Exposure and AUC ratio in 2-Year Carcinogenicity Study in Rats-I				
Dose in PPM	Dose(mg/kg)#	Route	AUC(µg.h/ml)	AUC ratio@
625	35	Feed	16.6	0.5
1250	69	Feed	29.9	0.8
2500	141	Feed	57.3	1.6

#Equivalent dose in mg/kg/day. @Based on an AUC value of 35 µg.h/ml for a recommended daily dose of 120 mg, tid.

CAC Concurrence: No
Restriction Paradigm for Dietary Restriction Studies: NA
Route of Administration: In diet
Frequency of Drug Administration: Food was offered ad libitum except during overnight fasts.
Dual Controls Employed: No
Interim Sacrifices: Yes (Please see mortality table below)
Satellite PK or Special Study Group(s): Yes, 5 rats/sex/group
Unscheduled Sacrifices or Deaths: Unscheduled animal necropsies were performed when the animals were moribund or found dead.

Deviations from Original Study Protocol: For weeks 26 and 80, all toxicokinetic blood samples from the control groups were analyzed rather than the 12- and 24-hour samples as indicated in the protocol. Terminal body weights were not collected for animals sacrificed early as required by the protocol.

STUDY RESULTS AND FREQUENCY OF MONITORING:
Clinical Signs and Mortality: Animals were observed once daily for mortality check. Rats showing signs of debility or if death appeared imminent were killed. There were no clinical signs considered to be attributable to treatment with nateglinide noted in any of the rats throughout the study. Treatment with nateglinide for 2 years had no effect on survival.

Effects of Nateglinide on Mortality in 2-Year Carcinogenicity Study in Rats-I								
Sex	Male				Female			
Dose@	Control	625	1250	2500	Control	625	1250	2500
Deaths#	22	31	25	24	35	31	31	31
% Survival	61	45	55	57	38	45	45	45
% Mortality	39	55	45	43	62	55	55	55
@Dose in ppm. # Indicates cumulative number of deaths for 104 weeks.								

Dose group:	Control		625 ppm		1250 ppm		2500 ppm	
No. Survivors	M	F	M	F	M	F	M	F
Initial no.	56	56	56	56	56	56	56	56
week 52	55	53	53	56	54	53	53	54
week 64	55	52	53	54	54	51	52	50
week 80	50	43	47	47	49	39	50	44
week 104	34	21	25	25	31	25	32	25

Body Weight: Body weight of each rat was recorded at the time of allocation of animals to groups on the day of initial treatment and once a week thereafter including the day of death or sacrifice. During the first 78 weeks of treatment, the bodyweight in males receiving 2500 or 1250 ppm was marginally lower than that of the controls. The differences were not dose-dependent and were consistent with food consumption data. Thus, the reduction in body weight as well as mortality data could not be used to establish the MTD was utilized in this study.

Hematology and Clinical Chemistry: Hematological evaluation was performed in Week 104 of treatment for the determination of total red blood cells and white blood cell counts, and showed no statistically significant differences between groups. Results from rats sacrificed during the treatment period showed no apparent treatment-related findings.

Macroscopic Pathology: Pathological examination of major organs was performed at termination and suspected treatment-related findings, as well as major post mortem findings of the remaining organs, were documented. In pancreas, masses were noted with increased incidence in female rats receiving 2500 ppm compared with female control rats. Histopathologic evaluation revealed that the masses were islet cell tumors. Other findings in lungs, mammary glands, and forestomach were found to have no significance since their frequency was unrelated to nateglinide treatment.

Microscopic Pathology:

The incidence of islet cell tumors was greater among female rats receiving 2500 ppm than in the controls as shown below. The intergroup differences did not attain statistical significance on pairwise comparison, but revealed a positive trend in the tumor incidence (p=0.039 on tailed test). The percentage incidence of pancreatic islet cell tumor in females of the high dose group (12.5%) was just above the historical range (0 – 12%) as shown below. The findings were not observed in males and the morphological features of the islet cell adenomas were similar in the controls and the treated groups, which suggested the positive findings in female rats, may not be significant. There were no treatment-related non-neoplastic findings.

Pancreatic Islet Cell Tumor in 2-Year Carcinogenicity Study in Female Rats-I				
Tumor Type	Control	625 ppm	1250 ppm	2500 ppm
Pancreas#	56	56	56	56
Adenoma	2	2	2	6
Carcinoma	0	1	0	1
Total Tumor	2	3	2	7

#Indicate number of pancreas examined.

Historical Control Data of Pancreatic Islet Cell Tumor in Female Rats-I										
Code	9006	9008	9009	9010	9011	9012	9105	9106	9107	9108
Pancreas#	59	50	49	55	59	49	50	50	60	60
Adenoma	3	1	2	1	1	1	0	5	2	0
Carcinoma	1	0	1	0	0	0	0	1	1	0
#Indicate the total number of pancreas examined.										

Conclusion and Comments:
The administration of nateglinide for 2 years at doses of 625, 1250 and 2500 ppm did not produce treatment-related non-neoplastic changes in rats. There was an increase in the incidence of pancreatic islet cell adenomas in female rats receiving 2500 ppm. The increase was not statistically significant on pairwise comparison with the control, although the statistical test indicated there was a positive trend. Nateglinide is not genotoxic; however, in view of the insulinotropic activity of nateglinide, it is conceivable that β-cell tumors could result from excessive stimulation of the pancreas by the compound.

A sustained hyperplastic response would be a prerequisite for tumor promotion. Pancreatic islet hyperplasia was not observed in either rat carcinogenicity study or any shorter term rat studies. The tumor incidence was at the high end of the historic control range of the laboratory which performed the study, and was not elevated in male animals. The incidence of pancreatic tumors was not affected in the second rat carcinogenicity study in which animals were exposed to higher (22 times) drug concentrations. It thus seems most probable that the elevation observed in the female rats in the initial rat carcinogenicity study was a chance finding.

CONCLUSION: Treatment of rats with nateglinide for 2 years was not associated with an increased incidence of neoplastic or non-neoplastic pathology. However, this study was considered invalid since doses were far below the MTD.

Study Title: Carcinogenicity Study in Rats (Experiment II)
Study Number/Report#/Document#: 96001
Volume Numbers/Page: 31/p5-1 – 5-230
Test Facility: Novartis Pharmaceuticals Corp., East Hanover, NJ
Study Date(s): 4/16/1996-4/20/1998
Date of Submission: June 24, 1999
GLP Compliance: Yes
QA Report- Yes (x) No ()
Study Type: Regular
Species/strain: Rat/Sprague-Dawley[Crl;CD(SD)BR]

Number of animals per group; age at start of study: 70/sex/group; 8 weeks old
Animal housing: Pair-housed in ——————— cages
Drug Lot/Batch number(s): 2G563-4, 2G635-8, 2G772-L, 2G773-4

Drug Purity / Stability / Homogeneity: The sponsor provided relevant analysis data of each batch. The analysis was performed by [redacted] Purity of all bathes was 99.4-100.6% with under 0.1% of L-isomer.

DOSES: Control, Placebo, Nateglinide (0, 100, 300, 600, or 900 mg/kg/day)
Basis of Dose Selection:
In a 13 week study in rats, decreased body weight gains were noted when nateglinide was administered in diet at 5000 ppm (17% and 12% for male and female rats), at 10000 ppm (25 and 27%, respectively), and at 20000 ppm (35 and 42%). Nateglinide concentrations with the three doses were approximately 364-438, 724-844, and 1522-1817 mg/kg/day. When rats were dosed with nateglinide orally by gavage for 13 weeks at 100, 300, or 1000 mg/kg/day, body weight gains were at least 10% below the control value only at the high dose (28% and 17% for males and females, respectively). Administration of nateglinide orally by gavage for 12 months resulted in body weight gains that were at 10% below the control value in males at 100 mg/kg/day and 26% and 18% reduced in males and females with the high dose of 500 mg/kg/day. The studies indicated that the maximum tolerated oral (gavage) dose was greater than 300 mg/kg/day and less than 500 mg/kg/day. Based on absorption data from dietary drug treatment, the high dose of 900 mg/kg/day in the diet provided systemic exposure to nateglinide that was comparable to that seen in this oral (gavage) dose range. The mid-high (600 mkd), mid (300 mkd), and low (100 mkd) doses were selected to evaluate dose-response relationship.

CAC Concurrence: Yes
Restriction Paradigm for Dietary Restriction Studies: NA
Route of Administration: In diet
Frequency of Drug Administration: Food was offered ad libitum except during overnight fasts.
Dual Controls Employed: No
Interim Sacrifices: Yes (Please see mortality table below)
Satellite PK or Special Study Group(s): Yes, 20 rats/sex/group
Unscheduled Sacrifices or Deaths: Unscheduled necropsies were performed when the animals were moribund or found dead.

Deviations from Original Study Protocol: For weeks 26 and 80, all toxicokinetic blood samples from the control groups were analyzed rather than the 12- and 24-hour samples as indicated in the protocol. Terminal body weights were not collected for animals sacrificed early as required by the protocol.

STUDY RESULTS AND FREQUENCY OF MONITORING:

Clinical Observations: In general, clinical signs of treated groups were not significantly different from the control groups. The incidences of rough coat and ataxia were slightly higher (not significant) in the treated groups, compared to the

control group. Other clinical signs were sporadic without any specific distribution pattern, and were attributed to the age of the rats.

Nateglinide Intake: The test article intake was calculated using the following formula: {[Drug in feed (mg/g)] x Mean daily food consumption (g/day)} / Mean weekly body weight (kg). For each drug treated group, individual weekly intake values were within 10% of the expected dose as indicated below.

Nateglinide Intake from Diet in 2-Year Carcinogenicity Study in Rats-II				
Animal Sex	Male		Female	
Dose	Mean Intake*	Expected(%)	Mean Intake*	Expected(%)
100 mg/kg/day	99	99	98	98
300 mg/kg/day	295	98	295	98
600 mg/kg/day	596	99	589	98
900 mg/kg/day	893	99	883	98

*The unit was in mg/kg/day.

The actual nateglinide intake at each dose group was compared with its systemic exposure in the second carcinogenicity study in rats. The table below shows the mean intake of the drug at 5 dose levels, and AUC values at week 80. The ratios of animal to human AUC values were calculated in the last column, based on human AUC value of 35 μg.h/ml for a daily dose of 120 mg, tid. Since nateglinide was not-genotoxic, the dose selections appear to be adequate based on AUC ratios > 25-30 times human exposure.

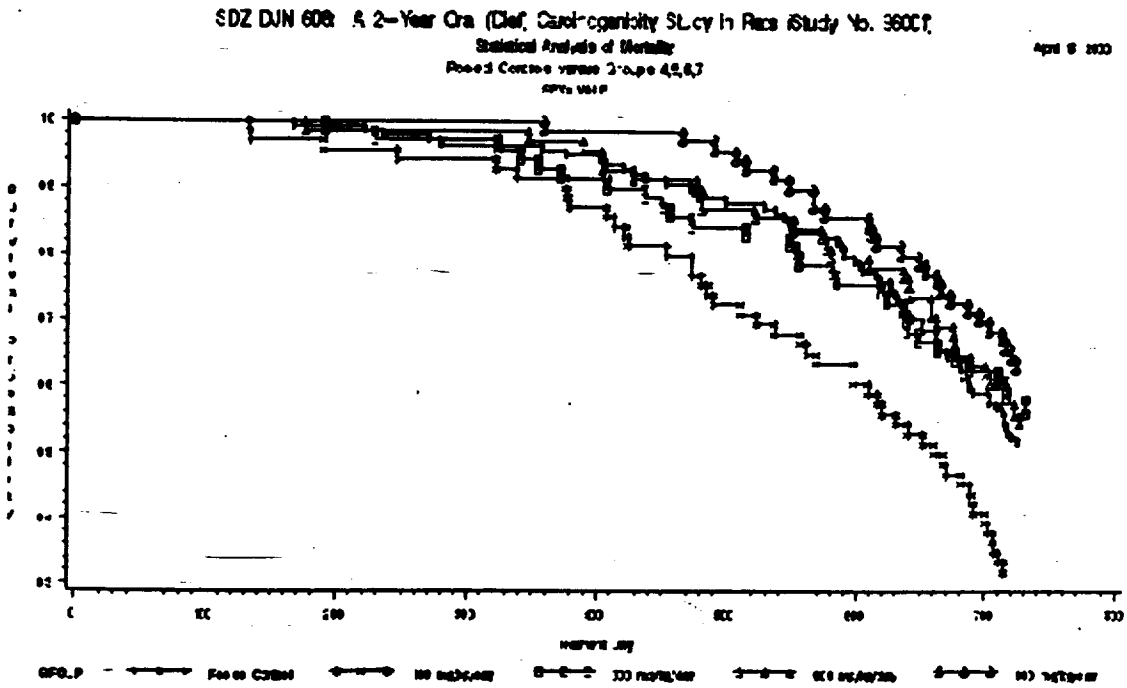
Exposure and AUC ratio in 2-Year Carcinogenicity Study in Rats-II				
Dose(mg/kg)	$AUC_{0\text{-}24\ hour}$		AUC ratio*	
	Male	Female	Male	Female
100	669	440	19	13
300	523	1000	15	29
600	873	1260	25	26
900	1780	1570	51	45

