Reproductive Toxicity Studies

1. **Fertility Study in Rats**

Testing Facility:

Study Numbers: ML-222C3 (contract Lab.'s No.)
000602 (sponsor's No.)

Study Dates: June, 1981 to September, 1981

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: JCL:SD strain rats, obtained from were housed individually in wire mesh bottom plastic wall cages with free access to CA-1 solid food. Males were about six weeks old (mean body weight 196 g) and females were nine to ten weeks old (mean body weight 216 g) at the initiation of the study.

Dose Levels and Treatment Regimen: 0, 30, 150 and 1000 mg/kg/day (24 males or 23-24 females per group). OPC-21 (Lot No.1F71) was suspended in 0.5% carboxymethylcellulose at appropriate concentrations and was administered once daily by oral gavage at a dose volume of 10 ml/kg. Control animals received vehicle alone.

Males were treated for 9 weeks prior to the start of pairing with treated females and continued through the pairing period till the day of copulation. Females were dosed for 14 days prior to pairing and continued through pairing period till day 7 of gestation. (The confirmed day of copulation, evidenced by the presence of a vaginal plug, was considered as day 0 of gestation.)

There was an additional group of 24 untreated females mated to the high dose group males. (The high dose males were first paired for 7 days with high dose females, and then again paired for 7 days with untreated females.) This additional female group will be referred hereafter in the review as the untreated female group.

[It is stated that the doses were selected based on the results of single dose and 28-day administration dose ranging studies in male and female rats of the same strain as used for the fertility study. In the single dose study, rats (10/sex) were given a single administration of the drug at the "highest feasible" dose of 5000 mg/kg by gastric intubation. None of the animals died during the 7 day observation period. There were no treatment-related findings.

For the multiple dose study (6 rats/sex/group), the drug was administered at 0, 500, 1000 and 2000 mg/kg/day for 28 days by gastric intubation. No deaths occurred during the study. There were no treatment-related findings except for significantly lower hemoglobin (4%) and RBC (7%) values for the 2000 mg/kg/day female group compared to concurrent control values. Although no remarkable toxicity findings were seen even at the highest dose (2000 mg/kg/day) tested in the study, it is stated that since "technical difficulty in continuously administering this high dose to pregnant animals was anticipated", a dose of 1000 mg/kg/day was selected as the high dose for the fertility study. No further explanation for high dose selection is provided.]

Observations and Measurements: Males were observed weekly for body weight and food and water consumption, and were killed after checking for pregnancy of paired females.

Females were examined once weekly before mating and once daily from day 0 to 20 of gestation for body weight and food and water consumption. They were also observed for clinical signs during the course of gestation.

At day 20 of pregnancy, females were killed and numbers of corpora lutea, implantation sites, resorption sites, dead embryos and live fetuses were counted. Live fetuses were weighed and examined for external abnormalities. About two-thirds of the fetuses were fixed in 95% alcohol, and their skeletons were cleared with KOH and stained with alizarin red S (Dawson's method) for the examination of skeletal abnormalities. The remaining fetuses were kept in 10% formalin until about 2 weeks prior to examination, then fixed in Bouin's solution, and examined for visceral abnormalities (Wilson's and Nishimura's methods).

Wilcoxon's rank sum test was used for the analysis of data on post-implantation loss, external, skeletal and visceral abnormalities of the fetuses, retarded ossification of metacarpals and metatarsals, and numerical variations of ribs and vertebrae. All other data were analyzed using the t test.

Results: No significant clinical symptoms were noted. Two males from the 30 mg/kg/day group (one in the third treatment week of the pre-mating period and the other on the third day of the mating period) were either dead or removed from the study due to dosing injuries. No significant treatment-related effect on body weight was seen during the 9 week pre-mating period in males or the 2-week pre-mating period in females. The mean body weights of all drug-treated female groups were higher than concurrent control weights during the entire gestation period, with statistically significant

differences noted at 150 and 1000 mg/kg/day during the first five days of gestation. No significant treatment-related effect on food or water consumption was noted.

Reproductive performance - The occurrence of copulation in females was determined by daily inspection for vaginal plugs. Copulation occurred within 6 days of pairing except for one female each from the 30 and 1000 mg/kg/day dose groups and untreated female group, and 2 females from the 150 mg/kg/day group. When females that failed to mate were paired again with males (of the same group) that had made other females pregnant, copulation occurred in the two females from the 150 mg/kg/day dose group. There was one pregnant female each from the 150 and 1000 mg/kg/day groups in which no vaginal plugs were seen as confirmatory evidence for mating. Since the treatment period in these 2 females did not accord with the protocol, the fetuses from these females were not evaluated.

