CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER:

21-187

PHARMACOLOGY REVIEW

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

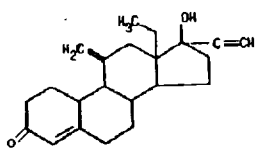
KEY WORDS:
Reviewer Name: Krishan L. Raheja
Division Name: DRUDP
HFD#: 580
Review Completion Date: 9-18-2000

Review number: 1
IND/NDA number: NDA 21-187
Serial number/date/type of submission: 000/12-28-1999/original submission
Information to sponsor: Yes ( ) No (X)
Sponsor (or agent): Organon Inc.
Manufacturer for drug substance: Diosynth BV, The Netherlands

Drug:

Code Name: Org 3226, Org OD 14
Generic Name: 3-keto-desogestrel, etonogestrel
Trade Name for drug product: NuvaRing (3-keto-desogestrel/ethinyl estradiol ring)
Chemical Name for 3-keto-desogestrel: 13-ethyl-17-hydroxy-11-methylene-18,19-dinor -17a-pregn-4-ene-20-yn-3-one
CAS Registry Number:
Molecular Formula/ Molecular Weight: 3-keto-desogestrel $C_{22}H_{28}O_2$/324.46; ethinyl estradiol $C_{20}H_{24}O_2$/296.41

Structure: Etonogestrel Ethinyl estradiol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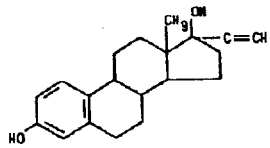
$C_{20}H_{24}O_2$

Relevant INDs/NDAs/DMFs: [redacted]

NDA 20-071 for Desogen tablets (desogestrel & ethinyl estradiol)

[redacted]

Drug Class: etonogestrel (Progestin)
ethinyl estradiol (estrogen)

Indication: Contraception

Clinical formulation: NuvaRing is a non-biodegradable, flexible, transparent, colorless to almost colorless combination contraceptive vaginal ring (CCVR) containing two active components, etonogestrel (3-keto-desogestrel, 3-KDSG) and ethinyl estradiol. When placed in the vagina, each ring releases 0.120 mg/day of etonogestrel and 0.015 mg/day of ethinyl estradiol over a 3-week period of use. NuvaRing is made of ethylene vinylacetate copolymers and magnesium stearate and contains 11.7 mg etonogestrel and 2.7 mg ethinyl estradiol. NuvaRing has an outer diameter of 54 mm and a cross-sectional diameter of 4 mm.

Route of administration: Vaginal. Each ring is to be used for one cycle; a cycle consists of 3 weeks of ring use followed by a one-week ring-free interval.

Proposed clinical protocol or Use: To be used for contraception

Previous clinical experience: It was stated that NuvaRing has been evaluated in adequate and well controlled studies involving 2322 healthy female subjects.

Disclaimer -- use of sponsor's material

Introduction and drug history: 3-keto-desogestrel is an active metabolite of desogestrel. Desogestrel in combination with ethinyl estradiol is a FDA approved marketed contraceptive as Desogen tablets under Organon NDA 20-071 and as Ortho-Cept 21 tablets and Ortho-Cept 28 tablets under Ortho Pharmaceutical NDA 20-301, respectively.

Desogestrel is rapidly and almost completely absorbed and converted into 3-keto-desogestrel, its biologically active metabolite. Following oral administration, the relative bioavailability of desogestrel, as measured by serum levels of 3-keto-desogestrel, is about 84% (PDR).

Studies reviewed within this submission: All studies not previously reviewed under sponsor's [redacted] These include

[redacted]

Studies not reviewed within this submission: All studies reviewed under Organon Inc. IND [redacted] for 3-keto-desogestrel (etonogestrel) and IND [redacted] for Desogestrel are not reviewed again. Instead copies of the reviews conducted under various IND submissions have been appended. Also pertinent studies related to the present formulation have been briefly summarized under the appropriate sections.

PHARMACOLOGY:

Pharmacology of 3-keto-desogestrel (etonogestrel) has been reviewed under the original IND [redacted] submission dated 1-30-1992 [redacted] Copy of the review dated 3-24-1992 is appended.

Mechanism of action: Combined hormonal contraceptives act by suppression of gonadotropins. Although the primary effect of this action is inhibition of ovulation, other alterations include changes in the cervical mucus (which increase the difficulty of sperm entry into the uterus) and the endometrium (which reduce the likelihood of implantation).

The following studies were conducted by the sponsor during 1977 to 1996 as non GLP studies.

Drug Activity Related to Proposed Indication:

Anti-fertility activity of desogestrel by oral administration and that of 3-keto-desogestrel by oral and subcutaneous administration was determined in female Cpb:ORGA rats under studies SDGRR Nos. 836, 1743 and 4553. The results are summarized in table below:

Table 1

Compound	Daily dose (mg/kg)	Number of rats with ovulation		
		Normal	Postponed	Inhibited
Control		46	1	0
Desogestrel (oral)	2 x 0.18	6	0	0
	2 x 0.37	5	9	4
	2 x 0.75	0	3	9
	2 x 1.5	0	0	18
Norethisterone (oral)	2 x 3	3	0	3
	2 x 6	1	2	2
	2 x 12	0	4	2
3-keto-desogestrel (oral)	O	11	1	0
	2 x 0.38	5	1	0
	2 x 0,75	4	2	0
	2 x 1.5	2	4	0
	2 x 3	0	0	5
3-keto-desogestrel (subcutaneous)	0	23	0	1
	2 x 0.006	6	0	0
	2 x 0.012	0	1	5
	2 x 0.024	0	0	6
	2 x 0.045	0	0	6
	2 x 0.09	0	0	3
	2 x 0.18	0	0	6
	2 x 0.38	0	0	6
	2 x 0.75	0	0	5

Results show that desogestrel and 3-keto-desogestrel administered orally to mature rats (236-386 g) displayed complete ovulation inhibition at twice daily dose of 1.5 mg/kg and 3.0 mg/kg, respectively. However, 3-keto-desogestrel administered subcutaneously displayed complete ovulation inhibition at twice daily dose of 0.024 mg/kg.

The anti-fertility activity of subcutaneous desogestrel, etonogestrel and their metabolites was determined by ovulation inhibition in Cpb.ORGA female rats under study SDGRR 4371. The results were as follows:

Desogestrel displayed complete ovulation inhibition at twice daily dose of 0.75 mg/kg
Etonogestrel displayed complete ovulation inhibition at twice daily dose of 0.024 mg/kg
3-keto-5a-H-DGS displayed no ovulation inhibition at twice daily dose of 0.75 mg/kg
3a-OH-5a-H-DSG displayed on ovulation inhibition up to twice daily dose of 1.5 mg/kg
3B-OH-5a-H-DSG displayed no ovulation inhibition up to twice daily dose of 3.0 mg/kg
3a-OH-DSG displayed complete ovulation inhibition at twice daily dose of 0.048 mg/kg
3B-OH-DSG displayed complete ovulation inhibition at twice daily dose of 0.096 mg/kg.

Results showed that the anti-ovulatory activity of 3-keto-desogestrel was about 30 times greater than desogestrel and 2 and 4 times as active as 3a-OH and 3B-OH metabolites, respectively. The activity of 5a-H-metabolites was 30 times lower than 3-keto-desogestrel.

No effect on transport and implantation of fertilized ova was reported for either oral administration of desogestrel or etonogestrel (SDGRR 836 & SDGRR 1743).

Anti-fertility activity of oral desogestrel and etonogestrel was also evaluated in female Dutch belted rabbits by determining the inhibition of coitus-induced ovulation (SDGRR 836 & SDGRR 1743). Reference substance was norethisterone.

The results were as follow:

Desogestrel displayed complete ovulation inhibition at 0.1 mg/kg
Etonogestrel displayed complete ovulation inhibition at 0.05 mg/kg
Norethisterone caused ovulation in 2/4 rabbits at a dose of 1.6 mg/kg.

The effect of desogestrel and etonogestrel on blocking spermatozoa migration through the cervix was evaluated in pseudopregnant rabbits. Rabbits were inseminated and ovulation was induced by i.v. injection of HCG. All ova were recovered and the % of unfertilized ova determined.

It was reported that an oral dose of 0.2 mg/kg/day of DSG and ENG displayed respectively, 100% and 96% inhibition. Norethisterone up to a dose of 3.2 mg/kg/day had no effect.

Ancillary Pharmacology Studies:

Progestational activity: Progestational activity of desogestrel and 3-keto-desogestrel and their metabolites on endometrium transformation was evaluated by the Clauberg-McPhail test in immature Cpb.CH rabbits (SDGRR 836, 1743, 4371 & 4553).

In rabbits estradiol-primed endometrium transformation takes place under the influence of progestational compounds. In the Clauberg-McPhail test, progestational agent was administered orally or subcutaneous.

Rabbits were primed with a daily dose of 0.002 mg estradiol benzoate for 8 days (days 1-8). Test compound was administered on days 8 to 13. Autopsy was performed on day 13.
Using this animal test model, progestational activity of desogestrel, 3-keto-desogestrel and their metabolites was studied. The results were as follows:

Oral Desogestrel was active at 0.030 mg/kg. Equivalent response was observed with 0.62 mg/kg NET
Oral Etonogestrel was active at 0.015 mg/kg. Equivalent response was observed with 0.5 mg/kg progesterone s.c.
Subcutaneous etonogestrel was active at 0.008 mg/kg. Reference substance was progesterone 0.5 mg/kg sc
Subcutaneous desogestrel was active at 0.060 mg/kg
Subcutaneous 3-keo-5a-H-DSG was active at 0.5 mg/kg
Subcutaneous 3B-OH-5a-H-DSG was active at 1.0 mg/kg
Subcutaneous 3a-OH-DSG was active at 0.016 mg/kg
Subcutaneous 3B-OH-DSG was active at 0.016 mg/kg
Reference substance was progesterone total dose 0.5 mg/kg sc on days 8-13.

Results thus showed that as regards to progestational activity, 3-keto-DSG, 3a-OH-DSG and 3-B-OH-DSG were the most active. 3-keto-DSG was about 8 times more active than DSG and 3a-OH-DSG and 3B-OH-DSG were a little less potent than 3-keto-DSG.
Progestational activity of intra-uterine desogestrel and etonogestrel on endometrium transformation was also determined in the McGinty test in immature Cpb:CH rabbits.
It was reported that while desogestrel was active at 0.005 mg/kg, etonogestrel was less effective. The effect of 0.01 mg/kg dose of etonogestrel was equivalent to that of 0.01 mg/kg progesterone.
Androgenic and anabolic activity: Desogestrel and 3-keto-desogestrel had lower andogenic activity compared to norethisterone in the Hersberger test in castrated immature male rats. Desogestrel, 3-ketodesogestrel and norethisterone and reference compound, methyl testosterone were administered orally. Also activity of 3-keto-desogestrel against methyl testosterone was tested when administered subcutaneously (SDGRR 836 & 1743). As shown in table below both desogestrel and 3-keto-desogestrel had lower androgenic activity compared to norethisterone.
Table 2

Compound	Daily oral dose mg/kg	Weight (% of controls)		
		Seminal vesicles	Ventral prostate	m.levator ani
Control (n=12)		100	100	100
Methyl testosterone (n=6) oral	1.25	128	240	99
	2.5	126	241	117
	5.0	159	341	128
	10.0	191	374	134
Desogestrel (n=6) oral	10	140	147	129
	20	162	224	155
	40	206	230	140
	80	**304**	310	182
Etonogestrel (n=6) oral	10	130	158	105
	20	166	177	147
	40	229	278	153
	80	**250**	266	166
Norethisterrone (n=6) Oral	10	223	181	118
	20	**260**	218	137
	40	291	199	129
	80	356	306	158
Methyl testosterone (n=6) subcutaneous	1.2	336	650	152
	2.5	370	600	137
	5.0	490	740	177

Etonogestrel (n=6) Subcutaneous	0.3	111	146	93
	1.2	141	184	126
	5.0	275	370	195

Androgenic effect is evaluated by an increased weight of seminal vesicles and testes, anabolic effect by increased weight of m. levator ani.