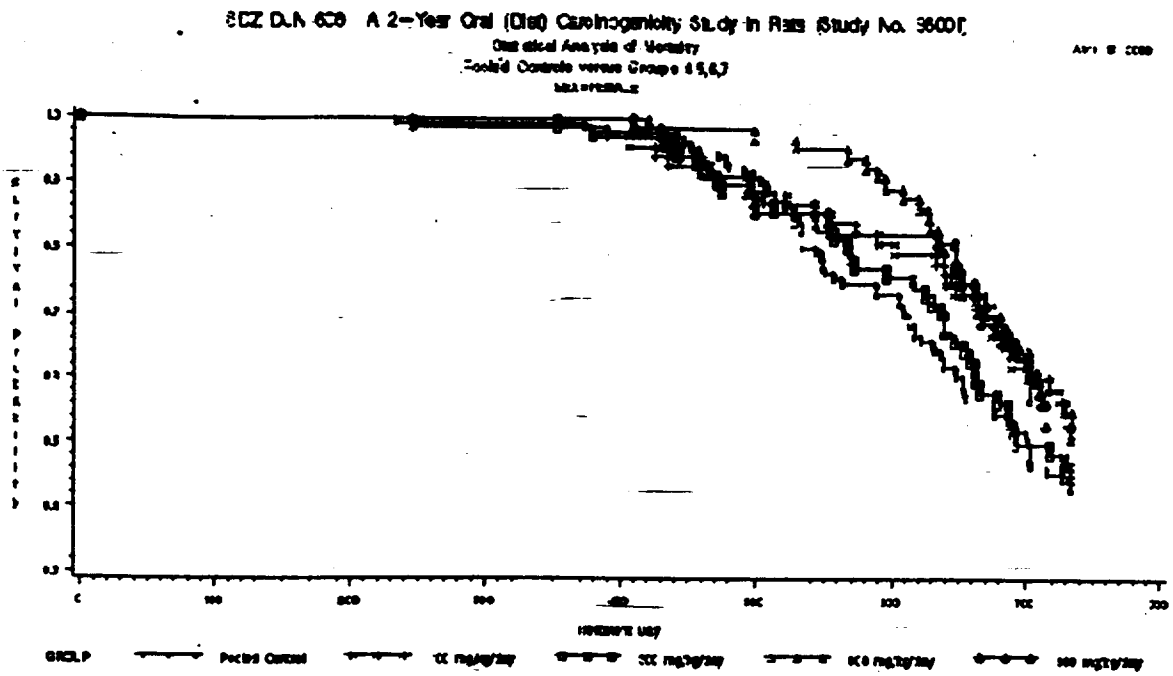
*Based on AUC_{rats}/AUC_{human} (35 μg.h/ml after 120 mg, tid) comparison.

Mortality: Unscheduled animal death was checked twice daily except weekends and holidays when the animals were checked once daily. There were no treatment-related differences in the mortality rate among the groups except for a trend toward increased survival in males at 900 mg/kg/day as shown below. The basis of increased survival in the high dose group is not clear.

Effects of Nateglinide on Mortality in 2-Year Carcinogenicity Study in Rats-II												
Sex	70 males per group						70 females per group					
Group	1	2	3	4	5	6	1	2	3	4	5	6
Died	28	21	29	17	19	15	8	14	8	16	12	10
Inter@	11	13	20	15	14	11	42	32	33	26	27	29
Term+	31	36	21	38	37	44	20	24	29	28	31	31
%*	44	51	30	54	53	63	29	34	41	40	44	44

*Groups 1 and 2 were control and groups 3, 4, 5 and 6 received Nateglinide at doses of 100, 300, 600, and 900 mg/kg/day, respectively. Inter@ and Term+ indicate animal numbers killed intercurrently and at study end, respectively. *Indicate survival (%).



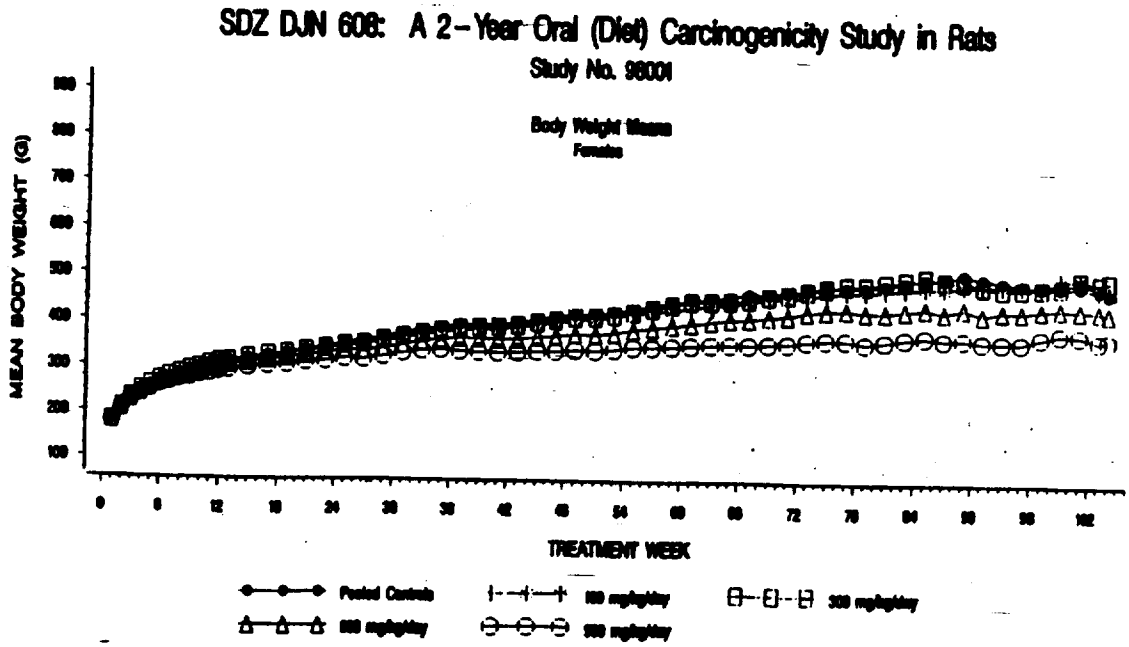


Body Weight: The parameter was checked once weekly during the first 3 months of study and once every 2 weeks for the remainder of study. There were no differences in mean body weights between the two control groups. Thus, drug-treated groups were compared to a combined control group (groups 1 and 2). In males, significant reduction in mean body weights started in weeks 11 and 6 at 600 and 900 mg/kg/day, respectively, and continued through the end of the dosing period. The parameter in the two high dose groups was reduced 13 and 15% from the combined control value by the end of treatment (Week 104) as seen below. The mean body weight reduction started at weeks 19 and 8 at 600 and 900 mg/kg/day groups in females, and continued to the end of study. At the terminal sacrifice (Week 104), the parameters were 9 and 21% lower than the combined control group at the two high dose groups.

The gradual reductions in body weight in males and females in 2-year carcinogenicity study are illustrated in two figures below. The effect of the two high doses of Nateglinide on the parameter was clearly demonstrated in the figures. The reduction in body weight in the high dose groups of both sexes was used as a justification for the maximum tolerated dose selection in the species, as presented earlier. The changes in body weight appear to be related to a reduction in mean food consumption in both sexes at the two high doses.

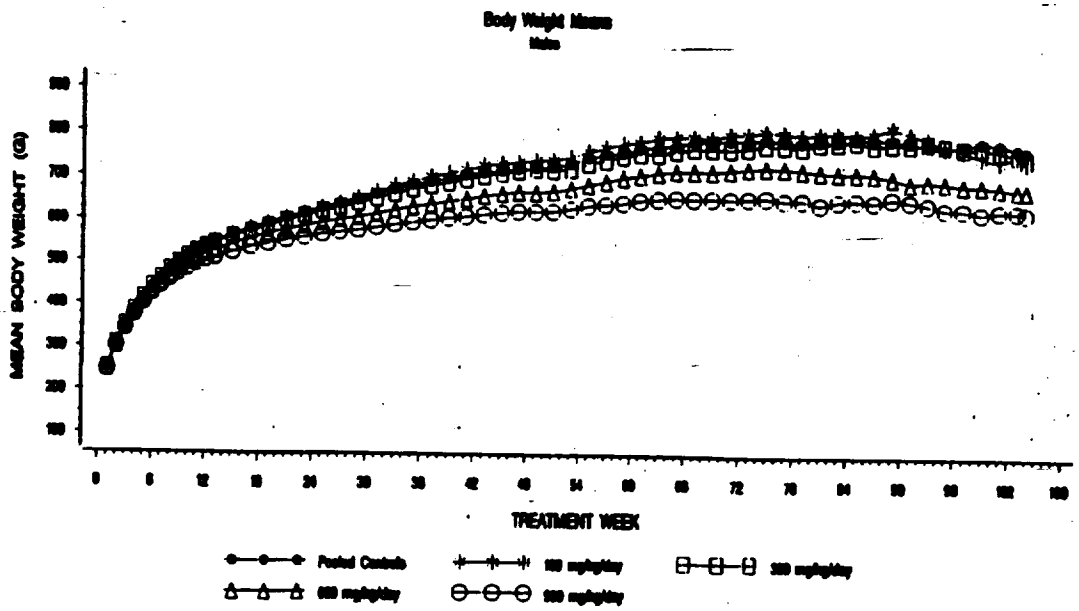
Effects of Nateglinide on Body Weight in 2-Year Carcinogenicity Study in Rat-II@										
Dose	C	100	300	600	900	C	100	300	600	900
Week	Male Rats					Female Rats				
0	186	185	185	185	185	149	150	150	150	149
12	540	538	537	519*	506*	310	308	312	301	293*
27	641	641	632	604*	573*	360	351	353	337*	319*
51	726	742	720	671*	625*	420	409	413	376*	340*
87	800	810	783	718*	654*	504	484	500	431*	372*
101	788	750	773	690*	636*	491	509	506	440	385*
103	778	763	764	685*	636*	482	484	506	440*	373*
104	748	747	755	684*	632*	474	479	504	433	375*

@Indicates absolute body weight in gram. # Indicates week after nateglinide treatment and *P<0.05, compared to control group(C).



SDZ DJN 608: A 2-Year Oral (Diet) Carcinogenicity Study in Rats

Study No. 98001



Food Consumption: Food consumption was calculated based on weekly consumption except for animals undergoing study defined fasting procedures. Since there were no differences in mean food consumption between the two control groups, drug-treated groups were compared to a combined control group. In both sexes at 600 and 900 mg/kg/day groups, significant reductions in mean food consumption were noted throughout most of the study as shown below. There were significant reductions in the parameter at 100 and 300 mg/kg/day. It appears that the two lower doses had little or no impact on food consumption. The decreased body weights in the two higher dose groups appear to be secondary to decreased food consumption. Therefore, reductions in body weight can not be used to establish the MTD.

Effects of Nateglinide on Food Consumption in 2-Year Carcinogenicity Study in Rat-II@										
Dose	C	100	300	600	900	C	100	300	600	900
Week	Male Rats					Female Rats				
0	24	25	24	24	24	18	17	18	17	17
12	26	26	25	25*	24*	19	18*	18	17*	17*
27	25	25	24	23*	22*	19	17*	17*	16*	16*
51	24	24	23*	22*	22*	18	17	17	16*	15*
87	25	25	24	23*	21*	19	18	18	16*	15*
101	24	21	23	21*	21*	19	19	19	17*	17
103	24	23	22*	22*	20*	18	19	16	16	16

104	24	22	21*	21*	21*	18	18	19	18	18

@Indicates average food consumed/day by week of study in gram. # Indicates week after nateglinide treatment and *P<0.05, compared to control (C).

Ophthalmoscopy: There were no remarkable testing article-related findings that were documented.

Hematology and Clinical Chemistry: Blood samples were collected in weeks 26, 52, 78, and prior sacrifice at the time of final dissection (week 104) from the first ten surviving animals/sex/group including control. Urine was collected during weeks 25/26, 51/52, 77/78, and 103/104. For the animals in a moribund status, blood samples were obtained before sacrifice as much as possible. Since there were no differences in mean values between the two control groups, drug-treated groups were compared to a combined control group.

Mean insulin values at 900 mg/kg/day were decreased by approximately 50% in weeks 78 and 104, although the values were quite variable within each group. Mean glucose concentrations were generally increased at doses >100 mg/kg/day, which appeared to dose-related until weeks 26. Significant decreases in alanine aminotransferase were observed in females at 900 mg/kg/day at weeks 78 and 104. There were transient and sporadic changes in serum creatinine, alkaline phosphatase activity, and total protein in females at 100 and 300 mg/kg/day during week 26, which appear unrelated to test article.

Urinalysis: Compared to pooled controls, there were slight reductions (not significant) in urine volume in females across all nateglinide-treated groups at week 78. The values returned to within the range of the pooled control group by week 104. It appears that nateglinide treatment was associated with an increase in mean total c-peptide and mean c-peptide/creatinine ratios at all time points in rats dose at >300 mg/kg/day. The significance is not clear because c-peptide levels in control male rats were also elevated at week 104.

Organ Weights:

Nateglinide did not have remarkable effects on organ weights in most of organs examined. Mild to moderate (15%) decreases in absolute pancreas weights occurred in 300 and 600 mg/kg male group. A similar reduction was noted in females in the two highest dose groups, although the reduction was not reflected in relative to brain weight values as shown below. There was no microscopic evidence of acinar or islet atrophy or other morphological finding that might have contributed to these weight reductions. The increases in the relative brain, liver, and kidney weights were attributed to the body weight reductions because the absolute weights were not significantly different from the control values.

