

104-Week Oral (Gavage) Carcinogenicity Study in the Rat

Key Findings: Two-years of daily oral administration of tolvaptan at 0, 100, 300 and 1000 mg/kg/day in males and 0, 30, 100, 300 and 1000 mg/kg/day in females did not increase the incidence of tumors in the rat.

Testing Facility: _____

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Study Numbers: Otsuka study number - 013784 (Report No.014253)
Contract lab's study number - 4285 (163-026)

Study Dates: Initiation of Dosing - January 21, 1999
Termination of Dosing - January 17 - 22, 2001

GLP Compliance: with Guidances and Ordinances of the Ministry of Health and Welfare, Japan

QA Report: yes

Drug Lot #s and % Purity: 6H99 - 7I93 - 99F94 - 99F94 - 99F94 - 99F94 - 67% purity for all lots

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Species/Strain: Rat/ CD (SD), 4-week-old, obtained from _____

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Number, Age and Weight at Start of Study: 55/sex/main study group (the 30 mg/kg/day group had only females); additional 18-30/sex/treatment group for toxicokinetic determinations; 5 weeks of age; males 117-153 g and females 105-133 g

Animal Housing: Animals were housed individually in aluminum cages with stainless steel wire mesh at the front and the floor, and had free access to the diet (Modified NIH Open Formula Rat and Mouse Ration - Oriental Yeast Co., Ltd.) and tap water.

Formulation: The test drug was suspended weekly in 1% hydroxypropyl methylcellulose at appropriate concentrations.

Drug Stability: The dose formulations were found to be stable for 8 days when stored in a cool place shielded from light. Hence formulations were prepared weekly, subdivided for each day of use and stored shielded from light in a refrigerator.

Methods

Doses: males - 0, 100, 300 and 1000 mg/kg/day
females - 0, 30, 100, 300 and 1000 mg/kg/day

Basis of dose selection: In a six month toxicity study in rats, there was excess mortality in females receiving 1000 mg/kg/day. It was noted that the mortality occurred only during the first week of the six month study and appeared attributable to dehydration of female rats (a consequence of the diuretic action of OPC-41061), the higher plasma concentrations OPC-41061 in females than males, and likely competition for water when animals are group housed. Similar toxicity was not observed in a 4 week study at doses of 300 and 1000 mg/kg/day, in which animals were housed 1/cage. Mortality ceased to be a problem in the six month study after the supply of water was augmented. Hence, the Exec. CAC, in their meeting on July 14, 1998, recommended dosages of 100, 300 and 1000 mg/kg/day for both sexes in the two year rat carcinogenicity study. (It is noted that the sponsor added an additional group, 30 mg/kg/day female group, to the study.)

Route of administration: Oral (gavage), 1 ml/100 g body weight

Frequency of drug administration: Once daily for 104 weeks

Interim sacrifices: None

Deviations from original study protocol: None

Statistical methods: The data on body weight, food consumption, food efficiency, hematological values and organ weight were statistically analyzed using the Dunnett's multiple comparison test. The survival data and the incidence of tumors were analyzed by the Log rank test and Fisher's exact test, respectively. Additionally, the incidence of tumors was also analyzed using the Cochran-Armitage test for determination of dose dependency. When the survival ratio was considered statistically significant between groups, it was then analyzed by Peto's test. The numbers of benign, malignant and total tumor were analyzed by the Dunnett's multiple comparison test.

Observations and Measurements

Clinical signs and mortality: twice daily (before dosing and within 3 hours postdose) or more frequently during early days of dosing; palpation was performed weekly for the presence of neoplastic lesions.

Body weight and food consumption: weekly

Hematology: Evaluations were performed on all surviving animals at the end of the treatment period. [parameters evaluated – RBC, WBC (total and differential) and platelet counts, hemoglobin, hematocrit, MCV, MCH and MCHC]

Clinical chemistry: not performed

Organ weights: Brain, heart, lungs, liver, kidneys, spleen, adrenals, testes and ovaries were weighed.

Gross pathology: At necropsy, all organs were examined macroscopically and gross findings were recorded.

The following organs/tissues were fixed in 10% neutral buffered formalin: skin, mammary glands, lymph nodes (mesenteric and mandibular), sublingual, mandibular and Zymbal's gland, sternum, femur, bone marrow (sternum and femur), thymus, trachea, lungs, heart, thyroid, parathyroid, tongue, pharynx, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, liver, pancreas, spleen, kidneys, adrenals, urinary bladder, seminal vesicles, prostate glands, testes, epididymis, ovaries, uterus, eyeballs (including optic nerve), Harderian gland, lacrymal gland, brain, pituitary, spinal cord, skeletal muscle, sciatic nerve, aorta and gross lesions.

Histopathology: All tissues (except Zymbal's gland, pharynx and lachrymal gland) from all control and high dose animals, all dead/moribund animals from all groups, and all gross lesions were examined microscopically.

Toxicokinetics: For the determination of parent and metabolite (DM-4103 and DM-4107) concentrations, blood was collected from all drug treated groups (3 animals/sex/group/time point) at 2 time points (2 and 4 hr post-dose) on day 1 and in weeks 4 and 26. Additionally, in week 4, blood was also collected from the 300 mg/kg/day group pre-dose and at 1, 6 & 24 hr post-dose.

Results:

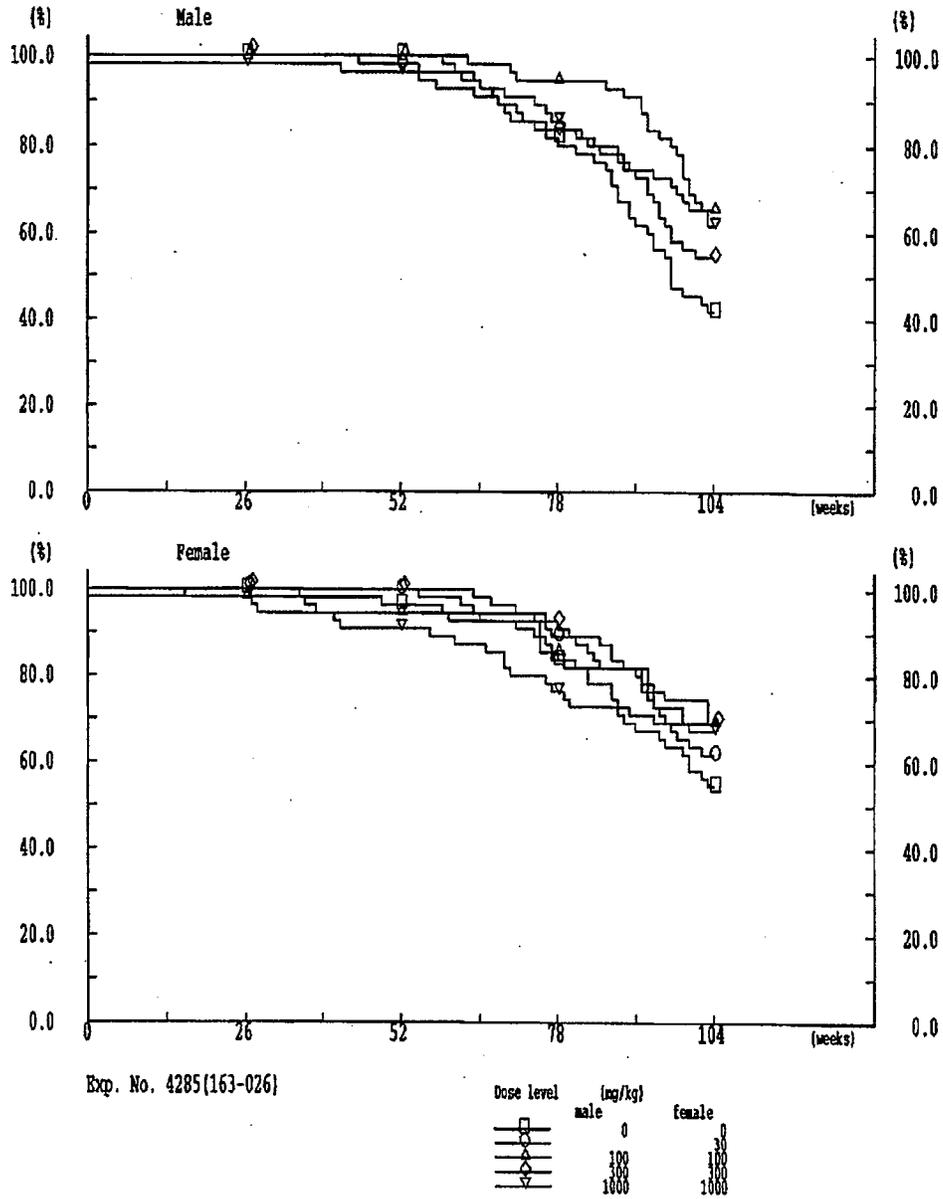
Mortality: The survival data over the course of the study is presented graphically in Figure 5. The percent mortalities at weeks 78 and 104 are given below.

Dose level (mg/kg/day)	<u>Males</u>				<u>Females</u>					
	0	100	300	1000	<u>Mortality (%)</u>					
	0	30	100	300	1000	0	30	100	300	1000
Week 78	18.2	5.5	16.4	14.5	16.4	10.9	14.5	7.3	23.6	
Week 104	58.2	34.5**	45.5	38.2*	45.5	38.2	30.9	30.9	32.7	

* $p \leq 0.05$ ** $p \leq 0.01$

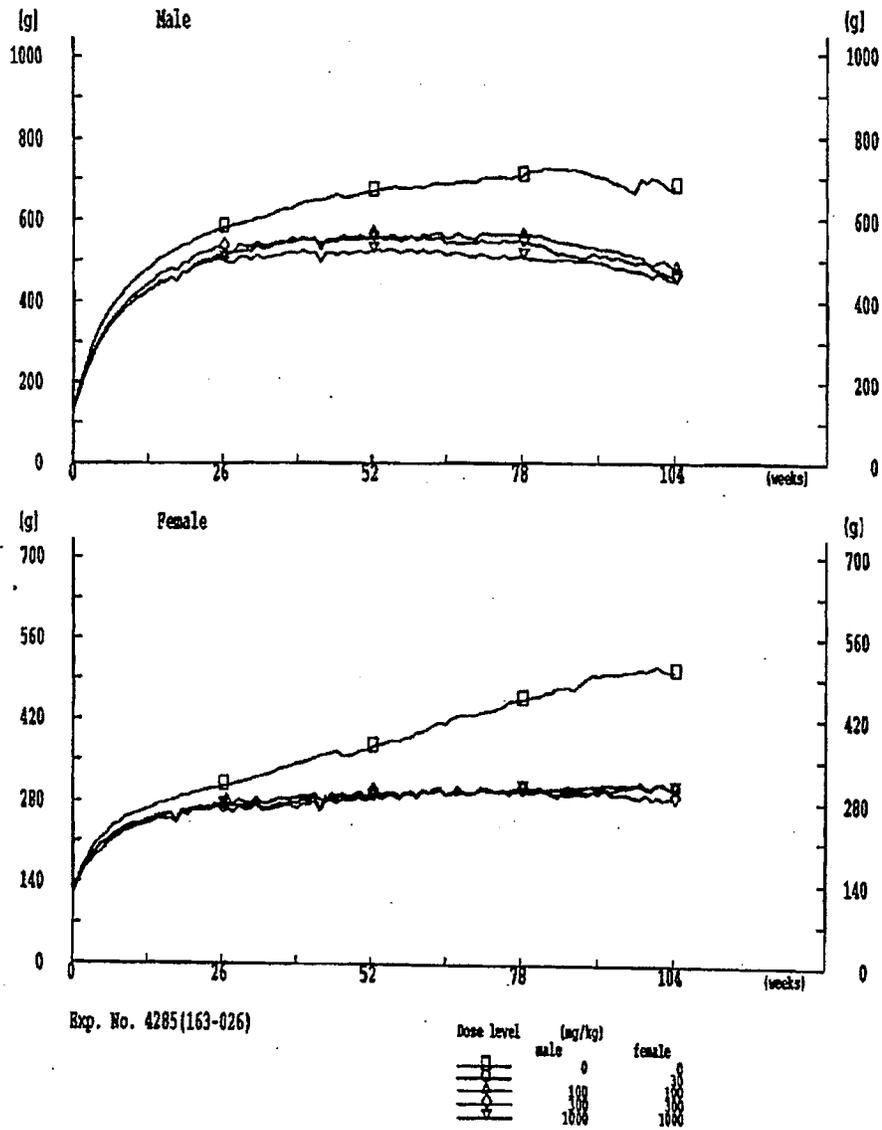
At the termination of the study, the mortality rates for males at 100 and 1000 mg/kg/day were significantly lower than control. For females, there were no significant differences in the mortality rates between control and treated groups.

Figure 5.



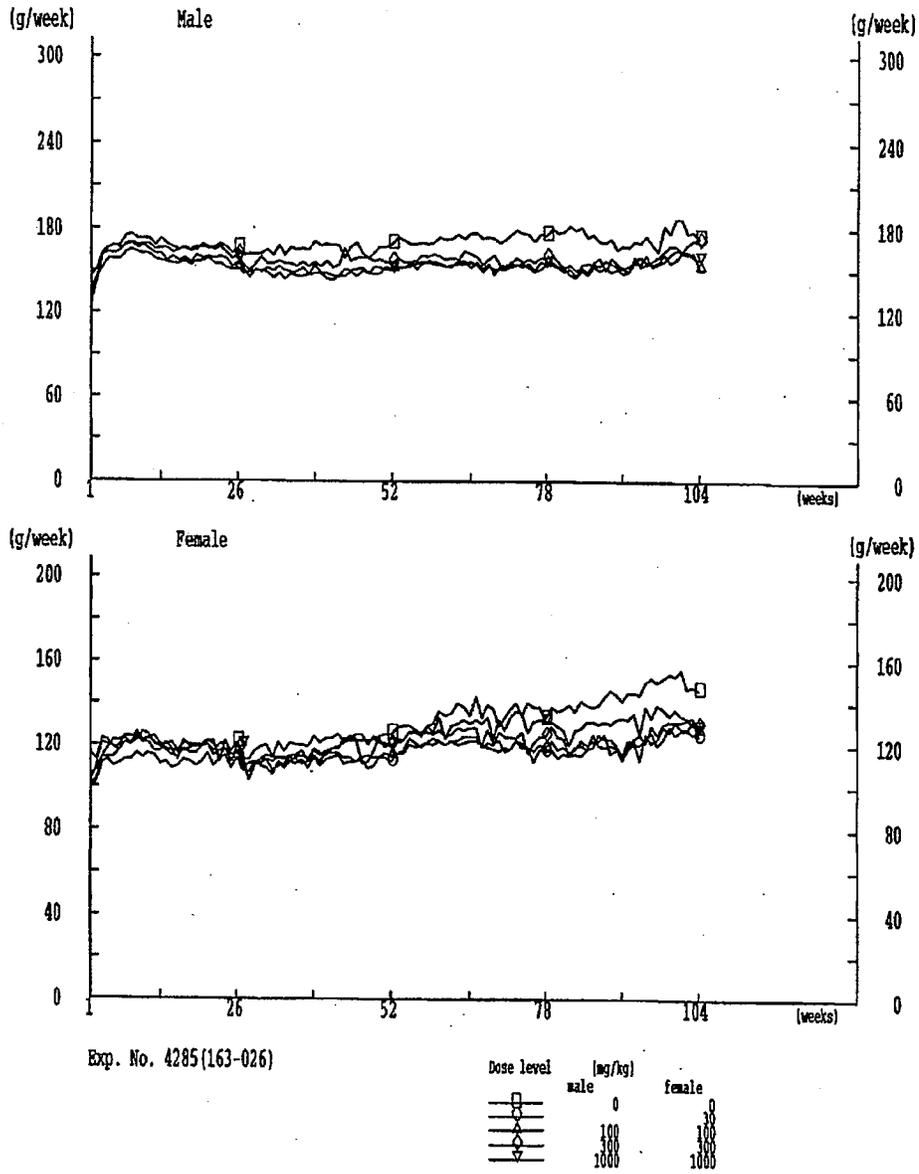
Survival ratio

Figure 6.



Body weight

Figure 7.



Exp. No. 4285(163-026)

Food consumption

Clinical signs: Treatment related clinical signs observed at increased incidence at the high dose beginning at week 1 of treatment included ptosis of the eyelid, decreased spontaneous motor activity, wasting and piloerection. Salivation was observed at the high dose beginning week 2, and was seen in all treatment groups by week 52. Other symptoms, observed in both treated and control animals, included tissue masses on body surface, subcutaneous tissue masses, loss of teeth, abnormal respiratory noise, urogenital hemorrhage, abdominal distention and staggering gait.

Body weights: Body weight data are presented graphically in Figure 6. The body weights for all treatment group rats were lower than control weights throughout the study. At the termination of the study, the mean body weights for males at 100, 300 and 1000 mg/kg/day were 29, 32 and 33% lower than control, respectively. For females, the body weights were 40, 40, 43 and 40 % lower than control at 30, 100, 300 and 1000 mg/ kg/day, respectively.

The body weight gain data for different treatment intervals are given below:

Dose levels (mg/kg/day)	<u>Body Weight Gain (g)</u>									
	<u>Males</u>					<u>Females</u>				
	0	100	300	1000	0	30	100	300	1000	
Treatment intervals (weeks)										
0 - 13	345	293	310	292	147	122	129	126	129	
0 - 52	532	423	424	388	252	171	171	174	168	
0 - 78	569	426	410	373	334	179	185	180	183	
0 - 104	541	342	322	315	382	182	183	167	183	

The body weight gain values for all treated groups were statistically significantly lower than respective control values for all treatment intervals ($p \leq 0.01$).

Food consumption: The food consumption data is presented graphically in Fig 7. The food consumption was lower in all treated groups, compared to control, during week 1 of dosing. Thereafter, the food consumption increased, and during treatment weeks 0 to 13, the food consumption was lower only in low dose male and female groups. For the entire treatment interval (weeks 0 - 104), the food consumption was lower than control in low and mid dose male groups and all treated female groups.

Hematology: There were increased RBC counts in the high dose male group and decreased white cell counts in all treated female groups (not dose-related).

Organ weights: Relative to control there was a decrease in absolute heart weights and an increase in relative heart weights for both sexes in all treated groups. Also, lower than control absolute and relative spleen weights were noted in treated males and higher than control absolute and relative lung weights were noted in treated females.

Gross pathology: Treatment-related increased incidences, compared to control, were noted for the following gross lesions in rats sacrificed terminally: atrophy of thymus and ovarian cysts in females, and atrophy of seminal vesicles in males; decreased incidences were noted for enlargement of spleen and kidneys (males) and pituitary hypertrophy (females).

In animals found dead or killed in moribund condition, higher than control incidences of adrenal hypertrophy in females and lower than control incidences of pituitary hypertrophy (both sexes) and parathyroid hypertrophy (males) were observed.

Histopathology: Neoplastic findings: The incidences of neoplastic findings observed in all control and high dose animals (found dead or sacrificed terminally or *in extremis*) are presented in Table 5. Sponsor's analysis, using Fisher's exact test, showed no statistically significant increased incidences of tumors in the high dose group of either sex. Peto's test revealed no dose response relationship in the incidence of tumor types tested.

Adenoma of the pituitary gland in males (control 32/55 vs HD 17/55) and females (control 44/55 vs HD 22/55) and pheochromocytoma in males (control 11/55 vs HD 1/55) showed decreased incidence in the high dose group compared to control. Other tumors occurring at a high incidence, in both treated and control groups, included fibroadenoma of the mammary glands, interstitial cell tumor of testes, endometrial stromal polyp and adenoma of the pancreatic islet.

Neoplastic lesions, such as adenoma of the pituitary gland, were considered to be the primary cause of death in both sexes during the study.

The FDA statistical analyses of the rat tumor data showed that the dose response relationship of the combined incidence of liver cholangiocellular adenoma and carcinoma in males (C 0/55, LD 0/55, MD 0/55 and 2/55) was statistically significant ($p=0.022$). However, a re-analysis of the data, using new software for the calculation of the exact p-value, indicated that the dose response relationship of the combined incidence of liver cholangiocellular adenoma and carcinoma in male rats is not statistically significant ($p=0.062$). The pairwise comparison between the control and high dose group was also not statistically significant.

