72 (41, 106)
Heart rate (beats/min)	73 (9.4)	72 (42, 120)	74 (9.4)	74 (40, 128)
Weight (kg)	67 (13.1)	65 (35, 148.8)	67.3 (13.5)	64.9 (35.6, 144.5)

Source: NDA 214154, ISS ADVS, Clinical Reviewer Analysis.

None of the group mean changes in blood pressure, heart rate or weight raised any new safety concerns. Transient increases in blood pressure occurred, however five participants experienced an adverse reaction of hypertension as discussed in Section 8.2.4. Hypertension is a known adverse reaction associated with CHC-use, and is included in labeling for DRSP/E4.

Electrocardiograms (ECGs)

No clinically relevant findings were observed in electrocardiogram and echocardiography examinations in studies C103, C201, and C202. In study C103, continuous Holter monitoring was performed from 1-hour pre-dose up to 24h post-dose on Day -1 and Day 28 of treatment. No abnormal findings were observed at any dose at any time and no specific labeling on ECG findings is needed.

QT

Studies C103 and C106 evaluated the potential of DRSP/E4 to cause delayed cardiac repolarization. Study C103 tested DRSP/E4 in a single dose as well as in 14-day daily doses of 3/15 mg, 6/30 mg, 12/60 mg, 15/75 mg (up to 5 times the therapeutic dose) compared to placebo. Study C106 tested single and multiple (10-day) dose DRSP/E4 compared to moxifloxacin 400 mg (active control). DRSP/E4 did not demonstrate a clinically relevant effect on ECG parameters.

Immunogenicity

Immunogenicity studies were not performed and not necessary for this hormonal contraceptive product.

8.2.5. Analysis of Submission-Specific Safety Issues

8.2.5.1. Serum Potassium Elevations

Documented serum potassium elevations above the threshold of 5.5 mmol/L occurred in a total of 189 participants in the overall Phase 3 trial population. More than two-thirds of these occurred in Study C302 (n=137, 72.5%). Almost half (n=82) occurred at the screening visit. Abnormal renal function at screening was exclusionary. The Applicant submitted additional details describing the sequence of events for each study participant with elevated potassium

levels in response to an Information Request sent by the Review Division on December 14, 2020.

Per the Applicant's protocols, any serum potassium value ≥6 mmol/L was automatically repeated. Eighteen participants had serum potassium levels of 6.0 mmol/L or greater during the course of the study (i.e., Visit 5 or Visit 7). Of these, 7 participants had serum potassium levels greater than or equal to 6.0 mmol/L. None of these 7 participants had their serum potassium levels repeated. Of the remaining 11 participants, repeat serum potassium values were within the normal range. No participants with elevated serum potassium values reported symptoms associated with hyperkalemia or required evaluation with ECG. All participants had documented normal renal function at baseline and at Visit 5. The Applicant did not document whether hemolyzed specimens were noted at the time of blood sample collection and analysis by the central lab.

Hyperkalemia

Eight participants' records documented the preferred terms "hyperkalemia" (n=6) and "blood potassium increased" (n=2). Of these, three participants had serum potassium elevations greater than 6 mmol/L at Visit 5 or Visit 7 that could reasonably be considered attributable to study drug. Three participants had serum potassium levels between 5.5 and 6.0 mmol/L at Visit 5 that returned to baseline at Visit 7. One participant (C302-(b) (6) had elevated serum potassium at screening and is therefore not considered treatment-emergent. One participant in Study 301 (C301-(b) (6)) had the reported preferred term "hyperkalemia" documented, but review of the dataset did not find any elevated serum potassium values. None of the study participants with reported preferred terms "hyperkalemia" or "blood potassium increased" indicated the presence of signs or symptoms associated with hyperkalemia or need for further evaluation.

Overall, the majority of elevated serum potassium levels noted during the clinical development program for DRSP/E4 appear to be incidental (possibly due to hemolyzed specimen) and unrelated to adverse effects of the study product. Consistent with labeling for other drospirenone-containing CHCs, product labeling for DRSP/E4 warns providers of the following: the contraindication for DRSP/E4 use in the presence of known renal impairment; the potential effects of the drospirenone component of DRSP/E4 on serum potassium levels; and the potential for drug-drug interactions with concomitant medications that may also increase serum potassium levels.

8.2.5.2. Endometrial Safety

Study PR3054

Study PR3054 was a Phase 1, partly randomized, open-label, multiple dose study to evaluate the safety, tolerability, pharmacokinetics, and pharmacodynamics of 3 doses of E4 and compare the lowest dose of 2 mg E4 to 2 mg of estradiol (E2) after daily oral administration for 28 days in healthy postmenopausal females. All endometrial biopsy samples showed atrophic/inactive

endometrium at screening and proliferative endometrium after receiving E4 10 mg for 28 days. All but one participant demonstrated return to baseline at follow-up.

MIT-Es0001-C301 – Endometrial Safety Substudy

Endometrial histology was evaluated in a substudy of the pivotal Phase 3 European/Russian study (MIT-Es0001-C301). The central laboratory initially selected by the Sponsor was (b) (4). The Sponsor was concerned by two cases of simple endometrial hyperplasia reported by this laboratory. These slides were reviewed by a well-recognized gynecologic pathologist in (b) (6) in a blinded fashion. Her diagnosis was benign secretory endometrium, cyclic type instead of hyperplasia. Based on this discrepancy the Sponsor met with the agency in a Type C meeting on April 29, 2019 and presented their plan to have all the slides re-read by a separate panel of three well-recognized pathologists. The agency found this plan acceptable. Results of re-read slides revealed no evidence of hyperplasia (with or without atypia) and no evidence of malignancy

The substudy screening and on-treatment endometrial biopsies were taken from participants in Finland, Germany and Poland. The on-treatment biopsies were taken between cycle days 12 and 19 from subjects who had at least 10 cycles of treatment. In total, 108 paired biopsies underwent histopathological analysis. The most commonly found subtypes of benign histology from the on-treatment biopsies revealed inactive endometrium (56.5%), proliferative endometrium (20.4%), atrophic endometrium (10.2%) and secretory endometrium (4.6%). This histologic pattern distribution is similar to what is seen in endometrial biopsies taken from females on combination hormonal contraceptives. In summary there is no signal for adverse endometrial findings from this combination product containing estetrol.

Table 45 below summarizes the endometrial biopsy findings for DRSP/E4 in the endometrial substudy of Study C301.

Table 45. Endometrial Biopsy Data Summary for DRSP/E4, Endometrial Substudy of Study MIT-Es0001-C301

	DRSP/E4 (3/15 mg) (N=108)	
Histological Category	**Screening Visit n (%)**	**Visit 7a n (%)**
No tissue	0	0
Tissue insufficient for diagnosis	1 (0.9)	9 (8.3)
Benign histology		
Atrophic	2 (1.9)	11 (10.2)
Inactive	7 (6.5)	61 (56.5)
Proliferative	76 (70.4)	22 (20.4)
Weakly proliferative	30 (27.8)	10 (9.3)
Active proliferative	24 (22.2)	1 (0.9)
Disordered proliferative	22 (20.4)	11 (10.2)
Secretory	22 (20.4)	5 (4.6)
Cyclic type	16 (14.8)	1 (0.9)
Progestational type (including stromal decidualization)	6 (5.6)	4 (3.7)
Menstrual type	0	0
Simple hyperplasia without atypia	0	0
Complex hyperplasia without atypia	0	0
Pre-malignant/Malignant histology		
Simple hyperplasia with atypia	0	0
Complex hyperplasia with atypia	0	0
Carcinoma	0	0

Source: NDA 214154, Clinical Study Report: MIT-Es0001-C301, Table 46. eCTD SN: 0001.
Only participants who signed the informed consent form for the Endometrial Safety Substudy and who had 2 biopsies (one at screening and one at Visit 7a) were included in this table.
Participants who were pregnant at the time that the biopsies were taken were excluded from the table.
Abbreviations: DRSP, drospirenone; E4, estetrol

After review, none of the endometrial changes were of clinical concern and no safety labeling is needed.

8.2.6. Clinical Outcome Assessment (COA) Analyses Informing Safety/Tolerability

Not applicable.

8.2.7. Safety Analyses by Demographic Subgroups

The US/Canada trial (Study C302) was the only study where demographic subgroups were included that were sufficiently similar to those in the US. In Study C302, the adverse event frequencies did not appear to differ by age although the majority of subjects in the US/Canada trial (89.9%) and in the pooled phase 3 studies (89.1%) were 35 years of age or less. In addition, serious adverse reactions and common adverse reactions did not appear to be different in Study C302 by BMI category, except that participants with BMI ≥25 and <30 kg/m^2 (overweight) or BMI greater than or equal to 30 kg/m^2 (obese) were more likely to report increased weight. Refer to Section 8.2.4 for further discussion.