Effects of Nateglinide on Organ Weights in 2-Year Carcinogenicity Studies in Rats-II@						
Organ	Sex	Control	100 mg/kg	300 mg/kg	600 mg/kg	900 mg/kg
Brain	Male	0.329	0.362	0.337	0.375*	0.393*
	Female	0.491	0.504	0.463	0.554	0.636*
Liver	Male	2.348	2.541	2.460	2.523	2.718*
	Female	2.737	2.559	2.758	2.799	3.234*
Kidney	Male	0.626	0.764	0.649	0.650	0.725*
	Female	0.677	0.746	0.659	0.745	0.886*
Pancreas #	Male	0.220	0.217	0.197	0.220	0.244
	Female	0.272	0.247	0.229	0.260	0.294

@ Indicate % of organ/body weight ratio. #The absolute weights were reduced in both sexes, which were not reflected in organ/body weight ratios. *P<0.05.

Gross Pathology:

There were no macroscopic observations at necropsy that were considered related to nateglinide treatment. Notably, the effects of nateglinide on nervous tissues in the rat were not remarkable as shown below.

Effects of Nateglinide on Neural Tissues in 2-Year Carcinogenicity Study in Rats-II*												
Sex	Males						Females					
Dose@	C	PB	100	300	600	900	C	PB	100	300	600	900
Brain#	70	70	70	70	70	70	70	70	70	70	70	70
Brain-N	61	51	54	58	51	51	59	59	65	63	60	65
Sciatic#	67	69	67	70	68	69	68	67	69	68	69	67
Sciatic-N	36	41	41	48	42	45	51	46	41	44	44	44
Axonal-D	28	23	25	18	20	20	16	18	27	23	24	23
Spinal#	70	70	70	70	70	70	70	70	70	70	70	70
Spinal-D	40	46	42	48	47	53	45	50	53	56	55	50

*Pathological examination was done at termination. @The unit was in mg/kg. # Indicate numbers of rats examined. N and D indicate normal and degeneration, respectively.

Histopathology: 82 organs or parts thereof were examined microscopically for histological evaluation for tumor or non-tumor incidences. Both neoplastic and non-neoplastic microscopic findings were considered to be spontaneous, most often age-related. There was no evidence of a statistically significant increase of any neoplasm, including pancreatic islet cell tumors in nateglinide-treated groups verses the pooled control group. In both sexes, the incidence of neoplasms was similar across all groups reflecting biological variation rather than a treatment effect.

Toxicokinetics: Blood samples were taken from the retrobulbar venous plexus from non-starved rats according to the following schedules. From 3 rats/sex/group, blood was collected at 1, 2, 3, 4, 7, and 24 hours after drug administration on week 4, 26, and 80. Drug exposure was confirmed in all test article-treated animals. In general, females exhibited 1.2 to 2.5 times higher plasma concentrations of nateglinide compared to males in all dose groups. This effect seemed more pronounced at doses above 300 mkd. Increases in exposure (AUC) across all doses were noted except for males in week 4. It appears that increasing duration of dosing from week 4 to 80 elevated AUC values in most of doses, which might suggest accumulation of plasma drug levels.

Summary of Nateglinide Toxicokinetics in 2-Year Carcinogenicity Study in Rats-II@							
Sex	Dose	Cavg/Doses(ng/ml)			AUC_{0-24h}(ng.h/ml)		
Time		Week4	Week26	Week80	Week4	Week26	Week80
Male	100	13.4	14.2	27.9	332	340	669
	300	10.9	15.4	21.8	261	370	523
	600	16.7	24.7	36.3	400	593	873
	900	15.2	31.0	49.1	366	744	1780
Female	100	12.2	18.3	18.3	292	438	440
	300	13.3	21.3	41.7	320	510	1000
	600	22.5	51.2	52.3	538	1230	1260
	900	37.3	65.3	65.3	896	1570	1570

OVERALL INTERPRETATION AND EVALUATION

The sponsor performed two sets of 2-year carcinogenicity studies in rats in addition to the standard mouse study. The first rat study was not valid because the top dose was not the MTD and the ratio of drug systemic exposure in animal to human (AUC ratio) was less than 2. There were no indications of the drug's adverse effects such as mortality, changes in body weight or food consumption.

A slight, non-significant increase in the incidence of pancreatic islet cell tumors was observed in high dose female rats. It was concluded that the pancreatic tumor findings were not clearly drug associated for the following reasons: 1) The percentage incidence of pancreatic islet cell tumor in females of the high dose group (12.5%) was just outside the historical range (0 – 12%), although the historical range seems to wide; 2) Immunohistochemically the tumors stained positive for insulin, indicating a β-cell origin, which is a common tumor type in rats; 3) The morphological features of the islet cell adenomas were similar in the controls and the treated groups; and 4)The findings were not observed in males.

The second 2-year carcinogenicity study in rat was conducted in an acceptable manner. The average AUC ratio was > 25 times human exposure in both male and female rats administered the two high doses. Nateglinide did not increase

mortality significantly. The high and mid-high doses of nateglinide reduced the absolute body weight significantly and the top dose reduced the parameter by 16% and 21% in male and female rats, respectively at the end of 2 years. The body weight reductions appeared to be secondary to significant reductions in food consumption in these groups. The findings in the second valid 2-year carcinogenicity study suggest that nateglinide was not carcinogenic.

Adequacy of the carcinogenicity studies and appropriateness of the test model:

Nateglinide purity, stability and homogeneity were well documented and verified to be acceptable. Sprague-Dawley rats [Crl:CD(SD)BR] have been used for the analysis of carcinogenic potential for many years. The animal number (70/sex/group) and housing conditions were acceptable. Acceptable doses were utilized since AUC ratio of drug systemic exposure in animal vs. human therapeutic AUC exposure approached 30 in the top dose groups. The mid-high, mid, and low doses were selected appropriately, which indicates the dose selection appears to be acceptable.

Evaluation of Tumor Findings:

There were no clear tumors in any groups of either sex in the second 2-year carcinogenicity studies in rats. However, the first 2-year carcinogenicity studies in rats suggested that the incidence of islet cell tumors was greater among female rats receiving 2500 ppm than in the controls. However, the intergroup differences did not attain statistical significance on pairwise comparison. The percentage incidence of pancreatic islet cell tumor in females of the high dose group (12.5%) was just outside the historical range (0 – 12%). Immunohistochemically the tumors stained positive for insulin, indicating a β-cell origin. The findings were not observed in males or in the repeat 2-year carcinogenicity study utilizing higher doses.

SUMMARY CONCLUSIONS AND RECOMMENDATIONS

The first 2-year carcinogenicity study was performed with extremely low systemic exposure condition in rats. Therefore, it is reasonable to disregard the outcome of the study. The second 2-year carcinogenicity study carried out under acceptable conditions in the same species and strain, utilizing acceptable doses revealed no tumor findings. The results of the 2-year bioassays indicate that nateglinide is not carcinogenic in standard 2-year oncogenicity studies in mice and rats.

Major Tumor Findings: There were no significant tumor findings in the 2-year mouse or 2-year rat carcinogenicity studies.
Non-neoplastic Findings: Nateglinide had no remarkable effects on histopathological parameters.
Biological Significance: None

Addendum 1:Histopathology Inventory for NDA#21-204

Study	94/0143	AJ078	96001	
Species	Mouse	Rat	Rat	
Adrenals	X	X	X	
Aorta	X	X	X	
Bone Marrow smear		X	X	
Bone (femur)	X	X	X	
Brain	x	x	x	
Cecum	X	X	X	
Cervix	X	X	X	
Colon	X	X	X	
Duodenum	X	X	X	
Epididymis	X	X	X	
Esophagus	X	X	X	
Eye	X	X	X	
Fallopian tube				
Gall bladder		X		
Gross lesions	X	X	X	
Harderian gland	X	X	X	
Heart	x	x	X	
Hyphophysis				
Ileum	X	X	X	
Injection site				
Jejunum	X	X	X	
Kidneys	X	X	X	
Lachrymal gland				
Larynx				
Liver	X	X	X	
Lungs	X	X	X	
Lymph nodes, cervical		X	x	
Lymph nodes mandibular	X	X	X	
Lymph nodes, mesenteric	X	X	X	
Mammary Gland	X	X	X	
Nasal cavity			X	
Optic nerves				
Ovaries	X	X	X	
Pancreas	X	X	X	
Parathyroid				
Peripheral nerve				
Pharynx				
Pituitary	X	X	X	
Prostate	X	X	X	
Rectum	X	X	X	
Salivary gland			X	
Sciatic nerve	X	X	X	
Seminal vesicles	X	X	X	

Skeletal muscle		X	X	
Skin	X	X	X	
Spinal cord		X	X	
Spleen	x	x	x	
Sternum		X	X	
Stomach	X	X	X	
Testes	x	x	X	
Thymus			X	
Thyroid	x	x	X	
Tongue	X	X	X	
Trachea		X	x	
Urinary bladder	X	X	X	
Uterus	X	X	X	
Vagina	X	X	X	
Zymbal gland				

V. IMMUNOTOXICOLOGY:

Study title: An active systemic anaphylaxis study in guinea pigs
Study No: 91002
Vol#40/Page#: 5/27-5/42
Site and testing facility: [redacted]
GRP compliance: No
QA- Report Yes (x) No ():
Lot and batch numbers: A-015

METHODS:
An active systemic anaphylaxis study of nateglinide was conducted in male [redacted] guinea pigs. A total 35 guinea pigs were immunized with the drug at a dose of 10 mg/kg, by subcutaneous injection with Freund's adjuvant for a total 4 times at 7 day intervals. At 14 days after the last immunization, nateglinide alone or its mixture with guinea pig serum albumin at a dose level of 10 mg/kg was intravenously administered as a challenging antigen. Bovine serum albumin was used as a positive control substance.

RESULTS:
No anaphylactic signs were observed in the nateglinide immunization group or the nateglinide-guinea pig serum immunization group. On the other hand, the BSA immunization group showed typical anaphylactic signs and death, which suggests that nateglinide does not cause anaphylactic reactions in guinea pigs.

Study title: An IgE antibody production study of nateglinide in mice
Study No:: 92-31
Vol#40, Page#: 5/43-5/64

Site and testing facility:

GRP compliance: No
QA- Report Yes (x) No ():
Lot and batch numbers: 2G121

METHODS:
Total 18 male BALB/c mice were immunized with nateglinide and IgE production was examined via the PCA reaction in rats. Nateglinide, together with aluminum hydroxide was administered IP to the mice, twice with an interval of 4 weeks at doses of 1 or 10 mg/kg. At 10 days after the last immunization the antiserum was administered to a rat by IV injection. A PCA test was performed by IV administration of nateglinide or its mixture with bovine serum albumin. Ovalbumin was used as a positive control substance.

RESULTS:
Antisera obtained from the nateglinide immunization groups showed negative PCA reaction. On the other hand, antisera obtained from all animals in the ovalbumin immunization group showed obvious patches of pigment, which suggests that no production of IgE antibodies to nateglinide were observed in the experiment.

Conclusions: The results from these investigations in mice and guinea pigs indicated that nateglinide has little or no immunogenic potential.

VI. SPECIAL STUDIES:

1. An ocular irritation study of nateglinide in rabbits(Study# 91008)

Methods: Nateglinide solution at the maximum soluble concentration of 4 mg/ml (pH=7.2) was applied once to the saccus conjunctivae of male New Zealand White rabbits. The dose volume was 0.1 ml. Three rabbits per group were assigned to 2 groups, consisting of a group with washing after application of the test substance to the eyes and a group without washing.

Results and Conclusions: There were no abnormal ocular findings up to 168 hours after 0.1-ml nateglinide solution (0.4 w/v%).

2. Effect of nateglinide on the blood level of glucose and peripheral nerves after dietary administration to old female mice(Study# 395602 and 395603)

Objective: The incidence of peripheral nerve degeneration characterized by axon degeneration and proliferation of schwann cells increased in the female mice of the highest dose group in 2-year carcinogenicity study in mice. The sponsor observed that drug-induced hypoglycemia was not responsible for the nerve

degeneration in a separate study(Study#394602). In the present study the sponsor explored the possibility that the development of the nerve lesions is related to age.

Methods: Two groups of 20 female B6C3F1 mice at 78 weeks of age were given 0.3% nateglinide in diet or unadulterated diet (control) for 42 weeks. The blood level of glucose was measured at 9:00 and 21:00 every two weeks for 10 weeks of treatment. At and after 12 weeks, blood glucose level was monitored at 3-hour intervals every four weeks. Five mice each from the two groups were sacrificed at 13 weeks. A third group of 20 female mice at 91 weeks of age was treated similarly and sacrificed after 29 weeks. The mice sacrificed or dying during the course of the study were subjected to histopathological examination of the sciatic nerve and brachial plexus.

Results and Conclusions: In this study, a long-term evaluation of peripheral nerve degeneration was performed because there were findings of sciatic and brachial nerve degeneration in the 2-year carcinogenicity study in mice. No clear lesions were noted in mice sacrificed at one year of age. In mice started on treatment at 78 and 91 weeks of age, no difference was found between the control and drug groups with respect to mortality. There were age-dependent increases in axon degeneration of peripheral nerve and Schwann cells. The incidence of severity of the lesions tended to be greater in the drug group than in the control group for mice treated from 78 weeks of age. However, the difference in the incidence was not significant. The same conclusions were obtained from the study with initiation of treatment at 91 weeks of age. The blood glucose level slightly decreased at 21:00 hrs, which appeared not to be responsible for the peripheral nervous degeneration. The present study does not add any new insight regarding the interpretation of original findings in 2-year carcinogenicity study in mice.

