4. **Developmental Toxicity Study in Rats**

Testing Facility:

Study Number: 000944

Study Dates: October 26, 1981 to May 21, 1982

GLP Compliance: Not addressed. However, it is stated that the study was inspected periodically, and the data and final report were audited by the Otsuka Quality Assurance Unit.

Animals: Fifty male and 200 female Sprague-Dawley rats (10-11 weeks old) were obtained from After a 9-day period of quarantine and acclimatization, the rats were mated. Females showing evidence of copulation (presence of vaginal plugs or sperms in vaginal smear) were considered to be pregnant and used for the study (40 pregnant females per group). The confirmed day of copulation was considered as day 0 of pregnancy.

On the day before mating, the body weight ranged from 372-434 g for males and 226-302 g for females.

Of the 40 pregnant rats per group, 25 were assigned for delivery by cesarean section and the other 15 for natural (spontaneous) delivery (with rearing of offspring to weaning).

Animals were housed individually except for F1 weanling offspring which were caged in groups of 4.

Dose Levels and Mode of Administration: 0, 30, 150 and 1000 mg/kg/day.

OPC-13013 (Lot No. IC-76M) was suspended in 0.5% aqueous solution of sodium carboxymethylcellulose at appropriate concentrations and was administered once daily by oral gavage, at a dosing volume of 10 ml/kg, on days 7 through 17 of gestation.

(Note: It is stated that doses were selected based on the results of a preliminary teratological study (0, 40, 200 and 1000 mg/kg/day on days 7 through 17 of gestation) in rats. In that study, at the highest dose, the body weight gain and food consumption of dams tended to be depressed, and retarded ossification was noted in the fetuses. However, since there was no increase in the number of resorptions or dead fetuses, the test compound at 1000 mg/kg/day

"was not considered to affect the progress of pregnancy". Hence, 1000 mg/kg/day was selected as the high dose for the definitive study. Median and low doses were set at 150 and 30 mg/kg/day, respectively.)

Observations and Measurements:

*Animals of Cesarean Section Groups* -

Dams were observed daily for clinical signs/general behavior 30 minutes post dose. Body weight was recorded on day 0 and from days 7 to 20 of gestation. Food consumption was recorded on day 0 and from days 6 to 19.

Dams were sacrificed on day 20 and uteri were examined. Total numbers of implantation and resorption sites, corpora lutea, and dead and live fetuses were recorded. Live fetuses were examined for sex and external anomalies. Placental weights and body weights of fetuses were recorded.

One-half of each litter of fetuses was fixed in Bouin's fluid, and were examined for visceral anomalies under a stereoscopic microscope. The heads of fetuses were examined by Wilson's method, chests by Nishimura's method and abdomens by conventional procedures. The other half of the litter was fixed in 95% ethanol, prepared by Dawson's method, and examined under a stereoscopic microscope for skeletal anomalies and progress of ossification.

*Animals of Spontaneous Delivery Groups* -

Dams were observed for clinical signs/general behavior 30 minutes post dose. Body weights were recorded on day 0, days 7-20 of gestation and on postpartum days 0, 7, 14 and 21. Food consumption was recorded on day 0, days 6-19 of gestation and on postpartum days 0, 7, 14 and 21.

Duration of gestation, delivery, live and stillborn pups, sex ratio, external anomalies and lactation were observed and/or recorded. One-half of the dead offspring were fixed in Bouin's fluid and examined for any visceral anomalies. (Sponsor does not say what, if anything, was done with the other half of the dead offspring.)

Dams were sacrificed on postpartum day 22 and the number of implantation sites were counted. Females that failed to deliver were sacrificed on day 23 of gestation, and grossly examined.

*F1 offspring* - Body weights of F1 offspring were recorded on days

0, 4, 7, 14 and 21 and at 4, 5 and 6 weeks of age. Food consumption was recorded at 3, 4, 5 and 6 weeks of age.

