# CENTER FOR DRUG EVALUATION AND RESEARCH

*APPLICATION NUMBER:*

**21-276**

# PHARMACOLOGY REVIEW

## REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

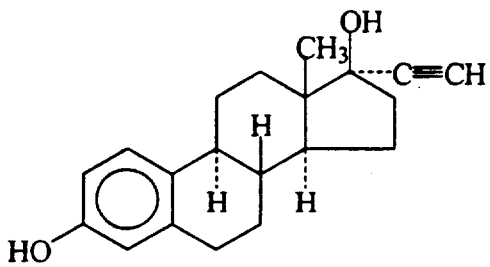
**KEY WORDS:** Estrostep®, ethinyl estradiol, norethindrone acetate, norethisterone acetate, oral contraceptive, acne vulgaris
**Reviewer Name:** Amy Nostrandt
**Division Name:** Division of Dermatologic and Dental Drug Products
HFD# 540
**Review Completion Date:** 3/9/01

**Review number:** 1
**IND/NDA number:** NDA 21-276
**Serial number/date/type of submission:** original submission, sent 6/30/00, received 7/3/00
**Information to sponsor:** Yes ( ) No (X)
**Sponsor (or agent):** Parke-Davis Pharmaceutical Research, Division of Warner-Lambert
**Manufacturer for drug substance:** [redacted]

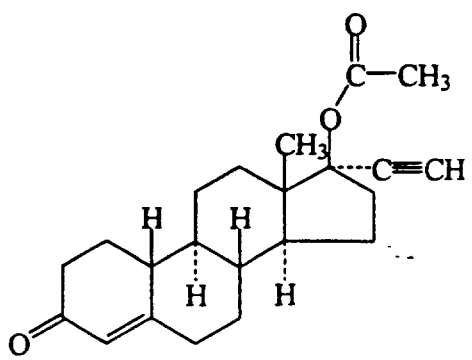
**Drug:**

**Code Name:** not provided
**Generic Name:** norethindrone acetate (NA) and ethinyl estradiol (EE)
**Trade Name:** Estrostep®
**Chemical Name:** ethinyl estradiol and norethindrone acetate; (17α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol and 7-(acetyloxy)-19-norpregn-4-en-20-yn-3-one
**CAS Registry Number:** not provided
**Molecular Formula/ Molecular Weight/Structure:**

ethinyl estradiol
$C_{20}H_{24}O_2$
MW=296.41




norethindrone acetate
$C_{22}H_{28}O_3$
MW=340.46



**Relevant INDs/NDAs/DMFs:** all from Parke-Davis

approved NDA 21-065 for femhrt 1/5 for osteoporosis
approved NDA's 17-876, 17-875, 17-354, and 17-355 for Loestrin®

**Drug Class:** combined oral contraceptive

**Indication:** for the treatment of moderate acne vulgaris in females between and 49 years of age, who have no known contraindications to oral contraceptive therapy, desire contraception, have achieved menarche and are unresponsive to topical anti-acne medications.

**Clinical formulation:**

Estrostep® tablets contain norethindrone acetate (NA) 1 mg and a graduated dose sequence of ethinyl estradiol (EE) over a 21-day period as follows:

5 tablets of 1mg NA/20 μg EE
7 tablets of 1mg NA/30 μg EE
9 tablets of 1mg NA/35 μg EE

Estrostep Fe® is packaged as 28 tablets as follows:

5 tablets of 1mg NA/20 μg EE
7 tablets of 1mg NA/30 μg EE
9 tablets of 1mg NA/35 μg EE
7 tablets of ferrous fumarate, 75 mg.

Inactive ingredients are listed as calcium stearate, lactose, microcrystalline cellulose and starch.

**Route of administration:** oral

**Proposed clinical protocol or Use**: The drug product was approved for the prevention of pregnancy in 1996. Tablets are taken once daily as graduated doses of ethinyl estradiol, as appropriate relative to the time of the patient's reproductive cycle.

Based on a body weight of 60 kg, the clinical doses would be 0.017 mg/kg norethindrone acetate and up to 0.58 μg/kg ethinyl estradiol (combined dose up to 17.6 μg/kg). These doses are used below for interspecies comparisons.

**Previous clinical experience:** Two six-month (six- cycle) blinded parallel-group clinical trials were performed in acne patients. The sponsor reports efficacy in the treatment of moderate acne vulgaris. Adverse events included those usually associated with oral contraceptive use, such as breakthrough bleeding, migraine, nausea, and breast pain/enlargement. Most adverse events were moderate or mild in intensity, but severe AE's were reported in a few patients (abdominal pain, headache, and migraine). On clinical chemistry evaluation, increases were seen

in total cholesterol and serum triglycerides. The sponsor states that there were no safety concerns in subjects aged 13-17.

**Disclaimer: Note that some information may come directly from the sponsor's submitted material.**

**Introduction and drug history:**

Estrostep was approved in 1996 for the prevention of pregnancy. The sponsor submitted [redacted] in 1998 for the investigation of the drug in the treatment of acne vulgaris in post-menarchal female patients.

**Studies reviewed within this submission:**

The only significant new information consists of a number of published references on genetic toxicology of the two drug substances; these are summarized and discussed in the section on Genetic Toxicology.

**Studies not reviewed within this submission:**

Estrostep® is currently approved for contraception and studies to support that indication have been previously reviewed. Information provided by the sponsor in the current submission is summarized in the appropriate sections below.

