CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER
21-204

Pharmacology Review(s)

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA

KEY WORDS: Nateglinide (Starlix), Diabetic complications, Insulin sensitivity and resistance, Insulin secretagogue, ATP-sensitive K^+channel blocker, Type II diabetes, and antidiabetic drugs

Reviewer Name: Herman Rhee, Ph.D., Pharmacology Reviewer
Division Name: Division of Metabolic and Endocrine Drug Products (DMEDP) HFD#510
Review Completion Date: Oct. 7, 2000
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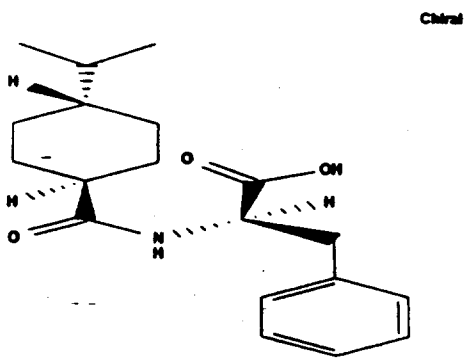
IND/NDA NUMBER: NDA21-204
Serial number/date/type of submission: 000/December 17, 1999/Regular
Information to sponsor: Yes () No (x)
Sponsor (or agent): Norvartis Pharmaceuticals Corporation, East Hanover, NJ (973) 781-7500
Manufacturer for drug substance:

DRUG: Nateglinide (Starlix)
Code Name: DJN-608, SDZ DJN 608, A-4166, AY4166
Generic Name: Nateglinide
Trade Name: Starlix™
Chemical Name: (-)-N-[(trans-4-isopropylcyclohexyl)carbonyl]-D-phenylalanine
CAS Registry Number: 105816-04-4

Molecular Formula/ Molecular Weight: $C_{19}H_{27}NO_3$ /317.43

Structure:



Relevant INDs/NDAs/DMFs: IND: IND
and NDA20-741 (Prandin/Repaglinide)
Drug Class: ATP-dependent K+ channel blocker
Indication: Antidiabetic agent

Clinical formulation: 60, 120, and 180 mg film-coated tablet
Route of administration: Oral
Proposed clinical protocol or Use: Nateglinide is intended for the oral treatment of type 2 diabetes as monotherapy and in combination with agents with a complimentary mechanism of action. The 12- and 24-week randomized human studies (Study# 202 and 302) were conducted in approximately 1000 diet controlled type II patients. The maximum recommended human dose is 180 mg (110 mg/m^2 based on 60 kg man). Nateglinide restores the physiologic insulin response to a meal that is lost in Type 2 diabetes. By restoring early insulin release while minimizing overall insulin exposure, nateglinide improves glucose tolerance with minimal risk of hypoglycemia, weight gain and deterioration of the lipid profile.

Previous clinical experience: Phase III studies in >1000 Type II diabetics
Disclaimer – use of sponsor's material: Relevant portions of the sponsor's IND submission may be used directly without modification as noted.

INTRODUCTION AND DRUG HISTORY:

Studies reviewed within this submission:

Study Title: Nateglinide- General pharmacological studies.....P03
Study Title: Nateglinide - Pharmacokinetic studies.....P09
Study Title: Nateglinide - Toxicological studies.....P16
Study Title: Nateglinide - Single-dose I.V. toxicity study in mice (96A005).....P17
Study Title: Nateglinide - Single-dose oral toxicity study in rats (9525).....P17
Study Title: Nateglinide - Single-dose oral toxicity study in dogs (B-1844).....P18
Study Title: Nateglinide- 13-week oral toxicity study in mice (YOC304).....P21
Study Title: Nateglinide- A 26-week oral (diet) toxicity study in mice (987069).....P23
Study Title: Nateglinide- 13-week oral toxicity study in rats (93.0317).....P26
Study Title: Nateglinide- A13-week oral toxicity study in Beagle dogs (B-1880).....P29
Study Title: Nateglinide –Toxicokinetic Study in Dogs by Repeated Oral administration for 13 Weeks.....P31
Study Title: Nateglinide- 12-Month oral toxicity study in rats (B-1978).....P34
Study Title: Nateglinide- A-12 month oral toxicity study in Beagle dogs (B-1976).....P39
Study Title: Nateglinide- A 2-year carcinogenicity study in mice ((94/0143).....P42
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Study Title: Nateglinide- A 2-year carcinogenicity study in rats-II (96001).....P54
Study Title: Immunotoxicology Study: An active systemic anaphylaxis studies in guinea pigs(91002).....P66
Special Toxicity Study: An ocular irritation study of nateglinide in rabbits (91008).....P67
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Study Title: Nateglinide- Reproductive segment I studies in rats (YM90114).....P68
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Study title: Nateglinide-Peri- and Postnatal Development (Segment III) in rats(YM91106).....P80
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Studies not reviewed within this submission:

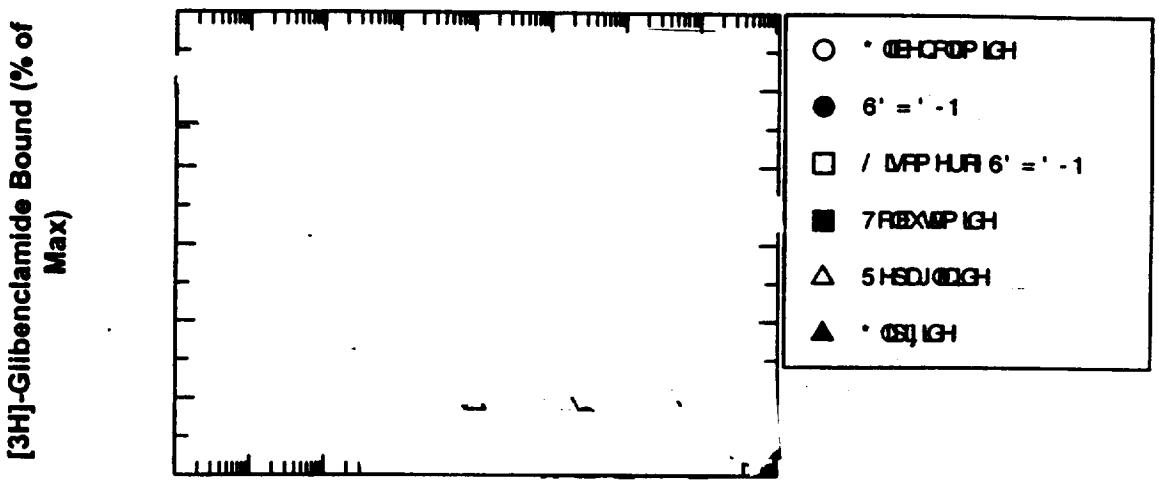
I. PHARMACOLOGY:

Mechanism of Action: The fundamental mechanism of nateglinide action has not been clearly established, although the following is the putative molecular model of the drug action. The primary pharmacology of nateglinide that results in its antidiabetic actions is the direct stimulation of insulin release from pancreatic ß-cells. The stimulation of insulin release by nateglinide is a consequence of the interaction of nateglinide with a membrane receptor/effector system known as the "sulfonylurea receptor" that comprises a ligand binding protein subunit and a pore-forming protein subunit, an ATP-sensitive K^+channel. Interaction of nateglinide with this membrane protein results in closure of the K^+_{ATP}-channel, membrane depolarization and entry of extracellular calcium *via* the voltage-gated (L-type) Ca^{++} channel. The elevated intracellular free Ca^{++} concentration acts as a stimulus for exocytosis of insulin-containing secretory granules. Many studies demonstrate that different ligands of this receptor/effector system can elicit differential effects on secretion in terms of kinetics, glucose-dependency and the influence of inhibitors of cellular nutrient metabolism and that these differential effects on secretion reflect differential effects on the receptor/effector system.

Drug Activity Related to Proposed Indication:

Nateglinide binds to sulfonylurea receptor with a high affinity, which initiates a series of electrophysiological changes such as ATP-dependent K^+ channel and voltage dependent Ca^{++} channel activities. The nateglinide-induced electrophysiological changes are the basis of its beneficial effects on glucose metabolism as outlined below.
Nateglinide displaces the sulfonylurea such as radiolabeled-glibenclamide from membranes prepared from rodent insulinoma cell line and other cell lines such as HIT-T15, and a hamster insulinoma cell line. Figure below (provided by the sponsor) shows displacement of ^{3}H-glibenclamide from the rat insulinoma cell line by several ligands. An analysis of the dose-binding curves indicate that the order of relative potency in displacement was glibenclamide> repaglinide> glipizide> nateglinide> L-isomer of nateglinide> tolbutamide.

Displacement of ^{3H}-Glibenclamide by Ligands in Rat Insulinoma Cell Lines



&RP SRXQG X0

IC_{50} values derived from the competitive binding studies for several ligands were also summarized in a table below. The estimated IC_{50} values vary with the cell system used, but generally the relative order of binding potencies of the various ligands is not altered. The binding affinity of nateglinide is relatively low. In this study Scatchard analysis of glibenclamide binding suggested two states/forms of binding sites: a high-affinity site with a K_d of 0.27 nM and a low-affinity site with a K_d of 25 nM. Each ligand displaced labeled glibenclamide in a concentration-dependent manner and when tested at high concentrations, each agent completely displaced labeled glibenclamide, suggesting all agents bound to the same site. The Hill coefficient for repaglinide was significantly different from those of the other ligands suggesting that there is heterogeneity in the high

affinity receptor populations and that repaglinide (but not the other ligands) binds with the two forms/states with different affinities.

Binding Parameters from Competitive Displacement Studies in Rat Insulinoma Cells Lines

Compound	IC_{50} (nM) ± SEM	Hill Coefficient (slope) ± SEM
Glibenclamide	1.1 ± 0.2	0.88 ± 0.09 (n=3)
Repaglinide	5.0 ± 0.6	0.60 ± 0.01 (n=3)
Glipizide	17 ± 0.9	0.85 ± 0.01 (n=3)
Nateglinide	490 ± 50	0.98 ± 0.06 (n=3)
L-nateglinide	12,000 ± 900	0.97 ± 0.05 (n=3)
Tolbutamide	76,000 ± 9600	0.95 ± 0.09 (n=3)

To assess the relevance of the binding studies that employed rodent cells to human insulin receptors, binding assays were also performed with membranes prepared from a human embryonic kidney cell line (HEK-239) transfected with recombinant human sulfonylurea receptors (h-SUR). As summarized in Table below, the order of potency of the ligands tested was consistent with that found using membranes prepared from rodent ß-cell lines. However, the affinity of each ligand for the human SUR was lower than that for the SUR expressed in rodent cells. In addition, although the dissociation kinetics of [^{3}H]-glibenclamide from the HEK-239/h-SUR membranes were biphasic, consistent with a two-state model, in steady-state binding assays none of the ligands had a Hill coefficient significantly less than one whereas the Hill coefficient of repaglinide in such studies using RIN cell membranes was significantly less than one. It is possible that another protein component not expressed in the HEK cells is required to observe the reduced Hill coefficient. Nonetheless, preservation of the relative affinities of agents for the SUR across species suggest that studies utilizing rodent tissues or performed *in vivo* in rodents are relevant to and will be predictive of activity in humans. The similarity of results from the human recombinant receptor (HEK cells) and the rodent receptor (RIN, HIT cells) indicate that experiments in rodents might be predictive of effects in humans.

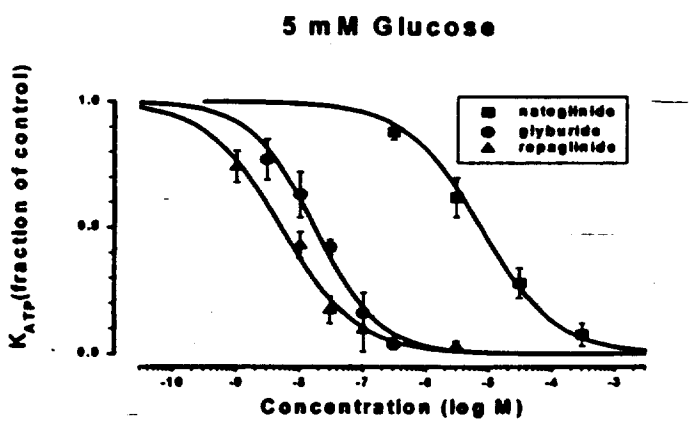
Binding Parameters obtained from Competitive Binding Studies Using Human Embryonic Kidney (HEK-239/h-SUR1) Cell Membranes

Compound	IC_{50} (nM) ± SEM	Hill coefficient (slope) ± SEM
Glibenclamide	4.8 ± 0.8	1.0 ± 0.05
Glimepiride	9.6 ± 1.9	1.1 ± 0.13
Glipizide	220 ± 50	0.82 ± 0.12
Repaglinide	500 ± 80	0.90 ± 0.07
Nateglinide	2800 ± 240	0.92 ± 0.03
L-nateglinide	40,000 ± 6000	0.80 ± 0.05
Tolbutamide	570,000 ± 36,000	1.05 ± 0.09

A physical demonstration of nateglinide-induced inhibition of K^+ channel activities was recorded electrophysiologically as the membrane potential of single pancreatic ß-cells isolated from normal rat pancreas (See Figure below). Nateglinide (10 µM) rapidly (<30 seconds) depolarized the membrane (resting potential = -68 mV) in the presence of sub-stimulatory glucose concentrations (2.8 mM), and the effects were rapidly reversed upon removal of nateglinide. Since the K^+_{ATP}-channel is the major determinant of resting membrane potential these finding suggest that nateglinide exerts a direct effect, as predicted from the binding studies. Recording of single channel current from cell-attached membrane patches confirmed a rapid and reversible effect of nateglinide to close the K^+_{ATP}-channel, with an IC_{50} of 0.23 µM. Under these conditions the IC_{50} of tolbutamide was 12.8 µM.

When the compounds were applied to the intracellular side of inside-out patches, the IC_{50}s of nateglinide and tolbutamide were 4.5 nM and 0.7 µM, indicating an increased potency of 51- and 18-fold, respectively. Since both agents were effective when applied to the external side of cell-attached membranes but their potency increased when applied to the internal surface this suggests that nateglinide (and tolbutamide) can diffuse into the lipid membrane. Additional studies by Akiyoshi, *et al. (Am.J.Physiol. 268:E185-193, 1995)* suggested that nateglinide was without affect on Ca^{++} channels (both L-type and T-type).

