Results:

- No mortality, no signs, ophthalmic effects, body weight effects, serum chemistry, urine chemistry, macroscopic or microscopic effects
- PTH decreased at 2h post dose beginning after first dose in all groups. PTH levels reduced in HDm and in MDf, HDf on Day28.
- Serum ionized calcium slightly reduced (17%) in MD, HD, on Day 27, at 2-4h post dose up to 8h post dose.
- No effects on serum or urine bone markers (resorption or formation)
- Increase in cortical bone area, decrease in trabecular bone area of femur in MDf and HDf
- Increase in total metaphyseal BMD of femur in HDf
- Increase in kidney weight in HDm
- Decrease in platelet count in HDf after recovery
- AUC parent: 21, 72, 220 ngxh/mL in LD, MD, HD (Day 1). AUC decreased after 27 days

Conclusion:
Potential changes in bone resorption/formation resulting in relative increase in cortical bone and increase in BMD at low AUC levels (100-200 ngxh/mL). This may be related to decrease in PTH and reduced bone resorption. Suppressed bone resorption in young animals may lead to growth effects or effects on bone marrow and hematopoiesis.

<u>28-day oral toxicity study in juvenile dogs with a 28-day recovery period (Study Nr. 101938)</u>
Methods:
Juvenile dogs (10 weeks, N=7/s/g) were dosed by oral gavage at 0, 0.5, 1.5, 5 mg/kg/day for 28 days, or for 28 days + 28 days recovery. The HD of 5 mkd was the NOAEL in the 28-day adult dog study. Plasma concentrations, ionized Ca, and PTH, and bone biomarkers (serum, urine) were measured. Femur was analyzed by pQCT.

Results:

- No mortality, no signs, ophthalmic effects, body weight effects
- No effects on hematology, coagulation, urinalysis, urine chemistry, organ weights, macroscopic findings
- No effects on bone markers or pQCT parameters (BMC, BMD, area)
- Slight increase in serum P in HDm on D27, slight decrease in ionized calcium in MD, HD on D0, D27. No clear effects on PTH
- Heart (post recovery): left ventricular arterial hypertrophy, in all groups including control but with increased incidence and severity in MDm, HDm,f. Multifocal left ventricular myocardial fibrosis with minimal arterial hypertrophy in 1/7 HDm
- AUC parent: dose-dependent exposure in all dose groups. AUC decreased on D27 as compared to D1 (autoinduction?). AUC values 4.5, 15.8, 62 ngxh/mL in LD, MD, HD. No sex difference.

Table 8. Summary of Cardiac Findings in the 28-Day Juvenile Dog Study at the Recovery Period

	Incidence							
	Male				Female			
Dose (mg/kg)	0	0.5	1.5	5	0	0.5	1.5	5
n	3	3	3	3	3	3	3	3
Left Ventricular Arterial Hypertrophy								
Minimal	1	1	1	1	1	0	0	1
Slight	0	0	1	0	0	0	0	1
Moderate	0	0	0	1	0	0	0	0

RAT AND MONKEY TOXICITY STUDIES OF ≥3-MONTH DURATION

A 26-WEEK ORAL GAVAGE TOXICITY STUDY OF AMG 099073-01 IN THE ALBINO RAT WITH A 4-WEEK RECOVERY

Project No. 88583; Sponsor Study Nr. 100082
Conducting laboratory: [redacted]
Study period: March – October 1998
GLP compliance: Y
QA report included
Lot Nr. 709001 , [redacted]. Purity: 99.7%
Assay of test compound (in vehicle-prepared dosing solution): HPLC
Vehicle/control: 0.5% methylcellulose in distilled water (batch nr. 126H1424)

METHODS

Sprague-Dawley CD (Crl:CDR(SD)BR) rats (*Rattus norvegicus*) (20 or 15/sex/dose group, 15 for experiment, 5 for recovery), age at start 6 weeks, body weight 130-220 gram, were dosed orally, by gavage, with **0, 5, 25, 100 mg/kg/day**, for 26 weeks (10 ml/kg/day). Additionally, 6/sex/grp were dosed for TK and ionized Ca evaluation. Feeding was ad libitum. pQCT scans of right distal femur (metaphysis, diaphysis) performed ex vivo in Wks 26, 31. Histopathology at sacrifice (see Appendix I). Toxicokinetics on Day 1, Wks 13, 26, 31: predose, 0.5, 1, 2, 4, 8, 12, 24h postdose. Samples used for assay of drug assay (LC-MS/MS) and ionized Ca determination.