Results thus suggest that androgenic activity of etonogestrel was about 25% of the norethisterone activity. The activity of desogestrel and 3-ketodesogestrel was similar.

Androgenic, anabolic, gonad inhibitory and glucocorticoidal activity of desogestrel and 3-keto-desogestrel were evaluated in study SDGRR 836 and 1743. In this study, 22-24 day old male and female rats were administered 20 mg/kg desogestrel orally or 3-keto-desogestrel orally or subcutaneously for 7 days. The androgenic activity was based on increased weight of seminal vesicles and testes; anabolic effect by increased weight of m. levator ani; gonad inhibitory activity by decreased weight of ovaries and testes and glucocorticoidal activity by decreased weight of thymus and adrenals. Comparisons were made with vehicle controls. Significant findings were follows:

Oral desogestrel in male rats decreased thymus, testes, seminal vesicle and ventral prostate weight and increased that of m. levator ani. In female rats weight of thymus and ovaries was decreased.
Oral etonogestrel decreased thymus, testes and seminal vesicle weights and increased that of m. levator ani. In females it decreased weight of the uterus.
Subcutaneous etonogestrel in male rats decreased thymus, testes and seminal vesicle weight and increased weight of m. levator ani.
Subcutaneous etonogestrel decreased adrenal, thymus and ovaries weights.

These results showed that desogestrel and etonogestrel had little androgenic but had anabolic activity. They had gonad inhibitory and glucocorticoidal activity. However, since no reference progestin was used, relative potency is not known.

Estrogenic activity: Estrogenic activity was determined in estrogen primed ovariectomized rats (SDGRR 1743). In this Allen-Doisy test, compounds with estrogenic activity induce development of the vaginal epithelium (especially cornification).
Table 3

Compound	Total dose/rat (mg/kg)	Ratio positive/total	
		Rat	Smear
Ethinylestradiol	0.04	6/16	11/64
	0.08	14/16	29/64
Desogestrel	0.04	0/8	0/32
	0.40	0/8	0/32
	4.00	0/8	0/32
	40.00	0/8	0/32

Etonogestrel	0.15	0/8	0/32
	0.30	0/8	0/32
	0.63	0/8	0/32
	1.25	0/8	0/32
	50	0/8	0/32

Results showed that oral administration of desogestrel and etonogestrel had no estrogenic activity

Anti-estrogenic activity: The anti-estrogenic activity of desogestrel and its metabolites administered sc was evaluated by their effect on vaginal cornification in estradiol-treated ORGA ovariectomized rats (SDGRR 4371). The results are shown in table below:

Table 4

No. of Animals	Test compound	Dose level (mg/kg)	% of smears with estrogen response
40	Estradiol	0.001	89
8		0.001	90
8	3-keto-5a-H-DSG	0.5	71
8	3a-OH-5a-H-DSG	0.25	79
		0.5	83
8	3B-OH-5A-H-DSG	0.25	90
8		0.5	57
8		0.5	92
8		1.0	92
8	3a-OH-DSG	0.008	78
8		0.016	68
8		0.032	56
8		0.25	0
8	3B-OH-DSG	0.016	81
16		0.032	53
8		**0.064**	14
8	DSG	0.060	90
8		0.24	82
8		**1.0**	39
8	Etonogestrel (ENG)	0.004	85
8		0.006	56
16		0.016	33
8		**0.032**	7

As with progestational activity, for anti-estrogenic activity, 3-keto-DSG, 3a-OH-DSG and 3B-OH-DSG were most active. 3-keto-DSG was more than 30 and 4 times more active than DSG and both 3-OH metabolites. 5A-H metabolites had minimal activity.

In another study (SDGRR 1743), anti-estrogenic activity was determined by inhibition of estradiol-induced transformation of vaginal epithelium in rats. While an oral dose of 8 mg/kg etonogestrel completely prevented the estrogen induced effect, similar effect was achieved with a dose of 0.06 mg/kg via the subcutaneous route of administration. The anti-estrogenic activity of etonogestrel and levonorgestrel was similar (SDGRR 4553).

Mineralocorticoid activity: The mineralocorticoid activity of desogestrel and etonogestrel was determined by the extent of protection afforded by these compounds in adrenalectomized rats. The results shown in table below suggest that while desogestrel had some mineralocorticoid effect, etonogestrel was devoid of such activity.

Table 5

Daily oral treatment (Starting on day 2)	# of rats	Number of survivors on day:						
		4	5	6	7	8	9	10
Vehicle	7	7	6	2	1	0		
Desogestrel (2.5 mg/kg)	8	8	8	8	6	3	1	0
Vehicle	7	6	1	0	0	0	0	0
Etongestrel (2.5 mg/kg)	8	7	1	0	0	0	0	0

Platelet aggregation inhibitory activity of desogestrel and etonogestrel was determined by their effect on rabbit platelet aggregation induced in vitro by ADP, collagen or thrombin (SDGRR 836).
It was reported that both desogestrel and etonogestrel had no effect at the 0.1 mg/ml dose level tested.

Receptor binding studies: The relative binding affinities (RBA) of etonogestrel (ENG), levonorgestrel (LNG), and levonorgestrel acetate (LN-ac) were assessed in vitro for progestagen (P) and androgen (A) receptors using cytosol of MCF7 cells (SDGRR 4553). The results were as follows:

Table 6

Compound	Progestagen receptor Gestodene=100% (n)	Androgen receptor Dihydrotestosterone=100(n)
Gestodene	100+20 (5)	0.79+0.44 (3)
LNG-ac	62+9 (5)	0.58+0.12 (3)
LNG	116+39 (4)	7.67+1.52 (3)
ENG	188+20 (4)	4.47+0.67 (3)

Results showed that the RBA of ENG for the progesterone receptor was approx. 3 and 1.6 times higher than that for LNG-ac and LNG, respectively. LNG-ac had essentially no binding to androgen receptor, while LNG and ENG had slight binding.

In vitro plasma protein binding: Using equilibrium dialysis and plasma from 49 healthy female volunteers, 4 female Beagle dogs and 15 female Wistar rats, binding of etonogestrel and desogestrel to plasma proteins in vitro was determined.

It was reported that protein binding of desogestrel to plasma proteins was high (approx 99.8%) in all 3 species and higher than the binding of etonogestrel to plasma proteins (approx. 98.4% in women plasma and approx. 97.3% in animal plasma). Binding was not concentration dependent. Addition of EE did not influence the plasma protein binding of DSG and ENG.

Summary of pharmacology: Etonogestrel's (3-keto-desogestrel) contraceptive action is attributed to its suppression of gonadotropins. Although the primary mechanism of this action is inhibition of ovulation, other alterations included changes in the cervical mucus (which increases the difficulty of sperm entry into the uterus). Etonogetrel has progestational activity after oral, subcutaneous and intra-uterine administration in rabbits. Etonogestrel has weak androgenic and anabolic activities after oral administration compared to subcutaneous administration and has no estrogenic activity but has strong anti-estrogenic activity in rats. Etonogestrel has ovulation inhibitory activity and causes inhibition of the migration of spermatozoa in rabbits after oral administration. The ovulation inhibitory effect of etonogestrel is stronger with subcutaneous

compared to oral administration. The biological activity of desogestrel is mediated by its transformation to 3-keto-derivative. 3-keto-DSG along with 3a-OH and 3B-OH-DSG, contribute to desogestrel's biological activity.

SAFETY PHARMACOLOGY: Essentially no safety studies are submitted.

Neurological effects: none submitted
Cardiovascular effects: were evaluated in 2 cats. The response of nictitating membrane to pre- and post-ganglionic sympathetic nerve stimulation was 34 and 19 % higher compared to control value in one cat and no increase in the other cat. Effect on blood pressure on peripheral vagus stimulation was –13% in one cat and +15% in the other cat, with noradrenaline it was –14% and –25%. Isoprenaline had no effect on effect on HR and BP in one cat and decreased 43% and 36% in the other. Thus the information is scanty and not consistent in 2 cats.
Pulmonary effects: none submitted
Renal effects: none submitted
Gastrointestinal effects: none submitted
Abuse liability: none submitted

Other: No significant anti-inflammatory activity, mineralocorticoid activity or effect on platelet aggregation was reported.

Conclusions: No conclusion can be drawn from the data submitted.

Summary: Safety pharmacology was not adequately tested.

PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

The pharmacokinetic studies submitted in the NDA submission were reviewed under IND [redacted] serial submission No. 038 dated 8-24-1998 on 5-19-1999. These studies include the following:

1. Pharmacokinetics of Org 3236 in Beagle dogs using chronic oral administration of 3-ketodesogestrel (etonogestrel) plus ethinyl estradiol and vaginal application of silastic rings releasing 3-keto-desogestrel plus ethinyl estradiol. SDG Release Report No. 2001.
2. Pharmacokinetics of 3-keto-desogestrel after oral dosing of desogestrel plus ethinyl estradiol to female Wistar rats. SDG Release Report No. 2411
3. Pharmacokinetic profiles of 3-keto-desogestrel and levonorgestrel under steady state conditions after oral administration of desogestrel, desogestrel plus EE, 3-ketodesogestrel, 3-

keto-desogestrel plus EE and levonorgestrel plus EE to female Beagle dogs. SDG Release Report No. 2678

4. Pharmacokinetics of 3-keto-desogestrel and ethinyl estradiol in Rhesus monkeys treated with contraceptive rings for 3 months. SDG Release Report No. 2932.
5. Pharmacokinetics of 3-keto-desogestrel in Rhesus monkeys after single and multiple oral dosing of desogestrel + EE after single intravenous administration of Org 3236 (3-keto-desogestrel) + EE and after implantation of a subcutaneous implant releasing 3-keto-desogestrel. SDG Release Report No. 2938.
6. A 24-month oncogenicity study with 3-keto-desogestrel in the rat via subcutaneous implants. Sub report on bioanalysis. SDG Release Report No. 4416
7. Release characteristics of subdermal 3-keto-desogestrel containing EVA implants (batch C.P.090.032) in mature female Beagle dogs: a 4.6-year study. SDG Release Report No. 4525
8. Plasma levels of 3-keto-desogestrel during a thirteen-week subcutaneous toxicity of 3-keto-desogestrel in dogs using polyethylene vinyl acetate/silastic implants releasing 3-keto-desogestrel
9. Comparative pharmacokinetic study with 3-keto-desogestrel and desogestrel in female wistar rats after oral administration and subdermal silastic implants releasing 3-keto-desogestrel

Of the above studies 2 studies, one in dogs (SDGRR # 2001) and other in monkeys (SDGRR #2932) where PK was determined using vaginal rings are pertinent to the present submission for NuvaRing. PK data for these 2 studies is summarized below:

In the dog study, three groups of female dogs (3/g) were used. One group was treated orally with 150 ug 3-keto-desogestrel plus 30 ug EE daily for 21 days. In the second and third groups, one and three prototype vaginal rings were inserted. Vaginal rings were allowed to stay for 21 days. Blood was collected on days 2, 3, 4, 9, 13 and 19 of treatment period. On the last day blood was collected just before tablet intake and at various time intervals up to 24 hours for all 3 groups. 3-keto-desogestrel was determined by RIA.
In vivo release rates calculated from the amount left in rings after 21 days was 165 and 440 ug 3-keto-desogestrel and 19 and 54 ug EE with one and three vaginal rings respectively.

The AUC for 3-keto-desogestrel during the whole treatment period was as shown in table below:
Table 7

Treatment (dose in umol)	AUC (pmol/ml.h) For total dose	AUC (pmol/ml.h) per umol dose
Oral tablet (9.72)	1094 + 172	112 + 17.9
1 vaginal ring (10.7)	1801 + 150	168 + 14.0
3 vaginal rings (28.5)	4491 + 596	158 + 20.0

Similar AUC/unit dose indicated that the release rate from the vaginal rings was linear for daily doses of 165 to 440 ug 3-keto-desogestrel. Also AUC/unit dose suggested that the relative bioavailability of 3-keto-desogestrel was 150% and 141% for the 1 and 3 rings respectively, taking the bioavailability of 3-keto-desogestrel tablets as 100%.