Litters were culled to 4 males and 4 females four days after birth. When number of litter mates of either sex fell below 4, the total litter size was standardized to 8. When the total litter size fell below 8, offspring from a different litter of the same group(same birth date) were used for standardization.

Culled offspring were fixed in Bouin's fluid, and control and high dose group pups were examined for visceral anomalies.

Offspring were weaned on postpartum day 21, and males and females were housed separately.

All offspring, including culled, were observed for detachment of the auricles. Animals that remained after culling were examined for emergence of abdominal hairs (postpartum day 8), eruption of incisors (postpartum day 11), separation of eyelids (postpartum day 14), descent of the testis (postpartum day 22) and the opening of the vagina (postpartum day 39).

Offspring were subjected to the following reflex function tests: ipsilateral flexor reflex (4 days of age), vibrissa placing reflex (7 days), cliff avoidance response (7 days), inclined plane test (11 days), visual placing reflex (21 days) and corneal reflex (21 days).

A learning ability test (conditioned avoidance response test) was performed on 10 males from each group at 6 weeks of age. (All males tested for learning ability were autopsied at 10 weeks of age.)

Reproductive performance was tested in F1 offspring. Twenty rats per sex per group were randomly selected, paired at 10 weeks of age and observed for 2 weeks for evidence of copulation. Females which failed to copulate during the 2 week period were mated again for another 10 days. Copulated females were isolated and weighed on days 0, 7, 14 and 20 of gestation, and food consumption was recorded on days 0, 7, 14 and 19. All F1 dams, including infertile animals, were cesarean sectioned on day 20 of gestation and uterine content was examined in the same way as done for F0 dams. One-half of the F2 fetuses were fixed in Bouin's fluid and the other half in 95% ethanol. These fetuses were not subjected to further examination.

Females which failed to copulate were autopsied after pairing, and the males used for the reproductive performance test were autopsied after cesarean section of F1 dams.

F1 offspring which were not used in the learning ability or reproductive performance tests were autopsied at 6 weeks of age.

Offspring which died before weaning were preserved in Bouin's fluid, and animals found dead after weaning were necropsied.

Data on body weight, food consumption, placental and fetal weights, number of caudal bodies, and response times in cliff avoidance and inclined plane tests were analyzed using t-test, and sex ratio, and copulation and conception rates by chi-square analysis. Data on avoidance rate and latency time for escape in the learning ability tests were evaluated by one-way lay out, and all other data by rank sum test.

Results:

*Animals of Cesarean Section Groups -*

No significant treatment-related clinical signs, changes in general behavior or effects on body weight were seen. There was a transient effect on food consumption at the high dose (significantly lower than control on days 7 and 9, and higher than control on days 13, 17 and 18).

Findings at cesarean section are presented in Table 12. The mean numbers of corpora lutea, implantation sites and live fetuses, and the sex ratios in treated groups were not significantly different from respective control values. No treatment related effect on early/late resorptions or total fetal loss was noted. At the high dose, female fetal weights were lower and placental weights were higher than respective control weights. No significant differences in fetal or placental weights were seen between control and low or mid dose groups.

(One animal from the low dose group and 2 from the mid dose group were non-pregnant.)

A statistically significant increased incidence of external anomalies (which included postaxial polydactyly of the right hind limb in 1 pup, unilateral anophthalmia in 2 pups and general edema in another) was seen at the high dose (control 0%, low dose 0.3%, mid dose 0.6% & high dose 1% of litters). General edema was seen in one fetus each from the low and mid dose groups.

The incidences of major visceral malformations are presented in Table 13. The number of fetuses with major malformations in the high dose group was significantly higher than in the control group. The major malformations observed in the high dose group included

ventricular septal defect, absence of aortic arch, aberrant right subclavian artery, presence of right aortic arch and unilateral anophthalmia. The animal with no aortic arch showed general edema. Ventricular septal defect was also noted at mid and low doses, the incidence being statistically significant at the mid dose.

