CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

22-252, Original 1

PHARMACOLOGY REVIEW(S)

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-252
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	7/6/09
PRODUCT:	Estradiol valerate/Dieogest (Qlaira)
INTENDED CLINICAL POPULATION:	Primary indication Prevention of pregnancy and secondary indication for the treatment of heavy and/or prolonged menstrual bleeding in women without organic pathology
SPONSOR:	Bayer HealthCare Pharmaceuticals, Montville, NJ
DOCUMENTS REVIEWED:	Volumes 26 and 27 of IND (b) (4)
REVIEW DIVISION:	Division of Reproductive & Urologic Products Drug Products (HFD- 580)
PHARM/TOX REVIEWER:	Krishan L. Raheja, D.V.M., Ph.D.
PHARM/TOX SUPERVISOR:	Alex Jordan, Ph.D.
DIVISION DIRECTOR:	Scott Monroe, M.D.
PROJECT MANAGER:	Pamela K. Lucarelli

Date of review submission to Division File System (DFS): 4-7-10

APPEARS THIS WAY ON ORIGINAL

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-252
Review number: 2
Sequence number/date/type of submission: 0000/7-2-09/original submission
Information to sponsor: Yes ( ) No (*)
Sponsor and/or agent: Bayer HealthCare Pharmaceuticals, Montville, NJ
Manufacturer for drug substance: Bayer Schering Pharma

Reviewer name: Krishan L. Raheja, D.V.M., Ph.D.
Division name: Reproductive and Urologic Products
HFD #: 580
Review completion date: 3-25-10

Drug:

Trade name: Qlaira
Generic name: Estradiol valerate/Dienogest
Code name: ZK 5104 (estradiool valerate and ZK00037659 (dienogest)
CAS registry number: 979-32-8 (for estradiol valerate) and 65928-58-7 (for dienogest)

Relevant INDs/NDAs/DMFs: IND (b) (4) and IND 64,809

Drug class: Estradiol is an estrogen and dienogest is a progestin

Intended clinical population: Prevention of pregnancy as a primary indication and for treatment of heavy and /or prolonged bleeding without organic pathology who choose to use an oral contraception as their method of contraception as a secondary indication.

Clinical formulation: Tablets

Route of administration: Oral

Studies reviewed within this submission: The following genotoxicity studies of 17α-cyanomethyl-17β-hydroxy-estraa-4,9(10)-dien-3-one (Dienogest, M18575) which were previously not included in the NDA review are summarized in this submission:

1. Study title: Chromosomal aberration study of M18575 using cultured mammalian cells.

This study report is a translation of a Japanese study conducted in 1989 at the (b) (4) . Berlex Study No. 98091

Chinese hamster lung fibroblasts (CHL) cells were used. Successive generations were cultured and stored frozen at -80C to provide cells for this trial.

A preliminary trial was run to determine 50% cell proliferation inhibition (ID50) value for setting the doses in the chromosomal aberration trial where dose levels of 400, 200, 100, 50 and 25 ug/mL were used.

In the trial where metabolic activation was not used, the ID50 value was 55 ug/mL. Therefore the highest dose used was 110 ug/mL and other doses were 55, 27.5 and 13.75 ug/mL. Two plates were used for each dose.

In the trial where metabolic activation was used, the ID50 value was 110 ug/mL. Therefore the highest dose used was 220 ug/mL and other doses were 110, 55, 27.5 ug/mL. Two plates were used for each dose.

Preparation of chromosomal samples: Chromosomal samples were prepared using standard method for both without and with metabolic activation.

Method of observing chromosomal samples: The prepared samples were observed randomly, in a blind method. Under the microscope with magnification of 1000 X, 100 middle mitosis period images were spread-out per sample i.e., 200 per dose were observed. As the structural chromosomal aberrations, gaps, breaks, exchanges, ring formations, and fragmentations were counted, and the number of polyploids were counted as a numerical aberration.

Positive controls: Mytomycin C was used for preparations without S-9 mix and benzopyrene was used in preparations with S-9 mix.

Statistical analysis: The frequency of appearance of the structural chromosomal aberrations and the frequency of appearance of polyploids were tested by the Chi Square test. The level of significance used was <5%.

Results: No significant increases in the number of cells with chromosomal aberrations were observed both in the absence and presence of metabolic activation. However, the numbers of cells with chromosomal aberrations were significantly increased compared to the control groups, when treatments were performed with 0.2 ug/mL mitomycin C, the positive control in the case in which metabolic activation was not used, and 20 ug/mL benzopyrene, the positive control in the case in which metabolic activation was used.

Conclusion: The results suggest that Dienogest does not have the property of inducing chromosomal aberrations.

2. Study title: Test for chromosomal aberrations by in vitro human lymphocyte metaphase analysis with Dienogest. Berlex Study No. 98096

This study was sponsored by (b) (4) and conducted in 1995 in accordance with GLP regulations by the (b) (4)

The purpose of this study was to check the genotoxic potential of Dienogest by investigating chromosomal aberrations induced in lymphocytes tested in vitro in accordance with OECD guidelines.

Cell preparation: Human lymphocytes, induced to divide in culture by phytohaemagglutinin A, were exposed to dienogest. Cell division was then blocked in metaphase by the addition of colcemide. The cells collected were subjected to hypotonic shock, fixed, spread on plates, and then stained. The chromosomes were then examined under the microscope. The tests were run with and without metabolic activation in order to demonstrate promutagens and direct mutagens.

Methods used:

Cells: Human lymphocytes
Vehicle: DMSO used because of low solubility of Dienogest in water.

Toxicity test: Mitotic index determination

Duration of treatment: 24 hours without S9- mix metabolic activation
4 hours with S9-mix activation.
Doses tested: 500, 200, 125, 62.5 and 31.3 without S9- mix
and same dose levels with S9- mix.

Mutagenicity test:

Duration of treatment: 24 hours without S9- mix
4 hours with S9- mix
Number of assays: 2
Number of cultures/dose: 2
Number of cells observed: structural aberration :200/dose
numerical aberration: 2000/dose

Doses tested in the first assay: 125, 62.5, 31.3 ug/mL without S9-mix
500, 250, 125 ug/mL with S9-mix
Doses tested in the second assay: 125, 62.5, 31.3 ug/mL without S9-mix
(24 and 48 hour sampling times)
500, 250, 125 ug/mL with S9-mix
(24 and 48 hour sampling times)

Factor limiting the maximum dose: Cytotoxicity for assay without S9-mix
Solubility for assay with S9-mix

Positive controls: Mitomycin without S9-mix (0.25 ug/mL)
Cyclophosphamide with S9-mix (5 ug/mL)

The clastogenic activity of Dienogest was determined when the compound was tested at the highest dose compatible with its toxicity (125 ug/mL without metabolic activation) or with its solubility in the system (500 ug/mL with metabolic activation) and two lower doses.

Results:

In the assay without metabolic activation, Dienogest provoked a strong cytotoxicity at the two highest concentrations i.e., 500 and 250 ug/mL, with mitotic index of 27.5 to 28.8% of control. A dose of 125 ug/mL showed moderate toxicity with a 41.3% mitotic index. Therefore a dose of 125 ug/mL was used as the high dose as the maximum dose in assay with S9-mix.

In the assay with metabolic activation, relatively low toxicity (72.7% mitotic index) was noted at the highest dose of 500 ug/mL and this dose was retained as the maximum dose in assay with S9-mix.

In the first assay both without and with metabolic activation, no clastogenic activity of Dienogest was observed. The frequency of structural chromosomal aberrations was the same in control and treated groups. Also the frequency of cells with abnormal number of chromosomes was the same in the control and treated groups.

In the second assay without and with metabolic activation at both 24 hour and 48 hour sampling times, no significant increase in the number of breaks per cell or in the frequency of aberrant cells excluding and including gaps only was found at any dose level tested. Also no significant effect in the frequency of cells with abnormal number of chromosomes was observed excepting the assay without metabolic activation at 48-hr sampling time when significant increase in the number of polyploids was observed at the top dose of 125 ug/mL and the lowest dose of 31.3 ug/mL. No increase was observed at the mid dose of 62.5 ug/mL. Since the effect was not dose-related and observed at relatively very high concentration relative to the therapeutic levels, was not considered significant in terms of genotoxic hazard due to polyploidy.

Conclusion: It was concluded that Dienogest induced no clastogenic activity and presented a weak aneugenic effect at very high doses.

3. **Study title:** Dienogest: measurement of unscheduled DNA synthesis in rat hepatocytes in vitro. Final report. Berlex Report # 98094

This study was conducted at the (b) (4) in for (b) (4), in 1995 in accordance with OECD Good Laboratory Practice regulations.

Dienogest was tested using an in vitro assay for its ability to cause DNA damage in cultured primary rat hepatocytes. Repair was measured as unscheduled DNA synthesis (UDS) by the uptake of radio-labeled thymidine assayed autoradiographically. [^{3}H] thymidine is incorporated into DNA only id UDS is occurring.

Two independent experiments were conducted using 3 male Wistar rats, 42-52 days old and weighing 221 – 264 g in each experiment. Rats were anesthetized and their livers perfused with collagenase to provide hepatocyte suspensions. The resulting hepatocytes were tested for viability and then exposed to the test article (Dienogest), solvent DMSO), positive control (2-acetamidofluorene) and [^{3}H] thymidine.

The concentration of dienogest used ranged from 0.016 ug/mL to 1250 ug/mL in the first experiment and 6.25 ug/mL to 250 ug/mL in the second experiment. At least 3 slides for dienogest and positive control and 5 slides for solvent control were prepared for autoradiography for nuclear and cytoplasmic grain counts to determine net nuclear grains (NNG) using 50 cells per slide. Mean NNG is obtained by subtracting the mean grains/cytoplasmic area from grains per nucleus.

Results:

UDS analysis: In both experiments 1 and 2, treatment with dienogest at concentrations up to 250 ug/mL yielded NNG values less than 0. The group mean values in the first experiment ranged from -6.6 to -0.6 with no more than1.2% cells were seen in repair at any dose of dienogest and ranged from-3.6 to -1.1 with no more than 6% cells seen in repair at any dose of dienogest in the second experiment.

Conclusion: Since a value greater than 0 NNG is required for appositive response, the data obtained in this study indicated that concentration of dienogest as high as 250 ug/mL a dose which was within the precipitating range, did not result in increased UDS in cultured hepatocytes treated in vitro.

4. **Study title:** In vivo/in vitro unscheduled DNA synthesis in rat hepatocytes with dienogest,

This study was conducted for (b) (4) by (b) (4) in 1996 in compliance with GLP regulations.

In this study 25 SD female rats were used (5/g) in 2 treatment groups as shown in table below:

Test group	Treatment (2 hours)	Treatment (16 hours)
Negative control (CMC 1% solution)		5
Low dose (200 mg/kg)		5
High dose (2000 mg/kg) mg/kg	5	5
Positive control 2-AAF (100 mg/kg)		5

The highest dose of 2000 mg/kg was non toxic and used in accordance with OECD guideline. The oral dose volume was 10 mL/kg b.w. The maximum dose of 2000 mg/kg is stated to be 60000-fold of the daily human dienogest dose of 2 mg.

After a treatment period of 2 and 16 hours, the animals were anesthetized and sacrificed by enzymatic liver perfusion. Primary hepatocyte cultures were established and exposed for 4 hours to ^{3}HTdR (methyl-^{3}H-thymidine), which is incorporated if UDS occurs.

Results:

Mean nucleus, cytoplasmic area and net grains for dienogest are given in table below:

	Grains per nucleus		Grains per cytoplasmic area		Net grains per nucleus	
Treatment period	**Mean***	**SD**	**Mean***	**SD**	**Mean***	**SD**
Dienogest 2000 mg/kg (2 hours)	8.79	+/-4.12	13.22	+/- 5.00	- 4.43	+/- 4.30
CMC 1% solution (16 hours)	6.07	+/- 2.54	11.30	+/-3.73	- 5.23	+/- 3.11
Dienogest 200 mg/kg (16 hours)	11.67	+/- 6.20	17.27	+/- 6.73	- 5.61	+/- 5.10
Dienogest 2000 mg/kg (16 hours)	14.60	+/-6.52	18.57	+/-7.64	- 3.97	+/- 5.91
2-AAF 100 mg/kg (16 hours)	20.66	+/- 9.45	15.30	+/- 6.21	5.37	+/- 9.31

2-AAF= 2-acetylaminofluorene * Mean of 100 cells

At treatment interval 2 hours, the values of nuclear and cytoplasmic grain counts were only slightly enhanced as compared to the vehicle control. The shift in the % distribution of higher values of nuclear grain counts was less pronounced than at the 16 hour treatment interval. The net grain value was negative and comparable to the value of the current vehicle control.

At treatment interval 16 hour, the nuclear as well as cytoplasmic grain counts were significantly enhanced after administration of Dienogest as compared to the vehicle control. However, the net grain values were consistently negative. The shift to cells with higher nuclear grain count values after treatment with dienogest at interval 16 hour were dose dependent. Since in parallel, the mean cytoplasmic grain per group were enhanced, it was assumed that replicative DNA-synthesis rather than UDS was causative for the results.

In vivo treatment with 2-AAF showed distinct increases in the number of nuclear and net grain counts.

Conclusion: Since the net grain values were consistently negative in all groups after administration of Dienogest, it was considered non-effective in inducing DNA damage leading to increased repair synthesis under the experimental conditions used. The enhanced values of nuclear grain counts and the shifts of the percentage distribution of the nuclear grain counts to higher values were explained by the induction of some replicative DNA-synthesis.

5. Study title: Dienogest, cyproterone acetate, chloromadinone acetate: Induction of preneoplastic enzyme-altered foci in rat liver foci bioassay. Final Report. No.B394

This study was conducted by (b) (4) in 1994 in accordance with the OECD Principal of GLP.

Background: The rat liver foci bioassay is stated to be a rapid and sensitive bioassay in vivo for chemical carcinogens. It employs foci of phenotypically altered cells as indicators of carcinogenesis. The preneoplastic, enzyme-altered foci are early precursor lesions of liver tumors, and have potential to progress to cancer. They can be identified by histologically detectable changes in the activities of various enzymes. This assay is used for the identification of initiating and promoting carcinogens. The initiation-promotion protocol uses the comparatively high sensitivity of weaning female rats for initiating carcinogens, combined with a strong promoting stimulus exerted by repeated treatment with polychlorinated biphenyls.

Study design: In the present study, as total of 48 females (21 day old) were randomly divided in 8 treatment groups of 6 animals each. The animals were treated for 5 consecutive days with the test substances, given p.o., suspended in olive oil. Beginning 3 days after the last application, a technical PCB mixture (Clophen A50) was given twice weekly for promotion for 11 consecutive weeks. Control animals were treated with PCB and the vehicle, respectively. For positive controls, a single dose of 10 mg diethylnitrosamine/kg b.w. was given. The dose volume was 2 mL/kg olive oil for dienogest, cyproterone acetate and chloromadinone acetate, which are insoluble in water. The positive control was given in water as a single dose at day 1 of the experiment at a dose volume of 2 ml/kg.
Animals were sacrificed 12 weeks after beginning the experiment and 3 days after the last application of PCB by decapitation. Pieces of liver were frozen on specimen holders at -70^{o} in isopentane cooled by liquid nitrogen. Serial sections of frozen samples of 8 um thickness were prepared. Histochemical staining for demonstration of ATPase was done using the lead salt method and for GGTase using λ-glutamyl-α-naphthylamide. A total of 8 sections were taken for ATPase staining and 4 for GGTase staining.

Results:

Boy weight: There was no treatment-related effect on body weight.

Relative liver weight: Treatment with dienogest, cyproterone acetate and chloromadinone acetate did not affect the relative lever weight compared to the promotion control group.

The relative liver weight was significantly enhanced in the groups treated with Clophen A50 for promotion, compared to the untreated control group.

Incidence of enzyme-altered preneoplastic foci: Treatment-related number and area of ATPase-deficient and GGTase-positive foci expressed as mean +/- SD is given in table below:

		ATPase		GGTase	
Dose (mg/kg b.w.)	**No. Of animals**	**Number n/cm^2**	**Total area mm^2/cm^2**	**Number n/cm^2**	**Total area mm^2/cm^2**
35 ng dienogest/PCB	**6**	**1.0 +/- 0.3**	**0.030 +/- 0.012**	**0.5 +/- 0.5**	**0.020 +/- 0.024**
70 mg dienogest/PCB	**6**	**0.8+/- 0.6**	**0.016 +/- 0.015**	**0.4 +/-0.5**	**0.009 +/- 0.011**
140 mg dienogest/PCB	**6**	**1.5 +/- 0.6**	**0.032 +/-0.015**	**0.7 +/-0.4**	**0.011 +/- 0.008**
100 mg cyproterone acetate/PCB	**6**	**10.4 +/- 2.2***	**0 208 +/- 0.067***	**7.0 +/- 1.6***	**0.109 +/-***
100 mg chloromadinone acetate/PCB	**6**	**1.1 +/- 0.5**	**0.024 +/- 0.013**	**0.4 +/- 0.3**	**0.002 +/- 0.001**
100 mg Diethynitrosamine/PCB	**6**	**44.8 +/- 5.5***	**0 942 +/- 0.280***	**30.7 +/- 4.3***	**0.727 +/- 0.297***
Promotion control (PCB)	**6**	**1.1 +/- 0.6**	**0.024 +/- 0.013**	**0.1 +/- 0.2**	**0.002 +/- 0.003**
Untreated control	**6**	**0.0**	**0.0**	**0.0**	**0.0**

* Significantly different from promotion control ($p \leq 0.001$)

Conclusion: It was concluded that cyproterone acetate has initiating activity as reported before and results do not indicate an initiating potency for Dienogest and chloromadinone in the liver of female rats.

The following genotoxicity studies were reviewed previously and included in the NDA review. Results of these studies indicated that Dienogest is not mutagenic.

1. Study title: Salmonella typhimurium reverse mutation assay with dienogest. Study No. (b) (4) Project 493601

2. Study title: Reverse mutation test of M 18575 using bacteria. Berlex Report No. 98089

3. Study title: Chromosomal aberration study of M 18575 using cultured mammalian cells. Berlex Report No. 98091

4. Study title: Mutation assay at the TK locus in L5178Y mouse lymphoma cells using a microtiter cloning technique (trifluorothymidine resistance) with dienogest. Berlex Report No. 98090

5. Study title: In vivo micronucleus assay in bone marrow cells of the mouse with dienogest. Berlex Report No. 98100.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22252	ORIG-1	BAYER HEALTHCARE PHARMACEUTICALS INC	Qlaira

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/s/

KRISHAN L RAHEJA
04/07/2010

ALEXANDER W JORDAN
04/07/2010

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

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	22-252
SERIAL NUMBER:	0000
DATE RECEIVED BY CENTER:	7/6/09
PRODUCT:	Estradiol valerate/Dienogest
INTENDED CLINICAL POPULATION:	Primary indication Prevention of pregnancy and secondary indication for the treatment of heavy and/or prolonged menstrual bleeding in women without organic pathology
SPONSOR:	Bayer HealthCare Pharmaceuticals, Montville, NJ
DOCUMENTS REVIEWED:	Electronic submission**.**
REVIEW DIVISION:	Division of Reproductive & Urologic Products (HFD- 580)
PHARM/TOX REVIEWER:	Krishan L. Raheja, D.V.M., Ph.D.
PHARM/TOX Team Leader:	Alex Jordan, Ph.D.
DIVISION DIRECTOR:	Scott Monroe, M.D.
PROJECT MANAGER:	Pamela K Lucarelli

Date of review submission to Division File System (DFS): 1-27-2010

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EXECUTIVE SUMMARY

I. Recommendations

A. Recommendation on approvability: Nonclinical data supports approval of NDA 22-252 for Estradiol valerate/Dienogest.

B. Recommendation for nonclinical studies: No additional nonclinical studies are required

C. Recommendations on labeling: The proposed Prescribing Information is in accordance with the PLR and presented in SPL format and is acceptable.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: The nonclinical safety assessment of Dienogest includes pharmacology studies, PK and TK studies, general toxicology, acute, subchronic and chronic studies, genotoxicity studies, reproductive toxicity studies, and carcinogenicity studies.

Toxicological findings in the general toxicology studies were generally similar to those reported previously for other approved progestins. No adverse neurological, cardiovascular, pulmonary, renal or gastrointestinal effects were observed in safety pharmacology studies. Dienogest was not mutagenic when tested in Ames assay, chromosomal aberration study using cultured mammalian cells, in mouse lymphoma test, and in in vivo mouse micronucleus assay. Fertility and early development and embryofetal development studies demonstrated no adverse effects on treated females or the fetuses. Carcinogenicity studies conducted in male rats and in male and female mice by oral administration of dienogest for 104 weeks demonstrated findings essentially similar to those with other progetins reviewed and approved previously. These studies were presented to and approved by the Exec-CAC.

B. Pharmacologic activity: Estradiol valerate is an estrogen and dienogest is a progestin

C. Nonclinical safety issues relevant to clinical use: none

2.6 PHARMACOLOGY/TOXICOLOGY REVIEW

2.6.1 INTRODUCTION AND DRUG HISTORY

NDA number: 22-252
Review number: 1
Sequence number/date/type of submission: 0000/7-2-09/original submission
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Bayer HealthCare Pharmaceuticals, Montville, NJ
Manufacturer for drug substance: Bayer Schering Pharma for both estradiol valerate and dienogest

Reviewer name: Krishan L. Raheja, D.V.M., Ph.D.
Division name: Reproductive and Urologic Products
HFD #: 580
Review completion date: 1-27-2010

Drug:

Trade name: Qlaira
Generic name: Estradiol valerate (EV) and dienogest (DNG)
Code name: ZK 5104 (estradiol valerate); ZK 00037659 & FS-10101-N (dienogest)
Chemical name estradiol valerate: Estra-1,3,5(10)-triene-3,17-valerate or estra-1,3,5(10)-triene-3,17-diol (17β),17 -pentanoate or 1,3,5(10)-estratriene-3,17β-diol-17-valerate

Chemical name for dienogest: 19-Norpregna-4,9-diene-21-nitrile, 17-hydroxy-3-oxo-17α-cyanomethyl-17β-hydroxy-estra-4,9-dien -3-one or 17α-hydroxy-3-oxo-19-norpregna-4,9-diene-21 -nitrile or 17β-hydroxy-3-oxo-19-nor-17α-pregna-4,9-fiene -21-nitrile or (17α)-17-hydroxy-3-oxo-19-norpregna-4,9-diene -21-nitrile

CAS registry number: 979-32-8 (for estradiol valerate) and 65928-58-7 (for Dienogest)
Molecular formula/molecular weight: Estradiol valerate ($C_{22}H_{32}O_3$/356.51)
Dienogest ($C_{20}H_{25}NO_2$/311.42)
Structure:

Relevant INDs/NDAs/DMFs: DMF 3379 (for estradiol valerate) and 14014 (for dienogest)

Drug class: Estradiol valerate is an estrogen and dienogest is a progestin

Intended clinical population: Prevention of pregnancy as primary indication and for the treatment of heavy and/or prolonged bleeding in women without organic pathology who choose to use an oral contraceptive as their method of contraception as secondary indication.