In the monkey study (SDGRR # 2932), eleven monkeys were used. Four monkeys in group 1 were inserted with drug-free vaginal rings during 2 period of 6 weeks each. Group 2 (4 monkeys)

and group 3 (3 monkeys) were inserted with vaginal (VR) releasing 80 ug/day 3-keto-desogestrel and 11 ug/day EE. Three was one week washout period between 2 six week insertion periods. Blood samples were taken on day 0 before ring insertion and then on days 7, 13, 42, 63 and at the end of insertion period.

Mean serum levels of 3-keto-desogestrel (ng/ml) and EE (ng/ml) were as shown table below:
Table 8

	3-keto-desogestrel				Ethinyl estradiol			
Group	1 Period		2		1 Period		2	
	Week		Week		Week		Week	
	2	6	2	6	2	6	2	6
2	2.71	0.99	1.31	0.96	0.29	0.21	0.32	0.26
3	1.89	0.76	1.16	0.89	0.26	0.24	0.24	0.21
Mean	2.30	0.88	1.23	0.92	0.28	0.22	0.28	0.24

It was stated that there was an initial burst with a mean 3-keto-desogestrel serum level of 2.8 ng/ml. Values of 3-K-DSG were higher in period 1 compared to period 2. For EE after an initial burst with a level of 0.37 ng/ml, mean + sd showed no significant difference between week 2 and week 6. One week washout between 2 insertions had no significant effect on serum levels of 3-kDSG and EE in the second period.

Pharmacokinetics in humans: Serum 3-keto-desogestrel concentrations (mean ± sd for 16 subjects) expressed as pg/ml observed with NuvaRing during a period week period are shown in table below:
Table 9

	1 week	2 week	3 week
3-keto-desogestrel	1578 +408	1476 + 362	1374 + 328
Ethinylestradiol	19.1 + 4.5	18.3 + 4.3	17.6 + 4.3

Mean + SD Cav serum value (pg/ml) for 3-keto-DSG in a multiple dose study with 150 ug DSG + 25 ug EE during cycle 3 in healthy women was 1603.7 + 650.03 (CP&B Review of NDA 21-090 dated 3-3-2000).

PK parameters of 3-keto-desogestrel and ethinyl estradiol determined during one cycle of NuvaRing in 16 subjects is given in table below. Values are mean + sd.
Table 10

3-keto-desogestrel				Ethinyl estradiol			
C_{max} (pg/ml)	T_{max} (hr)	$T_{1/2}$ (hr)	CL (l/hr)	C_{max} (pg/ml)	T_{max} (hr)	$T_{1/2}$ (hr)	CL (l/hr)
1716 + 445	200.3 + 69.6	29.3 + 6.1	3.4 + 0.8	34.7 + 17.5	59.3 + 67.5	44.7 + 28.8	34.8 + 11.6

Comparative pharmacokinetics: Comparison of pharmacokinetics of 3-ketodesogestrel (etonogestrel) after vaginal ring insertion in dog, monkey and humans is shown in sponsor's table (volume 19, page 0025)

Table 11

Species	Time after Insertion	Release rate Mg/day	Dose mg/kg/day	Mean 3-kDSG Serum concen-tration (pg/ml)
Dog	3 days 21 days	0.165** (vaginal ring)	0.016	1556 897
Dog	3 days 21 days	0.440** (3 vaginal rings)	0.044	4480 2277
Monkey	7 days1	0.080* (vaginal ring)	0.016	2800
Human	3 days 21 days	0.120* (vaginal ring)	0.002	1140 1436

* mean declared in vitro release rate ** mean in vivo release rate based on remnant ex-vivo content
1 study duration was 12 weeks, plasma samples were taken at steady state at Day 7 after insertion
Systemic exposure in the dog and monkey was just about 2 times the human systemic exposure with NuvaRing on day 21.

ADME

Absorption: Absorption of Desogetrel and Etonogestrel is very rapid. In the rat, dog and humans after oral administration, C_{max} is attained in 1-2 hours. With vaginal ring in dogs and monkeys C_{max} is reached in 3 and 7 days respectively. With subcutaneous implants in rats, dogs and monkeys, C_{max} is attained in 14, 7 and 1 day. In humans it was reported that etonogestrel released from NuvaRing is rapidly absorbed. C_{max} was reached in about 8 days.

Distribution: Etonogestrel is 98.4% bound to serum albumin in humans and 97.3% in rats and dogs. Binding was not concentration dependent and addition of ethinyl estradiol did not affect it.

Metabolism: Etonogestrel is completely metabolized by pathways of steroid metabolism.

A proposed biotransformation scheme based on the metabolites identified in the urine and feces in rats administered a single oral dose of [^{3}H]-desogestrel or a single oral or subcutaneous dose of [^{3}H]-etonogestrel is as follows (SDGRR # 4562):

For 3-keto-DSG
-reduction of the 3-keto moiety to a 3a or 3B hydroxy, followed by sulfation
-reduction of the ^{4} double bond to 5a or 5B substituent
-15a-hydroxylation, followed by glucuronidation
-epoxydation of the 11 methylene moiety
-formation of a 3a or 3 B hydroxy, followed by sulfation or the formation of a 3-keto
-2a-hydroxylation
-sulfation of the 17B-OH

Desogestrel was reported to undergo following reactions:

-form 3a or 3B hydroxy substituent, followed by sulfation or the formation of a 3-keto moiety
-all next steps are identical to the ENG route of metabolic reactions.

Urinary and fecal metabolites formed and identified are shown in sponsor table K9 appended.

In the dog proposed metabolic scheme based on a single oral or subcutaneous dose of [^{3}H]-ENG or single oral dose of [^{3}H]-DSG and metabolites identified in plasma, urine and feces is as follows (SDGRR # 4563):

For 3-keto-DSG
3a- or 3B-hydroxylation or formation of a 3-keto moiety
reduction of the ^{4} double bond to a 5aH or 5BH substituent

6a-hydroxylation
15a-hydroxylation
16-hydroxylation
D-homo-annulation to a 17 A-keto-D-homo metabolite
reduction of the 3-keto moeity to a 3a- or 3B-hydroxy substituent
hydroxylation of the ethyl substituent at C13
DSG was reported to undergo the following metabolic reactions:

3a or 3B hydroxylation or formation of a 3-keto moiety
all next steps are identical to the ENG route of metabolic reactions.

3 metabolites (P_1, P_2, P_3) were reported in dog's plasma with etongestrel and 5 (P_1, P_2, P_3, P_4, P_5) with desogestrel, when both were administered orally.

The in vitro metabolism of [^{3}H]-ENG and [^{3}H]-DSG in female Wistar rat, New Zealand White rabbit, Beagle dog and humans was determined using hepatic microsomes (SDGRR # 4597).

Both ENG and DSG were converted to several metabolites and some of the major metabolites of ENG were also main metabolites of DSG. ENG was a major metabolite of DSG with rabbit, dog and human hepatic microsomes and a minor metabolite of rat hepatic microsome.

The major metabolites of ENG formed by rat hepatic microsomes were:
-6B-hydroxy, 5a-H, 6a-hydroxy metabolite of ENG
-3a-hydroxy, 5a-H metabolite of ENG

The major metabolites formed by rabbit hepatic microsomes were:
-6B-hydroxy-13-hydroxyethyl metabolite of ENG
-6B-hydroxy metabolite of ENG
-13-hyroxyethyl metabolite of ENG

The major metabolites formed by dog hepatic microsomes were:
-15a-hydroxy metabolite of ENG and/or 13-hydroxyethyl metabolite of ENG

The major metabolites formed by human hepatic microsomes were:
-6B-hydroxy-13-hydoxyethyl metabolite of ENG
-6B-hydroxy metabolite of ENG and/or 3B-hydroxy, 5a-H, 6a-hydroxy metabolite of ENG
-15a-hydroxy metabolite of ENG and/or 13-hydroxyethyl metabolite of ENG

Using human liver microsomes in the in vitro metabolism of [^{3}H]-DSG, it was reported that the biologically active metabolite of DSG is ENG. The initial step in the bioactivation of DSG in vitro is catalyzed by the iso-enzyme CYP2C, which catalyzes the initial hydroxylation of DSG. Using [^{3}H]-ENG, it was observed that CYP3A4 is involved in the oxidative metabolism of ENG with the formation of 6B, 13-ethyl-dihydroxylated metabolites of as the major metabolites observed. The biological activity of oxidative metabolite of ENG is not reported.

Elimination: Following a single oral dose of [^{3}H]-DSG or a single oral or sc dose of [^{3}H]-ENG in the rat and dog, the cumulative (0-168 h) excretion of radioactivity was as shown in table below:

Table 12
Rat

Treatment	Urine (%)	Feces (%)	Total (%)
Oral ENG 52 ug/kg.	11.6 + 1.1	80.8 + 0.8	92.4 + 1.5
s.c. ENG 52 ug/kg	11.8 + 3.0	79.5 + 2.2	91.3 + 0.9
Oral DSG 56 ug/kg	15.6 + 3.9	74.7 + 1.4	90.3 + 3.5
Dog			
Oral ENG 52 ug/kg	18.5 + 2.2	68.3 + 3.2	86.7 + 3.2
s.c. ENG 62 ug/kg	19.5 + 2.5	67.9 + 2.2	87.3 + 1.7
Oral DSG 67 ug/kg	20.9 + 2.0	65.5 + 2.8	86.4 + 3.3

Values are mean + SD (n=3).

Other studies: Excretion of [^{3}H]-ENG and /or its metabolites in milk of lactating rats.

Three females were dose orally for 2 days a dose of 0.002 mg/kg and on the third day same dose of [^{3}H]-ENG was given. At 5.5 h after administration of radiolabeled dose of ENG, including 30 minutes of sucking, distribution of radioactivity in stomach contents and rest of the body was determined in 27 pups.
0.0013 % and 0.0007% of the maternal dose was recovered in stomach contents and rest of the body/pup, respectively.

Comments:

Summary: In dogs the release rate of 3-keto-DSG from the vaginal rings was linear for daily dose of 165 ug to 440 ug. The bioavailability of 3-keto-DSG was significantly higher by the subcutaneous route of administration compared to oral administration.
In the 12-week monkey toxicity study, serum 3-keto-DSG levels were lower with the second ring compared to first ring insertion. Serum levels determined on Day 7 of insertion were about 2 times higher than those observed in humans with the proposed vaginal ring. In humans serum levels of 3-keto-DSG were maintained for the 3-week vaginal ring application period.

3-keto-DSG was reported to be 97.3% bound to serum albumin in rats and dogs and 98.4% in humans. Under in vitro and in vivo conditions, DSG was metabolized to 3-keto-DSG and 5 other metabolites were detected. These included 3a-OH-DSG, 3B-OH-DSG, 3a-OH-5a-H-DSG, 3B-OH-5a-H-DSG and 3-keto-5a-H-DSG.
The excretion of 3-keto-DSG administered orally or subcutaneously and that of DSG administered orally in rats and dogs was mainly via feces and to less extent via urine.

TOXICOLOGY:

General comments: The following toxicity studies with 3-keto-desogestrel were reviewed under the original IND [redacted] submission dated 1-30-1992. A copy of the review dated 3-24-1992 is appended. Also toxicity studies for desogestrel reviewed under IND [redacted] are listed and copies of the reviewed appended. All the toxicity studies were conducted in 1970s and 1980s and therefore do not meet the current GLP requirements.

Studies reviewed under original IND [redacted] submission

- Acute toxicity studies with 3-KDSG in rats and mice

Repeat dose toxicity studies

- 13-week intravaginal and oral (tablet) toxicity study in the cynomolgus monkey followed by a 4 week withdrawal period with the combination of 3-KDSG and EE. SDG Release Report No. 2088
- A three month toxicity study in Rhesus monkeys with vaginal rings containing 3-KDSG + EE. SDG Release Report NO. 2732
- A 52-week oral toxicity study with 3-KDSG in Wistar rats. SDG Release Report No. 1683
- Twenty six-week oral toxicity study with 3-KDSG in Beagle dogs. SDG Release Report No. 1682

Since desogestrel is first metabolized to its active metabolite, 3-keto-desogestrel before it is further metabolized, sponsor has also supported the safety of 3-keto-desogestrel based on the toxicity studies conducted with desogestrel under IND [redacted] The desogestrel toxicity studies were conducted by the oral route of administration and toxicity data was used to support the approval of desogestrel for contraception under Organon Inc. NDA 20-071 for Desogen and under Ortho Pharmaceuticals NDA 20-301 for Ortho-Cept 21 tablets and Ortho-Cept-28 tablets, respectively.