VII. REPRODUCTIVE TOXICOLOGY:

Study title: Nateglinide-Reproduction toxicity study in rats (Segment 1)
Study No: and Report number: YM90114
Amendment #, Vol #47, and page#: 5/1-5/177
Site and testing facility[redacted]
GRP compliance: Yes
QA- Reports Yes (x) No ():
Lot and batch numbers: Lot A-015 and A-011
Protocol reviewed by Division Yes () No (x):

METHODS:
Species/strain: 103 male and 114 female Crj:CD(SD) rats
Doses employed: 100, 300, and 600 mg/kg
Route of Administration: Oral(Gastric tube)

Study Design: Combined fertility and pre- and postnatal study design. All the treated males and females received the test compound or the control solution orally once per day. The males were continuously treated from day 63 prior to mating until impregnation of either their treated or untreated allocated female or until the end of further mating attempts with two untreated females. The treated females received the test substances or the control solution during a 14-day pre-mating treatment period, during the mating period and throughout pregnancy and the 21-day lactation period. Administration was performed using a gastric tube in a volume of 10 ml/kg in the morning.

Number of animals/sex/dosing group:25/sex/group
Parameters and endpoints evaluated: Mating, sperm examination, examination at birth and during lactation, function tests, FI examination after weaning including behavior, sexual maturation, mating and fertility test, and autopsy of the parent and pups.
Statistical evaluations: Statistical evaluations were based on the assumption of a monotone dose-response relationship. Comparisons of dose groups and the control group at the 5% level were only carried out if significant effects were detectable in the highest dose group.

RESULTS:
Mortality: There were no treatment-article related deaths, although there were some accidental deaths in control as well as treated groups.
Clinical signs: Rats in the control group had no remarkable signs. All treated male rats had either salivation or salivation with gnawing activity. Gnawing and rubbing activities were noted in 3 days after initiation of drug treatment in the intermediate and high dose groups, while the rats in the low dose group manifested the activities in 30 days after the initiation of treatment. The salivation immediately before dosing was drug-dose dependent, which suggests it might not be simple reflex behavior. The males of the parental (Fo) generation did not show any impairment of behavior or general condition attributable to the various dosages of the test compound. There was also no impairment of behavior or general condition in the treated females.

Body weight: The mean absolute body weight of the males and treated females from the various groups was comparable with that of the respective control group during the pre-mating period. This was also true for the males as well as females during nateglinide treatment as shown below.

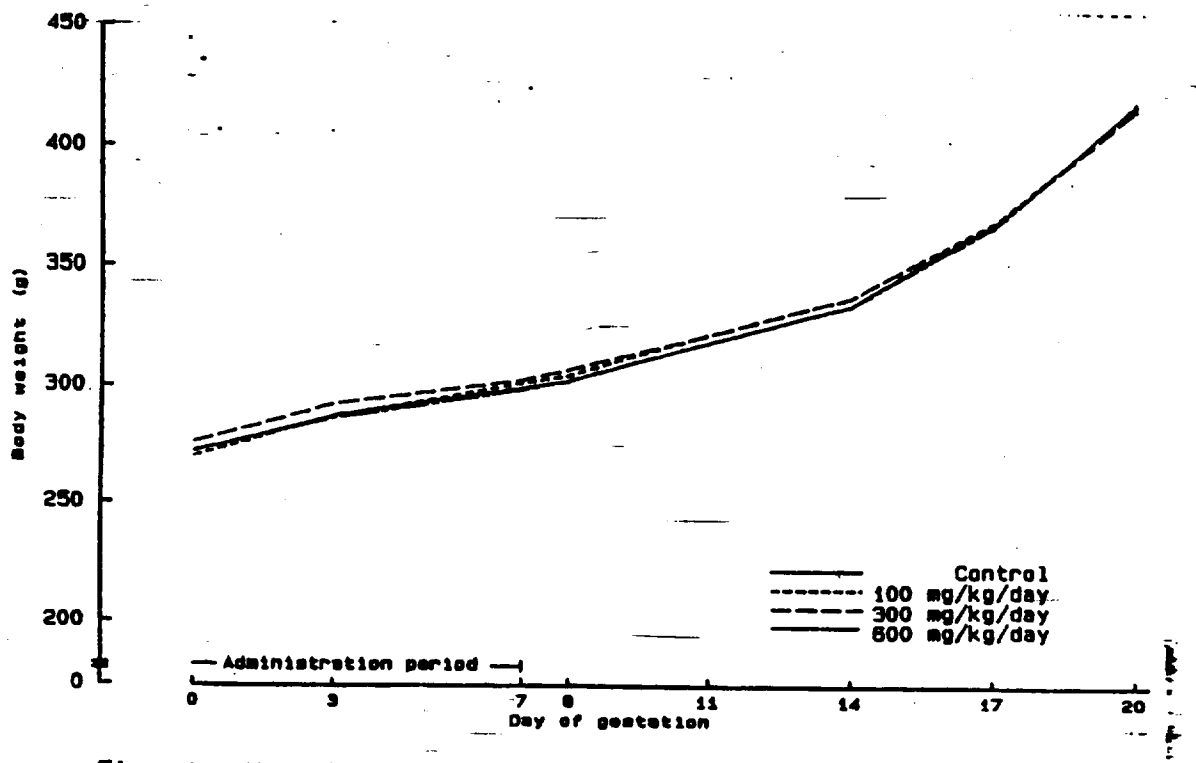


Fig. 4 Mean body weight in female rats administered AY4166 orally

Food consumption: The parameter of the males and treated females from the various groups was not impaired during the pre-mating treatment period. The animals in the test groups consumed amounts of food comparable to those consumed by the control animals. During pregnancy, no significant drug effects on food consumption were observed.

Gross pathology:
There were no abnormalities in any of the rats in the control or 100 mg/kg group. Atlectasis of a part of the left lung in an animal in the 300 mg/kg group and adhesion of a yellowish brown discolored area on the surface of the spleen to the adipose tissues in the abdominal cavity in a rat in the 600 mg/kg group were observed. The findings were considered to be incidental.

(- FERTILITY IN MALES): Most of the males in the various groups inseminated both their treated and untreated female partners. A few rats in each groups including the control were infertile. There were no differences between the control and treated groups in the copulation index, ferfility index or days required for copulation, indicating no adverse effects of the test article on male reproductive performance or fertility. Sperm was present in all the males with comparable numbers of sperm, which was counted on smear.

(- FERTILITY IN FEMALES):
The estrus cycle of treated and control female rats was normal since most rats had estrus in 4 days. There were no significant differences between the control and treated groups in the number of corpora lutea, indicating no remarkable adverse effects of the test article on ovulation.

Effects on Fetuses: No adverse effects of the test article were observed on the implantation index, fetal mortality, number of live fetuses, sex ratio, fetal weight or placental weight. Multiple external anomalies including agnathia, cleft lip and cleft palate were found in a fetus in the 100 mg/kg group. One fetus in the 300 mg/kg group had bloody amniotic fluid, which appeared to be not significant. There were no significant differences between the control and any of the treated groups in the incidence of visceral abnormalities, although ventricular septal defects or dilatations of the renal pelves were observed incidentally in animals of the control as well as treated groups.

Fusion of the vertebral arches and hypoplasia of the vertebral bodies in a fetus and wavy ribs in another were noted in the 100 mg/kg group. There were no other skeletal anomalies. There were no treatment-related skeletal variations. No significant differences between the control and treated groups in the number of ossified bones, indicating no remarkable adverse effects of the test article on bone ossification processes.

Conclusion: Nateglinide had no effects on body weight, mating and fertility of parent animals and the non-toxic dose for general toxicity in parent animals appeared to be less than 100 mg/kg, which is approximately 2 – 3 times human therapeutic exposure based on recommended daily dose of 120 mg tid.

Study title: Nateglinide-Reproductive (Segment II) and developmental toxicity study in rats
Study No: and Report number: YM91002
Amendment #, Vol #48, and page#5/1-5/357
Site and testing facility[ ]
GRP compliance: Yes
QA- Reports Yes (x) No ():
Lot and batch numbers: Lot A-015
Protocol reviewed by Division Yes () No (x):

METHODS: (The sponsor included some peri and postnatal development assessment in this study)
Species/strain: 68 male and 185 female Crj:CD(SD) rats
Doses employed: 100, 300, and 600 mg/kg
Route of Administration: Daily doses of control vehicle (0.5% aqueous solution of methylcellulose 400) or test article were given orally to rats on days 7-17 of gestation.

Study Design: Based on previous rat reproductive dose-finding studies, nateglinide was administered oral gavage at doses of 100, 300, and 1000 mg/kg on days 7 – 17 of gestation period.

Number of animals/sex/dosing group:25/sex/group
Parameters and endpoints evaluated:
Dam(F_o): Half the dams were killed on day 20 for caesarian section and the rest kept for natural delivery. The mortality, body weight, and food intake were measured and the duration of gestation was recorded. The day of completion of delivery was designated as day 0 of lactation. On day 22 of gestation dams were autopsied. The number of implantation sites was counted and the gestation index was calculated as follows: (number of dams with live newborn) / (number of pregnant animals) x 100.

Fetuses (F_1): The uterus of each dam was examined for the number of implantations, live and dead fetuses. The implantation index and fetal mortality were calculated. Live fetuses were used to examine for skeletal anomalies and variations, and for the number of ossified bones in the sacral and caudal vertebrae, sternebrae, and digits.

One-third of the live fetuses with no external anomalies from each litter were fixed in Boulin's fluid and examined for morphological anomalies in the internal organs. The other live fetuses were cleared and stained with alizarin red S to examine skeletal anomalies and variations. Implantation index, fetal mortality and sex ratio were calculated.

Postnatal Developmental Studies:

Offspring(F_1) were observed after birth until sexual maturity. At weaning, 3 offspring/sex/group were used for behavioral observation and the remaining 2 offspring/sex/group were used for reproductive function observation.

The number of live and dead newborn was counted and the delivery and birth indices were calculated. {Delivery index = (newborn#)/(implantation sites#) x 100; Birth index = (live newborn#)/(implantation sites#) x 100}. All the litters of greater than 8 offspring were adjusted to 8 animals (4 males and 4 females) at the age of 4 days and excess offspring were sacrificed. The number of live offspring was counted during the lactation period to calculate viability index on day 4 and weaning index.

Clinical signs, physical development, and early behavior of the offspring were recorded in addition to the physical development and reproductive function before weaning. The parameters were examined similarly after weaning in the selected offspring until the age of 70 days. During the period behavioral observations such as sensory function, emotionality, spontaneous motility, and conditioned avoidance responses were recorded.

Reproductive Function Studies:

Development of genital organs: The male offspring were observed for descent of the testes at the ages of 28 and 35 days and for development of the U-shaped penis at the ages of 49 and 56 days. The female offspring were observed for vaginal opening at the ages of 42 and 49 days. The phase of the estrous cycle in each female was determined. From the ages of 10-11 weeks males and females were paired for mating and housed together overnight. Copulation was determined on the following morning by the presence of a vaginal plug in the vagina or sperm in the vaginal smear. Copulation index was calculated by (animals# which mated/animals# paired) x 100.

Within 7 days after the caesarean section of females (F_1), the males were anesthetized with ether, bled for autopsy. Twenty-one days after the last day of the mating period, the females which failed to mate were anesthetized with ether, and autopsied. The females which mated were observed daily for clinical signs and weighed on days 0, 4, 7, 11, 14, 17 and 20 of gestation. On day 20 of gestation, they were examined in the same manner as F_0 dams. Fertility index was calculated by (Pregnant animals# /Animals# which mated) x 100. Observation of fetuses (F_2) on day 20 of gestation was performed in the same manner as that used in F_1 fetuses.

Statistical evaluations: The Chi-square test was used to analyze the following parameters: skeletal anomalies and variations observed using soft x-ray, genital organ development, sensory function, and the copulation, fertility and gestation indices. The duration of gestation, implantation index, sex ratio, fetal mortality, incidences of fetal external, visceral and skeletal anomalies and fetal skeletal variations, delivery and birth indices, viability index on day 4 of lactation, weaning index, early behavior and physical development were analyzed using the procedures described above for the Kruskal-Wallis's H test. All other analyses were performed by Bartlett's test. Differences from the control group were evaluated at the 5% level of significance and indicated as $P<0.05$.

RESULTS:

Effects of Dam (F_0):

Clinical signs: All rats in treated groups exhibited gnawing and rubbing of the low jaw against the cage immediately after dosing. The onset times of these signs were drug-dose dependent.

Body weight: There were no significant differences between the control and any of the treated groups during the gestation or lactation period, indicating nateglinide had no adverse effects on mean body weights.

Food consumption: The parameter was not basically affected by the treatment during gestation or lactation period. There were sporadic changes in the

parameter; for example, slight decrease in food intake in the 1000 mg/kg group during days 3-6 of administration (days 9-12 of gestation) was documented.
Other effects: No adverse effects were noted on the delivery, duration of gestation or gestation index in any of the treated groups. There were no abnormalities in nursing behavior.

Gross pathology: There were no abnormalities in any of the dams in the 100 and 300 mg/kg groups. In the control and 1000 mg/kg groups, dilatation of the renal pelvis and localized atelectasis were noted. Gross pathology of dams at weaning revealed no abnormalities in any dam in the control or 300 mg/kg group. Other findings such as a bleeding spot in the glandular stomach were considered to be incidental because they were not drug-dose dependent.

