Non-neoplastic histopathological findings expressed as % of affected animals:

Group #	1	2	3	4	5	6	7	8	9	10
# of animals	110	55	55	55	55	55	55	55	55	55
Focal hepatocellular hyperplasia	1	0	13	42	13	9	27	2	0	0
Centrilobular hypertrophy	0	0	35	53	4	11	24	0	2	2
Eosinophilic cell focus	5	9	27	40	13	22	25	2	4	4
Biliary duct proliferation	49	45	62	65	49	60	71	40	21	47
Biliary cystic change	2	2	4	7	5	5	9	4	5	4
Adrenal zona granulosa hypertrophy	1	4	13	15	0	4	0	4	33	60
Ovarian follicular atresia	0	0	0	87	16	7	7	15	78	55
Ovarian c.l	33	29	11	31	0	44	70	15	25	15
Uterus atrophy	16	18	13	87	16	7	7	15	25	15
stromal fibrosis	17	33	25	47	18	9	7	27	0	15
hemometra	4	2	0	0	4	15	24	0	0	0
endometritis	0	2	2	0	2	4	7	7	0	0
squamous metaplasia	0	0	0	0	0	5	5	2	0	0
endometrial hyperplasia	4	11	9	2	5	4	25	2	0	4
cervix epithelial atrophy	13	27	11	4	16	6	4	48	68	64
epithelial hyperplasia	6	2	0	0	2	0	13	0	0	0
Vaginal atrophy	12	15	0	0	15	2	2	35	60	42
mammary gland focal hyperplasia	23	16	15	11	19	15	34	16	15	27

Hyperplasia of the thyroid follicular epithelial cells was reported with all combination doses and after high DRSP dose.

The above drug pharmacodynamic profile with combination of EE and DRSP or either alone is possibly related to neoplastic changes in the affected organs as shown in table below:

Statistically significant neoplastic findings expressed as % affected animals are marked with asterisks:

Group #	1	2	3	4	5	6	7	8	9	10
Hepatocellular adenoma	1	2	2	11*	0	5*	9*	0	0	2
Carcinoma	0	0	2	2	0	0	2	2	0	0
Adrenal gland benign pheochromocytoma	1	0	0	0	2	2	0	0	0	4*
benign+malignant	3	0	0	0	2	2	0	0	0	7
Uterus endometrial adenoma	0	5	0	0	0	0	2	0	0	0
adenocarcinoma	8	2	0	0	7	27*	18*	2	2	0
Mammary gland adenocarcinoma	8	2	4	2	4	4	18*	0	4	0

Also compared to controls, adrenal glands cortical adenoma decreased in all treated groups. Also uterus adenocarcinoma and uterus and cervix stromal polyp were decreased in all combination groups and DRSP alone groups.

Percentage of animals with tumors per group and absolute number of tumors per group and animals along with comparison concerning the number of tumors per animal between control and the treated groups is given in the following table:

Text Table 14: Compilation of tumor incidence per group

Compound	Placebo	Ethinylestradiol + drospirenone			Ethinylestradiol			Drospirenone		
Dose (mg/kg/day)	0	0.003+0.3	0.03+3.0	0.1+10.0	0.003	0.03	0.1	0.3	3.0	10.0
Group	1	2	3	4	5	6	7	8	9	10
% of animals with neoplasms	86	84	69	73	84	84	85	80	76	78
Number of primary tumors										
- absolute number	176	64	54	55	66	71	82	61	53	58
- per animal	1.6	1.2	1.0	1.0	1.2	1.3	1.5	1.1	1.0	1.1
- % of control group	100	73	61	63	75	81	93	69	60	66
% of animals with more than one primary tumor	47	31	24	18	29	38	47	25	16	20
% of animals with metastases [1)]	7	2	2	2	5	18	24	5	0	0
Number of benign neoplasms										
- absolute number	147	55	49	48	54	48	48	55	48	51
- per animal	1.3	1.0	0.9	0.9	1.0	0.9	0.9	1.0	0.9	0.9
- % of control group	100	75	67	65	73	65	65	75	65	69
Number of malignant neoplasms										
- absolute number	29	9	5	7	12	23	34	6	5	7
- per animal	0.25	0.16	0.09	0.13	0.22	0.42	0.62	0.11	0.09	0.13
- % of control group	100	62	34	48	83	159	235	41	34	48

1) with the exception of the hemolymphoreticular system

These data showed that the average number of tumors per animal was reduced after all 3 DRSP dose levels given in combination with EE or alone. The mid and high dose of EE alone significantly increased the rate of malignant tumors/animal and was also reflected by increased rate of animals with metastasis.

The sponsor stated that experimental data suggested that in many cases a close correlation between neoplastic alterations and non-neoplastic alterations exists which might be related to pharmacodynamic mode of action of EE and DRSP.

In this study EE and DRSP doses administered were equivalent 5-170 times the proposed contraceptive dose of 0.03 mg EE plus 3 mg of DRSP/women/day. The maximum systemic load achieved in the test animals exceeded the human load by a factor of 0.1 to 1.1 with regards to mid and high doses of EE and by 0.6 to 12.3 with regards t DRSP. The low bioavailability of EE in rat compared to humans may be explained by the significant differences in PK parameters. Sponsor concluded that since significant effects were observed with the applied EE doses regimen, use of higher doses to further increase systemic exposure would not have

resulted in added advantage.

Most of the observed findings were attributed to PD of EE i.e., increased mortality due to EE related changes in reproductive tract, alopecia due to reduction in the size and number of hair shafts, retarded body growth, changes in hematology due to depression of erythropoietin production and iron utilization and to inhibition of erythropoietin activity. The occurrence of proliferative hepatocellular alterations including putative preneoplastic (foci of hepatocellular alterations) and neoplastic lesions were attributed to estrogenic effects. Cholestatic properties of estrogens was believed to cause adaptative bile duct proliferation.

Macroscopic changes of the ovarian sac and occurrence of ovarian cysts were considered EE induced effects but in the absence of histologic findings, it was not considered toxicologically relevant.

It was suggested that continuous EE stimulation of the uterus might have led to the increased incidence of squamous cell metaplasia and finally to adenocarcinomas in the mid and high dose groups. Similarly high incidence of focal hyperplasia and adenocarcinomas of the mammary gland may be via continuously increased estrogen stimulated prolactin secretion. DRSP antagonized the estrogen-induced uterine changes.

Antimineralocorticoid properties of DRSP which might have led to a compensatory activation of the aldosterone production in the adrenal cortex was suggested to have caused hypertrophy of zona glomerulosa. The incidence of pheochromocytoma was within the range of reported literature values.

High incidence of pituitary adenomas represents a common finding in the aging rat and values were within the limits of historical control data. The occurrence of 2 and 4% pancreatic adenoma in mid and high dose DRSP compared to 1% in controls was not statistically significant and was said to be within the limits of historical control values.

Hyperplasia of the thyroid follicular epithelial cells seen with all combination doses and after high DRSP dose was considered treatment-related and since these changes were not observed in the one year rat toxicity study, it indicated that life long exposure to these hormones may be required to induce these alterations.

Sponsor concluded that except for the development of estrogen

induced hepatocellular neoplasia to a similar extent as in the EE groups, no increases in any type of tumor was found after the administration of EE and DRSP in combination at a ratio of 1:100.

Z 30.595 (DRSP): Systemic tolerance study in female rats after daily per OS (intra gastric) administration over a period of about 27 weeks. Report NO. 8716

Groups of 25 female Wistar-Han Schering rats were treated daily for 27 weeks with DRSP alone at doses of 0.6, 3.0 and 15 mg/kg body weight or with an equivalent amount of vehicle. Concomitant PK studies were also performed. Four to five rats in each group were used for reversibility of drug effects during 3 weeks after treatment was stopped.

Results:

Mortality: 3 rats each in group 3 and 4 died due to gavage errors.

Food and water consumption: Food intake was significantly increased in groups 3 and 4 but water intake was not affected.

Body weight: Body weight gain for the control and 3 treated groups at the end of study was 84, 86, 106 and 124 g respectively.

Ophthalmologic examination revealed no treatment-related effects.

Hematology: Decreased RBC, Hb and monocyte count in groups 2 and 3 though significant were not considered treatment-related due to lack of dose and time response.
Bone marrow: No significant compound-related effects were observed.

Urinalysis: Significant changes (x/sd) during week 25 for 9-10 animals/g are shown in table below:

Parameter	Control	0.6	3.0	15.0
Urine vol (ml/24h)	4.1/1.2	3.9/1.9	6.1/2.6	6.5/2.7
PH	5.1/0.3	5.6/0.8	5.7/1.0	5.9/0.3
Na excretion(umol/24h)	446/135	454/201	665/236	751/268
K excretion(umol/24h)	628/148	511/171	685/242	967/299

Ca excretion(umol/24h)	67/20	38/19	52/22	22/12

Biochemistry: significant findings were a dose related increase in serum glucose concentrations in DRSP treated groups. It was stated that DRSP did not affect iv glucose tolerance test or insulin concentrations (data not provided). Without providing a reference sponsor also stated that progesterone also has a similar effect on serum glucose in the rat.

Blood coagulation: An increase in APTT in group 3 was not considered compound related due to lack of dose and time dependency.

Pharmacokinetics: Only mean plasma concentrations are given 24 hours after administration during weeks 1 through 26 and after 3 hour administration on week 27.

Dose (mg/kg/day)

week of study	0.6	3.0	15.0
	Mean plasma concentration (ng/ml)		
1	0.5	0.5	1.9
2	0.5	1.4	1.9
3	0.2	0.4	1.6
5	0.4	0.7	3.8
13	0.9	3.7	12.1
18	2.6	8.3	25.7
26	1.3	8.0	18.6

Results thus suggested drug accumulation on repeated administration. The respective values after 3 hours of administration on week 27 were 224, 316 and 772 ng/ml possibly representing Cmax.

Necropsy: Decreased incidence of hydrometra of uterus(proestrus changes) and of cystic dilatation of the ovarian sac were suspected to be treatment-related.

Organ weights: A significant increase in the absolute and relative weight of liver was considered treatment-related. Increases in absolute but not relative weights of kidneys, heart,

thyroid, pancreas and salivary gland in group 4 and decrease in relative weight but not in absolute weight of pituitary and adrenal in groups 3 and 4, uterus in group 4 and heart in group 3 were considered indirect effect of increased body weight.
Body weight in groups 3 and 4 after recovery was still significantly higher compared to controls.

Histopathology: The incidence of atretic follicles was 10/20, 10/20, 9/20 and 17/20 in the low, mid and high dose groups. The increased incidence in group 4 was considered pharmacologic rather than toxicologic effect.
Results were essentially similar to those observed in 14 week toxicity study.

Ethinyl estradiol and drospirenone: Systemic tolerance study in rats after intra gastric administration over a period of 52-53 weeks report NO. A544.