## PHARMACOLOGY:

The sponsor states that contraceptive steroids suppress gonadotropin secretion, which in turn decreases androgen production by the ovary. These drugs are also reported to increase the concentration of sex hormone binding globulin (SHBG), reducing the amount of unbound available androgen in the circulation. Since androgens are believed to trigger a rise in sebum production that contributes to the development of acne, reduction of their production and/or availability to receptors is expected to be beneficial in the treatment of acne.

## SAFETY PHARMACOLOGY:

There were no reports of safety pharmacology studies in the current submission.

## PHARMACOKINETICS/TOXICOKINETICS:

**Absorption:** The sponsor states that the oral bioavailability of ethinyl estradiol is 0.3-9% in most species studied (rat, rabbit, dog, and rhesus monkey), and may be up to 60% in the baboon. The oral bioavailability of norethindrone acetate in laboratory animals was reported to be 14-54%.

**Distribution:** Ethinyl estradiol was reported to exhibit a biexponential time course while the time course of norethindrone acetate was described to have a biphasic or triphasic decline.

**Metabolism:** Both drug substances were reported to be highly metabolized. Ethinyl estradiol was excreted in the urine of the baboon primarily as sulfate and glucuronide conjugates. Only a small amount was excreted unchanged in urine. Biliary excretion was predominant in the dog

and rat. High first pass extraction has been seen, due to metabolism in the gut wall and liver. Norethindrone acetate was highly metabolized primarily to glucuronide conjugates and non-hydrolyzable polar compounds in the rat and rabbit.

**Elimination:** The sponsor reports that plasma clearances of the drug substances are similar to hepatic blood flow; both have a high extraction ratio. For ethinyl estradiol, the terminal half-life was 1.1-10 hours after intravenous or intragastric administration. The terminal half-life of norethindrone acetate was 0.7-15.1 hours after intravenous or intragastric administration. Biliary excretion and enterohepatic recycling was described for norethindrone acetate, but was not mentioned in reference to ethinyl estradiol.

## TOXICOLOGY:

**Overall Toxicology Summary:**

In acute toxicology testing in rodents via the oral route, the $LD_{50}$ for ethinyl estradiol ranged from 1.7 to >5 g/kg, and the $LD_{50}$ for norethindrone acetate ranged from 1.4 to >4 g/kg. Signs of toxicity included apathy, abnormal breathing, abnormal gait, emaciation, and eventually convulsions. At the highest doses, lethality could occur within two weeks of dosing as a result of liver and kidney failure.

Repeated dose studies were conducted in multiple species with the individual drug substances. The sponsor states that the combination of ethinyl estradiol and norethindrone acetate has not been tested in subacute or subchronic studies, but that data from interim evaluations of long-term or carcinogenicity studies of the combination indicate that there are "no major adverse effects, other than the expected pharmacological actions" at those early time points. (*Reviewer's comment: The doses used in carcinogenicity studies are generally lower than those used in shorter term repeated-dose studies, to assure maximum survival toward the end of the study.*)

Studies of ethinyl estradiol have been conducted in rats, hamsters, rabbits, and dogs for durations of four to 95 days. Effects seen included decreased body weight gain and reduced food consumption. Additional effects that were considered to be related to the pharmacological activity of the drug included uterine hypertrophy, vaginal keratinization, mammary gland development, and testicular atrophy. Toxicity observed following administration of "non-physiological doses" included effects on the liver and hematopoietic system, such as cholestasis and inhibition of bile acid synthesis, biliary glutathione secretion, and bilirubin secretion. Cholestatic effects were seen in rats at >1 mg/kg/day (HED=0.17 mg/kg/day, or nearly 300 times the highest proposed human dose) for only a few days. Associated with cholestatic doses were increased liver weight and hepatocyte hypertrophy and hyperplasia. Periportal hepatocyte vacuolization was noted after chronic administration of 0.5 mg/kg/day to Sprague-Dawley rats, but not in Fischer rats at the same dose. The sponsor states that the NOEL for cholestasis in rats is 0.25 mg/kg/day when given for several days to weeks (HED=42 µg/kg/day, or approximately 70 times the highest clinical daily dose).

Rabbits treated with 0.015 mg/kg day ethinyl estradiol (HED=5 µg/kg/day, or 8.6 times the clinical dose) for several weeks exhibited hepatic toxicity. A dose of 0.06 mg/kg/day for eight weeks was lethal. In a 20-week study, ethinyl estradiol-treated rabbits had hepatic lesions including cholangiolar proliferation, bile duct proliferation, mononuclear cell infiltration, and fibrosis.

Dogs treated with ethinyl estradiol exhibited effects on the hematological system. Doses of 0.2 mg/kg/day (HED=0.1 mg/kg/day, or 172 times the clinical dose) for up to 95 days resulted in neutrophilia. Higher doses were reported to result in thrombocytopenia. Myeloid hyperplasia, depression of megakaryocytes and depression of erythropoietic activity were evident in bone marrow. The sponsor states that such effects on bone marrow were not seen in chronic studies in rats (2 years) or monkeys (10 years).

When rodents were treated with norethindrone acetate, effects included decreased weight gains, lower absolute organ weights and testicular and seminal vesicle atrophy. Cholestasis was seen in rats at 40 mg/kg/day (HED=6.7 mg/kg/day, or nearly 400 times the clinical dose) for 5 days (*Reviewer's comment: The sponsor states that the NOEL for this effect in rats has not been clearly defined.*)

**CARCINOGENICITY:**

Long term studies were conducted in rats, dogs, and monkeys for two, seven, and ten years, respectively.