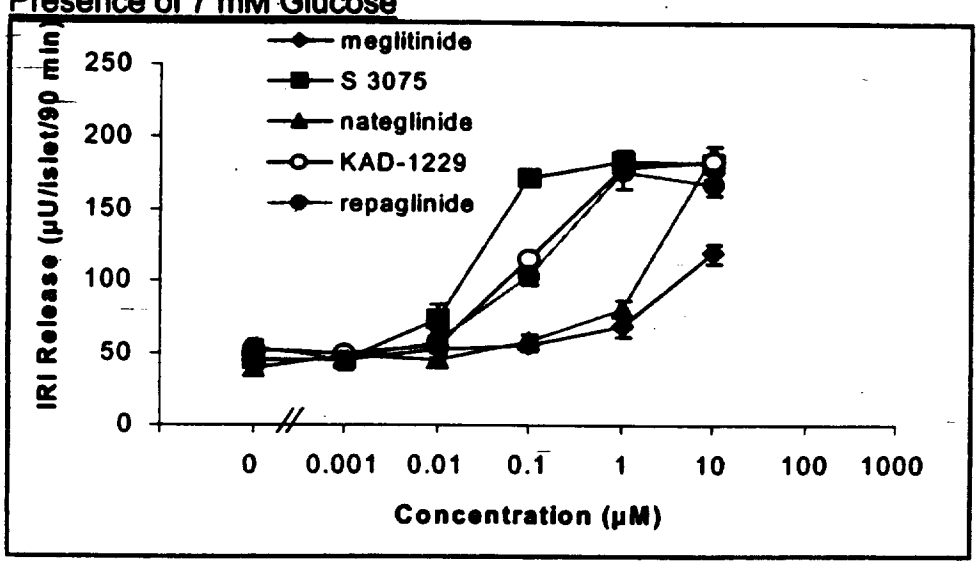
<u>Inhibition of K_{ATP} Current by Several Ligands in Rat Pancreatic β-Cells</u>



Nateglinide binds to sulfonylurea receptors and inhibits ATP-dependent K^+ currents, which will eventually release insulin from pancreatic β-cells by the mobilization of intracellular calcium. The effects of nateglinide on insulin release from isolated islets of Langerhans or from ß-cell lines during static incubations have been studied in several laboratories. This kind of experiment is well-suited for direct comparisons of several different treatments or compounds simultaneously using islets/cells from a single source tested under identical

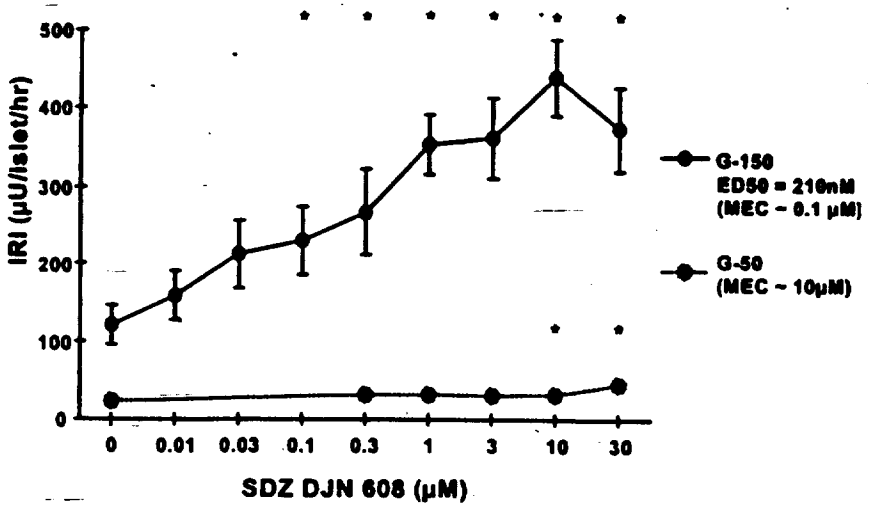
conditions. The effects of nateglinide, KAD-1229, meglitinide (the non-sulfonylurea moiety of glibenclamide) and its analogs, repaglinide and S 3075 were compared after a-90 minute incubation in pancreatic islets of normal rats (See figure below). In the presence of 7 mM glucose, the order of potency was S 3075 > KAD-1229 = repaglinide > nateglinide > meglitinide. The former three agents elicited similar maximum effects whereas the maximum effects of the latter two agents may not have been achieved at 10 µM, which was the highest concentration tested. It was reported that when tested at a concentration of 10 µM in the absence of glucose none of the compounds stimulated insulin release although each stimulated insulin release in the presence of the non-glucose nutrient, succinic acid monomethyl ester (SAM, 10 mM).

Relative Potencies of Several Ligands in Insulin Release from Rat Islets in the Presence of 7 mM Glucose



The stimulation of insulin release by nateglinide was also glucose-dose dependent. At an ambient glucose level of 50 mg/dl (2.8 mM) it required a concentration of 10 µM nateglinide to exert a small but significant increase of insulin release. In contrast, in the presence of 150 mg/dl glucose (8.3 mM), a concentration of 0.1 µM nateglinide exerted a significant stimulatory effect (Please see figure below). These data are in good agreement with the electrophysiology studies demonstrating that glucose sensitizes the ß-cell to nateglinide. The figure also demonstrates nateglinide-dose dependent release of insulin in isolated rat islets.

Dose-Response Curves for Nateglinide-induced Insulin Release from Isolated Rat Islets



The sponsor provided relevant information on the effect of nateglinide on its binding to SUR with relative good affinity and its inhibitory action on ATP-dependent K^+ current, which allows membrane depolarization. The increased depolarization of membrane action potential mobilized intracellular calcium, which was responsible for the release of insulin as demonstrated above. The role of released insulin for the normalization of carbohydrate metabolism is the main concern for the application of nateglinide in clinical settings. In human subjects, the effects of nateglinide and glyburide on the reduction of plasma glucose were compared (See the figures below). Nateglinide at a dose of 120 mg reduced blood glucose from 220 to 140 mg/dL in 2 hours after its treatment. Ten mg of glyburide also reduced the parameter slightly faster than the rate of glucose reduction with nateglinide and the maximum reduction of the parameter was greater with glyburide than that with a dose of nateglinide, 120 mg. Thus, the clinical potency of nateglinide in the reduction of plasma glucose appears to be consistent with its receptor binding data as well as its inhibitory effects on K^+_{ATP} current.

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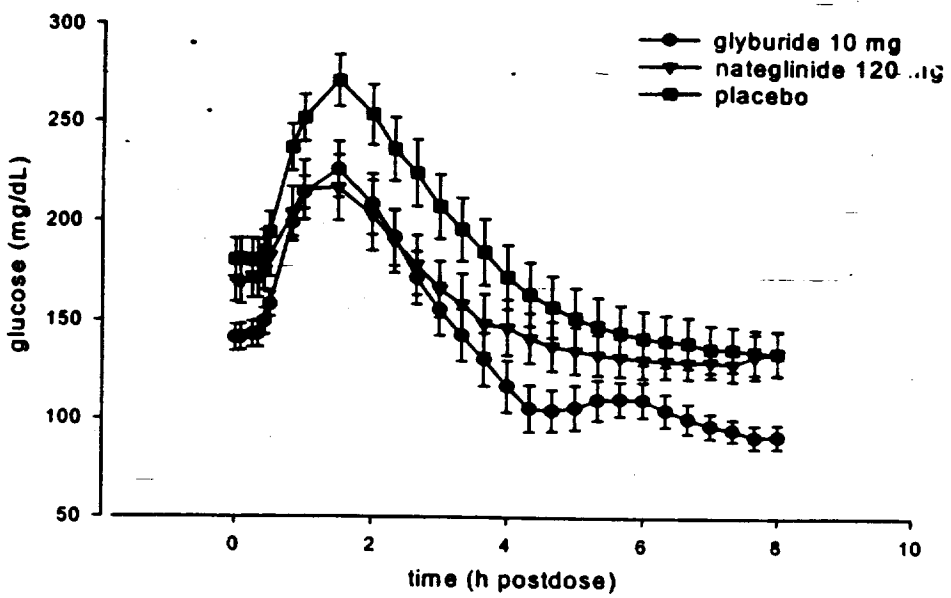

Summary of Pharmacology:

Sulfonylureas have been used as insulin secretagogues. In particular, the second generation of sulfonylurea such as glyburide, glipizide and gliclazide demonstrated their potency in insulin release. Glinides such as repaglinide (Prandin™) and mitiglinide are benzoic acid derivatives while nateglinide is a derivative of phenylalanine. It has been well documented that nateglinide binds to sulfonylurea receptors in vitro and in vivo, which inhibits ATP dependent K^+ channel as illustrated in several figures above. However, the binding affinity to the receptor as well as its ability to block the K^+ current were less than that of glibenclamide, glipizide, or repaglinide by several orders of magnitude. Likewise its ability to release insulin was also considerably less potent, compared to other insulin secretagogues. Nateglinide's insulin release activity was its dose and glucose-concentration dependent as demonstrated in isolated rat islets. It might have slightly rapid onset and shorter duration of action in insulin release as well as in glucose lowering effect, compared to other insulin secretagogues. Clear evidence for its advantages over other relevant agents on the market was not provided.

II. PHARMACOKINETICS/TOXICOKINETICS:

PK parameters:

Absorption and Bioavailability:

The absorption of orally administered nateglinide (solution, suspension) was rapid in animals including human. Peak concentrations of radioactivity and nateglinide were typically achieved between 0.25 and 1 h postdose, except

following high gavage doses in rats and capsule doses in dogs where the peak radioactivity was observed between 2 and 5 h post dose. Nateglinide was generally well absorbed (89-100%) after oral dosing. The absorption was independent of the dose and frequency of administration (single vs repeated) in rat and dog as shown below. When administered as a drug-diet mixture to mice and rats, nateglinide absorption was prolonged, consistent with continued feeding, and the extent of absorption was high (100%) in rats, but somewhat lower in mice. Based on several studies including radiotracer studies, actual Cmax and AUC values in selected animals including human are summarized below.

TOTAL RADIOACTIVITY CONCENTRATIONS (C_{max} AND AUC) IN BLOOD/PLASMA[a] FOLLOWING SINGLE AND MULTIPLE ORAL DOSES OF RADIOLABELED NATEGLINIDE

Species	Dose (mg/kg)	C_{max} (µgEq/mL)	AUC[b] (µgEq·h/mL)	Dose-Normalized	
				C_{max}	AUC
Mouse	100, M	115, 80.4[c]	188, 194	1.15,0.80	1.88, 1.94
Mouse	100, F	149, 76.4[d]	194, 151	1.49,0.76	1.94, 1.51
Rat	10	8.0	8.1 (0-.)	0.80	0.81
	30	23.8	38.8 (0-.)	0.79	1.29
	100	146.6	250.7 (0-.)	1.47	2.51
	52.4[e]	6.77	67.3 ($_{0\text{-}168h}$)	0.13	1.28
	300	143	552 ($_{0\text{-}168}$)	0.48	1.84
	30/day[f]	38	45 (0-24h)	1.27	1.50
Rabbit	150	242	1080 (0-96h)	1.61	7.20
Dog	3	13.06 [7.36]	65.46 (0-.) [43.96] (0-.)	4.35 [2.45]	21.8 [14.7]
	3/day[g]	[7.01]	[244.7] (0-.)	[2.33]	[81.6]
Human	1.43	6.36 [3.76]	20.3 [11.1] (0-.)	4.45 [2.63]	14.2 [7.76]

[a] Results in blood are given in brackets, [ ]
[b] Time interval indicated in parentheses
[c] Male B6C3F1 and CD-1 mice, respectively
[d] Female B6C3F1 and CD-1 mice, respectively
[e] Drug-diet, 24 h duration
[f] Multiple doses study, 21-day duration; values of C_{max} and $AUC_{0\text{-}24}$ are the mean of values after doses 1, 7, 14 and 21.
[g] Multiple dose study, 21-day duration; values of C_{max} and $AUC_{0\text{-}.}$ are those obtained after the 21st dose.

The terminal half-lives of nateglinide were: human, 1.7 hours; rabbit, 1.4 hours; dog, 3-5 hours; rat, 2.8 hours; male mice (B6C3F1 and CD-1), 2.0 hours, and female mice (CD-1), 2.6 hours, respectively. It appears that there were no significant sex differences in pharmacokinetic parameters as discussed subsequently.

Distribution:

Studies with rat, dog and human blood indicate that nateglinide uptake into blood cells is minimal and, therefore, drug concentrations in plasma were higher than in blood. The blood to plasma ratios were generally 0.7 in species tested after a single oral dose. As noted earlier, repeated dosing of [^{14}C] nateglinide in rat produced an apparent change in the blood to plasma distribution at 4 and 24 h postdose, which was attributed to the association of metabolite(s) to blood cells. This effect reached steady-state by the 7th daily dose.

In vitro binding of [^{14}C]-nateglinide to plasma proteins of mouse, rat, dog and human was determined using an ——— method. Based on the *in vitro* results, the percentage of radioactivity bound to plasma proteins was 94-96%, 94-95%, 96-97% and 98% for mouse, rat, dog and human, respectively, without any major change of binding in the concentration range between — µg/mL and — µg/mL. A gender effect was not observed in humans. Binding to human serum albumin (HAS: 0.2 mg/mL) remained relatively constant (~42-43%) over a concentration range of 0.025% to 1 µg/mL, but decreased to 37% and 24% at 2.5 and 10 µg/mL, respectively.

Whole-body autoradiograms of rats after a single oral or repeated daily oral doses for 21 days of [^{14}C] nateglinide showed distribution of radioactivity by 0.5 h essentially throughout the body, with levels higher than blood in the digestive tract contents, urine in urinary bladder, kidney and liver. Other tissues showed levels equal to or lower than in blood. Little or no radioactivity was observed in the eye or central nervous system. By 4 h, radioactivity levels had declined with only the digestive tract contents showing high concentrations. At 24 h, only the digestive tract contents had high concentrations of radioactivity and a trace of radioactivity was observed in the stomach wall. Some statistical data on nateglinide such as body clearance and volume of distribution at steady state are illustrated below.

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TOTAL BODY CLEARANCE AND STEADY STATE VOLUME OF DISTRIBUTION OF NATEGLINIDE

Species	Dose (mg/kg)	Clearance (L/h/kg)	V_{ss} (L/kg)
Mouse	5	1.1, 2.5[b] 2.0[c]	0.15, 0.73[b] 0.43[c]
Rat	5 30	2.68 0.9	0.67 1.3
Rabbit	5	0.16	0.11

Dog	1 3	0.07 0.06	1.1 1.3
Human	0.714	0.09	0.13

[a] Clearance and V_{ss} values were calculated from intravenous dose data in the referenced reports.
[b] Male B6C3F1 and CD-1 mice, respectively
[c] Female CD-1

Metabolism:

The biotransformation of nateglinide has been investigated in the mouse, rat, rabbit, dog and human. A major metabolic pathway of nateglinide in human involves hydroxylation of the isopropyl side chain to yield monohydroxylated metabolites, M1, M2 and M3. The site of hydroxylation in metabolite M1 is the methine carbon, while metabolites M2 and M3 represent diastereoisomers in which the hydroxyl is on one of the isopropyl methyl groups. Other minor metabolites include M7, which possesses an unsaturation in the isopropyl moiety, and metabolites M11 and M12, which are dihydroxylated with hydroxyls on both the methine and methyl carbons. As with metabolites, M2 and M3, metabolite M11 and M12 are diastereoisomers. The glucuronic acid conjugate of nateglinide, M4, with the carboxyl group as the point of attachment is also formed, as well as, its rearrangement isomers, M5 and M6. The major circulating metabolite in plasma is M1, with exposure (based on AUC_{0-12h} values) relative to parent drug of 9%. Metabolites M2, M3 and M7 are present in lesser concentrations with exposures of ~3-4% that of parent drug. Metabolites M11 and M12 are relatively minor in plasma, as are glucuronic acid conjugates, M4, M5 and M6. Nateglinide is extensively metabolized prior to excretion and the majority of the dose (84-87%) is eliminated in urine. The major urinary metabolite is also M1 representing 32-33% of the dose and approximately 16% of the dose is excreted as unchanged nateglinide. In urine, metabolites M2 and M3 collectively accounted for 7-8% of the dose, while all other metabolites each represented 5% of the dose. The main metabolite peaks found in plasma and excreta and the structures are shown below.