The pregnancy rates for control, 30, 150 and 1000 mg/kg/day dose groups were 95.8%, 95.4%, 87.5% and 100%, respectively. The untreated female group had a pregnancy rate of 100%

Fetal findings - The numbers of corpora lutea and implantation sites, the incidence of post-implantation loss, the number and weight of live fetuses, and the number of fetuses with external abnormalities are presented in Table 4. The mean number of corpora lutea was slightly higher in drug-treated groups (not dose-dependent) and in the untreated female group than in the control, the difference being statistically different at 150 mg/kg/day. The numbers of implantation sites and live fetuses and the weights of male and female fetuses were comparable between control and treated groups. The incidence of post-implantation loss appeared to be higher in the drug-treated and untreated female groups (6.3-8.7%) than in the control group (5.9%), however, the differences were not statistically significant.

The incidence of fetuses with external malformations for the control, 30, 150 and 1000 mg/kg/day dose groups was 0.0, 0.6, 0.4 and 0.3%, respectively. The untreated female group had a malformation rate of 0.3%. Multiple anomalies of the fore- and hind- limbs with bilateral microphthalmia, brachyury and mild edema were seen in one fetus from the 30 mg/kg/day dose group, and otocephaly with micrognathia, microstomia, unilateral microphthalmia and cleft palate were seen in another fetus from the same group. (The former fetus was sacrificed for skeletal examination and, at the time of abdominal dissection, was noted to have developed right aortic arch with complete transposition of the great vessels.) Mild edema was seen in one fetus from the 150 mg/

Table 4. Influence of OPC-21 on Number of Corpora Lutea and Implantations, Post Implantation Loss and Body Weight of Live Fetuses in Rats

Group and dose (mg/kg, ig) Male	Female	No. of mothers (M)	Total No. of corpora lutea (C) ( ):(C)/(M)	Total No. of implant. (I) ( ):(I)/(M)	Post implantation loss R.S. D.E. (D) ( ):(D)/(I)x100	Total No. of live fetuses (L) ( ):(L)/(M)	Body weight (g) Male Mean S.D.	Body weight (g) Female Mean S.D.	No. of fetuses with external malformations (X) ( ):(X)/(L)x100
Control	Control	23	374 (16.3)	341 (14.8)	20 0 (5.9%)	321 (14.0)	3.5 0.2	3.4 0.2	0 (0.0%)
30	30	21	359 (17.1)	335 (16.0)	20 1 (6.3%)	314 (15.0)	3.5 0.2	3.4 0.2	2(c) (0.6%)
150	150	20(a)	352 (17.6*)	309 (15.4)	21 3 (7.8%)	285 (14.3)	3.6 0.2	3.3 0.3	1(d) (0.4%)
1000	1000	22(a)	372 (16.9)	341 (15.5)	25 2 (7.9%)	314 (14.3)	3.6 0.3	3.4 0.2	1(e) (0.3%)
1000	Untreated	23	393 (17.1)	344 (15.0)	28 2 (8.7%)	314 (13.7)	3.6(b) 0.2	3.4(b) 0.3	1(f) (0.3%)

(a) One mother in which a vaginal plug had not been found was excluded from the data
(b) Calculated on 22 mothers
(c) 1:Multiple anomalies of the fore-and hind limbs with bilateral microphthalmia, brachyury and mild edema
1:Otocephaly with micrognathia, microstomia, unilateral microphthalmia and cleft palate
(d) Mild edema
(e) Vestigial digit of the right forelimb with hypoplastic paw of the left forelimb and kinky tail
(f) Edema
implant. : Implantations, R.S. : Resorption site, D.E. : Dead embryo
* : Significantly different from control, $P<0.05$
ig : Intragastrically

kg/day group; and vestigial digit of the right forelimb, kinky tail and plastic paw of the left forelimb were seen in one fetus from the 1000 mg/kg/day group. Edema was seen in one fetus from the untreated female group.

(Note: Historical control data from 21 studies performed at the facility where the present study was done, during the period from 1976 to 1981, showed that the incidence of fetuses with external malformations ranged from 0 to 1%.)

Skeletal findings observed were grouped under malformations, variations in the number of ribs and vertebrae, and retarded ossification.