Clinical formulation: The estradiol valerate/dienogest tablets constitute a combined oral contraceptive (COC) consisting of a four-phase (plus a placebo phase), 28 day sequential regimen given below:

Cycle days 1 to 2: 3.0 mg EV
Cycle days 3 to 7: 2.0 mg EV + 2.0 mg DNG
Cycle days 3 to 24: 2.0 mg EV + 3.0 mg DNG
Cycle days 25 to 26: 1.0 mg EV
Cycle days 27 to 28: Placebo

Estradiol valerate is a pro-drug that is rapidly hydrolyzed pre-systemically into 17β-estradiol and valeric acid. Currently EV is approved only as an injectable product (Delestrogen®) in US. However, it is stated that in many countries EV is widely used as a component of hormone therapy (HT) products and is an approved component of an oral contraceptive in Finland.

Dienogest is a progestin which is marketed in several counties in a dose of 2 mg in combination with 2 mg estradiol valerate (Climodien 2/2) for hormone replacement therapy (HRT). Dienogest (2 mg) is also marketed as an oral contraceptive in combination with 0.03 mg ethinyl estradiol (Valette® , Celimona®) in several countries. Sponsor has further stated that EV/DNG tablets, the subject of this NDA, were recently approved in over 20 European countries as a COC, and in some countries the product launch was initiated in May of 2009.

Thus in this four-phasic oral contraceptive, each phase contains a different dose of EV and a different dose of DNG. The proposed "dose-increased regimen A" starts and ends with estrogenic monophase. The 2 day application of 3 mg EV on day 1 and 2 is expected to effectively stimulate endometrial growth and to reduce intermenstrual bleeding. The treatment ends with an estrogenic monophase for 2 days. The rationale is to reduce the hormone withdrawal symptoms, such as headache, breast tension and mood changes to a minimum. It was further stated that since ovulation inhibition with estrogen only is not feasible, a progestogen is added in the early phases of the treatment cycle, starting on day 3 until day 24.

Route of administration: Oral

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

Data reliance : Except as specifically identified below, all data and information discussed below and necessary for approval of NDA 22-252 are owned by Bayer HealthCare Pharmaceuticals or are data for which Bayer HealthCare Pharmaceuticals has obtained a written right of reference. Any information or data necessary for approval of NDA 22-252 that Bayer HealthCare Pharmaceuticals does not own or have a written right to reference constitutes one of the following: (1) published literature, or (2) a prior FDA finding of safety or effectiveness for a listed drug, as described in the drug's approved labeling. Any data or information described or referenced below from a previously approved application that Bayer HealthCare Pharmaceuticals does not own (or from FDA reviews or summaries of a previously approved application) is for descriptive purposes only and is not relied upon for approval of NDA 22-252.

Studies reviewed within this submission: The sponsor has stated that their nonclinical testing strategy for the proposed fixed-combination drug product focuses mainly on the non-clinical qualification of dienogest, a new chemical entity (NCE), supplemented by studies of dienogest given orally in combination with either estradiol valerate (1:1) or ethinyl estradiol (200:3). Because estradiol is already approved in US, and because there is extensive worldwide experience with the use of EV by women over several decades, no new toxicology studies of EV were conducted or planned. Most of the nonclinical information for the NCE in this drug product, dienogest, is referred to IND (b) (4) original IND 64,809 submission dated 11-17-04 for oral contraception and IND (b) (4) The NDA review thus constitutes review of the original IND submissions.

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary: Dienogest, the progestin component of the EV/DNG tablets is a 19-norprogestin with strong progesterone receptor selectivity, leading to specific progestational action and to effective transformation of the endometrium. DNG lacks androgenic and antiestrogenic activities, has high bioavailability and does not bind to sex hormone binding globulin.

2.6.2.2 Primary pharmacodynamics

Non-clinical pharmacodynamics

The effects of the administration of DNG and EV, when given orally in a 1:1 combination to mice and rats were compared with the effects of oral administration of DNG and EV alone. The PD and PK profile were studied after once daily administration for 3 days and toxicological profile was studied after once daily administration for 13 weeks.

Pharmacodynamics (PD)**:** Daily administration of 0.4 mg/kg/day of dienogest, 0.4 mg/kg/day of EV or the combination of DNG + EV (0.4 + 0.4 mg/kg/day) to female Wistar rats significantly increased uterus and vagina weights and increased plasma angiotensinogen 1 levels compared with vehicle controls. Both DNG and EV lowered rat LH activity and had additive effect. In contrast neither hormone alone or in combination affected FSH.

Pharmacokinetics (PK) in adult ovariectomized rats given the above dosing regimen is shown in table 2 below:

Table 2

Analyte measured	Dienogest (DNG)		Estradiol (E2)		Estrone (E1)		Estrone sulfate (EIS)	
DNG (mg/kg/day)	0.4	0.4		0.4		0.4		0.4
EV (mg/kg/day)		0.4	0.4	0.4	0.4	0.4	0.4	0.4
Cmax (ng/ml)	68.3	83.5	57	61	60	97	0.35	0.47
AUC (ng.h/ml)	168	161	197	167	374	429	1.86	2.36
Tmax (h)	1	1	0.5	2	2	1	0.5	2

Other PK data are provided for 13-week repeated dose oral range finding study in CD-1 mice and rats. In these studies, 3 dose levels each of EV and corresponding 1:1 combination were tested, along with a single, high dose of DNG and an untreated control group were used.

In the mice study, administration of DNG (60 mg/kg/day), EV (0.6, 6.0, 60 mg/kg) or a combination of DNG and EV (0.6 + 0.6, 6 + 6, 60 + 60 mg/kg, respectively) produced the following PD effects:

With administration of EV or in combination with DNG, mainly estrogen effects were observed. These included decrease in body weight gain with a dose of 6 mg/kg EV, an increased incidence of endometrial/glandular hyperplasia and glandular dilatation in uterus, increased keratinization and decreased mucification of epithelium of the vagina, an increased incidence of cystic dilatation of the ovarian bursa and absent/sparse corpora lutea and increased glandular proliferation and secretary activity of mammary gland. An increased incidence of fusiform cell hyperplasia and decreased incidence of X-zone vacuolation and persistent X-zone was observed in adrenal gland. The absolute weight of ovary decreased with combination but increased with EV treatment alone. Also a dose-dependent increase in mean liver, pituitary, salivary gland and spleen were reported after administration of combination or EV alone. On histological examination, centrilobular hepatocellular hypertrophy and transitional cell/stromal hyperplasia were observed in the urinary bladder. An increased incidence of osteosclerosis of the sternum was observed in the mid and high doses of the combination and EV alone. Small decreases in RBC, leukocyte, eosinophil and lymphocyte counts were observed with combination and with both DNG and EV alone. Extramedullary hematopoiesis was increased in liver and spleen after administration of combination or EV alone. No overt organ toxicity was reported.

Administration of single high dose of DNG (60 mg/kg/day) produced alopecia in 4/12 rats, decreased body weight gain (11%) , decreased Hb, RBC count, swollen spleen, slightly increased incidence of cystic ovarian bursa, absent corpora lutea and ovarian atrophy and extramedullary hematopoiesis in liver and spleen.

Toxicokinetic (TK) parameters for DNG after administration of DNG alone or in combination (1:1) with EV to female CD-1 mice over 3 months are given in table 3 below:

Table 3

	Dose (mg/kg/day)			
DNG	**0.6**	**6**	**60**	**60**
EV	**0.6**	**6**	**60**	**-**
		AUC (ng.h/ml)		
Day 1	**14.1**	**385**	**13248**	**12760**
Day 85	**27.8**	**814**	**13631**	**6118**
		Cmax (ng/ml)		
Day 1	**41.1**	**1000**	**11664**	**13045**
Day 85	**69**	**1142**	**10364**	**7698**

TK parameters for estradiol and estrone after administration of estradiol valerate alone or in combination with dienogest are shown in table 4 below:

Table 4

	Dose (mg/kg/day)					
DNG	**0.6**	**6**	**60**	**-**	**-**	**-**
EV	**0.6**	**6**	**60**	**0.6**	**6**	**60**
			AUC 0-tlast (ng.h/ml)			
Estradiol						
Day 1	**0.179**	**4.58**	**07.4**	**0.241**	**3.37**	**129**
Day 85	**0.863**	**3.03**	**12.5**	**1.01**	**5.07**	**20**
Estrone						
Day 1	**0.206**	**5.88**	**131**	**0.296**	**1.89**	**124**
Day 85	**0.324**	**4.59**	**27.9**	**1.05**	**7.05**	**27.4**
			Cmax (ng/ml)			
Estradiol						
Day 1	**0.334**	**8.13**	**132**	**0.299**	**1.31**	**143**
Day 85	**0.336**	**0.919**	**8.32**	**1.15**	**4.45**	**8 12**
Estrone						
Day 1	**0.509**	**12.7**	**130**	**0.465**	**1.46**	**115**
Day 85	**0.494**	**2.49**	**18.1**	**1.41**	**7.36**	**9.48**

In the repeated dose oral range-finding study in rats, daily administration of DNG (10 mg/kg/day), EV (0.1, 1, 10 mg/kg/day) or a 1:1 combination of DNG + EV (0.1 + 0.1, 1 + 1, 10 + 10 mg/kg, respectively) for 3 months produced mainly estrogenic effects with EV alone or in combination with DNG. These included dose-dependent reduction in body weight and hair loss at dose of 1 mg and greater of EV alone or in combination with DNG. There was dose-dependent increases in adrenal and pituitary weights and decrease in ovary and thymus weights. Microscopically, centrilobular hepatocyte hypertrophy, renal cortical tubular dilatation and/or cortical tubular pigment, sinusoidal dilatation/congestion of the adrenal glands, luminal dilatation of the urinary bladder,

osteosclerosis in the femur and mastocytosis in the mesenteric lymph nodes were observed. As in mice mild hematological (decreased Hct, Hb, RBC, lymphocyte and platelet count) were observed when DNG and EV were administered alone or in combination at all doses. No overt organ toxicity was reported. A NOEL was not identified and MTD was defined as 1 mg/kg/day for EV. For the combination neither NOEL nor MTD was identified or defined.

TK parameters for DNG after administration of DNG alone or in combination with EV over 3 months are shown in table 5 below:

Table 5

	Dose (mg/kg/day)			
DNG	**0.1**	**1**	**10**	**10**
EV	**0.1**	**1**	**10**	**-**
AUC (ng.h/ml)				
Day 1	**9.28**	**73.3**	**6628**	**8898**
Day 85	**15.6**	**130**	**6351**	**9527**
Cmax (ng/ml)				
Day 1	**11.5**	**89.6**	**5183**	**7879**
Day 85	**28.8**	**66.4**	**4817**	**9241**

TK parameters for estradiol after administration of estradiol valerate alone or in combination with dienogest are shown in table 6 below:

Table 6

	Dose (mg/kg/day)					
DNG	**0.1**	**1**	**10**	**-**	**-**	**-**
EV	**0.1**	**1**	**10**	**01**	**1**	**10**
AUC 0-tlast (ng.h/ml)						
Estradiol						
Day 1	**NC**	**1.29**	**2.62**	**NC**	**2.08**	**214**
Day 85	**NC**	**0.67**	**95.1**	**NC**	**1.55**	**98.5**
Estrone						
Day 1	**NC**	**0.95**	**119**	**NC**	**1.39**	**124**
Day 85	**NC**	**0.97**	**54.4**	**0.33**	**1.27**	**59.9**
Cmax (ng/ml)						
Estradiol						
Day 1	**0101**	**1.59**	**182**	**0.067**	**2.03**	**142**
Day 85	**0058**	**0986**	**97.2**	**0.088**	**2.36**	**90.4**
Estrone						
Day 1	**0.037**	**1.01**	**75.2**	**0.466**	**1.07**	**79**
Day 85	**0.431**	**0.73**	**50.2**	**0.061**	**1.48**	**47.4**

Based on these data sponsor concluded that there is potential for induction of the metabolism of estradiol and estrone at doses of 1 mg/kg or greater. At low dose combination (0.1 + 0.1 and 1 + 1 mg/kg/day), there is potential for inhibition of the enzymes involved in the metabolism of dienogest. The ratio in the DNG alone dose group was unchanged.

Comparative PK parameters after oral administration of Dienogest in mouse, rat, cynomolgus monkey and human are given in table 7 below:

Table 7

Species	Mouse	Rat ----------Intact----------		OVX c	C.monkey	human
Report No	B364 a	A00565	B449 b	B441	A00565	B471
Dose (mg/kg)	10	1	10	40 d	1	0.05 e
Sex	Female	Female	female	Female	Female	female
N	55	3	45	8	3	15
AUC 0-inf (ug.hr/ml) f	0.729	0.76	12.0	164	4.0	0.90
Cmax (ug/ml)	731	536	7197	14360	700	81
Tmax (hr)	0.5	0.25	0.5	3.2	1.33	1.5
T1/2 (hr) g	2.6	0.66	8.1	5.3	10.6	11.8
Total Cl (ml/min) h	-	-	13.9	0.73	-	55.6
Total Cl (L/hr/kg)	-	1.33	-	-	0.25	-
Vd (L) i	-	-	9.7	0.33	-	-
Vd (L/kg)	-	1.3	-	-	3.8	-

a- report 98121 in IND (b) (4); day 5
b- report 98121 in IND (b) (4) day 5
c- rats ovariectomized
d- report 98115 in IND (b) (4) based on estimated 10 mg single dose/animal
e- based on 3 mg dose administered to 60 kg women
f- AUC=area under the concentration-time curve
g- t1/2 terminal half-life
h- Cl=clearance
i- Vd= volume of distribution

2.6.2.2 Primary pharmacodynamics

Mechanism of action: By ovulation inhibition

Drug activity related to proposed indication: The EV/DNG tablets regimen act as COC by inhibition of ovulation by its antigonadotropic action.

2.6.2.3 Secondary pharmacodynamics

2.6.2.4 Safety pharmacology

Neurological effects: Studies in mice and rats at oral doses of up to 30 mg/kg, dienogest had no analgesic action nor did it affect general behavior, sleep induction, spontaneous motor activity, coordinated movement, body temperature, or induced (chemical or electrical) convulsions. Also at this dose levels, dienogest had no effect on the rabbit electroencephalogram. At higher doses (up to 100 mg/kg) in mice, intraperitoneal administration of dienogest reduced food and water consumption and reduced spontaneous motor activity.

Cardiovascular effects: Following intraduodenal administration to rabbits (30 mg/kg), dienogest had no effect on systolic or diastolic blood pressure, heart rate, carotid artery flow, femoral artery flow, blood gases or electrocardiogram. When orally administered as a single dose to conscious, telemetered female monkeys (3, 10, 30 mg/kg), dienogest

did not effect mean blood pressure, heart rate or ECG parameters (PR, QRS, and QT intervals).

In vitro in isolated guinea pig papillary muscle, dienogest had no effect on the resting membrane potential, action potential amplitude, action potential duration at 50% and 90% repolarization (APD_{50} and APD_{90}, respectively) or V_{max} at concentrations up to 10^{-5} mol/L. However, dienogest significantly shortened the ADP_{50} by7% and significantly lengthened the ADP_{90} by 10% at 10^{-4} mol/L, respectively.

In vitro, dienogest, at concentrations ranging from 10^{-6} to 10^{-5} mol/L had no effect on the human HERG-mediated potassium channel.

Dienogest did not influence spontaneous rate of contractility in isolated rat atrial preparations (10^{-6} mol/L), but at concentrations ranging from 10^{-6} to 10^{-4} mol/L, dienogest moderately to completely inhibited the spontaneous contraction of smooth muscle in guinea pig atria and guinea pig or rat ileal preparations, respectively. Norepinephrine –induced contractions of isolated rat vas deferens were not effected at any concentration but significantly increased electrically-induced contractions at 10^{-4} mol/L. Dienogest increased spontaneous uterine contractions in female rats and even reversed progesterone-induced suppression of uterine contractions at concentrations ranging from 3 to 15x 10^{-4} mol/L. Dienogest significantly inhibited oxytocin-induced contractions of isolated rat uterus at 10^{-4} mol/L but had no effect at concentrations below that.
Pulmonary effects: When orally administered to female rats, dienogest reduced tidal and minute volume within 2 hours of dosing with doses greater than 10 mg/kg. A single iv dose of dienogest (2, 10, 20, 50 mg/kg) had no effect on arterial blood pressure or rate of respiration in female dogs, however, the magnitude of respiration was briefly higher after administration of a dose of 50 mg/kg.

Renal effects: In male rats, dienogest decreased urine volume and sodium excretion following intraperitoneal administration at dose levels of 10 or 100 mg/kg, with variable effects on potassium excretion.

Gastrointestinal effects: Dienogest did not affect gastrointestinal transit time in mice or rats at doses up to 30 mg/kg.

Abuse liability: none described

Other: none

2.6.2.5 Pharmacodynamic drug interactions: None described

2.6.3 PHARMACOLOGY TABULATED SUMMARY

None provided

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary: As described under section 2.6.2.2

2.6.4.2 Methods of Analysis
[see under individual study reviews]

2.6.4.3 Absorption: As shown in table below, dienogest was rapidly absorbed following oral administration in all species evaluated based on short time to reach Cmax:

Species	Mouse	Rat ----------Intact----------		OVX c	C.monkey	human
Report No	B364 a	A00565	B449 b	B441	A00565	B471
Dose (mg/kg)	10	1	10	40 d	1	0.05 e
Sex	Female	Female	female	Female	Female	female
N	55	3	45	8	3	15
AUC 0-inf (ug.hr/ml) f	0.729	0.76	12.0	164	4.0	0.90
Cmax (ug/ml)	731	536	7197	14360	700	81
Tmax (hr)	0.5	0.25	0.5	3.2	1.33	1.5
T1/2 (hr) g	2.6	0.66	8.1	5.3	10.6	11.8
Total Cl (ml/min) h	-	-	13.9	0.73	-	55.6
Total Cl (L/hr/kg)	-	1.33	-	-	0.25	-
Vd (L) i	-	-	9.7	0.33	-	-
Vd (L/kg)	-	1.3	-	-	3.8	-

a. report 98121 in IND (b) (4); day 5
b. report 98121 in IND (b) (4); day 5
c. ovariectomized
d. report 98115 in IND (b) (4) based on estimated 10 mg single dose/animal
e. based on 3 mg dose administered to 60 kg women
f. AUC=area under the concentration-time curve
g. t1/2 terminal half-life
h. Cl=clearance
i. Vd= volume of distribution

The absorption of dienogest following oral administration was almost complete, based on renal excretion of similar fractions of the administered dose following oral or intravenous administration. The oral bioavailability was 71% in rats administered ^{14}C-labeled dienogest and ranged from 85% to 90% in rabbits, Beagle dogs and baboons. The systemic exposure based on a comparison of $AUC_{0\text{-inf}}$ was similar to or greater than, those observed in women given the maximum dose (3 mg) in the clinical regimen. The T1/2 was generally shorter in animals except for monkeys compared to humans.

2.6.4.4 Distribution: The plasma protein binding was reversible and ranged from 97% to 95% in female rats, dogs, monkeys and healthy human volunteers and was not concentration dependent. The relative binding affinity to SHBG and CBG was <0.1% for dienogest in vitro. The main binding protein in plasma was albumin. In vitro, approximately 7% to 20% of total radioactivity was found in RBCs following incubation

of ^{14}C-dienogest with whole blood from female rats, dogs, monkeys and humans. Maximum tissue concentrations were detected both qualitatively and quantitatively at 1 hour after dosing. Qualitatively high levels of radioactivity were detected in contents of GI tract and bladder, and in adrenal gland, ovary and liver. Quantitatively, the highest levels of radioactivity were detected in the adrenal gland, liver, stomach, ovary and kidney. After repeated administration, maximum concentrations of radioactivity reached steady state by the 7th dose in most tissues. After 21st dose, concentrations of radioactivity in fat, skeletal muscle, cerebellum, blood, spleen and skin were >10-fold higher than after a single dose. Concentration in other organs increased 3 – 9-fold.

The % of radioactivity in the fetuses relative to maternal plasma radioactivity at 1 hour were similar (29% and 34%, respectively) after oral administration to dams on Day 12 or 18 of gestation. In general, radioactivity in the fetuses decreased in parallel with the radioactivity in maternal plasma during the study.

The mean concentrations of radioactivity in the milk of lactating rats administered 1 mg/kg of ^{14}C-dienogest on Day 10 postpartum was similar to, or slightly less than, the concentration in maternal plasma for all time points evaluated during the 72 hours after dosing and decreased in parallel with radioactivity in maternal plasma during the study.

2.6.4.5 Metabolism: Dienogest was poorly metabolized in vitro by microsomes or hepatocytes isolated from rats, dogs, monkeys or humans. Metabolites were detected after incubation with microsomes or hepatocytes after 0.5 or 24 hours, respectively, and 2 to 5 metabolites were isolated. The metabolite profile after incubation with monkey microsomes or hepatocytes was most like metabolite profile in humans. Also metabolism of dienogest in mouse and human micosomes produced a similar metabolite pattern.

In microsomes expressing human cytochrome P450s, CYP3A4 was the predominant isozyme catalyzing the metabolism of dienogest. The predominant metabolites observed were M1 (6β-OH-dienogest), M2 (1α-OH-dienogest) and A1 (8-OH-dienogest). Other isozymes (CYP1A1/2, CYP2A6, CYP2B6, CYP2D6 and CYP2E1) did not inhibit the formation of any of the dienogest metabolites. Dienogest was highly metabolized in all species, with <3% of the parent drug excreted in the urine of rats and dogs after a single dose.
The major metabolites in human urine were M1, conjugated dienogest and minor metabolites M2 and M3 (11βOH-dienogest). The major human metabolites were also observed in rat plasma, urine and feces. The in vivo metabolism in mice and guinea pigs was quantitatively similar to the rat.

2.6.4.6 Excretion The primary route of excretion in rats, rabbits, dogs, monkeys, baboons and humans was renal regardless of the route of administration or the radiolabel used. The secondary route of excretion in these species was generally the feces. About 45% of the dose was excreted in the bile following iv or oral administration of dienogest in bile duct-cannulated rats. Excretion was rapid and almost complete within 1 to 7 days after dosing in rats, rabbits, dogs, baboons and humans. Serum trough levels in monkeys treated from Day 5 to 20 of the menstrual cycle at doses of 0.3 and 1 mg/day,

demonstrated dose-dependent serum concentrations ranging from 2.3 to 4.4 ng/ml and 7.8 to 15.4 ng/ml at 0.3 mg and 1 mg/animal, respectively. Steady state concentrations were reached with the second dose and there was no evidence of accumulation after multiple doses.

2.6.4.7 Pharmacokinetic drug interactions: It was stated that in vitro drug-drug interaction studies did not indicate a potential for clinically-relevant drug-drug interaction.