Endocrine effects were seen in rats treated with ethinyl estradiol, norethindrone acetate, or a combination of the two. Albino rats were fed Norlestrin (NA:EE 50:1; same ratio as Estrostep tablets with lowest ethinyl estradiol dose) in the diet. Combined doses of 0.3 or 4 mg/kg/day (17 or 227 times the highest clinical dose, respectively) resulted in dose-related growth retardation, transient alopecia, mastopathy, liver hyperplasia, and gonadal atrophy. The sponsor states that the same effects were seen at the same doses of norethindrone acetate alone or with 50-fold lower doses of ethinyl estradiol. No pathological effects were noted. The sponsor states that there was no increase in the incidence of tumors usually associated with chronic administration of estrogenic and progestogenic agents, with the exception of high dose males in which an increased number of benign mammary tumors were seen. This finding was considered to be due to the altered endocrinological state of the animals. Higher doses (≥6 mg/kg/day; HED≥1 mg/kg/day; 57 times the combined clinical dose) of an 80:1 NA:EE combination resulted in increased incidence of liver nodules and hepatocellular carcinomas in female Wistar rats.

In dogs, long term administration of a 20:1 NA:EE formulation resulted in alopecia, cystic endometrial hyperplasia, and severe pyometra at doses between 21 and 525 µg/kg/day (HED≥10.5 µg/kg/day, or 0.6 times the combined clinical dose). The sponsor states that these animals had lower cholesterol levels than controls and had no evidence of mammary or hepatic nodules or tumors. There were dose-related decreases seen in erythrocyte counts, packed cell volume, and hemoglobin concentration in that study and in a study of Norlestrin (NA:EE 50:1) at doses up to 25 mg/kg/day (12.5 mg/kg/day, or approximately 700 times the clinical dose). In the latter study, the sponsor states that there was no evidence of mammary tumor development.

Rhesus monkeys treated with Norlestrin (NA:EE 50:1) at doses up to 2.55 mg/kg/day (HED=0.85 mg/kg/day, or 48 times the clinical dose) for cycles of 3 weeks followed by one week off of the drug for 10 years were reported to exhibit no significant toxicity or tumorigenicity. Effects seen were considered to be exaggerated pharmacological effects of the drug, such as uterine and ovarian atrophy and dilation of mammary acini and ducts. No tumor formation was reported. The sponsor did state that occasional mammary nodules or local mammary hyperplasia was seen in monkeys given "extremely high doses" of Norlestrin for 5 years.

In animal studies, tumors usually associated with chronic administration of estrogenic and progestogenic agents include those involving the pituitary gland, uterus, ovary, liver, and

mammary gland. The class label for oral contraceptive steroids describes the tumors associated with clinical use of these drugs, such as carcinoma of the reproductive organs and hepatic neoplasia.

**IMMUNOTOXICOLOGY:**

No data related to immunotoxicology was provided.

**REPRODUCTIVE TOXICOLOGY:**

Contraceptive effects include interruption of tubular transport of ova and hormone-induced uterine changes that are incompatible with implantation and embryonic development. The sponsor states that normal fertility is restored in animals within 6 months after discontinuation of treatment.

Pregnant rhesus monkeys given Norlestrin (NA:EE 50:1) at doses from 5-50 mg/kg/day (HED≥1.7 mg/kg, or 95 times the clinical dose) on gestation days 21-35, gestation days 33-46, or throughout organogenesis (gestation days 21-46), exhibited increased fetal mortality, but no teratogenesis. The sponsor states that *in utero* exposure to Norlestrin was not associated with any subtle effects on postnatal behavior or motor function.

The sponsor states that oral contraceptive steroids in general are embryolethal, but not teratogenic in animal species. They note that progestins alone may be teratogenic if given at sufficient doses at appropriate times. When norethindrone acetate was administered to pregnant mammals, the result was primarily female virilization. Other effects may include skeletal abnormalities, cryptorchidism, hydrocephalus, and club feet.

**Labeling Recommendations:**

The current label states that use of the drug should be discontinued in the event of a pregnancy. The drug is classified as Pregnancy Category X. No changes to the existing pregnancy label information are recommended.

**GENETIC TOXICOLOGY:**

The sponsor has submitted a number of journal articles describing studies of the genotoxicity of ethinyl estradiol, which together represent a complete genotoxicity test battery for that active ingredient. Articles were also submitted that represent a complete genotoxicity test battery for norethisterone acetate and a partial battery for the combination of those two drug substances. According to Martindale (via Micromedex Integrated Index), norethisterone acetate is the same as the sponsor's drug substance, norethindrone acetate.