Non-neoplastic findings: In terminally sacrificed high dose animals, there were statistically significantly higher than control incidences of accumulation of foamy cells in the lungs, granulomatous inflammation of the lungs and regeneration of epithelium of trachea (both sexes), dilatation of the esophagus (both sexes), focal necrosis of the

stomach (males), hypertrophy of hepatocytes (both sexes) and dilated pelvis of the kidney (females). The same group showed significantly lower than control incidences of myocardial degeneration (both sexes), cardiomyopathy (males), polyarteritis of the spleen (males), chronic nephropathy and fibrosis of kidneys (males), C-cell hyperplasia of the thyroid gland (both sexes) and hyperplasia of the medulla of the adrenal gland (males).

Table 5.

Summary of neoplastic findings with statistical analysis
(104 Weeks experiment)

Exp. No. 4285 (163-0)

Dose level (mg/kg)	No. of animals necropsied	Male animals		Female animals	
		Am 55	Bm 55	Af 55	Bf 55
CARDIOVASCULAR SYSTEM					
heart	=hemangioma	1	0	0	0
HEMATOPOIETIC SYSTEM					
bone marrow	#myelogenous leukemia	0	1	1	0
spleen	=hemangioma	1	0	0	0
	#hemangiosarcoma	0	1	0	0
RESPIRATORY SYSTEM					
lung	=alveolar/bronchiolar adenoma	1	0	0	0
nasal cavity	=sebaceous adenoma	1	-	-	-
DIGESTIVE SYSTEM					
stomach	=adenoma	0	0	0	1
exocrine pancreas	=adenoma	0	0	1	0
liver	=cholangiocellular adenoma	0	1	0	0
	#hepatocellular adenoma	2	3	4	5
	#cholangiocellular carcinoma	0	1	0	0
	#hepatocellular carcinoma	3	1	0	1
peritoneum	#histiocytic sarcoma	0	0	0	1
URINARY SYSTEM					
kidney	=adenoma	1	0	0	0
	=angliomyolipoma	1	0	0	0
urinary bladder	=transitional cell papilloma	1	1	0	0

Am: 0 Bm: 1000

Af: 0 Bf: 1000

=: benign #: malignant

Significant difference from control group by Fisher's exact test; * : P ≤ 0.05 ** : P ≤ 0.01

Table 5 continued

-continued Summary of neoplastic findings with statistical analysis
(104 Weeks experiment)

Exp. No. 4285 (163-026)

Dose level (ug/kg) No. of animals necropsied	Organ	Findings	Male animals		Female animals	
			Aa 55	Ba 55	Af 55	Bf 55
REPRODUCTIVE SYSTEM						
	mammary gland	=fibroadenoma	1	0	16	9
		#adenocarcinoma	1	1	2	3
	testis	=interstitial cell tumor	5	7	-	-
	uterus	=adenoma	-	-	0	1
		=endometrial stromal polyp	-	-	7	8
		=deciduoma	-	-	1	0
		=granular cell tumor	-	-	1	1
		#adenocarcinoma	-	-	1	0
		#leiomyosarcoma	-	-	1	0
	vagina	=squamous cell papilloma	-	-	2	0
		=granular cell tumor	-	-	5	8
	clitoral gland	=adenoma	-	-	1	0
		=squamous cell papilloma	-	-	0	1
ENDOCRINE SYSTEM						
	pituitary gland	=adenoma	32	17**	44	22**
		#adenocarcinoma	1	0	6	1
	thyroid gland	=C-cell adenoma	4	3	4	4
		=follicular cell adenoma	3	1	0	1
		#C-cell carcinoma	1	1	0	0
		#follicular cell carcinoma	2	1	0	0
	adrenal gland	=adenoma	4	0	1	0
		=pheochromocytoma	11	1**	0	0
		#adenocarcinoma	0	1	0	0
	pancreatic islet	=adenoma	14	10	7	4
		#adenocarcinoma	2	2	1	0

Aa: 0 Ba: 1000
Af: 0 Bf: 1000

=: benign #: malignant

Significant difference from control group by Fisher's exact test: * : P ≤ 0.05 ** : P ≤ 0.01

Table 5 continued

-continued Summary of neoplastic findings with statistical analysis
(104 Weeks experiment)

Exp. No. 4285 (163-026)

Dose level (mg/kg) No. of animals necropsied Organ Findings	Male animals		Female animals	
	Am 55	Ba 55	Af 55	Bf 55
NERVOUS SYSTEM				
brain				
=granular cell tumor	0	0	1	0
=astrocytoma	1	1	1	1
=malignant meningioma	2	0	0	0
spinal cord				
=malignant meningioma	0	1	0	0
trigeminal nerve				
=malignant schwannoma	1	-	-	-
SPECIAL SENSE SYSTEM				
ear				
=squamous cell papilloma	1	-	0	-
Zymbal's gland				
=carcinoma	0	-	0	1
INTEGUMENTARY SYSTEM				
skin				
=keratoacanthoma	0	2	0	0
=squamous cell papilloma	1	1	0	0
subcutaneous tissue				
=fibroma	1	0	1	0
=hemangioma	0	0	1	0
=lipoma	1	1	1	1
=fibrosarcoma	1	0	0	1
=hemangiosarcoma	0	0	1	0
=histiocytic sarcoma	0	0	1	0
=sarcoma, NOS	1	0	0	0
tail				
=fibrosarcoma	1	0	-	-
MUSCULOSKELETAL SYSTEM				
bone				
=osteochondroma	1	-	-	-

Am: 0 Ba: 1000
Af: 0 Bf: 1000

=: benign #: malignant

Significant difference from control group by Fisher's exact test; * : P ≤ 0.05 ** : P ≤ 0.01

In animals found dead or killed in moribund condition, increased incidences for the following non-neoplastic findings were noted: pericarditis of the heart (females), granulomatous inflammation of the lungs (both sexes), hypertrophy of hepatocytes (both sexes), hyperplasia of the pituitary gland (females) and hypertrophy of adrenal cortex (both sexes). Some lesions showed decreased incidences in treated animals.

Concentrations of OPC-156 and its metabolites in serum: Mean C_{max} values on day 1 and in weeks 4 and 26, and the AUC_{0-24hr} values at week 4 are given in Tables 6 and 7. On day 1, C_{max} values of OPC-156 and its metabolites increased in a dose-related manner. The C_{max} of OPC-156 decreased with time at all time points in males and up to week 4 in females. In week 26, the C_{max} values of OPC-156 and DM-4107 were lower and C_{max} values of DM-4103 were higher in males than their respective values in females at the same dosage. In week 4, the AUC_{0-24hr} values of the parent compound were higher and the metabolite values were lower in females compared to males at the same dosage.

The study appeared to be adequately performed using doses previously recommended by the ECAC. The highest dose employed in the study (1000 mg/kg/day) is about 160 times the maximum recommended human dose (MRHD), on a mg/m² basis; this dose resulted in systemic exposures that were about 3 times (males) and 7 times (females) higher than the exposures in humans at steady state at the MRHD of 60 mg/day. At least 23 males and 30 females in each group survived to scheduled termination.

The Executive CAC, in their meeting on April 15, 2008, agreed that both rat and mouse studies were acceptable, noting prior Exec CAC concurrence with the doses used. The Committee also concluded that studies were negative for treatment-related tumors.

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Table 6.

Mean Cmax($\mu\text{g/mL}$) in male rats				
Dose (mg/kg)	Analytes	Male		
		Day 1	Week 4	Week 26
100	OPC-156	0.816	1.102	0.742
	DM-4103	10.162	5.503	12.577
	DM-4107	3.736	1.949	4.007
300	OPC-156	1.727	0.761	0.768
	DM-4103	36.477	21.066	21.193
	DM-4107	13.390	5.208	8.210
1000	OPC-156	2.781	1.516	0.713
	DM-4103	37.532	25.604	30.003
	DM-4107	14.674	10.505	9.798

Mean Cmax($\mu\text{g/mL}$) in female rats				
Dose (mg/kg)	Analytes	Female		
		Day 1	Week 4	Week 26
30	OPC-156	0.646	1.155	1.834
	DM-4103	0.334	0.295	0.802
	DM-4107	0.229	0.359	0.808
100	OPC-156	5.117	3.109	3.228
	DM-4103	1.151	1.065	6.058
	DM-4107	1.185	1.167	5.309
300	OPC-156	9.685	4.455	3.211
	DM-4103	1.763	11.930	15.329
	DM-4107	2.320	9.352	9.989
1000	OPC-156	28.189	2.462	3.386
	DM-4103	3.152	16.102	24.768
	DM-4107	6.340	11.900	16.731

Table 7.

Table 2.6.6.5.1-2 Mean AUC_{0-24h} of Tolvaptan and its Principal Metabolites (DM-4103 and DM-4107) After Oral (Gavage) Administration of Tolvaptan to Rats for 4 Weeks						
Dose (mg/kg/day)	AUC _{0-24h} (µg·h/mL)					
	Tolvaptan		DM-4103		DM-4107	
	Males	Females	Males	Females	Males	Females
30 ^a	-	15.904	-	1.680	-	4.340
100 ^a	7.764	20.700	71.560	12.280	28.580	20.340
300	7.775	24.669	253.711	90.899	63.414	61.911
1000 ^a	12.716	33.449	581.170	169.460 ^b	297.910	131.510 ^b

^aData from Otsuka Report number 013774.

^bThis AUC was calculated using the concentration from 0 to 6 hours.

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REPRODUCTIVE TOXICITY STUDIES

Fertility Study of OPC-41061 in Rats

Key Study Findings: Oral administration of █████ OPC-41061 at 100, 300 and 1000 mg/kg/day to male and female rats during pre-mating, mating and up to gestational day 7 for females and day 20 for males was associated with reductions in body weight gain and food consumption (both sexes). The mean numbers of corpora lutea and implants at the high dose were significantly lower than control. The drug treatment did not produce any grossly observable fetal abnormalities.

b(4)

Testing Facility: Tokushima Research Institute
Otsuka Pharmaceutical Co., Ltd.
Tokushima 771-0192, Japan

Study Number: 014837 (Report No. 013066)

Study Dates: Initiation Date – August 27, 1998
Completion Date – September 1, 1999

GLP Compliance: The study was conducted in accordance with the GLP Standards for Safety Studies of Pharmaceutical Drugs, issued by the Ministry of Health and Welfare of Japan.

QA Report: Yes

Animals: Male and female █████ CD (SD) rats were obtained from █████. After a quarantine and observation period of 12 days, the animals were randomly assigned to 4 groups/sex of 25 animals each. The following day, drug treatment was started in males and the sampling of vaginal smear was initiated in females. Animals were mated on treatment day 63 and the day of confirmed copulation (presence of vaginal plug or sperm in the vaginal smear) was designated as day 0 of gestation. At the initiation of the study, the males were 6 to 7 weeks (224 – 257 g) and the females were 8 to 9 weeks (183 – 226 g) old. Before and after the mating period, animals were housed individually in stainless bracket cages, and commercial food (CRF-1, Oriental yeast Co., Ltd.) and in-house tap water were available *ad libitum*.

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Dose Levels and Mode of Administration: █████ OPC-41061 powder (Lot No. 6H99 █████ containing OPC-41061 and hydroxypropylcellulose (2:1), suspended in 1% hydroxypropylmethylcellulose 2910, was administered by oral gavage at a dose volume of 10 ml/kg. Dosage levels were 0, 100, 300 and 1000 mg/kg/day. Males were dosed for 9 weeks before mating with treatment continued through the mating period until the day before necropsy. Females were dosed for 2 weeks before mating with treatment continued through the mating period until day 7 of pregnancy. For non-mated females, treatment was continued till sacrifice. The test solutions were determined to be stable for up to 8 days after preparation when stored in a cool place protected from light.

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[Note: A fertility and early embryonic development study in rats, conducted with a "jet-milled" formulation of OPC-41061 at 0, 40, 200 and 1000 mg/kg/day, was previously reviewed by Dr. Sidney Stolzenberg (pharmtox review dated June 1, 1998). In that study, there were no treatment-related effects on fertility or mating performance of male or female rats or any adverse effects on the fetus. A low incidence of salivation after the fifth week of dosing and suppression of body weight gain during the first two weeks of treatment were observed in high dose males.

Comparative pharmacokinetic studies in healthy volunteers showed that after administration of equal doses of jet-milled or [redacted] formulations of OPC-41061, the C_{max} and AUC values were 4 to 5 fold higher for the [redacted] formulation than for the jet-milled formulation. Toxicokinetic data from rat studies (4-week oral toxicity study of jet-milled OPC-41061, and 26-week oral toxicity study of [redacted] OPC-41061) showed that the serum OPC-41061 level after 4 weeks administration of [redacted] formulation at 100 mg/kg/day was higher than the level obtained after 4 week administration of jet-milled formulation at 1000 mg/kg/day. Hence, in order to achieve a higher exposure level of OPC-41061 than obtained in the previous rat fertility study using jet-milled formulation, 1000 mg [redacted] OPC-41061/kg/day was selected as the high dose for the present study and 300 and 100 mg/kg/day were selected as mid and low doses, respectively.]

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Observations and Measurements: Males were observed once daily for their general condition from day 0 through the day before necropsy, at about 1-2 hr after drug treatment. Females were observed for their general condition once daily from day 42, when vaginal sampling was started, through the day of cesarean section. During the drug treatment period, females were observed at about 1-2 hr post-dose.

During the pre-mating period, body weight and food consumption were recorded weekly. During the mating period, body weight was recorded weekly only for determining the dosing volume (data not used for evaluation). For females, body weight was also recorded on days 0-7, 14 and 20 of gestation, and food consumption was recorded on days 0-7, 14 and 19 of gestation.

Vaginal smears were taken on days 42-62 and during the mating period till the day before copulation in order to determine the effect of treatment on the estrous cycle.

Cesarean section was performed on day 20 of gestation, pregnancy status confirmed, and the numbers of corpora lutea, implantations, early and late resorptions, and dead and live fetuses were recorded. Fetal weight, sex and external anomalies were noted. The placenta was examined grossly. If no visible implantation was observed, then the uterus was placed in 10% ammonium sulfide for further evaluation. The ovary and the uterus were preserved in 10% neutral buffered formalin. About one half of the fetuses were preserved in Bouin's solution and the remaining fetuses had their skeletons cleared with KOH and stained with alizarin red S.

Males were necropsied on day 97, after necropsy of the females. From males that did not mate or sire litters, smears were prepared from epididymides for sperm evaluation.

Data were analyzed using one-way analysis of variance (ANOVA) or Kruskal-Wallis test followed by Dunnett's test, or by using the Fisher-Irwin test.

Results

In-life Observations

Dose-related increased incidences of salivation were observed in males from all treated groups and in females from mid and high dose groups, the incidence being higher in males than in females. It was noted that the salivation occurred within one hour of drug administration and disappeared by about 4 hr post-dose.

Body weight gain for all treated male groups was significantly lower than control weight gain during the pre-mating period. Food consumption was reduced in all treated male groups during the first week of drug administration. In females, although there was no significant treatment-related effect on body weight, food consumption was decreased in all treated groups during the pre-mating period. During pregnancy, body weight gain and food consumption were reduced in all treated groups during the drug treatment period.

Dose-related increased incidences of abnormal estrous cycle (with prolonged diestrus; statistically significant at mid and high dose levels) were noted in treated groups.

The mating and pregnancy rates (%) are given in Table 8. There were no treatment-related effects on copulation or fertility indices, or on the mean number of days taken until mating.

Necropsy Findings

There were no treatment-related macroscopic findings in either males or females.

C-Section Findings

The mean numbers of early and late resorptions, dead and live fetuses, pre-and post-implantation losses and fetal body weights were unaffected by drug treatment (Table 9). A dose-related reduction in the mean number of corpora lutea was noted in treated animals, with values attaining statistical significance at the mid and high dose levels. The mean number of implants and the fetal sex ratio for the high dose group were lower than control. However, the above values (for the corpora lutea, implants and fetal sex ratio) were within the sponsor's historical control range (Table 10).

There were no significant treatment-related external anomalies observed in the study (Table 11).

The pharmacokinetic parameters of OPC-41061 and its metabolites (DM-4103 and DM-4107) were determined in pregnant rats after a single oral dose (on day 7 of gestation) or 11 consecutive daily oral doses (on days 7 to 17 of gestation) of OPC-41061 at doses of 100, 300 and 1000 mg/kg. After the single dose administration, the AUC values for OPC-41061 were higher than the values for metabolites in all treated groups. However, following repeated dose administration, the AUC values for the parent compound were lower than those after the single dose, and the AUC values for DM-4103 at mid and high doses and for DM-4107 at the high dose were higher than the parent drug values (Tables 12 & 13).

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Table 8.

Reproductive Performance
Fertility Study of **OPC-41061** Administered Orally to Rats

Dose (mg/kg)	A0	A100	A300	A1000
No. of Males Examined	25	25	25	25
No. (%) of Males Copulated	24 (96)	25 (100)	25 (100)	25 (100)
No. (%) of Fertile Males	23 (92)	22 (88)	23 (92)	22 (88)
No. of Females Examined	25	25	25	25
No. (%) of Females Copulated	24 (96)	25 (100)	25 (100)	25 (100)
No. (%) of Pregnant Females	23 (92)	22 (88)	23 (92)	22 (88)
No. of Days until Copulation (mean ± SD)	2.5 ± 0.8	2.6 ± 1.4	2.7 ± 1.3	3.0 ± 1.8

Values in the treated groups were not significantly different from the control values.

Table 9.

b(4)

Litter Data with Cesarean Section - Summary
Fertility Study of **██████████** OPC-41061 Administered Orally to Rats

Dose (mg/kg)	A0	A100	A300	A1000
No. of Dams	23	22	23	22
No. of Corpora Lutea				
Total	407	380	371	346
Mean ± SD	17.7 ± 1.9	18.4 ± 1.8	16.1 ± 2.3*	15.7 ± 1.6**
No. of Implants				
Total	384	340	359	332
Mean ± SD	16.7 ± 1.5	15.5 ± 1.5	15.6 ± 2.3	15.1 ± 2.0*
Prenatal Death				
Early Resorption				
Total	25	19	23	14
Mean ± SD	1.1 ± 1.3	0.9 ± 1.0	1.0 ± 0.9	0.6 ± 0.9
Mean ± SD (%)	6.7 ± 5.8	5.3 ± 5.8	6.7 ± 6.0	4.4 ± 6.4
Late Resorption				
Total	0	0	1	1
Mean ± SD	0 ± 0	0 ± 0	0.0 ± 0.2	0.0 ± 0.2
Mean ± SD (%)	0 ± 0	0 ± 0	0.3 ± 1.2	0.3 ± 1.3
Dead Fetuses				
Total	0	0	0	0
Mean ± SD	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Mean ± SD (%)	0 ± 0	0 ± 0	0 ± 0	0 ± 0
Total Death				
Total	25	19	24	15
Mean ± SD	1.1 ± 1.3	0.9 ± 1.0	1.0 ± 0.9	0.7 ± 0.9
Live Fetuses				
Total	359	321	335	317
Mean ± SD	15.6 ± 2.1	14.6 ± 1.3	14.6 ± 2.6	14.4 ± 2.2
Male/Female	1.11	1.07	1.03	0.78*
Pre-implantation Loss				
Mean ± SD (%)	5.09 ± 8.68	5.17 ± 6.15	3.23 ± 4.58	4.11 ± 6.57
Post-implantation Loss				
Mean ± SD (%)	6.70 ± 8.84	5.33 ± 5.84	6.83 ± 8.11	4.65 ± 6.58
Body Weight (g)				
Male	3.558 ± 0.250	3.588 ± 0.309	3.570 ± 0.247	3.685 ± 0.287
Female	3.373 ± 0.255	3.395 ± 0.288	3.438 ± 0.234	3.489 ± 0.228
Abnormality of the Placenta				
Total	0	0	2	0
Note			2 placentae fused in one dam	

* and **: Significantly different from the control values at p<0.05 and 0.01, respectively.

Table 11.

b(4)

External Anomalies of Fetuses (F1)
Fertility Study of ██████ OPC-41061 Administered Orally to Rats

Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses (Litters) Examined	359 (23)	321 (22)	335 (23)	317 (22)
No. of Fetuses (Litters) with External Anomaly	0 (0) [0 ± 0]	1 (1) [0.3 ± 1.3]	1 (1) [0.3 ± 1.3]	2 (2) [0.6 ± 1.8]
External Anomaly				
Anophthalmia	0 (0) [0 ± 0]	0 (0) [0 ± 0]	1 (1) [0.3 ± 1.3]	0 (0) [0 ± 0]
Agnathia	0 (0) [0 ± 0]	0 (0) [0 ± 0]	0 (0) [0 ± 0]	1 (1) [0.3 ± 1.3]
Anal atresia and vestigial tail	0 (0) [0 ± 0]	1 (1) [0.3 ± 1.3]	0 (0) [0 ± 0]	1 (1) [0.3 ± 1.4]

Values in the treated groups were not significantly different from the control values.
[]: %Mean±SD

b(4)

Table 12.