2.6.4.8 Other Pharmacokinetic Studies: None

2.6.4.9 Discussion and Conclusions

2.6.4.10 Tables and figures to include comparative TK summary

Species	Mouse	Rat ----------Intact----------		OVX c	C.monkey	human
Report No	B364 a	A00565	B449 b	B441	A00565	B471
Dose (mg/kg)	10	1	10	40 d	1	0.05 e
Sex	Female	Female	female	Female	Female	female
N	55	3	45	8	3	15
AUC 0-inf (ug.hr/ml) f	0.729	0.76	12.0	164	4.0	0.90
Cmax (ug/ml)	731	536	7197	14360	700	81
Tmax (hr)	0.5	0.25	0.5	3.2	1.33	1.5
T1/2 (hr) g	2.6	0.66	8.1	5.3	10.6	11.8
Total Cl (ml/min) h	-	-	13.9	0.73	-	55.6
Total Cl (L/hr/kg)	-	1.33	-	-	0.25	-
Vd (L) i	-	-	9.7	0.33	-	-
Vd (L/kg)	-	1.3	-	-	3.8	-

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

As shown in tables above [pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

General toxicology: General toxicology studies included single and repeat dose toxicity studies in various species as described under Toxicology section.

Genetic toxicology: Dienogest was not mutagenic when tested in the Ames assay, chromosomal aberration study using cultured mammalian cells, in the mouse lymphoma test, and in the in vivo micronucleus assay in bone marrow of the mouse.

Carcinogenicity: Carcinogenicity studies conducted in rats and mice by oral administration of dienogest for 104 weeks demonstrated findings essentially similar to

those with other progestins reviewed and approved previously. These studies have been presented to and approved by the Exec-CAC on 1/16/09.

Reproductive toxicology: Fertility and early embryonic development and embryofetal development studies demonstrated no adverse effects on treated females or the fetuses.

Special toxicology: None described.

2.6.6.2 Single-dose toxicity

Dienogest was well tolerated when given orally as a single dose to mice, rats, rabbits and dogs. Minimum lethal dose levels following oral administration were 1000 mg/kg in male rabbits, 2000 mg/kg in female mice and female rabbits, and 3000 mg/kg in male mice. No mortality was reported in male and female rat s or female dogs given dienogest orally at a dose of 2000 mg/kg. A single SC administration of dienogest at a dose levels up to 5000 mg/kg was well tolerated and was not lethal to male and female ABD_2F_1 mice. In male rabbits, deaths preceded by convulsions occurred when dienogest was administered intraperitoneally at dose level of 1500 mg/kg.

2.6.6.3 Repeat-dose toxicity

Study title: 91-day toxicity study of Dienogest (M18575) in female rats.

This study was conducted in accordance with GLP regulations by (b) (4) as study No. 0208. Berlex provided this study as Report No. 98017.

It was stated that Dienogest was developed by (b) (4) (former East Germany) and clinical trials are being performed in Germany as a drug for treating endometriosis. (b) (4) The review below is from a translation of the foreign language report.

Six-week old female Wistar rats weighing 110-122 g were used in this study. The dose levels used were 0.3, 3, 10 and 30 mg/kg/day administered once daily as suspension gavage (5 ml/kg) for 91 continuous days. There were 10 rats/group, with 10 more added to control and 30 mg/kg groups for 28-day recovery period, The high dose of 30 mg/kg/day was used as 30 mg/kg and greater resulted in a slight degree of anemia as well as reduced total cholesterol, increased relative liver weight, and mucous degeneration of the vaginal epithelial cells. A dose of 100 mg/kg caused suppression of body weight increases accompanied by a reduction in quantity of food consumed.

Observations:

General condition, body weight, and food and water consumption: General condition of the animals was observed daily. Body weight was recorded daily up to day 7 of administration, twice a week up to day 28 of administration and then weekly thereafter.

Food consumptions were recorded daily up to day 7 and then weekly thereafter. Water consumption was recorded before administrating and then on days 42 and 84 of administration and day 24 of recovery.

Sexual cycle: Microscopic examination of the vaginal smears was performed daily from day 77 to day 90 of administration and in the recuperation period.

Serologic and biochemical tests: Blood was collected on the day before the last day of administration and on day 28 of the recovery period. Tests included hematological parameters (RBC count, WBC count, platelet count, Hb, Hct. MCH, MCV, MCHC as well as WBC differential count). Also reticulocyte count and RBC osmotic fragility, PT, APPT and fibrinogen were measured.

Total protein, albumin, total cholesterol, phospholipids, neutral lipids, FFA, total bilirubin, GOT, GPT, glucose, creatinine and inorganic phosphorus, Na and K were determined.

Urinalysis: tested for pH, protein, glucose, ketone bodies, urobilinogen, occult blood and bilirubin.

Ophthalmologic and auditory tests: were performed at 12 weeks of administration and at 3 week of recovery.

Organ weights: On day after the final administration and on day 28 of recovery autopsies were performed and brain, pituitary gland, thyroid, submandibular gland, thymus, lungs, heart, liver, kidneys, spleen, adrenals, ovaries and uterus were weighed.

Histopathology: Various tissues were collected and preserved for histological examination. Livers and kidneys of 3 animals per group were fixed for electron microscopic examination.

The data were analyzed using Kruskal-Wallis ranked sum and Bartlett's tests. The standard of significance used was <5%.

Results:

General condition: No deaths occurred in any group. A dose and duration of treatment-dependent slight depilation of the lumbar region on the back was observed. This occurred in 1 or 2 animals in the 1 and 3 mg/kg dose groups in weeks 10 and 7, respectively and in 12-20 animals in the 30 mg/kg dose group in week 6. This was reversed during the recovery period and only one animal in 30 mg/kg dose group had during week 4 of recovery.

Sexual cycle: During day 77 to day 90 of the administration the mean number of periods of estrus in the 14 days were 2.8 in the control group and 2.2, 2.4, 2.1 and 0.1 in the 0.3, 1, 3, and 30 mg/kg groups, respectively. A pre-estrus period was observed for 2 – 5 days

in all animals in the 3 mg/kg group and a continuous estrus period was observed in all animals in the 30 mg/kg group. During weeks 3-4 of the recovery period the mean number of periods of estrus was 3.0 in the control group and 2.4 in the 30 mg/kg group.

Body weight and food consumption: There was suppression of body weight and food consumption during the first week in the 30 mg/kg group, but after that it was comparable to the controls.

Water consumption: In the 30 mg/kg group water consumption was significantly greater in weeks 6 and 12, while for other groups it was similar to control group. After the drug administration was stopped, the water consumption in the 30 mg/kg group was similar to that of the control group.

Serologic and biochemical tests: In the 30 mg/kg group, Hb, Hct., MCH, MCV was significantly decreased while platelet count and fibrinogen quantity were significantly increased. RBC fragility was decreased. Hb, Hct, MCH and MCV were also decreased in the 3 mg/kg group. No changes were seen in other groups.

Decreases in total cholesterol were observed in the 3 and 30 mg/kg groups. Significant increases in total protein, albumin, neutral lipids, total bilirubin, and alpha globulin fraction, decreases in the PL and glucose, and lowered GOT activity were observed in the 30 mg/kg group. No drug effects were seen in 1 mg and 0.3 mg/kg groups. All these changes showed recovery or tendency to recover during the recovery period.

Urinalysis: The urine volume increased in the high dose group. Also quantity of calcium excretion was increased in weeks 6 and 12 of the administration in the 30 mg/kg group (1.02 mg/day vs 0.59 mg/day in controls). Urine pH was lower in week 6 in the high dose group. These changes were not reported for the other groups. All these changes showed recovery during the recovery period.

Ophthalmologic and auditory tests: These were not affected by treatment.

Finding at autopsy: Although ovarian and follicular cystomas, uterine edema, protuberances in the diaphragm surface of the liver, and pale yellow color changes in the kidneys were observed sporadically in both the control and drug treated groups, the frequencies of appearance of these findings were not dose-related.

Organ weights: Relative liver weight was significantly higher in the 3 and 30 mg/kg groups, being 2.70, 2.70, 2.95, 3.07 and 4.11% for the control, 0.3, 1, 3, and 30 mg/kg groups, respectively. There was a significant decrease in the relative weights of thymus and ovaries and increases in the relative weights of the adrenals, kidneys, spleen, heart and lungs in the 30 mg/kg group. All changes showed recovery or tendency to recover after drug administration was stopped.

Histopathology:

Ovaries: Decreases in the number of corpora lutea were observed in all animals in the 30 mg/kg group. Decreases in follicles in the maturation process and increases in the closed follicles were observed in 3 and 1 animals, respectively in the 30 mg/kg group.

Vagina: Animals showing keratinized epithelial cells and estrus phenomena were seen in 5, 5, 5, 4 and 1 animal in the control, 0.3, 1, 3, and 30 mg/kg groups, respectively. Mucous degeneration of the epithelial cells was observed in 9 animals of 30 mg/kg group.

Uterus: Decreases in uterine glands were observed in 1 animal in the 3 mg/kg group and 10 animals in the 30 mg/kg group. Estrus phenomena in the endometrial epithelial cells were observed in 4, 4, 7, 9, and 7 animals in the control, 0.3, 1, 3, and 30 mg/kg groups, respectively. Mucous degeneration of the cervical epithelial cells was seen in 2 animals in the 3 mg/kg group and 5 animals in the 30 mg/kg group. Eight animals in the 30 mg/kg group showed vacuolization of the epithelial cells of the fallopian tubes.

Mammary glands: Four animals in the 30 mg/kg group showed accumulation of secretions in the ampullae's.

Adrenals: Decreased fat drops in the zona fasciculate were seen in 9 animals in the 30 mg/kg group and in 4 of these animals, decreases in fat drops were also seen in the zona glomerulosa.

Skin: No abnormal findings were observed in the skin at the depilated sites.

Note: All histological changes observed showed recovery or tendency to recover when drug administration was stopped.

Electron microscopy: No abnormal findings were reported any organ.

Conclusion: Significant treatment-related findings which could have clinical concern were increases in the platelet count and fibrinogen quantity, decreased erythrocyte osmotic fragility and increase in the neutral lipid value. Increased urine volume associated with increased calcium excretion may have bearing on bone density. Increased absolute and relative liver weight in the 30 mg/kg dose group, although not associated with increased transaminases and no histopathology findings needs to be watched in the longer term toxicity studies. Histological changes seen in the ovaries indicated suppression of ovulation. Sponsor stated that changes seen in the ovaries, uterus, vagina and mammary gland were similar to those reported with the administration of medroxyprogesterone acetate and gestrinone caproate.
Sponsor inferred from these results that the non-toxic dose in this study was 3 mg/kg/day. It seems that MTD was not defined.

Study title: 6 months oral toxicity study in rats with special attention to hepatotoxicity. Berlex Report No. 98015

This report is dated 9-5-1979 and was archived on 4-3-1998. It is not mentioned if the study was conducted in accordance with GLP regulations.

The study was conducted by (b) (4), Department of Pharmacology to obtain information about possible hepatotoxic properties of Dienogest.

Adult male and female Wistar rats were used in 4 treatment groups as stated below:

Controls	15 males/15 females
10 mg/kg Dienogest	15 males/15 females
10 mg/kg D-norgestrel	15 males/15 females
1 mg/kg Dienogest	10 males/10 females

Animals were housed in groups of 5 animals in plastic cages. The drugs were gavaged as suspension in 0.5% Tylose (vehicle) daily at a dose volume of 0.2 ml/100 g body weight for a period of 6 months.

Five animals/group and sex were bled and sacrificed after 6, 12 or 26 weeks of treatment. The parameter measured in serum included total protein, GPT, GOT, alkaline phosphatase, leucine aminopeptidase, BSP-elimination and bilirubin. Livers were weighed and triglycerides, cholesterol and glycogen content were determined. Livers were examined histologically.

Results:

No deaths were reported in any treatment group. Body weight and liver weight did not show any difference compared to the control group. Treatment had no effect on serum protein, and serum transaminases. Serum alkaline phosphatase was increased after 1 mg/kg of Dienogest in males in week 6. Also alkaline phosphatase and leucine aminopeptidase were increased in D–norgestrel treated females in week 6. There were no drug-related changes in BSP half-life determined within the first 8 minutes and BSP retention of 30 minutes after intravenous injection of 5 mg/kg BSP. Bilirubin levels were not affected by treatment. Liver contents of triglycerides, cholesterol and glycogen were not affected by treatment. There were no drug-related histological changes in the liver.

Based on no drug-related changes by treatment of rats with daily doses of 1 or 10 mg/kg Dienogest or 10 mg/kg D-norgestrel for 26 week on body weight, liver weight, total serum protein, serum transaminases, bromsulfalein half-life or retention, serum bilirubin, and on triglycerides and cholesterol contents of liver, sponsor concluded that oral administration of both steroids in doses up to 10 mg/kg/day for 26 weeks did not show any signs of hepatotoxicity.

Note: In the 91 day toxicity in rats the high dose used was 30 mg/kg, which was deemed not to be MTD or MFD. As such 10 mg/kg dose would not be expected to show hepatotoxicity.

Study title: 6 months toxicity study in the rat by oral administration. Berlex Report No. 98014

This study was conducted in compliance with the German testing guidelines available in 1977 by the (b) (4) . The study is dated 11-10-1978 and archived on 4-3-1998.

Groups of 16 Wistar rats/sex were gavaged daily with Dienogest suspended in 0.5% Tylose solution at dose levels of 0.1, 1.0 or 10.0 mg/kg. Another group of the same size received Tylose solution only and served as control group. Doses used represented 2.5, 25 and 250 times the human dose of 2 mg per woman (10 mg/kg dose is 40x the human dose of 2 mg on BSA basis). Four rats/s were killed after a recovery period of 2 months.

Observations:

Body weight was recorded weekly. Food consumption was recorded daily. Hematology and clinical chemistry parameters were determined in 8 animals/sex at week 1, 5, 14 and 24. Urine analysis was performed during week 26. At necropsy at day 191, all animals were examined macroscopically for any abnormalities and brain, thymus gland, lungs, heart, liver, spleen, kidney, adrenals, testes and ovaries were weighed. Thirty four organs were preserved for microscopic examination.

Results:

Mortality: Two females and 1 male in the 0.1 mg/kg dose group; 1 male and 1 female in the 1.0 mg/kg group and 2 males in the 10 mg/kg group died during the course of the study.

Body weight: body weight was not affected in the low and mid dose groups but was significantly decreased in males at the 10 mg/kg dose.

Hematology parameters: RBC count, Hb, Hct. and WBC count were not affected by treatment in any group.

Clinical chemistry: GPT, GOT, AP and blood glucose levels as will as the bromsulfalein (for liver function) and phenol red (for kidney function) retention in the serum were reported not affected by treatment and were within normal range.

Urinalysis: No abnormalities in protein, glucose or cells were reported in any treatment group.

Postmortem macroscopic findings: Smaller testes were reported in 11 dienogest treated animals.

Organ weight: Weight of testes was decreased at the mid and high doses. Weight of the ovaries, adrenals and spleen were higher in females at mid and high doses. With high dose in females, kidney weight was increased.

Autopsy was performed on 3 of the 7 reported dead during the course of the study. Two were considered due to congestive heart failure because of signs of haemostasis found in lungs, liver and kidneys. The third rat died of pneumonia.

Histopathology:

Liver: Hepatosis was found in 2 animals in the 10 mg/kg group. Deposition of fat, mostly in the form of central lobular fatty deposition was reported in 5 animals in the control group, 3 animals in the low dose, 4 animals in the mid dose and 8 animals in the high dose groups, respectively.

Thymus: The thymus showed significant involution at the higher doses, being 3, 1, 4 and 10 in control, low, mid and high dose groups, respectively.

Adrenals: The adrenals showed dose-dependent mobilization of lipids (3, 7 and 10 animals in the low, mid and high doses, respectively). The changes were more pronounced in the males, being 5 and 9 males in the mid and high doses, respectively).

Pituitary: There was an increase in the number of chromophobe cells (prolactin secreting cells) with higher doses, being 3 animals at 0.1 and 10 animals at 10 mg/kg doses, respectively.

Testes: The atrophy of the testes was dose-dependent associated with reduction of spermiogenesis. In females there was reduction of Graafian follicles in the ovaries.

Conclusion: Sponsor concluded that the oral administration of Dienogest for a period of 6 months only induced a slight lipid deposition in the liver of animals with a slight increase at higher doses. The effects on endocrine organs were suggested to be due to the anti-ovulatory effect of the compound.

Note: MTD or MFD was not defined.

Dienogest (M 18575): Toxicity to female rats by repeated oral administration for 52 weeks. Berlex Report No. 98007.

This study was conducted by (b) (4) in accordance with FDA's GLP regulations. The study was started on 7-8-1993 and terminated after completion of 52 weeks of treatment on 7-8-1994.

Study design: Sixty 6-week old Crl: CD(SD)BR female rats weighing 131-162 g were used in 4 treatment groups of 15 animals/g (3/cage) and gavaged daily at dose levels of 0

(control), 0.1, 1.0 and 10.0 mg/kg/day as suspension in 0.5% carboxymethyl cellulose at a dose volume of 5 ml/kg.

Observations and measurements:

Clinical signs and mortality: Individual animals were examined at least once daily

Body weight: Recorded at time of allocation of animals to treatment groups, on day of commencement of treatment and once weekly thereafter.

Food consumption: Was recorded daily. Efficacy of food utilization was calculated.

Ophthalmologic examination: Was conducted before treatment and then during weeks 26 and 52 of treatment.

Hematology and clinical chemistry: These parameters were checked during week 52. Under hematology, PCV, Hb, RBC,MCHC, MCV, MCH, total WBC, differential WBC counts and platelet count were determined.

Under clinical chemistry, total protein, albumin, urea nitrogen, creatinine, Na, K, Ca, P, Cl, total cholesterol, alkaline phosphatase, total bilirubin, GPT, GOT, gamma glutamyl transferase, LDH, total triglycerides, phospholipids and non-esterified fatty acids were determined.

Urinalysis: Comprised of determining urine volume, pH, specific gravity, protein, Na, K and Cl. Qualitative tests were conducted for glucose, ketones, bile pigments, urobilinogen and haem pigments. Microscopic examination of urine samples was also conducted.

Toxicokinetics: Blood samples were collected during week 26 for TK. The 15 animals per dose level were divided into 5 subgroups of 3 rats each for blood collection after dosing at 0.5, 1, 3, 6, and 24 hours.

Terminal sacrifice: On completion of 52 weeks of treatment, all animals were killed and examined visually and by palpation. Adrenals, brain, heart, kidneys, liver, lungs, ovaries, pituitary, submaxillary salivary gland, spleen, thymus, thyroid with parathyroid and uterus were weighed.

Histopathology: Routine tissues for histopathological examination were preserved. A 1 gram liver sample was collected from 5 rats from each group and processed for determination of total cholesterol, triglycerides and phospholipids.

Electron microscopy: One sample of liver and kidney from 3 rats in each group was processed and examined electron microscopically.

Statistical analysis: Bartlett, Kruskal-Wallis, Dunnett, Scheffe's tests and analysis of variance were used.

Results:

Mortality: There were no unscheduled deaths reported during the 52 week treatment period.

Clinical signs: Hair loss was observed in all groups. The incidence was similar in all groups including controls and increased with duration of treatment.

Body weight: There was no significant treatment effect. Expressed as % of the control, the values for the low, mid and high dose were 103, 102 and 94% during weeks 0 – 13 and 109, 112, and 97% during weeks 0 – 52.

Food consumption: Food consumption was not affected by treatment. Efficiency of food utilization was similar for control and all drug treated groups.

Hematology: The significant treatment-related changes were increased prothrombin time in the 10 mg/kg dose groups (15.6, 15.8, 16.0 and 17.6** seconds) associated with increased fibrinogen (157, 164, 175 and 186** mg/dl). Total WBC count was decreased in the 1.0 and 10 mg/kg dose groups (4.8, 4.1, 3.8* and 3.1** as $10^3/mm^3$) associated with significant decrease in the lymphocyte count in the 10 mg/kg dose group.
Note: * denotes $p<0.5$ and ** denotes $p<0.01$)

Biochemistry: Treatment-related significant changes were decreased albumin (3.7, 3.7, 3.7 and 3.5* g/dl) and GOT (63, 63, 51 and 42* mU/ml) in the 10 mg/kg dose group. Also total cholesterol and phospholipids were significant decreased and triglycerides increased in the 10 mg/kg dose group. NEFA were significant increased in all treated groups with values of 1.33, 2.02*, 1.81* and 2.96** meq/l.

Urinalysis: Urine volume was significantly decreased in the 10 mg/kg dose group in the 16 hour collection during week 52 of treatment and it was associated with significant decreased in Na, K and Cl expressed as total volume collected.

Note: In the 91 day toxicity study urine volume was reported to be increased with 10 mg/kg dose level.

Macroscopic pathology: Revealed a higher incidence of rats with corpora lutea in the group treated with 10.0 mg/kg/day compared to control rats.

Microscopic findings:

Liver: As shown in table 8 below a statistically significant increase was observed in the incidence of basophilic foci in rats receiving 10 mg/kg/day.

Table 8

Treatment-related findings	Control	0.1 mg/kg/day	1.0 mg/kg/day	10.0 mg/kg/day
Basophilic foci	3	2	7	12**
Periportl vacuolations	1	1	0	4
Periportal fat				
Total	11	9	7	13
Trace	11	6	5	6
Minimal	0	3	2	7**
Total number of animals examined	15	15	15	15

**= p< 0.01

Note: The increased incidence (12/15) of basophilic foci in the 10 mg/kg/day dose group exceeded the historical control range of 4/15, 7/19, 8/20, 7/20, 0/24 and 1/20 amongst SD female rats in 6 comparable 1 year studies at the (b) (4). Sponsor considered the increase as a treatment-related exacerbation of a spontaneous age-related finding and of no relevance as an indicator of tumerigenic potential of Dienogest.

Uterus and vagina: Thinning of the uterine wall associated with decreased uterine weight was seen in the 10 mg/kg dose group. Epithelial mucification of vagina was reported in all drug treated groups and severity was dose-related.

Ovaries: Corpora lutea were present in greater number of rats from all treatment groups when compared to controls. Also a low incidence of luteal cysts was seen in rats from all treatment groups but not in control animals. Because of low incidence of animals with corpora lutea in controls, these observations were considered not of toxicological importance.

Electron microscopy: In the liver of 2/3 rats receiving 10 mg/kg/day, lipid droplets were seen in more periportal hepatocytes than in controls. Livers of rats receiving 0.1 and 1.0 mg/kg/day were similar to the controls.

Note: Sponsor stated that at 10 mg/kg/day, there was evidence of a treatment-related effect on hepatic function. The changes seen in PT and fibrinogen were suggested to probably correlate with histopathological findings of slightly increased periportal fat deposition at 10 mg/kg/day, which in turn correlate with findings of increased triglycerides and NEFAs. It was stated that pattern of change is similar to that seen in humans with impaired liver function (Zilva and Pannall, 1979) and also in rats with experimental liver injury induced by number of agents (Plaa and Hewitt, 1989 and Zimmerman, 1978).