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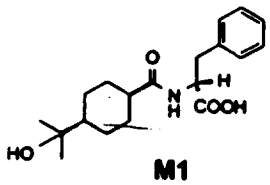
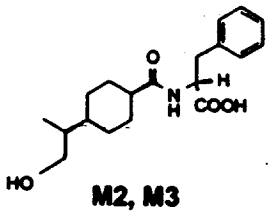
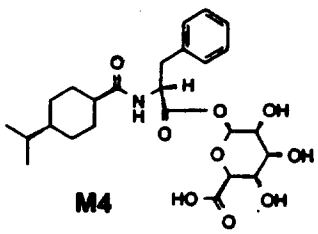
STRUCTURES OF THE IDENTIFIED METABOLITES OF NATEGLINIDE



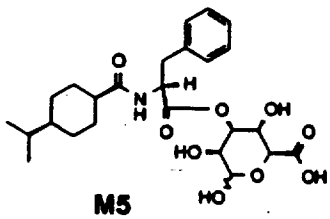
M1



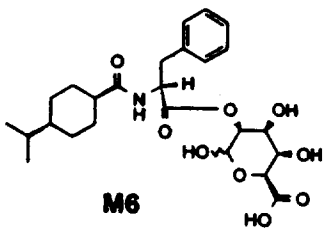
M2, M3



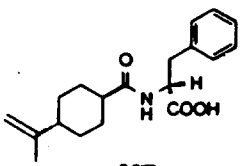
M4



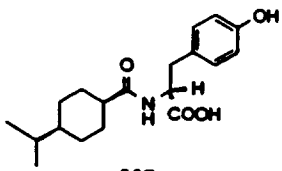
M5



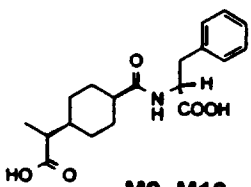
M6



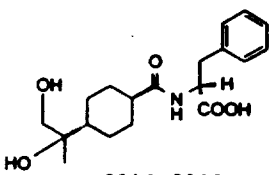
M7



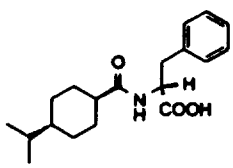
M8



M9, M10



M11, M12



Nateglinide

All of the human metabolites were identified in animals. Metabolites M8, M9 and M10 were found in animals. M8 is a phenolic metabolite of nateglinide, while M9 and M10 are carboxylic acids metabolites derived from further oxidation of M2 and M3. In general, mouse, rat and rabbit had a somewhat greater plasma exposure to metabolites (as a proportion of total radioactivity) as compared to humans, while dogs were exposed in plasma to mostly parent drug. The main metabolite peaks found in plasma and excreta are shown below.

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METABOLITES OF NATEGLINIDE AFTER ORAL DOSING IN VARIOUS SPECIES

Metabolite[a]	Metabolite Presence in Species and Sample Type[b]				
	Human	Mouse[c]	Rat	Rabbit	Dog
M11, M12	P,U	P,U	P,U	P,U	P,U
M1	P,U	P,U	P,U	P,U	P,U,B
M2, M3	P,U	P,U	P,U	P,U	U,B
M8	-[d]	P,F	-	-	P,B
M7	P,U	P,U	U	P,U	U,B
M9, M10	-	-	P,U,B	-	U,B
nateglinide	P,U	P,U,F[e]	P,U[e],B	P,U	P,U,B
M4, M5, M6	P,U	U[e]	U[e]	P	U,B

[a] Structures are found above.
[b] P = plasma; U = urine; B = bile, F = feces
[c] B6C3F1 and/or CD-1 strains
[d] Not detected
[e] Unchanged nateglinide and its glucuronide conjugate were present in urine in only trace amounts

Excretion:
The urinary and fecal excretion data following a single dose of radiolabeled nateglinide are summarized below. In all species, the renal elimination was relatively rapid and nearly complete within 24-h post dose. In bile-duct-cannulated rats, the respective excretion in bile, urine and feces was 58%, 36% and 4%, respectively. Approximately 40% of the biliary excretion in rat were found to be reabsorbed indicating significant enterohepatic recirculation in this species. In bile-duct cannulated dogs, excretion in bile, urine and feces by 48 h accounted for 53%, 31% and 10% of the dose, respectively. Biliary excretion in dog was rapid with ~80% occurring within 8 h of dosing.

Following an oral dose of [^{14}C] nateglinide to lactating rats, the transfer to milk of parent drug and metabolites was rapid. The milk to plasma ratio was ~1.4, based on AUC_{0-48h} values after a 300 mg/kg oral dose. Projecting the rat data to humans, it was estimated the maximum amount of nateglinide and/or metabolite(s) that a breast fed infant could be exposed to by ingesting one liter of milk per day was 1.2% of a 120 mg adult dose (12). The metabolite patterns in milk and plasma were similar. Unchanged drug was the predominant component in milk and the major metabolite was M1. Also present in milk were metabolites M2, M3, M7, M11 and M12, but at lower concentration. The relative proportions of parent drug and metabolites were similar to those seen in plasma.

EXCRETION OF NATEGLINIDE AND TOTAL RADIOACTIVITY FOLLOWING A SINGLE DOSE OF RADIOLABELED NATEGLINIDE

Species	Dose (mg/kg)	Dose Route	Amount Excreted (% of Dose)				
			Urine			Feces	
			Radioactivity		Nateglinide	Radioactivity	
			0-24 h	0-168 h	0-168 h	0-24 h	0-168 h
Mouse	5[a]	i.v.	23, 25	27, 35	0.4, 0	71, 27	78, 51
	5[b]	i.v.	16, 35	19, 39	0, 0	69, 48	77, 57
	100[a]	p.o.	39, 44	46, 56	0.7, 0.3	84, 33[a]	90, 41
	100[b]	p.o.	6, 26	12, 35	0.1, 0	50, 29	77, 43
Rat	5	i.v.	31.0	35.6	-[c]	51.1	61.1
	30	p.o.	47.2	47.8	nd[d]	48.0	49.3
	300	p.o.	47.2	53.6	-	47.1	56.7
Rabbit	5	i.v.	70.1	74.4	13.8[e]	0.99	1.21
	150	p.o.	71.5	75.3	11.6[e]	2.51	3.19
Dog	3	i.v.	20	22[f]	nd	55	75[f]
	3	p.o.	30	34[f]	3.5[g]	50	65[f]
Man	0.714	i.v.	86.3	86.9[f]	16.2[f]	0.1	8.2[f]
	1.43	p.o.	83.0	83.7[f]	16.3[f]	1.4	10.0[f]

[a] B6C3F1 male and female, respectively
[c] CD-1 male and female, respectively
[b] Trace or not detected
[d] Not determined
[e] 0-48 h
[f] 0-120 h
[g] 0-24 h

Summary: It appears that nateglinide was absorbed rapidly (Tmax < 1 hour) in many species after an oral administration. The maximum concentrations of nateglinide after an oral administration in diet were reached 2 to 5 hours postdosing. It is generally well absorbed (over 90%) after oral administrations, although the percent of drug absorption was low in mice. Drug absorption was independent of the frequency (single or repeated) of administration in rat and dog. The terminal half-lives of nateglinide were: human, 1.7 hours; rabbit, 1.4 hours; dog, 3-5 hours; rat, 2.8 hours; male mice (B6C3F1 and CD-1), 2.0 hours, and female mice (CD-1), 2.6 hours, respectively. The considerable level of drug was detected essentially throughout the body, with levels higher than blood in the digestive tract contents, urine in urinary bladder, kidney and liver within an hour after its administration. It is not clear the per centage of the drug that crosses the blood brain barrier.

There were three major metabolites (M1, M2, and M3) which are hydroxylated products, while glucuronic acid conjugates of nateglinide were minor metabolites that are eliminated in urine. Most of all the metabolites were found in human as well as in animals. Nateglinide was eliminated in urine in man and rabbit while fecal pathway was the main elimination route for other species such as mouse and dogs. Both elimination pathways were used equally in rats.

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 day 28, 189 and 370. The peak drug levels in serum were reached within a few hours after subcutaneous injection. Increased times to peak concentrations were noted in male with increasing dose as shown below (See table). Cmax as well as AUC were increased according to the size of doses. The two parameters were generally increased as a function of exposure duration. Gender dependent differences in pharmacokinetic parameters were not significant. Toxicokinetics data for other species and studies are reviewed in conjunction with the individual toxicity studies.

Pharmacokinetics of Nateglinide After Oral Administrations										
Parameters *		Tmax(hour)			Cmax(ng/ml)			AUC_{0-7h} (ng.h.ml)		
Sex	Dose@	28	190	370	28	190	370	28	190	370
Male	0	24	1	1	8.6	10.1	15.3	47	47	72
	72	1	1	1	28.0	23.4	34.8	64	71	106
	182	1	1	1	47.4	36.1	88.7	80	112	224
	455	1	1	1	32.2	76.9	159.0	101	251	539
Female	0	2	1	4	4.1	5.6	8.3	26	34	48
	72	1	1	1	5.56	19.4	28.3	28	49	60
	182	1	1	1	32.8	52.4	84.8	56	96	135
	455	1	1	1	54.4	124.0	169.7	98	196	344

*Indicated parameters were determined at 28, 190 and 370 days after the onset of study. @ Dose was in microgram/kg.

III. TOXICOLOGY:

General Comments:
The following toxicology studies were performed by
for Novartis Pharmaceuticals as indicated. The acute toxicity of nateglinide was vomiting and diarrhea at high oral doses. In repeated dose studies, target organs for toxicity were the gastrointestinal tract and the hepatobiliary system. Erosion or ulceration of the gastrointestinal tract observed in subchronic and chronic toxicity studies in rats

and dogs was considered to be the consequence of an irritant effect of nateglinide at high doses. A decrease in gastrointestinal motility was observed in rats treated with nateglinide at doses > 100 mg/kg. Nateglinide was not teratogenic and did not affect fertility, general reproductive performance, or development of the offspring of treated animals. In rabbits, an increased incidence of gallbladder agenesis occurred in pups at doses associated with embryotoxicity and maternal toxicity and mortality. In a supplementary study(rabbit teratology study), the livers and bile ducts appeared normal upon histopathological examination of the fetuses without gallbladders. Nateglinide demonstrated neither mutagenic nor carcinogenic potential. In the mouse carcinogenicity study an increased incidence of peripheral neuropathy was found in female B6C3F1 mice.

The Sponsor Performed the following Single-dose (Acute) Toxicity Studies

Species	Route	Dose (mg/kg)	Major findings
Mouse	iv	100 – 400	$LD_{50} \approx$ 200-400 mg/kg
Rat	oral (gavage)	500 - 2000	LD_{50} > 2000 mg/kg
Dog	oral (capsule)	100 - 2000	LD_{50} > 2000 mg/kg

Single-dose intravenous toxicity studies in mice (Study NO: 96A005)
Nateglinide (Lot no. 2G561, 100.5% purity) was administered by a single intravenous injection to — mice (5/sex/group) at doses of 0, 100, 200 or 400 mg/kg. After a 14-day observation period, the animals were euthanized and organs were examined macroscopically. The mice (Crj: CD-1) were approximately 6 weeks of age with body weight ranges of 30.0-34.4 g for males and 22.9-26.2 g for females on the day of dosing.
Immediately after dosing, 5 male and 4 female mice in the 400 mg/kg dose group showed tonic convulsions and died. One female animal in the 200 mg/kg dose group also experienced tonic convulsions. Other clinical observations included two male animals in prone and crouching positions with eyelid closure (200 mg/kg) and one female (400 mg/kg) in the crouching position. All signs disappeared within 2 hours after dosing in the surviving animals. No body weight changes attributable to AY4166 treatment were noted. In the nine animals that died, the findings at necropsy were hydrothorax, enlargement of the lung and mixed colored patches (red and orange) on the lung.
Based on the results of this study, the LD_{50} was between 200-400 mg/kg with 400 mg producing 90% mortality.

Study Title: Nateglinide- Single-dose oral toxicity study in rats
Study No: 9525
Amendment #, Vol #, and page #: Vol.15 and 16, p21 and 12-42
Conducting laboratory and location[ ]
Date of study initiation: June 26, 1990
GLP compliance: Yes
QA- Report Yes (x) No ()

METHODS:
Dosing: 500, 1000, and 2000 mg/kg
species/strain:.SD rat/ Crj: CD
#/sex/group or time point: 6 rats/sex/group
Age: 5 week old
Weight: Male: 164-184 grams; Female: 126-139 grams
Satellite groups used for toxicokinetics or recovery: None
Route, form, volume, and infusion rate: Oral (gastric tube)
Drug, lot#, radiolabel, and % purity: A-009, cold, and 100.11% pure
Formulation/vehicle: 0.5% methylcellulose 400

OBSERVATIONS AND TIMES:
Clinical signs: were recorded during the first day continuously, from Day 2 once per day for 14 days.

RESULTS:
Clinical signs: There were no deaths. Signs of acute toxicity observed for approximately 3 hours after dosing included salivation, decreased locomotor activity, loose stool and diarrhea in groups receiving 500 mg/kg or more.
Body weights: Decreased body weight gain (under 10% at Week 15) associated with a transient decrease in food consumption was observed 2 days after dosing in female rats in the 2000 mg/kg group; weight gain was similar to controls for the remainder of the study.
Gross pathology: Macroscopic examination on dissection of the animals killed at the end of the 2-week follow-up period revealed no morphological changes.
Conclusion: LD_{50} of nateglinide appeared to be greater than 2000 mg/kg.

Study Title: Nateglinide-Single-dose oral toxicity study in beagle dogs
Study No: B-1844
Amendment #, Vol #, and page #: Vol.16 and pp5-68-100
Conducting laboratory and location: [ ]
Date of study initiation: June 20, 1990
GLP compliance: Yes
QA- Report Yes (x) No ()

METHODS:
Dosing: 100, 500, 1000, 2000 mg/kg
Species/strain: Dog/Beagle
#sex/group or time point: 2 male dogs/group
Age: 5 months old
Weight: 10 kg
Satellite groups used for toxicokinetics or recovery: None
Route, form, volume, and infusion rate: Oral

Drug, lot#, radiolabel, and % purity: A-013, Cold, and 99.16%
Formulation/vehicle: White crystalline powder

OBSERVATIONS AND TIMES:
Clinical signs: were recorded during the first day continuously up to 6 hours after administration, and twice daily thereafter for mortality, signs of toxicity, the presence of abnormal behavior and stools for 14 days.

RESULTS:
Clinical signs: There were no deaths. Vomit containing white material (thought to be test substance) was observed in all animals in the 500, 1000 and 2000 mg/kg groups from 2 to 6 hours after administration. In the 100 mg/kg group, vomiting was not observed, but mucous stool containing a small amount of blood and white material was observed in one animal 4 hours after dosing. No clinical signs were noted from day 2 until termination. There were no changes in body weight, food consumption, gross pathological findings or organ weights.