8.2.8. Specific Safety Studies/Clinical Trials

MIT-Es0001-C201 – Endocrine Function, Metabolic Control and Hemostasis Study

Study C201 – a single-center, randomized, open-label, active-controlled, three-arm study – evaluated the effects of DRSP/E4 and two reference COCs (levonorgestrel or LNG 0.15 mg/EE 0.03 mg and DRSP 3 mg/EE 0.02 mg) on endocrine function, metabolic control and hemostasis during six treatment cycles. This was a study requested by the European regulatory authority.

Enrolled participants were healthy premenopausal females ages 18-50 years with baseline BMI 18.0-30.0 kg/m^2 inclusive, and primarily Caucasian (n=93 [95%]). A total of 88 participants completed the study; four participants discontinued due to adverse events. No formal sample size calculation or analysis was performed; data are descriptive only. The three products – DRSP/E4, DRSP/EE, and LNG/EE – did not differ significantly in their effects on endocrine, metabolic, and hemostatic parameters. The clinical significance of the study of these parameters is unclear.

Additionally, the Applicant submitted findings for 2 assays measuring Activated Protein-C Resistance (APCR) – activated partial thromboplastin time (aPTT) and endogenous thrombin potential (ETP). Although this may be used by other regulatory agencies, the clinical review team does not agree that findings from these studies are predictive of the risk of clinical thromboembolism. Therefore, these findings are interesting but will not be labeled.

8.2.9. Additional Safety Explorations

Human Carcinogenicity or Tumor Development

Human carcinogenicity studies were not performed although a substudy to assess endometrial changes was discussed previously in Section 8.2.5.2.

Human Reproduction and Pregnancy

There have been pregnancies that have been reported to have occurred during CHC use over the 60 years that these products have been on the market in the US. No specific reproductive concerns with CHC exposure have been identified. Observational data collected on pregnancies occurring during the clinical development of DRSP/E4 continued to support the safety of accidental exposure to a CHC and no evidence of teratogenicity or increased risk of congenital anomalies when participants determined they became pregnant during treatment. There is no reason to continue to use DRSP/E4 or any CHC once pregnancy has been identified.

Pediatrics Assessment

Studies in the pediatric population have not been conducted as this product is not indicated in males or premenarcheal females. It is expected that use in post-menarcheal adolescent females will have a similar effectiveness and safety profile to that of adult females using DRSP/E4 for contraception.

Overdose, Drug Abuse Potential, Withdrawal, and Rebound

Specific studies evaluating overdose symptoms with DRSP/E4 were not conducted. Based on general experience with combination oral contraceptives, overdose with DRSP/E4 is expected to cause nausea, vomiting, and vaginal bleeding. Information on overdose will be reflected in labeling.

There is no drug abuse potential for DRSP/E4.

Return to ovulation after treatment cessation was assessed in studies C301, C302, and C202 and no specific issues regarding discontinuing the product were identified that required further clinical assessment.

8.2.10. Safety in the Postmarket Setting

Safety Concerns Identified Through Postmarketing Experience

Estetrol (E4) is a novel estrogen and is not currently approved for marketing in any country. Therefore, postmarketing experience with E4 is not available.

Combination oral contraceptives containing DRSP and other estrogens including ethinyl estradiol (EE) have been associated with a potential for increased risk of thromboembolism - including VTE - compared to COCs containing other progestins such as LNG and norgestimate in the postmarket setting. This experience stretches over the past 50 years in the US. Postmarket experience with DRSP/E4 is not yet available to assess the risk of thromboembolism in the general population of females expected to use this product.

Expectations on Safety in the Postmarket Setting

There is limited clinical trial data to assess the risk of thromboembolism when DRSP/E4 is used in the general US population. The trial data did not appear to demonstrate a specific signal of increased risk in the overall patient population or in those with heavier weight. However, epidemiologic data indicates a potential increased risk of VTE associated with DRSP-containing CHCs and the risk with use of E4 (as a new estrogen) will require a large, observational study. Therefore, an additional study will be required under FDAAA to further assess the risk of thromboembolism with DRSP/E4 in the post-market setting.

8.2.11. Integrated Assessment of Safety

Overall, the safety of DRSP/E4 from the US/Canada safety population and from the pooled population of females from both the US/Canada and EU/R population (Integrated Safety Population) is clinically similar to the known safety profile of other progestin-estrogen combination hormonal contraceptives in terms of serious adverse events and common adverse reactions profile. The safety profile from Study C302 included more than 17,000 cycles of exposure and is sufficient for assessment of the safety profile of DRSP/E4. It is expected that the safety profile observed in Study C302 will be likely very similar to that seen in postmarketing with other approved CHC products.

During development of DRSP/E4, FDA recommended that the Applicant conduct one or two phase 3 clinical trials with at least 20,000 28-day treatment cycles to provide adequate safety and efficacy data. At least 400-500 participants who use the product for one year should be evaluated for safety and at least 200 participants should complete the study. At least 50% of the data should come from North America. With more than 17,000 cycles of exposure and 1016 participants completing Study C302, the Applicant submitted sufficient safety data for review and approval recommendations by the Review Division.

In addition, the totality of DSRP/E4 exposure in females includes more than 30,000 cycles of exposure which is more than sufficient to characterize most adverse reactions. However, thousands of subjects with chronic use are needed to assess the rare risk of thromboembolism, particularly to see whether this risk is significantly increased in the overweight and obese populations. Given the rise in overweight and obese patients of reproductive age in the US, a postmarketing requirement is necessary to further assess this serious risk in normal, overweight and obese patients to inform both patients and providers in making an informed decision about this product.

8.3. Statistical Issues

There were two statistical issues identified in study MIT-Es0001-C302 (Study C302):

1. The dropout rate was high.
2. A numerically higher Pearl Index was observed in Black/African American subjects.

In study MIT-Es0001-C302, 2,148 subjects were enrolled in the study, 1,939 of them aged 16 to 35 years. Among the 2,148 enrolled subjects, 1,864 (86.7%) took at least one dose of study medication, 1,016 (47.3%) completed the study. Similar patterns were observed for the 1,939 enrolled subjects with age 16 to 35 years old, 1,674 (86.3%) took at least one dose of study medication and were in the ITT Population for the efficacy analysis, 899 (46.4%) completed the study. See details in Section 8.1.2.

Unfortunately, such a high dropout rate (up to 50%) has been observed in previous trials of CHC products, and was observed in US/Canada Study C302 as well. There may be many possible reasons for this including: change in insurance status, changes in family situation (e.g., breakup with partner), tolerability (dissatisfied with unscheduled bleeding and/or amenorrhea), and unplanned pregnancy. It is unclear whether any or some of these factors or other unknown factors contributed to the high dropout rate. Although this high dropout rate is not desirable, there were a sufficient number of patients that completed the trial with a sufficient number of at-risk cycles to provide an effectiveness determination for this product, and Study 301 did not suffer from this problem and is strongly supportive of the effectiveness in Europe. Nevertheless, such a high dropout rates in US trials such as Study C302 is a major concern and needs to be reduced in future trials through better design and follow-up.

A numerically higher Pearl Index in the Black or African American subjects is observed in Study C302. The Pearl Index was 6.80 (95% CI: 3.26, 12.51) in the Black or African American subjects who had at least one at-risk cycle (N=278, 1,911 at-risk cycles). See details in Section 8.1.2. Since these subgroup analyses were exploratory and not controlled for multiplicity resulting from many analyses, the finding should be interpreted with caution. In addition, exposure data from PK studies conducted in support of this application indicate no difference in overall exposure to the study product between subgroups by race. Future efforts may include encouraging drug developers to further increase recruitment among racial subgroups to ascertain if there is differential effectiveness of combination hormonal contraceptives by race.

It is important to note that differences in the efficacy and safety of DRSP/E4 between demographic subgroups cannot be fully assessed without adequate number of subjects. Going forward, applicants will be encouraged to provide sufficient numbers of cycles in females with overweight and obese BMI as well as the African American subject group to ensure sufficient similarity to the current US population baseline characteristics and further assess whether the trend of increasing PI is related to ethnicity or BMI.

8.4. Conclusions and Recommendations

The overall efficacy and safety assessments of DRSP/E4 support approval. The single US Study C302 provided substantial evidence of effectiveness and the safety profile with supportive data from EU/Russia Study C301 was similar to other combined hormonal contraceptives. Although there were few thromboembolic adverse events in the clinical trials, a postmarketing study (4054-1) will be necessary to adequately assess VTE risk for this product as compared to other approved combined hormonal contraceptive products. . Additionally, the limited safety data in overweight and obese individuals raise uncertainties regarding whether there is a differential efficacy and safety of DRSP/E4 in these individuals. The postmarketing VTE study will be critical to determine if additional study (or studies) are necessary in these higher BMI groups .