Toxicity studies with desogestrel reviewed under original IND [redacted] submission dated 12-15-1988 were:

- Acute single dose toxicity in rats and mice
- Twenty-six week oral toxicity with desogestrel in rats
- Twenty-six week oral toxicity with desogetrel in Beagle dogs
- Desogestrel toxicity to rats in oral administration for 52 weeks followed by 7 week withdrawal period

- Oral toxicity of desogestrel plus ethinyl estradiol (in ratio of 125:50) to rats-52 week study (final report)
- Desogestrel oral toxicity study in beagle dogs (repeated dosage for 52 weeks followed by an observation period of 13 weeks)
- A 52-week oral toxicity study with the contraceptive combination of desogestrel + ethinyl estradiol (ratio 2.5 : 1) in Beagle dogs
- An oral toxicity study in beagle dogs with a combination of desogestrel and ethinyl estradiol in a ratio of 150/30 ug – One year interim final report
- An oral toxicity study in Rhesus monkeys with a combination of desogestrel and ethinyl estradiol in ratio of 150/30 ug – twelve month interim final report

- Potential tumorigenicity of desogestrel in repeated oral administration to mice for 81 weeks- Final report
- Potential tumorigenicity of desogestrel + ethinyl estradiol in a ratio of 125:50 in repeated oral administration to mice over 80 weeks – Final report
- Potential tumorigenic effects of desogestrel by prolonged oral administration to rats for two years- Final report
- Potential tumorigenic effects of desogestrel + ethinyl estradiol (in a ratio of 125:50) by prolonged oral administration for two years to rats- Final report

The following toxicity studies were reviewed under IND [redacted] serial submission No. 008 dated 10-5-1989:

- An oral toxicity study in beagle dogs with a combination of desogestrel and ethinyl estradiol in a ratio of 150/30 ug. Two year interim final report
- An oral toxicity study in Rhesus monkeys with a combination of desogestrel and ethinyl estradiol in a ratio 0f 150/30 ug. Two-year final report.

The following studies were reviewed under serial submission No. 035 dated 2-21-1991:

- An oral toxicity study in beagle dogs with a combination of desogestrel and ethinyl estradiol in a ratio of 150/30 ug. Three year final report
- An oral toxicity study in Rhesus monkeys with a combination of desogestrel and ethinyl estradiol in a ratio of 150/30 ug. Three year final report

The following toxicity studies were also reviewed under the original IND [redacted] submission

- Primary acute toxicity screening of biomaterials. The agar overlay test with poly-EVA implants containing 65 mg 3-KDSG. Study No. 1032/AO-35 (002813)
- Primary acute toxicity screening of biomaterials. Inhibition of cell growth by poly-EVA containing 65 mg 3-KDSG. SDG Release Report No. 2353
- Assessment of the systemic toxicity intravenously or intraperitoneally injected extracts of implanon in mice. SDG Release Report No. 2472
- A two-week subcutaneous local toxicity with 3-KDSG released from a poly-EVA implant in rats. SDG Release Report No. 2335

- A 13-week subcutaneous toxicity study with two types of implants releasing 3-KDS in rats. SDG Release Report No. 2321
- Evaluation of the toxicity caused by tissue implantation of poly-EVA implants containing 65 mg 3-KDSG in the rabbit. SDG Release Report No. 2353
- A 13-week subcutaneous toxicity study with two types of silastic implants releasing 3-KDSG in Beagle dogs. SDG Release Report No. 2344

Of all the toxicity studies conducted with 3-keto-desogestrel or with desogestrel, the following 3 studies in monkeys where either placebo vaginal rings were used to test their biocompatibility or vaginal suppositories and rings containing 3-keto-desogestrel + EE were used to determine the toxicity of drug administration by the vaginal route, are pertinent to the review of the NuvaRing.

Title: A chronic toxicity study in the Rhesus monkey with placebo vaginal rings. Final report.

This study has not been reviewed before. The study was conducted to determine the compatibility of the vaginal mucosae to the vaginal ring.

Study No.: SDGRR No. 3413
Amendment #, vol #, and page No.: vol 45/page 0002
Conducting laboratory and location: Organon International B.V. The Netherlands
Date of study initiation: 5-8-1992
GLP compliance: not indicated
QA Report: not indicated

Methods:
Dosing:
Species/strain: Rhesus monkey (Macca mulatta)
Age: young sexually mature adult females
Weight: 2.9 – 3.8 kg
Stellite groups used for toxicokinetic or recovery: none
Dosage groups in administered units: only placebo group (4 monkeys)
Route, form, volume, and infusion rate: intra-vaginal, ring, once
Drug, lot #, radiolabel, and % purity: IP 392/0076 (butyl Loctite adhesive)
IP 392/0075 (ethyl Loctite adhesive)
Formulation /vehicle: vaginal rings/placebo

Observations and times:
Clinical signs: twice daily
Detailed physical examination: pre-test and weekly thereafter
Vaginal and ring examinations: complete vaginal and ring examinations, including a digital palpation of the vagina were performed weekly on unanesthetized monkeys to insure that the ring was properly in place. Every third week ring was removed and reinserted in all monkeys to simulate a clinical situation.
Body weight: pretest and then weekly
Food consumption: 4 times/week

Ophthalmology: -
EKG: -
Clinical chemistry: -
Urinalysis: -
Organ weights: -
Gross pathology: yes
Organs weighed: no
Histopathology: All tissues routinely examined in a toxicity study were preserved. Uterus (body) with cervix and 2 levels of the vagina were examined. Also any tissue masses were examined
Toxicokinetics: no

Results:

Clinical signs: No abnormal clinical findings. All survived the 6-month study duration
Vaginal and ring examination: Initially butyl Loctite adhesive vaginal rings were inserted. Three of four monkeys had the ring break following a menstrual period (i.e. within 1-4 days after the last menses of the cycle between the period of 21 to 126 test days. The broken rings were replaced by rings connected with Loctite ethyl adhesive, which remained intact for a period of 2-3 months.
Body weights: no adverse affect
Food consumption: not affected
EKG: -
Hematology: -
Clinical chemistry: -
Organ weights: -
Gross pathology: no adverse findings
Histopathology: Pathologist's reported stated that examination of 2 levels of the vagina, cervix, and uterus revealed no histomorphological evidence of a tissue reaction to vaginal ring device. The reproductive tract tissues had morphological changes associated with apparently normal menstrual cycle. There was no gross tissue abnormality reported in any other tissue.
Key study findings:

Overall toxicology summary: Under the conditions of this test the vaginal ring device was found to cause no vaginal mucosal alteration and was compatible with the vaginal and cervical mucosa after long term exposure.

The following toxicity study conducted with vaginal rings in monkeys has been reviewed under IND [redacted] original submission dated 1-30-1992 and significant treatment-related findings are summarized here.

Title: 13-week intravaginal and oral (tablet) toxicity study in the cynomolgus monkey followed by a 4 week withdrawal period with the combination 3-keto-desogestrel (etonogestrel) and ethinyl estradiol): 10:1 Final Report.

This study was conducted in 1986 according to laboratory SOP and the report was audited by the QAU.
The experimental design was as follows:

Table 13

Group # Formulation	Route of adminstration	Group designation	Number of females	Dose level ug/kg/day 3-KDSG	EE	Multiples of human dose on BSA basis	Necropsy after 13 w	17 w*
1 suppository	Intravaginal	Control	8	0	0		6	2
2 suppository	Intravaginal	Low	6	5.0	0.5	0.65	6	
3 suppository	Intravaginal	Intermediate	7**	25.0	2.5	3.24	6	
4 suppository	Intravaginal	High	9**	62.5	6.3	8.11	6	2
5 tablet	Oral	High	8	250.0	25.0	32.43	6	2

* = 13 weeks treatment followed by a 4-week treatment-free period

** =one animal died during early treatment period and was replaced. The deaths were reported to be accidental.

Doses used were 2, 10, 25 and 100 times the HTD on body weight basis.

Significant treatment-related findings observed in hematology and clinical chemistry are shown in the following 2 tables:

Hematology results after 6 and 12 weeks of treatment and then at week 17 (i.e. 4 weeks after the recovery period).

Table 14

	Week 6				Week 12			
Group #	RBC (mil/mm^2)	Hb (g/dl)	PVC (%)	PT (sec)	RBC (mil/mm^2)	HB (g/dl)	PVC (%)	PT (sec)
1 ring control	7.2 + 0.5	13.3 + 0.5	43.5 + 1.7	9.6 + 0.2	7.0 + 0.8	12.8 + 1.0	42.1 + 3.2	9.7 + 0.3
2 ring low dose	7.3 + 0.5	13.4 + 1.3	43.3 + 3.5	9.5 + 0.4	7.2 + 0.4	13.2 + 0.7	42.3 + 3.0	9.4 + 0.3
3 ring mid dose	6.9 + 0.5	13.5 + 1.0	42.2 + 2.5	9.6 + 0.3	6.6 + 0.5	12.6 + 0.9	40.3 + 2.8	9.6 + 0.4
4 ring high dose	6.6 + 0.4*	12.6 + 0.5*	39.8 + 1.7	9.4 + 0.4	6.5 + 0.4	12.3 + 0.7	40.0 + 2.4	9.4 + 0.4
5 oral	6.6 + 0.4*	12.7 + 0.9	40.1 + 2.3*	8.9 + 0.4*	6.2 + 0.3*	12.0 + 0.7	38.3 + 2.1*	9.2 + 0.2*

*= significantly different from controls. Values are mean + SD.

Values for groups 4 and 5 after 4-week treatment-free period were not different when compared to untreated control group. All values were within +2sd of the reference values provided by the sponsor. Except prothrombin time determination, no other coagulation parameters were investigated.

Clinical chemistry findings after 6 and 12 weeks of treatment and then at week 17 (i.e. 4 week after treatment-free period)

Table 15

	Week 6								
Group	GTP (U/l)	AP (U/l)	Glucose mg/dl	Na+ mol/l	K mmol/l	Bilirubin mg/dl	Albumin G/dl	A/G Ratio	Cholesterol mg/dl
1	29.9+14.6	843.5 +371.0	54.4+11.3	166.4+6.1	6.2 + 0.4	0.2 + 0.1	5.7 + 0.5	1.7 + 0.2	166.8 + 21.0
2	61.2+41.8*	708.5 +123.9	60.7+9.2	166.6+5.3	6.4 + 0.8	0.2 + 0.0	5.4 + 0.5	1.5 + 0.2*	176.5 + 29.5
3	80.0+72.1	567.8 +181.7	80.2+15.2*	172.8 +10.3	5.7 + 0.6	0.2 + 0.0	5.5 + 0.5	1.4 + 0.2*	162.3 + 30.4
4	50.6+20.1*	532.4 +139.5*	84.4+46.7	163.5 +4.4	5.4 + 0.7	0.2 + 0.1	5.1 + 0.5*	1.4 + 0.2*	156.8 + 60.7
5	55.6)23.5*	517.6 + 158.1*	67.1+14.1*	170.5+6.2	5.7 + 0.7	0.2 + 0.1	5.3 + 0.3*	1.5 + 0.2*	97.5 + 14.6*
				Week 12					
1	35.9 +25.2	802.6 + 381.1	75.3+6.4	161.3+3.9	5.8+0.7	0.2+0.1	5.6+0.4	1.7+0.2	141.2 + 22.8
2	57.7+40.2	681.8 + 83.9	77.7+9.6	159.2+7.9	5.8+0.8	0.2+0.1	5.3+0.3	16.6+0.1	157.2 + 26.7

3	47.5+27.8	503.3 + 160.7	77.1+11.3	155.4+3.4*	5.8+0.9	0.3+0.1*	5.3+0.2	1.6+0.3	134.7 + 12.6
4	47.0+20.3	442.5 + 135.2*	71.1+10.0	153.7+2.4*	5.3+0.6	0.3+0.1*	5.0+0.5*	1.6+0.3	130.5 + 46.5
5	48.4+28.2	361.4 + 87.9*	65.7+4.2*	152.2+4.2*	5.2+0.4*	0.3+0.1*	5.1+0.4*	1.7+0.1	114.5 + 27.5

*= significantly different from controls. Values are mean + SD.
Values 4-weeks after drug-free period at week-17 in groups 4 and 5 were not different from values for control group 1. All values were within + 2SD of the reference values provided.
Glucose tolerance was normal in treated animals.