Toxicokinetics Study of [REDACTED] OPC-41061 Administered Orally to Pregnant Rats
 ITEM : Parameter - OPC-41061
 Sex : Female Day : G7

DOSE Item	A100	A300	A1000
Cmax ($\mu\text{g/ml}$)	11.028	19.910	96.219
Tmax (hr)	2	6	6
AUC ($\mu\text{g} \cdot \text{hr/ml}$)	119.828 (0-24hr)	264.300 (0-24hr)	458.234 (0-24hr)
A100 : Day7, 100mg/kg A300 : Day7, 300mg/kg A1000 : Day7, 1000mg/kg			

ITEM : Parameter - DM-4103
 Sex : Female Day : G7

DOSE Item	A100	A300	A1000
Cmax ($\mu\text{g/ml}$)	1.653	1.735	3.002
Tmax (hr)	6	6	6
AUC ($\mu\text{g} \cdot \text{hr/ml}$)	20.291 (0-24hr)	21.156 (0-24hr)	37.682 (0-24hr)
A100 : Day7, 100mg/kg A300 : Day7, 300mg/kg A1000 : Day7, 1000mg/kg			

ITEM : Parameter - DM-4107
 Sex : Female Day : G7

DOSE Item	A100	A300	A1000
Cmax ($\mu\text{g/ml}$)	1.733	2.845	6.198
Tmax (hr)	6	6	6
AUC ($\mu\text{g} \cdot \text{hr/ml}$)	21.184 (0-24hr)	33.672 (0-24hr)	71.025 (0-24hr)
A100 : Day7, 100mg/kg A300 : Day7, 300mg/kg A1000 : Day7, 1000mg/kg			

b(4)

Table 13.

Toxicokinetics Study of [REDACTED] OPC-41061 Administered Orally to Pregnant Rats				
ITEM : Parameter - OPC-41061				
Sex : Female				
DOSE Item	B100	B300	B1000	Day : G17
Cmax ($\mu\text{g/ml}$)	3.758	9.097	11.958	
Tmax (hr)	2	2	2	
AUC ($\mu\text{g} \cdot \text{hr/ml}$)	28.671 (0-24hr)	59.778 (0-24hr)	113.778 (0-24hr)	
B100 : Day7-17, 100mg/kg B300 : Day7-17, 300mg/kg				
B1000 : Day7-17, 1000mg/kg				
ITEM : Parameter - DM-4109				
Sex : Female				
DOSE Item	B100	B300	B1000	Day : G17
Cmax ($\mu\text{g/ml}$)	1.821	5.798	16.242	
Tmax (hr)	4	6	6	
AUC ($\mu\text{g} \cdot \text{hr/ml}$)	22.748 (0-24hr)	74.844 (0-24hr)	203.825 (0-24hr)	
B100 : Day7-17, 100mg/kg B300 : Day7-17, 300mg/kg				
B1000 : Day7-17, 1000mg/kg				
ITEM : Parameter - DM-4107				
Sex : Female				
DOSE Item	B100	B300	B1000	Day : G17
Cmax ($\mu\text{g/ml}$)	1.058	3.738	15.257	
Tmax (hr)	4	4	6	
AUC ($\mu\text{g} \cdot \text{hr/ml}$)	11.961 (0-24hr)	48.949 (0-24hr)	192.494 (0-24hr)	
B100 : Day7-17, 100mg/kg B300 : Day7-17, 300mg/kg				
B1000 : Day7-17, 1000mg/kg				

Embryo-Fetal Development Study of OPC-41061 in Rats

Key Study Findings: Oral administration of [REDACTED] OPC-41061 at 10, 100 and 1000 mg/kg/day to pregnant rats during organogenesis was associated with a reduction in maternal body weight gain and food consumption at 100 mg/kg/day and above, reduced fetal weight and delayed ossification of fetuses at 1000 mg/kg/day. Lower doses did not produce any significant adverse effects on the fetus. b(4)

Testing Facility: Tokushima Research Institute
Otsuka Pharmaceutical Co., Ltd.
Tokushima 771-0192, Japan

Study Number: 015468 (Report No. 013227)

Study Dates: Initiation Date – June 18, 1999
Completion Date – January 11, 2000

GLP Compliance: The study was conducted in accordance with the GLP Standards for Safety Studies of Pharmaceutical Drugs, issued by the Ministry of Health and Welfare of Japan.

QA Report: Yes

Animals: Nine-week-old male and female Sprague-Dawley rats [REDACTED] CD, SPF) were obtained from [REDACTED]. After a quarantine and observation period of 14 days, animals were mated and the day of confirmed copulation (presence of vaginal plug or sperm in the vaginal smear) was designated as day 0 of gestation. Mated females were randomly assigned to 4 groups of 20 animals each. On day 0 of gestation, females were 11 to 12 weeks of age and weighed 214-268 g. b(4)

Animals were housed individually in stainless steel bracket cages, and commercial solid food (CRF-1, Oriental Yeast Co., Ltd.) and in-house tap water were available *ad libitum*.

Dose Levels and Mode of Administration: [REDACTED] OPC-41061 powder (Lot No. 97I93 [REDACTED] containing 66.7% OPC-41061 and 33.3% hydroxypropylcellulose, suspended in 1% hydroxypropylmethylcellulose 2910 solution, was administered by oral gavage once daily on days 7 to 17 of gestation (period covering implantation to closure of hard palate) at a dose volume of 10 ml/kg. Dosages were 0, 10, 100 and 1000 mg/kg/day. The test solutions were prepared once every 6 or 7 days and stored at 4°C protected from light. Under these conditions, test solutions were found to be stable up to 8 days. b(4)

[Note: The doses for the present study were selected based on the results of a preliminary embryo-fetal development study in rats at oral doses of 0, 100, 300 and 1000 mg [REDACTED] OPC-41061/kg/day administered on days 7-17 of gestation. In that study, reductions in body weight and food consumption, increased incidence of post-implantation loss and high mean AUC value (35 times the AUC observed in humans after b(4)

the maximum dose of 60 mg/day) were observed at 1000 mg/kg/day. Therefore, 1000 mg OPC-41061/kg/day was selected as the high dose for the definitive study.]

b(4)

Observations and Measurements: Animals were observed once daily for general condition and for signs of toxicity. Females were weighed on days 0, 3 and 7-20 of gestation and food consumption was determined on days 0 and 6-19 of gestation.

All females were sacrificed on day 20 of gestation and complete necropsies were performed. All organs and tissues showing any abnormalities were preserved in 10% buffered neutral formalin. The numbers of corpora lutea, implantations, early and late resorptions and live and dead fetuses were counted. Placentas were examined grossly. Live fetuses were examined for external anomalies, weighed, and sex determined. About half of the live fetuses from each litter were fixed in Bouin's solution for visceral malformation examination, and the remaining live fetuses were fixed in 95% ethanol, after the removal of thoracic and abdominal organs, then cleared in potassium hydroxide, stained with Alizarin red S and examined for skeletal malformations and variations.

Data were analyzed using one-way analysis of variance (ANOVA) or Kruskal-Wallis test, followed by Dunnett's test, or by using Fisher-Irwin test.

Results

Maternal Toxicity

Three females (one control and 2 mid-dose animals) were found not pregnant.

There were no mortalities or abortions. Salivation was noted in one high dose animal.

Significant reductions in body weight gain were noted for high dose animals on days 8 to 19 of gestation and in mid dose animals on days 8 to 18 of gestation. No treatment related effect on body weight gain was noted for low dose animals. Food consumption was reduced in the high dose group on gestation days 7 to 11, 13 and 16, and in mid dose group on gestation days 7 to 11.

At necropsy, small thymus (HD - 2/20 & MD - 1/18) and enlarged adrenals (HD - 3/20 & MD - 1/18) were noted in high and mid dose animals. No other remarkable findings were observed at necropsy.

Developmental Toxicity

No significant differences were seen in the numbers of corpora lutea, implantations, resorptions and dead and live fetuses, sex ratio, and the incidences of post-implantation loss between treated and control groups (Table 14). The mean female fetal weight at 1000 mg/kg/day was significantly lower than the control female fetal weight.

The overall incidences of external, visceral and skeletal malformations and variations are summarized in Table 15.

The fetal and litter incidences of external anomalies are presented in Table 16. External anomalies were seen in one fetus each from the low (agnathia, astomia and aglossia) mid (polydactyly) and high (mandibular cleft) dose groups. There were no treatment related increased incidences of external anomalies observed in the study.

The incidences of visceral malformations and variations are given in Tables 17 and 18, respectively. Visceral malformations were observed in 5 low dose (4 litters) and 4 high dose (4 litters) fetuses. There were no malformations in mid dose and control groups. No significant differences were seen in the incidences of visceral malformations and variations between control and treated groups.

The incidences of skeletal malformations and variations are summarized in Tables 19 and 20, respectively. Skeletal malformations were seen in 1 low dose, 2 mid-dose (2 litters) and 1 high dose fetuses. No skeletal malformations were seen in controls. There were no significant treatment-related increased incidences of skeletal malformations or variations seen in the study.

Significant delayed ossification of cervical vertebral bodies and metacarpals, and increased incidences (not statistically significant) of non-ossified 5th and 6th sternbrae were observed at the high dose (Table 21).

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Table 14.

----- Embryo-Fetal Development Study of [REDACTED] OPC-41061 Administered Orally to Rats
 Item : Litter Data with Cesarean Section - Summary
 Statistical control : A0

	A0		A10		A100		A1000	
	19	20	19	20	18	18	20	20
No. of Corpora lutea								
Total	307	313	306	306	306	306	306	306
Mean±SD	16.2±1.2	15.7±1.6	17.0±2.4	17.0±2.4	17.0±2.4	17.0±2.4	17.0±2.4	17.0±2.4
No. of Implants								
Total	296	295	286	286	286	286	286	286
Mean±SD	15.6±1.1	14.8±2.2	16.4±1.9	16.4±1.9	16.4±1.9	16.4±1.9	16.4±1.9	16.4±1.9
Prenatal Death								
Early Resorption								
Total	13	19	17	17	17	17	17	17
Mean±SD	0.7±0.7	1.0±0.9	0.9±1.1	0.9±1.1	0.9±1.1	0.9±1.1	0.9±1.1	0.9±1.1
Mean±SD (%)	4.5±4.6	6.8±7.9	5.4±6.1	5.4±6.1	5.4±6.1	5.4±6.1	5.4±6.1	5.4±6.1
Late Resorption								
Total	0	0	0	0	0	0	0	0
Mean±SD	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Mean±SD (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Dead Fetuses								
Total	0	0	0	0	0	0	0	0
Mean±SD	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Mean±SD (%)	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
Total Death								
Total	13	19	17	17	17	17	17	17
Mean±SD	0.7±0.7	1.0±0.9	0.9±1.1	0.9±1.1	0.9±1.1	0.9±1.1	0.9±1.1	0.9±1.1
Live Fetuses								
Total	283	276	279	279	279	279	279	279
Mean±SD	14.9±1.4	13.8±2.4	15.5±1.5	15.5±1.5	15.5±1.5	15.5±1.5	15.5±1.5	15.5±1.5
Male/Female	1.04	1.12	1.01	1.01	1.01	1.01	1.01	1.01

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * ; p<0.05 . ** ; p<0.01 ; Significant difference from control value

Table 14 (continued)

Embryo-Fetal Development Study of [redacted] OPC-41061 Administered Orally to Rats
 Item : Litter Data with Cesarean Section - Summary
 Statistical control : A0

b(4)

Dose (mg/kg)	A0	A10	A100	A1000
No. of Dams	19	20	18	20
Body Weight (g)				
Male				
Mean	3.660	3.544	3.533	3.457
SD	0.228	0.290	0.313	0.242
Female				
Mean	3.480	3.948	3.355	3.247*
SD	0.242	0.251	0.272	0.252
Pre Implantation Loss (%)				
Mean	3.44	5.56	2.92	5.48
SD	4.96	11.03	4.93	7.76
Post Implantation Loss (%)				
Mean	4.50	6.81	5.42	5.74
SD	4.58	7.87	6.11	8.00

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : P<0.05, ** : P<0.01 : Significant difference from control value

Table 15.

b(4)

Embryo-Fetal Development Study of [redacted] OPC-41061 Administered Orally to Rats
 Item : Incidence of Fetal Anomaly (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
External Examination				
No. of Fetuses(Litters)	283 (19)	276 (20)	279 (18)	296 (20)
Anomaly				
No. of Fetuses(Litters)	0 (0)	1 (1)	1 (1)	1 (1)
% Mean±SD	0.0±0.0	0.3±1.5	0.3±1.4	0.3±1.5
Visceral Examination				
No. of Fetuses(Litters)	136 (19)	134 (20)	137 (18)	142 (20)
Variation				
No. of Fetuses(Litters)	14 (9)	13 (11)	9 (9)	15 (11)
% Mean±SD	10.1±12.4	10.1±11.2	6.4±6.6	10.5±11.2
Malformation				
No. of Fetuses(Litters)	0 (0)	5 (4)	0 (0)	4 (4)
% Mean±SD	0.0±0.0	4.1±9.8	0.0±0.0	2.7±5.5
Skeletal Examination				
No. of Fetuses(Litters)	147 (18)	142 (20)	142 (18)	154 (20)
Variation				
No. of Fetuses(Litters)	16 (8)	13 (8)	18 (9)	22 (19)
% Mean±SD	9.9±15.9	9.6±13.2	13.4±18.2	13.6±12.8
Malformation				
No. of Fetuses(Litters)	0 (0)	1 (1)	2 (2)	1 (1)
% Mean±SD	0.0±0.0	0.7±3.2	1.3±3.8	0.6±2.8

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : p<0.05 . ** : p<0.01 : Significant difference from control value

Table 16.

Embryo-Fetal Development Study of [REDACTED] OPC-41061 Administered Orally to Rats
Item : External Anomaly of Fetuses (F1) - Summary
Statistical control : A0

b(4)

Dose (mg/kg)	No. of Fetuses (No. of Litters)			
	A0 283 (19)	A10 276 (20)	A100 279 (18)	A1000 296 (20)
Agnathia				
Mean (%)	0(0)	1(1)	0(0)	0(0)
SD (%)	0.0	0.3	0.0	0.0
	0.0	1.5	0.0	0.0
Astomia				
Mean (%)	0(0)	1(1)	0(0)	0(0)
SD (%)	0.0	0.3	0.0	0.0
	0.0	1.5	0.0	0.0
Aplossia				
Mean (%)	0(0)	1(1)	0(0)	0(0)
SD (%)	0.0	0.3	0.0	0.0
	0.0	1.5	0.0	0.0
Mandibular cleft				
Mean (%)	0(0)	0(0)	0(0)	1(1)
SD (%)	0.0	0.0	0.0	0.3
	0.0	0.0	0.0	1.5
Polydactyly				
Mean (%)	0(0)	0(0)	1(1)	0(0)
SD (%)	0.0	0.0	0.3	0.0
	0.0	0.0	1.4	0.0

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
e : P<0.05 , es : P<0.01 : Significant difference from control value

Table 17.

b(4)

Embryo-Fetal Development Study of [REDACTED] OPC-1061 Administered Orally to Rats
 Item : Visceral Malformation of Fetuses (F1) - Summary
 Statistical control : AD

	AD 136(19)	A10 134(20)	A100 137(18)	A1000 142(20)
Dose (mg/kg)				
No. of Fetuses (No. of Litters)	136(19)	134(20)	137(18)	142(20)
Dilated cerebral ventricle	0(0)	1(1)	0(0)	1(1)
Mean (%)	0.0	0.7	0.0	0.7
SD (%)	0.0	3.2	0.0	3.2
Microphthalmia	0(0)	2(2)	0(0)	1(1)
Mean (%)	0.0	1.3	0.0	0.6
SD (%)	0.0	4.1	0.0	2.8
Ventricular septal defect	0(0)	2(2)	0(0)	1(1)
Mean (%)	0.0	1.7	0.0	0.7
SD (%)	0.0	5.4	0.0	3.2
Persistent atrioventri. canal	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	1.0	0.0	0.0
SD (%)	0.0	4.5	0.0	0.0
Aberrant right subclavian art.	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	0.7	0.0	0.0
SD (%)	0.0	3.2	0.0	0.0
Renal agenesis	0(0)	0(0)	0(0)	1(1)
Mean (%)	0.0	0.0	0.0	0.6
SD (%)	0.0	0.0	0.0	2.8
Absence of ureter	0(0)	0(0)	0(0)	1(1)
Mean (%)	0.0	0.0	0.0	0.6
SD (%)	0.0	0.0	0.0	2.8
Situs inversus totalis	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	1.0	0.0	0.0
SD (%)	0.0	4.5	0.0	0.0

AD : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : p<0.05, ** : p<0.01 ; Significant difference from control value

Table 18.

b(4)

Embryo-Fetal Development Study of [redacted] OPC-41061 Administered Orally to Rats
 Item : Visceral Variation of Fetuses (F1) - Summary
 Statistical control : A0

	A0		A10		A100		A1000	
	No. of Fetuses	(No. of Litters)						
Dose (mg/kg)								
Thymic remnant in neck	10(6)		5(5)		2(2)		5(5)	
Mean (%)	7.1		4.6		1.4		3.9	
SD (%)	11.9		8.6		4.0		6.8	
Abnormal lobation of lungs	0(0)		1(1)		0(0)		0(0)	
Mean (%)	0.0		1.0		0.0		0.0	
SD (%)	0.0		4.5		0.0		0.0	
Double renal vein	0(0)		0(0)		0(0)		1(1)	
Mean (%)	0.0		0.0		0.0		0.7	
SD (%)	0.0		0.0		0.0		3.2	
Left umbilical artery	1(1)		2(2)		3(3)		3(3)	
Mean (%)	0.8		1.5		2.2		2.2	
SD (%)	3.3		4.8		5.0		5.3	
Dilatation of ureter	2(1)		3(3)		3(3)		4(3)	
Mean (%)	1.5		2.3		2.1		2.6	
SD (%)	6.6		5.5		4.9		6.7	
Convolution of ureter	3(2)		0(0)		0(0)		2(2)	
Mean (%)	2.3		0.0		0.0		1.3	
SD (%)	7.2		0.0		0.0		8.6	
Diverticulum in intestine	0(0)		1(1)		0(0)		0(0)	
Mean (%)	0.0		0.8		0.0		0.0	
SD (%)	0.0		3.7		0.0		0.0	
Strayed artery into kidney	0(0)		1(1)		0(0)		0(0)	
Mean (%)	0.0		0.6		0.0		0.0	
SD (%)	0.0		2.8		0.0		0.0	

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : P<0.05 , ** : P<0.01 ; Significant difference from control value

Table 18 (continued)

b(4)

Embryo-Fetal Development Study of [redacted] OP0-41061 Administered Orally to Rats
 Item : Visceral Variation of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
No. of Fetuses (No. of Litters)	136 (19)	134 (20)	137 (18)	142 (20)
Strayed vein out of kidney	0 (0)	0 (0)	1 (1)	0 (0)
Mean (%)	0.0	0.0	0.7	0.0
SD (%)	0.0	0.0	2.9	0.0
Undescended ovary	0 (0)	0 (0)	0 (0)	1 (1)
Mean (%)	0.0	0.0	0.0	0.6
SD (%)	0.0	0.0	0.0	2.8

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : P<0.05 . ** : P<0.01 : Significant difference from control value

Table 19.

b(4)

Embryo-Fetal Development Study of [REDACTED] OPC-41061 Administered Orally to Rats
 Item : Skeletal Malformation of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
No. of Fetuses (No. of Litters)	147 (19)	142 (20)	142 (18)	154 (20)
Skull				
Incomplete Intermandibular J.	0(0)	0(0)	0(0)	1(1)
Mean (%)	0.0	0.0	0.0	0.6
SD (%)	0.0	0.0	0.0	2.6
Cervical vertebrae				
Absence of vertebral arch	0(0)	0(0)	1(1)	0(0)
Mean (%)	0.0	0.0	0.6	0.0
SD (%)	0.0	0.0	2.6	0.0
Fusion of vertebral arches				
Mean (%)	0(0)	1(1)	1(1)	0(0)
SD (%)	0.0	0.7	0.6	0.0
		3.2	2.6	0.0
Thoracic vertebrae				
Fusion of vertebral bodies	0(0)	0(0)	1(1)	0(0)
Mean (%)	0.0	0.0	0.6	0.0
SD (%)	0.0	0.0	2.6	0.0
Ribs				
Fused ribs	0(0)	0(0)	1(1)	0(0)
Mean (%)	0.0	0.0	0.6	0.0
SD (%)	0.0	0.0	2.6	0.0