9. Advisory Committee Meeting and Other External Consultations

Combined oral contraceptives (COCs) have been approved for use in the US market since the 1960's. These COCs include a variety of progestins, dosage strengths and regimes, some of whom contain drospirenone and ethinyl estradiol as active drug substances. The safety issues associated with COCs containing drospirenone with an estrogen component as regimes for prevention of pregnancy have been established and can be adequately labeled. Therefore, no Advisory Committee advice was necessary to make a regulatory determination on this application.

10. Pediatrics

An agreed iPSP was in place (b) (4) A partial waiver has been granted for studies in males and premenarcheal females given that DRSP/E4 is not indicated in these populations.

Efficacy of DRSP/E4 for prevention of pregnancy is not expected to differ in postmenarcheal female adolescents age 17 and under compared to adults. Therefore, efficacy findings for DRSP/E4 can be extrapolated from Study C302 to all post-menarcheal adolescents. (b) (4)

11. Labeling Recommendations

11.1. Prescription Drug Labeling

Labeling for the Prescribing information, Patient Package Information, Instructions for Use and Carton/Container have been completed on 04/12/2021. Recommendations from the Office of Prescription Drug Promotion (Review finalized 03/24/2021) and the Division of Medical Policy Programs (Review finalized 03/26/2021) have been incorporated into labeling.

As for all combination hormonal contraceptives, a Patient Package Insert (PPI) is required for all estrogen-containing contraceptive products (CFR 310.501). After review, the clinical review team and DMPP concur that the PPI is sufficient to inform patients of the benefits and risks of NEXTSTELLIS.

12. Risk Evaluation and Mitigation Strategies (REMS)

The Division of Risk Management (DRM) in the Office of Medication Error Prevention and Risk Management determined that a Risk Evaluation and Mitigation Strategy (REMS) is not necessary for this product. In their March 22, 2021 assessment, DRM indicates that the safety concern of increased VTE risk associated with use of DRSP/E4 has been well documented in previously approved CHCs. In general, healthcare providers who prescribe these medications (Obstetrician/Gynecologists, and family or general practitioners) should be familiar with the increased risk of VTE. Therefore, a REMS is not necessary to ensure the benefits outweigh the risks for DRSP/E4.

13. Postmarketing Requirements and Commitment

A postmarketing requirement (4054-1) for further evaluation of VTE risk associated with DRSP/E4 has been communicated to the Applicant. The Review Division requests a prospective, observational cohort study comparing the risks for fatal and non-fatal venous thromboembolism (VTE) and arterial thromboembolism (ATE) in new users of estetrol monohydrate (E4) and drospirenone to new users of combined oral contraceptives (COCs) containing drospirenone (first comparator) and new users of COCs containing other progestogens (second comparator) in U.S. females of reproductive age using COCs primarily for contraceptive reasons. This study should have sufficient confounding control for known risk factors of VTE including age, body mass index (BMI), and smoking status, among others. The study should be sufficiently powered to detect a 1.5 to 2.0-fold increase in risk of VTE in new users of DRSP/E4. Further, the study should be sufficiently powered for a stratified analysis by BMI to allow an assessment of the risk in obese females. Milestones for the postmarketing study were agreed to on 4/12/2021 and include the following:

- Draft Protocol Submission: 10/2021
- Final Protocol Submission: 12/2022
- Interim Study Report Submission: 10/2023
- Final Study Completion: 10/2026
- Final Report Submission: 06/2027

14. Nonclinical Pharmacology/Toxicology Secondary Review Comments

I agree with the nonclinical reviewer that there are no nonclinical concerns for the safety of this DRSP/E4 CHC product, and that Pharmacology/Toxicology supports the approval of Nextstellis for prevention of pregnancy in females under NDA 214154.

E4 is a New Molecular Entity (NME), and a full nonclinical battery of studies were completed to support the nonclinical safety of E4 as the estrogen component of this CHC. E4 shows selective estrogen receptor agonist activity in vitro, but is less potent than estrogens commonly used in CHCs such as EE and E2. E4 also has the same pharmacology as related estrogens and inhibits ovulation in animal models. Toxicology studies in rats and monkeys showed expected exaggerated pharmacologic effects of an estrogen, and both carcinogenicity and reproductive toxicity studies with E4 alone demonstrated consistent estrogen class effects with no unanticipated toxicities. A 13-week study in monkeys dosed with a combination of E4 and DRSP also showed exaggerated pharmacologic effects of a progestin/estrogen combination, with no unanticipated toxicities.

DRSP is an approved progestin component of contraceptive products, both alone and in combination with estrogens. To support the nonclinical safety of DRSP in Nextstellis, the

applicant conducted a relative bioavailability study to establish an adequate scientific bridge to the DRSP component in YAZ, and therefore is able to rely on the Agency's previous findings of safety for DRSP in YAZ as reflected in the product labeling. The nonclinical sections of labeling for Nextstellis will align with the DRSP information as represented in the YAZ® approved drug product labeling.

In conclusion, the nonclinical profile of E4 administered alone or in combination with DRSP is consistent with that of other estrogenic compounds or CHC estrogen/progestin products. There are no anticipated concerns for use of Nextstellis when used clinically in females of reproductive potential to prevent pregnancy.

15. Deputy Division Director (Clinical) Comments

I agree with the CDTL, Medical Officer, and the Clinical Pharmacology, ONDQA, and Statistical review teams that this combined oral contraceptive tablet product containing estetrol (E4) and drosperinone (DRSP) should receive an approval action.

I agree that Study C302 provides substantial evidence of efficacy with the DRSP/E4 regimen with an acceptable overall Pearl Index (2.65) and upper bound of the 95% confidence intervals (CI 3.88). BMI subgroup data in females ≥30 kg/m^2 was underrepresented as compared to the current US population. However, the limited data appeared to demonstrate a trend of increasing PI and upper bound of the 95% CI in females with increasing BMI., This trend is consistent with what has been reported with other CHC products and will be presented in labeling and a limitation of use statement to inform providers and patients. In addition, non-White patients were also significantly underrepresented in Study 301 which limited further ethnic subgroup analyses. The reason(s) for this difference are not clear and future trials need to ensure adequate representation to allow more precise estimates to ensure that providers and patients can be appropriately counseled.

In addition, based on the conclusions of the OSI inspection, the Medical Officer, the CDTL, and the Clinical Pharmacology and Statistical Reviewers believe that the submitted effectiveness and safety data can be relied upon to support efficacy for DRSP/E4 in females who will use this product for prevention of pregnancy and I agree.

The overall safety profile for DRSP/E4 was acceptable through data provided from Study C302 and is similar to other COC products containing drospirenone. The most common adverse events of interest included menorrhagia, mood disorders including depression and migraine with aura. The events reported in the US/Canada study (Study C302) and the pooled safety databases were similar to those seen with other combined oral contraceptive products. Tolerability related to unscheduled bleeding/spotting with DRSP/E4 use appears similar to other CHCs. Data from the Study C302 and the Integrated Safety population will both be labeled to inform risks/benefits for the individual female who may choose to use this DRSP/E4 product.

As with other COCs, cases of serious adverse events of thromboembolism occurred with use of this DRSP/E4 product and this risk will be included as class labeling for serious thrombotic events in the WARNINGS AND PRECAUTIONS section of labeling. In addition, there is no specific VTE signal in this database that warrants a CONTRAINDICATION in a specific population. However, caution is necessary to apply clinical trial thromboembolism rates to that of postmarketing use. In addition, an increased potential VTE risk has been identified during postmarketing in contraceptive products containing drosperinone. Therefore, I concur with the Medical Officer and CDTL that because of ongoing concerns of a potential increased risk of thromboembolism and specifically, VTE risk that has been identified with drosperinone-containing COCs from some observational epidemiologic studies, a postmarketing requirement will be necessary when this product is approved to further elucidate the impact of this new regimen on thromboembolic risk, particularly in overweight and obese females who already have an increased baseline risk.

The benefit/risk assessment for DRSP/E4 is favorable for a prevention of pregnancy indication and submitted clinical data from the US/Canada Study (Study C302) was sufficient to support approval with supportive safety data from the European/Russian study (Study C301). Prescriber and patient package insert labeling has been agreed to. In addition:

No advisory committee meeting was needed as no new efficacy or safety concerns outside of those expected for a combined hormonal contraceptive were identified in this submission that required committee input.