Conclusion: The lethal dose was estimated to be higher than 2000 mg/kg, although the accurate dose was not known due to vomiting at dose levels of 500mg/kg and above. The following table summarizes the repeat dose (subchronic and chronic) studies of nateglinide performed by the sponsors.

Species	Route	Duration	Dose	Major findings
Mouse	oral (diet)	13 weeks	0.5, 1, 2%*	NTEL = 0.5% Decreased body weight gain and low packed cell volume at 1% and 2%.
Rat	oral (diet)	13 weeks	5000, 10000, 20000 ppm**	All doses: decreased body weight gain. 20000 ppm: small increase in ALT No NOEL
Rat	oral (diet)	13 weeks	625, 1250, 2500 ppm***	NOEL = 2500 ppm(200-240 mg/kg)
Rat	oral (gav-age)	13 weeks	100, 300, 1000, 2000 mg/kg	NTEL = 100 mg/kg 1000 mg/kg: Stomach erosions/ulcers 2000 mg/kg: mortality (terminated on day 10)
Rat TK	oral (gav-age)	13 weeks	100, 300, 1000 mg/kg	Over-proportional increase in exposure above 300 mg/kg. No significant accumulation.
Rat	oral (gav-age)	52 weeks	30, 100, 500 mg/kg	NTEL = 100 mg/kg 500 mg/kg: Bulla formation or ulcer in fore-stomach and erosion in glandular stomach.
Rat	i.v.	5 days	6, 24, 48	MTD not achieved. 48 mg/kg: Local irritation and necrosis at injection site.
Rat	i.v.	2 weeks	6, 24	NTEL = 24 mg/kg; necrosis at injection

			mg/kg	site. Over-proportional increase in exposure; accumulation after 9 days

* 0.5, 1, 2% = 981/1352, 2643/2944, 6145/7067 mg/kg for males/females, respectively
** 5000, 10000, 20000 ppm = 364/438, 724/844, 1522/1817 mg/kg for males/females, respectively
*** 625, 1250, 2500 ppm = 52/58, 103/119, 201/240 mg/kg for males/females, respectively

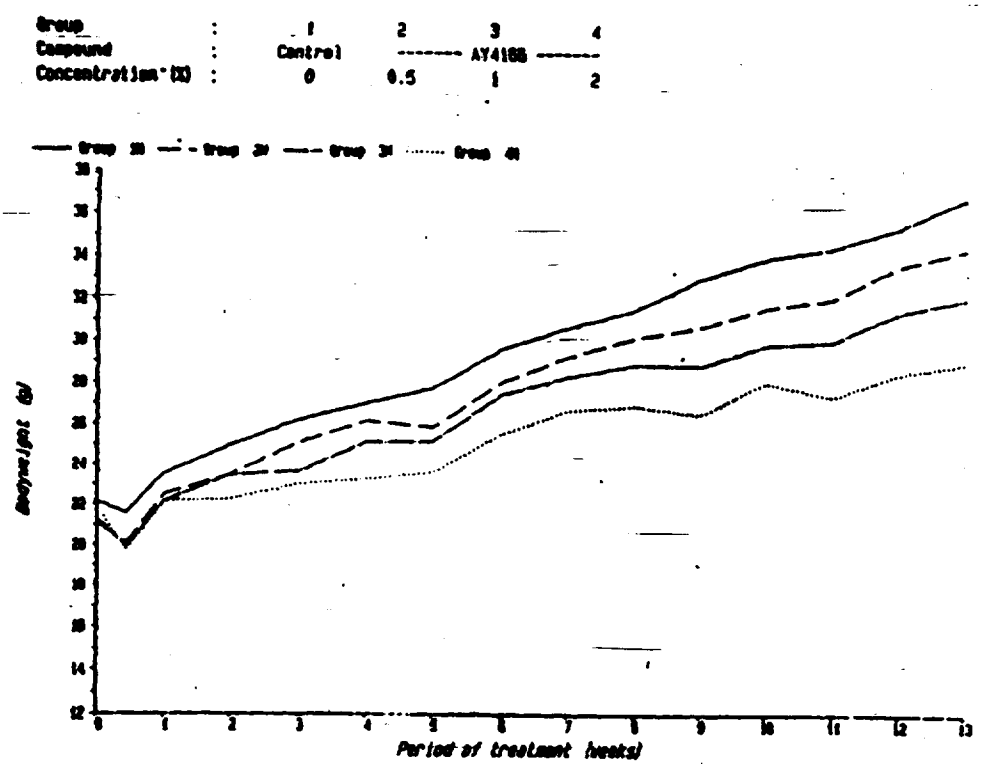
Repeat dose toxicity studies in non-rodent

Species	Route	Duration	Dose (mg/kg)	Major findings
Dog	oral (capsule)	13 weeks	10, 30, 100, 300	NOEL = 30 mg/kg; NTEL = 100 mg/kg 300 mg/kg: Mortality, increased ALT, AST, bilirubin. Bile plugs in liver; ulceration of duodenum, erosion of stomach.
Dog TK	oral (capsule)	13 weeks	10, 30, 100, 300	Rate and extent of exposure approximately linear up to 100 mg/kg, and less than proportional at 300 mg/kg
Dog	oral (capsule)	13 weeks, followed by up to 5 weeks recovery	300	Elevated ALT, AST, ALP, total bilirubin. Yellowish changes in organs, tissues & subcutis. Deposition of brown pigment in hepatocytes and hypertrophy of zona fasciculata in adrenals. Reversible single cell necrosis, fat deposition & cytoplasmic degeneration of hepatocytes; atrophy and ulcer of gallbladder mucosa and duodenal ulcers.
Dog	oral (capsule)	12 months	10, 30, 100	NTEL = 100 mg/kg
Dog	i.v.	acute and 5 days	5, 10, 20 (single dose) 20 (5 days)	MTD not achieved.
Dog	i.v.	2 weeks	4, 20	NTEL = 20 mg/kg; inflammatory lesions at injection site. Dose-proportional exposure; no accumulation

Study Title: Nateglinide- 13-week oral toxicity study in mice
Study No: LSR Schedule# YOC/034/4166/ LSR Report#91/YOC034/0923
Amendment #, Vol # 16, and page pp5-102/315
Conducting laboratory and location:[ ]
Date of study initiation: April 23,1991
GLP compliance: Yes
QA- Report Yes (x) No ()

Dosing: 0, 0.5, 1, and 2% of nateglinide in diet. In males, LD, MD, and HD were 981, 2643, and 6145 mg/kg/day while the LD, MD, and HD were 1352, 2944, and 7067 mg/kg/day, respectively, in females.
Species/strain: Mice/ B6C3F1
#Sex/group or time point: 10 mice/sex/group
Age: 26-28 days old
Weight: Approx. 18 g for males, and 14 g for females
Satellite groups used for toxicokinetics or recovery: None
Route, form, volume, and infusion rate: Oral, in regular diet
Drug, lot#, radiolabel, and % purity: 2G121 and A-015
Formulation/vehicle: Nateglinide was mixed with rodent diet[ ]Fixed amount diet was offered to each mouse and uneaten food was recorded to calculate the amount of drug consumed.

OBSERVATIONS AND TIMES:
Clinical signs: Observed at least twice a day for 13 weeks.
Body weights: Determined on pre-dose, day 3 and thereafter weekly.
Hematology: Determined in all animals before drug treatment and immediately before necropsy after 13 weeks treatment.
Clinical chemistry: Blood was drawn from the retro-orbital sinus of all animals before drug treatment and after 13-week.
Gross pathology: Necropsy was performed in all dead animals.
RESULTS:
Clinical signs: There were no deaths or signs attributable to drug.
Body weights: Overall body weight gains were significantly lower for males and females of the 1% and 2% dose groups. Numerical and per cent changes in bodyweight gain at the end of 13-week study are summarized in a table below. Food consumption was higher in males at 1% and both sexes at 2%, and the overall efficiency of food conversion was markedly inferior for males and females in both dose groups.



Group mean bodyweight gain in 13-week oral toxicity in mice at Day 90					
Sex	Dose(%)	0	0.5	1	2
Male	BW gain	14.7	13.4	10.9*	7.3*
	%, Control		91	74	50
Female	BW gain	8.1	8.3	6.5*	5.2*
	%, Control		102	80	64
*Indicate P<0.05. BW stands for body weight.					

Hematology: When packed cell volume was measured, significantly lower packed cell volumes were noted for males in all treatment groups and for females in the 2% group as indicated below.

Group mean packed cell volume in 13-week oral toxicity in mice at Day 90					
Sex	Dose(%)	0	0.5	1	2
Male	Cell Volume	48	46*	44*	44*
	%, Control		96	92	92
Female	Cell Volume	44	42	43	39*
	%, Control		95	98	87
*Indicate P<0.05.					

Clinical chemistry: Plasma ALT levels were reduced in treated males and AST was reduced in female after 13 weeks of dosing with >0.5% nateglinide.

Effects of Nateglinide on ALT and AST levels after 13-Week Treatment in Mice				
Drug Dose (% in Diet)	Male Mice		Female Mice	
	ALT	AST	ALT	AST
Control (0%)	120	135	67	106
0.5%	23*	54	49	71*
1.0%	34*	95	43	75*
2.0%	33*	57	23	18

*Indicate P<0.05 and the unit of enzyme activity was in IU/L.

Organ weights:

Absolute organ weights (g) of mice in 13-week oral toxicity study@								
Sex	Male				Female			
Dose(% in diet)	0	0.5	1	2	0	0.5	1	2
# Examined	10	10	10	10	10	10	10	10
Body Weight(g)	34.9	32.4	30.0*	26.8*	25.5	25.1	23.9	21.9*
Kidney Wt(g)	0.59	0.53	0.50	0.46*	0.41	0.39	0.36*	0.32*
Liver Weight(g)	1.65	1.64	1.55	1.58	1.40	1.45	1.42	1.45
@Indicates group mean values and *indicates P<0.05.								

Gross pathology: There was no treatment-article related change except organ weight as indicated above.
Histopathology: Not examined.
Toxicokinetics: Not performed in the study.
Conclusion: It appears that nateglinide treatment for 13-week was toxic (reduction in body weight) for both sexes of mice at or above 1% in diet, although animals tolerated 0.5% of the drug.

Study Title: Nateglinide-A 26-week oral (diet) toxicity study in female B6C3F1 mice
Study No: 987069
Vol #14, and page # pp5/1-264

Conducting laboratory and location: Novartis Pharmaceuticals Corp., East Hanover, NJ
Date of study initiation: July 28, 1998
GLP compliance: Yes
QA- Report Yes (x) No ()

Dosing: 0, 0.1, 0.3 and 1.0% of nateglinide in diet or 7.5 mg/kg acrylamide(positive control)
Species/strain: Mice/ B6C3F1
#Sex/group or time point: 20 female mice/group
Age: 9 weeks old
Weight: Approx. 18-24 g
Satellite groups used for toxicokinetics or recovery: 15 mice/group (The animals in this group were used as the fifth satellite group).
Route, form, volume, and infusion rate: Oral, in regular diet
Drug, lot#, radiolabel, and % purity: 2G621, 99.5% pure
Formulation/vehicle: Indicated % of nateglinide was mixed with rodent diet. Fixed amount diet was offered to each mouse and uneaten food was recorded to calculate the amount of drug. Animals in positive control group had aqueous acrylamide (0.5 to 2.0 mg/ml), which is known to cause peripheral nerve damage.

OBSERVATIONS AND TIMES:
Clinical signs: Mortality was checked twice daily on weekdays and once daily on weekends and holidays for 26 weeks. Clinical observations were checked once daily during pretest period. Animals were observed at least twice daily, once prior to dosing and approximately 2 hours postdose in acrylamide dosing group, and thereafter daily in all groups.
Body weights: Determined pre-dose, day 3, and weekly.
Food consumption: Weekly
Hematology: Determined in all animals before drug treatment and immediately before necropsy after 26 weeks treatment.
Toxicokinetics: Plasma concentrations of nateglinide were measured from the satellite animals during treatment weeks 2/3 and 26/27. During the week 2 and 26 sampling (0.2 to 0.5 ml) was obtained from retro-orbital venous plexus under anesthesia from the satellite animals in the morning.
Gross pathology: Necropsy was performed in all dead animals by ——— ——— at the Norvartis Preclinical Safety facility using standard procedures. In addition, sciatic nerve sections from all animals and tibial nerve sections from the control, acrylamide and 1% nateglinide-treated animals were examined.

RESULTS:
Clinical signs:
Mortality: There were no deaths in control or nateglinide-treated groups. There were 7 deaths in acrylamide treated group. Two animals, one each from the

control and the acrylamide treated-groups were sacrificed due to poor health condition. There was one animal death due to an accident as shown in a table below. The appearance and behavior of the animals such as, respiration, skin coat, eye, posture, locomotor activity, and stool were normal and there were no differences between the control and treated-groups. However, there were significant differences in the parameters in the acrylamide treated-group compared to the control or any other nateglinide-treated groups. It appears that the sponsor selected acrylamide because of its known toxicity in the central nervous system to compare the nateglinide potential for peripheral nerve degeneration. The dose of acrylamide was 7.5 mg/kg which was given by gavage.

Effects of Nateglinide on Mortality of Female Mice in a 26-Week Toxicity Study					
Dose(%)	0	0.1	0.3	1.0	Acrylamide
# Initial Rats	20	20	20	20	20
# Dead Rats	0	0	0	0	7
#Sacrificed	1	0	0	0	1
#Accident*	0	0	1	0	0
# Terminal	19	20	19	20	12

*Indicate the number of female rat that was killed by accident.

Body weights: There were no changes in body weights in the low and intermediate group animals from the relevant control animals over the 26-week study duration. The body weights of surviving animals in the acrylamide-treated animals were also not different from those of control animals at all points for 26 weeks. However, animals in the high-dose group lost weight (approximately 4%; $P<0.05$) in the first 5 weeks of treatment, which continued through week of 11. On week 12 the reduction in body weight in the top dose group was not significant, although there was a slight (3.4%) reduction in the parameter. The variable increases and decreases in the parameter continued to the end of 26 weeks.

Clinical chemistry: Relevant findings in control and all treated groups were not remarkable. Samples for clinical chemistry assessments were not collected from the acrylamide-treated group.
Urinalysis: Total C peptide levels and increased C peptide/creatinine ratios were observed during weeks 14 and 26 in the high dose group. Increased urine volume was noted at the dose. Urine was not collected from the acrylamide-treated animals.
Gross and microscopic pathology: Although the incidence of peripheral neuropathy in female mice of the highest dose group was somewhat higher than that of control animals, this was not considered to be remarkable.

Incidence of Peripheral Neuropathy in Female Mice treated with Nateglinide *

Dose Level Nateglinide*	26-Week Toxicity Study (B6C3F1)	
	Sciatic nerve	Sciatic + tibial
Control	2/19	11/19
0.1%	6/20	
0.3%	3/19	
1%	3/20	4/20
Acrylamide	10/13	13/13

*Animal no. with neuropathy/no. examined

Conclusions: A clear dose-related increase in peripheral neuropathy was not observed in nateglinide-treated female CD-1 mice at dietary concentrations up to 1% for 26 weeks.