Relative organ weight was decreased for spleen, kidneys, liver, heart and brain. All these were attributed to increased body weight with treatment.

Major macroscopic finding was an increase in uterus size of treated groups 3, 4 and 5 when compared to corresponding controls. Thickness of the myometrium and endometrium as determined by ocular micrometer is given in table below:

Mean uterine measurements

Group No.	1	2	3	4	5
Myometrium	120	109	101	125	115
Endometrium	65	57	117	116	101

To obtain actual measurements in micron all values should be multiplied by 37.4.

Thickened uterine wall was thus mainly due to increased thickness of the endometrium.

Microscopic examination revealed hypertrophy of the endometrial stromal cells, accompanied by edema and accumulation of secretions in the endometrial glands.
Other microscopic findings were thymic involution in 2/9 group 3, 6/9 in group 4 and 6/8 in group 5 females.
In cervix there was excessive mucus accumulation with distended endocervical canal and glands and thickened muscular layer in groups 4 and to lesser extent in groups 3 and 5. Morphology of cervix was similar in control and group 2 animals.

Vaginal epithelium in all animals from control and groups 2 was thick, stratified squamous and cornified.

In groups 3, 4 and 5 animals, ovaries were in post-ovulatory phases and in most cases they had no corpora lutea or old corpora lutea that were involuting.

There were no histologic changes seen in the liver or the spleen of the treated groups that could account for the decrease in the relative weights of these organs.

A benign adrenal cortical adenoma was found in a group 5 female which was administered 3-keto-DSG by oral administration. Sponsor described this a rare or unusual finding unrelated to treatment.

Thus **significant treatment-related findings** were hypertrophy of the endometerium, decreased prothrombin time, changes in sodium and potassium levels, increased bilirubin, increased GPT and decreased glucose. Adrenal cortical adenoma was reported in one group 5 female. Although the hematology and clinical chemistry changes were within + 2 SD of the reference values, these may have relevance to 3-keto-DSG clinical therapeutic use.

In another study (SDGRR No. 2127), 6 female cynomolgus monkey were used in 3 treatment groups (2/g). Monkeys of group 1 were treated with 3-KDSG + EE at dose level of 250 ug + 25 ug/kg/day by the oral route of administration. Those in groups 2 and 3 were treated with same dose as suppository by the intravaginal route. The monkeys in group 2 were restrained for 3 hours after dosing.

Results showed that leakage of carrier material after intravaginal administration happened more often in restrained than in unrestrained monkeys.

Microscopic findings reported in groups 2 and 3 females (intravaginal administration) were proliferation of epithelial cells, hornification, aggregation of leukocytes, intracellular edema, migration of micronuclear cells, hyperemia of papillary body with aggregation of lymphocytes, histiocytes and plasma cells in the vagina.

Addendum 1

Histopathology Inventory for carcingenicity study (NDA 21-187)

Study				
Species	Rat			
Adrenals	*			
Aorta				
Bone Marrow smear				
Bone (femur)	*			
Brain	*			
Cecum	*			
Cervix	*			
Colon	*			
Duodenum	*			
Epididymis	*			
Esophagus	*			
Eye	*			
Fallopian tube				
Gall bladder				
Gross lesions	*			
Harderian gland				
Heart	*			
Hypophysis	*			
Ileum	*			
Injection site	*			
Jejunum	*			
Kidneys	*			
Lachrymal gland	*			
Larynx				
Liver	*			
Lungs	*			
Lymph nodes, cervical				
Lymph nodes mandibular				
Lymph nodes, mesenteric	*			
Mammary Gland	*			
Nasal cavity				
Optic nerves	*			
Ovaries	*			
Pancreas	*			
Parathyroid	*			
Peripheral nerve				
Pharynx				
Pituitary	*			
Prostate	*			
Rectum	*			
Salivary gland	*			
Sciatic nerve				
Seminal vesicles	*			
Skeletal muscle	*			
Skin	*			
Spinal cord	*			
Spleen	*			
Sternum	*			
Stomach	*			
Testes	*			
Thymus	*			
Thyroid	*			
Tongue	*			
Trachea	*			
Urinary bladder	*			
Uterus	*			
Vagina	*			
Zymbal gland				

* organ weight obtained

CARCINOGENICITY:

Study Title: **A 24-month oncogenicity study of Org 3236 in the rat via subcutaneous implants**
Study Number: 92-2195
Volume Numbers: 45-52
Test Facility: [redacted]
Study Date(s): 12-22-1992 to 12-23-1994
Date of Submission: 12-28-1999
GLP Compliance/Quality Assurance: yes/yes
QA Report- Yes (*) No ( )
Study Type: 2-year subcutaneous implant study
Species/strain: CD [Sprague Dawley derived] (Crl: CD BR)
Number of animals per group; age at start of study: 50/s in groups 1, 3 and 4; 50 females in group 2; 28 days old
Animal housing: individually housed
Drug Lot/Batch number(s): Group 1: IPA 92025/1; group 2: IPA 92025/2; group 3: IPA 92025/3; group 4: IPA 92025/4
Drug Purity / Stability / Homogeneity: not provided
Doses: Doses used were as shown in experimental design below:
Table 16

Group	Test substance	Dose ug/day	# of rats		Terminal hematology		Terminal sacrifice		Microscopic pathology	
			male	female	Male	female	Male	female	Male	female
1	Placebo 2 EVA implants covered with silastic layer, length 19 mm	0	52	51	13	15	13	15	52	51
2	Placebo 2 EVA implants covered with silastic layer and adhered with ethyl loctite, length 19 mm	0	0	50	0	24	0	24	0	50
3	2 EVA implants covered with silastic layer releasing 3-keto-desogestrel length 11.5 mm	10	50	50	10	13	10	13	50	50
4	2 EVA implants covered with silastic layer releasing 3-keto-desogestrel length 19 mm	20	50	50	20	14	20	14	50	50

- Basis of Dose Selection: The high dose was the maximum feasible dose possible using subdermal implants of the largest dimensions compatible for a period of 2 years.

In earlier studies, implants releasing 10 ug 3-K-DSG/day in rats gave plateau serum levels of about 650 pg/ml. Assuming linear kinetics, implants releasing 5 ug/day and 20 ug/day, were predicted to give serum 3-keto-DSG levels of approximately 325 pg/ml and 1300 pg/ml, respectively.

In the original protocol 5 and 20 ug/day release implants were to be used. However, the low dose group rats were given two implants instead of one. Sponsor informed the division and it was agreed that dose levels of 10 and 20 ug/day instead of 5 and 20 ug/day were acceptable.

Human implants gave serum 3-K-DSG levels of approximately 324 pg/ml. Thus the proposed 10 and 20 ug/day implants in the rat carcinogenicity study will give 2 and 4 times the human exposure with the implant, [redacted] The exposure with the high dose implant will correspond to the human exposure with the vaginal ring, NuvaRing.

- Based on these observations, pharmacology informed the sponsor that the proposed dose of 10 and 20 ug/day release implants were acceptable. Sponsor was asked to determine plasma 3-keto-desogestrel every 3 month and values be maintained as close as possible to 650 and 1300 pg/ml for the 10 and 20 ug dose levels over the course of 2-year study. If the plasma levels fell below these values, the implants should be replaced. During the carcingenicity study, actual measurement of 3-K-DSG levels revealed that initial plasma levels were less than expected, 1000 in high dose female and 900 in high dose male rats.
- Relation to Clinical Use: Proposed human implant produces plasma 3-ketodesogestrel concentrations of 324 pg/ml. The proposed doses of 10 and 20 ug/day 3-keto-desogestrel implants used in the carcinogenicity study produced 1 and 3 times the human exposure with the implant. However, the vaginal ring produces plasma 3-keto-DSG levels of about 1300 pg/ml, approximately equal to the levels seen in the high dose rats.
-
- CAC Concurrence: no
- Restriction Paradigm for Dietary Restriction Studies: none
- Route of Administration: subcutaneous
- Frequency of Drug Administration: once at the initiation of the study
- Dual Controls Employed: yes
- Interim Sacrifices: none
- Satellite PK or Special Study Group(s): none
- Unscheduled Sacrifices or Deaths: Mortality and gross signs of toxicological or
- pharmacological effects were checked twice daily.
- Deviations from Original Study Protocol: none

Study Results and Frequency of Monitoring:

- Clinical Observations: Scabs were observed at the point of insertion of the implants during the first month of the study but were not seen 2 months following implantation. No other adverse effects were reported.
- Mortality: No treatment-related mortality was observed during the study. The percent survival after 24-months of treatment was comparable to or greater than the concurrent control groups as shown in table below:

Table 17

Percent survival rate at termination

Group	Males	Females
1 – 0 ug/day	13/52 (25%)	15/51 (29%)
2 – 0 ug/day	-	24/50 (48%)
3 – 10 ug/day	10/50 (20%)	13/50 (26%)
4 – 20 ug/day	20/50 (40%)	14/50 (28%)

- Body Weight: Body weight was taken twice pretest, weekly for 13 weeks and then monthly. Body weight and body weight gains of both males and females were greater for the drug treated groups compared with the concurrent controls. The changes were more pronounced in females than in males as shown in table below. Values expressed as means (gm) are shown only for weeks when significant changes occurred.

Table 18

Week	Male group 1	Male group 3	Male group 4	Female group 1	Female group 3	Female group 4
0	280 + 12	280 + 11	280 + 11	203 + 10	204 + 10	204 + 10
1	320 + 12	317 + 14	318 + 15	221 + 16	238 + 13**	239 + 15**
6	475 + 30	493 + 37*	483 + 39	290 + 27	347 + 28**	353 + 30**
45	724 + 90	794 + 91**	771 + 88**	433 + 74	509 + 70**	525 + 76**
81	787 + 140	855 + 131	884 + 107**	500 + 110	590 + 115**	623 + 108**
97	787 + 140	738 + 184	843 + 95	494 + 96	565 + 130	615 + 89**
104	757 + 120	750 + 91	807 + 80	510 + 93	580 + 115	576 + 118

From week 2 onwards, % difference from mean control body weight was approximately 15-20% in group 3 and over 20% in group 4 females. Percent weight changes for the treated males compared to mean control body weight were usually less than 10%. . *= $p < 0.05$ **= $p < 0.01$

Values for the control females receiving EVA implant covered with a silastic layer (group 1) or EVA implant covered with a silastic layer and adhered with ethyl loctite (group 2) were considered comparable.

Sponsor attributed the increase in body weight to hormonal activity of 3-keto-desogestrel since body weight increase has been reported with progesterone. Sponsor did not entertain the possibility that the increase could be due to possible androgenic effects of 3-keto-desogesterel.

- Food Consumption: Food consumption for both males and females treated with 10 or
- 20 ug/day was comparable of slightly lower than concurrent
- control values.
- Ophthalmoscopy: not conducted
- Hematology: Hematological determinations conducted just prior to termination showed that treatment did not reveal any drug-related adverse effects.
- Clinical Chemistry: none given

- Organ Weights: Organ weight data was not included in the submission.
- Gross Pathology: For animals found dead, killed accidentally, killed in moribund
- condition or killed at the scheduled sacrifice interval, no
- macroscopic pathological findings were reported.
- Histopathology: Histopathology was conducted on all tissues listed in table .

Non-Tumor: No macroscopic pathological findings were reported. Non-neoplastic findings consisted of a fibrous tissue capsule formation at the implant site in numerous rats and this was considered as a reaction to inert foreign material.

Microscopic findings which were considered to be the result of the pharmacological progestational activity of 3-keto-desogestrel were seen in the ovaries, uterus/cervix and vagina.

In the ovaries no corpora lutea were seen in any of the females in groups 3 and 4.

In the uterus, atrophy of the myometrium and/or endometrium along with eosinophilic material in the lumen of the uterus/cervix was reported more frequently in females from groups 3 and 4 compared to those in groups 1 and 2.