The safety database does not appear to demonstrate a unique safety issue or signal. The prescribing community is well aware of the benefits and risks of combined hormonal contraceptive products. Therefore, a risk mitigation and evaluation strategy (REMS) was not necessary for DRSP/E4 (Refer to the Division of Risk Management review dated 3/22/2021)

A postmarketing requirement (PMR) will be necessary to further define the risk of thromboembolism with use of DRSP/E4. The Division of Epidemiology concluded that ARIA was not sufficient to address the issue of thromboembolism because the database contains insufficient information on BMI to perform the necessary subgroup analyses (See ARIA Insufficiency Review dated 3/29/2021). No other PMRs or postmarketing commitments (PMCs) were determined to be necessary by the review teams. Milestones have been agreed to as follows:

- Draft Protocol Submission: 10/2021
- Final Protocol Submission: 12/2022
- Interim Study Report Submission: 10/2023
- Final Study Completion: 10/2026
- Final Report Submission: 06/2027

16. Appendices

16.1. References

Nonclinical References:

Abot, A., et al., The uterine and vascular actions of estetrol delineate a distinctive profile of estrogen receptor alpha modulation, uncoupling nuclear and membrane activation. EMBO Mol Med. 2014. 6(10): 1328-46.

Coelingh Bennink F, Holinka CF, Visser M, Cloelingh Bennink HJT. Maternal and fetal estetrol levels during pregnancy. Climacteric. 2008. 11(Suppl 1): 69-72.

Gérard, C., et al., Combined estrogenic and anti-estrogenic properties of estetrol on breast cancer may provide a safe therapeutic window for the treatment of menopausal symptoms. Oncotarget. 2015b. 6(19): 17621-36.

Hammond GL, Hogeveen KN, Visser M, Coelingh Bennink HJT. Estetrol does not bind sex hormone binding globulin or increase its production by human HepG2 cells. Climacteric. 2008. 11(Suppl 1): 41-46.

Montt-Guevara, M.M., et al., Regulatory effects of estetrol on the endothelial plasminogen pathway and endothelial cell migration. Maturitas. 2017. 99: 1-9.

Tulchinsky D, Frigoletto Jr. FD, Ryan KJ, Fishman J. Plasma estetrol as an index of fetal well-being. J Clin Endocrinol Metab. 1975. 40(4):560-7.

Visser M, Foidart JM, and Coelingh Bennink HJT. In vitro effects of estetrol on receptor binding, drug targets and human liver cell metabolism. Climacteric. 2008. 11(Suppl 1): 64-68.

16.2. Financial Disclosure

The Applicant certifies that no clinical investigator, or their spouse or dependent child, had disclosable financial interests in and/or arrangements with the sponsor of the covered clinical study as per 21 CFR part 54.

Covered Clinical Study (Name and/or Number): Study C301

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 63		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR		

54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

Covered Clinical Study (Name and/or Number): Study C302

Was a list of clinical investigators provided:	Yes ☒	No ☐ (Request list from Applicant)
Total number of investigators identified: 76		
Number of investigators who are Sponsor employees (including both full-time and part-time employees): 0		
Number of investigators with disclosable financial interests/arrangements (Form FDA 3455): 0		
If there are investigators with disclosable financial interests/arrangements, identify the number of investigators with interests/arrangements in each category (as defined in 21 CFR 54.2(a), (b), (c) and (f)): Compensation to the investigator for conducting the study where the value could be influenced by the outcome of the study: _____ Significant payments of other sorts: _____ Proprietary interest in the product tested held by investigator: _____ Significant equity interest held by investigator in S Sponsor of covered study: _____		
Is an attachment provided with details of the disclosable financial interests/arrangements:	Yes ☐	No ☐ (Request details from Applicant)
Is a description of the steps taken to minimize potential bias provided:	Yes ☐	No ☐ (Request information from Applicant)
Number of investigators with certification of due diligence (Form FDA 3454, box 3) 0		
Is an attachment provided with the reason:	Yes ☐	No ☐ (Request explanation from Applicant)

After review, the clinical reviewer concurred that there were no identified financial disclosure issues.

16.3. Nonclinical Pharmacology/Toxicology

Not applicable for this combination product

16.4. OCP Appendices (Technical Documents Supporting OCP Recommendations)

No additional supporting technical documents required.

16.5. Additional Clinical Outcome Assessment Analyses

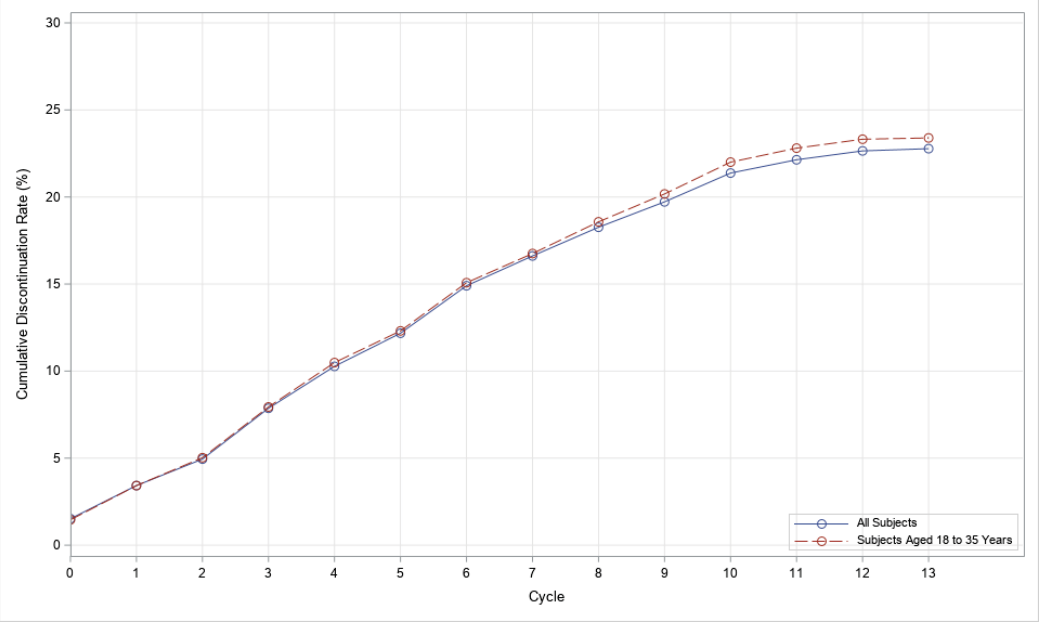
Not applicable.

16.6. Additional Analyses

Additional Efficacy Results (Study C301)

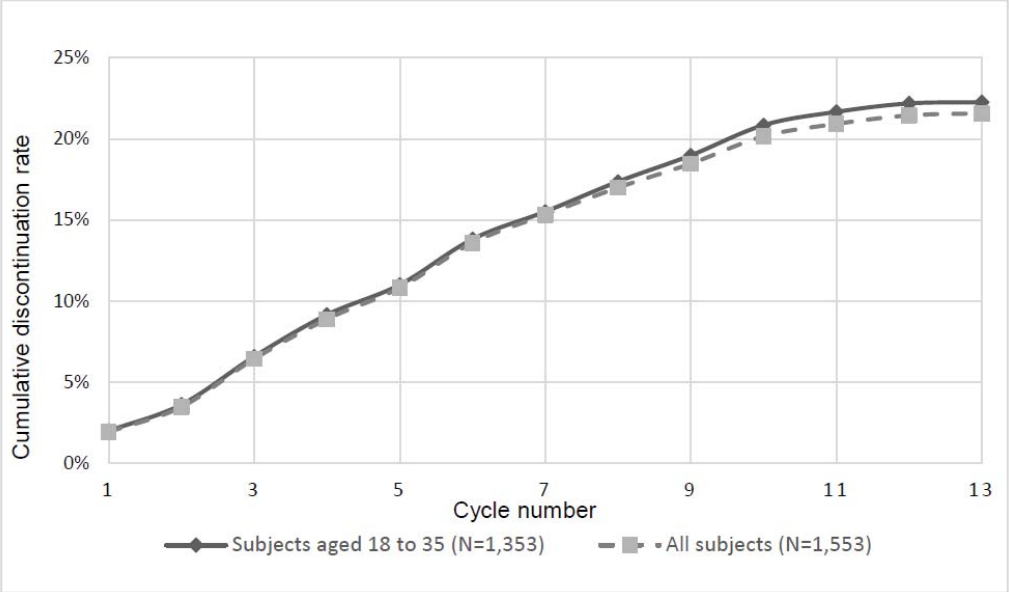
The Kaplan-Meier estimates of cumulative discontinuation rates based on the Enrolled Population and ITT population are displayed below.

Figure 7. Cumulative Discontinuation Rate, Enrolled Population, Study MIT-Es0001-C301



Source: Statistical reviewer's analysis.

Figure 8. Cumulative Discontinuation Rate, ITT Population, Study MIT-Es0001-C301



Source: Study MIT-Es0001-C301 clinical study report Figure 2 and confirmed by the statistical reviewer.
Abbreviations: ITT, intent-to-treat

Efficacy Results – Secondary and Other Relevant Endpoints

Life-table estimates of the cumulative pregnancy rates based on the on-treatment pregnancies and all cycles in subjects aged 18 to 35 years from the ITT Population are presented in Table 46. The cumulative pregnancy rate after 13-cycle of use was 0.45% (95% CI: 0.19%, 1.09%) for subjects aged 18 to 35 years in the ITT Population.