**for ethinyl estradiol:**

1. Hundal et al. (Mutation Research 389:173-181, 1997) evaluated three commonly used estrogens, including ethinyl estradiol *in vitro* and *in vivo*. In the Ames Salmonella assay, there was no effect of any of the three compounds on the number of revertant colonies with or without S9. Strains tested included TA100, TA1535, TA97a, and TA98. (*Reviewer's comment: A strain that would detect oxidizing mutagens, i.e. strain TA102 or E. coli WP2uvrA, was not included, but is recommended by the ICH guidelines.*)

The chromosomal aberration test was conducted with the same estrogens using human lymphocytes. Each compound was tested at 1, 10, and 100 μg/mL, with appropriate positive and

negative controls. Treatment durations of 0, 24, and 48 hours were evaluated without S9. Thirty-hour old cultures were exposed to a 6-hour treatment with the test chemicals and S9, followed by wash and centrifugation to remove the treatments and additional 36 hours incubation at 37°C. Fifty to one hundred metaphases were examined per treatment. Sister chromatid exchange was evaluated in similarly treated cultures that were also incubated with BrdU. At all doses of ethinyl estradiol, increased numbers of chromosomal aberrations and sister chromatid exchanges were induced in cultures in the presence or absence of S9.

The same compounds were evaluated in the mouse micronucleus assay. Swiss albino male mice were injected intraperitoneally with 1, 5, or 10 mg/kg (HED=83, 420, or 830 µg/kg, or 143, 724, or 1430 times the clinical dose of ethinyl estradiol alone) of the respective treatment or positive or negative control treatment. After 30 hours, the animals were euthanized, and bone marrow smears were made. One thousand polychromatic erythrocytes (PCE) were evaluated per animal. Sister chromatid exchange was evaluated in similarly treated animals that were also exposed to BrdU. Ethinyl estradiol, like the other estrogens tested, resulted in dose-dependent increases in frequencies of micronuclei and sister chromatid exchanges.

2. Wheeler *et al.* (Mutation Research 171:31-41, 1986) tested a number of chemicals, including ethinyl estradiol in Chinese hamster cells *in vitro*. They found that the estrogens tested caused mitotic arrest and aneuploidy in culture. After removal of the drugs from the culture medium, the cells were able to reorganize a spindle apparatus and enter anaphase. Ethinyl estradiol and the other drugs caused a significant increase in aneuploidy at high concentrations (75 and 100 µM) in recovering cell populations. The authors hypothesize that estrogens may have a mechanism of mitotic arrest similar to colchicine, which inhibits the polymerization of tubulin to form microtubules. They state that aneuploidy of DES and similar compounds may be related to carcinogenic potential.

3. Kochar (Toxicology Letters 29:201-206, 1985) studied the effects of ethinyl estradiol and other estrogens on CHO cells. All produced chromosomal aberrations in a dose-dependent manner at 10, 50, and 100 µM. These were most commonly chromatid-type aberrations, especially chromatid exchanges.

4. Reimann et al. (Environmental and Molecular Mutagenesis 28:133-144, 1996) tested the cytogenetic potential of 10 sex steroids, including ethinyl estradiol. Two chromosomal aberration tests in human lymphocytes were conducted at concentrations of 1-80 µg/mL with S9 and at concentrations of 1-40 or 50 µg/mL without S9. Duplicate cultures treated for 20-24 and 48 hours (two harvest times) were evaluated without S9. Forty-eight-hour old cultures were exposed to a 3-hour treatment with the test chemicals and S9, followed by wash and incubation for 17-25 hours and 27-45 hours (two harvest times). Colchicine was added three hours before harvest. One hundred metaphases were examined per replicate (200 per treatment). Three doses were scored at the first harvest, and two high doses were scored at the second. In the first experiment without S9, an increased frequency of polyploid cells was found at 20 and 30 µg/mL, relative to solvent control. That effect was confirmed in the second experiment at 20, 40, and 50 µg/mL. The authors state that the induction of polyploidy and mitotic arrest are common effects of endogenous and synthetic steroid hormones *in vitro*. (*Reviewer's comment: This finding is in agreement with those of Wheeler et al., #2 above*)

In the mouse micronucleus assay, female mice were administered two 1 g/kg doses 24 hours apart by gavage. Five animals were killed at each of 6, 12, 24, 48, 72 and 144 hours after the second treatment, and bone marrow smears were made. Two thousand polychromatic erythrocytes (PCE) were evaluated per animal. There was no increased incidence of micronucleated PCE's at any time point.

5. Shyama and Rahiman (Mutation Research 370:175-180, 1996) studied the effects of Lynoral tablets (0.05 mg ethinyl estradiol per tablet) *in vivo* in mouse bone marrow cells. Tests for chromosomal aberrations and micronuclei were performed. Suspensions of the tablets in water were prepared to yield doses of 0.002-0.2 mg/kg body weight (*Reviewer's comment: It is unclear if this dose is of ethinyl estradiol or of drug product).* The doses were administered by gavage daily for 15 days to mice. A separate time-response experiment was performed with a dose of 0.02 mg/kg. Animals were injected intraperitoneally with colchicine 1.5 hours before sacrifice. One hundred metaphases per animal were analyzed. There was a significant increase in chromosomal aberrations at doses of 0.120 mg/kg (HED=10 µg/kg; or 0.6 times the clinical dose) and above at 24 hours. These consisted primarily of breaks. In the time course experiment, there was an increase in breaks at 12 hours, but not at any other time point. There was no increase in micronucleated PCE's at any dose.