The incidences of minor visceral anomalies (presented in Table 14) were not significantly different between control and treated groups.

Table 12. **Effects on prenatal development in rats treated orally with OPC-13013**

| Exp. groups (mg/kg/day) | Control | 30 | 150 | 1000 |
|---|---|---|---|---|
| No. of dams | 25 | 24 | 23 | 25 |
| Total corpora lutea | 430<br>17.2±2.81[a)] | 407<br>17.0±1.52 | 390<br>17.0±1.43 | 438<br>17.5±1.92 |
| Total implants | 381<br>15.2±4.38[a)] | 374<br>15.6±2.06 | 365<br>15.9±1.77 | 400<br>16.0±2.60 |
| No. of resorptions and dead fetuses | | | | |
| early | 15<br>(7.5) | 11<br>(2.8) | 25<br>(7.2) | 30<br>(7.1) |
| late | 0 | 0 | 0 | 0 |
| dead | 0 | 0 | 0 | 0 |
| total | 15<br>(7.5) | 11<br>(2.8) | 25<br>(7.2) | 30<br>(7.1) |
| Total alive | 366<br>14.6±4.44[a)] | 363<br>15.1±1.99 | 340<br>14.8±2.30 | 370<br>14.8±2.33 |
| Sex ratio[b)] | 1.07<br>(189/177) | 1.20<br>(198/165) | 1.00<br>(170/170) | 0.89<br>(174/196) |
| Body weight (g) | | | | |
| male | 3.610±0.246[a)] | 3.653±0.184 | 3.622±0.220 | 3.528±0.203 |
| female | 3.478±0.145 | 3.477±0.149 | 3.398±0.240 | 3.364±0.180* |
| Placental weight (mg) | | | | |
| male | 476±77.5[a)] | 466±44.2 | 482±59.3 | 486±47.1 |
| female | 446±38.5 | 452±42.9 | 487±102.2 | 474±53.4* |
| No. of external anomalies | 0 | 1[c)]<br>(0.3) | 1[d)]<br>(0.6) | 4* e)<br>(1.0) |

a): Mean±S.D., b): Male/Female, ( ): %, *: P<0.05

c): General edema with hydrocephaly and bilateral anophthalmia.

d): General edema.

e): Postaxial polydactyly of hindlimb 1, unilateral anophthalmia 2 and general edema 1.

Table 13. Visceral major malformations observed in rat fetuses from dams treated orally with OPC-13013

| Exp. groups (mg/kg/day) | Control | 30 | 150 | 1000 |
|---|---|---|---|---|
| No of fetuses examined | 176 | 175 | 165 | 178 |
| No. of fetuses with major malformations | 1(0.6) | 6(3.6) | 5(3.9)* | 9(5.0)* |
| Hydrocephaly | 0 | 1(0.5)a) | 0 | 0 |
| Anophthalmia Bilateral | 0 | 1(0.5)a) | 0 | 0 |
| Unilateral | 0 | 0 | 0 | 2(0.9) |
| Ventricular septal defect | 1(0.6) | 6(3.6)a) | 5(3.9)* | 3(1.7)b) |
| Absence of the aortic arch | 0 | 0 | 0 | 1(0.6)b) |
| Right aortic arch | 0 | 0 | 0 | 2(1.1) |
| Aberrant right subclavian artery | 0 | 0 | 0 | 2(1.2) |

a, b): Observed in same fetus.

*: $p<0.05$ ( ): %

Table 14.