Toxicokinetics: The TK data is given under Berlex Report No. 98138 dated 6-30-1995. The Cmax, Tmax and AUC data for Dienogest for rats treated for 26 weeks in shown in a table below:

Table 9

Dose (mg/kg/day)	Cmax (ng/ml)	Tmax (minutes)	AUC 0-24 hr (ng.hr/ml)
0.1	14	30	18
1	124	30	22
10	2774	60	6307

Sponsor's conclusion: Sponsor concluded that 1.0 mg/kg/day could be considered as the non-toxic dose and suggested that present dosage could be considered for long term (carcinogenicity) repeat dose studies in the rat, since it is unlikely that the basophilic foci would develop into hepatocellular neoplasia.

P/T comments: The treatment-related hepatic changes seem to increase with the duration of Dienogest treatment. These will be checked in the 2-year carcinogenicity study and need to be monitored in the clinical trials. Seeing results of 91 day, 6-month and 12 month toxicity studies, in view of this reviewer MTD or MFD was not used in these studies.

Dienogest: 28 – 31 days toxicity in female Beagle dog by oral administration. Berlex Report No. 98029

This study was conducted in 1976 by (b) (4), certified in 1995 by the personnel who supervised the conduct of the study and was archived in 1998. The study was intended as a dose range-finding study for a subsequent chronic toxicity study in dogs of 6 month duration.

Eleven one year old female Beagle dogs weighing 9.5 – 13.5 kg were used in one control and 4 dienogest treatment groups. There were 3, 2, 1, 2 and 3 dogs in the control, 0.1, 0.5, 1.0 and 10.0 mg/kg/day dose groups, respectively. One dog from the control and 10 mg/kg groups was used for recovery and sacrificed on day 82. The compound was orally administered each day in gelatine capsules. Animals were sacrificed 24 hours after the last dose administration.

Observations and measurements:

Animals observed regularly for any adverse clinical findings and mortality. Hematology (erythrocytes, Hb, Hct, WBC, blood sedimentation rate, total blood clotting time, bleeding time, coagulation reaction, heat fibrin, thromboplastin time, recalcification time and partial thromboplastin time) and clinical chemistry (bilirubin, creatinine, urea, GOT, GPT, alkaline phosphatase, cholinesterase, bromsulfalein elimination, glucose tolerance test, total protein, triglycerides, b-lipoproteins, total cholesterol and phospholipids) were determined a week prior to treatment and then in week 1, 3, and 5 of treatment.

Urinalysis was conducted using test strips but time when conducted was not given.

Dissection and macroscopic examination was conducted immediately after animals were killed and organs (pituitary, brain, lungs, thymus, heart, liver, spleen, kidneys, adrenals,

ovaries, uterus and thyroid gland) were weighed. The organs subjected to histological examination were not specified.

Results:

Clinical findings: There were no treatment-related behavioral changes. During the recovery period, 14 days after the last dose administration, one dog in the 10 mg/kg dose showed hair loss, which returned to normal after 2 months.

Mortality: All animals survived to scheduled sacrifice.

Body weight: Body weight was not affected by treatment.

Hematology: None of the hematological parameters were affected by treatment except sedimentation velocity (mm), which was increased slightly in some dogs in the drug treated groups. This effect was not dose-related. The values were 7, 18 and 8 for controls, 27 and 15 for 0.1 mg/kg dose; 30 for 0.5 mg/kg, 76 and 65 for 1.0 mg/kg, and 11, 27 and 10 for the 10 mg/kg group, respectively.

Clinical chemistry: Phospholipids, total cholesterol and β-lipoproteins values were generally higher at the end of the study compared to values at the beginning of the study. Particularly higher values were reported for one dog in the 0.5 mg/kg dose group where PL values increased from 360 to 530, total cholesterol from 165 to 301 and β-lipoproteins from 1.1 to 5.0. These values were also increased in one dog in the 1 mg/kg dose (PL 530 to 670, TC 216 to 317 and β-LP 2.2 to 3.6 mg/dl) and one dog in the 10 mg/kg dose group (PL 390 to 670, TC 157 to 344 and β-LP 0.9 to 2.9 mg/dl).

Glucose tolerance test: During week 4 of treatment glucose tolerance test was performed on all dogs which received 1 g/kg of glucose intravenously. One dog in each of the treated groups showed slightly elevated glucose levels at 30 minutes but was returned to normal pre-dose values by 45 minutes.

Urinalysis: no treatment-related findings were reported.

Organ weights: Weight of the uterus was increased in one dog in the 1.0 mg/kg group and both dogs in the 10.0 mg/kg group.

Microscopic findings: The most prominent findings were focal hyperplasia of the acidophilic cells in the pituitaty after 10 mg/kg, hyperplasia of the mammary gland after 1 and 10 mg/kg, hyperplasia of the endometrium with secretion and glandular ectasis in 2 dogs after 0.1 mg/kg and in all dogs after 1 ad 10 mg/kg, missing corpora lutea in both dogs of 10 mg/kg and acanthose of the vagina. All these changes were attributed to progestational effect of Dienogest.

Conclusion: All treatment-related findings were seen in target organs for progestational agent. It was concluded that Dienogest doses up to 10 mg/kg/day for one month did not

induce any adverse effects in female dogs. It is not clear if maximum tolerated dose was used.

P/T comments: The treatment-related increased phospholipids and total cholesterol were also observed in the rat toxicity study with Dienogest. It was not indicated if liver was examined in the dogs. In the rat, treatment affected hepatic histopathology.

Study title: 91-day oral toxicity study of M18575 in female dogs. Berlex Report No. 98018.

This study was conducted by (b) (4) in 1989-1990. The report submitted is a translation of a foreign language report. It is stated that the operations of the trial were performed in conformity with GLP standards.

Experimental design: Nine month old female Beagle dogs weighing 7.7 – 10.9 kg were used in 4 treatment groups of 3 dogs each and administered Dienogest in 0.5% CMC at dose levels of 0.0 (control), 0.03, 0.3 and 3 mg/kg/day in gelatine capsules. The volumes administered were 0.3, 0.03 and 0.003 ml/kg. The drug lot number 020589 used in this study had purity 99.81%. One animal was added to control and high dose group for 28 day recovery period. The highest dose was based on results of a 6-month oral toxicity trial in dogs, where 1 mg/kg was reported to result in transitory anemia, increases in total blood cholesterol and neutral lipids, and hyperplasia of the clitoris.

Note: Based on sponsor criteria used, the 3 mg/kg/day may not be the MTD.

Observations and measurements:

The general condition of the animals was observed 3 times a day during the administration period and twice a day during the recovery period. Body weight was recorded once a week and food consumption was measured daily. Mammary glands were examined by palpation once a week. Body weights were measured daily for 7 days after the beginning of the recovery period.

Hematology and clinical chemistry parameters: These were measured before drug administration and then during week 4, 8 and 12 of treatment and then in week 4 of withdrawal. The hematological parameters included RBC count, Hb, Hct., MCV, MCH, MCHC, platelet count, reticulocyte count, WBC differential count. Also RBC osmotic fragility, prothrombin time, activated partial thromboplastin time and fibrinogen was determined.

The biochemical tests included total protein, albumin, total cholesterol, phospholipids, neutral lipids, total bilirubin, GOT, GPT, urea nitrogen, creatinine and inorganic phosphate. In addition Na, K, and Cl were measured.

Urinalysis: Urine samples were obtained before drug administration, in treatment weeks 4, 8 and 12 and in week 4 of recovery period. The determination included pH, protein,

glucose, ketone bodies, bilirubin, occult blood and urobilinogen and microscopic observation of sedimentation. 24 hour urine volume and specific gravity, Na, K, and Cl were measured.

Electrocardiograms: EKG was taken before drug administration, in week 12 of the administration and week 4 of recovery period in suspended vertical position. Pulse rate, mean electrical axis, PR, QRS, and QTc intervals as well as the R wave potential of lead II were measured.

Ophthalmoscopic examination was conducted before drug administration and then during week 12 of drug administration and week 4 of recovery.

Autopsies and organ weights: The animals were sacrificed under anesthesia on the day after the final administration day and on day 28 of recovery period. Macroscopic examination of organ was conducted and brain, pituitary gland, thyroid with parathyroid, submandibular gland, thymus, lungs, heart, liver, kidneys, spleen, adrenals, bladder, ovaries and uterus were weighed.

Histopathology: All organs weighed and other routinely required for chronic toxicity studies were processed for histopathological evaluation.

Results:

Clinical observations: No deaths occurred in any treatment group. Hypertrophy of the nipples was observed in weeks 1 – 3 of drug administration in all animals in the groups with doses of 0.03 mg/kg or more. These changes continued until weeks 3 – 6 and then tended to contract gradually after that. Two control dogs and 2 in the 3 mg/kg groups showed changes which appear to accompany estrus i.e., hypertrophy of the nipples, swelling and hemorrhaging of the external genitalia.

Body weight: There was a small increase in body weight in drug treated groups compared to controls. Weight increase for the control and 3 treated groups was 0.6, 0.9, 1.4, and 1.1 kg, respectively.

Food consumption: A slight decrease in food consumption was reported from day 14 of the recovery period but was recovered by week 4 of the recovery period.

Hematology: RBC count, Hb and Hct were decreased in dogs given doses of 0.3 and 3.0 mg/kg in week 8 of administration. Reticulocytes (%) was increased in the high dose group with values being 3.0, 3.3, 2.7 and 7.9, respectively before and then 4, 8 and 12 weeks of treatment. Also fibrinogen value of 151 mg/dl before treatment, increased to 160, 209 and 195 on weeks 4, 8, and 12, respectively. Coagulation time was slightly decreased in all drug treated groups at weeks 4, 8 and 12

Clinical chemistry: Drug effect on total cholesterol, phospholipids, triglycerides and lipoprotein fraction is shown in table 10 below. Values are mean of 4 animals in control and high dose and 3 animals in low and mid dose:

Table 10

group	Total cholesterol (mg/dl)				Phospholipids (mg/dl)				Triglycerides (mg/dl)			
week	B	4	8	12	B	4	8	12	B	4	8	12
1	181	163	174	177	334	317	327	320	36	26	36	34
2	152	161	193	225	297	311	346	382	36	24	34	31
3	176	157	202	218	324	295	358	355	33	30	44	46
4	172	166	237	244	321	324	409	395	31	29	35	48

group	HDL (%)				LDL (%)				VLDL (%)			
week	B	4	8	12	B	4	8	12	B	4	8	12
1	68.7	71.4	69.3	66.7	15.6	12.1	15.0	17.3	16.8	16.5	15.7	16.0
2	65.5	62.3	67.0	64.2	14.2	16.4	17.5	19.0	20.4	21.4	15.5	16.9
3	72.0	71.4	67.7	68.6	14.8	14.6	17.7	19.0	13.2	14.0	14.6	12.4
4	66.6	67.6	62.9	64.6	12.1	15.2	20.2	20.8	21.3	17.3	17.0	14.7

Thus there was tendency for total cholesterol, phospholipids and LDL fraction of lipoproteins to increase and VLDL fraction to decrease. These changes showed recovery on cessation of treatment.

Urinalysis: No changes related to drug treatment were observed.

Electrocardiograms: No effects of the drug administration on pulse rate or electrocardiograms were observed. Electrocardiograms were not included in the submission.

Ophthalmological examination revealed no drug-related findings.

Organ weight: No treatment-related effects were reported.

Histopathology:

Uterus: Hyperplasia of the endometrium was observed in 3 animals of the high dose group.

Mammary glands: Hyperplasia of the acini was observed in animals administered doses of 0.03 mg/kg and higher with dose dependency.

Liver: Focal necrosis was reported in one animal of the high dose group.

Spleen: A slight deposit of hemosiderin was observed in one high dose animal.

Note: All histological changes tended to recover on cessation of drug treatment.

Electron microscopy: No effect of drug administration was reported for the liver and kidney samples examined.

Conclusion: Sponsor suggested 0.3 mg/kg as the non-toxic dose considering that the hyperplasia of the mammary glands is species specific change in dogs.

P/T comments: The results suggest that Dienogest has possible adverse effects on lipid metabolism. The high dose of 3.0 mg/kg/day was not the maximum tolerated dose. Toxicokinetics was not conducted and as such the multiples of human exposure with human therapeutic dose (HTD) in this study is not known. Glucose tolerance test was not conducted.

Study title: Dienogest: 6 months toxicity study in beagle dogs by oral administration (in coated tablets)

This study was conducted in 1979 by the (b) (4) It was stated that the study was conducted in compliance with guidelines and regulations existing and valid for the territory of East Germany in 1979. It is not mentioned if the study results presented is translation of a foreign language.

The experimental design of the study is given in table below:

Group	Substance	Daily dose mg/kg b.w.	No. of dogs	Total dose in mg per animal	
1	Placebo	-	6F/5M	-	
2	Norgestrel	1.0	4F/4M	2390 (female)	2790 (male)
3	Dienogest	0.01	4F/4M	21 (female)	27 (male)
4	Dienogest	0.1	4F/4M	230 (female)	268 (male)
5	Dienogest	1.0	4F/4M	2430 (female)	2980 (male)

Dogs were dosed orally daily, 7 days per week. At the end of dosing, 3 male and 3 female of each group and 4 male and 4 female animals of control group were killed. One male and 1 female of each group were observed during the 2 month recovery period.

Justification of doses used:

0.01 mg/kg b.w.: as approximately the maximum daily dose intended in man
0.1 mg/kg b.w.: as a logarithmic intermediate dose between 0.01 and 1.0 mg/kg
1.0 mg/kg b.w.: as a dose expected to produce toxicologically relevant effects

Clinical observations: All animals were checked for general health daily, feed consumption was checked daily but not measured, body weight was recorded every 2 weeks and palpation of the mammary glands was conducted weekly.

Vaginal smear: Vaginal smears were prepared 5 weeks before the administration of the compounds and weekly during the 6 month of the experiment. Smears were stained with hematoxylin-eosin and 50 cells were microscopically counted and differentiated in to

parabasal, intermediate, superficial and plaque cells and ratio of plaque + superficial cells/ intermediate + parabasal cells were calculated.

Ophthalmological examinations: Before the beginning and at the end of the study.

Urinalysis: Urine was collected on week 15 from females and on week 23 from males.

Hematology and clinical chemistry: Blood was collected from all animals for these examinations.

Post-mortem examination: Seventeen organs were weighed and 49 organs were processed for histopathological examination.

Results:

Body weight: Contro as well as drug treated male and female groups gained weight' % increase for the control female group was 14% while for the norgestrel group was 28%. Respective increases for the low, mid and high dose dienogest were 9, 19 and 36%.

Food consumption: Weight increase in drug treated females was not accompanied by increased food intake.

General appearance: All dogs appeared healthy.

Mortality: There were no treatment-related deaths.

Palpation of mammary glands: Dienogest showed a dose-dependent enlargement of mammary glands. With norgestrel these changes were not distinct as compared to the controls.

Cytology of the vaginal smears: The increase in the ratio of plaque and superficial cells divided by intermediate plus basal cell (index Q) suggests a estrogen dominated situation. The index Q was decreased in the norgestrel and in the 0.1 mg/kg dienogest group. Norgestrel inhibited completely the appearance of heat during the experiment which was also diminished in 0.1 and 1 mg/kg dienogest groups. The effects on vaginal cytogram and inhibition of heat were considered as antigonadotropic effect.

Urinalysis: No treatment-related changes were described.

Hematology: In the high dose dienogest females, a statistically significant decrease in Hb and RBC count were observed from week 14. Also Hct was decreased significantly after 0.1 and 1 mg/kg dienogest in females in the week 14 and persisted until the end of the experiment.

WBC and differential blood count: No significant treatment-related changes were reported.

Other hematological findings: The number of thrombocytes was normal in all animals throughout the experiment. There were no changes in eh recalcification time and the thromboelastogram, the partial thromboplastin time, in plasmatic coagulation factors 2, 5 and 10. Thrombin time was shortened in all groups towards the end of the experiment and suggested that it could be due to an increase in fibrin, which was observed in all groups.

Clinical chemistry: Serum protein was normal in the males but in females there was a significant increase in the groups treated with 0.1 and 1 mg/kg dienogest groups. From the 3rd month onwards, a significant increase in serum cholesterol wit 1 mg/kg dienogest was observed in both sexes. Norgestrel however, produced a decrease in males. The increase in cholesterol after dienogest was accompanied by an increase of the b-lipopretein fraction containing large amount of cholesterol. The triglyceride concentration was unaffected in males of all groups. In females there was a significant increase in the groups treated with 0.1 and 1 mg/kg dienogest. No other changes were observed.

Endocrinological parameters: Values of greater than 30 pg/ml was observed at the end of experiment in 0/4 animals with Norgestrel, 0/4 in the 0.01 mg/kg dienogest, ¼ in the 0.1 mg/kg and ¾ in the 1 mg/kg dienogest. These results were interpreted as an incomplete blockage of estrous cycle by treatment with dienogest in contrast to norgestrel.
Progesterone values greater than 20 ng/ml which are typical for the luteal phase were not observed in any of the females, although in some animals of the control and dienogest groups values at the end were higher than at the beginning. Cortisone concentrations were reported slightly higher with high doses of dienogest in both sexes at the end of the experiment.

Bromsulphalein excretion: The normal BSP half-life time for Beagle dogs was stated to be < 5 minutes and the BSP retention after 30 minutes is < 5 -10%. In the present study, T1/2 or retention did not show drug related changes in males. In females the group mean for retention was 6.3 for controls, 9.3 for 0.1 mg/kg dose group and 11.0 for 1 mg/kg dose groups. These differences were not statistically significant.

Intravenous glucose tolerance: After iv glucose injection liberation of insulin increased in female animals in the norgestrel and high dose dienogest groups.

Organ weights: There was a dose-dependent decrease in the prostate weight in the dienogest group. The weight of the testes, epididymis and ovaries showed tendency to decrease. The relative weight of the uterus showed a decrease only in the norgestrel group and increase in females treated with 0.1 and 1 mg/kg dienogest, which was attributed to pyometra and mucometra.

Morphological findings: All treated animals showed hyperplasia and in high dose groups single cell hypertrophy of the prolactin-forming cells in the pituitary without enlargement of the organ. The prostate was dose-dependently atrophic. In the treated groups, there was follicle persistence and lack of corpora lutea with corresponding decrease of the

absolute and relative weights of the ovaries. In the uterus, dienogest prose a dose-dependent proliferation of the endometrium up to a glandular cystic hyperplasia with muco or pyometra. In the males, high dose of dienogest and norgestrel induced a labular alveolar hyperplasia and secretion of the mammary glands. Periarteriolitis of a branch of coronary artery and panarteritis of two branches of the testicular artery in the epididymis was reported in one high dose dog. Similar changes on the arteries were reported in one low dose and one mid dose female dogs in the spinal ganglia. Since these changes were observed in different organs and were not dose-dependent, their relationship to treatment was considered questionable.

Note: Most of the above changes are considered species specific for progestin effect in dogs and have been reported previously.

Recovery period: In order to study the mating behavior, males and females were of each group were housed together. The sexual behavior of treated animals housed together was normal. One female in the low dose dienogest group got pregnant after 1 week and brought forth a litter of 5 normal pups.

Post-mortem findings: The organ weights of the ovaries, uterus, prostate and testes were still below the organ weights of the control animals.

Atrophy of the zona reticularis of the adrenals of the females was completely reversible, prostate atrophy, hyperplasia of the mammary glands in males, persistence of follicles and lack of corpora lutea in the ovaries of females only partly reversed. Hyperplasia of prolactin-forming cells of the pituitary and arteriolitis were unchanged. In the uterus, hyperplasia of the endometrium had disappeared and the mucosa corresponded to an anestrus.

Study title: Toxicity study of 17α-cyanomethyl-17β-hydroxy-estra-4,9(10)-dien-3-one (Dienogest, M18575). 91-day oral toxicity study of M18575 in female monkeys. Berlex Report No. 98019.

This study was conducted by (b) (4). in 1993-. The report submitted is a translation of a foreign language report. It is not reported if the study was conducted in accordance with GLP regulations.

Experimental design: Fourteen 3 to 4 years old Chinese female breeding rhesus monkeys weighing 3.49 – 4.90 kg were used in this study (3 monkeys /group except 5 in the high dose group). They were given 120 gm of solid food and 50 g of apples daily. Drinking water was provided ad lib.

Dose selection and method of administration: Doses were selected based on the results of the 91-day toxicity in female dogs, where 0.03 mg/kg/day produced swelling of nipples (hyperplasia of the mammary glands) and a tendency toward elevation of the fibrinogen quantity. In addition, at 3 mg/kg/day, slight anemia and hyperplasia of the endometrium were observed. Furthermore, in a continuous oral administration, using one female

rhesus, an elevated fibrinogen and hyperplasia of the cervical glands were observed beginning at a dose of 10 mg/kg/day. Therefore, the highest dose was selected as 10 mg/kg/day and 1 and 0.1 mg/kg/day were intermediate and low dose levels. Control group was administered the solvent only. Monkeys were dosed using stomach tube with a dose volume of 0.5 ml/kg. After 91 day oral administration, a recovery period was provided for 28 days.

Observations: Animals were observed daily, 3 times during the administration period and twice a day during the recuperation period. Body weight was recorded weekly and food consumption daily.

Serologic tests: Blood samples were drawn from the cephalic vein for RBC count, Hb, Hct, MCV, MCH, MCHC, WBC count, platelet cont, reticulocyte count and WBC differential count. Prothrombin time, activated partial thromboplastin time and fibrinogen quantity as well as plasminogen and α_2- plasmin inhibitor were measured. In addition using whole blood, thromboelastograms were made and the reaction time (r), coagulation time (k), and maximum amplitude were obtained and fibrinolytic rate was calculated.

Blood biochemical tests: Plasma albumin, total and free cholesterol, free fatty acids, glucose, GOT, GPT, λ-GT, ALP, LDH, total bilirubin, urea nitrogen, creatinine, and inorganic phosphorus were measured. In addition, Na, K and Cl were measured. Serum was used to measure protein fraction and A/G ratio was calculated.

Urinalysis: Tests included pH, protein, glucose, ketone bodies, bilirubin, occult blood and urobilinogen were measured and microscopic examination of sediment was conducted at 4, 8 and 12 weeks of administration and at 4 weeks of recuperation. 24-hour urine quantity, specific gravity, Na, K, and Cl were measured.

Electrocardiograms: ECGs were recorded at 11 weeks of administration and at 4 weeks of recuperation under ketamine hydrochloride anesthesia, in a seated position with standard limb lead and the pulse rate, mean electrical axis, lead II PR, QRS,, and QT_c intervals and R wave potential were measured.

Ophthalmologic examination: was conducted before administration, at 11 weeks of administration, and week 4 of recuperation.