Table 46. Life-Table Estimate of Cumulative Pregnancy Rate, Study MIT-Es0001-C301

Variable	Subjects Aged 18-35 Years With All Cycles (N=1,353)
Number of subjects with at least 1 at-risk cycle	1,353
Number of cycles	15,343
Number of on-treatment pregnancies	5
Cumulative pregnancy rate (95% CI)	0.45% (0.19%, 1.09%)

Source: Study MIT-Es0001-C301 clinical study report Tables 14.2.2 and confirmed by the statistical reviewer.
Abbreviations: CI, confidence interval

Efficacy Results – Subgroup Analysis

The Pearl index by subgroups of race, age, BMI, ethnicity, and BMI, hormonal contraceptives use, and smoking status at baseline for subjects aged 18-35 years is presented in Table 47. The results by the subgroup are consistent with the overall analyses results, in general. The Pearl Indices were numerically higher in the smokers and Black or African American subjects. However, due to limited number of subjects in these subgroups, the interpretation of this observation need caution.

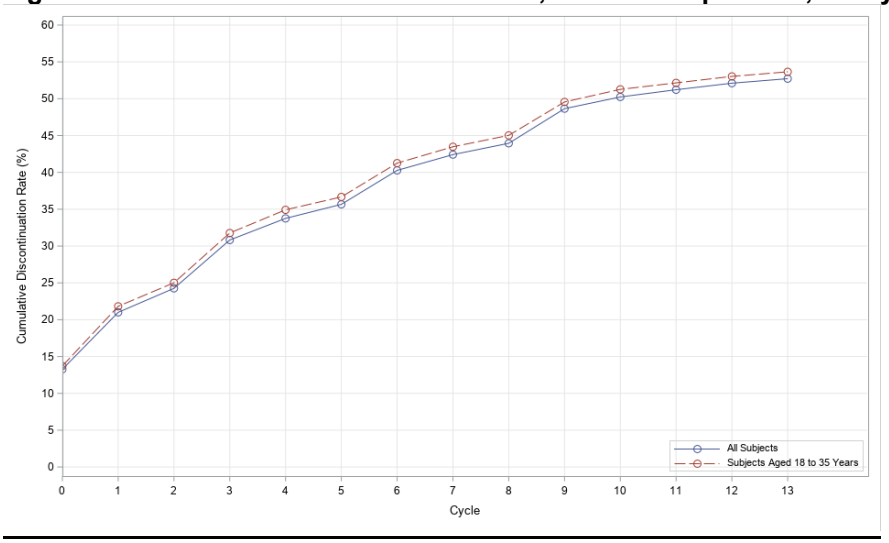
Table 47. Pearl Index by Subgroup, Study MIT-Es0001-C301

Subgroup	N	On-Treatment Pregnancies	At-Risk Cycles	Pearl Index (95% CI)
Race				
White	1,296	3	13,553	0.29 (0.06, 0.84)
Black or African American	6	2	45	57.8 (7.0, 208.7)
Asian	9	0	76	0
Other	2	0	18	0
Age (years)				
≥18 to ≤25	770	3	7,823	0.50 (0.10, 1.46)
>25 to ≤35	543	2	5,869	0.44 (0.05, 1.60)
BMI (kg/m^2)				
<30	1,240	5	12,906	0.50 (0.16, 1.18)
≥30	73	0	786	0
Hormonal contraceptives use				
Starters	500	1	5,113	0.25 (0.01, 1.42)
Switchers	813	4	8,579	0.61 (0.17, 1.55)
Smoking status at baseline				
Smoker	238	2	2496	1.04 (0.13, 3.76)
Non-smoker	1,075	3	11,196	0.35 (0.07, 1.02)

Source: Study MIT-Es0001-C301 clinical study report Tables 14.2.1.4 and confirmed by the statistical reviewer.
Abbreviations: BMI, body mass index; CI, confidence interval; N, number of subjects in subgroup

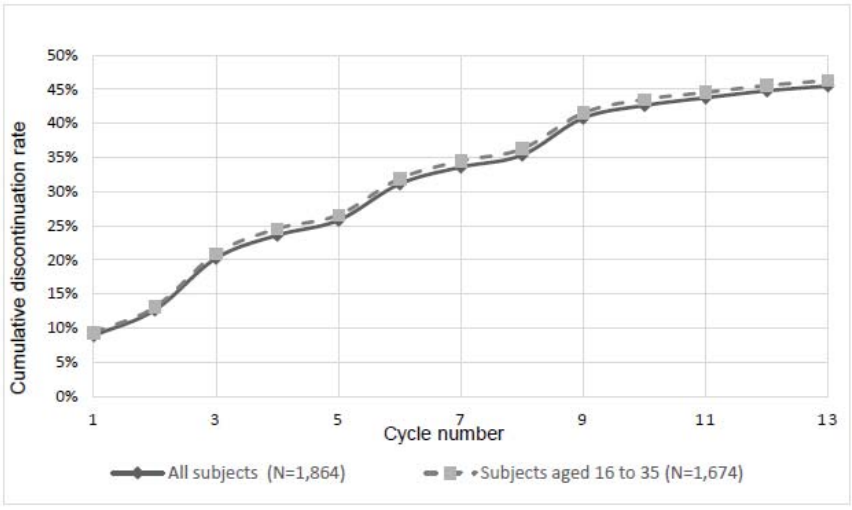
Additional Efficacy Results (Trial C302)

Figure 9. Cumulative Discontinuation Rate, Enrolled Population, Study MIT-Es0001-C302



Source: Statistical reviewer's analysis.

Figure 10. Cumulative Discontinuation Rate, ITT Population, Study MIT-Es0001-C302



Source: Study MIT-Es0001-C302 clinical study report Figure 2 and confirmed by the statistical reviewer.
Abbreviation: ITT, intent-to-treat