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : p<0.05, ** : p<0.01 : Significant difference from control value

Table 19 (continued)

b(4)

Embryo-Fetal Development Study of [REDACTED] OPC-41061 Administered Orally to Rats
 Item : Skeletal Malformation of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
No. of Fetuses (No. of Litters)	147 (19)	142 (20)	142 (18)	154 (20)
Ribs				
Fused costal cartilage	0 (0)	0 (0)	1 (1)	0 (0)
Mean (%)	0.0	0.0	0.7	0.0
SD (%)	0.0	0.0	2.9	0.0

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : P<0.05, ** : P<0.01 ; Significant difference from control value

Table 20.

b(4)

Embryo-Fetal Development Study of [redacted] OPC-41061 Administered Orally to Rats
 Item : Skeletal Variations of Fetuses (F1) - Summary
 Statistical control : A0

	A0	A10	A100	A1000
No. of Fetuses (No. of Litters)	147 (19)	142 (20)	142 (18)	154 (20)
Cervical vertebrae				
Cervical rib	0 (0)	1 (1)	1 (1)	0 (0)
Mean (%)	0.0	0.8	0.8	0.0
SD (%)	0.0	3.7	3.4	0.0
Thoracic vertebrae				
Dumbbell shaped vert. body	6 (9)	3 (3)	4 (4)	5 (4)
Mean (%)	3.9	2.0	2.9	3.1
SD (%)	9.4	4.8	5.6	6.8
Splitting of vertebral body				
Mean (%)	3.3	1.3	6.7	5.7
SD (%)	11.7	4.1	11.0	9.6
Lumbar vertebrae				
Splitting of vertebral body	0 (0)	0 (0)	0 (0)	1 (1)
Mean (%)	0.0	0.0	0.0	0.6
SD (%)	0.0	0.0	0.0	2.8
5 lumbar vertebrae				
Mean (%)	0 (0)	0 (0)	2 (2)	0 (0)
SD (%)	0.0	0.0	1.5	0.0
Ribs				
Shortened 13th rib	1 (1)	0 (0)	1 (1)	0 (0)
Mean (%)	0.6	0.0	0.6	0.0
SD (%)	2.5	0.0	2.6	0.0

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : p<0.05, ** : p<0.01 : Significant difference from control value

Table 20 (continued)

b(4)

Embryo-Fetal Development Study of [redacted] OFC-41061 Administered Orally to Rats
 Item: Skeletal Variations of Fetuses (F1) - Summary
 Statistical control: A0

Dose (mg/kg)	A0		A10		A100		A1000	
	147 (19)		142 (20)		142 (18)		154 (20)	
No. of Fetuses (No. of Litters)								
Ribs								
Unilateral 14 ribs	5 (4)		6 (4)		2 (1)		5 (5)	
Mean (%)	3.3		4.9		1.4		3.1	
SD (%)	7.0		10.7		5.9		5.5	
Bilateral 14 ribs	0 (0)		1 (1)		1 (1)		4 (3)	
Mean (%)	0.0		0.6		0.7		2.3	
SD (%)	0.0		2.5		2.9		5.9	
14 ribs	5 (4)		7 (4)		3 (1)		9 (6)	
Mean (%)	3.3		5.5		2.1		5.4	
SD (%)	7.0		11.4		8.8		9.6	
Rudimentary 14th rib	5 (4)		7 (4)		3 (1)		9 (6)	
Mean (%)	3.3		5.5		2.1		5.4	
SD (%)	7.0		11.4		8.8		9.6	
Non-articulation cost. cartil.	1 (1)		1 (1)		0 (0)		0 (0)	
Mean (%)	0.8		0.7		0.0		0.0	
SD (%)	3.3		3.2		0.0		0.0	
Sternum								
Fused sternbrae	0 (0)		0 (0)		0 (0)		1 (1)	
Mean (%)	0.0		0.0		0.0		0.6	
SD (%)	0.0		0.0		0.0		2.8	

A0: Control A10: 10 mg/kg A100: 100 mg/kg A1000: 1000 mg/kg
 s: p<0.05, se: p<0.01; Significant difference from control value

b(4)

Table 21.

Embryo-Fetal Development Study of [redacted] OPC-41061 Administered Orally to Rats
 Item : Ossification of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0		A10		A100		A1000	
	19	20	18	19	18	19	20	
No. of Dams	147	142	142	142	154	154	154	
No. of Specimens	147	142	142	142	154	154	154	
Cervical V. Bodies -Mean±SD-	0.58±0.42	0.75±0.65	0.72±0.53	0.22±0.27**	0.22±0.27**	0.22±0.27**	0.22±0.27**	
Caudal V. Bodies -Mean±SD-	3.95±0.42	3.96±0.39	4.01±0.60	3.70±0.42	3.70±0.42	3.70±0.42	3.70±0.42	
Sternum -non- n / Mean±SD								
1st	0	0	0	0	0	0	0	
2nd	0	0	0	0	0	0	0	
3rd	0	0	0	0	0	0	0	
4th	0	0	0	0	0	0	0	
5th	0	0	0	0	0	0	0	
6th	0	0	0	0	0	0	0	
Pubis -non- n / Mean±SD	0.0±0.0	0.7±3.2	0.7±2.9	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	
Ischium -non- n / Mean±SD	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	
Fore limb								
Metacarpal -Mean±SD-	7.48±0.62	7.29±0.58	7.41±0.65	6.76±0.57**	6.76±0.57**	6.76±0.57**	6.76±0.57**	
Phalanx Proximal -Mean±SD-	0.55±0.75	0.55±0.90	0.55±0.59	0.2±0.37	0.2±0.37	0.2±0.37	0.2±0.37	
Middle -Mean±SD-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
Hind limb								
Metatarsal -Mean±SD-	8.00±0.00	7.97±0.09	7.97±0.10	8.00±0.00	8.00±0.00	8.00±0.00	8.00±0.00	
Phalanx Proximal -Mean±SD-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	
Middle -Mean±SD-	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 * : p<0.05 . ** : p<0.01 : Significant difference from control value

Embryo-Fetal Development Study of OPC-41061 in Rabbits

Key Study Findings: Oral administration of ██████████ OPC-41061 at 100, 300 and 1000 mg/kg/day to pregnant rabbits from implantation to closure of the hard palate was associated with dose-related maternal toxicity (reductions in body weight gain and food consumption at all doses, and abortion at mid and high doses). At 1000 mg/kg/day, increased incidences of post-implantation loss, fetal microphthalmia, open eyelids, cleft palate, brachymelia and skeletal malformations were observed. Lower doses did not produce any adverse effects on the fetus.

b(4)

Testing Facility: Tokushima Research Institute
Otsuka Pharmaceutical Co., Ltd.
Tokushima 771-0192, Japan

Study Number: 015161 (Report No. 013174)

Study Dates: Initiation Date – June 8, 1999
Completion Date – December 28, 1999

GLP Compliance: The study was conducted in accordance with the GLP Standards for Safety Studies of Pharmaceutical Drugs issued by the Ministry of Health and Welfare of Japan.

QA Report: Yes

Animals: One hundred female and thirty male New Zealand White rabbits (Kbl:NZW, SPF, 17 and 20 weeks of age, respectively) were obtained from ██████████. After a quarantine and observation period of 12 days, the animals were mated. Eighty mated females (which showed the presence of sperm in the vaginal smear) were randomly assigned to four groups of 20 animals each. On day 0 of gestation (the day of mating), the females were 19-21 weeks of age and weighed 3.35 to 4.32 kg. Animals were housed individually in aluminum bracket cages, and had free access to commercial solid food (CR-1, Clea Japan Inc.) and tap water.

b(4)

Dose Levels and Mode of Administration: ██████████ OPC-41061 powder (Lot No.97193 ██████████ containing OPC-41061 and hydroxy-propylcellulose (2:1), suspended in 1% hydroxypropylmethylcellulose 2910, was administered by oral gavage from day 6 to day 18 of pregnancy, at a dose volume of 5 ml/kg. The doses employed were 0 (vehicle control), 100, 300 and 1000 mg ██████████ OPC-41061/kg/day.

b(4)

The test solutions were found to be stable for 8 days when stored in a cool place protected from light.

[Note: A developmental toxicity study in rabbits, conducted with ██████████ OPC-41061 at 0, 10, 30 and 100 mg/kg/day, was previously submitted to the agency and was reviewed by Dr. Sidney Stolzenberg (pharmtox review dated June 1, 1998). Although the

b(4)

sponsor had stated that the dose selection was appropriate, "as evidenced by observed signs of maternal toxicity (suppressed body weight gains and corresponding reductions in food consumption)", Dr. Stolzenberg noted that the body weight decrement at the high dose (100 mg/kg/day) occurred only after the initial oral dose and there was no sustained suppression of body weight gain throughout the treatment period. Hence, Dr. Stolzenberg suggested that the rabbit developmental toxicity study be repeated at higher doses than previously used, and recommended that a dose range-finding study be conducted to determine whether doses of 300 and 1000 mg OPC-41061/kg/day are tolerated by pregnant rabbits.

The dose range-finding developmental toxicity study conducted in rabbits at oral doses of 0, 100, 300 and 1000 mg ~~OPC-41061~~ OPC-41061/kg/day, administered on days 6 to 18 of gestation, was reviewed by Dr. Charles Resnick (review dated June 3, 1999). In that study, there was a transient, dose-dependent weight loss in treated animals (first day of dosing) and weights of the high dose animals had not caught up to concurrent control group weights by the end of the treatment period. Food consumption was significantly lower in the high dose group through gestation day 11 and significantly lower in the low and mid dose groups through gestation day 8. A dose dependent increasing trend for mean postimplantation loss across all groups, and retarded ossification, fetal malformations (including open eyelids, cleft palate, microphthalmia and limb defects) and an increased incidence of skeletal variations in the 1000 mg/kg/day group were noted. Based on the results of this study, the sponsor concluded that appropriate dose levels for the definitive study in pregnant rabbits would be 100, 300 and 1000 mg/kg/day. Dr. Resnick concurred with the sponsor's high dose selection for the definitive study based on the developmental toxicity associated with OPC-41061 in the rabbit.] b(4)

Observations and Measurements: Animals were observed once daily (1-2 hr post-dose on treatment days) for signs of toxicity. Body weights were recorded on days 0, 3 and 6 to 28 of pregnancy. Food consumption was recorded on days 0 and 5 through 27 of pregnancy.

All animals were necropsied on day 28 of pregnancy, major organs examined and organs showing any abnormalities preserved in 10% buffered neutral formalin. The numbers of corpora lutea, implantations, early resorptions (implantation scars, placental remnants or amorphous embryos), late resorptions (macerated fetuses with distinct extremities), dead (fetuses without maceration) and live fetuses were counted. The placentas were examined grossly. The live fetuses were weighed and examined for external anomalies. The live fetuses were then euthanized, dissected, and their sex determined. After examination of the brain, eyeballs and diaphragm, visceral organs were removed, fixed in 10% formalin and were examined for visceral malformations and variations using a stereoscopic microscope. The fetuses were then skinned, fixed in 95% ethanol, cleared in potassium hydroxide, stained with alizarin red S and examined for skeletal malformations and variations.

Data were analyzed using one-way analysis of variance (ANOVA) or Kruskal-Wallis test, followed by Dunnett's test using rank transformation.

Results

Maternal Toxicity

Nine females (1, 3, 3 and 2 animals from 0, 100, 300 and 1000 mg/kg/day dose groups, respectively) were found not pregnant.

There were no mortalities during the study. One mid-dose female aborted on day 26 of pregnancy, and a total of 5 high dose females also aborted, 1 on day 25, 2 on day 26 and 2 on day 27. Two high dose females had loose stools on days 25-27 and 2 others (not the ones which later aborted) showed vaginal hemorrhage on days 22 or 23 of pregnancy.

A significant reduction in body weight (relative to concurrent control) was noted for high dose animals from day 7 to 17 of gestation. Dose dependent reductions in body weight gain were noted for all treated groups. Body weight gains for the low dose group on days 7 through 16, for the mid dose group on days 7 through 19 and for the high dose group on days 7 through 20 of gestation were significantly lower than control.

Food consumption was significantly reduced in all treatment groups, compared to controls, on days 6 through 12 of gestation.

There were no remarkable findings observed at the scheduled necropsy.

Developmental Toxicity

The numbers of corpora lutea, implantations, early and late resorptions, and dead and live fetuses are presented in Table 22, and the body weights and mean pre- and post-implantation losses (%) are given in Table 23.

At 1000 mg/kg, the mean early resorption and the post-implantation loss values (22 and 27%, respectively) were higher than their respective control values (3 and 6%, respectively) and the historical control ranges for the laboratory conducting the study. The mean number of live fetuses in this group was lower than control. There were no treatment-related effects on fetal body weight or sex ratio.

The overall incidences of external, visceral and skeletal malformations and variations are summarized in Table 24.

The fetal and litter incidences of external anomalies are presented in Table 25. Although the incidences were not statistically significantly different from control, open eyelids and brachymelia were observed in 4 of 80 high dose fetuses [1 of 11 litters (doe number 10065)]. Of these 4 fetuses, 2 also had cleft palate. Flexion contracture of the wrist joint was observed in 2 of 146 mid dose fetuses [1 of 16 litters (doe number 10047)]. There were no external anomalies in low dose or control fetuses (17 and 19 litters, respectively).

The incidences of visceral malformations and variations are given in Tables 26 and 27, respectively. A significantly increased incidence of microphthalmia ($p < 0.05$) was observed at 1000 mg/kg/day [5 of 80 fetuses and 2 of 11 litters (doe numbers 10065 and 10077); of these 5 fetuses, 4 of them were the same fetuses that showed external anomalies noted above]. No microphthalmia was seen in other treated groups or in the control group. [The incidence of microphthalmia observed at the high dose (10.2%) was higher than the laboratory historical range (0–0.7%) for this anomaly]. There were no other significant treatment-related visceral malformations or variations in the study.

The incidences of skeletal malformations and variations are summarized in Tables 28 and 29, respectively. Skeletal malformations, including hypoplasia of ulna, radius, tibia and fibula, and bent radius, were limited to the high dose group and were observed in 4 of 80 fetuses [1 of 11 litters (doe number 10065)], "the same fetuses" in which external anomalies were seen. Some of these fetuses also had bent tibia and fibula and fused phalanx. An increased incidence of fused sternbrae (a skeletal variation) was noted in the high group (16% fetal incidence in the high dose group vs 1% in the control group and 4 of 11 litters at the high dose vs 2 of 19 litters in the control group). No other treatment-related skeletal malformations, variations or differences in ossification were observed in the study.

[Note: In a preliminary embryo-fetal development study in rabbits (oral administration of OPC-41061 to pregnant rabbits during organogenesis at 100, 300 and 1000 mg/kg/day), the highest dose was associated with increased incidences of embryo-fetal death, fetal microphthalmia, open eyelids, cleft palate, brachymelia, hypoplasia of ulna, radius, tibia and fibula, and bent ulna, radius, tibia and fibula. These findings were limited to 2 of 5 high dose litters.]

The pharmacokinetic parameters of OPC-41061 and its metabolites (DM-4103 and DM-4107) were determined in pregnant rabbits after a single oral dose (on day 6 of gestation) and 13 consecutive daily oral doses (on days 6 to 18 of gestation) of _____ OPC-41061. The C_{max} and $AUC_{0-24 \text{ hr}}$ values for OPC-41061, DM-4103 and DM-4107 were increased in a dose-related manner after the single dose as well as the 13-day repeated dose administration. After the single dose administration, the AUC values for the parent compound, at all dose levels (100, 300 and 1000 mg/kg), were lower than the values for the metabolites. The AUC values for OPC-41061 following repeated dose administration were lower, while these values for both metabolites were higher, compared to the values after the single dose (Tables 30-31).

b(4)

Table 22.

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Litter Data with Caesarean Section - Summary

Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Dams	19	17	16	13
No. of Corpora lutea				
Total	201	188	195	123
Mean±SD	10.6±1.7	11.1±2.2	12.2±1.8*	10.3±2.1
No. of Implants				
Total	172	153	168	103
Mean±SD	9.1±2.8	9.0±3.1	10.5±2.9	7.9±3.6
Prenatal Death				
Early Resorption				
Total	5	11	3	17
Mean±SD	0.3±0.6	0.6±0.5	0.2±0.5	1.3±2.6
Mean±SD (%)	3.2±7.3	7.0±6.5	1.5±4.1	21.9±37.1
Late Resorption				
Total	7	1	18	5
Mean±SD	0.4±0.8	0.1±0.2	1.1±2.0	0.4±0.9
Mean±SD (%)	3.1±7.0	1.0±4.0	9.9±17.6	4.9±12.1
Dead Fetuses				
Total	0	0	1	1
Mean±SD	0.0±0.0	0.0±0.0	0.1±0.3	0.1±0.3
Mean±SD (%)	0.0±0.0	0.0±0.0	0.5±1.9	0.6±2.3
Total Death				
Total	12	12	22	23
Mean±SD	0.6±1.0	0.7±0.6	1.4±2.1	1.8±2.5
Live Fetuses				
Total	160	141	146	80
Mean±SD	8.4±2.5	8.3±3.0	9.1±3.0	6.2±3.9
Male/Female	0.95	1.14	1.00	1.00

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05, ** : P<0.01 : Significant difference from control value

Table 23.

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Litter Data with Caesarean Section - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Dams	19	17	16	13
Body Weight (g)				
Male				
Mean	33.566	34.601	30.792	32.794
SD	5.907	5.551	3.979	5.191
Female				
Mean	33.024	34.086	31.253	32.497
SD	6.683	4.484	4.416	5.128
Pre Implantation Loss (%)				
Mean	14.93	15.24	14.06	17.63
SD	20.45	22.36	17.23	22.61
Post Implantation Loss (%)				
Mean	6.36	8.00	11.84	27.43
SD	9.40	6.66	18.54	35.41

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05, ** : P<0.01 : Significant difference from control value

Table 24.

----- Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)
 Item : Incidence of Fetal Anomaly (FI) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
External Examination				
No. of Fetuses (Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Anomaly				
No. of Fetuses (Litters)	0 (0)	0 (0)	2 (1)	4 (1)
% Mean±SD	0.0±0.0	0.0±0.0	2.5±10.0	9.1±30.2
Visceral Examination				
No. of Fetuses (Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Variation				
No. of Fetuses (Litters)	32 (14)	19 (12)	20 (12)	17 (8)
% Mean±SD	19.0±17.0	11.9±10.4	16.9±20.0	22.2±19.5
Malformation				
No. of Fetuses (Litters)	1 (1)	4 (1)	4 (2)	7 (4)
% Mean±SD	0.5±2.3	2.1±8.8	1.8±5.4	12.1±29.6
Skeletal Examination				
No. of Fetuses (Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Variation				
No. of Fetuses (Litters)	137 (19)	124 (17)	135 (16)	75 (11)
% Mean±SD	84.5±18.7	86.2±13.7	93.7±7.9	96.7±9.4
Malformation				
No. of Fetuses (Litters)	2 (2)	2 (2)	2 (2)	4 (1)
% Mean±SD	2.3±7.9	1.8±5.4	1.9±5.6	9.1±30.2

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05 , ** : P<0.01 : Significant difference from control value

Table 25.

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : External Anomaly of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses (No. of Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Open eyelids	0 (0)	0 (0)	0 (0)	4 (1)
Mean (%)	0.0	0.0	0.0	9.1
SD (%)	0.0	0.0	0.0	30.2
Cleft palate	0 (0)	0 (0)	0 (0)	2 (1)
Mean (%)	0.0	0.0	0.0	4.5
SD (%)	0.0	0.0	0.0	15.1
Brachymelia	0 (0)	0 (0)	0 (0)	4 (1)
Mean (%)	0.0	0.0	0.0	9.1
SD (%)	0.0	0.0	0.0	30.2
Flexion contract. wrist joint	0 (0)	0 (0)	2 (1)	0 (0)
Mean (%)	0.0	0.0	2.5	0.0
SD (%)	0.0	0.0	10.0	0.0

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : p<0.05 , ** : p<0.01 : Significant difference from control value

Table 26.

----- Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)
 Item : Visceral Malformation of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg) No. of Fetuses (No. of Litters)	AD			
	160(19)	141(17)	A300 146(16)	A1000 80(11)
Dilated cerebral ventricle	1(1)	0(0)	0(0)	0(0)
Mean (%)	0.5	0.0	0.0	0.0
SD	2.3	0.0	0.0	0.0
Microphthalmia	0(0)	0(0)	0(0)	5(2)
Mean (%)	0.0	0.0	0.0	10.2*
SD	0.0	0.0	0.0	30.0
Ventricular septal defect	0(0)	0(0)	1(1)	1(1)
Mean (%)	0.0	0.0	0.6	1.1
SD	0.0	0.0	2.3	3.8
Pulmonary hypoplasia	1(1)	0(0)	0(0)	0(0)
Mean (%)	0.5	0.0	0.0	0.0
SD	2.3	0.0	0.0	0.0
Retrocaval ureter	0(0)	4(1)	3(1)	1(1)
Mean (%)	0.0	2.1	1.3	1.0
SD	0.0	8.8	5.0	3.4
Diaphragmatic hernia	1(1)	0(0)	0(0)	0(0)
Mean (%)	0.5	0.0	0.0	0.0
SD	2.3	0.0	0.0	0.0
Postarterial ureter	0(0)	0(0)	0(0)	1(1)
Mean (%)	0.0	0.0	0.0	0.8
SD	0.0	0.0	0.0	2.7

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05 , ** : P<0.01 : Significant difference from control value

Table 27.

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)
 Item : Visceral Variation of Fetuses (F1) - Summary
 Statistical control : AD

Dose (mg/kg)	AD	A100	A300	A1000
No. of Fetuses (No. of Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Thymic remnant in neck	1 (1)	1 (1)	1 (1)	1 (1)
Mean (%)	0.5	0.5	0.4	2.3
SD (%)	2.1	2.2	1.7	7.5
Supernumerary coronary orifice	0 (0)	1 (1)	1 (1)	2 (2)
Mean (%)	0.0	0.5	0.9	3.4
SD (%)	0.0	2.2	3.6	8.1
Malbranching thoracic artery	3 (3)	0 (0)	2 (2)	6 (2)
Mean (%)	2.8	0.0	2.0	6.5
SD (%)	8.0	0.0	5.7	18.8
Small accessory lung	3 (1)	1 (1)	0 (0)	0 (0)
Mean (%)	1.6	0.7	0.0	0.0
SD (%)	6.9	3.0	0.0	0.0
DCVC, right predominant type	9 (6)	7 (4)	5 (5)	5 (5)
Mean (%)	4.8	4.9	4.3	8.3
SD (%)	9.7	9.6	7.2	11.6
DCVC, left predominant type	5 (4)	4 (4)	2 (2)	3 (3)
Mean (%)	3.0	2.2	1.0	4.1
SD (%)	6.1	4.2	2.7	8.0
CVC, left to descend. aorta	9 (5)	7 (7)	6 (6)	2 (2)
Mean (%)	5.0	4.2	5.0	2.1
SD (%)	9.6	5.4	7.3	4.8
Double renal artery	1 (1)	0 (0)	1 (1)	1 (1)
Mean (%)	0.6	0.0	0.8	1.0
SD (%)	2.5	0.0	3.1	3.4

AD : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05, ** : P<0.01 : Significant difference from control value
 DCVC = double caudal vena cava
 CVC = caudal vena cava

Table 27 (continued)

----- Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Visceral Variation of Fetuses (F1) - Summary
Statistical control : A0

Dose (mg/kg)	No. of Fetuses(No. of Litters)			
	A0	A100	A300	A1000
	160 (19)	141 (17)	146 (16)	80 (11)
Dilatation of renal pelvis				
Mean (%)	2 (1)	0 (0)	1 (1)	0 (0)
SD	1.2	0.0	1.3	0.0
	5.1	0.0	5.0	0.0
Convolution of ureter				
Mean (%)	0 (0)	0 (0)	2 (1)	0 (0)
SD	0.0	0.0	2.5	0.0
	0.0	0.0	10.0	0.0
Diverticulum in Intestine				
Mean (%)	1 (1)	0 (0)	0 (0)	0 (0)
SD	0.5	0.0	0.0	0.0
	2.3	0.0	0.0	0.0
Strayed artery into kidney				
Mean (%)	0 (0)	1 (1)	1 (1)	0 (0)
SD	0.0	0.5	1.3	0.0
	0.0	2.2	5.0	0.0
Adhered both ureters				
Mean (%)	1 (1)	0 (0)	0 (0)	0 (0)
SD	0.5	0.0	0.0	0.0
	2.3	0.0	0.0	0.0

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
* : P<0.05 , ** : P<0.01 : Significant difference from control value

Table 28

Embryo-Fetal Development Study of OFC-41061 Administered Orally to Rabbits (II)
 Item : Skeletal Malformation of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses (No. of Litters)	150 (19)	141 (17)	146 (16)	80 (11)
Skull				
Curved frontal bone	1 (1)	0 (0)	0 (0)	0 (0)
Mean (%)	0.5	0.0	0.0	0.0
SD (%)	2.3	0.0	0.0	0.0
Short incisive bone	1 (1)	0 (0)	0 (0)	0 (0)
Mean (%)	0.5	0.0	0.0	0.0
SD (%)	2.3	0.0	0.0	0.0
Fused frontal bone	1 (1)	0 (0)	0 (0)	0 (0)
Mean (%)	0.5	0.0	0.0	0.0
SD (%)	2.3	0.0	0.0	0.0
Fused incisive bone	1 (1)	0 (0)	0 (0)	0 (0)
Mean (%)	0.5	0.0	0.0	0.0
SD (%)	2.3	0.0	0.0	0.0
Fused nasal bone	1 (1)	0 (0)	0 (0)	0 (0)
Mean (%)	0.5	0.0	0.0	0.0
SD (%)	2.3	0.0	0.0	0.0
Cervical vertebrae				
Fusion of vertebral arches	0 (0)	1 (1)	0 (0)	0 (0)
Mean (%)	0.0	1.2	0.0	0.0
SD (%)	0.0	4.9	0.0	0.0

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05 , ** : P<0.01 : Significant difference from control value

Table 28 (continued)

----- Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Skeletal Malformation of Fetuses (F1) - Summary
Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses(No. of Litters)	160(19)	141(17)	146(16)	80(11)
Cervical vertebrae				
Hemivertebra	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	1.2	0.0	0.0
SD (%)	0.0	4.9	0.0	0.0
Thoracic vertebrae				
Fusion of vertebral bodies	1(1)	0(0)	0(0)	0(0)
Mean (%)	1.8	0.0	0.0	0.0
SD (%)	7.6	0.0	0.0	0.0
Fusion of vertebral arches	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	0.7	0.0	0.0
SD (%)	0.0	2.7	0.0	0.0
Lumbar vertebrae				
Hemivertebra	0(0)	1(1)	0(0)	0(0)
Mean (%)	0.0	0.7	0.0	0.0
SD (%)	0.0	2.7	0.0	0.0
Coccygeal				
Fusion of vertebral bodies	0(0)	0(0)	1(1)	0(0)
Mean (%)	0.0	0.0	0.7	0.0
SD (%)	0.0	0.0	2.8	0.0
Ribs				
Fused ribs	1(1)	0(0)	0(0)	0(0)
Mean (%)	1.8	0.0	0.0	0.0
SD (%)	7.6	0.0	0.0	0.0

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
* : P<0.05, ** : P<0.01 : Significant difference from control value

Table 28 (continued)

----- Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)
 Item : Skeletal Malformation of Fetuses (F1) - Summary
 Statistical control : A0

	Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses (No. of Litters)		160 (19)	141 (17)	146 (16)	80 (11)
Ribs					
Fused costal cartilage		0 (0)	0 (0)	1 (1)	0 (0)
Mean (%)		0.0	0.0	1.3	0.0
SD (%)		0.0	0.0	5.0	0.0
Forelimbs					
Hypoplasia of ulna		0 (0)	0 (0)	0 (0)	4 (1)
Mean (%)		0.0	0.0	0.0	9.1
SD (%)		0.0	0.0	0.0	30.2
Hypoplasia of radius		0 (0)	0 (0)	0 (0)	4 (1)
Mean (%)		0.0	0.0	0.0	9.1
SD (%)		0.0	0.0	0.0	30.2
Bent radius					
Bent radius		0 (0)	0 (0)	0 (0)	4 (1)
Mean (%)		0.0	0.0	0.0	9.1
SD (%)		0.0	0.0	0.0	30.2
Fused phalanx					
Fused phalanx		0 (0)	0 (0)	0 (0)	1 (1)
Mean (%)		0.0	0.0	0.0	2.3
SD (%)		0.0	0.0	0.0	7.5
Hindlimbs					
Hypoplasia of tibia		0 (0)	0 (0)	0 (0)	4 (1)
Mean (%)		0.0	0.0	0.0	9.1
SD (%)		0.0	0.0	0.0	30.2

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : P<0.05, ** : P<0.01 : Significant difference from control value

Table 28 (continued)

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Skeletal Malformation of Fetuses (F1) - Summary

Statistical control : A0

Dose (mg/kg)		A0	A100	A300	A1000
No. of Fetuses	(No. of Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Hindlimbs					
Hypoplasia of fibula					
Mean (%)		0 (0)	0 (0)	0 (0)	4 (1)
SD		0.0	0.0	0.0	9.1
		0.0	0.0	0.0	30.2
Bent tibia					
Mean (%)		0 (0)	0 (0)	0 (0)	3 (1)
SD		0.0	0.0	0.0	6.8
		0.0	0.0	0.0	22.6
Bent fibula					
Mean (%)		0 (0)	0 (0)	0 (0)	2 (1)
SD		0.0	0.0	0.0	4.5
		0.0	0.0	0.0	15.1
Fused phalanx					
Mean (%)		0 (0)	0 (0)	0 (0)	1 (1)
SD		0.0	0.0	0.0	2.3
		0.0	0.0	0.0	7.5

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg

* : p<0.05 , ** : p<0.01 : Significant difference from control value

Table 29.

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Skeletal Variations of Fetuses (F1) - Summary
Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses (No. of Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Skull				
Sutural bone	0 (0)	0 (0)	1 (1)	0 (0)
Mean (%)	0.0	0.0	0.7	0.0
SD (%)	0.0	0.0	2.8	0.0
Crooked thyreohyoid	2 (2)	1 (1)	0 (0)	0 (0)
Mean (%)	1.0	0.4	0.0	0.0
SD (%)	3.0	1.7	0.0	0.0
Cervical vertebrae				
Cervical rib	1 (1)	0 (0)	2 (2)	2 (1)
Mean (%)	0.5	0.0	2.1	2.3
SD (%)	2.3	0.0	5.9	7.5
Thoracic vertebrae				
Irregular shape of vert. body	0 (0)	1 (1)	0 (0)	0 (0)
Mean (%)	0.0	0.4	0.0	0.0
SD (%)	0.0	1.7	0.0	0.0
Unossified hemicentrum				
Unossified hemicentrum	0 (0)	1 (1)	0 (0)	0 (0)
Mean (%)	0.0	0.4	0.0	0.0
SD (%)	0.0	1.7	0.0	0.0
Lumbar vertebrae				
8 lumbar vertebrae	30 (11)	32 (11)	42 (10)	23 (8)
Mean (%)	17.7	20.6	33.2	24.2
SD (%)	24.3	23.5	31.7	24.0

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
* : p<0.05, ** : p<0.01 : Significant difference from control value

Table 29 (continued)

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)

Item : Skeletal Variations of Fetuses (F1) - Summary
Statistical control : A0

Dose (mg/kg)	A0	A100	A300	A1000
No. of Fetuses (No. of Litters)	160 (19)	141 (17)	146 (16)	80 (11)
Sacral vertebrae				
Lumbarization of sacral vert.	8 (8)	9 (6)	5 (5)	3 (3)
Mean (%)	4.5	5.7	2.9	4.2
SD	5.6	8.6	4.5	8.2
Coccygeal vertebrae				
Acentric vertebral body	1 (1)	2 (2)	4 (4)	3 (3)
Mean (%)	0.6	1.6	2.8	3.0
SD	3.3	4.7	5.6	5.1
Ribs				
13 ribs	136 (19)	114 (17)	134 (16)	73 (11)
Mean (%)	83.8	81.1	93.2	89.9
SD	18.4	19.3	8.9	23.1
Extra 13th rib	109 (19)	99 (17)	112 (15)	64 (10)
Mean (%)	65.5	69.8	77.1	75.4
SD	24.2	24.5	28.6	31.0
Rudimentary 13th rib	27 (13)	15 (10)	22 (8)	9 (6)
Mean (%)	18.3	11.3	16.1	14.5
SD	19.0	11.3	25.7	16.6
Non-articulation cost. carti.	5 (5)	17 (9)	5 (4)	2 (1)
Mean (%)	2.8	13.0	4.5	4.5
SD	4.9	17.0	10.4	15.1

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
* : P<0.05 , ** : P<0.01 ; Significant difference from control value

Table 29 (continued)

Embryo-Fetal Development Study of OPC-41061 Administered Orally to Rabbits (II)
 Item : Skeletal Variations of Fetuses (F1) - Summary
 Statistical control : A0

Dose (mg/kg)		A0		A100		A300		A1000	
No. of Fetuses (No. of Litters)		160 (19)	141 (17)	141 (17)	146 (16)	80 (11)			
Ribs	Shortened 12th rib	0 (0)	0 (0)	0 (0)	0 (0)	1 (1)			
	Mean (%)	0.0	0.0	0.0	0.0	2.3			
	SD (%)	0.0	0.0	0.0	0.0	7.5			
Sternum	Fused sternabrae	2 (2)	3 (1)	3 (1)	0 (0)	7 (4)			
	Mean (%)	1.1	1.5	1.5	0.0	15.5			
	SD (%)	3.3	6.1	6.1	0.0	30.4			
Splitting of sternabra	Splitting of sternabra	1 (1)	1 (1)	1 (1)	0 (0)	0 (0)			
	Mean (%)	1.1	0.7	0.7	0.0	0.0			
	SD (%)	4.6	2.7	2.7	0.0	0.0			
Split and asymmetric sternabra	Split and asymmetric sternabra	0 (0)	0 (0)	0 (0)	1 (1)	0 (0)			
	Mean (%)	0.0	0.0	0.0	1.3	0.0			
	SD (%)	0.0	0.0	0.0	5.0	0.0			

A0 : Vehicle control A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg
 * : p<0.05 . ** : p<0.01 : Significant difference from control value

Table 30.

Toxicokinetic Study of OPC-41061 Administered Orally to Pregnant Rabbit
 ITEM : Parameter - OPC-41061
 Sex : Female Day : 06

DOSE	A100		A300		A1000	
	Mean±SD	n	Mean±SD	n	Mean±SD	n
No. of animals	4	4	4	4	4	4
Cmax (µg/ml)	9.112±0.109	3	7.339±1.958	3	11.055±2.847	3
Tmax (hr)	1.33±0.36	3	2.33±1.55	3	2.67±1.15	3
AUC (µg·hr/ml) (0-24hr)	16.655±2.861	3	66.921±18.452	3	115.529±30.378	3

A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg

ITEM : Parameter - DM-4103
 Sex : Female Day : 06

DOSE	A100		A300		A1000	
	Mean±SD	n	Mean±SD	n	Mean±SD	n
No. of animals	4	4	4	4	4	4
Cmax (µg/ml)	3.310±2.111	3	10.622±3.952	3	20.153±9.276	3
Tmax (hr)	4.00±3.48	3	24.00±0.00	3	24.00±0.00	3
AUC (µg·hr/ml) (0-24hr)	50.648±30.954	3	187.710±60.966	3	295.763±113.209	3

A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg

ITEM : Parameter - DM-4107
 Sex : Female Day : 06

DOSE	A100		A300		A1000	
	Mean±SD	n	Mean±SD	n	Mean±SD	n
No. of animals	4	4	4	4	4	4
Cmax (µg/ml)	2.279±1.038	3	6.471±0.810	3	12.780±3.036	3
Tmax (hr)	1.33±0.58	3	2.67±1.15	3	10.00±12.17	3
AUC (µg·hr/ml) (0-24hr)	18.324±6.520	3	98.861±12.142	3	165.279±66.239	3

A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg

Table 31.

Toxicokinetic Study of OPC-41061 Administered Orally to Pregnant Rabbits									
ITEM : Parameter - OPC-41061									
Sex : Female									
DOSE	No. of animals	A100		A300		A1000		Day : G18	
		Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n
Cmax (µg/ml)		0.308±0.009	3	0.846±0.222	3	1.167±0.134	3		
Tmax (hr)		1.33±0.58	3	2.00±1.73	3	1.33±0.58	3		
AUC (µg·hr/ml) (0-24hr)		3.682±0.931	3	8.117±1.644	3	16.924±4.226	3		
A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg									
ITEM : Parameter - DN-4103									
Sex : Female									
DOSE	No. of animals	A100		A300		A1000		Day : G18	
		Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n
Cmax (µg/ml)		14.937±7.065	3	26.667±6.945	3	71.283±45.114	3		
Tmax (hr)		4.00±0.00	3	3.53±1.15	3	6.67±2.31	3		
AUC (µg·hr/ml) (0-24hr)		258.855±179.123	3	471.630±150.402	3	1601.561±1116.161	3		
A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg									
ITEM : Parameter - DN-4107									
Sex : Female									
DOSE	No. of animals	A100		A300		A1000		Day : G18	
		Mean±SD	n	Mean±SD	n	Mean±SD	n	Mean±SD	n
Cmax (µg/ml)		2.828±0.579	3	9.862±2.581	3	22.213±7.194	3		
Tmax (hr)		2.33±1.53	3	1.00±0.00	3	1.33±0.58	3		
AUC (µg·hr/ml) (0-24hr)		35.333±6.361	3	101.715±22.881	3	408.446±252.278	3		
A100 : 100 mg/kg A300 : 300 mg/kg A1000 : 1000 mg/kg									

Additional Studies With OPC-41061 Conducted in Pregnant Rabbits

A follow-up embryo-fetal development study was conducted in pregnant rabbits to confirm that fetal malformations are not produced at a dose of 300 mg OPC-41061/kg/day when administered during organogenesis. Results of this study showed that oral administration of █████ OPC-41061 at 300 mg/kg/day to pregnant rabbits on days 9 to 11 of pregnancy (maximum sensitive period of organogenesis) was not associated with the occurrence of fetal malformations. b(4)

In a separate study, the effects of OPC-41061 on various physiological parameters [urine volume, urine osmolality, urine electrolytes, water consumption, plasma osmolality, plasma electrolyte concentration, blood gas levels, blood pH, and plasma arginine vasopressin (AVP) levels] were determined in pregnant rabbits at a dose level (1000 mg/kg/day) that produced increased incidence of fetal malformations in a previous embryo-fetal development study. █████ OPC-41061 was administered orally to pregnant rabbits on days 9-11 of gestation at 0 and 1000 mg/kg/day and the above physiological parameters were examined on days 9 and 11. Increased urine volume (with decreased urine osmolality), water consumption (water consumption < urine volume), plasma osmolality, plasma sodium and chloride concentrations, and plasma AVP levels were noted at 1000 mg/kg/day, all these effects being attributed to the aquaretic activity of OPC-41061. It was hypothesized that the fetal anomalies associated with OPC-41061 administration might be related to exaggerated pharmacological effects (aquaretic action) of the drug and these fetal anomalies would be increased by water restriction to the dams during drug treatment. This hypothesis was tested in the following study. b(4)

OPC-41061 was administered orally to pregnant rabbits at 0 and 1000 mg/kg/day on days 9-11 of gestation, and half of the animals from each group were restricted to 75 ml/day of water supply and the other half of animals received water *ad libitum*. In the drug-treated animals, water restriction significantly increased plasma osmolality and electrolyte concentrations and post-implantation loss, and significantly reduced the number of litters with live fetuses. Since the number of live fetuses was so small, the effect of water restriction on the incidence of fetal anomalies could not be evaluated. Hence, it was considered necessary to re-examine the effect of water restriction on the incidence of fetal anomalies at a water consumption rate of more than 75 ml/day.

In a supplementary study, the test drug was administered to pregnant rabbits orally at 1000 mg/kg/day on days 9-11 of gestation, with animals receiving *ad libitum* or restricted supply of water (100 or 150 ml/day). The external and skeletal anomalies associated with the administration of OPC-41061 were aggravated in the water restriction groups, although the incidence was rather low in the more water-restricted group (100 ml/day). Moreover, no significant differences were observed in the plasma osmolality and electrolyte concentrations between the litters with and without fetal malformations. Therefore, the relationship between excessive pharmacological effects on dams and fetal malformations was not clearly evident from this study.