Study Title: Nateglinide: 13-Week Oral Toxicity and Toxicokinetic Study in Rats
Study No: 93.0317/Doc. No. 012525/ Report#93.0968
Amendment #, Vol #23, and page # p5/1-5/291
Conducting laboratory and location[ ]

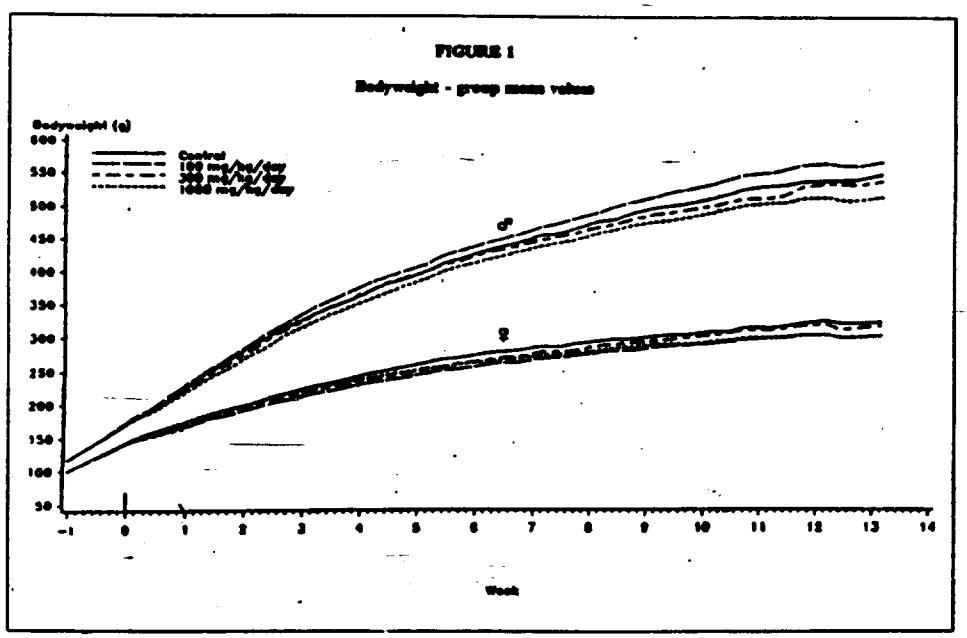
Date of study initiation: January 11, 1995
GLP compliance: Yes
QA- Report Yes (x) No ()

Species/strain: Rat/Crl: CD BR
#Sex/group or time point: 20 rats/sex/group
Dosing: 100, 300, or 1000 mg/kg
Age: 28 days old
Weight: 156-198 g for males and 136-169 g for females
Satellite groups used for toxicokinetics or recovery: 8 rats/sex/group
Route, form, volume, and infusion rate: Oral (gavage), Suspension, 10 mg/kg
Drug, lot#, radiolabel, and % purity: Batch 2G131, cold, 99.95%
Formulation/vehicle: 0.5% methylcellulose

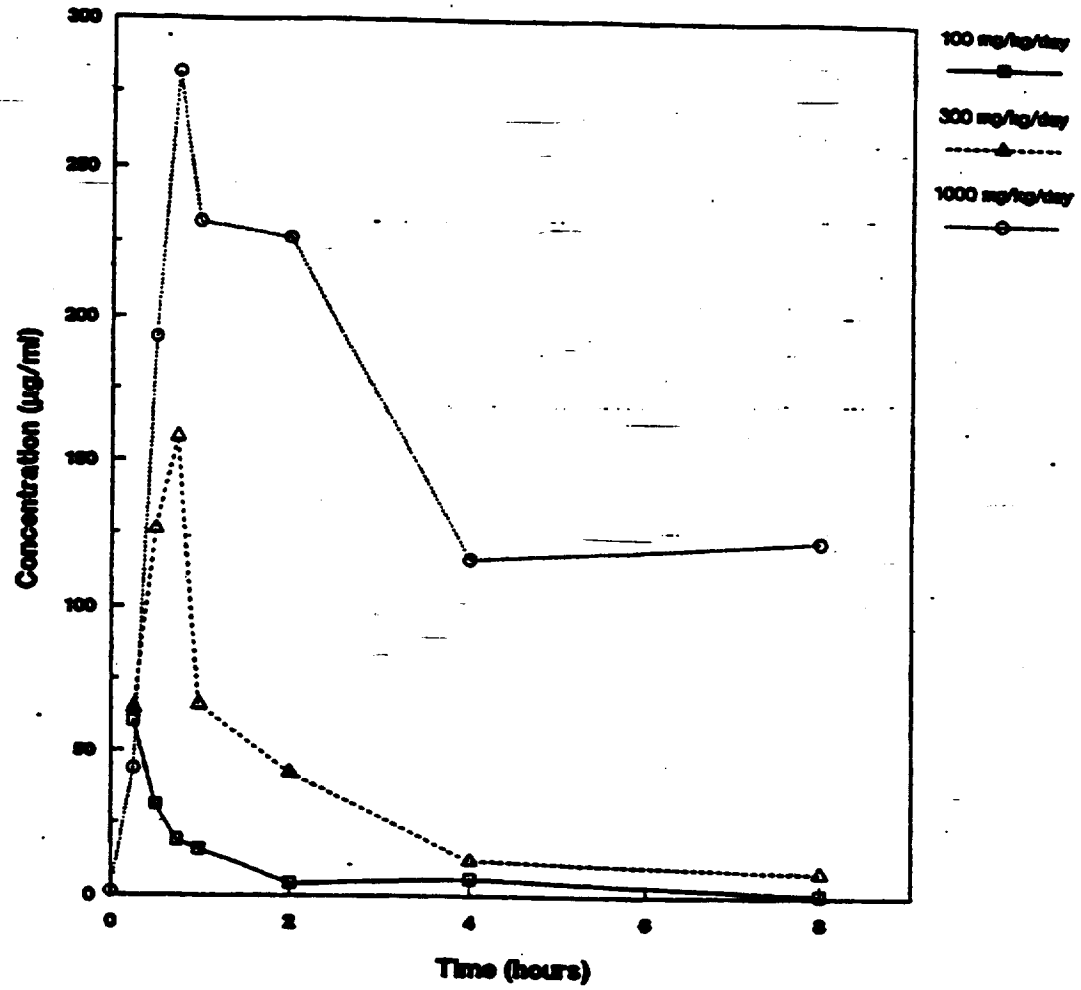
OBSERVATIONS AND TIMES:
Clinical signs: Observed at least once daily from pre-dose Day 7 to the day following admin.
Body weights: Determined on allocation to a group, on the day of commencement of treatment, and three times a week thereafter.
Food consumption: Food intake was checked weekly basis. Food intake per rat (g/rat/week) was calculated by the following formula: [(Total food given – Total food left)/Number of rat days] x 7.

Toxicokinetic Study: Blood samples were taken on Days 1, 30, and 86 of a 13-week toxicity study.
Hematology: Determined in animals twice before admin. and the day of termination.
Clinical chemistry: Blood was drawn from animals twice (Pre-dose Day 7 and the day of termination).
Gross pathology: Necropsy was performed in all dead animals.

RESULTS:
Clinical signs: There were no deaths, which were considered to be related to treatment. Four rats died in association with blood samplings. Salivation immediately after dosing was noted, which appeared not dose-related. Paddling motions of the forelimbs immediately after dosing were also noted, which appeared to be related to drug treatment since the rats in control and low dose groups did not show the behavior.
Body weights: During the 13 weeks of treatment, a slightly lower overall bodyweight gain was noted for males and females receiving 1000 mg/kg/day and females receiving 100 mg/kg/day. However, a level of statistical significance (176 vs 157 g at Day 91) was achieved only for the high dosage females as shown below.



Plasma Concentration: Nateglinide in blood level was determined on Day 86 of 13 weeks oral administration in rats. There were drug dose dependent increases in blood drug concentrations, with peak concentrations 30 to 60 minutes after oral administrations. The blood levels were rapidly reduced within 1 hour, and leveled off 4 hours after the treatment (See fig. below).



Hematology: Unremarkable
Clinical Chemistry: Unremarkable
Macroscopic pathology: There was no treatment-article related change.
Toxicokinetic Investigation: All surviving treated group animals were sampled on Day 1 and in Week 13 to determine TK parameters, of which results are summarized below. The rate and extent of systemic exposure of male and female rats to Nateglinide appeared to be characterized by dose-dependent (non-linear) kinetics. Increasing the dosage above 300 mg/kg/day is likely to result in a disproportionately higher extent of systemic exposure than that predicted from a linear relationship. Cmax and AUC are summarized in a table below.

Toxicokinetic Data in 13-Week Oral Toxicity Study of Nateglinide in Rats								
Dose (mg/kg /day)	Cmax (µg/ml)				AUC_{24} (µg.h.ml)			
	Day.1		Day 86		Day 1		Day 86	
	Male	Fem.*	Male	Fem.*	Male	Fem.*	Male	Fem.*
100	42	40	50	61	74	50	102	70
300	60	58	90	158	269	254	371	310
1000	116	154	266	282	1585	2085	2477	2199

*Fem. stands for female.

Study Title: Nateglinide –A 13-Week oral toxicity study in beagle dogs
Study No: B-1880
Amendment #, Vol. #37, and page #5/1-5/168
Conducting laboratory and location:

Date of study initiation: 3/30/1995
GLP compliance: Yes
QA- Report Yes (x) No ()

Species/strain: Beagle dogs
#Sex/group or time point: 3 dogs/sex/group
Age: 5 months old
Weight: Males (9.7-13.4 kg) and females (7.3-10.0 kg)

Dosage groups in administered units: Control (placebo), 10, 30, 100, and 300 mg/kg for 13 weeks.
Route, form, volume, and infusion rate: Oral
Drug, lot#, radiolabel, and % purity: 2G131 99.95% pure
Formulation/vehicle: Empty capsule
OBSERVATIONS AND TIMES:
Clinical signs: All signs of ill health or reaction to treatment were checked daily.
Body weights: This parameter was checked weekly throughout the study.
Food consumption: The quantity of food left by individual animals was recorded daily and food consumption was calculated weekly throughout the study.
Hematology: This parameter was checked at Weeks 5 and 13. Blood was taken from a jugular vein after overnight fast.
Clinical chemistry: This parameter was checked at weeks 5 and 13 from blood obtained from the hematological studies.
Gross pathology: After exsanguination, all dogs were necropsied and checked for visible abnormalities.
Organ weights: The parameter was determined at necropsy and organ to body weight ratio was calculated.
Histopathology: 36 tissues were preserved in appropriate fixative(see table p42).

RESULTS:

Clinical signs: There were no deaths or signs that were considered to be of toxicological significance. However, salivation and vomiting associated with dosing were noted. Isolated incidences of abnormal gait were observed in males receiving 300 or 100 mg/kg/day. Other signs such as hair loss, liquid feces and stained teeth were also recorded, which are not uncommon for laboratory dogs.
Body weights: There was considered to be no effect of treatment on bodyweight gain throughout the study except a decrease in male high dose group (12.8 vs. 11.6 kg).

Food consumption: There were no treatment-related changes in food consumption.
Electrocardiograms: There were abnormal findings that were related to treatment.
Ophthalmology: Unremarkable.
Clinical Chemistry: A decrease in blood glucose due to the pharmacological effect of the test article was observed at 10 mg/kg/day, which was not statistically significant compared to the control clinical chemistry. In Week 13, significantly higher ALT activity was noted males receiving 300 or 100 mg/kg/day, which was not drug-dose dependent. Alkaline phosphatase and bilirubin were increased in males of the high dose group as shown below.

Nateglinide Effects on Blood Chemistry in 13-Week Oral Study in Male Dogs					
Parameter	Nateglinide Dose (mg/kg/day)				
	Control	10	30	100	300
ALT (mU/ml)	26	32	28	48*	44*
AP(mU/ml)	128	117	126	131	167*
Bilirubin(mg/dl)	0.2	0.3	0.2	0.2	0.3*
*Indicates P<0.05.					

Urinalysis: A significant increase in the volume of urine was observed in males in the 300 mg/kg group in week 8 of treatment. It appears that the finding was incidental because there were sporadic changes in urine volume in other groups including the control group.

Terminal findings:
Pathological findings: Gross pathological examination performed following 13 weeks of treatment revealed no abnormalities considered to be attributable to nateglinide treatment.
Organ weight: Increases in adrenal, pancreas, and kidney weight were observed in males in the 300 mg/kg group without concomitant changes in histopathological changes. There were no other abnormal changes in organ weights that were related to the treatment.
Histopathology: There were no abnormal changes that were attributable to the treatment.

Conclusion: The 13-week study in dogs did not produce consistent findings that were attributable to nateglinide treatment except increases in adrenal, pancreas, and kidney weights in 300 mg/kg group in males. It appears that the top dose, 300 mg/kg, was too low to produce a frank toxicity after 13 weeks of administration.

Study Title: Nateglinide –Toxicokinetic Study in Dogs by Repeated Oral Administration for 13 Weeks
Study No: AJO 95/951761
Amendment #, Vol. #37, and page #5/169-5/353
Conducting laboratory and location

Date of study initiation: 3/30/1995
GLP compliance: Yes
QA- Report Yes (x) No ()

Species/strain: Beagle dogs
#Sex/group or time point: 3 dogs/sex/group except the high dose group that had 4 dogs/sex.
Age: 5 months old
Weight: Males (9.7-13.4 kg) and females (7.3-10.0 kg)

Dosage groups in administered units: Control (placebo), 10, 30, 100, and 300 mg/kg for 13 weeks.
Route, form, volume, and infusion rate: Oral
Drug, lot#, radiolabel, and % purity: 2G131 99.95% pure
Formulation/vehicle: Empty capsule
OBSERVATIONS AND TIMES: Please see the main studies.
Toxicokinetics: Blood sample (maximum 5ml) were collected from the jugular vein from all treated group animals on Days 1, 30, and 90 in order to determine Tmax, Cmax, C_{24}, AUC_{24}, $t_{1/2}$ and accumulation ratio. Blood samples were taken at the following time points after drug administration: 0 (pre-dose), 15, 30, 45 mins, 1, 2, 4, 8 and 24 hours. Blood samples (40 ml) were taken from all control dogs prior to termination.

RESULTS:
The rate and extent of systemic exposure of dogs to nateglinide were linearly related to drug dose in the range of 10 to 100 mg/kg/day as demonstrated in Cmax values. The approximately linear relationship did not hold true at the dose of 300 mg/kg/day as shown below. Treatment duration did not significantly affect Cmax values and accumulation ratio, which suggests nateglinide did not accumulate. Tmax was achieved in a few hours at low doses, but was observed later at high doses such as 100 or 300 mg/kg/day. There were no obvious gender differences in pharmacokinetic parameters.