Autopsies and organ weights: Autopsies were conducted after sacrifice by bleeding from the carotid artery under phenobarbital anesthesia on the day after the final administration and 28^{th} day of recuperation. Brain, pituitary gland, thyroid (including parathyroid), submandibular gland, thymus, lungs, heart, liver, kidneys, pancreas, spleen, adrenals, bladder, ovaries and uterus were removed and weighed, and expressed on body weight basis.

Histopathologic examination: All required organs were examined.

Results:

Mortality: There were no deaths in any group.

Clinical signs: Menstruation was stopped 2 days after the drug administration in the high dose group. In the control group, menstruation was observed in in one female. Menstruation was restored by stopping the drug administration, on days 4 – 5 of recuperation in both females.

Body weight and food consumption: Body weight changes were similar in the treated and control group. Final body weight was greater in all animals compared to their initial weight. Food consumption was not affected by treatment.

Serologic tests: Fibrinogen was elevated in one animal of 1 mg/kg dose group and 4animals in the 10 mg/kg dose group. The highest increase was at 4 weeks of administration. On the other hand plasminogen was increase in one animal in the 1 mg/kg dose group and 4 animals in the 10 mg/kg dose group. Again as for the fibrinogen, highest increase was observed at week 4 of administration. After drug withdrawal, values were similar to those before drug administration.

Blood biochemical tests: Lowered ALP activities were observed in all the animals of the 10 mg/kg group beginning in week 4 of administration and stayed elevated through the 12 weeks of administration. GPT was increased in one animal in this group only at week 4.

Urinalysis: It was not affected by treatment.

Electrocardiograms: Treatment had no effect on EKG.

Ophthalmologic examination: No abnormalities were observed in the anterior portion or fundus findings in any animal, including those in the control group.

Autopsies: No abnormalities were observed on examination of various organs.

Organ weights: The absolute and relative weight of the uteri was increased in all animals of the 10 mg/kg group and in one control animal. Also absolute and relative weight of left ovary was increased in these animals. The weights of the uteri after drug withdrawal were within the background data (absolute weights: 1.42 – 7.77 g and relative weights: 0.30 – 1.67 g/kg).

Histopathological examination:

Uterus: Hyperplasia and hypertrophy of the stromal cells of the endometrium were observed in 1 animal in the 0.1 mg/kg group, 2 animals in the 1 mg/kg group and all animals in the 10 mg/kg group. Edema of the functional layer was observed in 2 animals of the 0.1 mg/kg group, 2 animals of the 1 mg/kg group and all animals of the 10 mg/kg

group. Hypertrophy of the helicine arterial walls was observed in 1 animal in the 0.1 mg/kg group and 2 animals in the 10 mg/kg group. All these changes recovered when drug treatment was stopped.

Vagina: Slight to moderate atrophy of the epithelium was seen in 2 animals of the 0.1 mg/kg group and in all animals in the 1 and 10 mg/kg group. Slight to moderate edema was observed in one animal each in the 1 and 10 mg/kg groups. All changes were recovered after drug withdrawal.

Summary: Thus the principal changes due to administration of the drug were observed to be effects on blood coagulation and fibrinolytic system, effect on the uterus and the vagina, and a decrease in the ALP activity.

Effect on blood coagulation and the fibrinolytic system consisted of elevated fibrinogen quantity and elevated plasminogen activity. The observed effect on the reproductive system was an increase in the weight of the uterus in the 10 mg/kg/day group. The blood biochemical change involved a reduced ALP activity at the high dose. In a follow up study, sponsor reported that all of the isozyme fractions of the liver and bone type were reduced. It was stated that similar reduction of ALP activity was reported with norethindrone treatment. Progesterone and anti-estrogen activities reduced liver and bone type ALP activity. One animal of the high dose exhibited a tendency towards elevated GPT activity suggesting a transitory liver function disorder and was considered not serious enough to be accompanied by anomalies in the liver tissue findings.

No effects of the administration of dienogest were observed on the body weights, food consumption, urinalysis findings, electrocardiograms, or ophthalmologic tests.

Study title: Toxicity study of 17α-cyanomethyl-17β-hydroxy-estra-4,9(10)-dien-3-one (Dienogest, M18575); 52-week toxicity study of M18575 in female monkeys. Berlex report No. 98010.

This study was conducted in 1994-1995 by the (b) (4) The present report is a translation of a foreign language. No statement is made if the study was conducted in accordance with GLP regulations.

Animals used and rearing conditions: Twenty female rhesus monkeys, 3 – 5 years of age and weighing 2.87 – 4.76 kg were used in this study. They were kept individually in stainless steel cages and were given 120 g /day of solid feed and 50 g/day of apples as well as free access to tap water.

Basis of dose selection and mode of administration: In the 91-day oral toxicity study in rhesus monkeys described above, administration of 0.1 mg/kg/day or more caused discontinuation of menstruation, caused the endometrium appear as in pregnancy and caused the supravaginal epidermis appear as in menopause. Administration of 1 mg/kg/day produced increased in fibrinogen that did not affect blood coagulation or fibrinolysis capacity, as well as elevation of plasminogen activity. Administration f10

mg/kg/day caused decrease in blood ALP activity and an increase in uterine weight. Based on these findings, non-toxic dose was considered as 1 mg/kg/day. Accordingly, in this study, doses were set at 0.1, 0.3, 1.0 and 10.0 mg/kg/day. A control group was administered vehicle only at a dose volume of 0.5 ml/kg. Drug was administered daily for 52 week using an oral catheter. There were 4 females/group.

Observations:

General condition, body weight, and feed consumption: Animals were observed 3 times daily throughout the administration period. Body weight was recorded weekly and food consumption was recorded daily during the treatment.

Hematological tests: Blood samples were collected from the cephalic vein prior to drug administration and then after 3, 6, 9, and 12 months and prepared with added EDTA-2K and 3.8% sodium citrate respectively. The blood with EDTA-2K was used to measure RBC count, Hb, Hct, MCV, MCH, MCHC, leukocytes, platelets, reticulocytes, and differential leukocyte count. The blood with 3.8% sodium citrate was centrifuged and plasma was used to measure prothrombin time, activated partial thromboplastin time, fibrinogen, plasminogen and α_2- plasmin inhibitor activity. Whole blood was used to prepare thromboelastograms, reaction time, coagulation time (k), and maximum amplitude (ma) were determined, and the fibrinolytic rat [FLR=(1-ma$'$/ma) x 100] was calculated based on ma and maximum amplitude 1 hour after this maximum amplitude was reached (ma$'$).

Biochemical tests: Plasma was used to measure total protein, albumin, total and free cholesterol, phospholipids, neutral fats, FFA, glucose, GOT/GPT, λ-GT, ALP, LDH, total bilirubin, urea nitrogen, creatinine, Ca, inorganic phosphorus, Na and K. Serum was used for serum protein fractions and A/G ratios.

Urinalysis: Before drug administration and after 3, 6, 9 and 12 month for protein, glucose, ketone bodies, bilirubin, occult blood, and urobilinogen.

Electrocardiography: Electrocardiograms were recorded in sitting position before administration and after 6 and 12 months and electrical axis, PR, QRS and QTc interval and R potential were measured. Heart rate was calculated based on RR interval.

Ophthalmological examination: was conducted before and 6 and 12 months after drug administration.

Necropsy and organ weights: On the day after the final administration, the animals were sacrificed, gross examination was conducted and 15 organs were weighed and relative weights were calculated.

Histopathological examination: All routinely organs were processed for histopathological examination.

Results:

Mortality: No deaths were reported in either controls or drug treated groups.

General observations: In the drug administered groups, no menstruation was seen throughout the study period. In the control group, on the other hand largely periodic menstruation was observed in all cases throughout the administration period.

Body weight and food consumption: All animals gained in body weight during the study. There was no difference in feed consumption compared to the control group.

Hematological tests: One animal in the o.3 mg/kg group, two in the 1 mg/kg group and all in the 10 mg/kg group showed increases or increasing tendency in fibrinogen from month 3 of administration on. Two cases in the 0.3 mg/kg group, 2 cases in I mg/kg groups and all animals in the 10 mg/kg group showed increases or increasing tendency in plasminogen activity from month 3 of administration on. No other changes were reported.

Biochemical blood tests: LDH was decreased in all 10 mg/kg dose group from month 3 of administration onward. One high dose animal showed elevated GPT in month 6 and another showed increased PL from month 6 onward. Occasionally elevated LDH was reported in 0.2 and 3 mg/kg dose groups but did not show any dose-response relationship. One control group animal had elevated λ-GT in month 12 of administration.

Urinalysis: No treatment–related changes were reported.

Electrocardiogram findings: No treatment-related changes were observed.

Ophthalmoscopic examination: No abnormal treatment-related findings were revealed.

Autopsy findings: Thickening of the uterine intima was reported in one animal in the 0.3 mg/kg dose group, one in the 1 mg/kg group, and all of the animals in the 10 mg/kg dose group. Also hypertrophy of the uterus was observed in all of the 10 mg/kg group animals. No other gross findings were observed.

Organ weights: Increases in absolute and relative uterine weight was observed in 3 animals in the 10 mg/kg group.

Histopathological examination:

Uterus: In the intima, interstitial cell hyperplasia and hypertrophy was seen in 2 animals in the 0.1 mg/kg group and all animals in the 0.3 mg/kg or more. Thinning of the basal layer was seen in one animal in the 0.1 mg/kg group, 3 in the0.3 mg/kg group and in all animals in groups administered 1 mg/kg and above. Edema of the functional layer was observed in all animals in the 0.1 mg/kg group, 3 animals in the 0.3 mg/kg group, and all animals in the 1 mg/kg or above. The severity of increase of interstitial cell hyperplasia

and hypertrophy showed a dose-dependent tendency. In addition, thickening of the walls of the helicine arteries was seen in one animal in the 0.1 mg/kg group, 3 animals in the 0.3 mg/kg group, one animal in the 1 mg/kg group and all animals in the 10 mg/kg group. In one 10 mg/kg dose group, focal hemorrhagic necrosis of the intima and decreased uterine glands were observed.

Vagina: Epithelial atrophy was observed in all animals in the groups administered 0.1 mg/kg or more. The severity of this increased in a dose-dependent manner.

Ovaries: Increases in follicular atresia were seen in all animals in groups administered 0.1 mg/kg or more.

Conclusion: Drug-induced changes included effects on the blood coagulation and fibrinolysis systems, effects on the reproductive organs, and an action of decreasing LDH.

As for blood coagulation and fibrinolysis system, administration of 0.3 mg/kg or more caused increases in fibrinogen and elevation of plasminogen activity. In the present study as was seen in the 91-day toxicity study, no changes were seen in the extrinsic (prothrombin time) or intrinsic (activated partial thromboplastin time) coagulation times or thromboelastograms. Also as in the 91-day toxicity study, it was found that because of increased reactivity of coagulation inhibiting factors in response to promotion of coagulation in the form of elevated coagulation factor activity, the coagulation balance was maintained. The changes observed in the reproductive organs were attributed to hormonal action of the study drug. No drug effect was seen in the mammary glands.
Administration of dienogest had no effect on body weight, feed consumption, urinalysis, electrocardiogram findings, or on ophthalmological tests.
Based on results of this study, the nontoxic dosage of the study drug was assessed to be 1 mg/kg/day.

2.6.6.4 Genetic toxicology

Study title: **Salmonella typhimurium reverse mutation assay with dienogest**

Key findings : Dienogest was not mutagenic

Study no.: (b) (4) Project 493601

Volume #, and page #: Volume 26 of IND (b) (4) page 8 08663
Conducting laboratory and location: (b) (4)

Date of study initiation: 11-29-1994
GLP compliance: yes
QA reports: yes (*) no ( )
Drug, lot #, and % purity: 305041, purity 101.2%

Methods

Strains/species/cell line: S. typhimurium strains TA 1535, TA 1537, TA 98, TA 100, and TA 102.

Doses used in definitive study: 33.3, 100.0, 333.3, 1000.0, 2500.0, and 5000.0 ug/plate

Basis of dose selection: Toxicity was evaluated based on:

1. reduction in the number of spontaneous reversions
2. a clearing of the bacterial background lawn or
3. by degree of survival of treated cultures

Range finding studies: 5.0 – 5000 ug/plate
Test agent stability: performed by sponsor, not included in the study report.
Metabolic activation system: Aroclor 1254 induced rat liver S9
Vehicle/Negative controls: DMSO

Positive controls: 10 ug/plate Sodium azide for TA 1535 and TA 100
10 ug/plate4-nitro-o-phenylene-diamine for TA 1537 and TA 98
5.0 ug/plate Methyl methane sulfonate for TA 102
2.5 ug/plate 2-aminoanthracene for TA 1535, TA 1537, TA 98, TA 100 and TA102

Exposure conditions:

Incubation and sampling times: Assay was performed in 2 independent experiments, first according to plate incorporation and second with pre-incubation, both with and without liver microsomal activation. In the pre-incubation assay the test solution, S9 mix and bacterial suspension were mixed and incubated at 37 C for 60 minutes. After pre-incubation 2.0 ml overlay agar was added to each tube. The mixture was poured to minimal agar plates. After solidification the plates were incubated up side down for 48 hours at 37 C. Each concentration including the controls was tested in triplicate. Colonies were counted using the Autocount (Artek Systems Corporation, Biosys GmbH, F.R.G.)

Criteria for positive results:

Test article was considered mutagenic if in strain TA 100 and TA 102 the number of revertants was at least twice as high and in strains TA 1535, TA 1537, and TA 98 it was at least 3 times higher compared to the spontaneous reversion rate. Also a dose-dependent and reproducible increase in the number of revertants was regarded as an indication of possible existing mutagenic potential regardless whether the highest dose induced the above enhancement factors or not.

Results:

No toxic effects, evidenced by a reduction in the number of revertants occurred with of without metabolic activation. There was normal background lawn up to 5000 ug/plate with or without metabolic activation in all strains used.

No substantial increases in revertant colony numbers of any of the 5 tester strains were observed following treatment with dienogest at any concentration level, either in the presence of absence of metabolic activation. There was also n o increased tendency of higher mutation rates with increasing concentrations.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Appropriate strains of S. typhimurium were tested. In addition, all strains in DMSO performed within specified reversion frequency rates and all positive controls performed as expected. The test was considered valid.

Study outcome: There was no increase in reversion frequency when tested using pre-incubation and plate incorporation treatments, with and without metabolic activation.

Study title: **Reverse mutation test of M 18575 using bacteria**

Key findings: No evidence of mutagenicity with M 18575

Study no.: 98089

Volume #, and page #: Volume 26 of IND (b) (4) page 8 08699.
Conducting laboratory and location: This study was conducted by (b) (4) This is a repeat of the above study using S. typhimurium strains TA 1535, TA 1537, TA 1538, TA 98, TA 100 and E.Coli $WP2_{uvr}A$. Summary results provided are a translation of a foreign language report.

Results: The results showed no increase in the number of reverse mutation colonies, whether or not a metabolic activation during the treatment with dienogest up to maximum dose of 5000 ug/plate. Therefore, dienogest was considered to have no mutagenic potential.

Study title: **Chromosomal aberration study of M 18575 using cultured mammalian cells**

Note: This a translation of a foreign language report

Key findings: No evidence of clastogenicity was reported

Study No. 0209 Berlex Report No. 98091
Volume # and page #: Volume 26 of IND (b) (4), page # 8 08723

Conducting laboratory and location: [redacted] (b) (4)

Date of study initiation: September 1989
GLP compliance: yes
QA reports: yes (*) no ( )
Drug, lot #, and % purity: 020589, purity 99.81%

Methods

Strains/species/cell line: Chinese hamster lung fibroblasts (CHL) cells

Dose selection criteria:

Basis of dose selection: Measurement of 50% cell proliferation inhibition concentration (ID_{50} value)
Range finding studies: 25 – 400 ug/ml
Test agent stability: not given
Metabolic activation system: Aroclor 1254 induced rat liver S9
Negative control: DMSO
Positive controls: Mitomycin C (0.2 ug/ml) – without S9. benzo[a]pyrene (20 ug/ml) – with S9
Doses used in definitive study: 13.75, 27.5, 55 and 110 ug/ml without S9 and 27.5, 55, 110 and 220 ug/ml with S9

Incubation and sampling times:

Case in which metabolic activation was not used: 2 x 10^4 CHL cells cultured with 5 ml culture solution for 3 days at 37C. 25 ul culture solution removed and replaced with solution of dienogest. After culturing for 22 and 46 hours, 50 ul of a 20ug/ml colsemid solution added. After culturing for 2 hours, culture solution in plate transferred to test tube and 1 ml of 0.01 M PBPS containing 400 U/ml trypsin and 5 mM EDTA was added stripping the cells. Chromosomal samples were prepared by Giemsa-staining. DMSO as negative control and mitomycin as positive controls were used. Two plates were used for each dose.
Case in which metabolic activation was used: After 3 day culturing 525 ul of culture solution was removed and replaced by 500 ul of S9 mix and 25 ul of dienogest. After culturing for 6 hours, the culture solution was replaced and after culturing for 16 hours longer, 50 ul of a 20 ug/ml colsemid solution was added. After 2 hours cells were stripped and chromosomal samples were prepared DMSO as negative control and benzo[a]pyrene as positive control were used. Two plates were used for each dose.

Analysis:

No of replicates: 100 metaphase were scored for each of the duplicate cultures

Counting method: microscopic evaluation. As the structural chromosomal aberrations, gaps, breaks, exchanges, ring formations, and fragmentations were counted, and the number of polyploids were counted as a numerical aberration.

Criteria for positive results: none described

Results

No significant increases in the number of cells with chromosomal aberrations were observed both in the absence and presence of metabolic activation even when the M18575 concentration was twice the 50% cell proliferation inhibitory concentration. However, when treatment was performed mitomycin C or benzo[a]pyrene, the number of chromosomal aberrations was significantly increased.

Study validity (comment on replicates, counting method, criteria for positive results, etc.): Study as conducted is considered valid.

Study outcome: Dienogest did not exhibit clastogenic potential.

Study title: Mutation assay at the TK locus in L5178Y mouse lymphoma cells using a microtiter cloning technique (trifluorothymidine resistance) with dienogest

Key findings: Dienogest induced no mutagenic activity at the TK locus in L5178Y mouse lymphoma cell culture.

Study No: IPL-R 941210/ Dienogest/ (b) (4) Berlex Report No 98090
Volume #, and page #: volume 26 of IND (b) (4) page 8 08761
Conducting laboratory and location: (b) (4)
Date of study initiation: 1994
GLP compliance: yes
QA reports: yes
Drug Lot #, radiolabeled, and % purity: 100691
Formulation/vehicle: DMSO

Methods:

Strain/species/cell line: L5178Y mouse lymphoma cells obtained from (b) (4)

Dose selection criteria: solubility and cytotoxicity
Range finding studies: The heterozygous L5178Y $TK^{+/-}$ cells (5 x 10^6 cells) were exposed for 3 hours to the test article, in the presence or the absence of (S9) metabolic activation. The cells were then replated so as to determine their survival rate (counting with hemocytometer) and allow expression of the mutation. At the end of expression time (3 x 10^5 cells 2 days after treatment), cells were adjusted to 3 x 10^5 /ml and exposed to a

selective agent for $TK^{-/-}$ mutant cells: trifluorothymidine (TFT) and incubated for 10- 12 days for evaluation of mutant frequency. TFT is non toxic to the $TK^{-/-}$ mutant cells, which survive and multiply in its presence. In TK mutants resistant to TFT, a bimodal distribution of large and small colonies was observed.

From cytotoxicity assay, four doses ranging from the toxic dose (i.e., inferior to 20% relative total growth) or the dose provoking a slight precipitation after added to the culture medium, to the highest non-toxic dose (90 --100% relative growth) were selected.

Test agent stability : not described

Metabolic activation system: Aroclor 1254-induced rat liver S9

Negative control/Vehicle: DMSO

Positive controls: Methyl methanesulfonate 10 ug/ml without S9 mix and cyclophosphamide 2 ug/ml with S9 mix.

Doses used in definite study:

Without S9 mix: 148.1, 222.2. 333.3, 500 ug/ml in both assays 1 and 2
With S9 mix: 148.1, 222.2, 333.3, 500 ug/ml in both assays 1 and 2.

Study design: This was modification of the soft agar cloning method which has reported to be comparable to agar cloning method, not only for mutant frequency but also for the proportion of large and small colonies.

No. of replicates: duplicate cultures

Acceptance criteria for the results: The plating efficiency of negative control is higher than 50% and mutation frequency of the negative control is within a range of historical data of the laboratory.
The mutation frequency of the positive control is significantly increased compared to the solvent and values are close to those of historical positive controls.

Criteria for scoring mutation plates: Each well of the mutation plates (in selective medium containing TFT) is scored as containing either a small colony, a large colony or no colony

Criteria for a mutagenic activity: A multiplication by 2 of the number of spontaneous mutants has been provoked by at least one dose, a dose/effect relationship and results have to be reproducible in an independent study.

Results: In both with and without metabolic activation in 2 independent assays, mouse lymphoma cell culture treated at the highest dose compatible to its cytotoxicity i.e., 500 ug/ml dienogest induced no significant increase in the mean number of mutants. Also

dienogest produced no significant variation in the proportion of large and small colonies relative to the solvent control.

Conclusion: Dienogest induced no mutagenic activity at the TK locus in L5178Y mouse lymphoma cell culture.

Study title: In vivo micronucleus assay in bone marrow cells of the mouse with dienogest

Key findings: Dienogest is considered to be non-mutagenic in this assay.

Study No.: (b) (4) Project 493602. Berlex report No. 98100
Volume ##, and page #: Volume 27 of IND (b) (4), page 8 08942
Conducting laboratory and location: (b) (4)
Date of study initiation: 11-23-94
GLP compliance: yes
QA reports: yes
Drug lot #, radiolabeled, and purity %: 305041, not radiolabeled, purity 101.2%
Formulation/vehicle: 1% CMC
Methods:
Strain/species/cell line: NMRI mice

Dose selection criteria: Use of MTD that causes toxic reactions within 48 hours or the highest dose that can be formulated and administered or 2000 mg/kg as the upper limit.

Range finding studies: A small group of animals were gavaged dienogest and examined for acute toxic symptoms (i.e., death, reduced spontaneous activity, eyelid closure, apathy) at intervals of 1 h, 6 h, 24 h and 48 h after administration. Based on this pre-experiment 2000 mg/kg dose was selected as highest dose for the definitive study.

Test agent stability: not given

Metabolic activation system: none

Negative/vehicle controls: CMC 1%

Positive controls: Cyclophosphamide 30 mg/kg b.w.

Exposure conditions:

Doses used in definitive study: 0, 200, 600 and 2000 mg/kg b.w.

Study design: Dienogest was formulated in 1% carboxymethylcellulose and administered orally at a dose volume of 10 ml/kg body weight. Twenty four (200, 600 and 2000 mg/kg groups) and 48 hours (2000 mg/kg group) after single administration of dienogest, animals were sacrificed and bone marrow cells were collected for micronuclei analysis.