Effects on fetuses (F_1): There were no significant differences between the control and any of the treated groups in the corpora lutea, implantation index, fetal mortality, number of live fetuses, sex ratio, fetal weight or placental weight. One fetus in the 300 mg/kg group had multiple external anomalies including a short trunk, club foot and anal atresia. No gross abnormalities in the placenta or amniotic fluid were observed in any group. Visceral anomalies were observed in the control as well as the treated groups, but the incidences were not statistically significant(Table 12). This holds true for the case of skeletal anomalies as shown below(Please see Table 13).

Table 12 Visceral anomalies observed in fetuses (F_1)

		AT0166 (mg/kg/day)		
	Control	100	300	1000
Number of dams	24	23	24	24
Number of fetuses observed	124	115	122	123
Number of fetuses with anomalies: (%) [a]	11 (8.9)	9 [c] (7.8)	6 (4.9)	8 (6.5)
Number of fetuses with the following anomalies: (%) [b]				
Thymic remnant in neck	2 (2.4)	6 (5.2)	4 (3.3)	6 (4.9)
Ventricular septal defect	0 (0.0)	1 (0.9)	0 (0.0)	0 (0.0)
Persistent left umbilical artery	2 (1.6)	1 (0.9)	0 (0.0)	1 (0.8)
Hypoplasia of liver	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)
Dilatation of renal pelvis	5 (4.0)	2 (1.7)	2 (1.6)	1 (0.8)

Percentages were calculated as follows:
[a] (Number of fetuses with anomalies / Number of fetuses observed) X 100
[b] (Number of fetuses with the anomaly / Number of fetuses observed) X 100
[c] One with multiple anomaly (thymic remnant in neck and persistent left umbilical artery)

Table 13 Skeletal anomalies observed in fetuses (F1)

	Control	AT4166 (mg/kg/day) 100	300	1000
Number of dams	24	23	24	24
Number of fetuses observed	253	229	230	245
Number of fetuses with anomalies: (%) [a]	2 (0.8)	1 [c] (0.4)	2 (0.8)	0 (0.0)
Number of fetuses with the following anomalies: (%) [b]				
Absence of vertebral arch	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Fusion of vertebral arches	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Hypoplasia of vertebral body	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Absence of rib	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Fusion of ribs	0 (0.0)	1 (0.4)	0 (0.0)	0 (0.0)
Wavy rib	2 (0.8)	0 (0.0)	2 (0.8)	0 (0.0)

Percentages were calculated as follows:
[a] (Number of fetuses with anomalies / Number of fetuses observed) X 100
[b] (Number of fetuses with the anomaly / Number of fetuses observed) X 100
[c] Multiple anomaly (absence of vertebral arch, fusion of vertebral arches, hypoplasia of vertebral body, absence of rib and fusion of ribs)

Skeletal variations included cervical ribs, lumber ribs and shortening of the 13th rib in some fetuses in all groups, which were not significantly different between the control and any of the treated groups as shown (Table 14). There were also no significant differences between the control and any of the treated groups in the number of ossified bones such as sacral and caudal vertebra, sternebrae and digits, which may indicate no adverse effects of the test article.

Table 14 Skeletal variations observed in fetuses (F1)

	Control	AT4166 (mg/kg/day) 100	300	1000
Number of dams	24	23	24	24
Number of fetuses observed	253	229	230	245
Number of fetuses with variations: (%) [a]	11 (4.4)	28 (12.2)	13 (5.5)	23 (9.4)
Number of fetuses with the following variations: (%) [b]				
Cervical rib	1 (0.4)	5 (2.2)	4 (1.7)	5 (2.0)
Lumber rib	5 (2.0)	13 (5.7)	6 (2.5)	15 (6.1)
Splitting of vertebral body	3 (1.2)	5 (2.2)	1 (0.4)	0 (0.0)
Shortening of 13th rib	1 (0.4)	2 (0.9)	2 (0.8)	3 (1.2)
Splitting of sternebra	1 (0.4)	0 (0.0)	0 (0.0)	1 (0.4)
Asymmetry of sternebra	0 (0.0)	1 (0.4)	1 (0.4)	0 (0.0)
Twenty-five presacral vertebrae	0 (0.0)	4 (1.7)	1 (0.4)	2 (0.8)

Percentages were calculated as follows:
[a] (Number of fetuses with variations / Number of fetuses observed) X 100
[b] (Number of fetuses with the variation / Number of fetuses observed) X 100

Developmental Effects on Offspring (F_1): Litter size, delivery index, number of live newborn, birth index, body weight or sex ratio of newborn in the treated groups were not different from the control. All newborn had no external anomalies and no abnormal signs were observed in any offspring prior to weaning. Viability index, physical development (pinna unfolding, hair growth, incisor eruption or eyelid opening), and early behavior (geotaxis, pivoting, straight walking, back righting, cliff drop aversion, grasp reflex, bar holding, front placing, auditory startle, or mass reaction) in the treated groups were not different from the control. There were no gross abnormalities in any offspring in the control or

300 mg/kg group. One female in the 100 mg/kg group which had a thymic remnant in the neck and a female in 1000 mg/kg/day top dose group that had a dilated renal pelvis.

Effects on fetuses (F_1) after weaning: There were no abnormal clinical signs observed in any offspring after weaning. The body weights of the treated groups were not different from those of the control groups. There were no abnormalities in sensory function of any offspring such as visual placing response, papillary reflex, corneal reflex, or pain response including spontaneous motility and conditioned avoidance response.

Reproductive function on fetuses (F_1): In general, there were no remarkable abnormal clinical signs and there were no significant differences in body weight between the control and treated groups. Decent of the testes, development of a U-shaped penis or vaginal openings were normal in drug-treated groups. There was no abnormality in the estrous cycle of any animal. There were no differences between the control and any of the treated groups in the copulation index, fertility index or number of days required for copulation. There were no significant differences in the number of corpora lutea between the control and any of the treated groups.

Effects on fetuses (F_2): There were no significant differences between the control and any of the treated groups in the implantation index, fetal mortality, number of live fetuses, sex ratio, fetal weight or placental weight, indicating no adverse effects of the test article. Multiple external anomalies such as a short trunk, a vestigial tail and anal atresia were observed in a fetus in the 100 mg/kg group, which appeared to be incidental.

Summary and Evaluation: Fertility and pre- and postnatal studies with nateglinide were performed in CD rats at doses of 100, 300, and 1000 mg/kg. The dam had mild toxic signs in all treated doses. Thus, the non-toxic dose for dams might be at or near 100 mg/kg, which is approximately 2 times human therapeutic exposure based on recommended daily dose of 120 mg tid. Several fetuses (F_1) in each group had visceral and skeletal anomalies, which occurred at a low incidence in a non-dose dependent manner. The variations in skeletal anomalies and ossification of fetuses were not different between the control and the high dose groups, which had 1000 mg/kg, which is approximately 60 times human therapeutic exposure based on recommended daily dose of 120 mg tid. No remarkable drug-related effects on parental fertility, F_1 behavior, sexual maturation, mating, and autopsy findings, or F_2 fetal parameters.

Study title: Nateglinide-Oral (Gavage) Teratology Study in the Rabbit
Study No: Report number/Doc. No: /6845-555/11
Amendment #, Vol #49, and page#5/1-5/115
Site and testing facility:
GRP compliance: Yes

QA- Reports Yes (x) No ():
Lot and batch numbers: Lot# A-015
Protocol reviewed by Division Yes () No (x):

METHODS:
Species/strain: 64 mature New Zealand white rabbit (4-5 months old)
Doses employed: 0(0.5% methylcellulose), 50, 150, and 500 mg/kg/day
Route of Administration: Oral (gavage) once per day
Study Design: Potential embryotoxicity of nateglinide was tested in mated female rabbits. Prior to the start of the study, the females were mated with fertile males in the morning during estrus. To insure ovulation, the females were injected intravenously via the marginal ear vein with 10 IU of chorionic gonadotrophin ———————— Total 4 groups: one control placebo group and three treated groups (low, mid and high doses) were treated with the drug from the 6th to 18th day of pregnancy. Caesarian section and autopsy were performed on 29th day of pregnancy.

Number of animals/sex/dosing group: 16/group
Parameters and endpoints evaluated: Animals' behavior, physical condition, body weight, food consumption and clinical chemistry were monitored. Toxicokinetic evaluation was also performed. The uterus was open at caesarian section, and the live and dead fetuses; conceptuses undergoing resorption, placentas and corpora lutea in the ovaries were counted and examined macroscopically.

Statistical evaluations: Statistical evaluations were based on the assumption of a monotone dose-response relationship. Comparisons of dose groups and the control group at the 5% level were only carried out if significant effects were detectable in the higher dose group. The following statistical methods were used: analysis of variance and t-test for body weight, analysis of variance, regression analysis and Dunnett's test for body weight gain and mean food intake, Kruskal-Wallis, Terpstra-Jonckheere and Wilcoxon rank-sum tests for corpora lutea, implantations and loss, fetus number, and defects, and Cockran-Armitage and Fisher-Irwin exact test for the rest of pathological findings. The findings obtained at autopsy and skeletal examination of the fetuses were evaluated separately for the fetuses and for the litters by the exact Fisher test at significance levels of 5% and 1%. Frequencies of findings obtained at autopsy and the skeletal examination of the fetus were compared with those of corresponding findings in previous control groups.

RESULTS:
Mortality and morbidity: One animal in the high dose group was killed on day 19 of gestation having signs of an imminent abortion. Necropsy revealed all embryo-fetal tissue were non-viable, which might be related to the treatment. Another rabbit in the high dose group was killed on day 17 of gestation due to signs of distress. The signs included inertia from day 12, ataxia from day 15, weight loss

from day 16 and convulsions and red discharge from the vagina on day 17. Necropsy revealed no evidence of pregnancy and no macroscopic causative findings.

Clinical signs: Most animals behaved as expected. Clinical observations were minor and there were no indications of any effect of treatment.

Body weight: Mean body weight gain of animals in the high dose group was lower (20.5%) than control over the dosing period as shown below. The difference from control was significant from days 6 to 8 of gestation ($P<0.05$, Dunnett's test). Weight gains of the low and intermediate dose groups were comparable to controls. There were no remarkable differences between the treated and control groups in gravid uterus weight or body weight adjusted gravid uterus weight.

Group mean body weight (kg)

Test article	Control		AY4186	
Group	1	2	3	4
Level (mg/kg/day)	0	50	150	500

Date of printing : 12/MAR/91 Computer ID : T555011

Day of gestation	Group 1•	Group 2•	Group 3•	Group 4•	Group 4•
0	3.25	3.20	3.22	3.29	3.24
6	3.52	3.51	3.49	3.55	3.46
7	3.51	3.53	3.49	3.52	3.44
8	3.52	3.53	3.50	3.51	3.43
12	3.58	3.60	3.57	3.55	3.46
15	3.66	3.67	3.64	3.62	3.52
18	3.71	3.72	3.68	3.68	3.57
24	3.82	3.84	3.81	3.82	3.70
29	3.86	3.90	3.80	3.97	3.83
% body weight change days 0 - 29	18.8	18.9	20.5	20.7	18.2
% body weight change days 6 - 18	5.4	6.0	5.4	3.7	3.2

• including surviving females with total resorptions
• including females with live foetuses at day 29

Food consumption: Food intake of animals in the high dose group was lower (89.3% of control value) than control over the dosing period ($P<0.05$, Dunnett's test). Food intake in the low and intermediate dose groups was comparable to controls throughout the study.

Terminal and Necrosy evaluations: The number of corpora lutea and implants in the dams of the treated groups were slightly high, compare to those of the control dams. The pre-implantation embryonic death rate was rather high in nateglinide treated dams with the top dose as shown below. There were no appreciable

intergroup differences in sex ratio (% male fetuses). Litter weight was marginally lower than control in the high dose group. Mean fetal weight was higher in this group and the females showed an increasing trend with dose (P<0.05, Terpstra-J ckheere test), which was associated with the smaller litter size.

Summary of female performance

Test article	Control		AY4166	
Group	1	2	3	4
Level (mg/kg/day)	0	50	150	500

Number of animals:	Group 1	Group 2	Group 3	Group 4
In group	16	16	16	16
Total died/killed in study	0	0	0	2
Not pregnant	1	0	0	2
Died/killed	0	0	0	1
Pregnant (%)	15 (93.8)	16 (100.0)	16 (100.0)	14 (87.5)
Died/killed	0	0	0	1
With total resorption	0	0	0	2
With live fetuses on day 29	15	16	16	11

Examination of the fetuses: The live fetuses delivered by caesarian section in the treated group showed normal physical development. Their mean body weights and lengths did not differ appreciably from the corresponding values in the control group. The external and visceral malformations in the control, low and intermediate dose groups were not remarkable. In the high dose group, there were a significantly greater number of fetuses with gall bladder agenesis compared to the controls (P<0.05) as shown below. In addition, reduced gall bladder was associated with the visceral variation in the high dose group. Drug treatment did not significantly affect the incidence of fetuses showing other visceral and/or skeletal variations.

Effects of Nateglinide on Gall Bladder Agenesis in Teratology Study in Rabbits					
Dose(mg/kg/day)	Control	50	150	500	Control Data*
#Fetus examined	115	122	128	76	3115
#GB agenesis	0	1	0	9	4
% of Fetuses	0	0.8	0	11.8@	0.13

*Indicates cumulative control background data. #GB denotes the number of incidences of gall bladder agenesis.