The skeletal malformations are presented in Table 5. The incidences of malformations in drug-treated groups were not significantly different from the concurrent control. Dumbbell-shaped thoracic vertebral bodies were seen in all groups including control. Anomalies of thoracic and/or lumbar vertebral bodies (partial hypoplasia of the 10th thoracic vertebral body in one control fetus, absence of the 10th thoracic vertebral body in another control fetus, and partial absence of the 13th thoracic vertebral and the 2nd lumbar vertebral bodies with hypoplasia of the 2nd right lumbar vertebral arch in one fetus from the untreated female group) were seen in the control and untreated female groups. In the 30 mg/kg/day group, the fetus with otocephaly showed hypoplasia of the mandibula and unilateral orbita and cleft palate, and the other fetus from the same group with multiple anomalies of microphthalmia and brachyury showed fusion of the cervical arches and ribs with severe retardation of ossification. Wavy ribs with anomalies of the clavicula, scapula, humerus and thoracic vertebral body in one 150 mg/kg/day fetus and the absence of the 5th right metacarpal bone in one 1000 mg/kg/day fetus were observed.

Table 5. Skeletal Malformations in Live Fetuses of Rats Treated with OPC-21

Group and dose (mg/kg, ig)	No. of fetuses(F)	Skeletal malformations						
		Total(T) ( ):(T)/(F)x100	Dumbbell-shaped thoracic vertebral body	Hypoplasia of the mandibula	Fusion of the cervical vertebral archs	Anomaly of the thoracic vertebral body	Wavy ribs	Absence of the 5th metacarpal bone
Control	221	5 (2.3%)	3			2		
30	215	8 (3.7%)	6	1(a)	1(b)			
150	197	2 (1.0%)	1				1(c)	
1000	216	5 (2.3%)	4					1
Untreated	216	5 (2.3%)	4			1(d)		

(a) With hypoplasia of the unilateral orbita and cleft palate
(b) With fusion of the ribs and severe retardation of the ossification
(c) With anomalis of the clavicula, the scapula, the humerus and the thoracic vertebral body
(d) With anomaly of the lumbar vertebrae
ig : Intragastrically

The variations in the numbers of ribs and vertebrae are summarized in Table 6. A dose-dependent increase in the incidence of fetuses with 14 ribs was seen in drug-treated groups (statistically significant at 1000 mg/kg/day) compared to concurrent control incidence.

The incidence of ossification of metacarpals, metatarsals and caudal vertebrae are given in Table 7. Although not statistically significant, the incidence of less than 4 metacarpals on either or both side was higher than control in the mid and high dose groups and in the untreated female group.

Visceral malformations are presented in Table 8. The incidence of visceral malformations was higher in the untreated female group than in the concurrent control group, but the difference was not statistically significant. Abnormal origin of the left common carotid artery in one control fetus, ventricular septal defect and hypoplastic spleen in one high dose fetus, and absence of thyroid gland with abnormal origin of the left internal carotid artery and ventricular septal defect in one fetus from the untreated female group were observed.

Table 6. Number of Ribs and Presacral Vertebrae in Rat Fetuses

Group and dose (mg/kg, ig)	No. of fetuses	No. of ribs(a) 13/13	13/14	14/13	14/14	Presacral vertebrae 26	27
Control	221	169	16	14	22	218	3
			(23.5%)				(1.4%)
30	215(b)	143	25	12	34	213	1
			(33.2%)				(0.5%)
150	197	127	25	13	32	192	5
			(35.5%)				(2.5%)
1000	216	125	29	11	51	211	5
			(42.1%*)				(2.3%)
Untreated	216	150	24	14	28	213	3
			(30.6%)				(1.4%)

(a) Right side / Left side
(b) One fetus with severe retardation of the ossification was excluded from the data
* : Significantly different from control, $P<0.05$
ig : Intragastrically

Table 7. Observation on the Ossification of Rat Fetuses

Group and dose (mg/kg, ig)	No. of fetuses	Metacarpus(a) <4/4	Metacarpus(a) 4/4	Metatarsus(a) <4/4	Metatarsus(a) 4/4	Metatarsus(a) >4/4	No. of caudal vertebrae Mean	No. of caudal vertebrae S.D.
Control	221	109 (49.3%)	112	3 (1.4%)	218	0 (0.0%)	4.1	0.3
30	215(b)	91 (42.5%)	123	4 (1.9%)	210	0 (0.0%)	4.1	0.3
150	197	111 (56.3%)	86	2 (1.0%)	195	0 (0.0%)	4.0	0.3
1000	216(c)	128 (59.5%)	87	1 (0.5%)	214(d)	0 (0.0%)	4.1	0.3
Untreated	216	128 (59.3%)	88	4 (1.9%)	212	0 (0.0%)	4.1	0.4