Summary of mean toxicokinetic parameters of AY4166 following 13 weeks daily oral doses to dogs

Males

Dose level (mg/kg/day)	C_{max} (µg/ml)			T_{max} (hours)			C_{ss} (µg/ml)			AUC_{ss} (µg.h/ml)		
	Day			Day			Day			Day		
	1	30	90[a]	1	30	90[a]	1	30	90[a]	1	30	90[a]
10	22.6 (6.00)	23.0 (5.53)	25.3 (5.88)	3.33 (1.15)	1.17 (0.76)	2.92 (1.88)	2.40 (1.50)	2.96 (1.67)	3.34 (1.51)	155 (43.2)	201 (82.5)	233 (42.3)
30	87.4 (20.5)	79.7 (13.5)	87.9 (10.6)	2.00 (0)	2.67 (1.15)	2.00 (0)	7.08 (0.81)	9.46 (1.06)	12.5 (7.67)	617 (62.1)	793 (191)	836 (64.9)
100	248 (49.8)	236 (37.1)	183 (51.0)	2.00 (0)	4.00 (0)	3.33 (1.15)	8.76 (8.64)	16.0 (6.46)	26.9 (23.5)	1990 (196)	2290 (284)	2160 (803)
300	407 (66.9)	355 (151)	375 (43.2)	3.50 (1.00)	5.00 (2.00)	4.50 (2.52)	69.9 (33.6)	64.9 (38.2)	49.7 (29.4)	5480 (1440)	5030 (2210)	5240 (590)

Females

Dose level (mg/kg/day)	C_{max} (µg/ml)			T_{max} (hours)			C_{ss} (µg/ml)			AUC_{ss} (µg.h/ml)		
	Day			Day			Day			Day		
	1	30	90[a]	1	30	90[a]	1	30	90[a]	1	30	90[a]
10	19.6 (12.8)	19.2 (2.35)	27.8 (2.30)	2.92 (1.88)	3.00 (1.73)	2.67 (1.15)	0.55 (0.20)	1.47 (0.64)	1.12 (0.13)	117 (32.6)	136 (21.7)	164 (31.8)
30	92.1 (16.7)	82.3 (12.8)	101 (10.3)	2.00 (0)	2.00 (0)	2.00 (0)	7.54 (8.02)	5.77 (4.54)	8.17 (7.69)	510 (225)	557 (195)	727 (224)
100	225 (108)	226 (46.6)	194 (95.9)	3.33 (1.15)	4.00 (0)	10.0 (12.2)	6.24 (4.49)	7.66 (5.08)	38.8 (41.1)	1750 (871)	2040 (370)	1890 (749)
300	384 (10.7)	374 (92.6)	305 (189)	3.50 (1.00)	5.00 (2.00)	5.25 (3.40)	7.81 (4.46)	24.4 (15.3)	21.9 (17.0)	3930 (610)	4150 (1040)	3750 (2820)

Standard deviations in parentheses

[a] During Week 13

Results presented to 3 significant figures

Accumulation ratio[a] after repeated (Week 13) daily oral doses of AY4166 to dogs

Dose level (mg/kg/day)	Males		Females	
	Dog ID number	Accumulation ratio	Dog ID number	Accumulation ratio
10	267	1.7	268	1.4
	269	1.5	270	1.5
	271	1.4	272	1.3
	Mean[b]	1.5	Mean[b]	1.4
30	273	1.4	274	1.9
	275	1.4	276	1.3
	277	1.3	278	1.3
	Mean[b]	1.4	Mean[b]	1.5
100	279	0.9	280	0.9
	281	1.7	282	3.4
	283	0.7	284	0.5
	Mean[b]	1.0	Mean[b]	1.1
300	285	1.4	286	0.5
	287	0.9	288	1.3
	289	0.8	290	1.8
	291	0.9	282	0.2
	Mean[b]	1.0	Mean[b]	0.7

ID Identification
[a] Calculated from AUC_{24} (Week 13)/AUC_{24} (Day 1)
[b] Geometric mean

The terminal rate constants and terminal half-lives of nateglinide on Day 1, 30, and Week 13 of the 13-week study were quite variable due to limited sample size. The range of terminal half-lives was 3 to 7 hours, which did not appear significantly different as summarized below.

Terminal Rate Constants (k) and Half-Lives ($t_{1/2}$) of Nateglinide in 13-Week Oral Toxicokinetic Study in Dogs@							
Dose(m/k/d)	Parameter	Day 1		Day 30		Week 13	
		Male	Female	Male	Female	Male	Female
100	k	0.2043	0.1838	0.1459	0.1739	0.0914	0.1581*
	$t_{1/2}$	3.4	3.8	4.8	4.0	7.6	4.4*
300	k	0.0946	0.2050	0.1061	0.1497	0.1216	0.2282*
	$t_{1/2}$	7.3	3.4	6.5	4.6	5.7	3.0*
@Represents mean of 4 to 3 dogs. *Indicates value from a dog.							

Conclusion: The 13-week toxicokinetic study in dogs suggests that nateglindie is not likely accumulated in the system and there was no clear evidence on significant gender differences in major pharmacokinetic parameters.

Study Title: Nateglinide-A 12-Month Oral Toxicity Study in Rats
Study No: B-1978
Amendment #, Vol #24, and page # p001-534
Conducting laboratory and location:

Date of study initiation: April 8, 1991
GLP compliance: Yes
QA- Report: Yes (x) No ()

Species/strain: Crj: rat/ CD(SD)
#Sex/group or time point: 20 rats/sex/group
Age: 4 weeks old
Weight: 190-221 g (male) and 145-181 g (female)
Satellite groups used for toxicokinetics or recovery: None
Dosage groups in administered units: 0(Placebo control), 30, 100, and 500 mg/kg
Route, form, volume, and infusion rate: Oral (Gavage)
Drug, lot#, radiolabel, and % purity: 2G111, 100.1% pure
Formulation/vehicle: 0.5% methylcellulose in distilled water.
Stability: The 0.6 – 20% suspensions were confirmed to be stable for 10 days when stored under refrigeration.

OBSERVATIONS AND TIMES:
Clinical signs: This parameter was checked 4 times a day: before dosing, immediately after dosing, 2 hours after dosing and in the evening except holiday when two observations were made before and immediately after dosing.
Body weights: This parameter was checked twice a week for 13 weeks and once a week thereafter.
Food consumption: Weekly.
Ophthalmoscopy: Weekly, but examinations with mydratic agents were performed at 6 and 12 months.

Hematology: At termination.
Clinical chemistry: The blood samples collected for hematologic studies were used for the determination of the parameter at the end of treatment. Examinations were also conducted on moribund animals that were sacrificed, but the data were excluded from the statistical analysis.
Urinalysis: The urine was collected in a cage for 4 hours after withdrawal of food and water during the period. This parameter was checked at 6 and 12 months.
Auditory and ophthalmology: All surviving animals in the control and high dose groups were examined in month 6 and 12 of drug administration.
Gross pathology: Animals, which died, were dissected immediately as they were found.
Organ weights: The weights of 15 organs were determined at necropsy. The absolute weights of each organ and its weight ratio to final body weight (relative organ weights) were calculated based on 100 g body weight.

Histopathology: Complete histopathological examination was performed in all groups at termination as indicated in histopathology table on page 42 as per GRP. Organs or parts of them were submitted to microscopic examination in the case of the animals, which died intercurrently.

RESULTS:
Clinical signs:

Mortality: There were 17 deaths during the study as shown below. It appeared that most of deaths were due to accidental intubation. Three deaths, one from each dose group, were attributed to pituitary tumor, which is not uncommon in rats for one year study. The causes of three deaths in 100 mg/kg/day group were not determined, although one death was suspected to be vascular tumor in a female rat.

Mortality and Known Causes of Death in 12-Month Oral Toxicity Study in Rats#

Dose(mg/kg/day)	Sex	Death	Intubation$	Tumor@	Leukemia	Other*
30	Male	3	1	1	1	
	Female	2	2			
100	Male	3	1	1		1
	Female	3	1			2
500	Male	3	3			
	Female	3	1	1		1

and $ indicate number of dead or sacrificed rats and deaths due to intubation errs. @ and * indicate number of death due to pituitary tumor and unknown reasons, respectively.

Body weights: There were no significant differences in body weights between the control, low (30 mg/kg), and intermediate dose (100 mg/kg) groups. Body weights of males in the high dose group (500 mg/kg) were significantly ($P<0.05$) reduced from week 6 on wards (see fig.1 below). At the end of the study there was 20% reduction (765.9 vs 620.5) in the parameter in males of the high dose group. In females, the significant reduction in body weights in the high dose group females was not clear until week 32. The reduced body weights in females continued up to the week 40 when they were no longer significantly different from the control values. At the final week, the body weight of the control group was 439.9 ± 84.8 grams (N=20) while that of the high dose female group was 389.8 ± 64.9 grams (N=17), respectively.

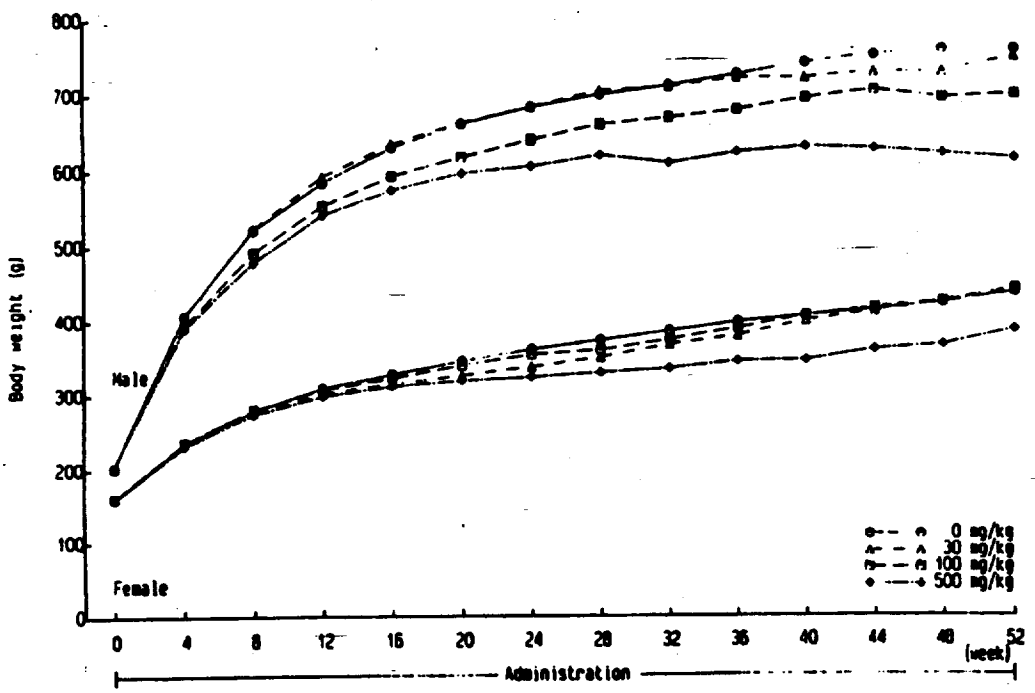


Fig.1 A 12-month oral toxicity study of AY4166 in rats
——Body weight——

Food consumption: Mean absolute and relative food consumption were not affected by the administration of the test article in either sex in the low and intermediate dose groups. The food consumption in the high dose male group was decreased (12%) at week 29 ($P<0.05$). The parameter fluctuated in males during the study period. The difference between the control and the top dose group was not significant, as indicated above, at most time points in males. At termination, there was approximately 8% reduction in the parameter in males of the top dose group. In females, the top dose (500 mg/kg) did not reduced the parameter except at the week 47 when the parameter was reduced approximately 9% ($P<0.05$).

Ophthalmoscopy: Inspection of the eyes did not reveal any macroscopically visible compound-related changes. There were also no clear changes in the auditory tests and scatoscopic examinations.
Hematology: Mild increases in hemoglobin and hematocrit was found in males of the 500 mg/kg group.

Clinical chemistry: Decreases in total cholesterol and total protein, an increase in glucose (males), and a tendency toward decrease in phospholipids (females) were observed in the 500 mg/kg group, without any correlating histopathological changes in the liver.

Urinalysis: In the 6 month urinalysis evaluation, a tendency toward an increase in ketones and a small decrease in the levels of sodium, potassium and chloride excreted observed in the 500 mg/kg group were considered to have little toxicological significance.

Organ Weights: No differences in organ weights were observed in the low or intermediate dose group of either sex. In the high dose groups, there were significant increases in heart weights (7.8%) and spleen weights (18.3%) in males, while the prostate weight was reduced (19.4%). In females, the heart weight was decreased (8.5%) while lung weights were increased (13.6%). The relative organ weights were significantly (P<0.05) increased in high dose males in almost of all organs examined except liver and spleen (Table below). The increased organ weights appear to be secondary to decreased body weights in this group. In high dose group females, the weights of pituitary, heart, spleen, kidney, adrenal, overy and uterus were not increased. An increase in relative organ weight of the adrenal glands was observed in high dose males and females.

A 12-month oral toxicity study of AY4166 in rats

Relative organ weight

Male

Dose mg/kg		Brain g%	Pituitary mg%	Salivary gl.(R) mg%	Heart g%	Lung g%	Liver g%	Spleen g%	Kidney (R) g%	Adrenal (L) mg%	Testis (R) g%	Prostate g%
0	No.	20	20	20	20	20	20	20	20	20	20	20
	Mean	0.32	4.0	60	0.24	0.28	2.46	0.13	0.26	5	0.24	0.18
	S.D.	0.03	6.0	5	0.02	0.03	0.22	0.01	0.02	1	0.03	0.04
30	No.	17	17	17	17	17	17	17	17	17	16(a)	17
	Mean	0.32	2.1	63	0.25	0.26	2.51	0.13	0.27	5	0.25	0.16
	S.D.	0.04	0.4	5	0.02	0.03	0.31	0.02	0.03	1	0.04	0.04
100	No.	17	17	17	17	17	17	17	17	17	17	17
	Mean	0.34	2.3	61	0.25	0.29	2.56	0.13	0.28	6	0.26	0.15
	S.D.	0.06	0.4	7	0.02	0.06	0.29	0.02	0.02	1	0.03	0.03
500	No.	17	17	17	17	17	17	17	17	17	17	17
	Mean	0.39**	2.7**	70**	0.28**	0.37**	2.66	0.13	0.31**	6*	0.30**	0.18
	S.D.	0.06	0.5	12	0.01	0.11	0.24	0.01	0.04	2	0.04	0.03

** : p<0.01 (Significant difference from control)

Gross pathology: Focal hepatization was seen in 4 males and 4 females in the top dose group. The focal hepatization was seen in one female in the control group, one male in the 30 mg/kg/day group, and one male and female in the 100 mg/kg/day group. Additionally white spots or patches were observed in control as well as the high dose group. In the stomach, dark red spots in the fore-stomach were noted in one male in the top dose group. Dark red spots in the glandular stomach were seen in 7 males and 5 females in the high dose groups. The findings appeared to be significant because there were only 2 female rats in the control group affected. Dark red spot at the border of the fore and glandular stomach was also seen in one female in the 30 mg/kg/day.

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Histopathology: There were no drug related histopathologic changes except mucosal and goblet cell hyperplasia in the trachea and lung, respectively. These findings were probably due to irritation associated with deposition of drug in the lungs secondary to gavage dosing.