Figure 11. Subjects Disposition, Study MIT-Es0001-C302

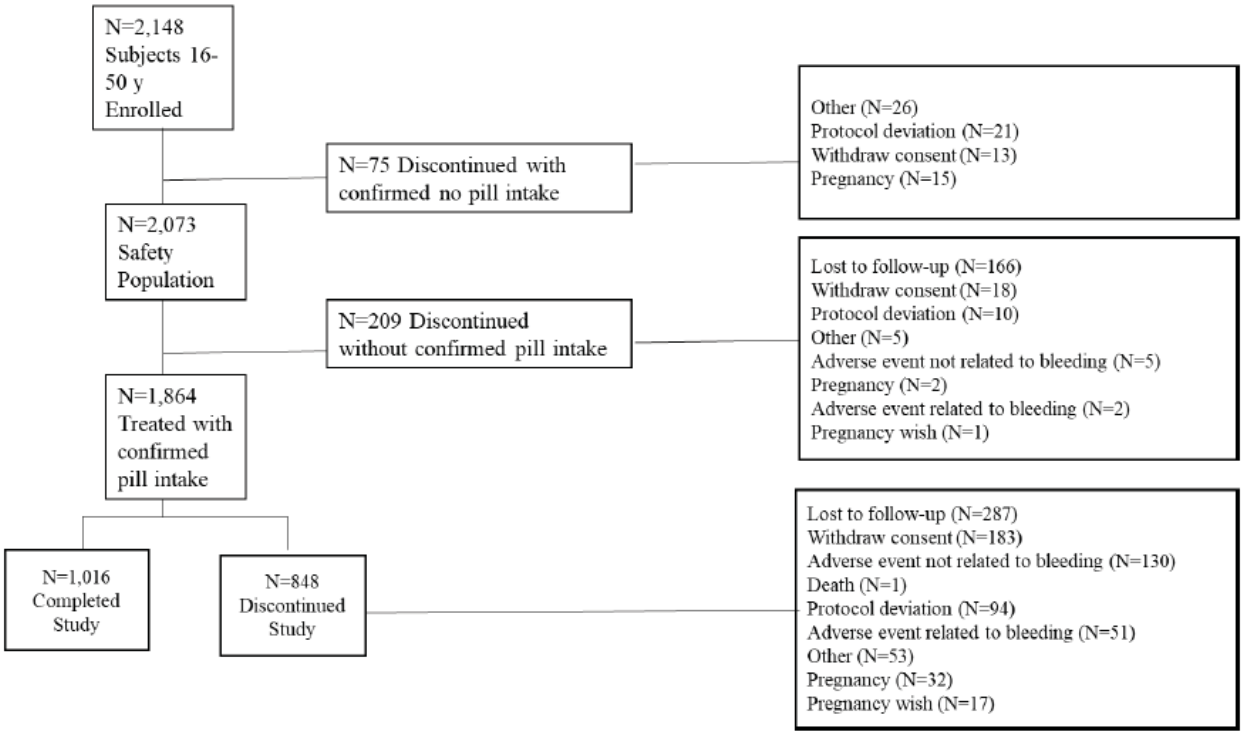
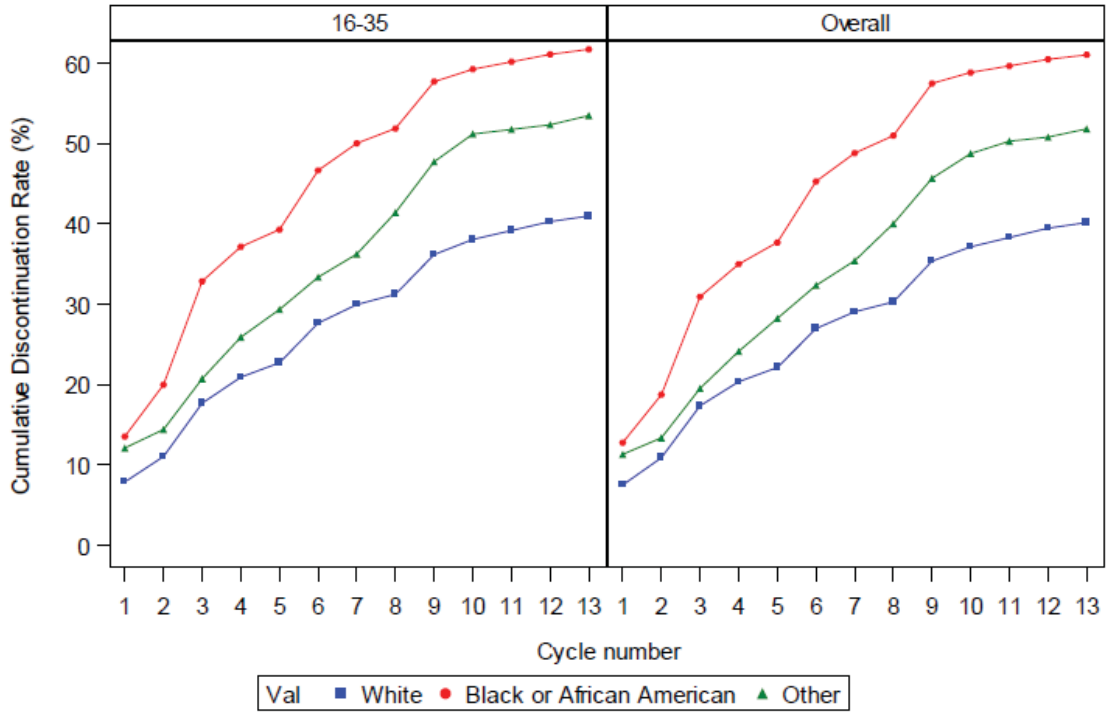
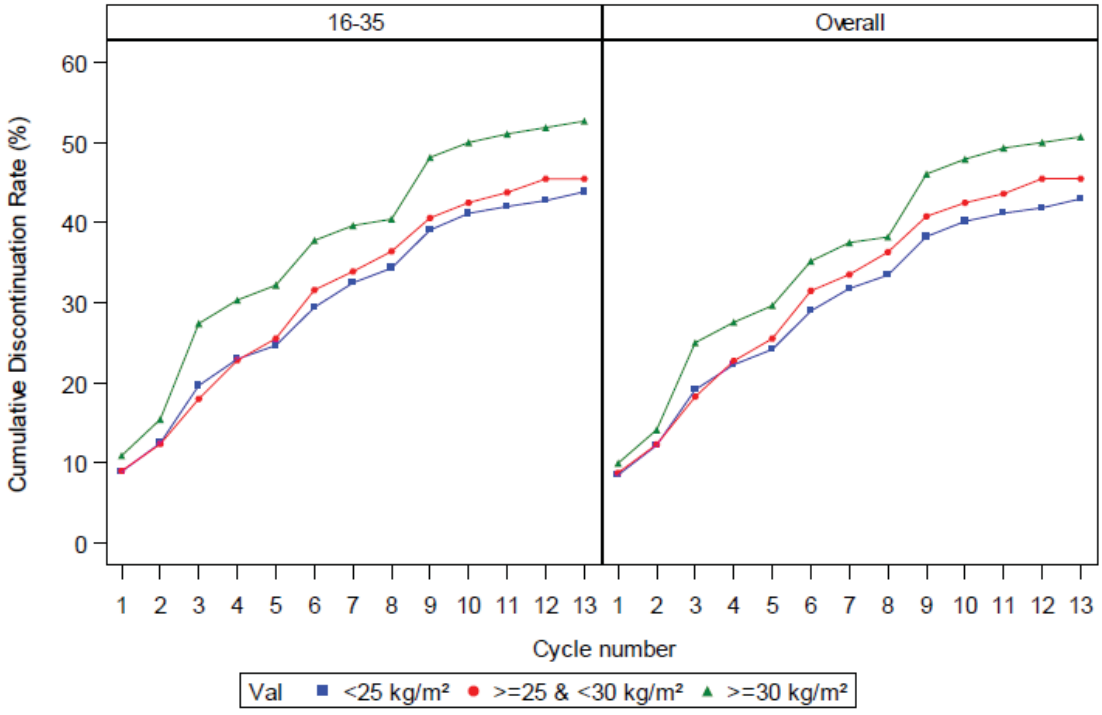


Figure 12. Cumulative Discontinuation Rate by Race, ITT Population, Study MIT-Es0001-C302



Source: The applicant's submission on 12/8/2020.
[a] "Other Races" included Asian, American Indian, or Alaska Native, Native Hawaiian or other Pacific Islander, and Other.
Abbreviation: ITT, intent-to-treat

Figure 13. Cumulative Discontinuation Rate by BMI, ITT Population, Study MIT-Es0001-C302



Source: The applicant's submission on 12/8/2020.
Abbreviations: BMI, body mass index; ITT, intent-to-treat

16.7. Pregnancy Adjudication

Table 48 below lists all participants with positive pregnancy tests during Study C302 and adjudication of each pregnancy.

Table 48. Adjudication of Positive Pregnancy Tests, Study MIT-Es0001-C302

Participant	Age	Gravidity/ Parity	Race	Pre/On/Post-Treatment Per Medical Officer	Pre/On/Post-Treatment Per Applicant
(b) (6)	22	G0P0	Black	On	On
	21	G1P1	White	On	On
	20	G1P1	White	On	On
	29	G1P1	White	On	On
	28	G1P0	Black	On	On
	27	G0P0	Black	On	On
	26	G7P3	American Indian	On	On
	35	G3P2	Asian	On	On
	24	G2P1	Black	On	On
	31	G2P2	White	On	On
	33	G3P3	White	On	On
	24	G3P3	White	On	On
	22	G0P0	White	On	On
	36	G0P0	White	On	On
	25	G0P0	Black	On	On
	21	G2P1	Black	On	On
	20	G0P0	Black	On	On
	19	G0P0	Black	On	On
	29	G2P2	White	On	On
	21	G0P0	Asian	On	On
	27	G2P0	White	On	On
	32	G3P2	White	On	On
	31	G5P4	White	On	On
	21	G2P2	White	On	On
	25	G0P0	Black	On	On
	36	G1P1	White	On	On
	23	G1P1	Black	On	On
	35	G2P2	White	On	On
	26	G2P2	White	Post	Post
	29	G3P3	Black	Post	Post
	26	G2P1	Black	Post	Post
	25	G1P1	Black	Post	Post
	20	G0P0	White	Post	Post
	25	G1P1	Black	Post	Post
	30	G3P2	White	Post	Post
	18	G0P0	White	Post	Post
	29	G0P0	White	Post	Post
	19	G0P0	White	Pre	Pre
	23	G3P3	Black	Pre	Pre
	29	G2P2	White	Pre	Pre
	23	G2P0	White	Pre	Pre
	23	G2P0	Black	Pre	Pre
	28	G2P1	White	Pre	Pre
	19	G1P1	Mixed race	Pre	Pre
	36	G3P3	White	Pre	Pre
	23	G3P2	White	Pre	Pre

Participant	Age	Gravidity/ Parity	Race	Pre/On/Post-Treatment Per Medical Officer	Pre/On/Post-Treatment Per Applicant
(b) (6)	27	G1P1	White	Pre	Pre
	32	G1P1	White	Pre	Pre
	25	G0P0	White	Pre	Pre
	25	G1P0	White	Pre	Pre
	28	G3P3	White	Pre	Pre
	34	G3P2	White	Pre	Pre
	24	G0P0	Black	Pre	Pre
	22	G0P0	White	Pre	n/a
	24	G0P0	White	Pre	Pre
	19	G2P1	White	Pre	pre
	29	G3P2	White	n/a	Post
	23	G2P0	Black	n/a	n/a
	21	G0P0	Black	n/a	n/a