Visceral minor anomalies observed in rat fetuses from dams treated orally with OPC-13013

| Exp. groups (mg/kg/day) | Control | 30 | 150 | 1000 |
|---|---|---|---|---|
| No. of fetuses examined | 176 | 175 | 165 | 178 |
| No. of fetuses with minor anomalies | 22(15.9) | 21(11.9) | 22(14.9) | 33(18.1) |
| Slightly dilatation of the lateral ventricle | 1(4.2) | 0 | 0 | 1(0.6) |
| Subcutaneous haemorrhage of the nose | 1(0.5) | 0 | 0 | 0 |
| Crack in the median lobe of the lung | 0 | 0 | 1(0.7) | 0 |
| Abnormal drainage of the esophagus [a] | 0 | 1(0.7) | 0 | 0 |
| Cul de sac in ventricular septum | 5(2.9) | 3(1.6) | 11(7.5) | 14(7.2) |
| Thymic remnant in the neck | 4(2.1) | 8(4.5) | 2(1.4) | 7(4.2) |
| Supernumerary right coronary orifice | 0 | 2(1.2) | 0 | 0 |
| Undescended testis | 0 | 1(0.5) | 0 | 1(0.6) |
| Hypoplasia of the caudate process of the liver | 1(0.6) | 0 | 0 | 0 |
| Supernumerary left kidney artery [b] | 1(0.5) | 1(0.6) | 1(0.7) | 0 |
| Supernumerary right kidney artery [c] | 0 | 0 | 1(0.5) | 0 |
| Supernumerary right kidney vein [d] | 0 | 0 | 1(0.5) | 0 |
| Dilatation of the renal pelvis grade(0) [e] | 0 | 1(0.5) | 2(1.2) | 1(0.5) |
| (+) [e] | 2(4.8) | 1(0.6) | 2(1.2) | 3(1.6) |
| (±) [e] | 5(2.8) | 2(1.1) | 2(1.2) | 8(4.4) |
| Dilatation of the ureter | 6(7.1) | 1(0.6) | 2(1.2) | 8(4.3) |
| Convolution of the ureter | 3(1.7) | 5(2.9) | 3(1.8) | 4(2.0) |
| Left umbilical artery | 1(0.5) | 1(0.5) | 1(1.4) | 2(1.1) |

a) : The esophagus located in the right of the trachea.

b) : Supernumerary left kidney artery arised from abdominal aorta and entered the renal hilus.

c) : Supernumerary right kidney artery arised from suprarenal artery and entered the parenchyma directly.

d) : Supernumerary right kidney vein arised from caudal vena cava and entered the parenchyma directly.

e) : grade(0)=no papilla and parenchyma slightly thin.
(+)=no papilla but parenchyma normally.
(±)=small papilla

( ) : %

Skeletal anomalies and variations are presented in Table 15. The incidence of skeletal abnormalities in the high dose group was significantly higher than in the control group. The anomalies observed in the high dose group included nodulated ribs and hypoplasia of the fibula and tibia. (The fetus with hypoplasia of the fibula and tibia showed polydactyly of the right hindlimb on external examination.) Wavy ribs at the low dose and the absence of palatine bone at the mid dose were noted.

A dose-related increased incidence of skeletal variations (statistically significant at the high dose), due primarily to the increased incidence of 14th rib, was seen in treated groups.

Findings on the progress of ossification in rat fetuses are presented in Table 16. Statistically significant increased incidences of unossified hyoid bone, first thoracic vertebral body and 2nd, 5th and 6th sternebrae, and incompletely ossified pubis were seen at mid and high doses. Moreover, at the high dose, increased incidences of unossified 4th sternebrae, incompletely ossified cervical vertebral arches, and reduced number of the distal phalanx of the hindlimb were also observed. Incidence of unossified hyoid bones was increased at the low dose.

*Animals of Spontaneous Delivery Groups* -

No treatment-related clinical signs, deaths or any effects on body weight were seen. A transient effect on food consumption (reduced food consumption) was noted at the high dose on days 7 and 9 of gestation.

No treatment-related effect on delivery or lactation was observed.

The findings at delivery, and the data on postnatal development of offspring are presented in Table 17.

The duration of gestation, number of implants, live births, delivery rates (number of newborns/number of implants) and sex ratio of offspring in treated groups were not significantly different from respective control values. No external anomalies of offspring were seen.