A 12-month oral toxicity study of AT4166 in rats

Histopathological findings (Survivors)

Male

Organs	Dose (mg/kg)	0					30					100					500				
	No. of animals	20					17					17					17				
-findings	Grade	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4	0	1	2	3	4
Spinal cord																					
-focal calcification in meninx		20					16	1				17					17				
Heart																					
-focal myocarditis		13	7				13	4				14	3				14	3			
-focal fibrosis		19		1			16		1			17					16	1			
Trachea																					
-hyperplasia of mucosal epithelium		18	1	1			14	2	1			14	2	1			10	5	2		
Lung																					
-granuloma		14		6			14	2	1			16			1		11	3	1	2	
-peribronchiolitis		18	2				17					17					13	4			
-goblet cell hyperplasia		17	2	1			16	1				16		1			13	1	4		
-mucoid material in bronchiole and/or alveoli		20					17					18	1				13	4			
-atelectasis		20					17					17					16			2	
-focal fibrosis		19	1				17					17					17				
-fibrous thickening of pleura		19	1				17					17					17				
-focal bronchiolar/alveolar hyperplasia		19		1			17					17					17				

0 : No remarkable changes 1 : Slight 2 : Mild 3 : Moderate 4 : Severe

In female rats, histopathological findings are not remarkable except a few incidence listed below. Mild necrosis in mucosal epithelium of trachea and bronchia in one female of the high dose group was observed. Mild hyperplasia of the mucosal epithelium was noted in control as well as treated groups. The observations were also true for goblet cell hyperplasia. Perilobular vacuolation of hepatocytes were seen many animals including control rats. Centrilobular vacuolation (one female in the 30 mg/kg group), proliferation of the bile duct in several animals in different group including control, focal fibrosis and focal hemorrhage were noted in control as well as treated animals without specific indications. Other minor findings in different organs such as heart (focal myocarditis), small intestine (granulomatous inflammation), pancreas (atrophy of the acinar cells), kidneys (chronic nephropathy), and female reproductive organ were scattered in control as well as the treated group so that the findings appeared to be incidental.

Toxicokinetics: The sponsor did not provide toxicokinetic data for this study. Please see the data in page 29 for TK data.

Conclusions: An oral administration (gavage) of 500 mg/kg of nateglinide over one year depressed body weight gains and decreased in food consumption in HD males. Some histopathological changes in lung were observed more frequently in the HD rats, probably secondary to lung irritation associated with chronic gavage dosing. The relative weights of a dozen organs in males were increased significantly, secondary to a decreased body weight. The intermediate and low doses of nateglinide did not produce any significant changes in most parameters evaluated. Thus, NOAEL of nateglinide administered for one year

appears to be 100 mg/kg/day, which is approximately 2 - 3 times human therapeutic AUC exposure based on recommended daily dose of 120 mg tid.

Study Title: Nateglinide –A 12-Month oral toxicity study in beagle dogs
Study No: [redacted]B-1976
Amendment #, Vol. #38, and page #5/83-5/344
Conducting laboratory and location: [redacted]
Date of study initiation: 4/10/1991
GLP compliance: Yes
QA- Report: Yes (x) No ()

Species/strain: Dog/Beagle
#Sex/group or time point: 4 dogs/sex/group
Age: 6 months old
Weight: Males (8.6 -10.0 kg) and females (7.7- 9.4 kg)

Dosage groups in administered units: Control (placebo), 10, 30, and 100 mg/kg
Route, form, volume, and infusion rate: Oral
Drug, lot#, radiolabel, and % purity: 2G111, 100.1% pure
Formulation/vehicle: Capsule/gelatin
OBSERVATIONS AND TIMES:
Clinical signs: Once a day before drug administration and 4 times a day after the administration except holidays and Saturdays when observations were made up to 2 hours after dosing only.
Body weights: Weekly up to 3 months, and bi-weekly from 3 months to study termination.
Food consumption: Weekly
Hematology: 2 and 1 week before the start of administration and in months 3, 6, 9, and 12 after the dosing. Blood samples were collected from the cephalic vein.
Clinical chemistry: This parameter was checked at 3, 6, 9, and 12 after the dosing.

Urinalysis: 2 and 1 week before the start of administration and in months 3, 6, 9, and 12 after the dosing.
Gross pathology: Autopsy was performed in pentobarbital-anesthetized dogs.
Organ weights: The parameter was determined at necropsy and organ to body weight ratio was calculated.
Histopathology: Tissues were fixed in 10% formalin or 4% glutaraldehyde for histopathological studies. Please see Inventory Table page 42 for tissues evaluated.
Toxicokinetics: The sponsor did not perform TK analysis for this study(see page 32 for dog TK data for 10, 30, 100 mg/kg/day doses).
Statistical analysis: Homogeneity of variance of data was tested using Barlett's test.

RESULTS:

Clinical signs: No mortality. Vomiting and salivation were noted in all groups including the control. Vomit and stools contained a white substance at times.

Body weights: There were no significant differences in body weight between the control group and treatment groups throughout the administration period.

Food consumption: A slight decrease in food consumption was sporadically seen, but no relation to administration of the test article was noted.

Ophthalmoscopy: There was no change in opacity of the refracting media of the eyes.

Electrocardiography: Not remarkable

Clinical chemistry:

A decrease in blood glucose due to the pharmacological effect of the test article was observed at ≥30 mg/kg. A significant reduction in CPK activity was seen in females in the 30 and 100 mg/kg groups in month 3 of administration and low values for α1-globulin in males in the 100 mg/kg group in month 6 were noted. The values were all within physiological range and were not reproduced at the various measurement times. In week 6 clinical pathological examinations, an increase in plasma GPT or GOT activity and increased bilirubin in the blood chemistry and qualitative urinalysis were observed in the high dose group. At the same time, an increase in GPT activity was noted in one animal in the 300 mg/kg group.

Plasma clearance of indocyanine green and phenolsulfonphthalein were not different from controls, indicating that nateglinide had no effect on liver and renal function. There were no remarkable abnormal changes in any of the other parameters.

Urinalysis: A significant decrease in specific gravity was seen in males in the 30 mg/kg group in month 12. The change was not dose-dependent because no changes were observed in the 100 mg/kg group. Thus, this finding was considered to be incidental.

Gross pathological findings: Gross and microscopic findings included yellowing of subcutaneous and constitutional fat and bile plugs in the bile canaliculi of the liver in one male and one female of the high dose group. Gallbladder distention was noted in two dogs at high dose. In addition, erosion of the stomach was observed in the female as well as ulceration in the duodenum of both animals in the high dose group. There were sporadic changes in 11 tissues in the control and treated groups without clear dose-dependent or treatment-related abnormalities. Atrophy of the right epididymis was seen in one male in the control group and a cyst (2 mm) in the pituitary in one male in the 30 mg/kg group was noted. White surface change (10 mm x 2 mm) in the spleen in one male of the 10 mg/kg group was also noted. They were all considered to be incidental due to the nature and rate of incidences. There were no remarkable gross pathological findings that were attributable to the treatment.

Organ Weights: The relative weight of the left epididymis in males in the 30 mg/kg group was low without dose-dependency. There were no changes that appeared to be treatment-related.
Histopathology: The sponsor evaluated the histopathological findings in the control and high dose groups. In males, there was moderate duodenal ulceration in 2 out of 4 high dose dogs. One of the dogs also had a mild mucosal atrophy in jejunum, ileum, cecum and colon. All animals had mild to severe glycogen content in hepatocytes, which was true in all females. In addition, three out of four HD females had mild adrenal hypertrophy of zona fasciculata.

Conclusions: There were no deaths and no consistent changes related to administration of the test article in body weights, food consumption, urinalysis, hematology, liver and renal function, ophthalmology, electrocardiograms, or gross pathology findings. Salivation was observed in the 100 mg/kg group and a reduction in blood glucose was noted as an extension of pharmacological action of nateglinide. Histopathologic findings observed frequently in high dose group included duodenal ulceration in 2/4 males and hypertrophy of the adrenal cortex in ¾ females. Nateglinide did not produce remarkable toxicities which were attributable to the drug in dogs. It appears that NOEL in dogs treated for a year was 30 mg/kg/day, which is approximately 25 times human therapeutic AUC exposure based on recommended daily dose of 120 mg tid.

Addendum 1:Histopathology Inventory for NDA#21-204

Study	B-1978		B-1976	B-1980
Species	Rat		Dog	Dog
Adrenals	X		X	X
Aorta			X	X
Bone Marrow smear			X	
Bone (femur)	X			
Brain			X	X
Cecum	X		X	X
Cervix				
Colon			X	X
Duodenum			X	X
Epididymis	X		X	X
Esophagus			X	X
Eye	X		X	X
Fallopian tube				
Gall bladder			X	X
Gross lesions			X	X
Harderian gland				
Heart	X		X	X
Hyphophysis				
Ileum			X	X
Injection site				
Jejunum			X	X

Kidneys	X		X	X
Lachrymal gland			X	
Larynx			X	
Liver	X		X	X
Lungs	X		X	X
Lymph nodes, cervical				
Lymph nodes mandibular			X	X
Lymph nodes, mesenteric			X	X
Mammary Gland	X			
Nasal cavity				
Optic nerves			X	X
Ovaries	X		X	X
Pancreas	X		X	X
Parathyroid			X	X
Peripheral nerve				
Pharynx				
Pituitary	X		X	X
Prostate	X		X	X
Rectum			X	X
Salivary gland			X	
Sciatic nerve			X	X
Seminal vesicles	X		X	X
Skeletal muscle				
Skin			X	X
Spinal cord	X		X	X
Spleen	X		X	X
Sternum	X		X	X
Stomach	X		X	X
Testes	X		X	X
Thymus			X	X
Thyroid	X		X	X
Tongue				X
Trachea	X		X	X
Urinary bladder			X	X
Uterus	X		X	X
Vagina			X	X
Zymbal gland				

IV. CARCINOGENICITY

Study Title: Nateglinide: 2-Year Carcinogenicity Study in Mice
Study#94/0143/ Schedule#YOC/036/Report#YOC036/0143
Volume Number/Page: 17/p0001– p1542
Test Facility: [ ]
Study Date(s): December 5, 1991 – December 13, 1993
Date of Submission: June 30, 1994
GLP Compliance: Yes

QA Report- Yes (x) No ()
Study Type: Regular
Species/strain: Mice/B6C3F1
Number of animals per group: 55/sex/group
Age at start of study: 4-5 weeks old mice

Animal housing: Individually in [redacted] cage [redacted] in an air-conditioned room.
Drug Lot/Batch number(s): Total 10 kg of batch# 2G121
Drug Purity / Stability / Homogeneity: 98-102% / 93-101% at 21°C as reported (YOC034/0923) / 99.2-102% in diet

DOSES: The animals were fed nateglinide at doses of 0.1, 0.3, and 1% for 23 weeks when the sponsor reduced the doses to 0.03, 0.1 and 0.3% due to excessive body weight loss. The doses for males were equivalent to 178, 535, and 1991 mg/kg/day and 233, 690, and 2552 mg/kg/day for females. After 24-week, the doses were 37, 124, and 390 mg/kg/day and 36, 123, and 409 mg/kg/day for males and females, respectively. The group means plasma levels of nateglinide were 1.2, 4.4, and 14.2 for males and 4.7, 9.0, and 47.5 μg/ml for females at the end of the treatment period.

Basis of Dose Selection:

In a 13-week toxicity study in B6C3F1 mice, the sponsor demonstrated that nateglinide reduced body weight at 1 and 2% concentrations in diet. The animals tolerated 0.5% of the drug in diet. Therefore, they selected 0.1, 0.3, and 1.0 % of nateglinide in 6-month toxicology study. There were no deaths in the drug-treated groups, although there were 8 deaths out of 20 rats in positive control (acrylamide) group. Therefore, they initially selected 0.1, 0.3, and 1.0 % in diet, which produced unacceptably high reduction in body weight. The findings forced them to decrease the doses to 0.03, 0.1, and 0.3% for the low, intermediate, and high doses, respectively at week 24.

There were drug-dose dependent decreases in survival rate in females, which appear to justify the high dose as the MTD. In males, the high dose actually increased the survival rate in a dose-dependent fashion. The top dose reduced body weight by approximately 10% in males. Other experimental designs or procedures appeared to be acceptable. The drug doses with 0.1, 0.3 and 1.0% in diet were equivalent to 178/233, 535/690, and 1991/2552 mg/kg in males/females, respectively. The amended doses of 0.03, 0.1 and 0.3% in diet were equivalent to 37/36, 124/123, 390/409 mg/kg in males/females.
Relation to Clinical Use: Starlix reduces plasma glucose level as an ATP dependent K+ channel blocker in laboratory animals. It might improve glucose tolerance with minimal risk of hypoglycemia and weight gain.

CAC Concurrence: No
Restriction Paradigm for Dietary Restriction Studies: NA
Route of Administration: Oral administration in diet
Frequency of Drug Administration: Daily

Dual Controls Employed: No
Interim Sacrifices: Yes
Satellite PK or Special Study Group(s): 15 male and 15 female mice served as satellite animals for toxicokinetic examinations.
Unscheduled Sacrifices or Deaths: Unscheduled animal death was processed for drug evaluation when the animals were moribund or dead.
Deviations from Original Study Protocol: Not noted.

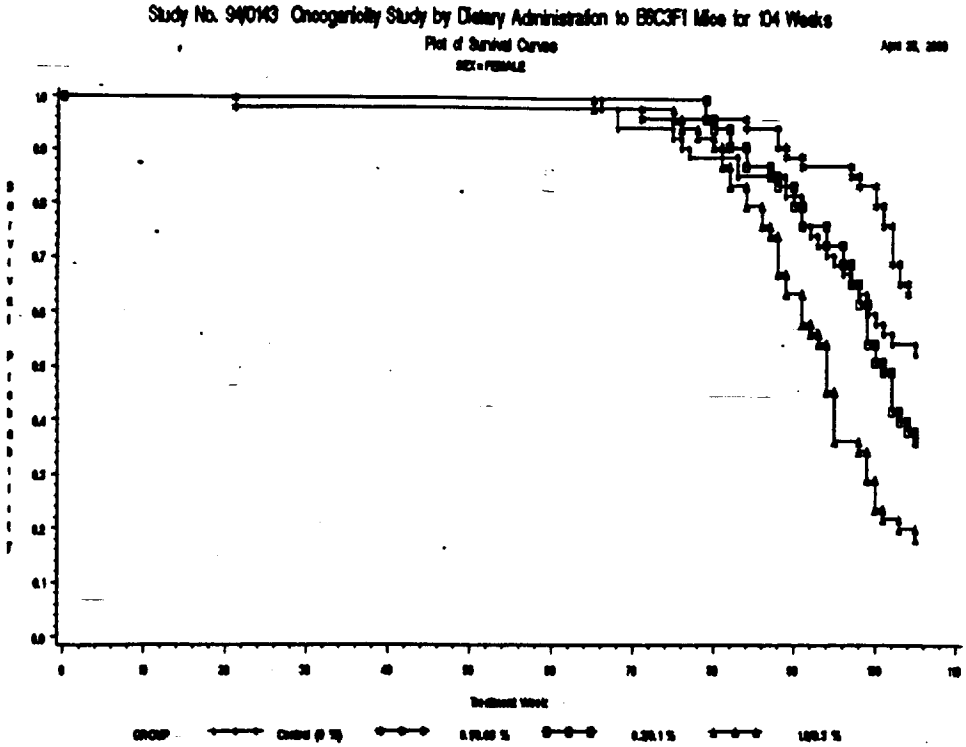
STUDY RESULTS AND FREQUENCY OF MONITORING:

Clinical Observations:
Animals were inspected at least twice daily for evidence of reaction to treatment or ill-health and mortality.
Mortality:
Debilitated animals were observed and animals judged to be in extremis were killed. Animals were also sacrificed to prevent unnecessary or prolonged suffering. Where possible, blood samples were taken ante mortem and analyzed for the characterizations. A complete necropsy was performed in all cases. There was a dosage-related increase in mortality among females receiving the intermediate or highest concentrations when compared to their controls. Please see the table below and subsequent figure. In case of males, nateglinide increased significantly the rate of survival in the high dose group as shown in a table below.