Abbreviations: n/a, never confirmed

16.8. Summary of Protocol Amendments: C302

The following protocol amendments and communications to investigators regarding clarifications of the protocol occurred during study C302 (Table 49):

Table 49. Protocol Amendments and Communications to Investigators, Study C302

Protocol Version	Protocol Date	Summary of Changes
Final version 1.1	June 7, 2016	Study initiated according to this version
Relevant safety communication	August 23, 2016	Clarification Memo – pill initiation instructions and need for use of condoms after intrauterine device (IUD) removal
Protocol amendment 1.0	December 14, 2016	Section 9.4.4.1 – instructions for pill initiation after IUD removal Section 9.4.4.3 – instructions in the case of vomiting or diarrhea Section 9.5.2 – 1. instructions to study participants desiring to discontinue to continue study product until early termination visit if possible 2. instructions for early termination visit Section 9.6.4.2 Population PK Substudy – instructions for timing of study drug dose will be determined by the time of the appointment Section 9.6.4.9 Contraceptive counseling
Protocol amendment 1.1	July 10, 2017	Section 9.5.2 – Subject enrollment: instructions to subject not on a hormonal contraceptive who is enrolled but has not started the investigational product; contact the study site if next menses is <21 days or >35 days since previous menses.
Clarification note on teratogenic medication	September 12, 2017	Subjects using any medication with known teratogenicity potential, e.g., methotrexate or isotretinoin, should not be enrolled in the study, or should be withdrawn from the study if already enrolled
Clarification note on contraceptive counseling	November 3, 2017	Contraceptive counseling to be performed at V6 or early termination visit, not V7

16.9. Serious Adverse Events/Deaths: Additional Information

The most frequently reported SAEs by system organ class included: Pregnancy, puerperium and perinatal conditions (n=11, 0.3%); Infections and infestations (n=7, 0.2%); Psychiatric disorders (n=6, 0.2%); Injury, poisoning, and procedural complications (n=5, 0.1%); Gastrointestinal disorders (n=3, 0.1%); Hepatobiliary disorders (n=2, 0.1%); and Neoplasms benign, malignant, and unspecified (n=2, 0.1). The most frequently reported SAEs by preferred term were spontaneous abortion (n=9, 0.2%), ectopic pregnancy (n=2, 0.1%), venous thrombosis (n=2, 0.1%), appendicitis (n=2, 0.1%), and depression (n=2, 0.1%).

Other SAEs by preferred term occurring with a frequency of one instance each (0.03%) included: abscess limb, bacterial pyelonephritis, campylobacter gastroenteritis, gastroenteritis, infection parasitic, affective disorder, alcohol withdrawal syndrome, bipolar disorder, psychotic disorder, suicidal ideation, accidental overdose, concussion, rib fracture, spinal column injury, upper limb fracture, abdominal pain, colitis, pancreatitis acute, cholecystitis, hepatic adenoma, acute myeloid leukemia, thyroid neoplasm, vertigo, pyrexia, drug hypersensitivity, migraine without aura, renal cyst, hemorrhagic ovarian cyst, and pneumomediastinum.

One Death Occurred in Study C302

Participant (b) (6) (Study C302) was a (b) (6) white female who enrolled in the study on (b) (6) The participant started treatment with 3 mg DRSP/14.2 mg E4 on (b) (6) No relevant medical history or concomitant medications were recorded at the time of enrollment. The participant attended a scheduled Visit 5/Cycle 7 appointment on (b) (6) She reported a recent non-serious event of urinary tract infection for which she was given antibiotics. The participant did not know the name of the antibiotic. The participant returned for fasting safety laboratory tests on (b) (6) per protocol. All labs were normal. The participant reported no changes in her health or medications.

On (b) (6) study day 185, the participant was found unresponsive. Cardiopulmonary resuscitation was attempted. The participant was pronounced dead at the scene. Hospital records indicated an accidental intravenous drug overdose. The autopsy report, dated (b) (6) documented pulmonary edema and puncture site in the left arm. The cause of death by the medical examiner indicated acute fentanyl and alprazolam toxicity. It was noted that the participant had a history of substance abuse, including abuse of heroin, cocaine, Xanax, and marijuana.

All unused study drug was returned by the participant's family on (b) (6). Based on study drug count, the Investigator determined the last dose of study drug was (b) (6)

The Investigator assessed the accidental overdose as severe in intensity and unrelated to study drug. The Applicant agreed with the Investigator's assessment.

The Review Division concludes that the event of accidental overdose is unlikely related to study product.

Serious Adverse Events

Venous Thrombosis (Case #1)

Participant (b) (6) (Study C301) was a 32 yo G1P0010 white female, baseline BMI 21.4 kg/m^2, who enrolled in the study on (b) (6) The participant started treatment with DRSP/E4 (3/14.2 mg) on (b) (6) At the time of enrollment, relevant medical history was significant for anxiety (ongoing), intermittent urticaria (ongoing), and history of surgical abortion. Concomitant medications included desloratadine and escitalopram.

On (b) (6) the participant attended a study visit (study day 87) and complained of calf pain since (b) (6) (study day 75). Blood pressure was documented as 128/55 mm Hg. The study site referred the participant to her workplace physician for further evaluation the same day. Physical exam revealed no calf tenderness or palpable cords, warmth or swelling, and a negative Homan's sign. A D-dimer test was performed and returned positive. On (b) (6) , the participant underwent lower extremity Doppler ultrasound examination which revealed a thrombus of the fibular vein from the proximal third of the calf, continuing distally for 15 cm. Other veins were unremarkable.

The study drug was discontinued as a result of this event. The participant's last dose of study drug was (b) (6) The participant was referred to an outpatient clinic for further management. Vital signs were: BP 117/73 mm Hg, HR 64 bpm, and O2 saturation 97%. Laboratory tests were significant for increased activated partial thromboplastin time and elevated factor VIII anti-thrombin III, and protein S levels. Testing for lupus anticoagulant, anticardiolipin antibodies, beta-2 glycoprotein antibodies, and activated protein C resistance were negative. Additional laboratory tests, including platelet count, were within normal limits and did not reveal a predisposition for thrombosis. Treatment with rivaroxaban was initiated.

A follow-up visit occurred on (b) (6) platelet count was unchanged at 229 x 10^9/L. The participant's lower extremities were symmetrical without tenderness or edema. The venous thrombosis was considered to be resolving. The participant requested a 13.5 mg LNG intrauterine system for contraception. On (b) (6) , the event was considered resolved, with the participant continuing anticoagulation as prescribed. Genetic testing revealed absent mutations for Factor V Leiden and prothrombin gene.

The Investigator assessed the event of venous thrombosis as moderate in severity, probably related to the study drug, and unexpected. The Applicant agreed with this assessment. The participant discontinued the study on (b) (6) . The reason for withdrawal cited is "adverse event."

The Review Division concludes that the adverse event of venous thrombosis is likely related to study drug.

Depression (Case #2)

Participant (b) (6) (Study C302) was a 21 yo G0 black female who experienced worsening of depression while on study drug, resulting in hospitalization. The participant enrolled in the

study on (b) (6) and began study treatment on (b) (6). The participant's medical history was significant for anxiety, depression, and dysmenorrhea. The participant reported taking ibuprofen at the time of the event.

On (b) (6), study day 125, the participant was involuntarily hospitalized for worsening depression. The participant was discharged home on (b) (6). The participant was subsequently voluntarily hospitalized from (b) (6). The participant was treated with lamotrigine, hydroxyzine pamoate, and venlafaxine for the event. Study drug administration was unchanged.

The participant withdrew from the study on (b) (6) and reported that the last dose of study drug was taken on (b) (6)(Cycle 5, Day 24). The participant's last menstrual period was (b) (6)and a urine pregnancy test was negative.

The Investigator assessed the event as severe and possibly related to study drug. The Applicant assessed the event of worsening of depression as unlikely related to study drug due to the timing of onset of worsening depression occurring 4 months after study drug initiation.

The Review Division concludes that the event of worsening depression is likely related to study drug. The Review Division disagrees that the timing of onset 4 months after study drug initiation eliminates relatedness.

Of note, the participant was treated for depression with lamotrigine, which has a known drug-drug interaction with hormonal contraception. However, the participant reported cessation of study drug on (b) (6), therefore it appears the risk of an adverse event was minimal.

Suicidal Ideation (Case #3)

Participant (b) (6) (Study C302) was a 19 yo Asian female with baseline BMI 21.6 kg/m^2 who enrolled in Study C302 on (b) (6) The first day of treatment was recorded as (b) (6) The participant reported a history of pre-existing anxiety, depression, and intermittent suicidal ideation. Additional medical history was significant for dysmenorrhea, and scoliosis. The participant was taking sertraline, lorazepam, and zolpidem at the time of enrollment. She had a prior history of oral contraceptive use and was a current smoker. On (b) (6) (Study Day 15), the participant reported insomnia and worsening suicidal ideation. Study drug was withdrawn, and the participant was discontinued from the study. The adverse event of suicidal ideation was reported as recovered/resolved on (b) (6). The adverse event of insomnia was reported as not recovered/not resolved.