The incidence and severity of mucification of the vagina and cervix was reported to be similar for groups 1 and 2 females but for groups 3 and 4, it was greater and dose-related. Hyperplasia and cornification of the vaginal epithelium and in several animals hyperplasia and cornification of the squamous epithelium lining of the cervix were seen only in group 1 and 2 females. The incidence of adrenal gland cortex granulosa hypertrophy/hyperplasia was 1, 4 and 3 in male groups 1,3, and 4 and 0, 0, 0, and 1 in female groups 1, 2, 3 and 4 respectively.

Tumor: Neoplastic findings consisted of mesenchymal neoplasms seen at the implant site in 4 rats. These were fibrosarcoma in 3 females (two from group 2 and one from group 3) and a fibroma in one male from group 3. This represented an incidence of 1.2% and the sponsor cited a reference (Russell et. al. Journal of the National Cancer Institute 23: 305, 1959), where the incidence ranged up to 40%.

As shown in table below, treatment with 3-keto-desogestrel via subcutaneous implant did not seem to have an oncogencic effect in the rat.

Table 19

	---------- Males----------			----------Females----------			
Group	1	3	4	1	2	3	4
Number of animals	52	50	50	51	50	50	50
Total primary neoplasms	65	64	72	97	110	102	100
Animals with one or more	38	39	40	49	47	47	47
Total benign neoplasms	50	48	62	72	78	77	82
Animals with one or more	33	34	39	46	44	46	44
Total malignant neoplasms	15	16	10	25	29	15	18
Animals with one or more	14	13	10	21	24	24	17

Thus various neoplasms occurred with comparable incidence in rats of treated and control groups. The sponsor stated that these

tumors have been seen in rats of this strain and age used in comparable studies conducted in their laboratory.

The incidence of pituitary adenoma, which was a major cause of unscheduled deaths, was 22/52, 24/50 and 31/50 in the male groups 1, 3 and 4 and 39/51, 35/50, 40/50 and 40/50 in the female groups 1, 2, 3, and 4 respectively. The incidence of other tumors shown in the appended histopathology tables was not affected by treatment.

- Toxicokinetics: Serum levels of 3-keto-desogestrel were determined pretest and after 1/2, 2, 3, 6, 9, 12, 15, 18, 21, and 24 moths. At each determination period, six different animals/sex/group were bled for blood drug level determination. At the 3 month interval, all animals which were bled at the 1/2 month interval were bled again, in addition to the animals which were already scheduled for that interval i.e. 12 animals/sex/group were bled instead of 6.

3-keto-desogestrel (etonogestrel) serum levels (pg/ml) expressed as mean + SD in female and male rats from group 3 (10 ug/day) and group 4 (20 ug/day) are given in the following table and also shown in appended Figures 1 and 2.

Table 20

	10 ug/day		20 ug/day	
Time post-implantation	Females	Males	Females	Males
2 weeks	670+133	550+67	1074+94	868+103
7 weeks	565+97	540+102	1089+210	1018+186
3 months	468+70	413+52	1049+114	737+82
6 months	495+55	364+84	812+231	767+132
9 months	426+167	364+105	844+95	769+101
12 months	415+45	330+68	734+226	617+181
15 months	412+66	351+60	736+224	603+145
18 months	359+176	313+58	817+154	655+189
21 months	253+95	271+74	578+98	506+92
24 months	260+69	190+51	571+97	465+203

Plasma 3-keto-desogestrel levels were maintained for about 18 months near the original levels, particularly in females of group 4. Values were somewhat less than those anticipated. Pre-dose serum 3 -keto desogestrel was less than 20 pg/ml, which was the lower limit of quantification.

In clinical trials with [redacted] release rate at one year was 46 ug/day and the plasma 3-keto-desogestrel concentration was 230 pg/ml. Plasma concentration decreased to 160 pg/ml at the end of 3 years. For ovulation inhibition, serum 3-keto-desogestrel concentrations of greater than 90 pg/ml are necessary.

Overall Interpretation and Evaluation

- Adequacy of the carcinogenicity studies and appropriateness of the test model: The model is considered appropriate since the subcutaneous (parenteral) route of administration used in the carcinogenicity study is similar to the clinical use of the implant and vaginal ring.
- Study as conducted is adequate for testing the carcinogenic potential of 3-keto-desogestrel based on maximum feasible dose of implants.
- Evaluation of Tumor Findings: Non-neoplastic findings seen in the ovaries, uterus/cervix and vagina were considered to be due to the pharamacological progestational activity of 3-keto-desogestrel.

- Neoplastic findings included mesenchymal neoplasms in 4 rats. The incidence of benign and malignant tumors was similar in treated and control rats of both sexes. Pituitary adenomas were the most prominent tumor, which was a major cause of unscheduled deaths. The incidence was similar in treated and control groups.

Summary Conclusions and Recommendations

- Acceptability of Study(s) or Overall Testing Approach: Overall testing approach is acceptable.
- Major Tumor Findings: Major tumor was pituitary adenoma in both treated and control animals
- Non-neoplastic Findings: Non-neoplastic findings involved ovaries, uterus/cervix and vagina and the changes were attributed to drug's pharmacologic progestational activity.
- Biological Significance: The systemic exposure observed in the carcinogenicity study was lower than that reported with the vaginal ring in women. The exposure level to 3-K-DSG with oral Desogestrel in women at doses approved under NDAs 20-071 and 20-301 for contraception was similar to that reported with the proposed combined contraceptive vaginal ring (CCVR) in humans.
-
- The safety of 30K-DSG is also supported by the rat carcinogenicity study with desogestrel. Desogestrel on oral administration is completely metabolized to 3-K-DSG, its biologically active metabolite. Although serum 3-K-DSG levels were not reported in the desogestrel carcinogenicity study, the high oral dose of 500 ug/kg/day desogestrel used will be expected to give serum 3-K-DSG levels greater than those observed in the present carcinogenicity using 3-K-DSG implants delivering 20 ug/day.

- One of sponsor's main concerns was to evaluate the toxicological effects of the EVA copolymer and the potential leachables from the vaginal ring, with or without a mixture of etonogestrel and EE. Results showed that that the ring was biocompatible and safe via the sub-cutaneous route of administration.

- Potential Clinical Implications of Findings: none expected
- Recommendations for Further Analysis: none

Addendum/Appendix Listing:

- Dose-Ranging Study Report:
- CAC Report:
- Alternative Study Protocols and CAC Report:
- Sponsor's Incidence of Histopathology Findings: tables appended
- List of Organs and Tissues Examined: listed in table No.
- Body Weight Changes versus Dose Level: given in table No.
  - Group Body Weight Summary: Compared to controls, both treated males and female gained more weight though the effect was much greater in treated females.
  - Individual Data Listing

IMMUNOTOXICOLOGY: none submitted

REPRODUCTIVE TOXICOLOGY: The following reproductive toxicity studies with 3-keto-desogestrel are reviewed under the NDA submission.

Study title: **Effect of 3-keto-desogestrel on the fertility of female rats mated after withdrawal from treatment**
Study No: Org/80/74786
Site and testing facility: [redacted]
GRP compliance: No GLP statement. Study conducted in 1975
QA-Reports: None
Lot and batch numbers: none mentioned
Protocol reviewed by Division: no

Methods:

Species/strain: rat/CFY strain
Doses employed: 4.0 mg/kg/day for 6 weeks
Route of administration: oral
Study design: Female rats were housed 5 to a cage. They had free access to food and water. Rats were weighed at weekly intervals during the dosing and withdrawal periods. Food consumption was recorded daily from week 3 up to mating. They were dose orally with 3-keto-desogestrel at a dose level of 4 mg/kg/day (equivalent to 260 times the HTD of 2.5 ug/kg on BSA basis) in 0.5% gelatine at 0.2 ml/100 body weight for a period of 6 weeks. Control females were dosed in a similar manner with the vehicle. Males were untreated.
On completion of treatment each group was divided into 3 batches:

Batch A mated 2 weeks after withdrawal from treatment
Batch B mated 4 weeks after withdrawal from treatment
Batch C mated 6 weeks after withdrawal from treatment

Vaginal smears were taken throughout the dosing and withdrawal phases. Mating females were caged for 14 days on a one-to-one basis with untreated males. The day sperm appeared in the vaginal smear was considered day 0 of pregnancy. Number of days between pairing and sperm detection was termed pre-coital time.

Number of animals/sex/dosing groups: 36/females/g with regular estrus cycles
Parameters and endpoints evaluated: Dams were sacrificed on day 20 of pregnancy and examined for:

Number of implantation sites
Number and distribution of fetuses in each uterine horn
Number and distribution of intra-uterine dead fetuses
Weight of ovaries

Number of corpora lutea
Signs of gross visceral changes in the dam
For each litter, pre- and post-implantation losses were calculated

Statistical evaluation: Results were statistically analyzed on a litter basis using Wilcoxon test and a two-tailed criterion.

Results:

Clinical signs: No adverse clinical signs were observed.

Mortality: none

Vaginal smears: As shown in table below, it is apparent that treatment with 3-KDSG was associated with a significant progressive suppression of estrus and pro-estrus. The effect persisted for almost 2 weeks after withdrawal before there was trend for a gradual but incomplete return towards regular cycling.

Table 21

Group	Total di-estrus smears during				
	Predosing period	Dosing period	Post-dosing period		
			Weeks 0-2	Weeks 2-4	Weeks 4-6
1	389/648	970/1512	349/504	216/336	107/168
2	419/648	1205/1512^{+++}	444/504^{++}	235/336	124/168
Total pro-estrus smears					
1	90/648	159/1512	71/504	46/336	30/168
2	79/648	138/1512	25/504^{+++}	44/336	28/168
Total estrus smears					
1	169/648	383/1512	84/504	74/336	31/168
2	150/648	169/1512^{+++}	35/504^{+++}	57/336	16/168^{+}

+= $p<0.05$ +++ $p < 0.001$

Body weight: No significant effect

Food consumption: No treatment effect

Mating performance: Slightly longer precoital time among test group animals at the first mating

Litter data: no significant treatment effect

Pre-and post-implantation losses: no treatment effect at any mating. Results were comparable with control values.

Viable young: unrelated to treatment. Higher litter size among treated animals at the third mating.

Abnormalities: one pup in a group 2 litter had SC edema and hemorrhage over whole body

Summary and evaluation: Results showed that suppression of estrus cycling with 3-KDSG treatment for 6 weeks was reversed on cessation of treatment. Only single dose level of 4 mg/kg/day and sponsor did not provide the basis of this dose selection.
Labeling recommendations: Fertility is returned after cessation of treatment

Study title: **Effect of 3-keto-desogestrel on pregnancy of the New Zealand White rabbit**

Study No.: ORG/104/7693
Site and testing facility: [redacted]
GRP compliance: none. Study was conducted in 1976
QA-Reports: none
Lot and batch numbers: Batch CH R3 ID 5519/75)
Protocol reviewed by Division: No

Methods:

Species/strain: Rabbit/New Zealand White

Doses employed: 0.005, 0.1 and 2.0 mg/kg/day, which amount to 0.65, 13 and 260 times the human therapeutic dose of 2.5 ug/kg/day, respectively, on body surface area basis. Control group was dosed with the vehicle

Route of administration: oral by intragastric intubation

Study design: Mature does were mated on a one-to-one basis with males of proven fertility. Does that successfully completed coitus were injected iv with 10 i.u. luteininzing hormone to ensure that ovulation took place. The day of mating was considered day 0 of pregnancy. All animals had free access to food and water. All animals were observed for signs of toxicity and weighed on days 1, 6, 10, 14, 19, 23 and 28.

Number of animals/sex/dosing groups: 20-21/females/control, 0.005, 0.1 and 2.0 mg/kg/day from day 6 to 18 of gestation. Dose volume was 1.0 ml/kg.

Parameters and endpoints evaluated: On day 29 of pregnancy the dams were killed by cervical dislocation and examined to determine:

Number and uterine disposition of young and resorption sites
Number of corpora lutea
Individual fetal weights
Individual placental weights
Examination of dams for gross lesions

Half of the fetuses in each litter were decapitated, preserved and examined for head abnormalities. The bodies of decapitated fetuses and remaining whole fetuses were eviscerated, preserved, and stained for skeletal examination. Early and late resorptions and abnormalities were classified.