Effects of Nateglinide on Survival Rate in 2-Year Carcinogenicity Study in Mice

Dose group:	Control		0.1/0.03%		0.3/0.1%		1.0/0.3%	
Sex of Mice	M	F	M	F	M	F	M	F
Initial no. of Mice	55	55	55	55	55	55	55	55
week 50	55	55	54	54	55	55	54	55
week 65	53	55	51	54	55	55	53	54
week 78	50	49	46	53	52	55	52	51
week 91	38	42	36	48	42	42	49	32
week 104	24	30	22	35	27	21	41*	11*
Survival (%)	44	60	40	64	50	38	75*	20*

*Indicates P<0.001, compared to the control group.



The animals killed or dying during the 2-year carcinogenicity study were examined to identify the causes of deaths. In both sexes, hepatocellular carcinoma, malignant lymphoma, and hemangiosarcoma were the most common cause of deaths. The incidences were comparable in control groups and treated groups, which indicated nateglinide was not responsible for the malignancies. However, there were 19 incidences of peripheral neuropathy in the high dose group of females (See table below). The increased incidence of peripheral neuropathy, characterized by axonal degeneration and Schwann cell proliferation, was reported in females treated at the intermediate (0.3/0.1%) and high (1.0/0.3%) dose levels.

Sustained, severe hypoglycemia can result in neuropathy. For example, continuous infusion of insulin into mice at doses which resulted in severe (in some animals lethal) hypoglycemia induced peripheral neuropathy in up to 60% of the animals within a 2-week treatment period. In the mouse carcinogenicity study, plasma glucose levels were retrospectively analyzed in samples collected for toxicokinetics. The results showed great variation, but a trend to decreased glucose levels was observed in the high dose females. Survival was also decreased in females in these dose groups. Although the incidence of peripheral neuropathy in male mice of the highest dose group was somewhat higher than that of control animals, this was not considered remarkable.

Findings as Causes of Death in 2-Year Carcinogenicity Study in Mice								
Nateglinide Dose	Control		0.1/0.03%		0.3/0.1%		1.0/0.3%	
Animal Sex	M	F	M	F	M	F	M	F
Hepatic cellular necrosis	0	0	0	2	0	0	0	0
Hepatic lobar necrosis	2	0	0	0	0	0	0	0
Hepatocellular carcinoma	6	7	4	4	3	3	2	2
Hepatic lobar torsion	0	0	1	0	0	0	1	0
Hepatic blastoma	0	0	1	0	0	0	0	0
Hemangiosarcoma	3	2	6	0	1	1	1	0
Malignant lymphoma	2	2	4	2	10	5	4	0
Pulmonary carcinoma	2	2	0	1	1	1	3	1
Peripheral neuropathy	0	2	0	0	0	0	3	19
Ovarian hemorrhage	0	0	0	0	2	1	1	0
Uterine abscess	0	0	0	0	1	0	0	0
Ovarian teratoma	0	0	0	0	0	1	0	0
Skin carcinoma	0	0	0	0	0	1	0	0
Skin sarcoma	0	0	0	0	0	2	0	0
Squamous cell carcinoma	0	0	0	0	0	0	1	0
Skin abscesses	0	0	0	0	0	1	0	0
Tongue keratoacanthomata	1	0	0	0	0	0	0	0
Tongue basal cell carcinoma	0	0	0	1	0	0	0	0
Number of mice examined	33	34	29	14	26	20	35	45

The high mortality in female mice is associated with high AUC ratio as shown below.

Dose in %(diet)	Dose in mg/kg	AUC(μg.h/ml)		AUC ratio*	
		Male	Female	Male	Female
0.03	37	28.8	112	0.81	3.2
0.1	124	106	216	2.99	6.1
0.3	400	341	1149	9.63	32.4

*Based on an AUC value of 35 μg.h/ml for a daily dose of 120 mg, tid.

Body Weight: The parameter was checked three days before treatment, on the first day of treatment, and at weekly intervals during the first 14 weeks and biweekly for the remainder of study. Over the first 22 weeks of treatment significantly ($P<0.01$) lower weight gains were observed in all treated groups except females receiving the low dose. Nateglinide dose was subsequently reduced due to its effect on body weight. From Week 24 high weight gains were noted in treated males receiving the intermediate and high doses. Over this period in females receiving the high dose, the parameter remained significantly low, while the low and intermediate doses had no clear effect on body weight. Overall weight gains for the two years were 12% and 29% lower than the control for males and females receiving the high doses respectively as shown below.

Therefore, the high doses in both sexes appear to be the maximum tolerated dose.

Effects of Nateglinide on Bodyweight of Male Mice in 2-Year Carcinogenicity Study*					
Treatment Duration(WK)	Nateglinide Dose(% in Diet)				% Reduction Bodyweight#
	Control	0.1/0.03	0.3/0.1	1.0/0.3	
0	21.3	21.2	20.7	20.8	2.4
5	28.0	27.1	26.0	25.8	7.9
10	33.1	31.8	30.2	28.9	12.7
20	41.0	39.4	37.8	34.9	14.9
30	44.9	44.6	43.7	40.2	10.5
40	48.0	47.6	46.8	43.8	8.7
50	50.3	4934	48.3	45.0	10.6
60	49.8	49.5	48.8	45.7	8.3
70	49.6	50.0	48.9	45.8	7.7
80	49.4	49.7	49.4	45.8	7.3
90	48.7	48.2	47.3	45.0	7.6
100	47.1	45.6	45.5	43.4	7.9

*Bodyweight in grams. # Denotes % reduction in mean bodyweight in the high dose group.

Food Consumption: The parameter was checked weekly. During the first 23 weeks of treatment there was no clear effect on the quantity of food consumed by treated animals. After the reduction of nateglinide in Week 23, the food consumption of males receiving the high dose was rather stable without fluctuation. The parameter in females fluctuated with 10 to 15% changes in both directions. Cumulative food consumption for each group is summarized in the table below. It appears that efficiency of food conversion in high dose of animals was not significantly different from controls in both sexes.

Effects of Nateglinide on Food Consumption in 2-Year Mice Carcinogenicity Study#								
Period Week	Sex of Animals and Nateglinide Dose in Diet(%)							
	M, 0	0.1/0.03	0.3/0.1	1.0/0.3	F, 0	0.1/0.03	0.3/0.1	1.0/0.3
1-23	934	894*	862*	924	972	940	917*	976
%	100	96	92	99	100	97	94	100
24-104	3407	3293	3288*	3215*	3422	3412	3283*	3281
%	100	97	97	94	100	100	96	96
1-104	4353	4209	4148*	4138*	4277	4362	4200*	4272
%	100	97	95	95	100	100	96	98

Indicate grams of food/animal and * $P<0.05$, compared to controls. M and F indicate male and female mice.

Achieved Dosage and Nateglinide Blood Level: The achieved dosages of nateglinide in treated groups are presented in the table below. The levels of drug intake decreased as the doses in diet were reduced in week 23.

Achieved Nateglinide Dosage in 2-Year Mice Carcinogenicity Study#								
Period Week	Sex of Animals and Nateglinide Dose in Diet(%)							
	M, 0	0.1/0.03	0.3/0.1	1.0/0.3	F, 0	0.1/0.03	0.3/0.1	1.0/0.3
1-23		177.5	535.1	1990.5		233.3	690.1	2551.6
24-104		36.9	123.7	389.9		35.7	123.2	408.8
1-104		68.0	214.6	743.9		79.4	248.6	882.7

Indicate mg/kg/day of Nateglinide.

Analysis of plasma samples taken after 104 weeks of treatment for nateglinide indicated positive absorption of the drug. The drug concentration was generally related to the dietary concentration; the plasma level was higher in samples taken at 0:00 hours than at 07:00 hours in all cases as shown below. The plasma levels were higher in females than in males on all but one occasion, indicating higher systemic exposure in females.

Nateglinide Plasma Level at the End(104 Weeks) of 2-Year Mice Carcinogenicity Study*						
Sex	Male			Female		
Dose	0.1/0.03	0.3/0.1	1.0/0.3	0.1/0.03	0.3/0.1	1.0/0.3
00:00@	1.2 ±0.7	4.2 ±2.2	14.2 ±2.9	4.7 ±1.7	9.0 ±4.3	47.5 ±10.5
07:00#	0.7±0.3	2.9 ±1.6	4.6 ±2.8	1.2 ±0.5	5.2 ±3.3	30.3 ±9.3

*The unit was in μg/ml plasma. @ And # indicate time (GMT) when the blood samples were taken.

Ophthalmoscopy: There was no remarkable observation in the parameter.
Hematology: This parameter was checked at day 0 and final week (105 and 107 weeks). In general, the hematological parameters were not affected remarkably by the treatment.
Clinical Chemistry: There were no remarkable blood chemical changes in treated animals.

Organ Weights:
Bodyweights at termination were reduced significantly in males (45.2 vs. 41.3 grams) and in females (56.1 vs. 45.9 grams). The absolute organ weight was determined at necropsy and relative organ weights were calculated. The absolute left and right kidney weights were reduced significantly in both sexes as indicated below. In males, the weights of seminal vesicles and the heart were also significantly reduced, compared to the controls. In females, spleen weight was reduced significantly. The findings were confirmed in the analysis of relative organ weight comparisons. The changes in absolute and relative organ weights

appeared significant and consistent, but the significance is questionable because there was no clear evidence that the changes were drug-dose related in both sexes.

Effects of Nateglinide on Absolute Organ Weight in 2-Year Mice Carcinogenicity Study@

Sex	Male Mice				Female Mice			
Dose	Cont.	0.1/0.03	0.3/0.1	1.0/0.3	Cont.	0.1/0.03	0.3/0.1	1.0/0.3
Mice#	22	21	26	41	29	35	20	10
Heart	0.274	0.256	0.261	0.248*	0.233	0.231	0.216	0.212
Kidney	0.916	0.890	0.864	0.794*	0.609	0.612	0.569	0.542*
Sem&	2.106	2.580	1.716	0.785*				
Spleen	0.169	0.129	0.128	0.212	0.353	0.415	0.479	0.197*

@ The weight in grams. # and & indicate number of mice available at termination and seminal vesicles, respectively. *P<0.05.

Gross Pathology: Dead animals or mice killed intercurrently on the day after the last dose was subjected to macroscopic exam. There were changes, which were not drug-dose related. For example, there were pale kidneys in 9 control mice and drug treatment reduced the incidence to 2, 3, and 3 in the low, intermediate and high dose groups. Ovarian weight was reduced in 5 control and high dose females. The incidence of fluid in abdomen was reduced in treated groups, of which significance is not clear. The incidence of thin build was higher among female mice, secondary to body weight decreases.

Histopathology and Tumor Findings: Standard microscopic examination was performed. There were no neoplastic findings, which were attributed to nateglinide treatment in either sex of mice.

Non-Tumor Findings: There was an increased incidence of peripheral neuropathy (axonal degeneration and Schwann cell proliferation) in the sciatic nerves of intermediate and high dose female mice as shown below. The findings were also observed infrequently in the brachial nerves of animals receiving the high dose.

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Effects of Nateglinide on Peripheral Neuropathy in 2-Year Mouse Carcinogenicity Study									
Dose(%)		C	0.1/0.03	0.3/0.1	1.0/0.3	C	0.1/0.03	0.3/0.1	1.0/0.3
Mice Sex		Male				Female			
L S N	#	55	55	55	54	55	53	55	55
	Minimal	9	4	10	11	9	10	14	4
	Slight	1	5	5	5	2	2	19*	23*
	Moderate	0	1	0	0	0	2	6*	20*
	Marked	0	0	0	0	0	0	0	1
	Total	10	10	15	16	11	14	39*	48*
B N	#	21	7	10	35	27	0	0	11
	Minimal	4	2	2	4	2	0	0	4*
	Slight	0	0	2	4	0	0	0	2
	Moderate	0	0	0	0	0	0	0	1
	Total	4	2	4	8	2	0	0	7*

LSN and BN stand for left sciatic nerve and brachial nerve. # Indicate number of mice examined.* P<0.05.

Toxicokinetics

OVERALL EVALUATION:

The 2-year carcinogenicity study in mice was performed under acceptable conditions. The doses for the female were justified based on mortality and body weight reduction was the basis of dose selection for the male. The 2-year carcinogenicity study in mice demonstrated nateglinide was not tumorigenic.

Adequacy of the carcinogenicity studies and appropriateness of the test model: In a 13-week toxicity study in B6C3F1 mice, the sponsor demonstrated that nateglinide reduced body weight at 1 and 2% concentrations in diet, while animals tolerated 0.5% of the drug. Therefore, doses of 0.1, 0.3 and 1.0 % of nateglinide were selected for the 6-month toxicology study. There were no deaths in the drug-treated groups in the 6-month study. Therefore, Novartis initially selected 0.1, 0.3 and 1.0%, which produced unacceptably high reductions in body weight. The body weight reduction resulted in decreasing the doses to 0.03, 0.1, and 0.3% as low, intermediate, and high doses at week 24. There was drug-dose dependent decrease in survival rate in female mice, which justified the dose selections for the 2-year carcinogenicity study. In males, drug treatment increased survival. The high dose reduced body weight by approximately 10% in high dose male mice establishing this dose as the MTD.

Conclusion:

There was no evidence of carcinogenicity in mice treated with nateglinide in the 2-year carcinogenicity study.

Major Tumor Findings: None
Non-neoplastic Findings: Findings observed appears significant only in sciatic nerve of female mice.
Biological Significance: None
Potential Clinical Implications of Findings: None

Study Title: Carcinogenicity Study in Rats (Experiment I)
Study Number/Report#/Document#: AJ078/942147
Volume Numbers/Page: 25: pp5-1/5-308
Test Facility:
Study Date(s): 5/2/1992-4/2/1994
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: 56/sex/group; 6 weeks old
Animal housing: 4 rats of same sex/cage, which was
cages.
Drug Lot/Batch number(s): 2G124
Drug Purity / Stability / Homogeneity: 98.37%

DOSES: Control, Placebo, Nateglinide (0, 625, 1250, 2500 PPM)

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%). At these doses, nateglinide absorptions 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 10% below the control level in males at 100 mg/kg/day and reduced in both sexes at 500 mg/kg/day (26% and 18% for males and females, respectively). The studies indicated that the maximum tolerated oral (gavage) dose was greater than 300 mg/kg/day and less than 500 mg/kg/day. However, the sponsor performed the first set of 2-year carcinogenicity study of Nateglinide at doses of 625ppm, 1250ppm and 2500ppm, which gave AUC ratios less than 2 times human exposure as shown below. The top dose appeared to be below the MTD.