The Investigator and Applicant assessed the events of worsening suicidal ideation and insomnia as unrelated to study drug.

The Review Division disagrees with the assessment that these events are unrelated to study drug. The onset of worsening suicidal ideation and insomnia could have been triggered by exposure to DRSP/E4 in a participant with a pre-existing history of mood disorder. Therefore, the Review Division concludes that the events of worsening suicidal ideation and insomnia are possibly related to study drug.

Ectopic Pregnancy (Case #4)

Participant (b) (6) (Study C302) was a 27 yo G7P3 mixed race female who was diagnosed with ectopic pregnancy on (b) (6) The participant enrolled in the study on (b) (6) and began treatment with study drug on (b) (6) Medical history was significant for acne (ongoing), amoxicillin and penicillin drug allergies (ongoing), dysmenorrhea (ongoing), positive HPV (ongoing), and seasonal allergies (ongoing). The participant was taking loratadine.

On (b) (6) the participant presented to her primary gynecologist for an annual examination with complaints of painless post-coital bleeding. The participant was treated presumptively for cervicitis with doxycycline for 7 days. Gonorrhea and chlamydia testing was performed, and results were negative. Pregnancy testing was not performed.

On (b) (6), the participant experience acute sharp right lower quadrant pain that gradually worsened. On (b) (6) (Study Day 227), the participant presented to the emergency room with sharp right lower quadrant pain, pelvic pain and nausea without vomiting. Physical examination revealed suprapubic and right lower quadrant abdominal tenderness without rebound or guarding. A urine pregnancy test was positive. A transvaginal ultrasound examination showed a 5 cm complex echogenic structure directly adjacent to the right ovary with free fluid in the pelvis. The participant was diagnosed with a right tubal pregnancy and was admitted to the hospital for surgical management. A laparoscopic right salpingectomy was performed without complications. At the time of surgery, approximately 50cc of blood was noted in the cul-de-sac and the right fallopian tube was not ruptured. The participant tolerated the procedure well and was discharged home on (b) (6)

Last use of study drug was on (b) (6). The participant reported the events to the study site on (b) (6) She also reported that she had restarted the study drug. The participant was advised to stop use of the study drug immediately and was discontinued from the study on (b) (6) due to the serious adverse event of ectopic pregnancy.

The Investigator assessed the adverse event of ectopic pregnancy as severe and possibly related to study drug. The Applicant disagreed with the Investigator's assessment of relatedness. The Applicant assessed the event of ectopic pregnancy as not related to study drug.

The Review Division concludes that the adverse event of ectopic pregnancy is unlikely related to study drug. Based on currently available evidence, combination oral contraceptives do not appear to increase the risk of ectopic pregnancy.

Deep Vein Thrombosis (Case #5)

Participant (b) (6) was a 54-year-old Caucasian postmenopausal female, baseline BMI 24.3 kg/m^2, who enrolled in Study MIT-Es0001-C106 – a randomized, double-blind parallel group with nested cross-over study to evaluate the pharmacokinetics and investigate the effects of therapeutic and supratherapeutic concentrations of DRSP/E4 on heart rate corrected QT interval (QTc). The participant reported no prior medical history and did not take any concomitant medications. Treatment began on (b) (6) with DRSP/E4 3/14.2 mg for 10

days, followed by 15/71 mg for another 10 days. The participant received study drug for a total of 20 days and completed treatment on (b) (6). The participant was diagnosed with deep vein thrombosis on (b) (6) three days after treatment completion.

On (b) (6) the participant experienced pain and mild swelling of her right lower leg and behind her right knee after travelling home from the clinic site seated in a car for several hours. She did not experience shortness of breath. The participant reported these symptoms to the study site during a follow-up call to inform the participant of the low platelet count on (b) (6) The participant went to the nearest Emergency Department for evaluation of possible DVT as per study site instructions. An ultrasound vein duplex of the lower right extremity was performed.

On (b) (6) the participant was diagnosed with deep vein thrombosis: ultrasound revealed an occlusive thrombus of the right proximal to distal superficial femoral vein and a non-occlusive thrombus within the right popliteal vein. The participant was prescribed apixaban 10 mg for 7 days followed by 5 mg twice daily for 21 days. The participant was discharged from the Emergency Department with instructions to follow-up with her primary care provider in one week.

On (b) (6) the participant reported the pain was resolving and swelling of the affected areas had decreased. The participant had completed study treatment at the time of the event. The adverse event of deep vein thrombosis was assessed as likely related to study drug. The participant was tested for Factor V Leiden and Prothrombin 20210GA mutations; both results were negative. The low platelet count of 81 thousand/µL on (b) (6) was not categorized as an AE or SAE.

The participant's platelet counts were as follows:

Table 50. Platelet Counts, Participant 1035, Study MIT-Es0001-C106

Date (Visit)	Platelet Count (Thousand/µL)
(b) (6) (screening visit)	166
(b) (6) (enrollment visit)	129
(b) (6)	121
	86
	81

The Review Division concludes that the adverse event of deep vein thrombosis is likely related to study drug. The low platelet count is likely due to consumptive coagulopathy and is unlikely to be related to study drug but rather is related to the event of deep vein thrombosis. Of note, the subject was taking a supratherapeutic dose (5x) of the study drug. At these high doses, causality cannot be definitively attributed to either the estrogen or progestin component.

Complicated Migraine (Case #6)

Participant (b) (6) was a 24-year-old Caucasian premenopausal female who enrolled in Study MIT-Es0001-C106 – a randomized, double-blind parallel group with nested cross-over study to evaluate the pharmacokinetics and investigate the effects of therapeutic and supratherapeutic

concentrations of DRSP/E4 on heart rate corrected QT interval (QTc). Relevant medical history included Lyme disease, menstrual pain and allergies to codeine and penicillin; she had no prior history of migraines. Concomitant medications at the time of the adverse event included ibuprofen. The participant reported a prior history of contraceptive pill use without adverse effects.

The participant began study treatment on (b) (6) The participant received 1 dose of moxifloxacin-placebo on (b) (6) (Day 1), followed by 10 days of DRSP 3 mg/E4 14.2 mg, and subsequently 10 days DRSP 15 mg/E4 71 mg (5x therapeutic dose) per protocol. The participant took the last dose of study treatment on (b) (6) (Day 20).

On (b) (6) 19 days after first study product dose, the participant experienced visual disturbances, hyperventilation, and difficulty communicating. She subsequently developed tingling of the right side of the mouth, right hand and arm, and frontal headache, and became less responsive. The participant was sent to the local hospital for further evaluation. Upon arrival at the Emergency Department, she was noted to be lethargic but arousable and able to cooperate with examination. Neurological examination revealed no significant findings.

The participant was admitted to the hospital for pain management and further evaluation. Computed tomography (CT) of the brain without contrast, computed tomography angiography (CTA) of the head and neck with IV contrast, magnetic resonance imaging (MRI) of the brain without contrast and magnetic resonance venography (MRV) of the head without contrast were performed and revealed no abnormalities. An echocardiogram showed a small right-to-left shunt at rest and with Valsalva. Laboratory tests were within normal limits. There was no evidence of infection. The participant received ketorolac, ondansetron, and acetylsalicylic acid (81 mg). The participant's symptoms resolved on the day of admission. She was discharged home the following day, (b) (6)

The participant did not complete the study due to the adverse event and therefore did not receive moxifloxacin-placebo on Day 21 as per protocol.

The Investigator and Sponsor classified the unexpected adverse event of "complicated migraine" as possibly related to the study medication. The Investigator and Sponsor assessed the event as possibly related to moxifloxacin.

The Review Division concludes that the adverse event of "complicated migraine" is likely related to study product given that the event occurred after receiving supra-therapeutic doses of DRSP/E4 and had no prior history of migraine.

The Review Division disagrees that the adverse event of "complicated migraine" is possibly related to moxifloxacin. The prescribing information for moxifloxacin does not indicate that migraine is an expected adverse reaction and it is a known adverse event related to both estrogen and combined hormonal contraceptive use. Additionally, there are no reports in the literature for migraine associated with moxifloxacin. Therefore, the Review Division considers this event unlikely related to moxifloxacin and related to use of NEXTSTELLIS.

This is a representation of an electronic record that was signed electronically. Following this are manifestations of any and all electronic signatures for this electronic record.

/s/

GERALD D WILLETT
04/14/2021 10:58:55 AM

AUDREY L GASSMAN
04/15/2021 08:44:10 AM

JANET W MAYNARD
04/15/2021 08:58:04 AM