6. Drevon *et al.* (Mutation Research 89:83-90, 1981) tested four estrogenic hormones, including ethinyl estradiol, in V79 Chinese hamster cells. Ethinyl estradiol was cytotoxic but not mutagenic in the absence of hepatocyte co-culture. In co-culture, the toxic effect was decreased, but no mutation was observed. The same results were obtained when V79 cells were treated in co-culture with hepatocytes from rats pre-treated with Aroclor to induce cytochrome $P_{450}$ enzyme activity. (*Reviewer's comment: Chromosomal aberrations were not evaluated, and this experiment was not one of the validated tests in the ICH battery.*)

7. Purdy and Marshall (Carcinogenesis 5:1709-1715, 1984) demonstrated that the main metabolite of ethinyl estradiol, 2-hydroxyethinyl estradiol, enhanced the mutagenicity of 2-acetylaminoflouorene. Phenobarbital- or 2-acetylaminoflouorene-induced S9 increased the formation of the ethinyl estradiol metabolite, relative to uninduced S9.

8. In a study by Wilpart *et al.* (Teratogenesis, Carcinogenesis, and Mutagenesis 6:265-273, 1986), ethinyl estradiol and mestranol were inhibitors of mutagenicity of the alkylating agent, MNNG, in Salmonella typhimurium strain TA1530 and of aminoanthracene (AA) in strain TA1538. The effect was concentration-dependent.

**for norethisterone acetate:**

1. Dhillon and Dhillon (Mutation Research 367:1-10, 1996) tested norethisterone acetate in the Ames *Salmonella* assay. There was no effect of concentrations of 1-10,000 µg/plate without S9 or 1- 1000 µg/plate with S9 on the number of revertant colonies, relative to appropriate controls. Strains tested included TA100, TA1535, TA97a, and TA98. (*Reviewer's comment: A strain that would detect oxidizing mutagens, i.e. strain TA102 or E. coli WP2uvrA, was not included, but is recommended by the ICH guidelines.*)

The chromosomal aberration test was conducted with norethisterone acetate using human lymphocytes. Each compound was tested at 1, 10, and 100 µg/mL, with appropriate positive and

negative controls. Cultures were incubated with the test chemical for 24, 48, and 72 hours without S9. Thirty-hour old cultures were exposed to a 6-hour treatment with the test chemical and S9, followed by wash and centrifugation to remove the treatments and additional 36 hours incubation at 37°C. Colchicine was added to cultures two hours before harvest. At least 50 to 100 metaphases were examined per treatment. Sister chromatid exchange was evaluated in similarly treated cultures that were also incubated with BrdU. There was a significant increase in chromosomal aberrations at all doses with and without S9 in cultured human lymphocytes, including both chromosomal and chromatid-type aberrations. The incidence in cultures with metabolic activation was significantly increased over those without S9. The authors state that this indicates that the drug and its metabolites were responsible for the clastogenic effect. The drug was more genotoxic *in vitro* with longer treatment durations. There was also a dose- and duration-dependent increase in sister chromatid exchanges, both with and without S9.

In the mouse micronucleus assay, Swiss albino male mice were injected intraperitoneally with a single dose of 100, 1000, or 10,000 µg/kg of the treatment or positive or negative control treatment. After 30 hours, the animals were euthanized, and bone marrow smears were made. One thousand polychromatic erythrocytes (PCE) were evaluated per animal. Sister chromatid exchange was evaluated in similarly treated animals that were also exposed to BrdU. Doses of 1000 (HED=0.08 mg/kg or approximately five times the clinical dose) and 10,000 µg/kg induced a significant increase in the number of micronucleated PCE's and sister chromatid exchanges relative to negative controls.

2. Shyama amd Rahiman (Mutation Research 300:215-221, 1993) evaluated Primolut N (norethisterone acetate) *in vivo* for production of chromosomal aberrations and micronuclei. Tablets were dissolved in water to prepare doses of 0.3-30 mg/kg (*Reviewer's comment: It is unclear whether the dose is described in terms of mg of drug substance or drug product per kg body weight.*) Doses were administered daily to Swiss albino mice for 15 days by gavage. Dose-response was evaluated at 24 hours after ending treatment for those doses, and time-response was evaluated for animals treated with 3 mg/kg/day for 6 hours to 3 weeks. For evaluation of chromosomal aberrations, colchicine was administered 1.5 hours before sacrifice, and 100 metaphase spreads per animal were analyzed. There was a significant increase in chromosomal aberrations at doses of 3 mg/kg (HED=0.25 mg/kg, or 15 times the clinical dose of norethindrone acetate alone) and higher. There was a dose-dependent increase in total aberrations. Stickiness and pulverizations were seen at all but the lowest dose (0.3 mg/kg). The results of the time-response experiment indicated that there was a significantly increased frequency of chromosomal aberrations at 24 hours, then the frequency of aberrations decreased. Stickiness and pulverizations were significantly increased at 24 hours, and translocations were significantly increased at 48 hours. Chromatid breaks were more frequent than chromosome breaks.

For micronucleus evaluation, animals were killed at different time intervals, and bone marrow smears were prepared. Two thousand polychromatic erythrocytes were evaluated for micronuclei per animal. There was no significant increase in micronuclei in the dose-response evaluation at 24 hours or at any time in the time-response experiment.