Summary and Evaluation:

Oral administration of nateglinide at doses of 50, 150, and 500 mg/kg during Day 6 to 18 of gestation did not produce severe teratogenic toxicity in New Zealand

White rabbits. No clear maternal toxicity and/or toxic effect on the intra-uterine development of the conceptuses was detectable in the low and intermediate dose groups. The intermediate dose (10 times of clinical dose, based on body surface comparison) had no remarkable effect on skeletal or visceral malformations including gall bladder agenesis.

The high dose, 500 mg/kg/day, slightly reduced body weight as well as food consumption significantly in the dams. The 500 mg/kg/day dose produced mortality in 2/16 rabbits. The high dose of nateglinide tested produced drug exposures in rabbits 32 times clinical exposure, based on body surface comparison. The top dose increased the incidence of gall bladder agenesis significantly above the cumulative control data as shown in table above. The increased incidence of the fetal abnormality occurred at the high dose where maternal toxicity was observed. Therefore, the association with maternal toxicity is unclear. However, the fact that the incidence was significantly above the laboratory's cumulative control background data compels to inclusion in labeling.

*Additional Note: The sponsor repeated the study in [ ] with 300 and 500 mg/kg/day in [ ] (Study# 392120). The repeat study confirmed the previous findings, although the mortality was high (15 out 20 rabbits) with 500 mg/kg/day dose. The high mortality in the study is inconsistent with the above study. However, the nateglinide lot#, animal source, and animal food were different from the previous study.

Labeling Recommendations: Please see the Labeling Section.

Study title: Nateglinide-Peri- and Postnatal Development in Rats (Segment III)
Study No: and Report number/Doc. No.: / YM91106
Amendment #, Vol #50, and page#5/1-5/292
Site and testing facility: [ ]
GRP compliance: Yes
QA- Reports Yes (x) No ():
Lot and batch numbers: A-011
Protocol reviewed by Division Yes () No (x):

METHODS:
Species/strain: 62 male and 134 female Crj:CD(SD) rats
Doses employed: 0 (vehicle), 100, 300, and 1000 mg/kg
Route of Administration: Oral (gastric tube) once a day.
Study Design: Based on Seg II study (YM91002), 1000 mg/kg was chosen as the high dose, and 300 and 100 mg/kg were selected including a control group (0.5% methylcellulose). Test compound was administered orally once daily between 10AM-12AM from day 17 of gestation until day 21 of lactation.
Number of animals/sex/dosing group: 24 female rats/group

Parameters and endpoints evaluated: Clinical signs, body weight of the dams, and their abnormalities in delivery, and examination at birth and during lactation were recorded. Gestation index was calculated using the following formula: {(#dams with live newborn/#pregnant rats) x100}. F_1 examination after delivery including external anomalies, weight and sex were documented. Delivery index, birth index, and sex ratio were calculated. Physical development, behavior, sexual maturation, mating and fertility test, and autopsy of the parent and pups were also evaluated.
Statistical evaluations: Statistical evaluations were based on the assumption of a monotone dose-response relationship. Comparisons of dose groups and the control group at the 5% level were only carried out if significant effects were detectable in the higher dose group. Relevant statistical tests such as Chi-square, Bartlett's, and Dunnet were used as needed.

RESULTS:
Effects on Dams (F_0):
Clinical signs: The rats in the 300 and 1000 mg/kg group exhibited gnawing and rubbing against the cage after dosing. The onset times and incidence appeared to be drug-dose dependent. Salivation was also observed immediately after dosing in the intermediate and high dose groups.

Body weight: This parameter from the treated groups was comparable with that of the control group during gestation. During lactation period, significantly increased body weight was noted on the day of delivery in the top dose group.
Food consumption: The animals in the test groups consumed amounts of food comparable to those consumed by the control animals. During pregnancy, the drug treatment did not effect food consumption.

Delivery, duration of gestation, and gestation index were not affected by drug treatment. There were no abnormalities in nursing in any of the dams. Atrophy of the thymus was found in several animals in all groups including the control group as shown below. Light gray small spots on the surfaces of the lungs and an erosion in the glandular stomach were observed in a rat in the high dose group, which appeared to be incidental.

Gross Pathological Findings in Dams (F_0) on Day 22 of Lactation				
Drug dose	Control	100 mg/kg/day	300 mg/kg/day	1000 mg/kg/day
Pregnant Dam#	24	24	24	24
Grey spots on lung	0	0	0	1
Thymus atrophy	3	2	1	4
Bleeding spot in stomach*	0	0	0	1
Erosions in stomach*	0	0	0	1

Fracture of 8th rib	1	0	0	0
Total # of dams with abnormalities	4	2	1	7
*Denote on glandular stomach.				

Effects on offspring (F_1): There were no abnormal signs in any offspring. Low body weight was noted from 11-22 days postpartum in males and from 14-22 days postpartum in females in the 1000 mg/kg group. There were no differences between the control and treated groups in the litter size, delivery index, number of live offspring, birth index or sex ratio of newborn as shown below. Viability index, weaning index or incidence of pinna unfolding, growth of hair, and incisor eruption including early behavior were the same in the control and treated groups.

There were no differences between the control and treated groups in the timing of descent of the testes (28 to 35 days) or vaginal opening (42 to 49 days). No abnormalities in estrous cycle were observed in any group. There were no drug-treatment related effects on copulation index, fertility index, or number of days required for copulation, or the number of corpora lutea.

Reproductive findings at the delivery of dams (F0) administered AY4166 orally from Day 17 of gestation through Day 21 of lactation

	Control	AY4166 (mg/kg/day) 100	300	1000
Number of pregnant dams observed in delivery	24	24	24	24
Number of dams which delivered	24	24	24	24
Number of dams with a gestation duration of				
21 days	2	1	0	0
22 days	22	23	24	24
Duration of gestation (days, Mean ± S.D.)	21.9 ± 0.3	22.0 ± 0.2	22.0 ± 0.0	22.0 ± 0.0
Number of dams with live newborns	24	24	24	24
Gestation index [a]	100.0	100.0	100.0	100.0
Number of implantation sites	392	400	399	412
(Mean ± S.D.)	(16.3 ± 1.9)	(16.7 ± 1.4)	(16.6 ± 2.0)	(17.2 ± 1.4)
Number of newborns	366	370	378	381
(Mean ± S.D.)	(15.3 ± 2.1)	(15.4 ± 1.6)	(15.8 ± 2.6)	(15.9 ± 1.9)
Delivery index [b]	93.4	92.5	94.7	92.5
Number of live newborns	356	365	371	363
(Mean ± S.D.)	(14.8 ± 2.3)	(15.2 ± 1.6)	(15.5 ± 2.7)	(15.1 ± 2.5)
Birth index [c]	90.8	91.3	93.0	88.1
Body weight in live male newborns (g, Mean ± S.D.)	6.34 ± 0.41	6.31 ± 0.44	6.38 ± 0.41	6.11 ± 0.31
Body weight in live female newborns (g, Mean ± S.D.)	5.96 ± 0.43	5.97 ± 0.47	5.96 ± 0.38	5.80 ± 0.31
Sex ratio of live newborns (Male : Female)	181 : 175	189 : 176	167 : 204	178 : 185
Males among live newborns (%)	50.8	51.8	45.0	49.0
Number of dead newborns	10	5	7	18
Stillb.ths	8	3	5	13
Deaths [e]	2	2	2	5
Cannibalism	0	0	0	0
Number of live newborns with external anomalies: (%) [d]	2 [f] (0.6)	1 [g] (0.3)	0 (0.0)	1 [g] (0.3)

Percentages and indices were calculated as follows:
[a] (Number of dams with live newborns / Number of pregnant dams observed in delivery) × 100
[b] (Number of newborns / Number of implantation sites) × 100
[c] (Number of live newborns / Number of implantation sites) × 100
[d] (Number of live newborns with external anomalies / Number of live newborns) × 100
[e] Newborns which died immediately after birth.
[f] One with vestigial tail, and one with multiple anomaly (cleft palate and transverse facial cleft)

Effects on fetuses (F_1): There were no drug-treatment related effects on implantation index, fetal mortality, number of live fetuses, sex ratio, fetal weight or placental weight. The only external anomaly was omphalocele in one control group pup.

Summary and Evaluation: Pre- and postnatal study was performed in Crj SD rats with doses of 100, 300, and 1000 mg/kg/day nateglinide. The top dose produced drug exposures in rats approximately 54 times of clinical exposure, based on body surface comparison. There were no remarkable drug-related effects on parental fertility, F1 sexual maturation, mating, and autopsy findings which suggests there would be little or no adverse effects of the test article on neonatal development.

VIII. GENETIC TOXICOLOGY:

I. Study Title: Nateglinide-Bacterial reverse mutation test
Study No/Document No/Report No: K01-0816/T-2324
Study Type: Mutagenicity test
Amendment #, Volume #51 and Page #5/1-1/26
Conducting Laboratory:
Date of Study Initiation/completion:4/23/1990; 6/29/1990
GLP Compliance: yes
QA- Reports Yes, No ():
Drug Lot Number: A-008
Study Endpoint: In vitro mutagenicity

METHODOLOGY:
Strains/Species/Cell line: Salmonella typhimurium TA100, TA1535, TA1537 and TA98 and E. coli WP2uvrA
Dose Selection Criteria: Drug-induced cytotoxicity
Basis of dose selection: In this dose finding study, the drug-induced toxicity was noted at 5000 µg/plate. Thus, 100, 500, 1000, 2000, and 5000 µg/ml were evaluated.
Range finding studies: Yes
Test Agent Stability: Stable in water, and stable against heat and light.
Metabolic Activation System: Phenobarbital pretreated rat liver extract

CONTROLS:
Negative Controls:
untreated control
solvent(water) control

Positive Controls:
Without S-9 fraction: Sodium azide (0.5 µg/plate for TA100 and TA1535), 9-aminoacridine (50µg/plate for TA1537), 2-nitrofluorence (0.1 µg/plate for TA98) and N-ethyl-N-nitro-N-nitrosoguanidine 0.01 µg/plate for WpuvrA).
With metabolic activation: 2-aminoanthracene (0.5 to 20 µg/plate) for TA98, TA100, TA1535 and TA1537, and WP2uvrA, respectively.

Exposure Conditions: Pre-incubated with medium and S9mix for 8 hours at 37°C
Incubation and sampling times: overnight
Doses used in definitive study: 4, 20, 100, 500, 2500, and 5000 µg/plate

STUDY DESIGN: Top agar was prepared for the Salmonella strains by mixing 100 ml agar (0.6%) with 10 ml of a 0.5 mM histidine-biotin solution. With E. coli histidine was replaced by tryptophan (0.5 mM, 2.5 ml). 0.1 ml of culture medium, 0.1 ml test compound suspension and 0.5 ml of S9-mix were added to 2 ml of molten top agar at 45°C. After mixing, the liquid was poured into a petri dish with a 25 ml layer of 1.5% agar, Vogel-Bonner E medium with 2% glucose. Colonies were counted after 48 hour-incubation at 37°C.

ANALYSIS: Colonies of his^+ and trp^+ revertants were counted with — counter for statistical evaluation.
No. slides/plates/replicates/animals analyzed: 3 plates per dose
Counting method: Bacterial colonies were counted microscopically.
Cytotoxic endpoints: The information was not provided by the sponsor.
Genetic toxicity endpoints/results: Not mutagenic in the absence or in the presence of the S-9 fraction.
Statistical methods: The information was not provided by the sponsor.

Criteria for Positive Results: 2-fold increase in the mean number of revertants per plate for vehicle control.

RESULTS: No significant increases in the number of revertant colonies was observed with any of the tester strains either in the absence or in the presence of S9-mix.
Study Validity: The studies appear valid since the data were reproducible and all positive control produced significant increases in the number of revertant colonies.
Study Outcome: Negative

SUMMARY:
Nateglinide was not mutagenic in the bacterial strains tested either with or without exogenous metabolic activation at the dose levels up to the limit dose of 5000 µg/plate in duplicate assays.

II. Study Title: **Nateglinide-In vitro mammalian chromosome aberration test in fibroblast derived from Chinese hamster lung (CHL) cell lines**
Study No/Document No/Report No: 2792
Study Type: Chromosome aberration test
Amendment #, Volume #51 and Page #5-151/168
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 1/10/1991-5/20/1991
GLP Compliance: yes
QA- Reports: (yes) or no ():
Drug Lot Number: A-015
Study Endpoint: Identification of chromosomal aberrations such gap, break, fragment, deletion, and/ or exchanges.

METHODOLOGY:
Strains/Species/Cell line: Fibroblast derived from Chinese hamster lung (CHL) cell lines
Dose Selection Criteria: Cell growth inhibition, which was determined by photometric measurements of cell cultures and solubility limit in DMSO.
Basis of dose selection: The maximum solubility of nateglinide in DMSO was 3.5 mg/ml. The highest dose reduced the survival rate to 0 to 6% in the presence and absence of metabolic activation. Approximately 50% inhibition of cell growth was achieved at doses of 0.4 mg/ml and 0.8 mg/ml in the presence and absence of metabolic activation, respectively.
Range finding studies: There was no dose range finding study.
Test Agent Stability: Stable in cool place.
Metabolic Activation System: Frozen S-9 fraction was used (Lot no. RAA-244), which were prepared from male SD rats after Phenobarbital treatment.