(a) Right side / Left side
(b) One fetus with severe retardation of the ossification was excluded from the data
(c) One fetus with absence of the 5th metacarpal bone was excluded from the data of metacarpus
(d) Skeletal specimen in one fetus that failed to clear was not examined
ig : Intragastrically

Table 8. Visceral Malformations in Live Fetuses of Rats Treated with OPC-21

Group and dose (mg/kg, ig)	No. of fetuses(F)	Visceral malformations			
		Total(T) ( ):(T)/(F)x100	Thymic remnant in the neck	Abnormal origin of the left common carotid artery	Ventricular septal defect
Control	100	5 (5.0%)	4	1	
30	99	4 (4.0%)	4		
150	88	1 (1.1%)	1		
1000	98	2 (2.0%)	1		1(a)
Untreated	98	11 (11.2%)	10		1(b)

(a) With hypoplastic spleen
(b) Absence of the thyroid gland with abnormal origin of the left internal carotid artery
ig : Intragastrically

2. **Preliminary Teratological Study in Rats**

Testing Facility:

Study Number: 210521-0812

Study Dates: July 10, 1981 to August 3, 1981

GLP Compliance: The study was conducted in compliance with GLP regulations.

Animals: Male and female Sprague-Dawley rats (10-11 weeks-old) were obtained from The males were housed in metal cages and the females in plastic cages, with free access to solid food. After a week of quarantine and acclimatization, the animals were mated. The confirmed day of copulation was considered as Day 0 of gestation.

Dose Levels and Mode of Administration: 0, 40, 200 and 1000 mg/kg/day (5 pregnant females per group). OPC-21 (Lot No.1F95M) was suspended in 0.5% sodium carboxymethylcellulose at appropriate concentrations and was administered to the dams once daily by oral gavage, at a dose volume of 10 ml/kg, on days 7 through 17 of gestation.

Observations and Measurements: The dams were observed for clinical signs twice daily (30 min and 2 hr post-dose) during the treatment period. The body weights were recorded on day 0 and days 7 through 20 of gestation.

On day 20 of gestation, the dams underwent cesarean section, and the numbers of corpora lutea, implantation sites, early and late resorption sites and live and dead fetuses were counted. The live fetuses were sexed, weighed, placental weights taken, and examined for external abnormalities (including oral cavity abnormalities). The dams were subjected to gross pathological examination.

About half of the live fetuses were fixed in 95% ethyl alcohol, cleared with KOH and stained with Alizarin red S(Dawson's method) for the examination of skeletal abnormalities and progress of ossification. The remaining fetuses were fixed in Bouin's solution and examined for visceral abnormalities, the head by Wilson's method and the thoracoabdominal part by Nishimura's method.

Skeletal and visceral examinations were performed using a binocular stereoscopic microscope.

Data on maternal weight, food consumption, numbers of corpus lutea, implantations and live fetuses, fetal weight, placental weight and the number of caudal vertebral bodies were analyzed using the t test and the data on sex ratio by the X^2 test. All other data were analyzed using Wilcoxon's rank sum test.

Results: No notable clinical signs were observed throughout the treatment period. No treatment-related effect on body weight or food consumption was observed except for a slight (and not statistically significant) reduction of the body weight gain and a decrease in food consumption at the high dose during the first 2 or 3 days of the treatment period.

Results of the cesarean section are presented in Table 9. No significant differences in any parameters (numbers of corpora lutea, implantation or resorption sites, dead or live fetuses, and body weight or placental weight) between control and treated groups, or any dose-dependent findings were seen. The only external anomaly observed was a hematoma in one fetus at 200 mg/kg/day.

The skeletal findings are presented in Table 10. Skeletal anomalies observed in the study included waved and knobbed ribs with shortened and misshapen femur in one mid-dose fetus, and waved and knobbed ribs in one low-dose fetus.

No dose-dependent variations were seen except for the cervical ribs with incidence rates of 0, 0, 2.6 and 6.9% for control, low, mid and high dose fetuses, respectively. Fourteenth ribs were seen in all treated and control groups, the incidence being highest in the low dose group, significantly higher than in controls.

A greater than control incidence of non-ossified cervical vertebral bodies was observed in all treated groups, the difference from control being statistically significant at low and mid doses. Dose-related increases in fetuses with non-ossified 5th and 6th sternebrae (statistically significant for the 6th sternebrae at the high dose) were seen. The number of fetuses with less than 4 metacarpals was also increased dose-relatedly. No treatment related effects were noted in the number of caudal vertebrae or in the number of fetuses with reduced metatarsals.