Four groups of 20 female Han:Wist Schering rats each were administered daily orally with a combination of EE/DRSP at doses of 0.003/0.03, 0.03/3.0 and 0.1/10.0 mg/kg body weight or an equal volume of vehicle at a rate of 10 ml/kg. Dose selection was based on the results of 13 week dose-range finding study and represented 5, 50 and 170 times the human contraceptive dose of 0.03/3.0 (EE/DRSP)/woman/day. PK parameters were determined during weeks 27 and 53.

Results:

Mortality: there were no compound-related deaths.

Clinical observations: consisted of alopecia and thinning of fur which was attributed to estrogenic effect.

Food and water consumption: Food intake was decreased in mid and high dose groups from week one onwards but water intake was not affected.

Body weight: No compound-related effect was observed.

Ophthalmology: ophthalmologic examination revealed no treatment-related effects.
Hematology: treatment-related changes consisted of a decrease in Hb in the mid and high dose groups and increase in absolute and relative reticulocyte in high dose group. These were related to estrogen's depression of erythropoietin production and iron utilization and inhibition of erythropoietin activity.

Bone marrow: No significant treatment-related effects were reported.

Urinalysis: Treatment related effects were most significant at 26 weeks for which values are shown below:

parameter	Group 1	Group 2	Group 3	Group 4
Volume (ml)	5.5	6.8	9.8	10.8
K excretion (umol/24h)	871	957	1323	1408
Na excretio (umol/24h)	629	787	1104	1261
Ca excretio (umol/24h)	53	49	67	75
Cl excretio (umol/24h)	618	808	1195	1303

Biochemistry: Significant treatment-related changes were:
1) A dose related increase in ALP, considered due to bile duct proliferation,
2) An increase in GPT in mid and high dose groups, possibly correlated to hepatocellular hypertrophy,
3) A decrease in total cholesterol in all treated groups,
4) An increase in urea nitrogen attributed to protein metabolism in mid and high dose and glucose in high dose group due to decreased peripheral utilization,
5) An increase in urinary K, Na and chloride in mid and high dose groups, and decrease in plasma Ca in high dose and Cl in all groups was attributed to mineralocorticoid effect,
6) An increase in total protein associated with increased fibrinogen values, and
7) A decrease in albumin and increase in globulins resulting in decreased albumin/globulin ratio.

Blood coagulation: Only consistent significant change over time was an increase in plasma fibrinogen contents. The average mean values for the control and 3 treated groups were 267, 312, 362 and 412 mg/dl. The decrease in thromboplastin time and APTT and increase in thrombin time observed in group 4 were not observed at all time intervals.

Biochemical determination in liver microsomes and liver tissue demonstrated a dose-dependent induction of monooxygenase

activities expressed as reaction product/microsomal protein/20 minutes as shown below:

group	Aromatic Hydroxylase	O-demethylase p-nitroanisole	N-demethylase Ethylmorphine	aminopyrine	benzphtamine
cont	6.9	22.4	24.8	46.6	39.6
LD	7.9	24.4	32.4	55.8	49.0
MD	8.1	27.0	37.5	63.9	58.2
HD	8.5	29.0	47.8	74.3	72.3

This reviewer could not locate any literature reference for enzyme induction with marketed estrogen progestin combination contraceptives. Probably this is unique property of DRSP.

Pharmacokinetics: Using plasma concentration from 0-4 h and extrapolating it to 0-24 h, exposure multiple for EE with respect to human 0-24 h exposure with proposed contraceptive dose was 2.0 for the high dose and 0.1, 0.7 and 3.1 for DRSP for the low, mid and high dose groups. Data suggested that no drug accumulation occurred beyond week 27. Low DRSP concentration were suggested due to ex-vivo metabolism of DRSP.

Necropsy: only finding was occurrence of alopecia.

Organ weights: Treatment effect on relative weight (mg/100 g b.wt) of various organs at 53 week sacrifice is shown below:

Organ	Group 1	Group 2	Group 3	Group 4
Liver	2.6	2.7	3.1	3.8
Pituitary	5.2	4.7	4.6	4.0
Uterus	0.37	0.28	0.26	0.17
Pancreas	0.33	0.33	0.35	0.39
Brain	0.71	0.74	0.76	0.77

Decrease in pituitary weight was suggested to be due to negative feed back mechanism and that of uterus by blockage of all cyclic

activities. Although the sponsor suggested that the increase in pancreas weight in high dose was not treatment related, in light of increased serum glucose levels, it may worsen with time and may be become more obvious in the 2 year carcinogenicity study.

Microscopic examination: Microscopic findings in the liver consisted of bile duct proliferation, signs of increased glycogen storage, hepatocellular hypertrophy and foci of hepatocellular alteration (eosinophilic) which is considered pre-neoplastic. Except for bile duct proliferation, these findings were observed in mid and high dose groups. Although severity of these conditions was not different in mid and high dose groups, the incidence increased with dose. Ovarian follicular atresia possibly due to reduced FSH secretion was increased with dose and its severity was greater in the high dose. Dose-related increased incidence was observed for uterine atrophy of all uterine layers and moderate to marked atrophy of endometrial glands was observed only in all high dose animals. Dose-related increased incidence was observed for cervix and vaginal epithelial mucification.

ZK 30.595: Systemic tolerance study in female monkeys (maraca fascicularis) after daily per OS (intra gastric) administration over a period of about 27 weeks. Report No. 8717

Four groups of 4 monkeys each were treated daily with doses of 0.2, 2.0 and 10.0 mg DRSP/kg body weight. Control group was dosed with equivalent volume of vehicle containing 1% Klucel, 0.9% NaCl, 0.085% Myrj and distilled water.

Dose selection was based on PK data shown on page 4 of this review (report NO. 9037).

Results:

Mortality: No compound-related deaths were reported. Only one monkey died in study week 8 due to septicemia.

General observations and palpation of mammary glands: No drug-related changes were observed.

Body weight, food and water consumption: were not affected.

Ophthalmoscopy, EKG, heart rate and blood pressure: were not affected by treatment.

Hematology: Although Hct and Hb were decreased with treatment, because of a lack of dose and time response, these were not considered treatment-related.

Bone marrow: no compound related changes were reported.

Urinalysis: Only significant effect was increased sodium excretion attributed to drug's anti-aldosterone action.
Biochemistry: no time or dose related changes were reported.

Blood coagulation: Compared to controls, thrombin time was shortened significantly in all treated groups with values being 19.8 seconds for the control and 17.8, 18.4 and 18.1 seconds for the low, mid and high dose treated groups. Also fibrinogen values were increased being 362, 434, 454 and 475 mg/dl for control and 3 treated groups. These values were said to be in the range of historical data, which the sponsor has been requested to submit.

Pharmacokinetics: Mean plasma concentrations (ng/ml) 24 hours after drug administration over time ranged from 5.0 to 11.7, 28.1 to 60.0 and 109 to 275 ng/ml in low, mid and high dose groups respectively.

Necropsy: But for one monkey which died of septicemia, no compound-related effects were reported.

Organ weight: Both absolute (g) and relative (g/10 kg b.wt) weights of liver expressed as mean+sd were decreased in mid and high dose groups as shown below:

Group #

Group #	1 (control)	2 (0.2)	3 (2.0)	4 (10.0)
Absolute	72+5	72+7	60+8	60+4
Relative	288+15	280+6	237+19	234+17

Absolute and relative weights of kidneys were decreased in the high dose group, the respectively values were 12.3+0.6 and 48.2+6.5 compared to control values of 14.5+1.2 and 57.7+3.2. Relative weight of pituitary was decreased in group 4 (0.15+0.03) compared to control (0.19+0.018). Both abs. and rel. weights of adrenals were increased in mid and high dose group but were not statistically significant, values were as shown below:

Absolute	0.32+0.04	0.34+0.13	0.43+0.03	0.42+0.06
Relative	1.27+0.13	1.31+0.42	1.70+0.15	1.64+0.29

No significant organ wt changes were reported in the endocrine target organs, in uterus or ovary in spite of compound related histological alterations.

Microscopic findings: decreased liver wt was associated with decreased glycogen contents. However, serum glucose and glucose tolerance were said to be not affected. Increased adrenals wt was not accompanied by any microscopic alterations. There were no histological alterations in kidneys or pituitary.

Uterus: Uterine endometrial changes consisted of stromal edema and increased vascularization in 3/4 animals in low dose and all in mid and high dose. This was associated with diminution of the surface epithelium and glandular atrophy in 1/4 in low dose and in all monkeys of mid and high dose groups. Some monkeys had pseudodecidual alterations. Degree of mucus secretion was increased in cervix uteri.

Vagina: epithelial cornification was not observed in 2/4 low dose and in all mid and high dose animals.

Ovaries: degree of follicular maturation decreased and the incidence of atretic follicles increased. Increased condensed ovarian stroma was seen in one low dose and in all mid and high dose animals.
Mammary glands: while all controls showed juvenile mammary development, lobular alveolar maturation was observed in 2/4, 3/4 and 3/4 animals in the low, mid and high dose groups.

Thus all findings were expected from a progestogenic compound with aldosterone-antagonistic properties.

Comments: In spite of its distinct pharmacological properties compared to other available marketed compounds, sponsor has repeatedly stated that DRSP is a typical progestogenic compound and should not pose any more risks to human than other comparable compounds which are already marketed, a statement without providing comparative data.
Also without specifying conditions, sponsor has stated that DRSP having aldosterone-antagonistic and anti-androgenic properties might be useful for the endocrine treatment.

Since these statements imply DRSP superiority over other progestogenic compound, this reviewer has requested the sponsor to provide clinical data to support their claim of superiority.

Ethinyl estradiol and drospirenone: Systemic tolerance study in monkeys (macacus fasicularis) after intra gastric administration over a period of 53-54 weeks. Report NO. A418.

Eight groups of 5 female monkeys/g were used. Group 1 animals were administered the vehicle at a dose volume of 10 ml/kg.

Animals of groups 2, 3 and 4 were administered daily combination of EE/DRSP at dose levels of 0.03/0.3, 0.3/3.0 and 1.0/10.0 mg/kg respectively. Animals in groups 5 and 6 received EE alone (0.3 and 1.0 mg/kg) and those in groups 7 and 8 DRSP alone (3 and 10 mg/kg). Dose selection was based on results of 27 week toxicity study and on PK determinations in a previous study (Schering report No. 9912).

Results:

Mortality and clinical observations: Two monkeys in the mid dose were sacrificed during the first 3 months. One in moribund condition had multifocal dermatitis and marked purulent endometritis and myometritis which were considered treatment-related. Two animals in the low dose group showed an inflammatory reaction in the cervix and the endometrium. One monkey in the combination mid and high dose had reddening of the mamillae and swelling.

Menstrual cycle: Treatment with EE and DRSP either alone or in combination led to cessation of cyclic activity at all doses used.