Analysis:

No of replicates: Ten animals (5/sex) per test group were evaluated for occurrence of micronuclei.

Counting method: Evaluation of slides was performed using NIKON microscopes. 1000 polychromatic erythrocytes (PCE) were analyzed per animal for micronuclei. To describe a cytotoxic effect the ratio between polychromatic and normochromatic erythrocytes (NCEs) was determined in the same sample and expressed in normochromatic erythrocytes per 1000 PCE.

Acceptance criteria: The study is considered valid if the negative controls are in the range of historical control data (0.04 – 0.22% PCEs with micronuclei) and positive controls show statistically significant increased values.

Results:

The highest dose of 2000 mg/kg animals exhibited slight toxic reactions.
After treatment with diengest the number of NCEs was not increased as compared to the corresponding negative controls indicating dienogest had not cytotoxic effect in the bone marrow.
In comparison to the corresponding negative controls there was no significant increase in the frequency of the detected micronuclei at any preparation interval after administration of dienogest with any dose level used.
Positive control cyclophosphamide administration per os showed statistically significant increase of induced micronucleus frequency.

Study validity: Study is considered valid as controls performed as expected.

Conclusion: Under the experimental condition used, dienogest did not induce micronuclei as determined by the micronucleus assay with bone marrow cells of the mouse.
Therefore, dienogest is considered to be non-mutagenic in this assay.

2.6.6.5 Carcinogenicity

Study title: **Dienogest: Oncogenicity study by oral (gavage) administration to CD-1 mice for 104 weeks.**

Key study findings: The only significant treatment-related non-neoplastic changes were adrenal cortex spindle cell hyperplasia, mineralization of the iris and mesenteric lymph node histiocytosis. In females there was ovarian atrophy, decreased incidence of uterine adenomyosis and increased vaginal epithelial basal cell proliferation. Neoplastic changes in males consisted of an increased although not statistically significant, incidence of

malignant lymphoma. In females the incidence of uterine stromal polyps was significantly increased.

Adequacy of the carcinogenicity study and appropriateness of the test model: Test model is adequate and appropriate

Evaluation of tumor findings:

Study no. (b) (4) Report No. JPH01294
Volume #, and page #: volume 24 of IND 64,809 Page 8-3309
Conducting laboratory and location: (b) (4)
Date of study initiation: August 10, 1995
GLP compliance: yes
QA report: yes (*) no ( )
Drug, lot #, and % purity: Lot # 505034 (weeks 1 – 81), purity 99.91%. Lot #412014 (weeks 82 –termination), purity 99.80%.
CAC concurrence: No

Method;
Doses: 5, 15 and 50 mg/kg/day for males and 10, 30 and 100 mg/kg/day for females.

Basis of dose selection (MTD, MFD, AUC etc.): Dose selection was based on UC values determined in 2 preliminary studies. Under study JPH00195, three groups of 55 male and 55 female mice received Dienogest at dosage of 2, 10 or 50 mg/kg/day for 5 days. After the fifth administration, blood was collected and PK parameters were calculated. For animals receiving 50 mg/kg/day the AUC of Dienogest was 8820 ng.h/ml for males and 3307 ng.h/ml for females, which was about 19 times (males) and 7 times (females) of human AUC of 455 ng.h/ml after a dose of 2 mg. In a second study reported under JPH04394, 12 male and 12 female mice received 5, 25, and 125 mg/kg/day for 13 weeks. Liver weights were higher in animals receiving 125 mg/kg/day. Seminal vesicle weight in males given 125 mg/kg/day and uterus and cervix weight in female mice given 25 and 125 mg/kg/day were low. Ovary weight was lower in females given 125 mg/kg/day. Microscopic examination revealed periaciner hepatocyte hypertrophy of the liver of treated males, particularly those receiving 125 mg/kg/day.

Comments: The systemic exposure attained in the first preliminary study was particularly low in females. Also in the second study no organ toxicity or systemic adverse findings were observed. Based on these findings it would seem that the doses used were probably not MTD or MFD for a carcinogenicity study, particularly for female mice. The high dose used in this study based on AUC which gave a low multiple of the human therapeutic dose and induced no adverse effects is probably not adequate to establish that dienogest has no carcinogenic potential. Based on these findings, it is not clear why high dose of 125 mg/kg/day was not selected.

Species/strain: mice/CD-1
Number/sex/group (main study): As shown in table 1 below:

Table 1

Group	Treatment	Dosage	Number of animals			
			Maim study		Satellite study	
			Male	Female	Male	Female
1	Vehicle control	0	52	52	6	6
2	Untreated control	Not dosed	52	52	0	0
3	Dienogest	5	52	0	6	0
4	Dienogest	10	0	52	0	6
5	Dienogest	15	52	0	6	0
6	Dienogest	30	0	52	0	6
7	Dienogest	50	52	0	6	0
8	Dienogest	100	0	52	0	6

Route, formulation, volume: oral, graded concentrations in 0.5% aqueous Tylose MH300/ 5 ml/kg body weight/day.

Frequency of dosing: once daily

Satellite groups used for toxicokinetics or special groups: yes as shown in table 1 above.

Age: 29 -36 days old

Animal housing: 4 of one sex/cage (main study) and 3 of one sex/cage (satellite study).

Restriction paradigm for dietary restriction studies: free access

Drug stability/homogeneity: formulation was stable for 7 days and was homogenous

Dual controls employed: yes

Interim sacrifices: none

Deviations from original study protocol: Study was planned for 78 weeks and subsequently extended to 104 weeks on the basis of good survival of the animals.

Observation times

Mortality: Twice daily

Clinical signs: Inspected twice daily. Detailed weekly examination

Body weights: Recorded during the acclimation period, on day treatment commenced, weekly for the first 14 weeks and then once every 4 weeks and at the end of week 104 at necropsy
Food consumption: Recorded weekly for first 14 weeks and then every 4 weeks

Hematology: During week 102 of treatment before terminal necropsy, blood samples were collected from all surviving animals via the retro-orbital sinus. All samples were analyzed for the following:
Packed cell volume (PCV)
Hemoglobin concentration (HB)
Erythrocyte count (RBC)
Total and differential leukocyte count (WBC)
Platelet count (PLAT)
Mean cell hemoglobin concentration (MCHC)
Mean cell hemoglobin (MCH)
Mean cell volume (MCV)

Organ weights: The following organs from each animal were weighed:
Brain, epididymides, heart, kidneys, liver, lungs, ovaries, prostate, salivary glands (submandibular), seminal vesicles, spleen, testes, thymus, and uterus with cervix

Tissues preserved for histopathology: All tissues as listed in histopathology inventory table.

Histopathology: Peer review: yes ( ), no ( ) not indicated

Toxicokinetics: During week 53 of treatment, blood samples were collected 4 hours after dosing from the surviving satellite animals. The number of animals in male groups 1, 3, 5 and 7 were 4, 6, 6, and 5, respectively. For female groups 1, 4, 6 and 8 the numbers were 4, 6, 6, and 6, respectively.

Results

Mortality: Statistical analysis of the data indicated that mortality was not affected by treatment. The distribution of death by group was as given in table 2 below:

Table 2

Group/sex	1M	2M	3M	5M	7M	1F	2F	4F	6F	8F
Dosage (mg/kg/day)	0	0	5	15	50	0	0	10	30	100
Number of decedants	30	38	35	34	27	22	22	26	27	19

Thus a total of 164 males and 116 females died or were killed during the treatment period.

Clinical signs: There were no adverse clinical signs related to treatment. As shown in table 3 below, the incidence, multiplicity and mean time of onset of palpable swelling were unaffected by treatment. Numbers include swelling which regressed or were not positively identified at post mortem examination.

Table 3

Group/sex	Multiplicity[1]					Number of animals with swellings	Total number of swellings	Mean time of onset[2]
	0	1	2	3	4 or more			
1M	26	14	10	2	0	26	40	55
2M	26	13	7	4	2	26	47	52
3M	24	18	7	3	0	28	41	54
5M	26	15	7	3	1	26	42	56
7M	32	16	1	2	1	20	30	63
1F	47	5	0	0	0	5	5	77
2F	45	6	0	0	1	7	10	96
4F	40	10	1	1	0	12	15	85
6F	44	6	1	0	1	8	15	72
8F	44	8	0	0	0	8	8	78

1 Expressed as number of animals bearing the indicated number of swellings
2 In weeks to onset of first swelling including those found at necropsy examination

Body weights: As shown in table 4 below, males receiving 15 or 50 mg/kg/day gained less body weight during the first 78 weeks of treatment when compared to their controls. The overall gains during the 104 week dosing period were similar to controls. Treatment had no effect on body weight gains in females.

Table 4

Weeks of treatment	Group and sex				
	1M	2M	3M	5M	7M
0-50	22.6	23.9	21.0[c]	20.9c	19.8[ac]
As % of group 1 (vehicle control)	95	106	93 88	92 87	88 83
As % of group 2 (untreated cont)					
0-78	21.6	23.7	21.2	20.0[d]	19.8[c]
As % of group 1		110	98	93	92
As % of group 2	91		89	84	84
0-104	20.4	19.5	20.0	18.0	18.9
As % of group 1		96	98	88	93
As % of group 2	105		103	92	97
	1F	2F	4F	6F	8F
0-50	15.1	17.7	15.4	15.8	16.5
As % of group 1		117	102	105	109
As % of group 2	85		87	89	93
0-78	17.5	20.1	16.4[d]	16.5[d]	16.9[d]
As % of group 1		115	94	94	97
As % of group 2	87		82	82	84
0-104	16.3	18.0	15.9	16.8	17.4
As % of group 1		110	98	103	107
As % of group 2	91		88	93	97

Significant when compared with group 1: a=p<0.05
Significant when compared with group 2: d=p<0.05; c=p<0.01

Food consumption: Food consumption was not significantly affected by treatment. The total consumption for the treatment period 1-102 weeks for the male groups 1, 2, 3, 5 and 7 was 1518, 1523, 1470, 1469 and 1416 g, respectively. These values ranged from 93 to 97 % compared with respective controls. The total consumption for the female groups 1, 2, 4, 6 and 8 for the period 1 -102 weeks was 1279, 1361, 1334, 1288 and 1308 g, respectively. These values represented 95 to 104% compared with their respective controls. Food conversion efficacy was reported similar in all groups compared to control determined during the first 14 weeks of treatment.

Hematology: Hematological parameters were determined on blood samples collected during week 102 of treatment. In comparison to controls, low packed cell volumes, Hb concentrations and RBC count and high mean cell Hb and mean cell volumes were reported in male55s receiving 50 mg/kg/day. Mean cell Hb and mean cell volumes were also high for males receiving 15 mg/kg/day and for females receiving 100 mg/kg/day.

Organ weights: Analysis of organ weights of animals killed after 104 weeks of treatment revealed in comparison with the controls demonstrated effects on prostate, seminal vesicles and epididymides in males and on uterus and liver in females. Changes expressed as mean organ weight relative to body weight in males and females are shown in table 5 below:

Table 5

Sex	**Male**				
Group	**1**	**2**	**3**	**5**	**7**
Number	**22**	**14**	**17**	**18**	**25**
Prostate	**0.0811**	**0.0783**	**0.0686**	**0.0685**	**0.0626[a]**
Seminal vesicles	**04.0358**	**3.7442**	**3 1269**	**2.4567[a]**	**1.7369[b]**
Epididymides	**0.2727**	**0.2837**	**0.2447[b]**	**0.2676[b]**	**0.2409[b]**

Sex	**Female**				
Group	**1**	**2**	**4**	**6**	**8**
Number	**30**	**30**	**26**	**25**	**33**
Uterus-cervix	**2.4320**	**1.6550**	**1.5232**	**1.4229**	**1.1470[a]**
Liver	**5.062**	**5.014**	**5.423**	**5.566[a]**	**5.699[b]**

[a]= $p < 0.05$ [b]= $p < 0.01$

Comments: The changes observed in the male and female reproductive system may be attributed to pharmacological activity of Dienogest. The dose-related increase in liver weight only in females and not in males may suggest treatment-related adverse effect; it does not establish that high dose was MTD or MFD.

Gross pathology: No consistent dose-related macroscopic findings were observed. There was decreased incidence of cystic ovaries, pallor of the skin and incidence of cystic uterus in treated females. Incidence of spleen enlargement though not statistically significant, was higher in high dose males. The incidence of these findings for mice dying

or killed during the treatment period is given in table 6 below. Similar trend was seen when data for all animals was reviewed.

Table 6

Organ and incidence	1F	2F	4F	6F	8F
Ovaries-cystic	18/22	15/22	17/26	16/27	8/19[a]
Skin- pale	10/22	13/22	13/26	17/27	3//19[a]
Uterus- cystic	12/22	10/22	9/26	16/27	5/19
L.N. Pancreatic- enlarged	3/22	1/22	2/26	9/27[d]	2/19
	1M	2M	3M	5M	7M
Spleen – enlargement	1/30	1/38	1/35	1/34	4/27
L.N. pancreatic- enlarged	2/30	2/38	1/35	1/34	5/27

[a]= significant when compared to group 1 p<0.05 = significant when compared with group 2 p<0.05

Comments: The macroscopic findings suggested no significant adverse treatment-related effect with the doses used.

Histopathology:

Non-neoplastic: Significant treatment-related findings were observed in adrenals in males and in reproductive organs of females. As shown in table 7 below, an increased incidence and severity of spindle cell hyperplasia and a reduced incidence of focal cortical hypertrophy were recorded in males given Dienogest at a dose of 50 mg/kg/day.

Table 7

Group/sex	**1M**	**2M**	**3M**	**5M**	**7M**
Dosage (mg/kg/day	**0**	**0**	**5**	**15**	**50**
Adrenal cortex number examined	**50**	**52**	**52**	**52**	**51**
Spindle cell hyperplasia					
Minimal	**4**	**3**	**6**	**8**	**4**
Slight	**7**	**6**	**7**	**10**	**14**
Moderate	**2**	**2**	**2**	**0**	**4**
Marked	**0**	**0**	**0**	**0**	**2**
Total	**13**	**11**	**15**	**18**	**24[ac]**
Focal cortical hypertrophy	**9**	**6**	**7**	**8**	**1[b]**

Significant when compared to group 1: a= p<0.05 b= p<0.01
Significant when compared with group 2: c= p<0.01

Changes observed in the ovaries, uterus and vagina are shown in table 8 below:

Table 8

Group/sex	**1F**	**2F**	**3F**	**5F**	**7F**
Dosage (mg/kg/day	**0**	**0**	**10**	**30**	**100**
Ovaries number examined	**52**	**52**	**52**	**52**	**52**
Ovarian atrophy					
Minimal					
Slight	**1**	**0**	**2**	**6**	**2**
Moderate	**5**	**4**	**8**	**4**	**5**
Marked	**3**	**6**	**3**	**7**	**9**
Total	**9**	**1**	**13**	**17**	**16**
Cysts	**40**	**43**	**37**	**35**	**29[ac]**

Uterus number examined	**52**	**52**	**52**	**51**	**51**
Cystic endometrial hyperplasia					
Slight	**2**	**10**	**7**	**6**	**5**
Moderate	**10**	**5**	**3**	**9**	**15**
Marked	**7**	**1**	**1**	**0**	**2**
severe	**1**	**1**	**0**	**0**	**0**
Total	**20**	**17**	**11**	**15**	**22**
Adenomyosis					
Minimal	**0**	**4**	**2**	**1**	**1**
Slight	**7**	**9**	**8**	**8**	**2**
Moderate	**4**	**8**	**4**	**1**	**1**
Marked	**3**	**3**	**0**	**1**	**0**
total	**14**	**24**	**14**	**11[d]**	**4[af]**
Polyluminal dilatation	**6**	**11**	**18[b]**	**14[a]**	**12**
Distended endometrial glands	**14**	**12**	**8**	**9**	**5[a]**
Vagina-Epithelial basal cell proliferation	**7**	**6**	**3**	**4**	**16[d]**

Significant when compared to group 1: [a]=p<0.05, [b]= p<0.01
Significant when compared to group 2: [d=] p<0.05; [c]= p<0.01; [f]=p<0.01

Note: All changes most likely due to hormonal effects of dienogest and not expression of toxicity.

Other significant treatment-related findings were mineralization of the iris in the eyes of males given 50 mg/kg/day. However, this was also observed in control group 2. The incidence of sinus histiocytosis of the mesenteric lymph nodes was significantly higher in the 50 mg/kg/day dose group as shown in table 9 below.

Table 9

Group/sex	**1M**	**2M**	**3M**	**5M**	**7M**
Dosage (mg/kg/day	**0**	**0**	**5**	**15**	**50**
Mineralization of the iris (number examined)	**1 (52)**	**5 (38)**	**1 (35)**	**1 (34)**	**8[a] (52)**
Mesenteric lymph node histiocytosis	**2 (52)**	**2 (35)**	**8 (41)**	**0 (33)**	**10[a] (51)**

Significant when compared with group 1: [a]= p<0.05

Neoplastic: Treatment-related findings were observed in the uterus as shown in table 10 below.

Table 10

Group/sex	**1F**	**2F**	**3F**	**5F**	**7F**
Dosage (mg/kg/day	**0**	**0**	**10**	**30**	**100**
Uterine Stromal polyp (number examined)	**5 (52)**	**9 (52)**	**15[a] (52)**	**12 (51)**	**24[cc]**

Significant when compared with group 1: [a]= p<0.05; [c]= p<0.01
Significant when compared with group 2: [c]= p<0.01

As shown in table 11 below there was a treatment-related increase in the incidence of malignant lymphoma in males given 50 mg/kg/day dose. Also pituitary tumors were seen only in treated males.

Table 11

Group/sex	1M	2M	3M	5M	7M
Dosage (mg/kg/day	0	0	5	15	50
Malignant lymphoma (number examined)	6 (52)	4 (38)	3 (36)	2 (34)	12 (52)
Pituitary adenoma	0 (50)	0 (45)	0 (51)	2 (52)	1 (50)
Pituitary adenoma, pars intermedia	0 (50)	0 (45)	0 (51)	1 (52)	0 (50)
Testes interstitial cell tumor	1 (52)	1 (52)	0 (52)	3 (51)	4 (52)

Note: sponsor stated that although the incidence of malignant lymphoma in males (23%) did not reach statistical significance, it was above the background level normally seen in male mice of this age at this laboratory. The background incidence was reported to be 0 - 14.3% in males and 5 – 26.9% in females.
Also although incidence of pituitary tumors did not reach statistical significance, it was above the background level normally seen in males of this age. The background incidence was 0% for males and 0 -5 % for females.

The background incidence rate of interstitial cell tumor was stated to be 1.7 to 7.1%. There was also no treatment-relationship in the incidence of interstitial cell hyperplasia.

Toxicokinetics: PK parameters of dienogest after 104 weeks of treatment is shown in 12 table below:

Table 12

	3M	4F	5M	6F	7M	8F
Dose (mg/kg/day0	5	10	15	30	50	100
Cmax (ng/ml)	399.91	672.47	780.31	3143.47	7387.67	7332.40
Tmax (hour)	0.5	0 5	0.5	0.5	0.5	0.5
T1/2	1.57	2.20	1.41	2.51	1.53	2.21
AUC 0-24 h (ng.h/ml)	394.43	972.38	1120.81	3192.70	7529.38	9539.15
CL/F (L/hour/kg)	12.68	10 28	13.38	9.40	6.64	10.48

Considering AUC of 682 ng.h/ml in human with a therapeutic dose, the systemic exposure in high dose males and females was 11 and 14 times higher compared to human systemic exposure.

Study title: **Carcinogenicity study of Dienogest (M18575) in rats.**

Key study findings: Treatment-related significant findings were observed in the ovary, uterus and vagina.

Adequacy of the carcinogenicity study and appropriateness of the test model:

Evaluation of tumor findings: The decreased treatment-related incidence of corpora lutea in ovaries, increased incidence of uterine hyperplasia, vacuolization of the endometrial cells and vacuolization of the glandular epithelial cells as well as increased mucification

of the vaginal epithelial cells with decreased incidence of atrophy of the vaginal epithelial cell could be attributed to pharmacological effect of drug product and not an indication of drug toxicity.

Study no. 0343
Volume #, and page #: volume 25 of IND 64,809 Page 8-3746
Conducting laboratory and location: (b) (4)

Date of study initiation: 6-25-1998
GLP compliance: No statement regarding Compliance with Good Laboratory Practice is made. The report is an english translation of a foreign language and the translator has signed that the report is acceptable as to content. Translator's notes described the report as follows:

"The test was written in a very cumbersome style with excessive and unnecessary use of bracketed information making some sentences impossible to read. I have in places therefore incorporated the bracketed information in a more natural style into sentences or used square brackets to try and make things clearer where there were brackets within brackets".

It was also stated by the Regulatory Affairs that the report was adequate for regulatory purposes.

Note: It is not clear to this reviewer if this study was conducted in compliance with GLP regulations**.**

QA report: yes ( ) no ( ) With regards to Quality Assurance, statement is made by the QAU that this study was conducted in accordance with Ordinance on Standards for the Conduct of Non-Clinical Studies on the Safety of Medicinal Products (26.3.1997, MHW ordinance 21), and that method of the study is accurately described and the raw data moreover accurately reflected in the final report.

Drug, lot #, and % purity: 090691, purity 99.5% - 99.7%

CAC concurrence: No

Methods

Doses: 0, 1, 3 and 10 mg/kg/day. Each concentration of prepared test sample was administered daily, seven days a week for 104 weeks.

Basis of dose selection (MTD, MFD, AUC etc.): The dose levels were set with reference to the findings of a 12 month toxicity study with M18575 in rats where treatment at 10 mg/kg/day led to target organ toxicity and significant variations in clinical pharmacological parameters which included increased foci of basophilic tigroid altered hepatocytes in the liver, longer prothrombin time, increased plasma fibrinogen and

decreased total cholesterol and phospholipids. The medium dose was set at 3 mg/kg/day as a level anticipated to provide AUC (660 – 1900 ng.hr/ml) compared to AUC_{0-12} of 341 ng.hr/ml x 2 at an estimated clinical dose in man (1 mg/body b.i.d.) and the low dose of 1 mg/kg/day corresponding to one tenth of the high dose. Control groups 1 and 2 were given only the 0.5% CMC vehicle.

Note: Sponsor has cited at other places AUC values of 455 and 682 ng.hr/ml with a therapeutic dose of 2 mg in humans.

Species/strain: Rat/Crj:CD(SD)IGS

Number/sex/group (main study): Only female rats were used as shown in table 1 below:

Table 1

Test substance	Dose level (mg/kg/day)	Conc. of drug suspension (mg/ml)	Dosing volume (ml/kg)	Number of females Main study	PK
Vehicle control 1	0	0	2	65	5
Vehicle control 2	0	0	2	65	0
Dienogest	1	0.5	2	65	5
Dienogest	3	1.5	2	65	5
Dienogest	10	5	2	65	5

Route, formulation, volume: oral, graded concentrations in 0.5% aqueous Tylose MH300/ 2 ml/kg body weight/day

Frequency of dosing: once daily

Satellite groups used for toxicokinetics or special groups: yes as shown in table above

Age: 3 weeks old Animal housing: housed individually

Restriction paradigm for dietary restriction studies: free access

Drug stability/homogeneity: formulation stable for 7 days/homogenous

Dual controls employed: yes

Interim sacrifices: none

Deviations from original study protocol: no significant deviations

Observation times

Mortality: Checked twice daily

Clinical signs: Clinical signs were monitored for general condition 3 times daily and were palpated for nodules or masses once a week from week 27 of treatment.