CONTROLS:
Vehicle: Cell culture medium was minimal essential medium with Hanks-salts and 25 mM Hepes-buffer.
Negative Controls:
Untreated control
Solvent controls-0.5% DMSO
Positive Controls:
Without S-9 fraction: Mitomycin C(MMC) (Batch 655AIK) 0.05 µg/ml
With metabolic activation: N-nitrosodimethylamine (DMN) (Batch ECM 0811) 0.4 mg/ml

Exposure Conditions: Chinese hamster lung fibroblast cells in 10% bovine serum were exposed to nateglinide for 24 and 49 hours for the direct method and 6 hours for the metabolic activation in the presence of 4% CO_2 at 37°C in plastic flasks.
Incubation and sampling times: 24 and 48 hours after onset of treatment with nateglinide and 2 more hours followed by washing and staining.
Doses used in definitive study: 0, 100, 200, 400, and 800 µg/ml with/out S9-mix

STUDY DESIGN: The highest dose should reduce the survival rate to 20-50%. The solvent control data are within the laboratory's normal control range for mutant frequency and the positive controls should cause a significant increase the frequency of aberrations.
No. slides/plates/replicates/animals analyzed: Only metaphases with 22+/- 1 chromosomes are included in the analysis.
Counting method: Chromosomal aberrations were counted and classified.
Cytotoxic endpoints: Statistically significant decrease in cell survival with highest dose > 50% as shown below.

Results of the cell growth inhibition test
Test substances: AY4166

Dose (mg/ml)	Direct method (%)		Metabolic activation method (%)	
0.109	98 100	(99)	98 95	(96)
0.219	93 95	(94)	86 90	(88)
0.438	41 42	(42)	93 81	(87)
0.875	9 7	(8)*	63 59	(61)
1.75	0 0	(0)	40 22	(31)
3.5	1 0	(1)	2 10	(6)

Note:
Each value was calculated based on solvent controls cell's growth ratio regarded as 100%.
Used solvents: DMSO
*: the died cells

[Estimated of 50% cell growth inhibition dose]
direct method: 0.4mg/ml
metabolic activation method: 1.0mg/ml

Statistical methods: Biometry of the results was performed with a one-sided Fisher-Exact test.

Criteria for Positive Results: Test article would be positive if 1) it induces a reproducible statistically significant increase in the aberration rate (>10%) with one or more of the concentrations tested, and 2) there is a reproducible concentration-related increase in the aberration rate. The test result would be negative if the incidence of aberration cell in each group was < 5%.

RESULTS: Nateglinide was not mutagenic in this chromosome aberration test in vitro with cells of Chinese hamster lung cell line.
Study Validity: It appears that the chromosomal aberration test was performed under acceptable conditions. Positive controls responded appropriately and the high dose resulted in greater than 50% inhibition of cell growth.
Study Outcome: Nateglinide was not clastogenic in this chromosome aberration test in vitro with Chinese hamster lung cell line.
SUMMARY: The sponsor performed standard chromosome aberration test with doses of 100, 200, 400 and 800 µg/ml of nateglinide. The procedures of experiments, criteria for positive results, and analysis methods were acceptable. Nateglinide was not mutagenic in this in vitro Chinese hamster lung cell line.

III. Study Title: Nateglinide-Micronucleus Study in Mouse
Study No/Document No/Report No: 2793
Study Type: Clastogenicity test
Amendment #, Volume #51 and Page #5-233/244
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 2/22/1991-/3/20/1991
GLP Compliance: yes
QA- Reports Yes
Drug Lot Number: Batch No. A-015

METHODOLOGY:
Strains/Species/Cell line: Six male Slc: ddY mice
Dose Selection Criteria: Animal toxicity
Basis of dose selection: 2000 mg/kg was selected as the highest level according to the guidelines of "Standard for Safety Study of Drugs". Four dose levels were established by dilution of the highest concentration with a common ratio of 2 into 1000, 500 and 250 mg/kg.

Range finding studies: No mortality was observed at 2000 mg/kg, full study was performed using 500, 1000, and 2000 mg/kg levels.
Test Agent Stability: Stable under cool and light-resistant condition
CONTROLS:
Vehicle: 0.5% Methylcellulose
Negative Controls: a). untreated control; and b). solvent controls
Positive Controls: 0.1 mg/ml of mitomycin C
Exposure Conditions: Please see "Incubation" below.
Incubation and sampling times: Bone marrow samples were collected at 12, 24 or 48 hours after treatment.

Doses used in definitive study: 0, 500, 1000, and 2000 mg/kg
STUDY DESIGN: The test substance and positive control were given to the animals. Individual bone marrow preparation was fixed on slides and the specimen was observed microscopically. Micronucleated polychromatic erythrocytes were counted in 1000 polychromatic erythrocytes/animal.

ANALYSIS: Incidences of micronuclei in the test compound group were compared with positive and negative control groups.
No. animals analyzed: 6 mice/group
Counting method: Microscopically with 1000 x magnification.
Cytotoxic endpoints: Mouse lethality.
Genetic toxicity endpoints/results: Micronuclei formation.
Statistical methods: Significance in the incidence was analyzed by Katernbaum and Bowman test based on binomial distribution with significance levels of 1 and 5%.

Criteria for Positive Results: The test article was classified as mutagenic if it induced significant increases in micronucleated polychromatic erythrocytes, compared with negative control groups.

RESULTS: The mean incidence of micronuclei in the 500, 1000, and 2000 mg/kg groups and in the negative control groups were 0.2%, 0.2%, 0.1% and 0.3%, respectively. Per cent of polychromatic erythrocytes in total erythrocytes in 500, 1000, and 2000 mg/kg groups were 61.2%, 66.6% and 53.8% as shown below. The high dose produced bone marrow depression, piloerection and dacryohemorrhea. Mean incidence of micronuclei and PCE% in the positive control group was 4.2% and 49.3%, respectively.

Dose	Animal No.	Weight (g)	MNPCE (%)	Mean Minimum	± /	S.D. Minimum	Number of PCE (1000 Erythrocytes)	PCE%	Mean Minimum	± /	S.D. Maximum	Judgment
0.5% MC (10ml/kg)	3	38.2	0.3	0.3	±	0.1	695	69.5	64.9	±	4.4	
	11	38.3	0.3				658	65.8				
	24	40.1	0.2				618	61.8				
	34	40.2	0.4				584	58.4				
	36	41.6	0.1	0.1	/	0.4	689	68.9	58.4	/	69.5	
	38	41.5	0.2				659	65.9				
500mg/kg	14	41.3	0.4	0.2	±	0.2	625	62.5	61.2	±	4.8	
	15	41.5	0.5				606	60.6				
	19	38.9	0.0				653	65.3				
	20	38.4	0.2				588	58.8				
	27	38.9	0.1	0.0	/	0.5	548	54.8	54.8	/	65.3	
	39	37.2	0.2				649	64.9				
1000mg/kg	16	39.3	0.5	0.2	±	0.2	613	61.3	66.6	±	2.9	
	22	42.4	0.2				703	70.3				
	25	38.7	0.0				667	66.7				
	28	41.2	0.1				678	67.8				
	31	41.3	0.2	0.0	/	0.5	669	66.9	61.3	/	70.3	
	40	39.8	0.1				666	66.6				
2000mg/kg	2	42.0	0.0	0.1	±	0.1	566	56.6	53.3	±	4.7	
	5	38.4	0.1				587	58.7				
	6	40.2	0.2				556	55.6				
	7	39.7	0.2				469	46.9				
	33	37.6	*	0.0	/	0.2	*	*	46.9	/	58.7	
	37	37.2	0.2				512	51.2				
MMC (1.0mg/kg)	1	37.9	4.0	4.2	±	0.4	515	51.5	49.3	±	2.9	
	4	41.2	4.5				443	44.3				
	9	37.0	4.0				482	48.2				
	17	40.0	3.7				511	51.1				
	18	41.4	4.8	3.7	/	4.8	519	51.9	44.3	/	51.9	
	42	39.6	3.9				487	48.7				

* : Dead animal
PCE : Polychromatic erythrocytes
MNPCE : Micronucleated polychromatic erythrocytes
PCE% : Polychromatic erythrocytes per 1000 Erythrocytes (%)
MC : Methyl cellulose
MMC : Mitomycin C

Study Validity: This study was conducted in acceptable conditions since the positive control group produced an expected increase in polychromatic erythrocytes while the negative control had no effects.
Study Outcome: The results lead to the conclusion that nateglinide is not mutagenic in the in vivo mouse micronucleus test.

IV. Study Title: Nateglinide-Mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells
Study No/Document No/Report No: ML-1
Study Type: Gene mutation test
Amendment #, Volume #51 and Page #5-72/146
Conducting Laboratory: [redacted]
Date of Study Initiation/completion: 4/28/1995-5/5/1995
GLP Compliance: yes
QA- Reports yes (x), no ():
Drug Lot Number: Batch No. 2G453
Study Endpoint: Identification of absolute increase in mutant colonies relative to the solvent control

METHODOLOGY:
Strains/Species/Cell line: Mouse lymphoma L5178Y cells, $tk^{+/-}$ clone 3.7.2C were grown in RPMI-10 medium at 37 °C under 5% CO_2 in air.
Dose Selection Criteria: Cytotoxicity

Basis of dose selection: In the presence and in the absence of an S9-mix a cytotoxicity experiment was performed with 6 doses. Based on the results five concentrations producing between 10 to 20 up to 100% relative survival were chosen for the mutagenicity experiment.
Range finding studies: Yes
Test Agent Stability: The sponsor provided statements that indicated the product stability for at least 24 hour, which appeared to be acceptable.

CONTROLS:
a. Vehicle: Deionized water
b. Negative Controls: Untreated control and Vehicle
c. Positive Controls:
1) Without S9-mix
Methyl methanesulfonate (MMS), 10 µg/ml, was incubated with mouse lymphoma cells for 3 hours.
2) With S9-mix
Benzo(a)pyrene was dissolved in DMSO at 150 µg/ml and incubated with mouse lymphoma cells for 3 hours to have final concentration of 1.5 µg/ml.
Exposure Conditions: Under 4% CO_2 at 37°C
Incubation and sampling times: 4 hours and viability plates were scored after 7 days.
Doses used in definitive study: 10, 25, 50, and 100 µg/ml

STUDY DESIGN: Two independent assays for mutation to 6-thioguanine resistance were performed in the absence of metabolic activation and three assays were performed in the presence of S9-mix. In the absence/presence of S9-mix, dose levels of 10, 25, 50, and 100 µg/ml were employed in all mutation assays.
ANALYSIS: Number of mutant colonies was counted in the absence/presence of S9-mix and mutation frequency (mutant colonies per 1 million cells) was calculated for statistical analysis.
No. slides/plates/replicates/animals analyzed: 6 wells per dose were examined.
Counting method: Mutant colonies of more than 50 cell were counted microscopically.
Cytotoxic endpoints: Nateglinide was cytotoxic at doses ranging from 20 to 600 µg/ml. Cytotoxicity was found at concentrations >38 µg/ml and >75 µg/ml in the absence and in the presence of an S9-mix, respectively.
Genetic toxicity endpoints/results: Please see the section of "Results" below.
Statistical methods: The biometry of the results was performed using ——— for fluctuation assay. Survival, viability, and mutant frequency were calculated by equating the fraction of wells without growth to the term of the Poisson distribution.
Criteria for Positive Results: The test article was classified as mutagenic if 1)one or more of the mutant frequencies in the treated groups with acceptable toxicity levels is statistically significantly ($P<0.05$) larger than the corresponding solvent control value, 2) there is a significant ($P<0.05$) dose relationship indicated by the

linear trend analysis, and 3) the drug effects are reproducible under valid assay condition.

RESULTS: The sponsor performed 3.experiments. In the first experiment, five doses were tested ranging from 75 to 250 µg/ml without S9 and from 65 to 225 µg/ml with S9. The two highest dose groups in each of these experiments had mean relative total growth (RTG) values <0.09, which is below the acceptable range of cytotoxicity values as shown below.

Experiment 1

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	102.30		0	100.00	1.00	143.99	
75.3	82.18	0.99	129.18	NS	65.1	122.39	1.03	139.58	NS
101.6	73.34	0.71	133.92	NS	130.2	53.79	0.70	153.92	NS
137.2	85.75	0.811	129.711	NS	156.3	55.45	0.751	159.801	NS
185.2	19.44	0.05	304.37	*	187.5	28.57	0.09	681.85	*
250	10.01	0.02	280.70	*	225	11.82	0.06	510.25	*
Linear trend				**	Linear trend				*
MMS 10	73.54	0.45	863.26		B(a)P 1.5	53.46	0.35	1444.04	

In the second experiment, RTG values and the % relative adaptive survival (RS) corresponded well in the presence of S9. The highest doses giving valid mutagenicity data were 140 and 172 µg/ml, which yielded 82% (RTG 0.55) and 19% (RTG 0.19) relative adapted survival in the absence and in the presence of an S9. Due to the steep drop in %RS and RTG in the two experiments without S9 the third experiment was performed using a dilution factor 1.1 to obtain the different test compound concentrations in the range between 130 and 190 µg/ml, which resulted in desired range of cytotoxicity values. No relevant reproducible enhancement of the mutant colonies or mutant frequency over the range of the solvent control was found with any of the concentrations used in the presence of S9-mix in the second and final experiment. It can be concluded that nateglinide did not show evidence of a mutagenic potential under acceptable experimental conditions.