Food and water consumption: Food consumption was not affected but water intake was increased and was attributed to diuretic effect of DRSP.

Body weight: wasn't affected by treatment.

Ophthalmoscopy: revealed no treatment-related changes.

EKG, heart rate and BP: were not altered by treatment.

Hematology: decreased RBC count and Hb in the mid and high dose EE groups was attributed to decreased number of nucleated bone marrow cells and decreased erythropoietic cell counts. Neutrophil counts were increased in EE and eosinophil count decreased in DRSP alone treated groups.

Biochemistry and urinalysis: Increased serum glucose levels with glucosuria in the mid and high dose combination groups was suggested to be related to a decrease in the peripheral glucose utilization. Decrease in serum albumin and increase in globulin were observed as seen in the 27 week toxicity study. There were transient increases in GPT and GOT but were not accompanied by any liver alterations. Hyperglycemia was not observed with DSRP alone in the 27 week toxicity study.

Comments: Trimegestone which like DRSP exhibits antimineralocorticoid activity, caused hyperglycemia in monkeys treated with TMG in combination with conjugated estrogen. Since hyperglycemia has been observed with combination of EE/DRSP in the 54 week toxicity study but was not seen in the 27 week study with DRSP alone, it would suggest that either it is the duration of treatment or estrogen/progestin interaction.

Blood coagulation: There was no significant treatment effect. DRSP alone increased fibrinogen and EE increased factor X. Antithrombin III was increased in all treatment groups.

Liver microsomal enzymes: N-demethylase was increased by high dose combination treatment. High dose of DRSP increased and that of EE decreased cytochrome P-450. Combination had no effect.

Pharmacokinetics: AUC ratios monkey/human are given in table below:

Compound EE + DRSP EE DRSP
Dosage(mg/kg/day)

Group	2	3	4	5	6	7	8
EE-ratio AUC(0-24h)monkey/ AUC(0-24h)human	1.0	11.8	25.9	16.3	92.3		
DSRP-ratio Total AUC monkey/ total AUC human	0.7	3.2	19.0			5.1	12.9

For calculation of the multiples of human exposure, a mean AUC of 1807 ng.h/ml for DRSP and 923 pg.h/ml for EE (0-24h) for human contraceptive dose (Schering report No. 9274) was used. The differences in the multiples of the human systemic burden after administration of the single component and those after the combination were considered to be related to enzyme inhibition by EE and induction by DRSP. Based on trough levels, the steady state was reached within a week after the start of treatment.

Necropsy: Ovaries were diminished in size in all treated groups but incidence was greater in EE alone treated animals. Uterus was thickened/enlarged in all animals in combination groups associated with thickened myometrium. Thickened inner surface of endometrium was observed in DRSP alone treated animals. Cervix was of soft consistency in combination groups only and thymus was diminished in size in all groups but more so in combination groups.

dated 23 Nov. 1992. Pharma Research Report No. A595

It was stated that since pharmacodynamically effective dosage of the hormones would preclude the establishment of pregnancy due to inhibition of ovulation, treatment in this study was restricted to 6 week during premating. This allowed examination of return to fertility and the course of the following pregnancy.

Experimental design: 4 groups of female rats (25/g) were treated daily orally with combination of EE/DRSP. Groups 2, 3 and 4 were dosed at doses of 0.05/5.0, 0.15/15.0 and 0.45/45.0 mg/kg, while rats in group 1 were treated with vehicle control at a volume of 10 ml/kg/day. 25 untreated males were allotted to 4 groups.

Estrous cycles were monitored during treatment period, mating period and after insemination until pregnancy was confirmed. Dams were sacrificed on day 20 of gestation and gross necropsy was performed. Reproductive data of parental females and examination of fetuses for developmental status, external, visceral and skeletal anomalies were assessed. Blood was drawn at 0.5 hour post-administration (5 rats/g) when Cmax for EE is expected and at 2 hours p.a. (5 rats/g) when Cmax for DRSP is expected on days 1 and 36 of treatment.

Results: Cmax for EE and DRSP were as shown below:

Compound	dosing day	time p.a	Group 2	group 3	group 4
EE(pg/ml)	1	0.5	197	360	1530
		2.0	82	132	405
	36	0.5	138	209	164
		2.0	62	94	134
DRSP(ng/ml)	1	0.5	166	555	725
		2.0	101	314	1410
	36	0.5	160	398	396
		2.0	157	600	822

It thus showed that there was a dose-dependent increase in Cmax for both EE and DRSP and no accumulation on repeated administration. In a clinical study (A470), it was reported that Cmax with EE/DRSP contraceptive dose on day one was 36-40 ng/ml and after 21 day treatment 60 ng/ml. Respective values for EE were 115 and 144 pg/ml. In an other single dose DRSP study (No.9274), Cmax was 46 ng/ml. Sponsor also reported that with

similar doses given from day 14 to 21 post copulation in rats, total AUC for DRSP was at least 2-14 times and for EE 2-10 times the human exposure in pregnant rats.

Results: During treatment with low dose only 13/25 rats showed regular cycles and the duration of the cycles was prolonged. With mid and high dose there was cessation of cyclic activity in the majority of animals and only one female in each group showed regular cycle which had prolonged duration.
After treatment there was an increased mean number of mating days and number of matings to successful insemination. In the high dose, number of fetuses/dam and fetal weights were reduced which indicated persistent disturbances of ongoing pregnancy despite nearly complete return to fertility.
Fetal defects consisted of one fetus in control group with a filamentous tail. In group 2 at one implantation site living twin terat were found. One externally malformed fetus of group 3 showed anasarca (generalized massive edema). In another group 3 fetus scoliosis was detected. All malformations were considered incidental. No malformations were detected in group 4.

Ethinyl estradiol plus drospirenone: Evaluation of the report entitled "Z 4.944 + Z 30.595 -study of the course of gestation in rats after daily intra gastric administration from day 0 to day 6 of gestation: Report No. A592.

Four groups of 25 inseminated female rats were treated daily with EE/DRSP at doses of 0.01/1.0, 0.03/3.0 and 0.1/10.0 mg/kg from gestation days 0-6. Five additional rats/g were dosed similarly for serum hormone determination 0.5 h p.a. on days 0 and 6 post-copulation.

Results:

EE and DRSP serum levels increased in a dose-dependent manner. EE levels on days 0 and 6 for group 4 were 637 and 333 pg/ml respectively. At these time intervals DRSP levels were 507 and 484 ng/ml respectively.

Mean body weight for the control and 3 treated groups on day 6 of gestation were 264, 253, 249 and 239 g respectively.
Body weight gain was decreased in a dose-dependent manner from gestation day 0-20 in the mid and high dose dams and was related to a dose-dependent increases in pre-implantation losses (17, 13, 21 and 28%) and post-implantation losses (9, 7, 11 and 30%). This led to reduction in the mean number of implantations (14, 15, 12 and 11) and live fetuses/dam (13, 14, 11 and 7). Incidence of intra-uterine deaths was higher in mid and high dose dams.

Reduced body weight in mid and high dose dams was due to reduced number of fetuses.
Fetal defects: In control group, 4 fetuses from one litter showed external malformations such as exencephaly, gnathoschisis, chilschisis and/or open eyes. Three of these also had cleft palates.
In group 2, two fetuses from 2 litters showed external malformations as anasarca or edema at the head.
In group 3, one fetus had visceral malformation showing internal hydrocephaly, anophthalmia, microphthalmia and aphakia. Two other fetuses from one litter showed external malformations as exencephaly, open eyes or displaced ear.
In group 4, dead twin terta were found which had 2 palves with 2 hind limbs and a tail each and additional forelimbs on the back.

It was concluded that although pregnancy index was reduced, no teratogenic effects attributable to treatment were observed.

Drospirenone embryotoxicity including teratogenicity study in the rat after daily intra gastric administration from day 6 to 15 of gestation. Pharma Research Report No. 9918

Four groups of 36 inseminated female rats/g (Han:Wist) were dosed daily from day 6-15 of gestation with the vehicle or aqueous solution of DRSP at dose levels of 5, 15 and 45 mg/kg. DRSP alone was used because of the low threshold dose for embryolethal effects of EE. Based on available PK data, low dose of 5 mg/kg was expected to give systemic exposure equal to that in humans on contraceptive dose. 24/36 rats were sacrificed on day 21 post copulation. Other 12 were sacrificed at weaning. 5 additional rats were used for serum DRSP determination on days 6 and 15 p.c. Selected pairs of F1 rats were mated in each group and inseminated females were sacrificed on day 21 p.c. and F2 fetuses were examined. Functional and behavioral development including reproductive performance of the F1 generation was tested.

Results:

At the low dose no treatment-related effects were seen. At the mid and high doses there was slight decrease in body weight of P generation over the treatment period. A little increase in post-implantation and prenatal losses were reported in P-generation. The incidence of unossified feet bones in F1 fetuses was slightly increased. A small increase in number of F1 fetuses with visceral anomalies in high dose group was due to increase in fetuses with severely dilated renal pelves. In this group number of fetuses with incomplete ossified bones of skull was also increased. There was no indication of teratogenic effect. No compound-

related influence on reproductive performance, including prenatal development of the F2 generation was observed in any of the dosed groups.
Serum DRSP Cmax in the low, mid and high dose groups compared to Cmax for humans on therapeutic contraceptive dose was 6-8, 25-33 and 24-41 times respectively.

Drospirenone - embryotoxicity including teratogenicity study in the rabbit after daily intra-gastric administration from day 6 to day 18 of gestation. Report No. 9998

NZW female rabbits (20/g) were treated daily orally by gavage from day 6-18 of gestation with DRSP at dose levels of 10, 30 and 100 mg/kg (groups 2, 3 and 4). Animals in group 1 were gavaged with vehicle at a volume of 10 ml/kg/day. Five additional similarly treated rabbits/g were used for PK parameters determination. Doses were based on the results of previous studies (reports 9274 and 9392) where a single 5 mg dose in rabbits gave AUC equal to and Cmax 4 times of that observed in humans in 4-12 week treatment.

Results:

At the low dose no drug-related effects were observed.
At 30 mg/kg dose, one fetus had hypoplasia of the cerebellum. One fetus had acaudia which was also observed in historical controls (data not presented).
At 100 mg/kg there was a significant increase in the number of abortions and one fetus was severely growth retarded with hypoplasia of the cerebellum. It was stated that no incidence of hypoplasia of cerebellum was reported in the historical control data (data not submitted).
There were 6 abortions in group 4 in contrast to 1, 2 and 2 in groups 1, 2 and 3.
Other observations consisted of delays in ossification of feet bones, stemebrae and vertebrae as well as multiple fusion of the ribs. 9.5% of fetuses in group 4 compared to 2.4, 2.7 and 0.9% in groups 1, 2 and 3 had visceral anomalies such as small vessels branching from great vessels at the base of heart. The incidence of skeletal anomalies as incompletely ossified skull bones was 17.1,24.3, 14.0 and 30.2% in groups 1, 2, 3 and 4 respectively. The visceral and skeletal anomalies were considered indicative of retardation of fetal development.
There were no differences in the number of corpora lutea, implantation sites and pre and post-implantation losses in the 4 groups. Serum Cmax and AUC (0-24) values are shown below:

	Cmax (ng/ml)	AUC (ng.h/ml)

	Day 6	Day 18	Day 6	Day 18
group 2	251	204	1747	1603
group 3	388	503	2676	2232
group 4	725	1310	8545	8906

With human AUC of 1300-1759 ng.h/ml and Cmax of 70 ng/ml, the multiples of exposure compared to humans was 1.5 with mid dose and 5-7 with high dose . With respect to Cmax the multiples were 5-7 for mid dose and 10-18 for high dose respectively.