Body weights: Weighed on the first day of treatment, once a week in weeks 1 – 13 of treatment, in week 16 and thereafter every 4 weeks.

Food consumption: Measured once a week in weeks 1 – 13 of treatment, in week 16 and thereafter every 4 weeks.

Hematology: RBC and WBC counts were determined from the terminal sacrifice in the main study. Blood smears for morphological examination of the hemocytes were prepared but not examined since no tumors of the hematopoietic system were found histopathologically.

Femoral marrow was collected from moribund and terminal sacrifice animals and bone marrow slides were prepared. However, the bone marrow cells were not examined as no tumors of the hematopoietic system were found histopathologically

Necropsy and organ weights: Following blood sample collection, moribund sacrifice and terminal sacrifice animals were exsanguinated and checked visually for the presence of any gross or visceral abnormalities. The following organs from each animal were weighed:
Brain, pituitary, thyroid with parathyroid, submaxillary gland including sublingual gland, thymus, heart, lung including bronchus, liver, kidney, spleen, adrenal, ovary and uterus.

Tissues preserved for histopathology: All organs and tissues from all animals in the main study as listed in the histopathology inventory table were process for histopathological examination.

Histopathology: Peer review: yes ( ), no ( ) not indicated

Toxicokinetics: Blood was collected from the caudal vein 0.25, 0.5, 1, 2, 4, and 24 hours after the dose on the first day of treatment, then one hour after the dose in weeks 17 and 34 of treatment and just before the dose and 0.25, 0.5, 1, 2, 4, and 24 hours after the dose in week 52. The plasma concentrations of M18575 were determined by HPLC using (b) (4) as the internal standard substance.

Statistical analysis: Comparisons of body weight, food consumption, organ weights at the scheduled time of sacrifice, relative organ weights and hematology parameters were made between vehicle control 1 and each M18575 group and vehicle control 2 and each drug treated group. Bartlett, Dunnett and Fisher exact tests were used with significance level set at less than 0.5%.

Results

Mortality: Animal mortality and survival rates are shown in table 2 below:

Table 2

	Control 1	Control 2	M18575 (mg/kg/day)		
			1	3	10
Number of animals Total provided	65	65	65	65	65
Moribund sacrifice	33	36	38	36	38
Mid-term death	10	4	9	10	6
Terminal sacrifice	22	25	18	19	21
Mean survival time (weeks)	91+/-14[a]	89+/- 17	87 +/-17	89 +/-16	90 +/- 16
Final survival rate (%)	33.8	38.5	27.7	29.2	32.3

[a]= Mean +/- SD

Statistical analysis of the data indicated that mortality was not affected by treatment.

Clinical signs: The clinical symptoms observed in dead, moribund sacrificed and terminal sacrificed were described mainly as those associated with aging or symptoms seen when moribund. Clinical observations in moribund and dead animals in all groups including the vehicle control group were alopecia, red secretions around the eye lids, soiling around the nostrils and lower abdomen, reduced fecal volume, pallor of the ears and conjunctiva, reduced spontaneous activity, reduced body temperature, abdominal respiration, emaciation, lying prone, paralysis of the limbs, tachypnea, dyspnea, and lacrimation. Symptoms described at terminal sacrifice in all groups including the vehicle controls included red secretions around the eyelids and alopecia. A few animals showed soiling around the nostrils, pallor of the ears and conjunctiva, genital bleeding, reduced fecal volume, lacrimation, scratched foot pads, external injury and hematuria. No correlation with dose level was reported for the frequency of any clinical symptom.

Food consumption: Food consumption (g/rat/day) at various intervals during the course of 104 weeks was not affected by treatment as is shown in table 3 below:

Table 3

Week	Control 1	Control 2	M18575 (mg/kg/day)		
			1	3	10
1	17.6	18.0	17.7	17.9	17.6
12	18.8	19.4	19.3	19.3	19.3
24	18.2	18.7	18.4	18.8	19.8
36	19.1	19.6	19.0	19.3	19.6
48	19.5	20.3	19.9	20.3	20.1
60	20.3	21.0	20.5	20.5	19.9
72	21.4	21.9	21.2	21.1	21.0
84	22.3	21.9	22.4	21.1	21.1
96	22.2	23.2	22.4	21.5	21.1
104	21.7	23.3	22.4	19.6[a]	22.7
0 -104[b]	14017 +/-1550 (22)	14783+/-1222 (25)	14677+/-1383 (18)	13632+/-1128 (19)	14486+/-1183 (21)

[a]=$p<0.01$ vs control 2. [b]= cumulative food consumption (mean +/- SD) for number of animals examined.

Body weight: Weight gain was significantly suppressed in the high dose dienogest group compared to vehicle control groups 1 and 2 during experimental weeks 4 - 88. Body weight patterns in the low and mid dose groups were similar to the control group. Changes in body weight at various time intervals during the course of 104 weeks experimental period is shown in table 4 below;

Table 4

Week	Control 1	Control 2	M18575 (mg/kg/day)		
			1	3	10
0	130.4	130.8	129.8	131.0	130.5
1	1623	163.2	162.2	162.7	160.0
4	231.8	232.7	232.8	231.8	223.9* (96.6)
12	306.7	309.2	309.7	307.0	290.3**## (94.6, 93.9)
24	347.0	353.2	352.1	349.7	329.8## (93.4)
36	388.5	395.3	392.8	390.8	367.6 # (93.0)
48	423.0	435.8	428.6	432.0	399.3## (91.6)
60	446.7	460.0	456.4	456.5	416.2## (90.5)
72	482.6	492.7	477.2	480.7	441.4*## (89.6)
84	517.1	509.3	502.6	493.3	452.4*## (87.7, 88.9)
96	517.7	495.2	500.4	507.1	457.7
104	509.2	479.3	498.4	479.3	440.8
0 -104[a]	380.2+/-117.0 (22)	357.0+/-96.0 (25)	373.4+/-143.0 (18)	353.2+/-82.8 (19)	306.2+/-78.4 (21)

[a]=body weight gain (mean +/- SD) with number of animals in parentheses
*= p<0.05; **= p<0.01 vs control 1; # p<0.05; ## p<0.01 vs control 2

Based on decreased body weight gain of >10% for the high dose group compared to respective controls at weeks 72 and 84, the high dose could be considered as the MTD.

Hematology: As shown in table 5 below, no significant treatment-related changes in the RBC and WBC count were observed.

Table 5

	Control 1	Control 2	M18575 (mg/kg/day)		
			1	3	10
No. of animals examined	22	25	17	18	21
RBC ($x10^4/mm^3$)	549+/-150	504+/-130	529+/-126	588+/-135	512+/-158
No. of animals examined	20	24	14	18	19
WBC ($x10^2/mm^3$)	65+/-25	93+/-48	82+/-57	59+/-23	66+/-27

Values are expressed as mean +/- SD

Palpable nodules, masses or tumors: The incidence of palpable nodules or masses reported upon necropsy is given in table 6 below:

Table 6

	Control 1	Control 2	M18575 (mg/kg/day)		
			1	3	10
No. of animals examined	65	65	65	65	65
No. of animals with palpable tumors/palpable nodules or masses	44/54	51/56	51/56	51/56	51/60
Total number	118/174	129/191	114/172	102/143	94/155
Mean time (weeks) of onset of palpable tumors	63.5	70.0	71.1	73.8	81.3

The incidence of palpable tumors/palpable nodules or masses did not increase as a result of treatment with M18575. Also the mean time of onset for palpable masses did not shorten as a result of treatment.

Organ weights: Absolute organ weights and organ weights relative to body weight which were significantly affected by treatment at terminal sacrifice are given in table 7 below. The increased relative weights in the high dose group were attributed to decreased body weight with treatment in this group.

Table 7

	Control 1	Control 2	M18575 (mg/kg/day)		
			1	3	10
No. of animals examined	22	25	17	18	21
Body weight (g)	508.0+/-117.6	483.8+/-101.3	499.6+/-150.0	481.3+/-85.3	438.9+/-79.5
Absolute organ weights					
Lungs (g)	1.52+/-0.17	1.68+/-0.20	1.63+/-0.17	1.47+/-0.14$^{\#\#}$	1.56+/-0.18
Ovaries (mg)	86.4+/-52.9	76.4+/-51.4	55.3+/-21.4	69.9+/-33.0	57.6+/-50.5**
Uterus (mg)	945+/-457	1035+/-507	999+/-228	855+/-231	629+/-119$^{**\#\#}$
Organ weight relative to body weight					
Submandibular glands (mg%)	107+/-24	121+/-30	119+/-23	114+/-20	135+/-23**
Heart (g%)	0.31+/-0.05	0.34+/-0.08	0.34+/-0.08	0.31+/-0.04	0.36+/-0.05**
Liver (g%)	3.21+/-0.56	3.76+/-0.49	3.73+/-0.57*	3.56+/-0.57	4.03+/-0.54**
Adrenals (mg%)	31.9+/-28.4	132.2+/-456.6	42.5+/-28.0	65.9+/-119.1	55.8+/-64.5*
Kidneys (g%)	0.64+/-0.15	0.76+/-0.24	0.75+/-0.30	0.64+/-0.13	0.78+/-0.15*
Ovaries (mg%)	17.6+/-10.4	16.2+/-11.7	11.6+/-4.0	15.2+/-8.1	14.1+/-15.3*
Uterus (mg%)	198+/-105	221+/-106	232+/-131	182+/-55	148+/-40$^{\#\#}$

Values are mean +/-SD * $p<0.05$, ** $p<0.01$ vs control 1, $^{\#\#}$=$p<0.01$ vs control 2

Comments: Treatment resulted only in decreased ovaries and uterus weight in the high dose groups attributed to dienogest hormonal effect.

Gross pathology: At necropsy findings common to all groups including the control groups were subcutaneous nodules or masses; enlargement , nodules/masses and reddish spots/patches in the pituitary, reddening, reddish spots/patches or nodules/masses/enlargement in the adrenal; enlargement of lymph nodules, spleen and heart; ovarian cysts and dilatation/hydrometra of the uterus. The incidence of discoloration/pale for bone marrow was 4, 3, 8, 8, and 12 and that for liver 6, 5, 11, 8 and 15, respectively for the 2 control and 3 treated groups. Sponsor concluded that no effects due to treatment with M18575 were seen in the incidence of necropsy findings and they were spontaneous changes seen in old rats of the strain used.

Comments: The macroscopic finding suggested essentially no significant adverse treatment effect at doses used.

Histopathology:

Non-neoplastic lesions: Non-neoplastic findings which had significantly higher incidence in the drug treated groups compared to controls involved ovary, uterus and vaginal as shown in table 8 below:

Table 8

Organ/finding	Control 1	Control 2	M18575 (Dienoogest)		
			1	3	10
Animals examine	65	65	65	65	65
Ovary					
Absent corpora lutea	39	42	42	46	62**##
Uterus					
Endometrial hyperplasia	2	1	0	5	8#
Vacuolization of endometrial cells	0	4	1	6*	16**##
Vacuolization of glandular epithelial cells	1	2	0	6	12**##
Vagina					
Mucification of epithelial cells	31	41	58**##	61**##	62**##
Atrophy of epithelium	35	30	6**##	1**##	3**##

*p<0.05, **p<0.01 vs control 1; ##p<0.01 vs control 2.

The incidence of submaxillary lymph node hemorrhage was higher in mid and high dose groups with incidence of 4, 4, 4, 7, and 10 for controls 1 and 2 and three treated groups. Panarteritis in stomach was reported in the drug-treated groups with values of 0, 0, 1, 1, and 2 for control 1 and 2 and low, mid and high dose dienogest groups.

Neoplastic: The incidence of neoplastic tumors observed in various groups is given in table 9 below:

Table 9

Organ/finding	Control 1	Control 2	M18575 (Dienoogest)		
			1	3	10
Animal examined	62	65	64	61	62
Mammary gland					
Adenocarcinoma	25	25	27	34	33
Adenoma	4	4	6	4	7
Fibroadenoma	36	36	32	30	24
Adenoma + adenocarcinoma	29	29	33	38	40
Pituitary					
Adenocarcinoma- anterior lobe	7	6	3	2	5
Adenoma –anterior lobe	42	44	43	44	38
Adenoma + adenocarcinoma	49	50	46	46	43
Uterus					
Endometrial stromal sarcoma	0	0	0	1	2
Endometrial stromal polyp	3	5	5	7	4
Pancreas					
Islet cell adenoma	1	3	4	2	3
Islet cell carcinoma	0	2	0	1	3
Adenoma + adenocarcinoma	1	5	4	3	6
Number of primary tumors	144	149	140	148	148
Total benign	103	105	100	102	96
Total malignant	41	44	40	46	52

The data demonstrated that there was no significant difference between the drug-treated groups and control groups for the incidence of the observed tumors.

Toxicokinetics: Plasma concentration of dienogest after the first dose and in weeks 17, 34 and 52 are sown in table 10 below:

Table 10

		M18575 (Dienogest) mg/kg/day		
	Control 1	1	3	10
Number of animals	**5**	**5**	**5**	**5**
Week 0				
1 hour values (ng/ml)	**0.0 +/- 0.0**	**77.3 +/- 31.4**	**288.7 +/- 126.1**	**1638.5 +/-562.0**
$AUC_{0\text{-}24hr}$ (ng.hr/ml)	**0.0 +/-).0**	**118.1 +/- 36.9**	**518.4 +/- 238.0**	**2892.7 +/- 732.4**
Week 17				
1 hour values (ng/ml)	**0.0 +/- 0.0**	**74.0 +/- 43.3**	**140.1 +/- 19.7**	**1470.6 +/- 732.4**
Week 34				
1 hour values (ng/ml)	**0.0 +/- 0.0**	**57.8 +/- 35.9**	**224.6 +/- 52.4**	**1724.1 +/- 557.4**
Week 52				
1 hour values (ng/ml)	**0.0 +/- 0.0**	**97.3 +/- 47.4**	**401.2 +/-115.1**	**2303.6 +/- 1072.8**
$AUC_{0\text{-}24\ hr}$ (ng.hr/ml)	**0.0 +/- 0.0**	**164.2 +/- 60.6**	**1227.8 +/- 299.8**	**5502.8 +/-1850.5**

One animal in control group died at week 51 and values are mean of 4 animals. Data are expressed as mean +/- S.D. Taking AUC of 682 ng.h/ml for humans with therapeutic dose of dienogest, the AUC of 5503 ng h/ml in high dose group, gives a multiple of 12.

With the first dose, dienogest was detected in the plasma after 15 minutes in all treated groups, and thereafter disappeared 4 hour after the dose in the low dose group and at 24 hours in the medium and high dose groups. No statistical analysis of the data was provided. AUC seems to be marked higher at 52 week compared to week 0 values.

Histopathology inventory (optional)

Study	JPH01 294	0343		
Species	Mouse	Rat		
Adrenals	X	X		
Aorta	X	X		
Bone Marrow smear		X		
Bone (femur)	X	X		
Brain	X*	X		
Cecum	X	X		
Cervix	X			
Colon	X	X		
Duodenum	X	X		
Epididymis	X*			
Esophagus	X	X		
Eye	X	X		
Fallopian tube				
Gall bladder	X			
Gross lesions		X		
Harderian gland	X	X		
Heart	X*	X		
Ileum	X	X		
Injection site				
Jejunum	X	X		
Kidneys	X*	X		
Lachrymal gland	X			
Larynx				
Liver	X*	X		
Lungs	X*	X		

Lymph nodes, cervical				
Lymph nodes mandibular	X			
Lymph nodes, mesenteric	X	X		
Mammary Gland	X	X		
Nasal cavity				
Optic nerves	X	X		
Ovaries	X*	X		
Pancreas	X	X		
Parathyroid	X	X		
Peripheral nerve				
Pharynx				
Pituitary	X	X		
Prostate	X*			
Rectum	X	X		
Salivary gland	X*			
Sciatic nerve	X	X		
Seminal vesicles	X*			
Skeletal muscle	X	X		
Skin	X	X		
Spinal cord	X	X		
Spleen	X*	X		
Sternum	X	X		
Stomach	X	X		
Testes	X*			
Thymus	X*	X		
Thyroid	X	X		
Tongue	X			
Trachea	X			
Urinary bladder	X	X		
Uterus	X*	X		
Vagina	X	X		
Zymbal gland				

X, histopathology performed
*, organ weight obtained

Note: Both the rat and the mouse carcinogenicity studies have been presented and approved by the Exec-CAC. The Exec-CAC minutes are given below:

Executive CAC
Date of Meeting: January 16, 2007

Committee: David Jacobson-Kram, Ph.D. OND 10, Chair
Joseph Contrera, Ph.D., OPS, Member
Abby Jacobs, Ph.D. OND 10, Member

Bayo Laniyonu, Ph.D. DMIHP, Alternate Member
Lynnda Reid, Ph.D., DRUP, Team Leader
Krishan Raheja, D.V.M., Ph.D., DRUPP, Presenting Reviewer

Author of the Draft: Krishan Raheja

The following information reflects a brief summary of the committee discussion and its recommendations.

IND #: 64,809
Drug name: Dienogest
Sponsor: Berlex Inc. P.O.Box 1000, Montville, NJ 07045

Background

Dienogest is a 19-norprogestin, a NME under investigation combined with estradiol valerate for use as an oral contraceptive. Dienogest in combination with ethinyl estradiol is marketed in Europe as Valette for oral contraceptive, and in combination with estradiol valerate as Climodin for hormone replacement therapy (HRT).

Rat Carcinogenicity Study Protocol and Dose Selection: Neither the dose range-finding studies results nor the protocol for the carcinogenicity study were submitted for Exec-CAC review and concurrence. Doses of 1, 3 and 10 mg/kg/day were used based on results of 12- month toxicity study, where treatment at 10 mg/kg/day led to target organ toxicity as increased foci of basophilic tigroid altered hepatocytes in the liver. In the carcinogenicity study body weight gain was decreased more than 10% compared to respective controls. Only females were used in this study.

Mouse Carcinogenicity study Protocol and Dose Selection: Neither the dose range-finding studies results nor the protocol for the carcinogenicity study were submitted for the Exec-CAC review and concurrence. Doses of 5, 15 and 50 mg/kg/day in males and 10, 30 and 100 mg/kg/day in females were used based on systemic exposure in relation to human exposure. These doses were found to be acceptable based on observed exaggerated pharmacodynamic effects.

Executive CAC Recommendations and Conclusions:

For the rat carcinogenicity study the committee agreed to accept the study doses as adequate. The Committee found the study was negative for statistically significant dose-related neoplasms pending confirmation that the study was conducted under GLP and the tumor data and the incidence tables were correct regarding identical numbers of mammary adenocarcinomas and adenomas in the two control groups.

Note: The sponsor has confirmed that the study was conducted in accordance with GLP guidelines and that the number of mammary tumors reported for the two control groups was accurate.

For the mouse carcinogenicity study the committee agreed that the study was adequate, reaching a pharmacologic endpoint. The committee found that the study was positive for uterine stromal polyps in high dose females.

David Jacobson-Kram, Ph.D
Chair, Executive CAC

Cc:\

Division File, DRUP
Lynnda Reid, Team Leader, DRUP
Krishan Raheja, Reviewer, DRUP
Nenita Crisostomo, PM, DRUP
A.Seifried, OND 10

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: Reproductive function and fertility study by oral route in female rats.

Key study findings: Pre- and post-implantation losses were increased significantly at the 3 mg/kg/day dose but not at the 0.3 and 1 mg/kg/day dose levels. No external abnormalities were seen at any dose level.

Study no.: Berlex study No. 98069

Volume #, and page #: Volume 20 of 84 page 06451
Conducting laboratory and location: (b) (4)

Date of study initiation: 8-18-92

GLP compliance: yes

QA reports: yes (*) no ( )

Drug, lot #, and % purity: not indicated

Methods

Doses: 0, 0.3, 1 and 3 mg/kg/day from day 15 before mating and until day 7 of pregnancy. Dose levels were selected based on results of the dose range-finding study No. 98068.

Species/strain: SD rats/Crl CD (SD) BR

Number/sex/group: 24 females/g

Route, formulation, volume, and infusion rate: oral, solution in corn oil, 5 ml/kg/day

Satellite groups used for toxicokinetics: none

Study design: After 15 days treatment, the females were paired. Dosing was continued until day 7 of pregnancy. On day 20 of pregnancy, the females were sacrificed, examined macroscopically and fetuses removed by caesarean section.

Parameters and endpoints evaluated: Litter parameters recorded included number of corpora lutea, implantation sites, resorptions, dead and live fetuses. Fetuses were weighed, sexed and submitted to external examination.

Results

Mortality: No deaths occurred during the study

Clinical signs: No clinical signs were observed

Body weight: Except for a slightly lower mean body weight change in the 3 mg/kg/day dose group from days 13 to 20 of pregnancy (61 g vs 92 g). This was attributed to lower number of fetuses observed at this dose level. There was treatment effect with other dose levels.

Food consumption: Food consumption was similar in females of the control and treated groups during the mating and pregnancy periods.

Toxicokinetics: Not conducted.

Necropsy:

Fertility parameters (mating/fertility index, corpora lutea, preimplantation loss, etc.):

Mating index was 100%, 100%, 100% and 95.8% for the control and low, mid and high dose groups.

The fertility index was 87.5%, 83.3%, 91.7% and 43.5%. The fertility index for the high dose was significantly decreased (P<0.001).

Caesarean section data: As shown in table below significant treatment-related changes were observed in the high dose group. These included increased pre and post-implantation losses. No other treatment-related significant findings were reported.

Dose (mg/kg/day)	0	0.3	1	3
Pregnant females alive at term	19	20	22	10
Dams with all resorptions	0	0	0	3
Dams with all dead fetuses	0	0	0	0
Corpora lutea (No. per animal)	19.3	21.4	19.7	16.1
Implantation sites /animal	15.7	17.1	16.4	5.6**
Preimplantation loss (%)	10.5	19.9	16.6	65.2**
Live fetuses/animal	15.5	16.8	16.0	4.5**
Resorptions (early+late)/animal	0.2	0.3	0.4	1.1*
Resorption (early)/animal	0.1	0.2	0.4	1.0*
Post-implantation loss (total)	5	7	10	11**
Post-implantation loss/animal	0.3	0.3	0.5	1.1*
% of viable male fetuses	52.0	52.1	49.0	64.4
% of vaibale female fetuses	48.0	47.9	51.0	35.6

*=p<0.05 **=p<0.001

Fetal external examinations: No malformations were noted in fetuses of any group.