Pre- and Post-Natal Development Study of OPC-41061 in Rats

Key Study Findings: Oral administration of OPC-41061 at 10, 100 and 1000 mg/kg/day to pregnant rats from day 7 of gestation through day 21 postpartum was associated with reductions in maternal body weight gain (100 mg/kg/day and above) and food consumption (10 mg/kg/day and above); one dam died at the high dose. Increased perinatal death and suppressed body weight gain of offspring were noted at this same dosage level. F0 maternal drug treatment had no significant effect on the physical development, reflex functions, learning ability or reproductive performance of the F1 progeny.

Testing Facility: Tokushima Research Institute
Otsuka Pharmaceutical Co., Ltd.
Tokushima, Japan

Study Number: 018790 (Report No. 015403)

Study Dates: Initiation Date- August 28, 2002
Completion Date – June 03, 2003

GLP Compliance: The study was conducted in compliance with the GLP Standards for Safety Studies on Drugs, issued by the Ministry of Health and Welfare of Japan.

QA Report: Yes

Animals: Nine to ten-week-old male and female Sprague-Dawley rats — CD, SPF) were obtained from ————. After a quarantine and observation period of 12 days, animals were mated and the day of confirmed copulation (presence of vaginal plug or sperm in the vagina) was designated as day 0 of gestation. Mated females were assigned to 4 groups of 23 animals each. On day 0 of gestation, females were 10 to 12 weeks of age and weighed 219 to 290 g.

b(4)

Animals were housed individually in stainless bracket cages before mating, and in plastic cages with paper chips during gestation and lactation periods. Weaned pups were housed individually. Commercial solid food (CRF-1, Oriental Yeast Co. Ltd.) and in-house tap water were available *ad libitum*.

Dose Levels and Mode of Administration: ———— OPC-41061 powder (Lot No.020625), containing 66.7% OPC-41061 and 33.3% hydroxypropylcellulose, suspended in 1% hydroxypropylmethylcellulose 2910 solution, was administered by oral gavage once daily from day 7 of gestation through day 21 postpartum, at a dose volume of 10 ml/kg. Dosage levels were 0, 10, 100 and 1000 mg/kg/day. Test solutions were prepared at intervals of 7 days or less and stored at a cool place (below 10°C) protected from light. Under the above conditions, test suspensions were found to be stable for 8 days. Analyses of the samples from the first and fourth preparations showed that the concentrations of the test formulations were within 95 to 107% of the nominal values.

b(4)

(Note: The doses for the present study were selected based on the results of the embryofetal development study of OPC-41061 in rats. In that study, suppression of body weight gain and decreased food consumption were observed in dams at 1000 mg/kg/day.)

Observations and Measurements

F0 Maternal Animals

Animals were observed for their general condition once daily from day 1 of gestation through postnatal day (pnd) 21. On each day of drug administration, animals were observed for clinical signs 1 to 2 hours post-dose. Body weights were recorded on days 0, 3 and 7 through 20 of gestation, and on pnd 0, 4, 7, 10, 14, 17 and 21. Food consumption was recorded on days 0 and 6 through 19 of gestation, and on pnd 1, 7, 14 and 21.

Females were allowed to deliver litters. After parturition was completed, females were weighed. The day when parturition had completed was designated as day 0 postpartum.

On pnd 22, all F0 dams that had viable pups were necropsied. All internal organs were grossly examined and the number of implantation sites was recorded. Any organs that showed abnormalities in the treated animals, along with the corresponding organs from controls, were fixed in 10% buffered neutral formalin. Animals that died or had no live pups were also necropsied.

F1 Progeny

The number of dead and live pups was recorded after the completion of parturition. The live pups were weighed, sex determined and examined for external anomalies. The dead pups were preserved in 10% formalin solution.

On pnd 4, the size of each litter was adjusted to eight (4 per sex, where possible). Litters with less than 8 pups were not adjusted. The culled pups were necropsied and preserved in 10% formalin solution.

The pups were weighed on pnd 4, 7, 14, 21, 28, 35 and 42.

During the lactation period, the pups were examined for physical development as follows: pinna detachment on pnd 5, hair growth on pnd 8, incisor eruption on pnds 11 to 13, and eyelid opening on pnds 15 to 17. The pups were also examined for ipsilateral flexor reflex (on pnd 5), righting reflex (on pnd 7), cliff avoidance response (on pnd 8), inclined plane test (on pnd 11), pinna reflex (on pnd 15), and visual placing and auricular startle reflexes (on pnd 21).

After weaning on day 21, twenty pups/sex/group (1 pup/sex/litter) were randomly selected for post-weaning examinations and rearing to sexual maturity. (An additional 20 females/group were also selected for possible supplementary mating; however, they were

not used and, hence, were necropsied.) The pups that were not selected were necropsied, internal organs examined grossly, and organs that showed abnormalities were fixed in 10% buffered neutral formalin along with corresponding organs from controls.

The selected pups were observed daily for general condition. Males were examined for preputial separation (pnd 43-45) and females for vaginal opening (pnd 35). Learning ability assessment (conditioned avoidance response test) was performed on 10 males/group (pnd 59-67).

When the F1 animals reached about 81-89 days of age, each female was mated with a male from the same group (not from the same litter) for up to 14 days and the copulation was confirmed by the presence of vaginal plug or sperm in the vagina. The day of confirmation of copulation was designated as day 0 of gestation.

Cesarean sections were performed on day 13 of gestation and the uterus was removed with the ovaries. Pregnancy status and the numbers of corpora lutea, implantation sites, early and late deaths and live embryos were recorded. When no visible implantation was noted in the uterus, the uterus was placed in 10% ammonium sulfide to detect any trace of implantation, and the ovary was preserved in 10% buffered neutral formalin.

The F1 females that showed no evidence of mating were necropsied 14 days after the end of the mating period.

The F1 males were sacrificed at 109-119 days of age, after the completion of cesarean section of females. From the males that failed to impregnate females, samples of fluid from the cauda epididymides were collected and examined microscopically for the presence of sperm. Also from these males, the epididymides, prostates, coagulating glands, seminal vesicles and any abnormal tissues (with corresponding tissues from controls) were fixed and preserved in 10% buffered neutral formalin and the testes were fixed in formalin-sucrose-acetic acid solution and preserved in 10% buffered neutral formalin.

Data were analyzed using one-way analysis of variance (ANOVA) or Kruskal-Wallis test followed by Dunnett's test, or by using the exact rank-sum test or the Fisher-Irwin test.

Results

F0 Maternal Animals

One low dose female was found not pregnant.

One high dose animal (# 10072) showed hypoactivity on day 9 of gestation and was found dead on day 10. At necropsy, no gross lesions were noted in this animal. During the gestation period, one low dose dam showed alopecia. There were no other clinical signs in any animals from other groups during gestation or lactation period.

The mean body weight was significantly lower than concurrent control for the mid dose group on gestation days 8, 9 and 11 and for the high dose group on gestation days 8 to 12 and 18 through 20. The body weight gain was significantly reduced for the mid and high dose groups on gestation days 8 through 20. Although the mean body weights for the mid and high doses were lower than control at the beginning of the lactation period (postpartum day 4), the body weight values for these groups were higher than control at the end of the lactation period (day 21 postpartum). Food consumption was reduced in all groups on gestation days 7 to 11 and in the high dose group on postpartum days 7 and 14. However, the food consumption was higher than control in the high dose group on postpartum day 21.

The duration of the gestation period in treated groups was comparable to that of control (Table 24). On day 23 of gestation, one high dose dam (#10084) delivered one pup, but during the examination after the completion of parturition, the pup was missing, suggesting cannibalization by the dam. The necropsy of this dam revealed no significant findings. Another high dose dam (#10071) did not gather pups after delivery and 9 of 12 pups in the litter died on postpartum day 0. However, on postpartum day 1 onwards, the dam had collected the remaining pups for nursing. There were no abnormal maternal behaviors in the mid and low dose groups.

The necropsy of the F0 females showed no remarkable findings except the red foci noted in the gastric mucosa of a mid dose animal.

F1 Progeny

Although statistically not significant, the number of dead pups at birth at the high dose was higher and the birth index was lower compared to concurrent control. The viability index on pnd 4 for the high dose group was significantly lower than control, but the mid and low dose group values showed no treatment related effects. The weaning indices were similar across control and treated groups (Table 32).

There were no external anomalies in any pups at birth.

The pup body weights for both sexes were generally similar across control and treated groups on pnd 0. From pnd 4 through pnd 42, the pup body weights at 1000 mg/kg/day were significantly lower than control for both sexes. The pup body weights at mid and low dose levels were comparable to the corresponding values in the control group.

The incidences of pups with eyelids open on pnd 15 were significantly higher in treated groups than in control. There were no significant differences in the eye opening on pnd 16 and 17 between treated and control animals (Table 33). No treatment-related effects on pinna detachment, hair emergence, incisor eruption or vaginal opening were observed. A statistically nonsignificant delay in preputial separation was noted in high dose males compared to concurrent controls.

There were no treatment-related effects on reflex functions or learning ability.

The mean number of days taken for mating, and the copulation and fertility indices were similar across control and treated groups (Table 34).

There were no significant differences in the numbers of corpora lutea, implants, live embryos, early and late embryonic deaths and post implantation loss that were considered to be related to F0 maternal drug treatment (Table 35). Statistically non-significant and non-dose related increases in preimplantation losses were noted at the mid and high dose levels.

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Table 32.

Prenatal and Postnatal Development Study of OPC-41061
Administered Orally to Rats
Item: Litter Data With Natural Delivery - Summary
Statistical control: A0

	A0	A10	A100	A1000
Females				
Pregnant	N 23	22	23	22
Delivered	N 23	22	23	22
Delivered Live Pups	N 23	22	23	21
Duration of Gestation (Days)				
Mean±SD	21.7±0.5	21.7±0.5	22.0±0.2	22.0±0.4
N	374	356	371	358
Mean±SD	16.3±1.5	16.2±2.1	16.1±2.4	16.3±3.0
Pups at Birth				
Alive	N 350	339	333	314
Mean±SD	15.2±1.4	15.4±2.1	14.5±2.7	14.3±4.1
Ratio	1.06	0.93	0.92	1.00
Dead	N 2	6	7	12
Mean±SD	0.1±0.4	0.3±0.5	0.3±1.1	0.5±1.0
Total				
N	352	345	340	326
Mean±SD	15.3±1.4	15.7±2.0	14.8±2.5	14.8±4.0
Gestation Index				
%	100.0	100.0	100.0	95.5
Birth Index				
Mean±SD	93.9±7.9	95.3±4.9	90.2±12.0	85.0±21.3
Viability Index	Mean±SD 98.7±4.0	99.2±2.1	99.4±2.1	92.0±16.6*
Weaning Index	Mean±SD 100.0±0.0	99.4±2.7	99.5±2.6	97.8±7.6

A0: Control A10: 10 mg/kg A100: 100 mg/kg A1000: 1000 mg/kg
 Mean±: Mean of percentages of occurrence with litter
 Gestation Index: 100x(No. of females delivered live pups)/(No. of females pregnant)
 Birth Index: 100x(No. of live pups at birth)/(No. of implants)
 Viability Index: 100x(No. of live pups on Day 4 before culling)/(No. of live pups at birth)
 Weaning Index: 100x(No. of live pups on Day 21)/(No. of live pups on Day 4 after culling)

*: P<0.05. **: P<0.01: Significant difference from control value

Table 33.

Prenatal and Postnatal Development Study of OF0-41061
Administered Orally to Rats
Item : Physical Development (F1) - Summary(All days)
Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
Pinna Detachment (day 5)				
Pups/Litter Examined	184/23	175/22	184/23	163/21
Pups Positive	184	175	184	163
Mean±SD	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Pups/Litter Examined (day 6)				
Pups Positive	±	±	±	±
Mean±SD				
Hair Emergence (day 8)				
Pups/Litter Examined	184/23	175/22	184/23	163/21
Pups Positive	184	175	184	163
Mean±SD	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Incisor Eruption (day11)				
Pups/Litter Examined	184/23	175/22	184/23	162/21
Pups Positive	184	149	157	145
Mean±SD	89.1±17.0	85.1±20.6	85.3±23.4	86.9±25.6
Pups/Litter Examined (day12)				
Pups Positive	184/23	175/22	184/23	162/21
Pups Positive	184	175	184	162
Mean±SD	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0
Pups/Litter Examined (day13)				
Pups Positive	184/23	175/22	184/23	162/21
Pups Positive	184	175	184	162
Mean±SD	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
* : P<0.05. ** : P<0.01 ; Significant difference from control value

Table 33 continued

Prenatal and Postnatal Development Study of OPC-41061
Administered Orally to Rats
Item : Physical Development (FI) - Summary (All days)
Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
Eyelids Opening (day15)				
Pups/Litter Examined	184/23	175/22	183/23	161/21
Pups Positive	177	175	183	161
Mean±SD	96.2±10.9	100.0±0.0*	100.0±0.0*	100.0±0.0*
(day16)				
Pups/Litter Examined	184/23	175/22	183/23	161/21
Pups Positive	183	175	183	161
Mean±SD	99.5±2.6	100.0±0.0	100.0±0.0	100.0±0.0
(day17)				
Pups/Litter Examined	184/23	175/22	183/23	161/21
Pups Positive	183	175	183	161
Mean±SD	99.5±2.6	100.0±0.0	100.0±0.0	100.0±0.0
Preputial Separation (day43)				
Pups/Litter Examined	20/20	20/20	19/19	19/19
Pups Positive	8	12	5	2
Mean±SD	40.0±50.3	60.0±50.3	26.3±45.2	10.5±31.5
(day44)				
Pups/Litter Examined	20/20	20/20	19/19	19/19
Pups Positive	14	16	15	19
Mean±SD	70.0±47.0	80.0±41.0	78.9±41.9	68.4±47.8
(day45)				
Pups/Litter Examined	20/20	20/20	19/19	19/19
Pups Positive	20	19	19	16
Mean±SD	100.0±0.0	95.0±22.4	100.0±0.0	84.2±37.5
Vagina Opening (day35)				
Pups/Litter Examined	20/20	20/20	20/20	20/20
Pups Positive	20	20	20	20
Mean±SD	100.0±0.0	100.0±0.0	100.0±0.0	100.0±0.0

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
* : p<0.05. ** : p<0.01 ; Significant difference from control value

Table 34.

Prenatal and Postnatal Development Study of OPC-41061
 Administered Orally to Rats
 Item : Reproductive Performance - Summary
 Control : A0

Dose (mg/kg)	A0	A10	A100	A1000
Copulated/Paired	18/20 90.0	17/20 85.0	16/20 80.0	18/20 90.0
Fertile/Copulated	13/18 72.2	12/17 70.6	15/16 93.8	16/18 88.9
Duration of Pairing (Days)				
Mean±SD	4.2±3.1	5.4±4.3	3.6±3.1	3.2±1.4

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
 Fertile: For males, impregnating female; and, for females, being pregnant
 * : P<0.05 , ** : P<0.01 : Significant difference from control value

Table 35.

Prenatal and Postnatal Development Study of OPC-41061
Administered Orally to Rats
Item : Litter Data with Caesarean Section(F1) - Summary
Statistical control : A0

Dose (mg/kg)	A0	A10	A100	A1000
Dams Examined	13	12	15	16
Corpora Lutea	214	193	240	269
Mean±SD	16.5±1.6	16.1±2.2	16.0±2.2	16.8±2.1
Implants	196	186	196	223
Mean±SD	15.1±3.3	15.5±3.4	13.1±4.8	13.9±6.0
Preimplantation Loss				
Mean±SD	8.5±17.4	4.4±13.3	19.5±24.4	19.1±32.8
Live Embryos	181	175	185	209
Mean±SD	13.9±3.3	14.6±3.2	12.3±5.1	13.1±5.6
Embryonic Deaths				
Early Deaths	13	11	11	14
Mean±SD	1.0±0.8	0.9±0.8	0.7±0.7	0.9±0.7
Mean±SD	7.0±5.4	5.6±5.0	8.7±12.7	5.3±4.1
Late Deaths	2	0	0	0
Mean±SD	0.2±0.6	0.0±0.0	0.0±0.0	0.0±0.0
Mean±SD	1.0±3.5	0.0±0.0	0.0±0.0	0.0±0.0
Total Deaths	15	11	11	14
Mean±SD	1.2±0.8	0.9±0.6	0.7±0.7	0.9±0.7
Postimplantation Loss				
Mean±SD	8.0±5.1	5.7±5.0	8.7±12.7	5.3±4.1

A0 : Control A10 : 10 mg/kg A100 : 100 mg/kg A1000 : 1000 mg/kg
Mean±SD : Mean of percentages of occurrence with litter
* : P<0.05 . ** : P<0.01 : Significant difference from control value

GENOTOXICITY STUDIES

In Vitro Mouse Lymphoma L5178Y Cell TK Assay

Key findings: OPC-156 tested negative in the mouse lymphoma assay. However, the sponsor has not performed a confirmatory study (with a 24-hour treatment without metabolic activation) required when negative results are obtained with short-term (3-4 hr) treatments.

Study no: 10135

Study type: In vitro mutagenesis and clastogenesis

Volume #, and page #: 003 & 040

Conducting laboratory and location: _____

Date of study initiation: October 5, 1995

GLP compliance: Yes (UK and OECD codes of GLP)

QA report: yes (x) no ()

Drug lot #, and % purity: 2B76SM, & 99.75%

Formulation/vehicle: Test solutions were prepared by dissolving OPC-156 in DMSO immediately prior to use.

Method: Microtiter version of the mouse lymphoma assay

Cell line: Mouse lymphoma L5178Y cells (TK^{+/+})

Dose selection criteria: A preliminary cytotoxicity range finding study was conducted at concentrations ranging from 10 to 1000 µg/ml. Upon addition of the test article to the cultures, precipitate was observed at the top three levels (200, 500 and 1000 µg/ml) and the precipitate was still evident after the 3 hour incubation period. No dose-related toxicity was observed following treatment with OPC-156. Relative survival values at the top dose (1000 µg/ml) were 94.4 and 91.8% with and without S-9, respectively (Table 36).

Table 36
Raw plate counts and % relative survival for OPC-156
in the cytotoxicity range-finder

Treatment µg/ml	In the absence of S-9		In the presence of S-9	
	Survival ¹ at day 0*	% Relative survival	Survival ¹ at day 0*	% Relative survival
0	64	100.0	68	100.0
10	70	110.9	73	115.0
20	66	105.9	59	77.4
30	66	105.9	71	109.2
100	59	88.8	55	69.0
200	44	58.8	70	119.6
500	54	75.2	61	81.9
1000	61	91.8	66	94.4

*- 96 wells scored
*- 1.6 cells/well plated

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Based on the above results, the 200 µg/ml (the lowest precipitating) concentration was selected as the top dose for the main study.

Test agent stability: The test agent and test solutions were determined to be stable throughout the test period.

Metabolic activation system: Aroclor 1254 induced rat liver post-mitochondrial fraction (S-9)

Controls: Vehicle – DMSO

Negative controls – comprised treatment with the solvent DMSO diluted 10-fold in the treatment medium

Positive controls – 4-nitroquinoline 1-oxide (NQO) – without S-9
Benzo(a)pyrene (BP) – with S-9

Exposure conditions: Cell cultures (2 cultures/concentration for the test drug and single culture/concentration for positive controls) containing at least 10^7 cells in a volume of 18.8 ml of culture medium (RPMI 10) were placed in each of a series of sterile centrifuge tubes. The vehicle, test drug (0, 20, 35, 50, 70, 100, 140 and 200 µg/ml) or positive control solution (0.2 ml), and one ml of S-9 mix or 150 mM KCl were added to each tube. After 3 hours of exposure at 37°C, tubes were centrifuged, cells washed and resuspended in culture medium. Cell densities were determined using a Coulter counter or hemocytometer (due to the presence of precipitate at 200 µg/ml) and the cell concentrations were adjusted to 2×10^5 /ml. Cells were then transferred to flasks for growth through the expression period (2 days), or were diluted to 8 cells/ml to be plated for survival at a volume of 0.2 ml per well of two 96-well microtitre plates (2 plates/culture). These microtitre plates were incubated at 37°C for 8 days and wells containing viable clones were identified by naked eye using background illumination and counted.

At the end of the expression period, the cultures were plated for viability (as described above for plating for survival) and 5-trifluorothymidine (TFT) resistance (indicating mutation, induced by the test compound, at the TK locus). [Note: Only the top six doses were selected for plating to determine viability and TFT resistance.]