There were 4 stillbirths at the low dose and 3 at the high dose (no stillbirths in the control and mid dose groups). Three of the 4 stillborn at the low dose were litter mates, delivered by a dam which showed poor nursing behavior. Visceral examination revealed diaphragmatic hernia in one high dose stillborn; the other stillborns did not show any abnormalities.

Table 15. Skeletal findings in rat fetuses from dams treated orally with OPC-13013

| Exp. groups (mg/kg/day) | Control | 30 | 150 | 1000 |
|---|---|---|---|---|
| No. of fetuses examined | 190 | 188 | 175 | 192 |
| No. of fetuses with abnormalities | 0 | 1(0.5) | 1(0.5) | 5(2.6)* |
| Wavy ribs | 0 | 1(0.5) | 0 | 0 |
| Nodulated ribs | 0 | 0 | 0 | 4(2.1) |
| Hypoplasia of the fibula and tibia | 0 | 0 | 0 | 1(0.5) |
| Absence of the palatine bone[a)] | 0 | 0 | 1(0.5) | 0 |
| No. of fetuses with variations | 32(16.1) | 30(15.9) | 37(21.3) | 59(31.3)* |
| Bifurcation of the cervical arch | 0 | 0 | 0 | 1(0.5) |
| Splitting of ossfication centers of the thoracic vertebral bodies | 1(0.5) | 3(1.5) | 1(0.6) | 1(0.7) |
| Dumbbell shaped vertebral bodies | 10(5.1) | 16(8.9) | 11(6.2) | 12(6.0) |
| Extra lumbar vertebra | 0 | 0 | 1(0.5) | 0 |
| Splitting of the sternebrae | 0 | 0 | 1(0.5) | 0 |
| 14th rib | 21(10.5) | 17(8.7) | 28(16.3) | 51(26.9)** |
| Shortening of the 13th rib | 1(0.5) | 0 | 0 | 0 |

*a): Lamina horizontalis*

* : $p<0.05$, ** : $p<0.01$

( ): %

Table 16. Progress of ossification in rat fetuses from dams treated orally with OPC-13013

| Exp. groups (mg/kg/day) | | Control | 30 | 150 | 1000 |
|---|---|---|---|---|---|
| No. of fetuses examined | | 190 | 188 | 175 | 192 |
| Unossified | | | | | |
| Hyoid bone | | 13(10.0) | 34(17.1)* | 50(28.0)* | 59(29.0)** |
| Cervical vertebral bodies | | 106(56.6) | 99(51.5) | 105(60.7) | 110(57.4) |
| First thoracic vertebral body | | 1(0.5) | 3(1.7) | 19(11.6)** | 29(15.1)** |
| Sternebrae | 1st | 0 | 0 | 2(1.1) | 1(0.5) |
| | 2nd | 1(0.5) | 3(1.6) | 7(4.1)* | 8(4.0)* |
| | 3rd | 0 | 0 | 1(0.5) | 2(1.1) |
| | 4th | 0 | 0 | 1(0.5) | 4(4.1)* |
| | 5th | 49(28.5) | 54(29.1) | 101(57.6)** | 115(59.8)** |
| | 6th | 47(27.6) | 54(28.6) | 97(56.0)** | 113(58.9)** |
| Forelimb | Metacarpal(under 4) | 18(12.4) | 19(9.5) | 31(17.7) | 35(17.7) |
| | Proximal phalanx | 50(28.4) | 36(19.4) | 57(33.0) | 55(30.0) |
| | Distal phalanx(under 5) | 4(5.9) | 4(2.1) | 2(1.0) | 4(2.0) |
| Hindlimb | Metatarsal(under 5) | 116(62.3) | 94(50.5) | 113(65.8) | 153(79.7) |
| | Proximal phalanx | 132(70.5) | 115(61.8) | 116(67.4) | 148(77.5) |
| | Distal phalanx(under 5) | 1(0.6) | 6(4.1) | 2(1.1) | 11(6.6)** |
| Pubis | | 0 | 0 | 0 | 2(1.1) |
| Incompletely ossified | | | | | |
| Cervical vertebral arches | | 0 | 1(0.5) | 2(1.1) | 4(2.1)* |
| Pubis | | 1(4.2) | 3(1.6) | 8(4.7)* | 25(12.4)** |
| Ischium | | 0 | 0 | 2(1.1) | 3(1.6) |
| No. of caudal vertebral bodies | | 3.90±0.54 a) | 4.03±0.31 | 3.82±0.43 | 3.77±0.36 |

a): mean±S.D.