**for ethinyl estradiol and norethisterone acetate combined:**

1. Shyama et al. (Mutation Research 260:47-53, 1991) investigated Anovlar 21 (a combined oral contraceptive containing ethinyl estradiol and norethisterone acetate) in an *in vivo* chromosomal aberrations test and a micronucleus test. Doses of 0.08 to 8.0 mg/kg/day were administered daily

by gavage for 15 days to adult female Swiss albino mice. (*Reviewer's comment: It is not clear if these doses represent weights of drug product or combined weights of the drug substances. The relative proportions for the two actives in this formulation are not provided. Anovlar 21 is not listed in the PDR.*) Bone marrow preparations were made 24 hours after the final dose. For chromosomal evaluations, colchicine was administered 1.5 hours prior to sacrifice. A time-response experiment was conducted in mice dosed with 0.8 mg/kg/day. One hundred metaphases per animal were evaluated for chromosomal aberrations. Two thousand polychromatic erythrocytes per animal were evaluated for micronuclei. There was a statistically significant increase in chromosomal aberrations in animals administered doses of 0.4 mg/kg (HED=33 µg/kg, or approximately two times the clinical dose) or greater. Aberrations were dose-dependent and consisted of breaks, gaps, translocations, centric fusions, stickiness and pulverizations. Stickiness and pulverizations were most frequent and increased at higher doses. Centric fusions were significantly increased at 4.8 and 6.4 mg/kg. Breaks were significantly increased only at 8 mg/kg. Chromatid breaks were seen more often than chromosome breaks; the authors state that this indicates that the break occurred in the DNA strand at late S-phase or after DNA had replicated. In the time-response experiment, the maximum frequency of aberrations was seen at 24 hours, then frequency decreased with time. The drug did not induce a significant increase in the number of micronuclei in bone marrow erythrocytes at any dose or time.

**Summary:**

From the data provided, ethinyl estradiol was not mutagenic in *Salmonella* strains tested. The tests performed did omit a strain (TA102) that would be sensitive to oxidizing mutagens that is currently recommended by the ICH guidelines.

One *in vitro* test of ethinyl estradiol in the chromosomal aberrations assay in human lymphocytes indicated increased incidence of chromosomal aberrations and sister chromatid exchanges at concentrations ≥1 µg/mL, both with and without metabolic activation. Another chromosomal aberrations assay in human lymphocytes found only an increased incidence of polyploidy; that finding was replicated in another laboratory in Chinese hamster cells. Another test for chromosomal aberrations in CHO cells was positive for chromosomal aberrations, particularly chromatid aberrations, including chromatid exchanges. Evaluation of chromosomal aberrations in mouse bone marrow cells after *in vivo* treatment indicated that chromosomal aberrations were increased at 24 hours at doses of 0.120 mg/kg (HED=10 µg/kg) and above, and at 12 hours at 0.02 mg/kg (HED=1.7 µg/kg).

Dose dependent increases in the frequency of micronucleated polychromatic erythrocytes and sister chromatid exchanges were seen in one study of mice treated with single intraperitoneal doses of ≥1 mg/kg (HED=80 µg/kg, or 143 times the clinical ethinyl estradiol dose alone). Another study of 15 days treatment with lower doses by gavage was negative for induction of micronuclei. A third study of two 1 g/kg oral doses spaced 24 hours apart was also negative.

Additional studies indicated that ethinyl estradiol may modulate the genotoxicity of other compounds.

Norethisterone (norethindrone) acetate was not mutagenic in *Salmonella* strains tested. The tests performed did omit a strain (TA102) that would be sensitive to oxidizing mutagens that is currently recommended by the ICH guidelines.

One test of norethisterone (norethindrone) acetate in the *in vitro* chromosomal aberration assay in human lymphocytes demonstrated an increased incidence of chromosomal aberrations

and sister chromatid exchanges at concentrations ≥1 μg/mL, both with and without metabolic activation. The incidences in cells with metabolic activation were significantly greater than those without metabolic activation. Effects were dose- and duration-dependent. In a second study of chromosomal aberrations, bone marrow cells of mice treated orally *in vivo* for 15 days with 3 mg/kg (HED=250 μg/kg) and higher had increased incidences of chromosomal aberrations.

In the mouse micronucleus assay a single intraperitoneal dose of 1000 (HED=83 μg/kg, or approximately 5 times the clinical norethindrone acetate dose alone) or 10,000 μg/kg resulted in increases in the frequency of micronucleated polychromatic erythrocytes and sister chromatid exchanges. A second study of 15 days treatment with doses up to 30mg/kg by gavage was negative for induction of micronuclei.

One laboratory conducted *in vivo* chromosomal aberrations testing and micronucleus testing in mice using a commercial preparation of the two drug substances in combination. A significant and dose-dependent increase in chromosomal aberrations was seen at doses of 0.4 mg/kg (HED=33 μg/kg) and above. There was no increase in the incidence of micronucleated polychromatic erythrocytes.

The sponsor states that positive results in *in vitro* tests of genotoxicity occurred at concentrations much higher than physiologic levels. This appears to be true, as clinical pharmacokinetic studies indicate that plasma drug levels are in the ng/mL range for norethindrone acetate and in the pg/mL range for ethinyl estradiol. However, positive results were also noted in *in vivo* assays for chromosomal aberrations and micronuclei at doses within the same order of magnitude of the clinical dose after normalization for total body surface area.

## SPECIAL TOXICOLOGY STUDIES:

No data from special toxicology studies were submitted.

## OVERALL SUMMARY AND EVALUATION:

**Introduction:** The drug product was approved previously for use as an oral contraceptive agent. The currently proposed use in the treatment of acne and the patient population are consistent with approved labeling, with the possible inclusion of younger adolescent patients.