Statistical evaluation: Results were statistically analyzed on a litter basis using Wicoxon and a two-tailed criterion.

Results:

Clinical signs: No adverse clinical signs were observed.

Mortality: Five rabbits died. Three due to intubation errors and remaining two mortalities occurred, one in control and one in low dose groups

Body weight: No significant treatment effect

Food consumption: Food consumption was consistently lower in the high dose groups. Food consumption (g/rabbit/day) between days 23-28 was 140, 145, 132 and 118 for the control, low, mid and high dose groups.

Toxicokinetics: none

Pregnancy rate: Pregnancy rate as assessed by the number of animals becoming pregnant (14,17, 17 and 15 in control and 3 treated groups) and pre-implantation losses (17.0, 15.5, 18.3 and 16.1% for the control and 3 treated groups) did not suggest any adverse effect of treatment.

Litter data: Among test groups, litter size tended to be higher (7.2. 8.2, 7.9 and 7.9 viable young for control and 3 treated groups) and fetal loss lower (11.3, 8.4, 5.1 and 7.2% respectively) than concurrent controls. Values for mean litter and pup weights and placental weights for treated were not different from control values.
Major malformations and minor anomalies: Four major malformations were observed; 1/139 (0.7%) in low dose which had right kidney small and displaced to position adjacent to bladder and 3/134 (3.2%) in mid dose group (one with lanticular opacity of left eye, second with umbilical hernia, severe left forelimb flexure, undescended left testis and bilateral anterior and posterior polar opacity and third with heart displaced to right side of thorax). None reported in 101 control and 119 high dose fetuses examined. The differences in minor anomalies and skeletal variants were not related to treatment.

Summary and evaluation: No dose range-finding studies were conducted and it is not clear what was the basis of dose selection. Since no abortion or total resorption occurred in any treated group, it would seem that high dose used was not the MTD. Although incidence of major malformation was increased in mid dose group animals, a dose-related effect was not seen since no abnormality was seen in the high dose group.
Labeling recommendations: none

Study title: **Effect of 3-keto-desogestrel on pregnancy of the rat**
Study No. Org 107/7692
Site and testing facility: [redacted]
GRP compliance: not indicated. Study was conducted in 1976.
QA-Reports: none
Lot and batch numbers: none given
Protocol reviewed by Division: no

Methods:

Species/strain: rat/CFY strain

Doses employed: 0.005, 0.1 and 2.0 mg/kg/day. These doses translate to 0.32, 6.5, and 130 times the human therapeutic dose of 2.5 ug/kg/day on body surface area basis.

Route of administration: oral

Study design: The day of mating was considered day 0 of pregnancy. Rats were dosed during gestation days 6 to 15 by intragastric intubation of drug suspension in 0.5% gelatine at a volume of 1 ml/kg. Control animals were dosed in a similar manner with the vehicle. All animals had free access to food and water. Dams were observed daily for signs of any adverse treatment effect and were weighed on days 1, 3, 6, 10, 14, 17 and 20 of pregnancy.
Number of animals/sex/dosing groups: 20/female/group

Parameters and endpoints evaluated: On day 20 the rats were killed and ovaries and uterine contents examined to determine:

Number of corpora lutea
Number and position of live young
Number and position of resorption site (early and late)

Individual fetal weights
Individual placental weights
Any gross abnormality in the dam

Fetal abnormalities – All pups examined externally. Half preserved in Bouin's solution for examination of visceral abnormalities by the Wilson technique and remaining half preserved in alcohol to detect skeletal abnormalities.

Statistical evaluation: Results were analyzed on a litter basis using Wilcoxon test and a two-tailed criterion

Results:

Clinical signs: No adverse treatment effects observed

Mortality: no deaths at any dose

Body weight: body weight changes were comparable for all groups

Food consumption: no significant treatment-related changes

Toxicokinetics: none

Pregnancy rate: All 20 rats in each group became pregnant

Litter data: no instances of total litter loss occurred. Only significant finding was a decrease of viable young males in group 4 (6.6, 5.7, 5.9 and 5.4). The total number of viable young (males + females) was not different (11.5, 11.8, 10.9 and 11.4 respectively for control and3 treated groups)

Litter and mean pup weight: no significant treatment effect

Placental weights: no treatment effect

Major malformations and minor anomalies: Major malformation occurred as follows:

1/229 (0.4%) among controls showing transposition of the aortic arch and

4/228 (1.7%) pups at 2.0 mg/kg dose distributed among 3 litters, one with gastroschisi, one with diaphragmatic hernia and two with high interventricular septal defect

Sponsor stated that it is within the laboratory incidence rate of 0-2.4% but provided no historical control data.

The incidence of minor visceral and skeletal anomalies and skeletal variants were unaffected by treatment.

Summary and evaluation: Higher incidence of major malformations in the high dose group was within the laboratory incidence rate. In the clinical trials with NuvaRing, 22 pregnancies were reported. Of these 16 were electively terminated and 6 were live births. No fetal abnormalities were reported. Thus based on clinical experience there does not seem to be any safety concern.

Labeling recommendations:

The following reproductive toxicity studies with desogestrel alone or in combination were reviewed under IND [redacted] original submission dated 12-15-1988:

- Effect of desogestrel on pregnancy of the rat
- Effect of desogestrel + ethinyl estradiol (ratio 2.5:1) on pregnancy of the New Zealand White rabbits
- Embryotoxicity and teratogenicity studies with desogestrel in pregnant rabbits

- Embryotoxicity study with the oral contraceptive combination of desogestrel + ethinyl estradiol (ratio 2.5:1) in rats
- Effect of desogestrel on the fertility of the female rat and maturation of the F1 generation
- Effect of desogestrel on the lactating rat and maturation of F1 generation
- Effect of desogestrel on external sexual differentiation in the offspring of rats

GENETIC TOXICOLOGY:

Study Title: **Etonogestrel (Org 3236) Micronucleus test in CD-1 mice**
Study No: 6995
Study Type: in vivo mutagenicity test
Amendment #, Volume # and Page #: NDA 21-187, vol 33, page 0098
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 3-16-1999/5-18-1999
GLP Compliance: yes
QA- Reports Yes (*) No ( )
Drug Lot Number: Batch R
Study Endpoint: Induction of micronuclei in the polychromatic erythrocytes in treated mice

Methodology:

- Strains/Species/Cell line: CD-1 mice
- Dose Selection Criteria: used highest recommended dose
  - Basis of dose selection: was based on results of a preliminary toxicity study
- Range finding studies: in preliminary study single dose level of 2000 mg/kg was
- used
- Test Agent Stability: Certificate of analysis indicated that the expiry date as 5-29-1999
- Metabolic Activation System: -
- Controls:
  - Vehicle: [redacted]
  - Negative Controls: administered orally vehicle only
  - Positive Controls: Mitomycin-C
  - Comments:
- Exposure Conditions:
  - Incubation and sampling times: 24 hours (all groups) and 48 hours (control and 2000 mg/kg only)
- Doses used in definitive study: etonogestrel at doses of 500, 1000 and 2000
- mg/kg in vehicle as a single oral dose, mitomycin-C at a dose of 2 mg/kg in distilled water administered intraperitoneally as a single dose.

- Study design: 5 per sex/dose/time period, 70 mice in total. All animals except positive control group, were fasted overnight before treatment
- Analysis:
  - No. slides/plates/replicates/animals analyzed: 5 mice/sex/group
  - Counting method: at least 2000 PCE analyzed/animal
- Cytotoxic endpoints: ratio of normochromatic erythrocytes/polychromatic erythrocytes (NCE/PCE)
  - Genetic toxicity endpoints/results: increase in micronucleated PCE
- Statistical methods: A modified Chi-square method used to compared treated and control groups.
- Other:
- Criteria for Positive Results: marked increases in the frequency of micronucleated PCEs with treatment over the control value

Results: Results are shown in table below:

Table 22

Treatment	Dose level mg/kg	Number of mice Male	Female	Incidence of micronucleated PCEs/1000 cells	NCE/PCE Mean ratio
24-hour sampling time					
Vehicle	20 ml/kg	5	5	0.8 + 0.2	1.37
Etonogestrel	500	5	5	1.1 + 0.3	1.25
Etonogestrel	1000	5	5	0.9 + 0.2	1.55
Etonogestrel	2000	5	5	1.2 + 0.2	1.86
Mitomycin-C	2.0	5	5	56.2 + 2.2***	1.14
48 hour sampling time					
Vehicle	20 ml/kg	5	5	1.3 + 0.2	0.92
Etonogestrel	2000	5	5	1.3 + 0.2	2.39

PCE= polychromatic erythrocytes NCE= normochromatic erythrocytes *** = $p < 0.001$
Data combined for male and female mice since there were no sex differences. Vales are means + SE

From these results it was concluded that under the experimental conditions used, etonogestrel administered orally as a single dose at dose levels of 500, 1000 and 2000 mg/kg body weight to both male and females mice, did not induce micronuclei in the polychromatic erythrocytes of treated mice. Slight increase in the ratio of NCE/PCE compared to the vehicle control at the 24 hour sampling time for female animals from all dose groups indicated that etonogestrel exerted a mild toxic effect on the bone marrow cells.
Treatment did not affect body weight. Clinical observations consisted of swollen abdomen, reduced activity and dirty uro-genital area in low dose group; piloerection and swollen abdomen in mid and high dose groups on day following treatment.

- Study Validity: Study seems valid
- Study Outcome: etonogestrel is negative in the mouse micronucleus assay.

Summary: Etonogestrel used at the maximum recommended dose caused slight toxic effect on the bone marrow cells but did not induce micronuclei in the polychromatic erythrocytes of the treated mice.

Study title: **Etonogestrel (Org 3236) Chromosomal aberrations in Chinese hamster ovary cells in vitro**
Study No.: 6996
Study type: In-vitro mutagenicity test
Amendment #, volume # and page #: NDA 21-187, vol33, page 0153
Conducting Laboratory: [redacted]
Date of study initiation/completion: 4-13-1999/6-1-1999
GLP compliance: Yes
QA-report: Yes
Drug Lot number: Org 3236 batch R
Study endpoint: chromosomal aberrations

Methodology:

Strain/species/cell line: Chinese hamster ovary cells
Dose selection criteria: Based on the solubility and cytotoxicity of etonogestrel.
Basis of dose selection: Concentration causing moderate toxicity i.e., reduction of viable cells to approximately 50%. Sponsor stated that mitotic index (# of metaphases /1000 cells) may be determined in order to have additional information on the cytotoxicity of treatment. Up to one dose level at which precipitation is observed may be included in the treatment series. Also osmolality and pH of treatment media checked at the highest dose level.
Range finding studies: A dose range finding study using dose levels of 250, 125, 62.5, 31.3, 15.6, 7.8, 3.9 and 2.0 ug/ml in DMSO both in the absence and presence of D9 mix.

Test agent stability:
Metabolic activation system: Phenobarbital and B-naphthoflavone-induced liver S9 mix
Controls:
Vehicle: [redacted]
Negative controls: [redacted]
Positive controls: a) Mitomycin-C 0.05 to 0.3 ug/ml in water (in the absence of metabolic activation)
b) Cyclophosphamide 15 ug/ml in water (in the presence of metabolic activation)
Comments:
Exposure conditions:
Incubation and sampling times: a) 3 hour exposure with 20 hour and 31 hour harvest, 20 hour exposure and harvest and 31 hour exposure and harvest.
b) 3 hour exposure with 20 hour and 31 hour harvest
Doses used in definitive study: a) 62.5, 125 and 250 ug/ml in culture media
b) 62.5, 125 and 250 ug/ml in culture media
Study design: 100 metaphase spreads were scored for chromosomal aberrations from each culture. Total number of aberrations (chromatid type and chromosomal type) including and excluding gaps were scored.
Analysis:
No. slides/plates/replicates/animals analyzed: 2 replicate culture

Counting method: 100 cells/culture
Cytotoxic endpoints: a decrease in relative cell count and in mitotic index
Genetic toxicity endpoints/results: an increase in chromosomal aberrations
Statistical methods: Fisher's Exact Test

Other:

Criteria for positive results: 1. Statistically significant increases in the incidence of cells bearing aberrations are observed at any dose-level over the concurrent control. Evaluation based on set of results which exclude gaps and the type of aberrations observed are taken in to consideration.
2. The increases are reproduced in both replicate cultures.