This study was repeated (study report No. A807) to investigate reproducibility of hypoplasia of cerebellum using single dose level of 30 mg/kg.
Examination of 164 and 182 dams with 531 and 622 fetuses in control and treated groups respectively did not show any alteration of the cerebellum. A small increase in number of abortions was reported. It was concluded that occurrence of hypoplasia of cerebellum in the above study was a spontaneous finding not associated with treatment.

Ethinyl estradiol plus drospirenone: Evaluation of the report entitled " Z 4.994 + Z 30.595 - oral (gavage) embryotoxicity including teratogenicity study in the cynomolgus monkey" and the appertaining"supplementary histopathology report" Report NO. A740

Four groups (12/g) of ultrasonically confirmed pregnant monkeys were gavaged daily from day 20 to 90 of gestation with EE/DRSP at dose levels of 0.01/1.0, 0.03/3.0 and 0.1/10.0 mg/kg (groups 2, 3 and 4). Control group was gavaged with the vehicle at a volume of 10 ml/kg. Pregnancies were terminated on day 100 of gestation by caesarian section. Blood samples were collected for drug conc determination.
Dose selection was said to be based on results of a previous study where a single 1 mg dose in monkeys gave an AUC of 4060 ng.h/ml and Cmax of 368 ng/ml and respective values for 10 mg dose being 30367 ng.h/ml and 1846 ng/ml respectively.

Results:

Clinical observations: Mild drug toxicity was suggested as 4, 2 and 2 animals in the low, mid and high dose respectively occasionally vomited. The occurrence of abortion in groups 2, 3 and 4 were 4, 4 and 7 respectively. These occurred late in pregnancy. In addition embryonic death occurred in one animal of the high dose group where placental remnants were found at caesarian section. In control group one aborted and it was

considered due to impaired condition of the animal.
11, 8, 8 and 4 fetuses were examined in groups 1, 2, 3 and 4 respectively. No malformations and morphological and histological examinations revealed no indications of hormone-related changes in genital organs in any of the fetuses. Sponsor stated that teratogenic potential could not be definitely assessed because of only 4 fetuses were available in group 4.

Z 4.944 + Z 30.595 -preliminary oral perinatal and postnatal toxicity study in the rat. Report No. 9570.

This study was conducted to investigate the possibility that progestin may interfere with parturition.
Ten inseminated rats were treated with EE/DRSP dose levels of 0.15/15.0 and 0.45/45.0 from day 15 of gestation to day 3 post partum.

Results: 9/10 rats were seen to be pregnant in each group of which 3 and 1 animals respectively had normal delivery. The remaining had prolonged or incomplete delivery and 4 and 5 animals were unable to deliver up to day 26 of gestation.

In light of the above results, it was decided to interrupt the treatment for a period of few days before delivery. In this study (report no. A709), therefore, 3 groups of 35 inseminated rats were gavaged at EE/DRSP dose levels of 0.05/5.0, 0.15/15.0 and 0.45/45.0 mg/kg daily from days 15 to 18 and days 1 to 22 post partum. Additional subgroups of 5 rats were used for serum drug levels determination. All females were allowed to deliver and rear offspring (F1a generation). After weaning they were not treated. One male and one female/litter (F1 generation) were mated at maturity. Because of high incidence of inseminated but non pregnant F1 females in the high dose group observed at interim evaluation, further matings were not initiated and instead remaining males and females were cross mated with naive partners from breeding pool.

Results:

Treatment decreased body weight gain of P generation during gestation and early lactation.

Treatment effect on the F1 offspring consisted of a dose-dependent increased post-natal loss from day 5 to weaning; reduced pup weight during lactation and at sexual maturity; delayed physical and functional development and delay in testes decent. These effects were more pronounced in high dose group. F2 fetuses did not reveal compound-related effects.

Effects in F1a offspring consisted of decreased birth index; increased early post natal loss from birth to day 4; increased incidence of total litter loss during the lactation period; increased mortality rate during growth period; increased incidence of morphological changes in the male reproductive organs and histological evident tubular atrophy and depletion of sperms in epididymides of 6/22 F1 males.
Results showed that there was impairment of reproductive function of F1 animals as evident by the increased incidence of inseminated but no-pregnant females. It was attributed to male reproductive incompetence as there was decreased insemination, fecundity and fertility indices. There were no treatment-related effects on F2 generation. It was concluded that this occurred primarily at the high dose and there was no indication that treatment of the P females at low and mid dose affected the fertility or general reproductive performance of F1 animals. Serum DRSP concentrations on day 15 at two hours p.a. in group 1, 2, 3 and 4 were n.e (not evaluable), 80, 626 and 1680 ng/ml. On day 22 corresponding values at 2 hr were 0.15, 148, 1580 and 2720 and at 0.5 hr 0.9, 767, 1300 and 1830 ng/ml respectively. EE values (pg/ml) on day 15 at 2 hours were 27, 96, 193 and 389. Values on day 22, two hr p.a were 26, 43, 70 and 123 and 0.5 hr p.a. were n.e., 125, 135 and 367 pg/ml respectively (A-379).

In the above study reduced fecundity was observed for male offspring of dams treated with high dose of 0.45/45.0 mg of EE/DRSP/kg/day. This effect was interpreted to indicate antiandrogenic effect of DRSP. So in this study (report No. A145), same dose levels was used from day 14 to 21 post copulation to cover period of sexual organ development.

Results: Aside from significant decrease in fetal weight in the mid and high dose groups, there was statistically significant increases in the relative lengths of the urethra and in the anogenital distances in female fetuses and decreases in male fetuses. Also at the mid and high doses there was a significant decrease in uterus weight.

Based on 0-24 hour AUC it was reported that calculated AUC for DRSP was 2-14 times and for EE 1-10 times the AUC with the proposed HTD. Dose-dependent maternal and embryotoxic effects were attributed to estrogen component. Dose-dependent feminization in male fetuses was suggested to be due to progestin's anti-androgenic property and virilization of female fetuses due to paradoxical masculinizing effect of estrogens.

Mutagenicity studies:

Z 30.595 - Evaluation in the Ames Salmonella/microsome mutagenicity test. Report No. 8467
This study was conducted to determine if DRSP can induce point-mutations in 5 histidine-dependent salmonella typhimurium strains. Strains TA 1535 and TA 100 for the detection of base pair substitutions and TA 1537, TA 1538 and TA 98 for the detection of frame shift mutations when tested at a dose of 0.05 to 5.0 mg/plate in the presence and absence of S-9 mix using direct plate incorporation procedure. Appropriate negative and positive controls were used.

Results: Under the conditions of the assay, none of the strains showed increased reversion to prototrophy either in the presence or absence of S-9 mix. Growth inhibition of the background lawn was observed at 5 mg/plate dose and precipitation of the agar was found at 2 high doses. Data thus did not indicate that DRSP is a mutagenic in this test.

The test was repeated (report No. 8494) using doses ranging from 0.025 mg to 2.5 mg/plate and with preincubation for 60 minutes at 37 C. Results were in agreement with those with direct plate incorporation procedure.

Z 30.595 - Evaluation in a bacterial mutagenicity test with Escherichia coli strain wp2 uvrA. Report No. 9211.

Test was conducted with this tryptophan dependent strain both in the absence and presence of S-9 mix at concentrations ranging from 0.1 to 5.0 mg/plate using both direct plate incorporation and pre-incubation modification.

Results: E.coli did no show increased reversion to prototrophy either in the absence or presence of S-9 mix. Growth inhibition of the background lawn was not observed and partial agar precipitation was seen at 2.5 and 5.0 mg/plate concentrations.

Z 30.595 -Evaluation of gene mutations in mammalian cells in culture: HGPRT-test with V79 cells. Report No. 9313.

In 2 independent experiments, mutagenic potential of DRSP was examined in a mammalian HGPRT (hypoxanthine guanine phosphoribosyl transferase) system using Chinese hamster cell line V79 in the absence (with concentrations of 0.05 to 125 ug/ml) and in the presence (with concentrations of 1.25 to 500

ug/ml) of S-9 mix. Highest dose was limited by clear toxic effect. Negative and positive controls were included.
This mutagenicity test in eukaryotic cells involves the induction of mutants at the HGPRT locus. This gene is located in man hamster and other animals at the X chromosome and gene product HGPRT enzyme which is not vital for the cell, activates purine analogs to toxic products. Mutants which do not synthesize active enzyme are resistant to high concentrations of 6 thioguanine or 8 azaguanine. A simple destruction of a gene is sufficient to produce mutants (forward mutation).

Results: Neither assay showed a mutagenic effect in the cultures when compared to negative controls.

Z 30.595 -Evaluation of the clastogenic potential in the human lymphocyte test. Report No. 8495.

These cells have been shown to be sensitive indicators of both in-vivo and in-vitro induced chromosomal structural changes and offer scorable morphological evidence of damage to the genetic material. To detect chromosomal aberrations one needs dividing cells to analyze chromosomes in the metaphase.
Concentrations of 5, 10 and 25 ug/ml were used in the absence and 10, 50 and 100 ug/ml in the presence of S-9 mix. The highest concentration in assay without S-9 mix was limited by 47% reduced mitotic index, an indication of cytotoxicity and limited by solubility limit in the presence of S-9 mix assay.

Results: DRSP did not show clastogenic potential.

Evaluation of the report: Unscheduled DNA synthesis in primary hepatocytes of female rats in-vitro with drospirenone (Z 30.595). Report No. A934.

An increase in UDS (excision repair) is regarded as evidence of a genotoxic potential i.e. ability of the test substance to damage the DNA.

Two experiments using identical procedures were conducted.
Freshly prepared hepatocytes were exposed to DRSP for 18 hours in the presence of 3HTdR (methyl-3H-thymidine). Uptake of radioactivity was determined by autoradiography using 100 cells.
In the first experiment doses ranging from 5 to 50 ug/ml and in the second experiment 5 to 60 ug/ml were used. These doses were said to have previously shown a dose-related toxic effects.

Results: As shown for first experiment in table below there was a dose-related increase in the mean number of nuclear and net grain counts.