Pathology: Macroscopic examination revealed no treatment-related findings.
Microscopic evaluation of the ovaries, uterus and vagina showed morphological changes indicative of regular estrous cycle in 3/3 examined control females, in 3/4 examined females given 0.3 mg/kg/day, in 2/2 examined females given 1 mg/kg/day and in 6/14 examined females given 3 mg/kg/day.
Condensation of the endometrial stroma and /or vacuolization of the endometrial epithelium in uterus was noted in 6/14 females given the high dose and mucification of the vaginal epithelium was observed in 7/14 females of the same group. Sponsor attributed these changes to the progestogenic activity of dienogest.

It was concluded that the doses used did not induce any toxic effects in females, did not induce any effects on fertility of females, did not disturb the outcome of the pregnancy and did not induce any external fetal malformations at dose levels of 0.3 and 1.0 mg/kg/day.

At dose of 3 mg/kg/day significant changes were increased pre- and post-implantation losses. No fetal malformations were observed. The NOEL was considered as 1 mg/kg/day.

Embryofetal development

Study title: Toxicities studies of 17 a-cynomethyl-17b-hydroxy-estrra-4,9(10)-dien-3-one (Dienogest, M18575): Study of M18575 in rabbits during the organogenetic period.

Note: This study is translation of a Japanese language report

Key study findings:

Study no.: Berlex Report No. 98080
Volume #, and page #: Volume 24 of 84 IND (b) (4)
Conducting laboratory and location: (b) (4)
Date of study initiation: September 1990
GLP compliance: This study was conducted in accordance with GLP Standards of Pharmaceutical Affairs Bureau, Ministry of Health and Welfare, Japan
QA reports: yes (*) no ( )
Drug, lot #, and % purity: Lot No. 020589, purity 99.98%

Methods
Doses: 0, 0.3, 1 and 3 mg/kg/day

Species/strain: NYW rabbits

Number/sex/group: 14-16 females/group

Route, formulation, volume, and infusion rate: Oral, suspension in 0.5% MC, 1 ml/kg

Satellite groups used for toxicokinetics: None

Study design:

Procurement of pregnant rabbits: To produce pregnant animals, decoy females were paired with males which showed copulatory behavior, and semen was collected artificial vaginas; semen was examined for admixture of foreign substances, sperm count and sperm motility. Pooled suitable semen was used for artificial insemination where 1.0 mL seminal fluid was injected into vagina of each rabbit that showed signs of estrus. After artificial insemination, 25 IU of HCG were administered through the auricular vein in order to induce ovulation. The date on which artificial insemination was performed was taken as day 0 of pregnancy.

Dose selection: In a preliminary trial (Berlex Report No. 98075) rabbits were administered dienogest in the organogenetic period. A tendency of depressed body weight in the surviving fetuses was observed at dose of 1 mg/kg group and in the 10 mg/kg group, all the animals had only absorbed fetuses and no surviving fetuses. Based on these observations, a dose of 3 mg/kg/day was selected as the high dose, with 1.0 mg/kg/day and 0.3 mg/kg/day as the mid and low dose, respectively administered once a day using stomach catheter for a total of 13 days, from day 6 to day 18 of pregnancy.. Control group received only solvent at a dose volume of 1 ml/kg.

Parameters and endpoints evaluated:

Observations for female parents: On day 29 of pregnancy, the dams were sacrificed by iv injection of sodium pentobarbital, their chests and abdomens were opened, the presence or absence of abnormalities of the internal organs was observed grossly and the ovaries and uteri were removed. The number of corpora lutea and implantations, absorbed and dead fetuses, and surviving fetuses were recorded and post-implantation death rates were calculated. In addition, organs and tissues which showed abnormalities were fixed in a 10% buffered formalin solution and processed for histopathological examination.

Observation for the fetuses: The body weights and placental weights of the surviving fetuses were measured and they were examined for external malformations. Their abdomens were opened and their sexes were determined. The presence or absence of abnormalities in the chest organs and large blood vessels of the chest was observed with a stereoscopic microscope. In addition, the brains, hearts and kidneys were removed. Internal images of the heart were obtained and cut surfaces of the brain and kidneys were examined. Furthermore, skeletal specimens of all of the fetuses were made and used in skeletal examination.

Results

Litter data at cesarean section of rabbits dams treated orally with dienogest (M18575) is given in table below:

Parameter	Dienogest treatment (mg/kg)			
	control	0.3	1.0	3.0
No. of dams	14	15	14	16
No. of dead dams	0	1[a]	0	2[a]
No. of dams with abortion	1	1	1	3
No. of dams with complete resorption	0	0	2	8[*]**[*]
No. of dams with live ffetuses	13	13	11	3 [**]
Maternal body weight gain (g) Days 6-19 of gestation	215+/-18[b]	201 +/-21	118 +/ 36	-183+/-71[**]
Matenal body weight gain Days 0- 29 of gestation	641+/-76	598+/-63	638+/-48	220+/-132[**]
Maternal food consumption (g/rabbit) Days 7-19 of gestation	2248+/-60	2172+/-81	2083+/-151	1343+/-197[**]
Maternal food consumption (g/rabbit) Days 1-29 of gestation	4821+/-180	4687+/-81	4928+/-149	3417+/-431
No. of corpora lutea per litter	9.7+/-0.4	9.4+/-0.4	9.8+/-0.5	11 3+/-1.0
No. of implantations per litter	6.8 +/-0.9	7.2+/-0.6	6.5+/-0.7	8.2+/-0.6
Total number of absorbed or dead fetuses	6	6	8	70
Post-implantation loss (%)[c]	6.1	5.8	20.7	78.4[**]
No of live fetuses/litter	6.3+/-0.9	6.7+/-0.5	5.8+/-0.9	1.8+/-1.0[**]
Sex ratio (male/female)	33/49	48/39	42/34	11/9
Fetal body weight per litter (g)				
Male	45.44+/-1.95	41.50+/-1.17	40.35+/-2.01	39 53+/-2.60
Female	43.50+/-1.12	40.42+/-1.17	42.14+/-2.00	36.71+/-2.60
Placental weight per litter (g)	5.76+/-0.29	5.78+/-0.28	5.89+/-0.26	5.04+/-0.25
Total number of fetuses with external malformations	0	1 (0.9)[d]	1 (1.1)[e]	0

a) These dams died likely due to dosing error
b) Mean +/_ S.E.
c) Post-implantation loss is expressed as [(No. of implantations)-(no. of live fetuses)/(No. of implantation] x 100
d) Oligodactyly of hind limb
e) Cranioschisis, syndactyly of forelimbs and micrognathis

Figures in parentheses indicate average percentage to one litter
Significant differences from control are marked: * ($p<0.05$), ** ($p<0.01$)

Visceral examinations of fetuses

Parameter	Dienogest treatment (mg/kg)			
	control	0.3	1.0	3.0
No. of litter	13	13	11	3
No. of fetuses examined	82	86	75	20
Total no. of fetuses with anomalies	2 (1.6)	0	1 (1.0)	1 (5.6)
Type and prevalance of anomalies				
Dilatation of 3rd ventricle and lateral ventricle	1 (0.8)	0	0	0
Dilatation of lateral ventricle	1 (0.9)	0	0	0
Riding aorta with ventirclar septal defect	0	0	1 (1.0)	0
Riding aorta	0	0	2 0	1 (5.6)*
No other treatment related findings were reported.				

Figures in parentheses indicate average percentage to one litter
Significant difference from control is marked: * ($p< 0.05$)

Conclusion: In the 3 mg/kg group following was observed:
Tendencies for the number of cases in which hemorrhaging of external genitalia occurred and the number of aborting animals increased. There was a decrease in body weight accompanied by a decrease in the quantity of food consumed. Also increases in the number of dams with only absorbed fetuses and the rates of deaths after implantation was observed. There was a tendency for fetal body weight to be depressed. However, there was no teratogenic activity observed for dienogest.
The non-toxic dose was considered as 1 mg/kg/day.

Prenatal and postnatal development (check)

Study title: Toxicity studies of 17α-cyanomethyl-17β-hydroxy-estra-4,9(10)-dien-3-0ne (Dienogest, M18575). Study of M18575 in rats during perinatal and nursing periods.

Note: This is a translation of a foreign language report.

Key study findings: Dienogest had no adverse effects on F_0 and F_1 generations reproductive performance. Effects on dams (F_0) consisted of significant decrease in body weight and food consumption and lengthening of the pregnancy period at the high dose of

1 mg/lg day. No effects of treatment were observed in the 0.3 and 0.1 mg/kg groups. With regards to the F_1 generation, in the 1 mg/kg group autopsies showed atrophy of ovaries and histological examination showed increase in closed follicles and decrease in corpora lutes. The weight of the pituitary was significantly increased. Effects on live-born offspring (F_2) included decrease in number of neonates and decreased birthrate. No effects were observed in the 0.3 and 0.1 mg/kg groups.

Study no.: Berlex Report No. 98081
Volume #, and page #: 25 of 84 of IND (b) (4) pg 8 08139
Conducting laboratory and location: (b) (4)

Date of study initiation: December 1991 – September 1992
GLP compliance: Yes
QA reports: yes (*) no ( )
Drug, lot #, and % purity: 020589, 99.81%

Methods
Doses: 0, 0.1, 0.3 and 1 mg/kg/day

Basis of dose selection: The high dose of 1 mg/kg/day was based on the results of a preliminary trial of drug administration during the perinatal and nursing periods, using the same strain of rats as used in this study, a lengthening of the pregnancy period and an increase in the number of stillborn fetuses were observed when 1 mg/kg or more was administered.

Species/strain: Wistar-Imamichi

Number/sex/group: 23 pregnant dams/group

Route, formulation, volume, and infusion rate: Oral gavage, drug in 0.5% aqueous solution of CMC given at a volume of 5 mL/kg

Satellite groups used for toxicokinetics: None

Study design: The mating was performed by allowing male-female pairs to cohabitate; copulation was established by the presence of vaginal plugs or the presence of semen in vaginal smears. The day on which copulation was confirmed was taken as day 0 of pregnancy. M18575 was administered to the pregnant rats during the perinatal (day 17 to day 21) and nursing period in order to investigate effects on the parents (F_0) and the offspring (F_1, F_2). Dams were allowed to give birth naturally. The parturition rates [(number of females bearing live offspring/number of pregnant females) x 100] were calculated.
On the 21st day after parturition or when all of the neonates had died, the dams were sacrificed and the presence or absence of abnormalities in the internal organs were observed, The number of implantation traces were obtained, and the birth rates [(number of live offspring/number of implantation traces) x 100] were calculated.

Parameters and endpoints evaluated: Observation of dams, observations of live-born offspring (F_1) which consisted of examination before weaning, examination at the time of weaning and post-weaning examination and observation of live offspring (F_2).

Results

F_0 in-life: In the 1 mg/kg group, a significant decrease in body weight gain was observed during gestation days 17-21 of gestation (61.6 gm for control vs 52.2 gm for the treated) or for days 0 -21 of gestation (177.7 for controls vs 169.9 for the treated). Also a significant decrease in food consumption (g/rat) was observed on days 18 - 21 of pregnancy (103 for controls vs 93 for the treated group). In addition there was a significant lengthening of the pregnancy period (22.2 days for controls vs 22.5 days for the treated dams). No effects on the dams were observed in the 0.1 and 0.3 mg/kg groups. A spontaneous case of renal failure was reported in the 0.1 mg/kg group.

F_0 necropsy: No remarkable necropsy findings in any group.

Histopathological findings involving uterus and kidney were reported for female in the 0.1 mg/kg group. For the uterus, these included hemorrhage of endometrium (slight), inflammatory cell infiltration into the endometrium (severe). For the kidney these included bilateral necrosis of cortex (very severe) and bilateral calcium deposition (moderate).

F_1 physical development: There were 23 litters/group, except for the 0.1 mg/kg group where there were 22 litters. Developmental landmarks (days) examined included ear unfolding, incisor eruption, fur appearance, eye opening, testicular descent and vaginal opening. None of these parameters were affected by treatment.

F_1 functional observation: There were 177, 172, 177 and 176 offspring were evaluated in control, low, mid and high dose, respectively for Irwin's test (score) i.e., pina reflex, corneal reflex, air righting reflex, ipsilateral flexor reflex, pain response. Also papillary reflex (%), preyer's reflex (%), visual placing response (%), inclined plane test, negative geotropism (%), traction test (%), rotor rod test (number of falls) and motor co-ordination (%).
None of these parameters were affected by treatment of F_0 dams.

F_1 behavioral evaluation: No treatment adverse effects were reported on the nervous systems, perceptions or behavioral functions. Learning ability in water-filled T-maze for both male and female offspring determined on 1st, 2nd and 3rd day was unaffected by treatment of F_0 dams.

F_1 reproduction: It was reported that no effects of the drug administration were observed on the times of sexual differentiation and sexual activity. It was stated that in the 1 mg/kg group, tendencies for the number of estrus to decrease and for the copulation and impregnation rates to fall were observed. At autopsy atrophy of the ovaries was observed

in 13 out of 34 animals, and the histopathological examination showed increases in closed follicles in 17 out of 21 animals and decreases in corpora lutea in 11 out of 21 animals. No effects on reproductive ability were seen among the male live-born offspring. In the 0.3 and 0.1 mg/kg groups, no effects of drug administration were observed on the reproductive abilities of the male and female live-born offspring.

Some of the parameters which were significantly affected by in F_1 offspring of F_0 treated dams is given in table below:

	Control	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg
Number of dams pregnant	21	21	20	12
Number of dams delivering	21	21	20	12
Without live offspring	0	0	0	2
Gestation index (%)	100	100	100	100
Gestation period (days)	22.3	22.4	22.4	22.7
Maternal body wt (days 0-21 of gestation)	177.5	168.4	157.0	129.3**
Number of implantation per litter	16.3	15.7	13.4	7.5**
Number of offspring born alive/litter	14.1	12.5	11.3	6.2**

But for significant F_0 dams treatment effect on F_1offspring on maternal body weight during gestation days 0-21, significantly decreased number of implantation/litter, and number of offspring born alive/litter, no other adverse effects were observed. Absolute pituitary weight was significantly greater in the 1 mg/kg groups with values being 11.4, 11.7, 12.0 and 15.1** mg, respectively.

Effects on live-born offspring (F_2) findings: In the 1 mg/kg group, there was a significant decrease in the number of neonates and tendency for birth rate to decrease. In the 0.3 and 0.1 mg/kg groups, no effect was observed on the live-born offspring.

2.6.6.7 Local tolerance: None described

2.6.6.8 Special toxicology studies: None described

2.6.7 TOXICOLOGY TABULATED SUMMARY

None provided by the sponsor. Reviewer tabulated summaries are provided under individual studies.

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Integrated summary: The estradiol valerate/dienogest tablets constitute a combined oral contraceptive consisting of four-phase (plus a placebo phase), 28 day sequential regimen.

Estradiol valerte (EV) is a pro-drug that is rapidly hydrolyzed pre-systemically into 17β-estradiol and valeric acid and dienogest (DNG) is a progestin, which is marketed in

several formulations for hormone replacement therapy and as oral contraceptive in many countries though not in the USA.
Dienogest, the progestin component of the EV/DNG tablets is a 19-norprogestin with strong progesterone receptor selectivity, leading to specific progestational action and to effective transformation of the endometrium. The mechanism of the contraceptive effect of DNG is by inhibition of ovulation by its antigonadotropic action.

Under this NDA toxicity studies were conducted only for Dienogest, which is a new molecular entity.

Under safety pharmacology studies in mice and rats, dienogest had no neurological effects, it exhibited no adverse cardiovascular effect as it did not affect mean blood pressure, heart rate or ECG parameters (PR, QRS, and QT intervals) in female monkeys. In vitro in isolated guinea pig papillary muscle, dienogest had no effect on the resting membrane potential, action potential amplitude, action potential duration at 50% and 90% repolarization or V_{max} at concentrations up to 10^{-5} mol/L. In vitro, dienogest, at concentration s ranging from 10^{-6} to 10^{-5} mol/L had no effect on the human HERG-mediated potassium channel. Dienogest did not influence spontaneous rate of contractility in isolated rat atrial preparations at dose of 10^{-6}mol/L, but at concentrations ranging from 10^{-6} to 10^{-4} mol/L, it moderately to completely inhibited the spontaneous contraction of smooth muscle in guinea pig atria and guinea pig or rat ileal preparations, respectively. Dienogest's pulmonary effects consisted of reduced tidal and minute volume within 2 hours of dosing with oral doses greater than 10 mg/kg in female rats. A single i.v. injection of dienogest up to doses of 50 mg/kg had no effect on arterial blood pressure or rate of respiration in female dogs. Dienogest did not exhibit adverse renal effects in rats or gastrointestinal effects in rats and mice.

General toxicology studies included single and repeat dose toxicity studies in various species. Dienogest was well tolerated when given orally as single dose to mice, rats, rabbits and dogs. No mortality was reported in female rats and female dogs given dienogest orally at a dose of 2000 mg/kg.

In the 91-day toxicity where dienogest was administered once daily as suspension at dose levels of 0.3, 3, 10 and 30 mg/kg, clinical significant treatment-related findings included increases in platelet count and fibrinogen quantity, decreased erythrocyte osmotic fragility and in crease in the neutral lipid value. Urine volume was increased with increased calcium excretion at the highest dose level.

In the 6-month oral toxicity study where dose levels of 0.1, 1.0, or 10.0 mg/kg/day were used, the observed effects on endocrine organs were suggested to be due to anti-ovulatory effect of the compound. In another 6-month study using dose levels of 1 or 10 mg/kg/day conducted with special attention to hepatotoxicity did not exhibit adverse effects.

In the 52-week oral toxicity in female rats where dose levels of 0.1, 1.0 and 10.0 mg/kg/day were used, significant hematological findings included increased prothrombin time associated with increased fibrinogen in the high dose group. Biochemical changes

included decreased albumin and GOT and significantly increased NEFA in all dienogest-treated groups. On electron microscopic examination, livers of 2/3 rats receiving the 10 mg/kg/day dose, exhibited lipid droplets in periportal hepatocytes.
It was stated that at the 10 mg/kg dose, there was evidence of treatment-related effect on hepatic function. The changes seen in PT and fibrinogen were suggested to probably correlate with histopathological findings of increased periportal fat deposition, which in turn correlate with findings of increased triglycerides and NEFAs. It was stated that the pattern of changes is similar to that seen in humans with impaired hepatic function and in rats with experimental liver injury induced by number of agents.

In the 4-week toxicity study in female dogs where dose levels of 0, 0.1, 0.5, 1.0 and 10.0 mg/kg/day were used significant clinical chemistry findings included increased phospholipids, total cholesterol and B-lipoproteins. The microscopic examination findings included focal hyperplasia of the acidophilic cells in the pituitary at the high dose, hyperplasia of the mammary gland after 1 and 10 mg/kg, hyperplasia of the endometrium with secretion and glandular ectasis in all treated groups and missing corpora lutea in the high dose.
In the 91-day toxicity study in female dogs, desogestrel was administered in gelatine capsules at dose levels of 0.03, 0.3 and 3 mg/kg/day (3 dogs/g). Hematology findings included increased reticulocytes (%) and fibrinogen value with slight decrease in coagulation time.

In the 91-day and 52-week toxicity studies in female monkeys dose-related drug effects were seen in hematological tests where increase in fibrinogen and plasminogen activity was observed.

Dienogest was not mutagenic when tested in the Ames assay, chromosomal aberration study using cultured mammalian cells, in the mouse lymphoma test, and in the in vivo micronucleus assay in bone marrow of the mouse.

Carcinogenicity studies conducted in rats and mice by oral administration of dienogest for 104 weeks demonstrated findings essentially similar to those with other progestins reviewed and approved previously. These studies were presented to and approved by the Exec-CAC.

Fertility and early embryonic development and embryofetal development studies demonstrated no adverse effects on treated females or the fetuses.

Conclusions: The preclinical toxicology studies with Dienogest showed that the observed findings were essentially similar to those observed with other approved progestins.

Unresolved toxicology issues (if any): None

Recommendations: Based on review of the nonclinical toxicology studies submitted, P/T recommends approval of NDA 022-252.

Suggested labeling: Draft label is in accordance with PLR and submitted in SPL format and is acceptable.

Application Type/Number	Submission Type/Number	Submitter Name	Product Name
NDA-22252	ORIG-1	BAYER HEALTHCARE PHARMACEUTICALS INC	Qlaira

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

KRISHAN L RAHEJA
01/27/2010

ALEXANDER W JORDAN
01/27/2010

45 Day NDA Meeting Checklist
Pharmacology/Toxicology

NDA Number: 22-252 **Date: 7-15-09**
Drug Name: Estradiol valerate/Dienogest **Reviewer:** Krishan L. Raheja
Sponsor: Bayer HealthCare Pharmaceuticals

Date CDER Received: 7-6-09
Filing Date: 8-11-09
User Fee Date: 5-6-2010
Expected Date of Draft Review:

On initial overview of the Pharm/Tox portion of the NDA application

ITEM		YES / NO	COMMENTS
1)	On its face, is the Pharm/Tox section of the NDA organized in a manner to allow substantive review to begin? 0	Yes	It was agreed at the meeting on 18 Dec. 2007, that Nonclinical reports would not be included in this submission. Instead sponsor will provide cross-reference to prior IND submissions.
2)	Is the Pharm/Tox section of the NDA indexed and paginated in a manner to allow substantive review to begin?	NA	
3)	On its face, is the Pharm/Tox section of the NDA legible so that substantive review can being? Has the data been presented in an appropriate manner?	NA	
4)	Are all necessary and appropriate studies for this agent, including special studies/data requested by the Division during pre-submission communications/discussions, completed and submitted in this NDA?	Yes	All provided under prior INDs submissions
5)	If the formulation to be marketed is not identical to the formulation used in the toxicology studies (including the impurity profiles), has the Sponsor clearly defined the differences and submitted reviewable supportive data?	NA	

6)	Does the route of administration used in animal studies appear to be the same as the intended human exposure? If not, has the sponsor submitted supportive data and/or an adequate scientific rationale to justify the alternative route?	Yes	.
7)	Has the sponsor submitted a statement(s) that all the pivotal Pharm/Tox studies have been performed in accordance with the GLP regulations (21 CFR 58) or an explanation for any significant deviations?	Yes	
8)	Has the sponsor submitted a statement(s) that the Pharm/Tox studies have been performed using acceptable, state-of-the-art protocols which also reflect agency animal welfare concerns?	Yes	
9)	Has the proposed draft labeling been submitted? Are the appropriate sections for the product included and generally in accordance with 21 CFR 201.57? Is information available to express human dose multiples in either mg/m^2 or comparative serum/plasma AUC levels?	Yes Yes Yes	
10)	From a Pharm/Tox perspective, is this NDA fileable? If not, please state in item #11 below why it is not.	Yes	
11)	Reasons for refusal to file:		

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Krishan L. Raheja
7/20/2009 10:34:32 AM
PHARMACOLOGIST

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