Experiment 2

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	175.32		0	100.00	1.00	241.77	
96.5	98.59	0.84	148.53	NS	124.1	50.93	0.53	211.73	NS
115.7	89.63	0.85	173.61	NS	148.9	21.46	0.24	320.18	NS
138.9	82.10	0.55	199.70	NS	156.3	21.19	0.33	238.58	NS
166.7	42.90	0.04	1862.91	*	164.1	21.08	0.25	263.45	NS
200	26.63	0.02	2553.99	*	172.3	18.64	0.19	232.65	NS
Linear trend				NS	Linear trend				NS
MMS					B(a)P				
10	85.87	0.69	982.79		1.5	53.97	0.55	1062.13	

§ 5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS Relative adaptive survival adjusted by post treatment cell count factor
! Based on one replicate only
NS Not significant
*,**,***Significant at 5%, 1% and 0.1% level respectively

MF: Mutation Frequency
RTG: Relative Total Growth
MMS: Methyl Methane Sulfonate
B(a)P: Benzo(a)pyrene

Experiment 3

Treatment (µg/mL)	-S-9 %RS	RTG	MF§		Treatment (µg/mL)	+S-9 %RS	RTG	MF§	
0	100.00	1.00	195.75						
129.8	72.66	0.82	186.32	NS					
142.7 SS	56.31	(0.46)	(351.27)						
157	45.91	0.64	196.50	NS					
172.7	52.28	0.58	202.79	NS					
190	29.40	0.38	245.77	NS					
Linear trend				NS	Linear trend				
MMS 10	60.13	0.67	1140.40						

§ 5-TFT resistant mutants/10^6 viable cells 2 days after treatment
%RS Percent relative survival adjusted by post treatment cell count factor
$ Not plated for viability / 5-TFT resistance
SS Treatment excluded due to excessive heterogeneity
NS Not significant

MMS: Methyl Methane Sulfonate
RTG: Relative Total Growth
MF: Mutation Frequency

Study Validity: The mutant frequency of the solvent control fell within the normal range of 60 to 300 x 10^{-6}. The positive control induced the number of colonies by 3 to 10 times of relevant controls. Thus, the study was performed under valid experimental conditions.

Study Outcome: The results lead to the conclusion that nateglinide was not mutagenic in the mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells.

Overall Genotoxicity Summary: Nateglinide was negative in the standard battery of genotoxicity tests such as bacterial reverse mutation, mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells, chromosome aberration study using Chinese hamster lung cell lines, and micronucleus study in mouse.

NDA Summary:
Nateglinide stimulates insulin secretion as a result of its interaction with the sulfonylurea receptor. This interaction results in closure of the K^+_{ATP} channel with subsequent membrane depolarization and elevation of intracellular Ca^{++} levels.

The elevated intracellular-free Ca^{++} concentration stimulates insulin secretion. The effect is characterized by a rapid onset of activity and rapid reversal upon removal of the drug, in contrast to other insulinotropic agents.

Nateglinide was well absorbed and rapidly excreted from testing animals. Clearance was slowest in the dog, a species in which hypoglycemic effects were seen in repeat dose studies. Investigations in rodent models of diabetes indicated that effects were more marked in animals with the highest remnant β-cell activity. Nateglinide is metabolically cleared, and at least two cytochrome 450 enzymes are involved in the metabolism. One active metabolite is formed (M7), which is equipotent with the parent compound; however, exposure to this metabolite is low, so it does not contribute significantly to the overall pharmacological activity.

Nateglinide treatment was without effects on the cardiovascular system, central nervous system, pulmonary, and renal parameters at doses which were 5 to 50 times higher than the clinical dose, based on body surface area comparison. The acute toxic potential was low. In repeat dose studies target organs for toxicity were the GI tract and the hepatobiliary system. Safety factors based on comparative exposure levels were adequate, and only sporadic incidences of GI intolerance or hepatobiliary effects have been reported in human studies.

The 2-year carcinogenicity study in mice was performed under acceptable conditions. The 2-year carcinogenicity study in mice clearly demonstrated nateglinide was not carcinogenic at doses up to 10 and 32 times maximum human therapeutic exposure in males and females, respectively, based on AUC ratio.

The sponsor performed two 2-year carcinogenicity studies in rats. In the first study, nateglinide was administered at doses of 625, 1250 and 2500 ppm. There was an increase in the incidence of pancreatic islet cell adenomas in female rats receiving 2500 ppm, which produced drug exposure two times maximum human therapeutic exposure, based on AUC ratio. The increase was not statistically significant on pairwise comparison with the control, although the statistical test indicated there was a positive trend. It was concluded that the pancreatic tumor findings were not clearly drug associated for the following reasons: 1) The percentage incidence of pancreatic islet cell tumor in females of the high dose group (12.5%) was just outside the historical range (0 – 12%), 2) Immunohistochemically the tumors stained positive for insulin, indicating a β-cell origin, which is a common tumor types in rats; 3) The morphological features of the islet cell adenomas were similar in the controls and the treated groups; and 4)The findings were not observed in males.

The second 2-year carcinogenicity study in rat was conducted with doses of 100, 300, 600 and 900 mg/kg/day under acceptable condition. The average AUC ratio was > 25 times human exposure in both male and female rats administered the

two high doses. Nateglinide did not increase mortality significantly. The mid- and high doses of nateglinide reduced the absolute body weight significantly with the top dose reducing the parameter by 16% and 21% in male and female rats, respectively at the end of 2 years. The body weight reductions appeared to be secondary to significant reductions in food consumption in these groups. No significant tumor findings were observed, suggesting that nateglinide was not carcinogenic.

Fertility and pre- and postnatal studies with nateglinide were performed in CD rats. The nateglinide top dose was 600 mg/kg, which was approximately 32 times clinical exposure, based on body surface area comparison. There were no remarkable drug-related effects on parental fertility, F_1 sexual maturation, mating, and autopsy findings, and F_2 development. In offspring(F1), viability index, weaning index or incidence of pinna unfolding, growth of hair, and incisor eruption including early behavior were the same in the control and treated groups. No remarkable drug-related effects on parental fertility, F_1 behavior, sexual maturation, mating, and autopsy findings, or F_2 fetal parameters.

Oral administration of nateglinide at doses of 50, 150, and 500 mg/kg during Day 6 to 18 of gestation did not produce severe teratogenic toxicity in New Zealand White rabbits. No clear maternal toxicity and/or toxic effect on the intra-uterine development of the conceptuses was detectable after the low and intermediate dose groups of nateglinide administration. The intermediate dose, which was 10 times of clinical dose based on body surface area comparison, had no remarkable effect on skeletal or visceral malformations including gall bladder agenesis.

The high dose, 500 mg/kg/day, reduced body weight as well as food consumption significantly in the dams. The high dose of nateglinide tested was 32 times of clinical dose, based on body surface area comparison. The top dose did not increase malformations except gall bladder agenesis, which was significantly above the cumulative control data. Since the increased incidence of the fetal abnormality occurred at a level where maternal toxicities was observed, the possibility of their association with maternal toxicity might be real. However, the fact that the incidence was significantly above the laboratory's cumulative control background data compels to inclusion in labeling.

Nateglinide was tested for its potential genotoxicity according to the standard ICH battery tests. The sponsor performed bacterial reverse mutation tests, mutation assay at the thymidine kinase locus of mouse lymphoma L5178Y cells, chromosome aberration study using Chinese hamster lung cell lines, and micronucleus study in mouse under acceptable conditions. The results lead to the conclusion that nateglinide was not mutagenic.

1 pages redacted from this section of the approval package consisted of draft labeling

IX. OVERALL SUMMARY AND EVALUATION:

Introduction:

Nateglinide (Starlix™) is a secretagogue of insulin like repaglinide. The basic mechanism is to block ATP-dependent potassium channel. Nateglinide binds to sulfonylurea receptors with a high affinity, which initiates a series of electrophysiological changes to release insulin from the pancreatic β-cells. Nateglinide's insulin release activity was dose- and glucose-concentration dependent as demonstrated in isolated rat islets. Oral absorption of nateglinide was rapid (30 min) in most species and it was distributed throughout the body. There are several major metabolites such as monohydroxylated compound (30%), diastereoisomers (8%), and other minor metabolites such as glucuronic acid conjugates. The drug is eliminated via bile, urine, and feces.

Safety Evaluation:
Data suggest that nateglinide appeared to have little systemic toxicity as an extension of its pharmacodynamic action. Neurological, renal, or cardiovascular effects were not observed in rats at doses up to 300 mg/kg (approximately 10 times the human therapeutic exposure with a recommended dose of 120 mg, tid). Similarly, no effects were observed in dogs with doses up to 100 mg/kg (approximately 55 times the human therapeutic exposure with a recommended dose of 120 mg, tid). The acute toxic effects of nateglinide were vomiting, stomach ulcers, and diarrhea at high oral doses. In repeat dose studies, target organs for toxicity appeared to be the gastrointestinal tract and the hepatobiliary system because duodenal ulcers and slight to moderate increases in ALT and total bilirubin were observed in dogs, treated with nateglinide at dose of 300 mg/kg (approximately 130 times the human therapeutic exposure with a recommended dose of 120 mg, tid).

Nateglinide demonstrated neither mutagenic nor carcinogenic potential. In the mouse carcinogenicity study, the only drug-related finding was an increased incidence of peripheral neuropathy in female B6C3F1 mice. The sponsor performed two sets of 2-year carcinogenicity studies in rats in addition to the standard mouse study. The first rat study was not valid because the top dose studied was not the MTD and the ratio of drug systemic exposure in animal to human (AUC ratio) was less than 2. A slight, non-significant increase in the incidence of pancreatic islet cell tumors was observed in high dose female rats. It was concluded that the pancreatic tumor findings were not clearly drug-treatment related. The second 2-year carcinogenicity study in the same strain of rats was conducted under acceptable manner (AUC ratio was > 25 times human exposure in both male and female rats) with no evidence of tumorigenic effects.

The sponsor performed fertility, teratology, and prenatal and postnatal reproductive studies in rats and in New Zealand White rabbits. In Crj:CD (SD) rats, nateglinide had no clear effect on fertility, general reproductive performance

or development of the offspring of animals treated with up to 600 mg/kg (approximately 16 times the human therapeutic exposure with a recommended dose of 120 mg, tid). In rabbits, nateglinide was not teratogenic at dose up to 500 mg/kg (approximately 30 times the human therapeutic exposure with a recommended dose of 120 mg, tid). However, an increased incidence of gallbladder agenesis occurred at doses associated with embryotoxicity and maternal toxicity and mortality. This finding was reflected in "Pregnancy Category" as indicated previously in Label Section. In a supplementary study, the livers and bile ducts appeared normal upon histopathological examination of the fetuses without gallbladders. Nateglinide was not genotoxic in Ames test, mouse micronucleus assay, gene mutation test at the thymidine kinase locus of mouse lymphoma L5178Y cells, and chromosomal aberration test in fibroblast derived from Chinese hamster lung cells.

Clinical Relevance of Safety Issues:

In 2-year mouse carcinogenicity study, an increased incidence of peripheral neuropathy was found in female B6C3F1 mice of the intermediate and high dose groups (0.1 and 0.3% drug in diet). The doses were equivalent to 250 and 880 mg/kg (approximately 7 and 24 times of maximum clinical exposures, based on body surface area comparison), respectively. The left sciatic nerve was mainly involved with little effect in other nerve such as brachial nerves. The sponsor suggests the effect was secondary to sustained hypoglycemia at these high doses. The relevance of the animal finding to human is not known.

Conclusions:

According to the sponsor's data presented in the NDA, nateglinide had minimal toxicities in systemic pharmacology and toxicology studies in laboratory animals. Reproductive, genetic, and carcinogenicity studies were performed acceptably, and demonstrated that the potential toxic risk is small. The 2-year carcinogenicity studies in mice and rats were negative. Nateglinide had no effects on fertility, fetal development, or pre/post-natal development in rats. However, there was an increased incidence of gallbladder agenesis at doses associated with embryotoxicity, and maternal toxicity and mortality in rabbits. Nateglinide was not genotoxic.

COMMUNICATION REVIEW:
Labeling Review (NDA): Please see Label Recommendation on page 97.
Investigator's Brochure/Informed consent review (IND): NA

RECOMMENDATIONS:
Internal comments:

[redacted]

External Recommendations (to sponsor): None
Future development or NDA issues: None
Reviewer signature/team leader signature [concurrence/Non-concurrence]

/S/ /S/ 10/23/00

Herman M. Rhee, Ph.D.
Pharmacology Reviewer

Jeri El Hage, Ph.D.
Pharmacology Team Leader

Cc: Original NDA
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Draft date (# of draft): 7/24/2000(0)
Memorandum of Non-concurrence (if appropriate, attached): None
Addendum to review (if necessary): None
Appendix/attachments:
1) Original IND
2) Executive CAC report
3) Statistical Review and Evaluation

Redacted 22

pages of trade

secret and/or

confidential

commercial

information

MEMORANDUM
Dec. 15, 2000

TO: John K. Jenkins, M.D.
Leah Ripper
FROM: Kenneth L. Hastings, Dr.P.H.

SUBJECT: NDA 21-204 (Starlix®; nateglinide)

I have reviewed the Pharmacology/Toxicology information and concur with the approval of this NDA. The labeling is acceptable with one comment: it is inconsistent to designate nateglinide as "Pregnancy Category C" and include in the label a statement that "Starlex should not be used during pregnancy". Unless there is data demonstrating a risk to the fetus, the label should state that [redacted]

/S/
Kenneth L. Hastings, Dr.P.H.
Acting Associate Director for Pharmacology/Toxicology