Treatment	Grains/ Nucleus	grains/cyto Plasm area	net grains/ nucleus
-tive control:medium	17	20	-3
solvent control:DMSO	17	22	-5
solvent control +2AAF(2.23ug/ml)	64	29	35
DSRP 5 ug/ml	21	26	-5
DRSP 10 ug/ml	25	24	-1
DRSP 20 ug/ml	46	22	24
DRSP 30 ug/ml	64	17	46
DRSP 40 ug/ml	42	12	30
DRSP 50 ug/ml	57	11	46

Similar results were obtained in the second experiment. Sponsor concluded that the observed positive response was reproducible and dose-dependent at concentrations range of 10-60 ug DRSP/ml and not related to cytotoxic effects. DRSP therefore can be considered to possess a genotoxic potential in primary hepatocytes of female rats in-vitro.

Studies on the mutagenic potential of Z 30.595 in the mouse micronucleus test. Report No. 8724.

This test determines any induced chromosome breakage or malfunction of the spindle apparatus leading to aneuploidy in-vivo measured by the increase in micronucleated erythrocytes in mice.
NMRI mice(15-18/s) were given once by gavage 0.25, 0.5 or 1.0 g DRSP/kg body weight. An equal number of control mice were given vehicle composed of NaCl, Myrj 53, Klucel and water at a volume of 10 ml/kg. Positive control groups were included. Treated and control mice (5/s) were killed 14, 48 and 72 hours post-dosing. The positive controls were killed after 24 hours. Femur bone marrow smears were prepared and stained using May-Gruenwala and Giemsa solutions. Micronuclei in 2000 polychromatic (young cells stained blue) and 1000 normochromatic (matured cells stained red) and ratios of PC/NC erythrocytes were examined.

Results: High dose was toxic as half of the animals showed slight to moderate apathy. Compared to controls, treatment resulted in neither biologically relevant nor statistically significant differences. Positive controls compounds triaziquone and cyclophosphamide showed significant increases of micronucleated PCE and NCE counts and a significant decrease of the ratio of PCE to NCE. DRSP was thus showed no evidence of mutagenic potential.

DNA adduct analysis in liver of male and female rats after daily intra gastric administration of chlormadinone, megestrol acetate, drospirenone, ethinyl estradiol, norethisterone acetate, gestodene, estradiol or progesterone over a period of 14 days. Report No. AG18.

Three rats/s were used in each treatment group. Livers were removed 24 hours after dosing and adduct analysis was performed with DNA isolated from pooled sample of 3 animals. Following DNA hydrolysis and nuclease P1 enrichment, nucleotides were 32P-postlabeled and the DNA adducts separated by multidirectional thin layer chromatography.

Results: The relative adduct labeling (RAL) values are shown in the following table. Values are mean + sd for 2 determinations:

Steroid	Dose	RAL-values Add./10 nucleotides Sex	
	Mg/kg/day	Female	Male
CMA	10.0	4.96 + 0.61	2.95 + 0.15
MGA	10.0	30.1 + 3.1	Below the quantification limit
DRSP	10.0	5.26 + 0.12	2.36 + 0.96
EE2	0.2	-	-
NET-Ac	10.0	-	-
GEST	0.5	-	-
E2	2.0	-	1.81 + 0.10
P	100.0	-	-
Control	vehicle	-	-

The adduct levels were shown to be sex specifically distributed. From the 4 rat liver tumorigenic steroids i.e., EE, NET, CMA GEST

only CMA showed adduct formation. The sponsor concluded that data did not indicate a correlation between the DNA adduct formation and tumorigenic potential of these compounds.

Previous human experience with DRSP:

Sponsor has conducted 15 phase 1 and 3 phase 2 studies involving 450 subjects (63 healthy young men, 36 of those received DRSP; 375 women of child bearing age, 334 of whom received DRSP and 12 postmenopausal women, 6 of whom received DRSP).

In various studies, PK of DRSP or DRSP/EE, effects of DRSP or combination with EE on sex hormones and on ovulation, on renin-angiotensin-aldosterone (RAA), on contraceptive efficacy, cycle control and menstruation as well as on safety and tolerance were determined.

With single dose administration at doses of 1 mg and 100 mg, Cmax was seen at 3 hour, the first post-administration time point. The AUC increase was dose-related.

The calculated bioavailable fraction after administration of non-enteric coated and enteric coated tablets was 0.762 and 0.65 respectively.

With HTD, ethinyl estradiol did not significantly affect DRSP PK. Repeated daily administration of DRSP resulted in an accumulation of the drug by a factor of 2.5 times concentration and was reported to be expected by linear processes.

In subjects receiving HTD, initial levels of DRSP were 15-25 ng/ml and remained constant from day 12 of treatment until last day of treatment. Mean levels were 27.9-32.6 ng/ml. Drug levels fell quickly during the 7 day tablet free interval. Free fraction of DRSP in serum was calculated to be 3%.

In a PK study with 35 subjects, data from which was used to calculate multiples of human exposure for toxicity studies, AUC (mean + SD) on day 21 of first cycle was 754 + 259 and for third cycle 814 + 268 ng.h/ml. A mean of these 2 values (784 ng.h/ml) was used for calculations. Tmax was 2.2 to 2.7 hours and terminal T1/2 was 28 to 30 hours.

In artificially estrogen primed cycles, a total DRSP dose which produced endometrial transformation was determined to be between 40 to 60 mg. From these studies it was concluded that a dose of DRSP of 2 mg or greater would appear to suppress follicular development and ovulation and on this basis a proposed OC dose of

3 mg DRSP + 0.03 mg of EE was selected.

In various studies, decreases in RBC count, Hb, and Hct was observed during treatment. In 2 studies, serum glucose was increased accompanied in one by insulin secretion.

Adverse effects were stated to be similar (i.e., breast tension, nausea, tiredness and acne) to those associated with combination oral contraceptives.

Proposed clinical program: A study protocol No. 96049 entitled as " An open-label multicenter study to evaluate the efficacy and safety of a monophasic oral contraceptive preparation, containing drospirenone 3 mg and Ethinyl estradiol 30 ug" is planned. Approximately 300 women will be enrolled and treated for 13 cycles.

Labeling: Investigator's brochure is consistent with labeling for combination oral contraceptives.

Correspondence with the sponsor: The sponsor had stated that dose selection in the 27 week rat and monkey studies was based on estimated 100 fold AUC for high dose in relation to human exposure with therapeutic contraceptive dose of 2 mg/woman/day. Since this did not seem correct, sponsor was called on January 24 to provide data on the basis of which AUC was calculated. Sponsor said that they will reexamine the calculations and submit the results. Data tables faxed on January 27 showed that multiple of human exposure was about 12 in both rats and monkeys studies rather than 100 as stated in the submission. On March 11, metabolite pattern of DRSP for mouse, rat, rabbit and monkey as well as mouse exposure level for carcinogenicity study was requested. Sponsor informed on March 21 that these data will be available during the month of April. According data was submitted and reviewed under submission serial NO. 010 dated 4-18-97.

Comments: Sponsor suggested that DRSP will have the advantage of a natural progesterone with anti-mineralocorticoid and anti-androgenic properties, implying that DRSP is better than other marketed progestins.

It should be pointed out that DRSP is a 17-a-spironolactone derivative and its aldosterone-antagonistic activity is greater than spironolactone and thus may pose greater risk of drug-drug interaction. Spironolactone which is an approved aldosterone-antagonist as Aldactone has a boxed warning in PDR that it has been shown to be tumorigenic in rats and its unnecessary use should be avoided. There are also numerous warnings and

precautions stated for Aldactone in PDR.

Drospirenone is a new progestin which has a strong antimineralocorticoid, anti-androgenic effects and causes enzyme induction, properties which are not mentioned in PDR for any of the marketed progestin. As such safety profile of Drospirenone may not be similar to that of natural progesterone as implied by the sponsor.

Patient consent form: is not included in the submission.

Summary: Drospirenone is a derivative of 17-a-spironolactone which has progestational, aldosterone-antagonistic and anti-androgenic properties.

After oral administration, it is rapidly absorbed, bioavailability being dose-dependent and approached 100% at high doses in rats and mice. Cmax and AUC increased with dose in mice, rats, and rabbits. Multiple dosing did not suggest systemic accumulation. DRSP is rapidly eliminated in the mouse, rat and rabbit because of its short MRT and T1/2 and increased total metabolic clearance. Elimination was complete in 24, 72 and 96 hours in the mouse, rabbit and rat respectively. In the monkey it took 15 to 20 days, total metabolic clearance being about 25% of the plasma flow rate to the liver. Excretion was primarily in the feces in rat, mouse and monkey and equal in feces and urine in rabbits.

The toxicologic profile of DRSP, EE or combination of the two (100:1 or 10:1) were evaluated in female animals in acute, single and repeat dose systemic tolerance, carcinogenicity, reproduction studies and in genetic toxicology studies.

LD50 after a single dose by oral gavage in rats and mice was 1250 mg/kg and by ip 500 and 250 mg/kg in mice and rats respectively.

In dogs after a single oral dose of 250 mg/kg, plasma fibrinogen was increased in all animals on days 2, 4 and 8. Also serum free Hb was also increased. Surprisingly no further studies were conducted using dogs.

Daily administration of DRSP by gavage in rats for 7 days at doses of 10, 50 and 100 mg/kg caused induction of N-demethylase activity at all dose levels. Also microsomal P-450 content was increased from mid dose onwards.

In a 14 week study in rats with EE/DRSP combination at dose of 0.01/1.0, 0.03/3.0 and 0.1/10.0, enzyme induction was seen at all

dose levels and increased P-450 at mid and high dose levels. Liver weight was increased. Significant decrease in Hb and MCHC was observed in combination and EE alone groups. Increased urinary sodium excretion was attributed to DRSP anti-mineralocorticoid effect.

Organ weight changes and microscopic findings were consistent with hormonal effect of EE and DRSP. Body weight was increased significantly at all doses of DRSP alone. Since even at the low combination dose certain metabolic changes were observed, sponsor concluded that higher doses in carcinogenicity study were not justified.

Using DRSP alone at a high dose of 15 mg/kg/day in the 27 week study, similar results were obtained as in the 14 week toxicity study.

In the 52 week rat toxicity study, body weight was not affected in contrast to 14 ad 27 week studies. Hematology and biochemical parameters as well enzyme induction and organ weight changes were observed as in 14 and 27 week toxicity studies.

In the 27 week DRSP study in monkeys, body weight was not affected. Hematologic and urinalysis changes were similar to those seen in rats. Thrombin time was decreased and fibrinogen values were increased. Treatment decreased relative weights of liver, kidney and pituitary and increased that of adrenals. Progestogenic effects were seen in uterus, ovaries and mammary gland.

In the 53 week EE/DRSP toxicity study with monkeys, glucosuria and increased urinary K excretion in high dose groups was reported. High dos combination caused enzyme induction and DRSP increased and EE decreased cyt. P-450. Liver weight was increased as in the rats. Other expected hormonal effects were observed.