For the determination of TFT resistance, the cell densities in selected cultures after the expression period were adjusted to 1×10^4 /ml. TFT was then added to give a final concentration of 3 µg/ml, and 0.2 ml (2000 cells) of culture was placed into each well of four 96-well microtitre plates (4 plates/culture). Plates were incubated for 12 days and wells containing clones were counted. In addition, the number of wells containing large and small colonies was scored for the negative and positive controls.

The above experiment was repeated using drug concentrations at 0, 50, 70, 100, 140 and 200 µg/ml.

Analysis: The percentage relative survival (% RS) in each test culture was determined by comparing plating efficiencies (PE) in test and control cultures as follows:

$$\% \text{ RS} = [\text{PE (test)/PE(control)}] \times 100$$

Mutant frequency (MF; mutants per 10^6 viable cells) was calculated as follows:

$$\text{MF} = [\text{PE (mutant cells)/PE (viable cells)}] \times 10^6$$

Mutant frequency data were analyzed for statistical significance. The control log mutant frequency (LMF) was compared with LMF from each treatment dose based on Dunnett's test for multiple comparisons with the same control. The data were also analyzed for a linear trend in mutant frequency with dose using weighted regression.

The assay was considered valid if the following criteria were met:

1. the mutant frequencies in the negative (solvent) control cultures fell within the normal range (not more than 3 times the historical mean value)
2. at least one concentration of each of the positive control chemicals induced a clear increase in mutant frequency

The test article was considered to be mutagenic if:

1. the assay was valid
2. the mutant frequency at one or more doses was significantly greater than that of the negative control
3. there was a significant dose-relationship as indicated by the linear trend analysis
4. the effects described above were reproducible

Summary of study findings: There was no significant increase in osmolality (>50 mOsm/kg) in cultures treated with OPC-156 at the top dose of 200 $\mu\text{g/ml}$ (Experiment 1.)

In the first experiment, precipitation was noted at the top dose (200 $\mu\text{g/ml}$) when the test article was added to the cultures, and this was still evident at the end of the 3-hr incubation period. The top dose yielded 104.4% and 103.6% relative survival in the absence and presence of S-9, respectively.

The relative survival values (%) and the mutant frequencies for experiments 1 and 2 are summarized in Table 37.

No statistically significant increases in mutant frequency, compared to negative controls, were obtained following treatment with OPC-156 at any dose level in experiments 1 and 2 in the absence or presence of S-9. However, a linear trend ($p < 0.05$) was noted in the presence of S-9 in experiment 1, but it was not reproduced in experiment 2.

Table 37.

Summary table of results

Experiment 1

Treatment (µg/mL)	URS	-S-9 Mutant frequency#	Treatment (µg/mL)	URS	+S-9 Mutant frequency#
0	100.0	210.54	0	100.0	175.40
20 S	142.7		20 S	123.7	
35	102.9	194.29 NS	35	92.5	173.61 NS
50	78.4	220.32 NS	50	94.5	204.60 NS
70	76.6	197.66 NS	70	82.4	210.80 NS
100	85.8	214.32 NS	100	87.3	217.32 NS
140	93.0	183.38 NS	140	91.2	202.45 NS
200	104.4	220.61 NS	200	103.6	246.96 NS
Linear trend		NS	Linear trend		*
NQO			BP		
0.05	79.6	460.50	2	73.2	642.03
0.1	57.6	709.14	3	52.2	1292.33

Experiment 2

Treatment (µg/mL)	URS	-S-9 Mutant frequency#	Treatment (µg/mL)	URS	+S-9 Mutant frequency#
0 S\$	100.0	183.80	0	100.0	188.73
50	95.2	133.44 NS	50	95.2	139.16 NS
70	71.0	188.46 NS	70	68.9	131.82 NS
100	73.6	144.82 NS	100	75.2	125.45 NS
140	70.5	178.68 NS	140	92.5	155.20 NS
200	99.3	177.19 NS	200	103.4	123.59 NS
Linear trend		NS	Linear trend		NS
NQO			BP		
0.05	78.2	423.41	2	106.6	458.79
0.1	54.0	751.76	3	69.4	697.24

Per 10⁶ viable cells
 \$ Not plated for viability / S-TFT resistance
 \$\$ Treatment has high heterogeneity, but is included in analysis
 NS Not significant
 *, **, *** Test for linear trend: χ^2 (one-sided), significant at 5%, 1% and 0.1% level respectively

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For negative controls, the proportion of small colony mutants in the absence and presence of S-9 ranged from 66 to 67% in experiment 1 and from 43 to 45% in experiment 2. Good recovery of small colony mutants was observed following treatment with positive control chemicals (Table 38).

Table 38

**Small and large colony mutant frequencies for
negative and positive controls**

Experiment	Concentration ($\mu\text{g/mL}$)	S-9	Mutant frequency*		Proportion small colony mutants
			Small colony	Large colony	
1	0	-	134.9	69.0	0.66
	NQO 0.05		336.6	90.1	0.79
	NQO 0.1		562.1	110.6	0.84
	0	+	110.6	54.7	0.67
	BP 2		452.8	137.0	0.77
	BP 3		986.5	236.3	0.81
2	0	-	72.5	97.6	0.43
	NQO 0.05		199.0	173.6	0.53
	NQO 0.1		322.7	270.1	0.54
	0	+	77.7	96.1	0.45
	BP 2		184.7	168.5	0.52
	BP 3		306.0	236.5	0.56

* Per 10^6 viable cells

Study validity: The assay is considered valid since the mutant frequencies of the negative cultures were within the historical control range and positive control chemicals induced marked increases in mutant frequency.

Study outcome: Although a linear trend was observed in the presence of S-9 in experiment 1, since there were no statistically significant increases in mutant frequency, compared to negative control, at any dose levels of OPC-156 in either experiment with or without S-9, and also because the linear trend was not reproduced in the second experiment, the linear trend observed in the first experiment is considered to be a chance occurrence with no biological significance.

According to ICH Guidance for Industry, for the mouse lymphoma tk assay, "a continuous treatment without metabolic activation for approximately 24 hours is needed in case of a negative result for the short treatment without metabolic activation." Therefore, it is recommended that a confirmatory study with OPC-156 be performed

using a 24-hour treatment regimen in the absence of an exogenous metabolic activation system.

[At an End-of-Phase 2 (EOP 2) meeting with the sponsor on August 26, 2003, the Division recommended that the sponsor conduct a confirmatory test for the mouse lymphoma assay or provide justification for not following the ICH Guidance. In their response dated January 28, 2004, the sponsor stated that since the results of the three standard battery of tests (the test for gene mutation in bacteria, an in vitro test with cytogenetic evaluation of chromosomal damage with mammalian cells, and an in vivo test for chromosomal damage using rodent hematopoietic cells) were all negative, a mouse lymphoma is not considered necessary to further evaluate the genotoxicity of the compound. According to the sponsor, "the 24-hour continuous treatment would be necessary if the mouse lymphoma assay were substituted for the cultured mammalian cell chromosomal aberration test within the three recommended standard battery tests. However, this is not the case." Hence, they did not consider that "additional mouse lymphoma assay with a continuous treatment of 24 hours is necessary." The Division, in their reply dated April 9, 2004, notified the sponsor that the justification for not conducting the confirmatory test for the mouse lymphoma assay is acceptable provided

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**In Vivo Bone Marrow Erythrocyte Micronucleus Test in Sprague-Dawley Rats
Following 3-Day Oral Administration of OPC-41061**

Key findings: OPC-41061 tested negative in the *in vivo* bone marrow micronucleus test.

Study no: 011591

Study type: *In vivo* bone marrow micronucleus assay

Volume # and page #: 1 & 01

Conducting laboratory and location: Department of Toxicology, Tokushima Research Institute, Otsuka Pharmaceutical Co., Japan

Date of study initiation: January 22, 1996

GLP compliance: Yes (GLP Standard for Safety Studies on Drugs – issued by the Ministry of Health and Welfare of Japan)

QA report: yes (x) no ()

Drug lot #, and % purity: 5174 [redacted] mixture of OPC-41061 (66.7%) and hydroxypropyl cellulose-SL at ratio of 2:1]

Formulation/vehicle: [redacted] OPC-41061 was suspended in 1% hydroxypropyl methyl cellulose 2910 (HPMC) aqueous solution (test suspensions were confirmed to be stable for 8 days at 4°C) **b(4)**

Methods: Strain/species: [redacted] CD Sprague-Dawley rats (5/sex/group) **b(4)**

Dose selection criteria: In a previous bone marrow erythrocyte micronucleus test, no male or female Sprague-Dawley rats died, nor were any marked signs of toxicity seen, after oral administration of jet-milled OPC-41061 at 2000 mg/kg/day for 3 days, and no increase in the micronucleus frequency was noted. Also, in a single dose oral toxicity study in the same rat strain, no deaths were observed after administration of 2000 mg [redacted] OPC-41061/kg. In a previous 4-week repeated dose oral toxicity study of [redacted] OPC-41061 at 1000 mg/kg/day, no deaths were noted. Therefore, the high dose was set at 2000 mg/kg/day (limit dose) and 1000 and 50 mg/kg/day were chosen as mid and low doses, respectively. **b(4)**

Controls: Vehicle – HPMC 1% aqueous solution

Negative control – Hydroxypropyl cellulose-SL – dissolved in 1% HPMC solution to a concentration of 10%

Positive control – Mitomycin C (MMC)– dissolved in sterile water to a concentration of 0.5 mg/ml

Exposure conditions: Doses used in definitive study – 0, 500, 1000 and 2000 mg/kg/day.

Study design – Animals, fasted for about 5-6 hr prior to dosing, received test suspension or negative control solution by oral gavage once daily (at 24-hr intervals) for 3 days. Positive control group animals (3/sex) received a single iv injection of MMC solution (2 mg/kg).

Sampling times – Animals were sacrificed about 24 hours after the last dosing. Bone marrow cells were harvested from the femur and smears were prepared.

Analysis: Counting method – The bone marrow slides were stained with acridine orange and examined under an epifluorescent microscope. Two thousand polychromatic erythrocytes (PCE) per animal were counted for micronuclei evaluation and 1000 erythrocytes per animal were counted for determination of polychromatic to normochromatic erythrocyte (NCE) ratio.

Data were statistically analyzed using Jonckheere test, Dunnett's test and Cochran-Armitage test.

The study was considered valid if the following criteria were met:

1. at least the lowest two doses of OPC-41061 induced no mortality
2. the micronucleus frequency and the ratio of PCE to total erythrocytes in the negative control group were within acceptable range (not more than 3 times the historical mean value)
3. the micronucleus frequency was significantly higher in the positive control group

The test drug was considered to produce a positive response if:

1. the micronucleus frequency was significantly higher in any test drug group than in the negative control group
2. a dose-dependent increase in micronucleus frequency was noted in drug-treated groups

Summary of study findings: The incidences of micronucleated polychromatic erythrocytes (MNPCEs) and the percentage of PCEs are summarized in Table 39. No significant increase in micronucleus frequency or any significant decrease in the ratio of PCE to total erythrocytes, compared to negative control, was noted in male or female test drug treated animals. Positive control animals showed significant increase in MNPCEs ($p < 0.01$)

Study validity: The study was considered valid since: 1) the micronucleus frequency and the ratio of PCE to total erythrocytes in the negative control group were within acceptable historical control range, 2) the positive control significantly increased the micronucleus frequency, and 3) previous pharmacokinetic studies demonstrated systemic exposure at the dose levels used in the study.

Study outcome: OPC-41061 tested negative in the *in vivo* bone marrow micronucleus test.

Table 39

Micronucleus test of OPC-41061 in bone marrow erythrocytes of SD rats following 3-day-repeated oral administration (2)
 Item : Bone Marrow - Micronucleus Count
 SEX : Male

Dose	Statistical control : A0				
	A0	A500	A1000	A2000	
XPCe	(MEAN,SD)	57.9±9.6	56.1±2.0	58.8±7.4	54.7±2.5
	(MIN-MAX)	44.4-71.4	52.9-58.3	50.0-68.7	51.4-57.3
XMPCE	(n)	5	5	5	5
	(MEAN,SD)	32/10000	20/10000	14/10000	13/10000
(MIN-MAX)	(n)	1.0-4.5	2.0±0.7	1.4±0.5	1.3±0.7
	(n)	5	1.5-3.0	1.0-2.0	0.5-2.0
					168/6000**
					28.0±10.7
					19.5-40.0

SEX : Female

Dose	Statistical control : A0				
	A0	A500	A1000	A2000	
XPCe	(MEAN,SD)	63.5±8.9	52.2±7.4	62.3±7.9	60.2±5.4
	(MIN-MAX)	54.9-75.0	44.3-59.5	54.0-74.2	51.9-67.1
XMPCE	(n)	5	5	5	5
	(MEAN,SD)	16/10000	29/10000	20/10000	22/10000
(MIN-MAX)	(n)	0.5-2.5	2.9±1.8	2.0±0.4	2.2±0.9
	(n)	5	1.0-5.0	1.5-2.5	1.0-3.0
					116/6000**
					19.7±2.9
					17.5-23.0

A0 : 10%HPC A500 : OPC-41061 SDP 500mg/kg
 A1000 : OPC-41061 SDP 1000mg/kg A2000 : OPC-41061 SDP 2000mg/kg
 * : P<0.05 . ** : P<0.01 : Significant difference from the control (one tailed)
 † : P<0.05 . †† : P<0.01 : Significant dose dependency (one tailed)

B2: Mitomycin C - 2 mg/kg, iv (single dose)

[The Reverse mutation assay (Ames test) and the in vitro chromosomal aberration test were reviewed earlier by Dr. Sidney J. Stolzenberg and his review is provided below (review dated July 3, 1996).]

Reverse Mutation Assay

Performing Laboratory: Tokushima Research Institute
Otsuka Pharmaceutical Co., Ltd.
Tokushima, Japan

Report No: 006851
Study No: 007986

Date Study Initiated: 6/22/92

Quality Assurance: A statement of compliance in accordance with Japanese Ministry of Health standards for GLP is included.

Procedure: Strains used were *S. typhimurium* TA 1535, TA1537, TA100, TA98 and *E. coli* WP2uvrA, with and without metabolic activation with S9 from livers of male rats pre-treated with phenobarbital. Five concentrations of OPC-41061 (Lot No. 2B76SM, dissolved in DMSO before treatment) ranged from 20 to 5000 ug/plate. The tests were done in triplicate and two experiments were performed. Positive controls for each test system are indicated in the tables which follow.

Results: Because of precipitation problems which at the 2 highest concentrations resulted in thin lawns with growth inhibition of bacteria, the test is apparently valid only at concentrations up to 1250 ug/plate. No indications of mutagenicity were observed in the presence or absence of S9. Positive controls confirmed that the tests were valid for each of the strains tested.

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Mutagenicity study of OPC-41061 in TA1535

Compound	Dose (ug/plate) S9	EXP. 1			EXP. 2			Mean	Plate-1	Plate-2	Plate-3	Mean
		Plate-1	Plate-2	Plate-3	Plate-1	Plate-2	Plate-3					
DMSO	-	11	15	12	13	9	10	11	10	11	10	10
OPC-41061	70	11	12	10	11	10	13	0	10	10	10	10
	156	9	10	12	10	9	9	11	9	11	10	10
	313	P 12	P 10	P 12	11	P 12	P 9	P 10	P 10	P 10	10	10
	625	P 14	P 10	P 14	13	P 10	P 10	P 13	P 10	P 10	11	11
	1250	P 12	P 10	P 16	13	P 10	P 10	P 15	P 10	P 10	12	12
	2500	P* 10	P* 12	P* 10	11	P* 11	P* 8	P* 12	P* 10	P* 12	10	10
	5000	P* 13	P* 12	P* 11	12	P* 12	P* 10	P* 13	P* 10	P* 13	12	12
WATER	-	9	10	11	10	12	9	10	9	10	10	10
Mean	0.5	347	337	360	340	330	305	369	364	369	364	364
DMSO	-	11	13	12	12	11	13	13	12	13	12	12
OPC-41061	70	14	12	10	12	12	14	13	12	14	13	13
	156	10	12	12	11	13	10	15	13	10	13	13
	313	13	12	16	14	9	9	13	10	13	10	10
	625	14	15	11	13	14	10	10	10	10	11	11
	1250	P 11	P 12	P 15	13	P 13	P 15	P 12	P 15	P 12	13	13
	2500	P* 13	P* 12	P* 14	13	P* 11	P* 12	P* 16	P* 12	P* 16	13	13
	5000	P* 14	P* 12	P* 12	13	P* 10	P* 13	P* 10	P* 13	P* 10	11	11
2AA	2	222	232	242	232	246	265	260	257	260	257	257

P : precipitation

* : unable to judge the extent of growth inhibition (thin lawn) due to heavy precipitation

Mutagenicity study of OPC-41061 in TA1537

Compound	Dose (µg/plate) S9	EXP. 1			EXP. 2			Mean	Plate-1	Plate-2	Plate-3	Mean
		Plate-1	Plate-2	Plate-3	Plate-1	Plate-2	Plate-3					
OPC-41061	-	9	6	9	7	4	10	7				7
	70	9	5	9	5	10	5	5				5
	150	5	6	7	7	4	4	5				5
	313	P	P 10	P 5	P 7	P 7	P 5	6				6
	625	P 5	P 5	P 7	P 6	P 9	P 8	6				6
	1250	P 0	P 6	P 6	P 4	P 5	P 7	5				5
	5000	P* 4	P* 4	P* 6	P* 5	P* 5	P* 5	5				5
	P* 9	P* 5	P* 3	P* 6	P* 5	P* 4	5				5	
ICR-191	1	1703	1725	1792	1740	1660	1722	1600	1660	1722	1692	
DMSO OPC-41061	-	15	10	12	12	15	11	12				12
	70	10	11	11	11	14	11	11				11
	150	10	14	15	13	0	14	13				13
	313	0	7	7	7	9	14	11				11
	625	10	11	8	10	6	11	11				11
	1250	P 11	P 0	P 0	9	P 6	P 0	0				0
	5000	P* 0	P* 10	P* 0	9	P* 10	P* 7	7				7
	P* 14	P* 6	P* 6	9	P* 9	P* 4	6				6	
2AA	2	190	161	151	167	309	315	252	309	315	292	

P : precipitation

* : unable to judge the extent of growth inhibition (thin lawn) due to heavy precipitation

Mutagenicity study of OPC-41061 in TA100

Compound	Dose (ug/plate)	S9	EXP. 1				Mean	EXP. 2				Mean
			Plate-1	Plate-2	Plate-3	Mean		Plate-1	Plate-2	Plate-3	Mean	
DMSO	-	-	141	129	129	130	127	140	134	134	134	
	70	-	131	150	134	130	123	125	134	127		
	156	-	136	125	131	131	120	120	111	120		
	313	-	P 135	P 151	P 130	139	P 130	P 129	P 131	133		
	625	-	P 142	P 133	P 134	136	P 117	P 110	P 120	118		
	1250	-	P 120	P 135	P 124	126	P 124	P 117	P. TL 101	114		
2500	-	-	Pk:144	Pk:120	Pk:122	129	Pk:125	Pk:126	Pk:115	122		
	5000	-	Pk:120	Pk:123	Pk:119	121	Pk:93	Pk:102	Pk:103	99		
AF-2	0.01	-	501	572	642	590	520	520	516	521		
DMSO	-	+	152	162	174	163	130	160	130	140		
	70	+	107	163	169	173	143	155	145	140		
	156	+	179	194	174	182	174	166	149	163		
	313	+	150	156	147	154	150	144	163	152		
	625	+	164	177	149	163	141	137	120	135		
	1250	+	P 151	P 177	P 147	150	P 146	P 125	P 125	132		
2500	+	Pk:156	Pk:169	Pk:151	159	Pk:120	Pk:146	Pk:125	133			
	5000	+	Pk:149	Pk:146	Pk:156	150	Pk:123	Pk:130	Pk:130	128		
2AA	1	+	010	020	020	042	763	763	709	745		

P : precipitation TL : thin lawn
 #: unable to judge the extent of growth inhibition (thin lawn) due to heavy precipitation

Mutagenicity study of OPC-41061 in TA98

Compound	Dose (ug/plate)	S9	EXP. 1				EXP. 2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	-	-	21	23	19	21	24	16	21	20
OPC-41061	70	-	27	16	23	22	19	21	21	20
	156	-	16	19	22	19	21	26	16	21
	313	-	P 10	P 20	P 20	19	P 26	P 25	P 15	22
	625	-	P 20	P 20	P 24	21	P 20	P 10	P 15	10
	1250	-	P 21	P 23	P 20	21	P 16	P 16	P 16	16
	2500	-	P* 21	P* 10	P* 16	10	P* 16	P* 15	P* 15	15
	5000	-	P* 10	P* 10	P* 17	10	P* 24	P* 15	P* 10	19
AF-2	0.1	-	436	459	410	438	499	533	479	504
DMSO	-	+	29	30	30	32	31	43	34	36
OPC-41061	70	+	32	37	32	34	31	24	31	29
	156	+	31	30	10	26	30	23	27	29
	313	+	27	29	26	27	25	32	31	29
	625	+	20	33	17	26	27	26	42	32
	1250	+	P 25	P 20	P 26	24	P 35	P 26	P 22	20
	2500	+	P* 31	P* 30	P* 30	30	P* 25	P* 31	P* 24	27
	5000	+	P* 23	P* 26	P* 21	23	P* 31	P* 26	P* 22	26
2AA	0.5	+	390	407	301	393	333	321	327	327

P : precipitation

* : unable to judge the extent of growth inhibition (thin lawn) due to heavy precipitation

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Mutagenicity study of OPC-41061 in WP2uvrA

Compound	Dose (ug/plate)	S9	EXP. 1				EXP. 2			
			Plate-1	Plate-2	Plate-3	Mean	Plate-1	Plate-2	Plate-3	Mean
DMSO	-	-	22	18	19	20	22	20	22	21
OPC-41061	70	-	17	15	17	16	15	20	25	20
	156	-	22	19	23	21	20	18	19	19
	313	-	P	P	P	22	P	P	P	17
	625	-	P	P	P	20	P	P	P	17
	1250	-	P	P	P	17	P	P	P	17
	2500	-	P#	P#	P#	17	P#	P#	P#	22
	5000	-	P#	P#	P#	16	P#	P#	P#	15
AF-2	0.01	-	266	230	250	264	210	213	241	224
DMSO	-	+	28	22	25	25	19	18	21	20
OPC-41061	70	+	30	19	22	24	25	27	26	26
	156	+	29	20	24	24	17	19	26	21
	313	+	37	21	21	25	29	22	29	27
	625	+	16	33	21	23	15	33	25	24
	1250	+	P	P	P	25	P	P	P	20
	2500	+	P#	P#	P#	20	P#	P#	P#	19
	5000	+	P#	P#	P#	20	P#	P#	P#	13
2AA	10	+	1505	1383	1471	1453	1633	1502	1509	1548

P : precipitation
 #: : unable to judge the extent of growth inhibition (thin lawn) due to heavy precipitation

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In Vitro Chromosomal Aberration Test With CHL Cells

Performing Laboratory: Tokushima Research Institute
Otsuka Pharmaceutical Co., Ltd.
Tokushima, Japan

Report No: 006856
Study No: 008029

Date Study Initiated: 5/20/92

Quality Assurance: A statement of compliance in accordance with Japanese Ministry of Health standards for GLP is included.