The visceral findings are presented in Table 11. No dose-dependent increase in visceral abnormalities and no significant difference between control and treated groups was noted. Visceral anomalies observed only in treated groups included ventricular septal defect (high dose), left umbilical artery (high dose), post-ureteric uterus (mid dose), ureteric convolution with dilatation (mid dose), subcutaneous hematoma (mid dose) and polydactyly (low dose).

Table 9.

Effects on prenatal development in rats treated with OPC-13013

- Preliminary study -

Exp. groups (mg/kg/day)	Control	40	200	1000
No. of dams	5	5	5	5
Total corpora lutea	81 16.2±1.79[a)]	86 17.2±0.84	90 18.0±2.55	79 15.8±2.17
Total implants	69 13.8±4.09[a)]	72 14.4±1.82	76 15.2±3.03	59 11.8±5.50
No. of resorptions and dead fetuses				
early	2 (2.9)	9 (12.5)	3 (3.9)	1 (1.7)
late	0	0	0	0
dead	0	0	0	0
total	2 (2.9)	9 (12.5)	3 (3.9)	1 (1.7)
Total alive	67 13.4±4.51[a)]	63 12.6±2.30	73 14.6±3.58	58 11.6±5.55
Sex ratio	1.48[b)] (40/27)	1.03 (32/31)	0.78 (32/41)	1.07 (30/28)
Body weight (g)				
male	3.552±0.158[a)]	3.565±0.231	3.527±0.211	3.619±0.070
female	3.332±0.135	3.372±0.178	3.269±0.356	3.459±0.185
Placental weight (mg)				
male	486±41.9[a)]	465±34.4	472±51.4	494±35.3
female	456±46.4	468±57.6	499±96.5	488±49.7
No. of external anomalies	0	0	1 (1.4) hematoma	0

a): Mean±S.D.

b): Male/Female

Table 10. Skeletal findings in rat fetuses treated with OPC-13013 - Preliminary study -

Exp. groups (mg/kg/day)	Control	40	200	1000
No. of fetuses examined	35	33	39	29
Anomalies				
Waved and knobbed ribs with shortened and misshapen femur	0	0	1 (2.6)	0
Waved and knobbed ribs	0	1 (3.0)	0	0
Variations				
Cervical rib	0	0	1 (2.6)	2 (6.9)
Hypoplasia of cervical arches	0	0	1 (2.6)	0
14th rib	1 (2.9)	5 (15.2)*	2 (5.1)	1 (3.4)
Ill-shaped vertebral bodies	6 (17.1)	3 (9.1)	3 (7.7)	1 (3.4)
Progress of ossification				
No ossified cervical bodies	27 (77.1)	32 (97.0)*	38 (97.4)*	27 (93.1)
Non-ossified 1st thoracic body	0	0	8 (20.5)	4 (13.8)
Non-ossified sternebrae				
2nd	0	0	2 (5.1)	0
3rd	0	0	0	0
4th	0	0	1 (2.6)	0
5th	11 (31.4)	13 (39.4)	26 (66.7)	20 (69.0)
6th	10 (28.6)	17 (51.5)	22 (56.4)	21 (72.4)*
Reduced metacarpals	13 (37.1)	12 (36.4)	22 (56.4)	20 (69.0)
Reduced metatarsals	0	0	1 (2.6)	0
No. of caudal bodies	4.08±0.31[a)]	3.76±0.63	3.34±0.78	3.96±0.11

a) : Mean±S.D., * : $p<0.05$, ** : $p<0.01$, ( ) : %

Table 11. Visceral findings in rats fetuses treated with OPC-13013 - Preliminary study -

Exp. groups (mg/kg/day)	Control	40	200	1000
No. of fetuses examined	32	30	34	29
No. of fetuses with visceral findings	12 (37.5)	2 (6.7)	15 (44.1)	4 (13.8)
Ventricular septal defect	0	0	0	1 (3.4)
Postureteric uterus	0	0	1 (2.9)	0
Polydactyly	0	1 (3.3)	0	0
Renal hematoma	1 (3.1)	0	0	0
Thymic remnant in neck	4 (12.5)	0	5 (14.7)	1 (3.4)
Ureteric anomalies				
Convolution with dilatation	0	0	3 (8.8)	0
Convolution alone	7 (21.9)	1 (3.3)	10 (29.4)	2 (6.9)
Left umbilical artery	0	0	0	1 (3.4)
Subcutaneous hematoma	0	0	1 (2.9)	0

( ) : %