In the rat carcinogenicity study, systemic exposure of DRSP in rat was about 12 fold higher then in humans on therapeutic dose. Significant DRSP-related changes were increase in body weight and hematologic changes as seen in other rat toxicity studies. Non-neoplastic histopathological changes consisted of adrenal gland zona granulosa hyperplasia, cervix and vaginal epithelial atrophy. Significant neoplastic findings were increase in adrenal gland benign pheochromocytoma as well as benign + malignant incidence. Ovarian and uterine changes seen at necropsy, and non-neoplastic histopathologic findings observed in liver (hepatocellular neoplasia), ovaries, uterus were EE-related.

During reproductive toxicity studies, administration of EE/DRSP for 6 weeks during premating in rats prolonged duration of regular cycles or resulted in cessation of the cyclic activity. There was complete return to fertility. Dosing during day 0-6 of gestation resulted in dose-dependent increase in pre and post-implantation losses but no teratogenic effects.

In segment 2 in rats, no teratogenic effect of treatment was reported. Treatment increased the number of fetuses with incompletely ossified bones.

In rabbit segment 2, there was increase in number of abortions at the high dose and one fetus had hypoplasia of the cerebellum which was not observed on repeat experiment. Delay in ossification of bones was observed. Visceral and skeletal anomalies were increased in the high dose group.

In the monkey teratology study, no teratogenic effect was observed but the number of fetuses examined was small.

In the perinatal and postnatal toxicity study in rats, there was increased pup postnatal loss during weaning, reduced body weight during lactation and indication of delayed physical and functional development.

Ames mutagenicity test, HGPRT-test with V79 cells, evaluation of the clastogenic potential in the human lymphocyte test, and mouse micronucleus test did not reveal a mutagenic potential for DRSP.

DRSP did form DNA adduct in liver but using various steroids, it was shown that there was no correlation between the DNA adduct formation and tumorigenic potential as shown in a table on p.39.

In 2 experiments using identical procedure positive response observed in the unscheduled DNA synthesis was reproducible and dose-dependent and not related to cytotoxic effect. Based on these findings, sponsor concluded that DRSP possess a genotoxic potential in primary hepatocytes in vitro.

Recommendations: Based on the results of the preclinical studies conducted by the sponsor along with sponsor's clinical experience with drospirenone outside the USA, Pharmacology considers it reasonably safe and has no objection to the initiation of the proposed clinical study.

NDA 21-098

Attachment 5 to NDA review: Pharmacokinetic data for animal toxicity studies.

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption						
Pharmaco-kinetics and Absorption	Mouse (Han NMRI (SPF)), 3 to 6 females, 24.6 to 34.0 g	Drospirenone (Batch No. 20048170) or Ethinyl Estradiol, Oral (intragastric), Single dose, 48 hours	Drospirenone*: 3.0, 10.0, 30.0 mg/kg *added to samples to prevent in vitro metabolism of drospirenone* Ethinyl Estradiol: 0.1, 0.3 mg/kg	None	Drospirenone: All dose levels: • Plasma concentrations peaked within 0.5 hours irrespective of dose level • Terminal half-lives at 3.0, 10.0 and 30.0 mg/kg were 1.7, 1.4 and 1.5 hours, respectively • Mean residence times (MRT) of 2.2, 1.7 and 2.7 hours, respectively, were calculated for dose levels of 3.0, 10.0 and 30.0 mg/kg • The C_{max} and AUC increased with dose • AUC values at 3.0, 10.0 and 30.0 mg/kg were 89, 627 and 6522 ng•hour/mL, respectively • Bioavailability at dose levels of 3.0, 10.0 and 30.0 mg/kg were 18%, 38% and 132%, respectively Ethinyl Estradiol: All dose levels: • Plasma concentrations peaked within 0.5 hours irrespective of dose level • The C_{max} and AUC increased with dose • Bioavailability at dose levels of 0.1 and 0.3 mg/kg were 7% and 6%, respectively	Report A705, Vol. 36, Page 5 12586

explan. included in

attachment 4 5

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)	Mouse [Han NMRI (SPF)], 3 to 6 females, 23.7 to 32.3 g	Drospirenone (Batch No. 20048170) or Ethinyl Estradiol, Oral (Intragastric), Once daily for 14 days	Drospirenone*: 3.0, 10.0, 30.0 mg/kg *to samples to prevent in vitro metabolism of drospirenone* Ethinyl Estradiol: 0.1, 0.3 mg/kg	None	Drospirenone: All dose levels: • Plasma concentrations peaked within 0.5 hours of the last administration, irrespective of dose level • The C_{max} and AUC increased with dose • AUC values at 3.0, 10.0 and 30.0 mg/kg were 85, 893 and 8401 ng•hour/mL • Terminal half-lives at dose levels of 3.0, 10.0 and 30.0 mg/kg were 1.5, 1.6 and 1.5 hours, respectively • Mean residence times (MRT) of 2.1, 1.9 and 2.6 hours, respectively, were calculated for dose levels of 3.0, 10.0 and 30.0 mg/kg • Bioavailability at dose levels of 3.0, 10.0 and 30.0 mg/kg was 17%, 54% and 170%, respectively • No evidence of accumulation was observed Ethinyl Estradiol: All dose levels: • Plasma concentrations peaked within 0.5 hours irrespective of dose level • The C_{max} and AUC increased with dose • Bioavailability at dose levels of 0.1 and 0.3 mg/kg was 6% and 5%, respectively • No evidence of accumulation was observed	Report A705, Vol. 36, Page 5 12586 (Cont'd)

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)	Mouse, [Han NMRI (SPF)], 3 to 5 females, 24.0 to 33.7 g	Drospirenone (Batch No. 20048170) or Ethinyl Estradiol, Intravenous, Single dose, 24 hours	Drospirenone*: 1.0 mg/kg *to samples to prevent in vitro metabolism of drospirenone* Ethinyl Estradiol: 0.1 mg/kg	None	Drospirenone: • Plasma concentrations peaked within 0.08 hours • Terminal half-life estimated to be 1.1 hours • Mean residence time (MRT) = 0.3 hours • AUC = 165 ng·hour/mL • Volume of distribution (V_z) = 9.68 L/kg Ethinyl Estradiol: • Plasma concentrations peaked within 0.08 hours • $AUC_{(0\text{-}16\ hours)}$ = 14814 pg·hour/mL • Volume of distribution (V_z) = 14.4 L/kg	Report A705, Vol. 36, Page 5 12586 (Cont'd)
	Rat [Wistar-Han (SPF)], 27 to 30 females, 170 to 190 g	[^{14}C]Drospirenone [^{14}C-ZK 30595 (Batch No. 2320-10-1)], Oral (intragastric), Single dose, 48 hours	[^{14}C]Drospirenone: 1, 10 mg/kg (1.56 Mbq/mg)	^{14}C	Oral: All dose levels: • Plasma radiolabel and unchanged drug concentrations peaked at 1 - 1.5 hours, irrespective of dose level • The bioavailability at dose levels of 1 and 10 mg/kg was 67% and 100%, respectively • Terminal half-lives at dose levels of 1 and 10 mg/kg were 3.2 and 2.3 hours, respectively • Mean residence times (MRT) of 4.5 and 5.3 hours, respectively, were calculated for dose levels of 1.0 and 10.0 mg/kg • The C_{max} and AUC increased with dose, in a manner which was not dose proportional • AUC values at 1 and 10 mg/kg were 346 and 5230 ng·hour/mL, respectively	Report 9518, Vol. 36, Page 5 12684

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)		[^{14}C]Drospirenone [^{14}C-ZK 30595 (Batch No. 2320-10-1)], Intravenous, Single dose, 48 hours	[^{14}C]Drospirenone: 0.66 mg/kg (1.56 Mbq/mg)	^{14}C	Intravenous: • Volume of distribution (V_z) = 8.77 L/kg • Total metabolic clearance rate was 32 mL/minute/kg Oral or intravenous: • Levels of radioactivity and unchanged drug decreased at similar rates	Report 9518, Vol. 36, Page 5 12684 (Cont'd)
	Rat (Han:Wistar, SPF), 3 females, 160 to 200g	Drospirenone (Batch No. Pe 5988), Oral (intragastric), Single dose, 2 days	Drospirenone: 1, 10 mg/kg	None	Oral: All dose levels: • Plasma concentrations at 1 and 10 mg/kg peaked at 0.4 and 2.7 hours, respectively, with secondary peaks in plasma concentration observed 2 to 4 hours after dosing • Terminal half-lives at 1 and 10 mg/kg were 4.7 and 3.1 hours, respectively • Mean residence times (MRT) of 5 and 6 hours respectively, were calculated for dose levels of 1 and 10 mg/kg • The C_{max} and AUC increased with dose • AUC values at 1 and 10 mg/kg were 495 and 7919 ng•hour/mL, respectively • Bioavailability at dose levels of 1 and 10 mg/kg were 70% and 110%, respectively • Liver, brain and pituitary concentrations were generally higher than plasma concentrations • Unchanged drospirenone was eliminated in the feces with only a minor amount excreted in urine • Amount of unchanged drospirenone excreted in the feces was similar after administration of 1 or 10 mg/kg	Report A154, Vol. 36, Page 5 12705

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)		Drospirenone (Batch No. Pe 5988), Intravenous, Single dose, 2 days	Drospirenone: 1 mg/kg	None	Intravenous: • Plasma concentrations peaked at 0.8 hours, with secondary peaks in plasma concentration observed 2 to 4 hours after dosing • Terminal half-life was 4.0 hours • MRT was approximately 4.4 hours and total metabolic clearance rate was 22 mL/minute/kg • Volume of distribution (V_z) was approximately 7.7 L/kg • Liver, brain and pituitary concentrations were generally higher than plasma concentration • Unchanged drospirenone was eliminated in the feces with only a minor amount eliminated in the urine	Report A154, Vol. 36, Page 5 12705 (Cont'd)
	Rat (Wistar-Han SPF), 3 females, 200 to 220 g	Drospirenone (Batch No. 11053863), Oral (intragastric), Single dose, 48 hours	Drospirenone*: 1, 10 mg/kg *to samples to prevent in vitro metabolism of drospirenone*	None	Oral : All dose levels: • Plasma concentrations at 1 and 10 mg/kg peaked at 1.5 and 2 hours with secondary peaks in plasma concentration observed 4 and 8 hours after dosing, respectively • Terminal half-lives at 1 and 10 mg/kg were estimated to be 2.6 and 3 hours, respectively • The AUC and C_{max} increased with dose • AUC values at 1 and 10 mg/kg were 510 and 8641 ng·hour/mL, respectively • Mean residence time (MRT) was calculated to be 3.1 and 5.5 hours for dose levels of 1 and 10 mg/kg, respectively • Bioavailability at dose levels of 1 and 10 mg/kg was 99% and 167%, respectively • Terminal half lives and mean residence times were low indicative of rapid elimination • Pharmacokinetics were non linear but dose-dependent	Report AE68, Vol. 36, Page 5 12734

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)		Drospirenone (Batch No. 11053863), Intravenous, Single dose, 48 hours	Drospirenone*: 1 mg/kg *to samples to prevent in vitro metabolism of drospirenone*	None	Intravenous: • Plasma concentration peaked at 0.08 hours • Terminal half-life was 6.5 hours • AUC was calculated to be 517 ng ·hour/mL • MRT was calculated to be 3.2 hours • Volume of distribution (V_z)=18.0 L/kg • Metabolic clearance rate 32.3 mL/minute/kg	Report AF68, Vol. 36, Page 5 12734 (Cont'd)
	Rabbit (New Zealand White), 5 females, 3.0 to 4.1 kg	Drospirenone (Batch No. 20048170), Oral (intragastric), 2 Single doses, 96 hours Drospirenone (Batch No. 20048170), Intravenous, Single dose, 96 hours	Drospirenone: 0.5 and 5 mg/kg Drospirenone: 0.5 mg/kg	None	Oral: All dose levels: • Plasma concentrations of 0.5 and 5 mg/kg peaked at 1.2 and 2.5 hours, respectively • Dose proportional pharmacokinetics were observed at the tested dose levels • Average absolute bioavailability approximately 40% following administration of the tested dose levels • Mean total clearance rate from plasma (approximately 35 mL/minute/kg) was slightly more than the reported hepatic plasma flow rate for the rabbit (26 mL/minute/kg) • AUC values at 0.5 and 5 mg/kg were 105 and 993 ng·hour/mL, respectively • Terminal half-life at dose levels of 0.5 and 5 mg/kg was 5.9 hours • Mean residence times (MRT) of 6.8 and 7.2 hours, respectively, were calculated for dose levels of 0.5 and 5 mg/kg Intravenous: • Volume of distribution (V_z) = 19.7 L/kg	Report 9392, Vol. 36, Page 5 12757

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)	Monkey (Cynomolgus), 5 females, 3.3 to 3.9 kg	Drospirenone (Batch No. Pe 5988), Oral (intragastric), 3 days Drospirenone (Batch No. Pe 5988), Intravenous, 3 days Animals received two intragastric (2 weeks apart) and one intravenous dose (4 weeks later)	Drospirenone: 1, 10 mg/kg Drospirenone: 0.5 mg/kg	None	Oral: All dose levels: • Plasma concentrations at 1 and 10 mg/kg peaked on average at 2 and 3 hours, respectively • Mean terminal half-lives were 10.8 and 19.6 hours at 1 and 10 mg/kg, respectively • Mean residence times (MRT) of 13.0 and 28.3 hours, respectively, were calculated for dose levels of 1.0 and 10.0 mg/kg • AUC values at 1 and 10 mg/kg were 4060 and 30367 ng·hour/mL, respectively • At dose levels of 1 and 10 mg/kg, bioavailability was 74% and 55%, respectively • Pharmacokinetics were not dose-proportional following oral administration Intravenous: • The mean terminal half-life was 11.4 hours • The volume of distribution (V_z) was 3.0 L/kg • The mean total metabolic clearance rate was 3.3 mL/minute/kg Oral or intravenous: • Less than 1.5% and 3.3% of the administered dose was excreted unchanged in the urine and feces, respectively, independent of dose and route of administration	Report 9037, Vol. 36, Page 5 12819

Pharmacokinetics, Absorption, Distribution, Metabolism and Excretion (Cont'd)

Type of Study	Species (Strain), Number and Sex/Group and Body Weight	Compound, Route, Frequency of Administration and Duration of Study	Compound(s): Dose Level Information	Isotope	Notable Findings	Reference
Pharmacokinetics and Absorption (Cont'd)						
Pharmaco-kinetics and Absorption (Cont'd)	Monkey (Cynomolgus), 3 to 4 females, 3.0 to 4.0 kg	[^{14}C]Drospirenone [^{14}C-ZK 30595 (Batch No. 2320-17)], Oral (Intragastric), 96 hours [^{14}C]Drospirenone [^{14}C-ZK 30595 (Batch No. 2320-17)], Intravenous, 96 hours Each animal received one intravenous dose and two oral doses approximately 3 to 7 weeks apart	[^{14}C]Drospirenone: 1 and 10 mg/kg (0.7 and 0.07 Mbq/mg, respectively) [^{14}C]Drospirenone: 0.5 mg/kg (1.4 Mbq/mg)	^{14}C	Oral: All dose levels: • Plasma concentrations peaked at 2 and 4 hours after administration of 1 and 10 mg/kg, respectively • Bioavailability was estimated to be 67% and 43% of administered dose at dose levels of 1 and 10 mg/kg, respectively • The apparent mean terminal half-lives of dose levels of 1 and 10 mg/kg were 15.9 and 13.4 hours, respectively • DRSP AUC values at 1 and 10 mg/kg were 2513 and 21230 ng·hour/mL, respectively • The mean terminal half-life of the ^{14}C-label following administration of 1 or 10 mg/kg was 29.5 or 32.2 hours, respectively Intravenous: • Secondary peaks in plasma levels of drug were observed 1.5 to 2.0 hours after dosing • The DRSP AUC value was 1881 ng·hour/mL • The mean terminal half-life was 13.2 hours • The mean terminal half-life of the ^{14}C-label was 33.7 hours	Report A376, Vol. 37, Page 5 12839

TABLES OF MEAN PHARMACOKINETIC PARAMETERS OF YASMIN

Table I

Drospirenone
Mean (%CV) Values

Cycle / Day	Cmax (ng/mL)	tmax (h)	AUC(0-24h) (ng•h/mL)	AUC (ng•h/mL)	$t_{1/2}$ (h)
1 / 1	36.5 (29)	2.5 (56)	308 (23)	NA	NA
1 / 21	59.5 (32)	2.4 (49)	754 (34)	1502 (36)	28.3 (23)
3 / 1	39.6 (30)	2.2 (64)	376 (33)	NA	NA
3 / 21	60.4 (33)	2.7 (51)	814 (33)	1675 (36)	29.5 (21)
1/1	36.9 (13)	1.7 (47)	287.9 (25)	NA	NA
1/21	87.5 (59)	1.7 (20)	826.5 (23)	1888.9 (43)	30.9 (44)
6/21	84.2 (19)	1.8 (19)	930 (19)	2228(42)	32.5 (38)
9/21	81.3 (19)	1.6 (38)	957 (23)	2290 (47)	31.4 (39)
13/21	78.7 (18)	1.6 (26)	968 (24)	2343 (48)	31.1 (36)

NA = Not available

Table II

Ethinyl Estradiol
Mean (%CV) Values

Cycle / Day	Cmax (pg/mL)	tmax (h)	AUC (0-24h) pg•h/mL	AUC pg•h/mL	$t_{1/2}$ (h)
1 / 1	121.6 (32)	2.2 (34)	975 (50)	NA	NA
1 / 21	145.7 (40)	2.3 (42)	1175 (52)	NA	NA
3 / 1	107.7 (25)	2.4 (42)	900 (48)	NA	NA
3 / 21	143.3 (26)	2.6 (52)	1320 (42)	NA	NA
1/1	53.5 (43)	1.9 (45)	280.3 (87)	NA	NA
1/21	92.1 (35)	1.5 (40)	461.3 (94)	NA	NA
6/21	99.1 (45)	1.5 (47)	346.4 (74)	NA	NA
9/21	87 .0 (43)	1.5 (42)	485.3 (92)	NA	NA
13/21	90.5 (45)	1.6 (38)	469.5 (83)	NA	NA

NA = Not available

NDA 21-098
Yasmin® 28 Tablets (drospirenone/ethinyl estradiol)
Berlex Laboratories, Inc.

There was no DSI (GLP) Inspection.

/S/
4/10/04

APPEARS THIS WAY
ON ORIGINAL

NDA 21-098
Yasmin® 28 Tablets (drospirenone/ethinyl estradiol)
Berlex Laboratories, Inc.

Statistical review of carcinogenicity studies was completed during the first review cycle.

4/18/01

Statistical Review and Evaluation
Carcinogenicity

DEC 2 1 1999

Date:

NDA No:	21-098
Applicant:	Berlex Laboratories, Inc
Drug Name:	Drospirenone 3 mg and Ethinyl Estradiol 0.030 mg Tablets
Data Source:	two diskettes - mice and rats
Pharmacologist:	Dr. Krishan Raheja (HFD-580)
Statistical Reviewer:	Moh-Jee Ng (HFD-715)

1. Introduction

Two animal carcinogenicity studies (one in mice and one in rats) were included in this IND submission. The goal of these two studies was to evaluate the carcinogenic potential of drospirenone alone, ethinyl estradiol (EE) alone and the combination of drospirenone and ethinyl estradiol in the oral administration in the diet to mice and rats for two years. The pharmacology reviewer, Dr. Raheja requested the Division of Biostatistics II to perform a statistical review and evaluation of these studies.

2. The Mouse Study

2.1 Design

This study included only female mice. There were 10 groups in this experiment; one control group and 9 treated groups. Groups 2, 3 and 4 received low, medium and high doses of the combination of drospirenone and ethinyl estradiol; groups 5, 6, and 7 received low, medium and high doses of the ethinly estradiol alone; and groups 8, 9 and 10 received low, medium and high doses of drospirenone alone. The dose levels were 1+0.01, 3+0.03, 10+0.1 mg/kg for the combination of drospirenone and ethinyl estradiol, 0.01, 0.03, 0.1 mg/kg for ethinyl estradiol, and 1, 3, 10 mg/kg for drospirenone alone. The control group consisted of 110 females and each treated group consisted of 55 females. The terminal sacrifice was performed during experimental 106 to 110 weeks. All animals were necropsied at termination and gross and histopathological examinations were conducted.

2.2 Sponsor's Analyses

The sponsor used the Cox proportional hazards model (Cox, D.R., 1972) and Tarone's partition of the Chi-square tests (Tarone, RE., 1951) to analyze the inner-group differences in mortality, and used the Kaplan-Meier and the Product Limit estimation (Kaplan, E.L., and Meier, P., 1958) to adjust mortality rates. The sponsor also used the methods described in Peto et al (1980) to analyze tumor incidence data.

The sponsor's analyzed data of groups 2-4 (the combination of drospirenone and ethinyl estradiol dose groups), groups 5-7 (ethinyl estradiol dose groups) and groups 8-10

(drospirenone dose groups) separately versus group 1 (control group). All tests were conducted at the 5% level of significance.

The sponsor listed the following findings in the reports.

In survival analysis:

- Significant dose-mortality trends among the doses of ethinyl estradiol alone and among doses of the combination of drospirenone and ethinyl estradiol were detected.