Procedure: The maximum concentration of 40 ug OPC-41061/ml for the 24 and 48 hour treatment tests was based the ID₅₀ obtained in a preliminary cytotoxicity test with CHL cells (fibroblast cell line derived from Chinese hamster lung, obtained from [REDACTED], both in the presence and absence of S9. The maximum concentration for the 6 hour treatment (with an 18 hour recovery period), set at 100 ug/ml, was based solubility limitations at 62 ug/ml and higher. OPC-41061 (Lot No. 2B76SM, dissolved in DMSO before treatment), was tested in each test system, both with and without S9 that was derived from male rat liver after pre-treatment with phenobarbital. Positive controls for each test system are indicated in the tables which follow.

b(4)

Results: There were no increases in frequencies of cells with structural aberrations, chromatid gaps or breaks or polyploidy. The positive controls with and without S9 indicated that the test was valid.

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Cytotoxicity of OPC-41061

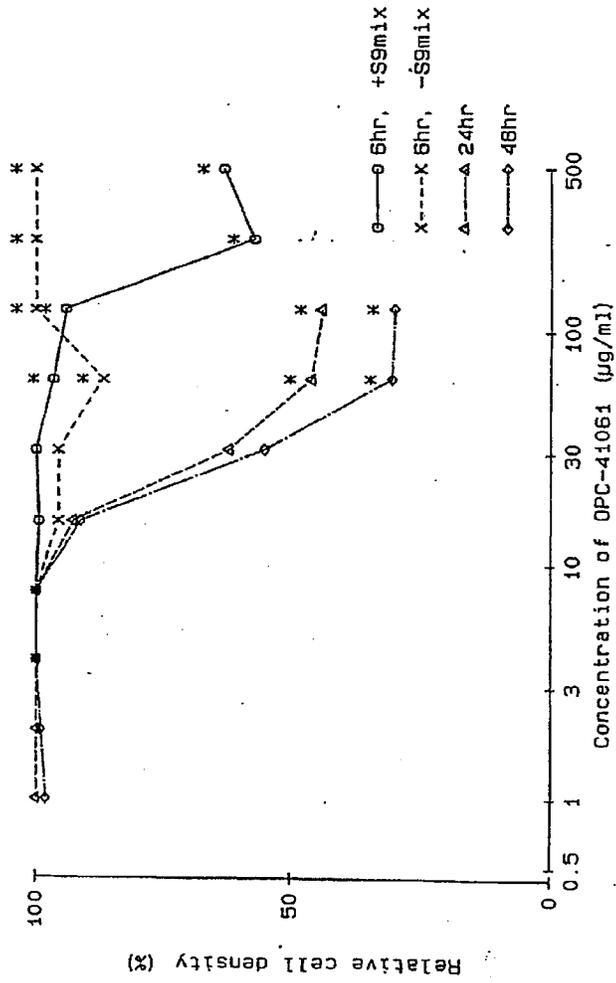


Fig. 1 CHL cells were treated with OPC-41061, fixed and stained with crystal violet. Optical absorbance was measured with * : Precipitation was observed.

b(4)

Table 1 The cytogenetic effect of OPC-41061 on cultured CILL cells

Treatment	Concentration ($\mu\text{g}/\text{ml}$)	Time (hr)	SSmix (+/-)	No. of cells analysed	clg	ctb	cle	csg	csb	cse	mul	Frequencies (%) of cells with SA -Gap	Frequencies (%) of cells with Other Poly
DMSO	-			200	0	0	0	0	2	0	1	1.0	1.0
OPC-41061	25			200	1	2	0	0	0	1	0	1.5*	1.5*
OPC-41061	50	6-18	+	200	0	1	0	0	1	0	0	1.0*	1.0*
OPC-41061	100			200	0	1	0	0	2	0	0	1.5*	1.5*
CP	10			200	0	20	55	0	0	1	1	35.0*	35.0*
DMSO	-			200	0	1	0	0	0	0	0	0.5	0.5
OPC-41061	25			200	0	0	0	0	0	0	0	0.0*	0.0*
OPC-41061	50	6-18	-	200	0	0	0	0	0	1	0	0.5*	0.5*
OPC-41061	100			200	0	0	0	0	0	1	0	0.5*	0.5*
CP	10			200	1	1	1	0	0	0	0	1.5*	1.0*
DMSO	-			200	0	0	1	0	1	0	0	1.0	1.0
OPC-41061	10			200	0	0	1	0	2	0	0	1.0*	1.0*
OPC-41061	20	24-0	-	200	0	1	0	0	0	0	0	0.5*	0.5*
OPC-41061	40			200	1	0	0	0	0	0	0	0.5*	0.0*
MHC	0.04			200	5	55	69	0	2	1	2	51.0*	49.0*
DMSO	-			200	0	1	0	0	1	1	1	1.5	1.5
OPC-41061	10			200	0	1	0	1	0	1	0	1.5*	1.5*
OPC-41061	20	48-0	-	200	0	0	0	0	0	0	0	0.0*	0.0*
OPC-41061	40			196	0	1	1	0	1	0	0	1.5*	1.5*
MHC	0.04			200	1	115	143	0	1	0	51	84.5*	84.5*

Time: treatment-recovery. SA: structural aberrations. clg: chromatid gap, ctb: chromatid break, cle: chromatid exchange, csg: chromosome gap, cse: chromosome break, csb: chromosome exchange, mul: multiple aberration; more than 5 aberrations. -Gap: gaps are included in aberrations. +Gap: gaps are excluded from aberrations. Other: other aberrations except structural aberration. Poly: polyploidy. DMSO: dimethylsulfoxide (0.5% in medium). CP: cyclophosphamide, MHC: mitomycin C. Significant differences from solvent control (DMSO) at $p < 0.05$ (†) or not (N) by Fisher's exact test (one-sided).

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SPECIAL TOXICOLOGY STUDIES

[Sponsor's summaries of special studies are provided below.]

2.4.4.7.1 Antigenicity/immunotoxicity

The antigenic potential of tolvaptan was evaluated in guinea pigs.¹²⁷ The study was appropriately designed, conducted in conformance with GLP regulations, and evaluated doses up to 10 mg/kg. Guinea pigs sensitized subcutaneously and intramuscularly with tolvaptan as a mixture with Freund's complete adjuvant showed no evidence of active systemic anaphylaxis following intravenous challenge with tolvaptan. Additionally, there was no evidence of passive cutaneous anaphylaxis in naive guinea pigs challenged intravenously with tolvaptan after intradermal administration of diluted sera from tolvaptan-sensitized guinea pigs.

A 4-week oral study of T-cell dependent antibody response in rats¹²⁸ was conducted to determine the potential immunotoxicity of tolvaptan. The study was appropriately designed and was conducted in conformance with GLP regulations. Tolvaptan did not adversely affect the T-cell dependent humoral immune response to sheep red blood cells in rats treated at doses of 30, 100 or 1000 mg/kg/day indicating that the humoral response was unaltered by treatment with tolvaptan. In addition, the immunotoxic potential of tolvaptan was assessed by standard hematologic and morphologic endpoints in repeat-dose toxicity studies. There were no drug-related morphologic changes in lymphoid or hematopoietic tissues of rodents or dogs in the repeat-dose studies. Based on the lack of evidence for drug-related effects on immune system function in the nonclinical studies, additional nonclinical immunotoxicity testing was unnecessary.

2.4.4.7.2 Coagulation

In the 4-week repeat dose toxicity study in rats, coagulation parameters were altered in male and female rats given 300 mg/kg/day (prolonged APTT) and 1000 mg/kg/day (prolonged PT and APTT) and in those given 1000 mg/kg/day there was also a significant decrease in the activity of vitamin K-dependent coagulation factors II, VII, IX and X in

both sexes. Investigative studies were conducted to evaluate the potential for tolvaptan to alter coagulation.¹²⁹ The effects of tolvaptan on PT and APTT were investigated in male rats using ████████ tolvaptan at a dose of 1000 mg/kg/day for 2 weeks. The results of this study demonstrated that the prolongation of PT and APTT occurred only under fasting conditions and was reversed by vitamin K supplementation. It was concluded that the slight prolongation of PT and APTT induced by tolvaptan at 300 mg/kg/day in the rat 4-week repeated-dose toxicity study was caused by depletion of vitamin K-dependent coagulation factors. In addition, the effect of tolvaptan on prolonged PT and APTT induced by warfarin¹³⁰ was investigated in male rats using ████████ tolvaptan at doses of 10 and 100 mg/kg/day. Tolvaptan had no notable effect on the serum concentration of warfarin or on the prolonged PT and APTT induced by warfarin. Therefore, the mechanism of the PT and APTT prolongation, observed in tolvaptan-treated rats and exacerbated under fasted conditions, was not considered to be relevant in humans since, unlike rats, humans can utilize both vitamin K₁ and K₂. For these reasons and because changes in PT/APTT have not been observed in any other species tested, it was determined that there is no nonclinical evidence of tolvaptan-induced coagulopathy relevant to humans.

b(4)

2.4.4.7.3 Hormones

An investigative study was conducted to evaluate the potential for tolvaptan to alter levels of AVP and adrenal cortex-related hormones.¹³¹ In a 52-week toxicity study of tolvaptan in beagle dogs, hypertrophic changes of the adrenal cortex were noted in some treated animals. Since the changes were similar to those one would observe as a stress response and because no effects were noted in the adrenal glands in other toxicity studies (up to 26 weeks in rats and 13 weeks in dogs), a direct effect of tolvaptan on the adrenal cortex was considered unlikely. To further investigate the potential effect of tolvaptan on the adrenal cortex, glucocorticoid production in dogs was assessed by determining the effects of tolvaptan on plasma AVP, ACTH, serum cortisol and urinary 17-OHCS levels during 7-day (from Day 0 to Day 6) repeated oral administration of 1000 mg/kg of tolvaptan. As expected, increases in water consumption and urine volume as well as a marked decrease in urine osmolality were noted after tolvaptan administration due to its pharmacological effect (aquaresis). This effect was generally consistent during the treatment period. Plasma AVP was increased after tolvaptan administration and seemed to return to the control level prior to the next daily dose. This increase was considered to be related to the pharmacological profile of tolvaptan, since an increase in endogenous AVP might be attributable to a feedback regulation of the endocrine system against a V₂-receptor

antagonist, tolvaptan. Plasma ACTH was significantly increased after tolvaptan administration compared to that of the controls however biological significance of this change was questionable because the magnitude of increase was small when compared to the pre-values. Changes in serum cortisol could not be evaluated in this study because the values were mostly below the detection limit of the assay. Urinary 17-OHCS excretion, a parameter of cortisol production, was increased in the treated group, suggesting that tolvaptan marginally enhanced adrenal cortex function. Since several studies in dogs^{132,133,134,135} showed that infusion of AVP transiently increased the corticosteroid level, increased AVP level by tolvaptan administration was considered as one of the cause of the enhanced adrenal cortex function. The hypertrophic changes of adrenal cortex observed in the 52-week toxicity study in dogs were considered to be due to a hyper-function of the adrenal cortex in which increased cortisol production was sustained at least partly by increasing endogenous AVP after tolvaptan administration. For humans, more than 50 pg/mL of AVP was required to increase serum cortisol levels¹³⁶ and this AVP level is much higher than that observed in the dogs given 1000 mg/kg of tolvaptan. Therefore, an increase in endogenous AVP by tolvaptan administration would not cause a notable increase in serum cortisol levels in humans.

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2.4.4.7.4 Phototoxicity

The phototoxicity of tolvaptan was examined using *in vitro*¹³⁷ and *in vivo*^{138,139} test systems. In the *in vitro* assay using mouse (BALB/c) embryonic (fibroblast-like) cell line cells, cell survival was reduced at precipitating concentrations of 0.13 mg/mL and higher. The concentration of tolvaptan inhibiting relative survival by 50% (IC₅₀) was 0.31 mg/mL under non-irradiated conditions and 0.14 mg/mL under irradiated conditions which represents a ratio (photoirritation factor, PIF) of 2.2 and a 'probably phototoxic' classification. The phototoxicity of tolvaptan was considered to be weak because the PIF in the dose-range finding assay was 0.61 and cytotoxicity was noted at concentrations at which precipitation of the compound occurred. In addition, the IC₅₀ for irradiated conditions was 370-times higher than the tolvaptan plasma C_{max} (374 ng/mL) achieved in humans administered tolvaptan at 60 mg. In an *in vivo* phototoxicity study in guinea pigs,¹³⁸ no phototoxicity was observed. In an *in vivo* phototoxicity study in rabbits,¹³⁹ no phototoxicity was observed at a serum level of tolvaptan which is 7-times the mean human serum level (Table 2.4.4.7.4-1).

	Mean Serum (µg/mL)		
	Guinea pig at 2000 mg/kg	Rabbit at 1000 mg/kg	Human at 60 mg ^a
Tolvaptan	0.94 ^b	2.6 ^b	0.37 ^c
DM-4103	1.1 ^b	48 ^b	2.2 ^d (3.2)

() = Highest value

^aFrom OMRI Study No. 156-95-305; Sample Analysis Report (██████████ Project No. 44427) entitled, 'The Determination of OPC-41061 and its Metabolites (DM-4103, DM-4104, DM-4105, DM-4107, and DM-4111) by HPLCMS/MS in Human Plasma Samples for Otsuka Maryland Research Institute Study No. 156-95-305'

^b2 hours after final dosing

^cAfter single dose

^dAfter 28-day repeated dose (at the presumed steady state)

b(4)

2.4.4.7.5 Metabolites

The toxic potential of DM-4103 and DM-4107, the major metabolites of tolvaptan in humans, was investigated in single-dose,^{140,141,142,143} genotoxicity,^{144,145,146,147} and phototoxicity studies in rats.^{148,149} Because DM-4103 is insoluble in water and re-crystallizes when mixed with serum¹⁵⁰ or injected intravenously,¹⁴³ it was dissolved in dimethylsulfoxide(DMSO) and then administered to male rats by single subcutaneous

injection at doses of 100 and 500 mg/kg. DM-4103 did not show any notable toxicity in rats given 100 mg/kg (C_{max} : 54 $\mu\text{g/mL}$, $\text{AUC}_{0-24\text{h}}$: 743 $\mu\text{g}\cdot\text{h/mL}$) or 500 mg/kg (C_{max} : 26 $\mu\text{g/mL}$, $\text{AUC}_{0-24\text{h}}$: 319 $\mu\text{g}\cdot\text{h/mL}$). The reversed exposure observed in this study was due to the reversed absorption amount, higher at 100 mg/kg and lower at 500 mg/kg, due to re-crystallization of DM-4103 at the injection site. DM-4107 was also dissolved in DMSO and subcutaneously administered to male rats at single doses of 100 and 500 mg/kg. No toxicity was noted and the serum level of DM-4107 increased with dose elevation, with a C_{max} 310 $\mu\text{g/mL}$ and an $\text{AUC}_{0-24\text{h}}$ of 3906 $\mu\text{g}\cdot\text{h/mL}$. The genotoxic potential of DM-4103 and DM-4107 was evaluated in both forward and reverse mutation studies and both metabolites were determined to be non-mutagenic in these studies. In addition, the phototoxicity potential of DM-4103 and DM-4107 was investigated in *in vitro* and *in vivo* phototoxicity studies. In the *in vitro* phototoxicity study of DM-4103, cell survival was reduced under non-irradiated conditions at precipitating concentrations of 0.50 and 1.0 mg/mL. The IC_{50} was 0.99 mg/mL under non-irradiated conditions and 0.0061 mg/mL under irradiated conditions, which represents a PIF of 162 and resulted in a classification of 'phototoxic' under *in vitro* conditions.¹⁴⁸ For DM-4107, cell survival was reduced only at the highest concentration of 1.0 mg/mL, 77.9% and 51.1% under non-irradiated and irradiated conditions, respectively. Therefore, a PIF could not be calculated and the compound was classified as 'phototoxicity indeterminable.' Since the cytotoxic effects were almost the same under both non-irradiated and irradiated conditions, it is thought that DM-4107 is unlikely to be phototoxic. In the *in vivo* phototoxicity studies in guinea pigs¹³⁸ and rabbits¹³⁹ in which tolvaptan was administered to the animals, no phototoxicity was observed. In the rabbit study, no phototoxicity was observed at a serum level of DM-4103 which is 22-times the mean human serum level and a serum level of tolvaptan which is 7-times the mean human serum level at the MRHD.

2.4.4.7.6 Isomers

The toxicity of (R)-(+)- and (S)-(-)-OPC-41061, the optical isomers of tolvaptan, was investigated in single dose toxicity studies in female rats given 500, 1000 or 2000 mg/kg tolvaptan orally (gavage)^{151,152} and in *in vitro* genotoxicity studies.^{153,154,155,156} In the single dose toxicity studies, deaths occurred in animals receiving 500 mg/kg or higher of the (R)-isomer and in those receiving 1000 mg/kg or higher of the (S)-isomer. Racemic tolvaptan did not cause any deaths at up to 2000 mg/kg. Toxicokinetic data showed that serum levels of tolvaptan (as sum of (R)-(+)-OPC-41061 and (S)-(-)-OPC-41061) in rats given 500 mg/kg of (R)-isomer and those given (S)-isomer were respectively higher than

and equivalent to the serum levels in rats given 2000 mg/kg of racemic tolvaptan. The difference in exposure was considered to be due to the particle size of each powder, with the particle size of each isomer being smaller than that of racemic tolvaptan. It was therefore concluded that the difference in mortality between the racemic and isomeric tolvaptan was due to a difference in exposure level reflecting the different particle sizes of the formulations. The genotoxic potential of (R)-(+)-OPC-41061 and (S)-(-)-OPC-41061 was evaluated in both forward and reverse mutation studies and both isomers were determined to be non-mutagenic in these studies.

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