( ): %

*: $p<0.05$, **: $p<0.01$

Table 17. Effects on postnatal development in rats treated orally with OPC-13013

| Exp. groups (mg/kg/day) | | Control | 30 | 150 | 1000 |
|---|---|---|---|---|---|
| No. of dams | | 14 | 15 | 15 | 15 |
| Length of gestation (day) | | 21.3±0.47[a)] | 21.4±0.51 | 21.6±0.51 | 21.5±0.52 |
| No. of implants | | 233<br>16.6±2.31[a)] | 245<br>16.3±1.18 | 229<br>15.3±2.76 | 241<br>16.1±1.62 |
| No. of offspring | | | | | |
| At birth | alive | 213<br>15.2±1.81[a)] | 229<br>15.3±1.94 | 210<br>14.0±3.02 | 231<br>15.4±1.45 |
| | dead | 0 | 4 | 0 | 3* |
| Sex ratio[b)] | | 1.24<br>(118/95) | 0.94<br>(111/118) | 1.12<br>(111/99) | 1.01<br>(116/115) |
| At day 4 | pre-culling | 208<br>14.9±1.79[a)] | 226<br>15.1±2.49 | 202<br>13.5±2.88 | 225<br>15.0±1.46 |
| | post-culling | 112 | 120 | 120 | 120 |
| At weaning | | 84 | 97 | 111 | 103 |
| Delivery rate | | 91.9 | 95.1 | 91.3 | 97.2 |
| Viability rate[c)] | | 97.7 | 98.2 | 96.6 | 97.4 |
| Weaning rate | | 75.1 | 81.1 | 92.6 | 85.9 |
| Body weight (g) | | | | | |
| At birth | male | 5.78±0.37[a)] | 5.75±0.64 | 6.12±0.41* | 6.05±0.51 |
| | female | 5.49±0.38 | 5.46±0.54 | 5.77±0.40 | 5.67±0.49 |
| At day 7 | male | 15.1±3.15 | 15.4±1.93 | 17.5±1.42* | 16.5±1.91 |
| | female | 14.8±2.78 | 15.2±1.44 | 16.6±1.57* | 15.5±1.98 |
| At day 14 | male | 34.2±3.78 | 34.7±3.28 | 34.9±3.64 | 35.6±3.67 |
| | female | 32.5±5.36 | 33.8±3.09 | 33.5±2.49 | 34.0±3.61 |
| At day 21 | male | 54.9±6.01 | 54.5±5.13 | 55.0±3.61 | 55.4±6.91 |
| | female | 53.5±4.93 | 53.1±4.21 | 52.3±3.35 | 54.2±6.36 |
| No. of external anomalies | | 0 | 0 | 0 | 0 |

a): Mean±S.D., b): male/female

c): (No. of live offspring at day 4 before culling / No. of offspring born alive) x 100

* : $p<0.05$

*F1 offspring:* Survival rates at postpartum day 4 and weaning rates at postpartum day 21 were not significantly different between treated and control groups. The mean body weights for mid dose males at birth and for mid dose males and females at 7 days of age were higher than respective control weights. No significant weight differences between control other treated groups were seen from birth to 21 days of age.

No treatment-related effects on body weight or food consumption were seen in offspring from 3 to 6 weeks of age (time points when these indices were measured).