In tumor data analysis:

Combination of drospirenone and ethinyl estradiol:

- A significant positive-dose response relationship was observed in adenoma in pituitary, and statistically differences in adenocarcinoma in uterus and in carcinoma in mammary gland were observed when the medium dose was compared with the control group.

Ethinyl estradiol alone:

- Significant positive-dose response relationships were observed in carcinoma, carcinosarcoma and adenoacanthoma in the mammary gland, in adenoma in pituitary, and in adenocarcinamo and stromal polyp in uterus.

2.3 Reviewer's Analyses

This reviewer performed independent analyses on the survival and tumor data submitted by the sponsor, using the programs developed by Dr. Ted Guo of Division of Biostatistics II. The primary statistical methods used were described in Peto *et al* (1980), and Lin and Ali (1994). These methods adjust differences in animal mortality and take contexts of observation of the tumors into consideration. The intervals used for the adjustment of mortality were 0-52, 53-78, 79-91 and 92-106 weeks and terminal sacrifice for females.

The statistical analyses of carcinogenicity study data consist of two parts, namely, the survival data analysis and the tumor data analysis. The survival data analysis is: 1) to examine the differences in survival distributions among the treatment groups (homogeneity test); and 2) to determine if there is a positive linear trend in the proportion of deaths with respect to the dose levels (Linear trend test). Two statistical tests were used in the survival data analysis: the Cox test and the generalized Kruskal-Wallis test. The theoretical background of these tests was described in Lin and Ali (1994) and Thomas *et al* (1977).

The tumor data analysis is: 1) to determine if there is a positive linear trend in the proportions of a selected tumor type in a selected organ/tissue with respect to the dose levels. The tumors were classified as either fatal (lethal) or non-fatal (non-lethal), according to Peto *et al*(1980). The reviewer applied the death-rate method to fatal tumors and the prevalence method to non-fatal tumors. For tumors that caused death for some, but not for all, animals, a combined test was performed.

A rule for adjusting the effect of multiple tests proposed by Haseman (1983) can be used in control-high pairwise comparisons. Haseman's rule says that rare tumors should be tested at 0.05 level of significance and common tumors should be tested at 0.01 level of significance. A similar rule proposed by the Divisions of Biometrics, CDER/FDA for trend tests was used in this review for tests for positive trend. The rule states that in order to keep the overall false-positive rate at the nominal level of approximately 0.1, rare tumor types should be tested at 0.025 significance level, otherwise (common tumors) at 0.005 significance level (Lin and Rahman, 1988). A tumor type with spontaneous rate of 1% or less is defined as rare, and as common, otherwise.

2.3.1. Survival Data Analysis

Figures 1a, 1b and 1c in the attachment present plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for female mice.

The cumulative mortality rates and the survival at terminal sacrifice for each dose group are summarized in Tables 2a, 2b and 2c in the attachments.

The dose-mortality trend test for survival rates of four groups (Control, Low, Medium, and High) were performed separately for the combination of drospirenone and ethinyl estradiol, ethinyl estradiol alone, and drospirenone alone for female mice using the Cox test and the generalized Kruskal-Wallis test. Results of the tests are given in Tables 3a, 3b and 3c in the attachments.

The results of this reviewer's analysis are consistent with the sponsor's survival analysis results. Neither the Cox test nor the generalized Kruskal-Wallis test showed a statistically significant dose-mortality trend among the doses of drospirenone alone at 0.05 level. However, the results showed significant dose-mortality trends among the doses of ethinyl estradiol alone and among doses of the combination of drospirenone and ethinyl estradiol.

2.3.2 Tumor Data Analysis

This reviewer's analyses used the procedures described in Peto et al (1980) and Lin and Ali (1994). The results are summarized in Tables 4a, 4b and 4c in the attachments. The tumor types for all mice showing significant trends or differences at α=0.05 are summarized in the following table.

Table 1a

Dose	Control	Low	Medium	High	P-Values
Adenoma in pituitary					
Combined drospirenone and Ethinyl Estradiol	4	11	13	23	<0.005**
Ethinyl Estradiol alone	4	7	27	41	<0.005**
Adenocarcinoma in uterus					
Ethinyl Estradiol alone	0	4	10	9	<0.005**
Carcinoma in mammary gland					
Ethinyl Estradiol alone	2	0	6	5	0.002**
Carcinosarcoma in mammary gland					
Ethinyl Estradiol alone	0	1	1	2	0.024*
Carcinoma in harderian glands					
Drospirenone alone	0	0	1	3	0.009*

* Indicate statistically significant at level 0.025.
** Indicate statistically significant at level 0.005.

The results of this reviewer's tumor analysis are as follows:

For doses of the combination of drospirenone and ethinyl estradiol:

- The positive-dose response relationship in adenoma for pituitary was significant ($p<0.001$).

For doses of ethinyl estradiol alone:

- Significant positive-dose response relationships were observed in carcinoma ($p=0.002$), and carcinosarcoma ($p=0.024$) in the mammary gland, in adenoma in pituitary ($p<0.001$), and in adenocarcinoma in uterus. No significant positive-dose response relationships were observed in adenoacanthoma in the mammary gland ($p>0.005$), and in endometrial stromal polyp in uterus ($p>0.005$) after adjusting the effect of multiple testings.

For doses of drospirenone alone:

- The positive-dose response relationship in carcinoma in harderian glands was significant ($p=0.009$).

It should be noted that this review did not compare the tumor rates of the medium dose group with the control group.

3. The Rat Study

3.1 Design

This study included only female mice. There were 10 groups, one control group and 9 treated groups. Groups 2, 3 and 4 received low, medium and high doses of the combination of drospirenone and ethinyl estradiol; groups 5, 6, and 7 received low, medium and high doses of the ethinly estradiol alone; and groups 8, 9 and 10 received low, medium and high doses of drospirenone alone. The dose levels were 0.3+0.003, 3.0+0.03, 10.0+0.1 mg/kg for the combination of drospirenone and ethinyl estradiol, 0.003, 0.03, 0.1 mg/kg for ethinyl estradiol, and 0.3, 3.0, and 10.0 mg/kg for drospirenone alone. The control group consisted of 110 females and each treated group consisted of 55 females. The terminal sacrifice was performed during experimental 106 to 110 weeks. All animals were necropsied at termination and gross and histopathological examinations were conducted.

3.2 Sponsor's Analyses

The statistical methods used by the sponsor in the analysis of data of the rat study were described in the mouse study (see section 2.2).

The sponsor listed the following findings in the reports.

In survival analysis:

- A significant dose-mortality trend among the doses of drospirenone alone was detected but not in either among the doses of the combination of drospirenone and ethinyl estradiol or among the doses of ethinyl estradiol alone.

In tumor data analysis:

In the combination of drospirenone and ethinyl estradiol:

- There was a significant positive-dose response relationship in hepatocellular adenoma in liver. There were decreasing trends in the adenocarcinoma and endometrial stromal polyp tumors in uterus, in the adenocarcinoma in mammary gland, and in cortical adenoma in adrenal gland.

In ethinyl estradiol alone:

- There were significant positive-dose response relationships in hepatocellular adenoma in liver, in adenocarcinoma in uterus, and in adenocarcinoma in mammary gland. However, there were decreasing trends in endometrial stromal polyp in uterus and in cortical adenoma in adrenal gland.

In drospirenone alone:

- Significant negative-dose response relationships were observed in adenocarcinoma in uterus, in endometrial stromal polyp in uterus, in adenocarcinoma in mammary gland, and in cortical adenoma in adrenal gland. There were significant positive-dose response

relationships in benign pheochromocytom in adrenal glands, and combined malignant-benign pheochromocytom in adrenal glands.

3.3 Reviewer's Analyses

This reviewer performed independent analyses on the survival and tumor data submitted by the sponsor and used the programs developed by the statistician, Dr. Ted Guo of Division of Biostatistices II. The primary statistical methods used were described in Peto *et al* (1980) and Lin and Ali (1994). The methods allow for the differences in animal mortality and the contexts of observation of tumors. The time intervals used for the adjustment of mortality were 0-52, 53-78, 79-91, 92-109 weeks and terminal sacrifice.

3.3.1 Survival Data Analysis

Figures 1d, 1e and 1f in the attachment present plots of Kaplan-Meier estimates of the survival distributions of the treatment groups for female rats.

The cumulative mortality rates and the survival at terminal sacrifice for individual dose groups were summarized in Tables 2d, 2e and 2f in the attachments.

The dose-mortality trend test for survival rates of four groups (control, low, medium, and high) were performed separately for the combination of drospirenone and ethinyl estradiol, ethinly estradiol alone and Drospirenone alone for female mice using the Cox test and the generalized Kruskal-Wallis test. Results of the above tests were presented in Tables 3d, 3e and 3f in the attachments.

The results of this reviewer's analysis were not consistent with the sponsor's results. The receiver's tests showed that for all treatment groups in rats, there was a statistically significant ($p<0.05$) positive linear trend in mortality of drospirenone alone. However, no positive linear trends in the combination of drospirenone and ethinyl estradiol and ethinyl estradiol alone were detected.

3.3.2 Tumor Data Analysis

This reviewer's analyses used the procedures described in Peto *et al* (1980) and Lin and Ali (1994). The results were summarized in Tables 4d, 4e and 4f in the attachments. The incidence rates and p-values of all tumors showing significant positive or trends or differences at 0.05 level of significance are summarized in the following table.

Table 1b

Dose	Control	Low	Medium	High	P-Value
Hepatocellular Adenoma in Liver					
Combined drospirenone and EE	1	1	1	6	0.001*
EE alone	1	0	3	5	0.005*

* Indicate statistically significant at level 0.025.

The results of this reviewer's tumor analysis are as follows:

For doses of the combination of drospirenone and ethinyl estradiol:

- A significant positive dose-response relationships ($p<0.025$) in incidence rate of hepatocellular adenoma in liver was detected.

For doses of ethinyl estradiol alone:

- A significant positive dose-response relationships ($p<0.025$) in incidence rate of hepatocellular adenoma in liver was detected.

For doses of drospirenone alone:

- No significant positive trends or increase s in tumor incidence rate in the high dose group over the control group were deteched.

4 Conclusion

In the 2-year mouse study, there was a statistically positive-dose response relationship in adenoma in pituitary among doses of the combination of drospirenone and ethinyl estradiol. There were statistically positive-dose relationships in carcinoma and carcinosarcoma in the mammary gland, in adenoma in pituitary, and in adenocarcinoma in uterus among doses of ethinyl estradiol alone. There was a statistically positive-dose relationship in carcinoma in harderian glands among doses of drospirenone alone.

In the 2-year rats study, there was a statistically positive-dose response relationship in hepatocellular adenoma in liver among the doses of the combination of drospirenone and ethinyl estradiol. There was a significant positive dose-response relationship in incidence rate of hepatocellular adenoma in liver among the doses of ethinyl estradiol alone.

References

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