**Safety Evaluation:** The only new nonclinical safety information provided includes data from multiple tests for genetic toxicity of both active ingredients. While neither drug substance appears to be mutagenic in bacteria, both exhibited positive results in chromosomal aberration assays in mammalian cells and in *in vivo* mouse micronucleus assays. In some cases, the positive results were repeated in different studies and/or test systems.

**Clinical Relevance of Safety Issues:** Currently approved labeling already addresses the possibility of neoplasia associated with long-term oral contraceptive use.

**Other Clinically Relevant Issues:** none at this time

**Conclusions:** From a pharmacology/toxicology standpoint, this application is approvable. Some labeling changes are suggested below.

**Communication Review:**

- Labeling Review (NDA):

New information related to genetic toxicology should be added to the label. Wording is recommended below.

There is evidence in from *in vitro* studies in the literature (Jurima-Romet et al. Hum. Exp. Toxicol. 16:198-203, 1997) that retinoids induce cytochrome P450 3A enzymes, which also are responsible for the metabolism of contraceptive steroids. It is possible that this interaction may result in an increased contraception failure rate, and put the fetus at risk of retinoid-related teratogenicity. The review team may wish to consider addition of this information to the label. Wording is suggested below.

**PRECAUTIONS**

**8. Drug interactions**

...

**Other**: Ascorbic acid and acetaminophen may increase plasma ethinyl estradiol concentrations, possibly by inhibition of conjugation. A reduction in contraceptive effectiveness and increased incidence of breakthrough bleeding has been suggested with phenylbutazone.

[redacted]

**10. Carcinogenesis, Mutagenesis**

See **WARNINGS** section.

[redacted]

- Investigator's Brochure/Informed consent review (IND): not applicable

**RECOMMENDATIONS:**

**Internal comments:** The proposed use of Estrostep in the treatment of acne is consistent with its use as labeled for the prevention of pregnancy. From a pharmacology/toxicology standpoint, the application is approvable. Labeling changes are recommended, as described above.
**External Recommendations/Draft letter Content for Sponsor:** none
**Future development or NDA issues:** none at this time

Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

cc:
NDA
HFD-340
HFD-540
HFD-540/PHARM/Nostrandt
HFD-540/TLPHARM/Jacobs
HFD-540/MO/Porres
HFD-540/CHEM/Pappas
HFD-540/PMS/Cintron

Concurrence Only:
HFD-540/DD/WILKIN

HFD-540/TLPHARM/JACOBS

Draft date (# of drafts): 3/9/01 (1)

**REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:**
**Addendum to Review**

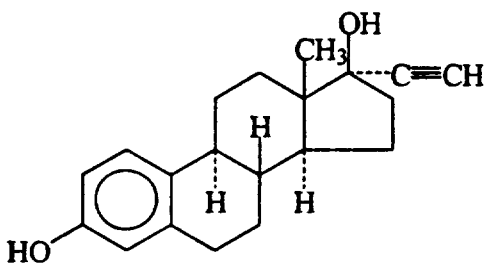
**KEY WORDS:** Estrostep®, ethinyl estradiol, norethindrone acetate, norethisterone acetate, oral contraceptive, acne vulgaris
**Reviewer Name:** Amy Nostrandt
**Division Name:** Division of Dermatologic and Dental Drug Products
HFD# 540
**Review Completion Date:** 4/10/01

**Review number:** addendum #1 to review #1
**IND/NDA number:** NDA 21-276
**Serial number/date/type of submission:** original submission, sent 6/30/00, received 7/3/00
**Information to sponsor:** Yes ( ) No (X)
**Sponsor (or agent):** Parke-Davis Pharmaceutical Research, Division of Warner-Lambert
**Manufacturer for drug substance:** [redacted]

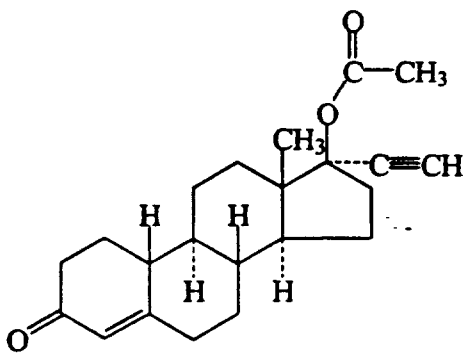
**Drug:**

**Code Name:** not provided
**Generic Name:** norethindrone acetate (NA) and ethinyl estradiol (EE)
**Trade Name:** Estrostep®
**Chemical Name:** ethinyl estradiol and norethindrone acetate; (17α)-19-norpregna-1,3,5(10)-trien-20-yne-3,17-diol and 7-(acetyloxy)-19-norpregn-4-en-20-yn-3-one
**CAS Registry Number:** not provided
**Molecular Formula/ Molecular Weight/Structure:**

ethinyl estradiol
$C_{20}H_{24}O_2$
MW=296.41




norethindrone acetate
$C_{22}H_{28}O_3$
MW=340.46



**Relevant INDs/NDAs/DMEs:** all from Parke-Davis

approved NDA 21-065 for femhrt 1/5 for osteoporosis
approved NDA's 17-876, 17-875, 17-354, and 17-355 for Loestrin®

**Drug Class:** combined oral contraceptive

**Indication:** for the treatment of moderate acne vulgaris in females between and 49 years of age, who have no known contraindications to oral contraceptive therapy, desire contraception, have achieved menarche and are unresponsive to topical anti-acne medications.