Data on postnatal growth and differentiation of F1 offspring are presented in Table 18. The detachment of auricles in females and the separation of eye-lids in males and females from mid and high dose groups were significantly progressed compared to control animals. No treatment-related effects were seen on the emergence of abdominal hair, eruption of incisor, descent of the testes and the opening of the vagina.

No drug-related effects were seen in reflex function or learning ability tests.

(Autopsy of the males used for the learning ability test revealed renal pelvises that were slightly dilated in one animal each from the control and mid dose groups and 2 from the high dose group.)

Reproductive performance findings at cesarean section (day 20 of gestation) of F1 offspring are presented in Table 19. The average mating period, and the rates of copulation and conception were not different between treated and control groups. No treatment-related effects were seen on the numbers of corpora lutea, implantation and resorption sites, dead and live fetuses, or on sex ratio or fetal and placental weights. External anomalies included general edema in 2 low dose fetuses and vestigial tail in 1 mid dose fetus. No external anomalies were seen in control and high dose groups.

Autopsy of F1 dams at cesarean section revealed an increased incidence of dilatation of the renal pelvis at the high dose.

Autopsy of F1 males used for mating revealed dilatation of renal pelvis in 1 control animal and in 2 each from mid and high dose groups.

Increased incidence of dilatation of the renal pelvis was also observed in mid and high dose F1 offspring sacrificed at 6 weeks of age.

Table 18. Effects on postnatal differentiation in rat offspring from dams treated orally with OPC-13013

| Exp. groups (mg/kg/day) | Detachment of auricles Day 4[a)] | Emergence of abdominal hair Day 8[a)] | Eruption of incisors Day 11[a)] | Separation of eye-lids Day 14[a)] | Descent of testes Day 22[a)] | Opening of vagina Day 39[a)] |
|---|---|---|---|---|---|---|
| Male | | | | | | |
| Control | 104/117 ( 90) | 54/54 (100) | 24/48 ( 49) | 13/44 ( 33) | 31/42 ( 73) | |
| 30 | 109/109 (100) | 59/59 (100) | 23/50 ( 51) | 23/47 ( 45) | 32/47 ( 63) | |
| 150 | 109/109 (100) | 61/61 (100) | 32/60 ( 52) | 39/59 ( 66)* | 46/57 ( 81) | |
| 1000 | 112/112 (100) | 59/59 (100) | 25/54 ( 43) | 34/53 ( 67)* | 48/53 ( 88) | |
| Female | | | | | | |
| Control | 77/91 ( 87) | 53/53 (100) | 30/46 ( 65) | 21/45 ( 46) | | 42/42 (100) |
| 30 | 116/117 ( 99) | 58/58 (100) | 31/53 ( 61) | 30/50 ( 61) | | 49/50 ( 97) |
| 150 | 93/93 (100)* | 57/57 (100) | 37/56 ( 68) | 46/55 ( 80)* | | 54/54 (100) |
| 1000 | 113/113 (100)* | 56/56 (100) | 31/51 ( 62) | 43/51 ( 84)* | | 50/50 (100) |

a): Examined age
( ): % (mean)
* : $p<0.05$

Table 19. Effects on prenatal development in rats($F_1$) from dams($F_0$) treated orally with OPC-13013