Results: At the dose of 250 ug/ml precipitation of etonogestrel in the media was observed. On this basis dose of 250, 125 and 62.5 ug/ml were selected.
A summary of the results giving the incidence of cells bearing aberrations (excluding gaps) and the viable cell count for each test point is given in a table below.

Table 23

Sampling time: 20 hours

		Presence of S9 mix		Absence of S9 mix		Absence of S9 mix Continuous treatment	
Treatment	Dose (ug/ml)	%CA	Rel .cell count	%CA	Rel. cell count	%CA	Rel. cell count
Untreated	-	0.0	100	1.0	133	0.0	261
Solvent	1%	0.5	100	0.0	100	0.5	100
Etonogestrel	250	0.5	57	0.0	78	1.0	100
Etonogestrel	125	1.0	54	1.0	74	1.0	109
Etonogestrel	62.5	0.0	78	0.5	81	0.0	83
Cyclophosphamide	15.0	26.5***	86	-		-	-
Mitomycin-C	0.30	-		47.3***	61	-	
Mytomycin-C	0.10	-		-		36.0***	58
Sampling time: 31 hours							
Untreated	-	0.0	90	1.0	119	0.0	123
Solvent	1%	0.0	100	0.0	100	0.0	100
Etonogestrel	250	2.5	46	1.0	48	0.0	54
Etonogestrel	125	0.5	51	0.0	83	0.5	80
Etonogestrel	62.5	0.0	100	0.6	88	1.0	77
Cyclophosphamide	15.0	25.0***	63	-		-	-
Mitomycin-C	0.10	-		4.0	74	-	72
Mitomycin-C	0.05	-				34.0***	93

% CA = % of cells bearing aberration (excluding gaps) - = not tested of not selected for the scoring of aberrations

Rel. cell count = Viable cell count relevant to solvent controls (percent) *** = statistically significant at P< 0.001

Evaluation: No statistically significant increase in the incidence of cells bearing aberrations (both excluding and including gaps), compared with the relevant control values was reported at any dose level in the presence or absence of S9 mix with the exception of one replicate culture treated at the dose of 250 ug/ml in the presence of S9 mix. In this culture statistically significant increase in the number of cells bearing aberrations including gaps was observed at the 31 hour sampling time. Sponsor considered this positive result not biologically relevant as it was only observed in one of the duplicate cultures and in the set of data which included gaps.

No increase in the number of cells bearing aberrations, excluding gaps in one culture treated with mytomycin-C at the 31 hour sampling time was considered to be a technical artifact as many

metaphases of this culture were observed to be too spread out suggesting that hypotonic fixing treatment may have affected the quality of metaphases selectively excluding the heavily damaged cells.
Endoreplicated cells were observed in all treatment series. Polyploid cells (3/200 cells scored) were also observed mostly at the 31 hour sampling time at the highest dose level of 250 ug/ml in the presence of S9 mix i.e. the same conditions where one culture showed increased chromosomal aberrations including gaps (13/200 cells scored) and excluding gaps (5/200.cells scored).

Study validity: seem valid (doses based on precipitation and cytotoxicity of test substance).
Study outcome: Etonogestrel was negative in this assay under the experimental conditions used.

Summary: Etonogestrel did not produce chromosomal aberration in Chinese hamster ovary cells.
Study title: **A salmonella microsome mutagenicity test (Ames test) with Org 3236 (Etonogestrel)**
Study No: SDG Release report No. 2453
Study type: mutagenicity study
Amendment #, volume # and page #: volume 39, page 0320
Conducting laboratory: Organon Pharmaceutical, Netherlands
Date of study initiation/completion: study conducted in 1988-89. Report dated 4-17-1991
GLP Compliance: In compliance with OECD regulations
QA-reports: yes
Drug lot number: I.P.No. 388/0160, 389/0050 and 389/0086. Batch No. G, H and H respectively
Study endpoint: Induction of gene mutation

Methodology:
Strains/species/cell line: Salmonella typhimurium strains TA98, TA100, TA1535, TA1537, TA1538
Dose selection criteria:
Basis of dose selection: Maximum recommended dose of 5000 ug/plate
Range finding studies: A precipitate was formed at doses of 500, 1000 and 5000 ug/plate
Test agent solubility: 100 mg/ml in DMSO. In aqueous solution < 250 ug/ml.
Metabolic activation system: S9-mix
Controls:
Vehicle: [redacted]
Negative controls: [redacted]
Positive controls: 2-acetylaminofluorene (2-Ac-AF, 2-aminoanthracene (2-AA) and benzo (a) pyrene (BP)
Comments:
Exposure conditions:
Incubation and sampling time: incubation at 37 ^{0}C for 48 hours
Doses used in definitive study: For etonogestrel 0, 8, 40, 200, 1000, 5000 ug/plate in the first study both with and without S9 mix. In an additional study, doses of 31.25, 62.5, 125, 250, 500 and 1000 ug/plate with S9-mix were used. For positive controls 5 ug/plate was used for

strains TA98 and TA 1538 and 2 and 20 ug/plate respectively for strains TA 1535 and TA100. For strain TA 1537 BP was used at concentration of 5 ug/plate.

Study design:

Analysis:

No. slides/plates/replicates/animals analyzed: 3 plates/dose in the first experiment and 6 plates/dose in the second additional experiment.

Counting method: his$^+$revertants counted

Cytotoxic endpoints: bacterial cell killing due to etonogestrel

Genotoxic endpoints/results: induction of gene mutation by etonogestrel

Statistical methods:

Other:

Criteria for positive results: 1) presence of a relationship between dose and the number of his$^+$ revertants; and
2) a relative increase in the number of his$^+$ revertants; a twofold increase was used for positive response

Results: Results are shown in table below:

Table 24

Maximum number of his$^+$ revertants with any dose/plate

		First expt		Additional expt
Test strain	Treatment	No of his$^+$ revertants/ Plate without S9mix	No of his$^+$ revertant/ Plate with S9mix	No of his$^+$ revertants/ Plate with S9mix
TA98	DMSO	21	31	23
	Etonogestrel	27	46	24
	2-Ac-AF(5 ug/plate)	24	322	246
TA100	DMSO	125	134	
	Etonogestrel	135	146	
	2-Ac-AF(20 ug/plate)	93	306	
TA1535	DMSO	20	31	
	Etonogestrel	20	31	
	2-Ac-AF(2 ug/plate)	41	318	
TA1537	DMSO	10	12	
	Etonogestrel	15	19	
	BP(5 ug/plate)	16	87	
TA1538	DMSO	24	25	20
	Etonogestrel	22	44	23
	2-Ac-AF(5 ug/plate)	19	262	248

Thus in the first study a slight increase in the number of his$^+$ revertants was observed in strains TA 98 and TA1538 in the presence of S9-mix. It was not reproducible in the additional second study.

Study validity: was valid

Study outcome: Etonogestrel was not mutagenic in the Ames test.

Summary: Etongestrel did not induce an increase in the number of his$^+$ revertants in any of the Salmonella typhimurium strains. All positive controls induced mutagenic response in all strains.

SPECIAL TOXICOLOGY STUDIES: none submitted

OVERALL SUMMARY AND EVALUATION:

Introduction: NuvaRing is a non-biodegradable, flexible combined contraceptive ring containing etonogestrel (3-keto-desogestrel) and ethinyl estradiol. 3-keto-desogestrel is an active

metabolite of desogestrel and desogestrel in combination with ethinyl estradiol is a FDA approved marketed contraceptive as Desogen tablets under Organon NDA 20-071 and as Ortho-Cept 21 tablets and Ortho-Cept 28 tablets under Ortho Pharmaceutical NDA 20-301, respectively.

NuvaRing is made of ethylene vinyl acetate copolymers and magnesium stearate and contains 11.7 mg 3-keto-desogestrel and 2.7 mg ethinyl estradiol. It has an outer diameter of 54 mm and a cross-sectional diameter of 4 mm.

When placed in vagina, each ring releases 120 ug/day of 3-keto-desogestrel and 15 ug/day of ethinyl estradiol over a 3-week period of use.

Each ring is to be used for one cycle, which consists of 3 weeks of ring use followed by a one-week ring-free interval.

Safety Evaluation: Desogestrel is rapidly and almost completely absorbed and converted into 3-keto-desogestrel (etonogestrel), it's biologically active metabolite. Following oral administration, the relative bioavailability of desogestrel, as measured by the serum levels of 3-keto-desogestrel is reported to be about 84%.

Since desogestrel is first metabolized to its active metabolite, 3-keto-desogestrel before it is further metabolized, the safety of 3-keto-desogestrel is also supported by the toxicity studies conducted with desogestrel by the oral route of administration as well as from those conducted with 3-keto-desogestrel administered by the intravaginal and oral routes.

Most nonclinical toxicity studies except the mutagenicity studies and rat carcinogenicity study were conducted in the 1970s and therefore do not meet the current GLP regulations and ICH guidance for toxicity studies.

The chronic toxicity of placebo vaginal rings was determined in a 6-month study in Rhesus monkeys. It was reported that no histopathological evidence of a tissue reaction to vaginal rings was observed in the vagina, cervix and uterus. There was no gross tissue abnormalities reported in any other tissue. The vaginal ring was considered compatible with the vaginal and cervical mucosa on long term exposure.

In the 13-week intravaginal and oral toxicity studies in cynomolgus monkeys with the combination of 3-keto-desogestrel and ethinyl estradiol, at doses up to 32 times the HTD on BSA basis, significant treatment-related findings were hypertrophy of the endometrium in all treated monkeys. Statistically significant hematological changes were a reduction in RBCs and Hb in the high dose vaginally treated group after 6 weeks of treatment and in the orally treated animals both after 6 and 12 weeks. Prothrombin time was reduced at both time intervals with oral 3-KDSG. Other significant findings were increased GPT at 6 weeks in the high dose vaginal and orally dosed monkeys and decreased alkaline phosphatase in both groups at both time intervals. Bilirubin was increased in mid and high dose vaginal treated groups as well as in the tablet administered group at the 12 week determination.

3-keto-desgestrel was not genotoxic in the in-vivo mouse micronucleus test or in the in-vitro chromosomal aberration assay in Chinese hamster ovary cells or in the Ames test.

3-keto-desogestrel was not carcinogenic in the 2-year study in rats via subcutaneous implants.

The systemic exposure in humans with Nuvaring is greater than 1300 pg/ml. The high dose in the rat carcinogenicity study maintained serum 3-KDSG concentrations between 1089 and 571 pg/ml in female and 1018 and 465 pg/ml in male rats throughout the 2-tear study period. As such the maximum systemic exposure in the carcinogenicity study ranged from about the same to 1/3 the systemic exposure observed with the proposed NuvaRing.

Clinical Relevance of Safety Issues: No consistent adverse treatment-related findings were observed across species which could have clinical relevance.

Other clinically relevant observations were: Reduced prothrombin time, increased GPT and bilirubin in vaginally treated monkeys. No histopathological evidence of liver toxicity.

Other Clinically Relevant Issues: Implanon in women gives a systemic 3-KDSG exposure of 250 pg/ml and the sponsor has reported that an exposure of 90 pg/ml is the threshold for contraceptive efficacy. NuvaRing use results in an exposure of approximately 1300 pg/ml or 14 times the reported minimum effective contraceptive dose.

Communication Review:
Labeling Review (NDA): Label is satisfactory as written.

RECOMMENDATIONS: Pharmacology recommends approval of NuvaRing for contraception.

Internal comments: none
External Recommendations (to sponsor): none
Draft letter Content for Sponsor: none
Future development or NDA issues: none
Reviewer signature/team leader signature [Concurrence/Non-concurrence]

/s/ 9/20/2000
/s/ 9/20

cc: list
Draft date (# of drafts):
Memorandum of Non-concurrence (if appropriate, attached):
Addendum to review (if necessary):
Appendix/attachments: Carcingenicity study presented to Exec-CAC
Exec-CAC comments and recommendations