**Clinical formulation:**

Estrostep® tablets contain norethindrone acetate (NA) 1 mg and a graduated dose sequence of ethinyl estradiol (EE) over a 21-day period as follows:

5 tablets of 1mg NA/20 µg EE
7 tablets of 1mg NA/30 µg EE
9 tablets of 1mg NA/35 µg EE

Estrostep Fe® is packaged as 28 tablets as follows:

5 tablets of 1mg NA/20 µg EE
7 tablets of 1mg NA/30 µg EE
9 tablets of 1mg NA/35 µg EE
7 tablets of ferrous fumarate, 75 mg.

Inactive ingredients are listed as calcium stearate, lactose, microcrystalline cellulose and starch.

**Route of administration:** oral

**Proposed clinical protocol or Use**: The drug product was approved for the prevention of pregnancy in 1996. Tablets are taken once daily as graduated doses of ethinyl estradiol, as appropriate relative to the time of the patient's reproductive cycle.

Based on a body weight of 60 kg, the clinical doses would be 0.017 mg/kg norethindrone acetate and up to 0.58 µg/kg ethinyl estradiol (combined dose up to 17.6 µg/kg).

**Previous clinical experience:** Two six-month (six-cycle) blinded parallel-group clinical trials were performed in acne patients. The sponsor reports efficacy in the treatment of moderate acne vulgaris. Adverse events included those usually associated with oral contraceptive use, such as breakthrough bleeding, migraine, nausea, and breast pain/enlargement. Most adverse events were moderate or mild in intensity, but severe AE's were reported in a few patients (abdominal pain, headache, and migraine). On clinical chemistry evaluation, increases were seen in total cholesterol and serum triglycerides. The sponsor states that there were no safety concerns in subjects aged 13-17.

**Disclaimer: Note that some information may come directly from the sponsor's submitted material.**

**Introduction and drug history:**

Estrostep was approved in 1996 for the prevention of pregnancy. The sponsor submitted [redacted] in 1998 for the investigation of the drug in the treatment of acne vulgaris in post-menarchal female patients.

GENETIC TOXICOLOGY:

Articles submitted to this NDA containing genetic toxicology data were previously reviewed and recommendations were made to add the new information to the updated label along with the new indication for moderate acne vulgaris. It has since been determined that the guidance document for the class label for oral contraceptives in the Division of Reproductive and Urologic Drug Products does not allow for label updates or the addition of current scientific information specific to the drug product in question. Dr. Alex Jordan, pharmacology/toxicology supervisor in the DRUDP, has indicated through the DRUDP clinical reviewer that no new information should be added to the class label, and that addition of material specific to these drug substances would necessitate addition of that same information to all other oral contraceptive products, including those with different active ingredients, as part of revised class labeling.

OVERALL SUMMARY AND EVALUATION:

**Introduction:** The drug product was approved previously for use as an oral contraceptive agent. The currently proposed use in the treatment of acne and the patient population are consistent with approved labeling, with the possible inclusion of younger adolescent patients.

**Safety Evaluation:** The only new nonclinical safety information provided includes data from multiple tests for genetic toxicity of both active ingredients. While neither drug substance appears to be mutagenic in bacteria, both exhibited positive results in chromosomal aberration assays in mammalian cells and in *in vivo* mouse micronucleus assays. In some cases, the positive results were repeated in different studies and/or test systems.

**Clinical Relevance of Safety Issues:** Currently approved labeling only addresses the possibility of neoplasia associated with long-term oral contraceptive use.

**Other Clinically Relevant Issues:** One of the journal articles submitted in support of genetic toxicology testing referred to multiple articles in which lymphocytes cultured from blood obtained from women taking oral contraceptives had an increased incidence of chromosomal aberrations and sister chromatid exchanges relative to lymphocytes obtained from subjects not taking oral contraceptives. Another cited study indicated that chromosomal abnormalities were observed in six of eight spontaneous abortions in women who became pregnant after they stopped taking oral contraceptives.

**Conclusions:** From a pharmacology/toxicology standpoint, addition of genetic toxicology information would bring the label up to current standards. However, the DRUDP guidance on the class label for these drugs does not allow for such updates to the labels of individual products.

**Communication Review:**

- Labeling Review (NDA):

Wording previously recommended to include genetic toxicology information for these two drug substances will not be added to the label.

It was also previously recommended that published *in vitro* data on the induction of cytochrome P450 3A enzymes by retinoids be added to the drug interactions section of the label. However, since that study did not examine specifically P450 3A enzymes involved in the metabolism of these drug substances, that information may not be relevant. Additionally, the DRUDP guidance for the class label for oral contraceptives does not allow for addition to the label of current scientific information. The paragraph related to these data should not be added to the label.

- Investigator's Brochure/Informed consent review (IND): not applicable

**RECOMMENDATIONS:**

**Internal comments:** If, at some time in the future, the DRUDP guidance for the class label for oral contraceptives is amended to allow for updated scientific information or for information specific to a drug product, it is recommended that genetic toxicology information as described in the ICH guidelines, be added to the label for Estrostep®.

**External Recommendations/Draft letter Content for Sponsor:** none
**Future development or NDA issues:** none at this time

Amy C. Nostrandt, D.V.M., Ph.D.
Pharmacologist/Toxicologist

cc:
NDA
HFD-340
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HFD-540/CHEM/Pappas
HFD-540/PMS/Cintron

Concurrence Only:
HFD-540/DD/WILKIN

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Draft date (# of drafts): 4/10/01 (1)