Dose groups

Group	1	2	3	4
	control	LD	MD	HD
Dose (mg/kg/day):	0	5	25	100
N/sex/group (main study)	20+6	15+6	15+6	20+6
N/sex (terminal sacrifice)	15	15	15	15
N/sex (recovery)	5	0	0	5
N/sex (PK, Ca)	6	6	6	6

RESULTS

Mortality – None

Signs – Main: Salivation and abnormal respiratory sounds in MD and HD

Body weight -

Body weight (g)	control	LD	MD	HD
Day –1				
males	194	196	191	197
females	155	155	156	155
Day 182				
males	617	624	578	460*
females	329	326	331	271*
Day 210 (Recovery)				
males	616	-	-	503*
females	352	-	-	303*

*statistically significantly different from control

Body weight gain (g) (average)	control	LD	MD	HD
Day (–1)-182				
males	423	428	387 (91%)	263 (62%)
females	174	171	175 (100%)%)	116 (67%)

Food consumption - reduced by 0-5% in MDm, and by 10-20% (g/animal) in HDm and HDf throughout treatment period; no significant differences in recovery period

Ocular finding - Cataracts (small, multifocal opacities in anterior cortex) in almost all Group 4 animals, on Day 88/87, Day 172/171, and recovery, similar at all times

Hematology

	Neutrophils (%)		MPV		PT		APTT	
Wk27	m	f	m	f	m	f	m	f
Control	14.6	14.0	8.5	8.4	14.6	14.0	18.5	15.7
LD	14.5	11.7	8.6	8.6	15.1	14.5	19.0	16.5
MD	13.4	12.6	8.6	8.6	15.7*	14.6	19.3	16.7
HD	21.5	26.2	8.1*	7.7*	15.8*	15.3*	19.4	19.7*
Wk31								
Control	15.6	20.8	8.5	8.2	1.4	14.5	20.1	16.7
HD	31.0	16.2	8.2	8.2	16.7	15.3	20.0	16.1

*statistically significantly different from control

Clinical Chemistry

	BUN		creatinine		ALT		CK		Ca		P	
Wk27	m	f	m	f	m	f	m	f	m	f	m	f
Control	11.5	11.8	0.6	0.6	34	39	733	729	9.7	10.2	6.2	5.7
LD	13.7	12.3	0.6	0.7	32	47	588	588	9.6	10.2	6.9*	6.4
MD	12.4	14.3*	0.6	0.7	36	54	608	347*	9.0*	9.7*	7.8*	7.1*
HD	18.0*	18.2*	0.7*	0.8*	52*	64*	295*	280*	6.8*	6.6*	10.8*	11.0*
Wk31												
Control	13	15.4	0.6	0.6	34	78	452	255	9.8	10.8	6.2	6.0
HD	16*	14.9	0.6	0.6	34	41*	254	191	10.0	10.4	7.5	7.7*

Other finding: glucose decreased slightly but significantly in HD groups, in Wk 13 and Wk 27 in males, in Wk27 in females

Urine electrolytes (meq/L)

	Ca		Na		K	
Wk27	m	f	m	f	m	f
Control	11	46	77	77	222	211
LD	22*	43	81	64	237	181
MD	38*	93*	57	42*	277*	210
HD	54*	83*	44*	49*	151*	142*
Wk31						
Control	9	38	71	43	224	190
HD	16	36	77	70*	195	226*

Serum PTH

- Males: Serum PTH decreased from ca. 60-80 pg/mL (predosing) to < 20 pg/ml (wks 1-13) or <40-50 pg/mL (wk26) @2hrs after dosing, in non-dose-related manner.
- Females: Decrease from 40-80 pg/mL (predosing) to <20 pg/mL (wks 1-4), or <50 pg/mL (wk 13), or <60 pg/mL (wk 26) @2hrs post dose, also non-dose-related manner.
- Predose levels were slightly lowered in Wks 4-13 in HD groups, but returned to control levels in all dose groups by Wk 26. Apparently, some adaptation in PTH response occurred.