| Exp. groups (mg/kg/day) | Control | 30 | 150 | 1000 |
|---|---|---|---|---|
| No. of female examined | 20 | 20 | 20 | 20 |
| No. of copulated | 20 (100) | 20 (100) | 18 (90) | 19 (95) |
| Period of mating (day) | 2.35±1.42[a)] | 2.95±1.43 | 2.50±1.25 | 3.05±3.15 |
| No. of conceived | 16 (80) | 16 (80) | 16 (89) | 17 (89) |
| Total corpora lutea | 263 16.4±2.10[a)] | 256 16.0±1.46 | 286 17.9±2.80 | 296 17.4±2.00 |
| Total implants | 229 14.3±2.24[a)] | 242 15.1±1.31 | 250 15.6±1.78 | 262 15.4±3.06 |
| No. of resorptions and dead fetuses | | | | |
| early | 14 | 14 | 21 | 17 |
| late | 1 | 2 | 0 | 0 |
| dead | 0 | 0 | 0 | 0 |
| total | 15 (6.4) | 16 (6.6) | 21 (8.2) | 17 (6.2) |
| Total alive | 214 13.4±2.22[a)] | 226 14.1±1.54 | 229 14.3±1.89 | 245 14.4±2.81 |
| Sex ratio | 1.04 (109/105)[b)] | 0.93 (109/117) | 0.78 (100/129) | 0.94 (119/126) |
| Body weight (g) | | | | |
| male | 3.603±0.223[a)] | 3.460±0.368 | 3.596±0.173 | 3.540±0.258 |
| female | 3.411±0.201 | 3.315±0.341 | 3.452±0.154 | 3.357±0.251 |
| Placental weight (mg) | | | | |
| male | 457±37.4[a)] | 483±61.1 | 468±58.7 | 472±95.1 |
| female | 441±40.1 | 468±54.7 | 455±51.0 | 466±93.7 |
| No. of external anomalies | 0 | 2 (0.9) Edema | 1 (0.4) Vestigial tail | 0 |

a): Mean±S.D., b): Male/Female, ( ): %

Visceral abnormalities observed in F1 offspring (all animals, 6 weeks of age or older) are summarized in Table 20. A dose-dependent increased incidence of visceral abnormalities, mostly attributed to increased incidence of severe dilatation of the renal pelvis, was observed in treated groups. The incidence of total visceral abnormalities and the incidence of dilatation of the renal pelvis at the high dose were both statistically significant.

Visceral findings in culled offspring, on day 4 after birth, are presented in Table 21. Diaphragmatic hernia, a major malformation, was seen in one high dose animal (high dose 1%, control 0%). The number of animals with minor visceral abnormalities was significantly higher in the high dose group than in the control group.

Table 20

Visceral abnormalities observed in rat offspring at necropsy

| Exp. groups (mg/kg/day) | Control | 30 | 150 | 1000 |
|---|---|---|---|---|
| No. of offspring examined | 83 | 97 | 111 | 103 |
| No. of offspring with visceral abnormalities | 1 (1.0) | 1 (1.1) | 3 (2.5) | 12 (10.9)* |
| Hypoplasia of the papillary process of the liver | 0 | 0 | 0 | 1 (1.1) |
| Absence of the external right lobe of the liver | 0 | 0 | 0 | 1 (0.8) |
| Projection of the external right lobe and caudate process of the liver into the thoracic cavity | 0 | 0 | 0 | 1 (1.1) |
| Severe dilatation of the renal pelvis | 1 (1.0) | 1 (1.1) | 3 (2.5) | 9 (7.9)* |

* i.p<0.05,. ( ): %

Table 21. Visceral findings in rat offspring culled at day 4 after birth

| Exp. Groups (mg/kg/day) | Control | 1000 |
|---|---|---|
| No. of offspring examined | 96 | 105 |
| No. of offspring with visceral major malformations | 0 | 1 (1.0) |
| Diaphragmatic hernia | 0 | 1 (1.0) |
| No. of offspring with visceral minor anomalies | 10 (10.9) | 20 (19.4)* |
| Cul de sac in the ventricular septum | 6 (6.3) | 15 (14.6) |
| Thymic remnant in the neck | 1 (0.8) | 1 (1.1) |
| Adhesion between liver and diaphragm | 0 | 1 (1.0) |
| Supernumerary right kidney artery | 0 | 1 (1.0) |
| Dilatation of renal pelvis | | |
| severe | 1 (1.4) | 0 |
| slight | 3 (3.2) | 2 (1.8) |
| Convolution of the ureter | 1 (1.0) | 2 (1.7) |
| Dilatation of the ureter | 2 (2.0) | 1 (1.0) |

( ): %

* : $p<0.05$