Ionized calcium

Ionized Ca (mg/dL) (not normalized for pH)

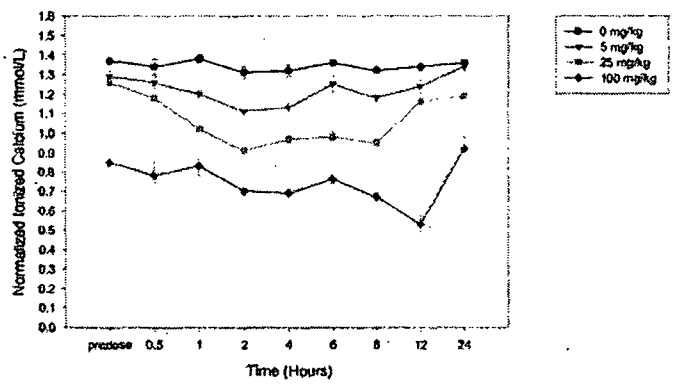
	predose	2h	8h	24h		Predos	2h	8h	24h	

						e				
MALES										
Day1					Wk26					
Control	5.5	5.7	5.1	5.5		5.2	5.0	5.0	5.2	
LD	5.3	4.6	4.4	5.2		5.0	4.1	4.5	4.9	
MD	5.2	3.9	3.6	5.2		4.6	3.6	3.7	4.6	
HD	5.3	3.6	3.3	4.0		3.2	2.7	2.6	3.4	
FEMALES										
Day1					Wk26					
Control	5.4	5.3	4.9	5.2		5.2	5.0	5.1	5.2	
LD	5.1	4.7	4.3	5.2		4.8	4.5	4.7	5.2	
MD	5.4	4.1	3.6	4.9		4.8	3.5	3.6	4.6	
HD	5.4	3.5	3.1	4.0		3.2	2.5	2.4	3.6	

*statistically significantly different from control

- Ionized Ca values minimal at ca. 6-12h post dose.
- 24h and pre-dose levels remained lower throughout study in treated groups.
- Ionized Ca values recovered at Wk30.

Figure 1. Mean Normalized Ionized Calcium Levels in Male and Female Rats Administered Cinacalcet HCl Over a 24 hr Period at Week 26



Bone effects

		m	F
Bone	Reduced bone length	HD	HD
	Increased bone diameter	HD	HD
	Increased trabecular BMD (distal femur)	HD*	
	Decreased cortical BMD (distal femur)	HD*	HD*

Femur metaphysis (Wk 27)

MALES	Length (mm) (femur)	Total		Trabecular		Cortical			
		Area (mm2)	BMD	Area	BMD	Area	BMD	Thickness (mm)	Endo.C (mm)
Control	43	20.7	634	10.1	197	9.2	1132	0.66	12.0
LD	43	22.0	624	11.1	188	9.6	1144	0.66	12.4
MD	43	21.5	619	11.0	183	9.3	1146	0.65	12.4
HD	42*	23.0	601	11.7	271*	9.0	1050*	0.60*	13.2*
FEMALES									
Control	37	15.9	666	6.1	263	7.9	1030	0.65	10.0
LD	37	16.5	680	6.7	280	7.9	1068*	0.64	10.4
MD	37	16.2	659	7.0	249	7.6	1075*	0.62	10.4
HD	36	17.4*	666	5.7	292	9.2	964*	0.75	10.0

Shorter bone (both sexes), trabecular and cortical BMD and area/thickness effect in different directions in m and f. Unclear effects on bone.

Femur diaphysis (Wk 27)

MALES	Total		Cortical			
	Area (mm2)	BMD	Area	BMD	Thickness (mm)	Endo.C (mm)
Control	16	963	10.7	1353	0.96	8.2
LD	16	965	10.7	1358	0.95	8.2
MD	16	956	10.6	1354	0.94	8.3
HD	17	982	11.5	1358	1.01	8.3
FEMALES						
Control	10.5	960	7.1	1324	0.79	6.5
LD	11.3*	938	7.5	1323	0.79	7.0*
MD	11.7*	937	7.7*	1326	0.80	7.1*
HD	12.2*	954	8.1*	1329	0.83*	7.1*

Thicker cortical bone (particular in f) at diaphysis level, but also increased endocortical circumference

Organ weights (relative-to-body weight)

	control	LD	MD	HD		control	LD	MD	HD
Wk26									
Body weight	598	600	552	423*		311	301	313	251*
Liver	2.4	2.3	2.4	2.6*		2.3	2.5	2.6	3.3*
Lungs	0.3	0.3	0.3	0.36*		0.42	0.44	0.44	0.51*
Gonads	0.61	0.58	0.62	0.79*					
Prostate	0.24	0.24	0.26	0.30*					
Adrenals	0.009	0.009	0.009	0.012*		0.019	0.02	0.019	0.025*
Brain	0.38	0.37	0.41	0.51*		0.65	0.68	0.66	0.80*

Not recovered: (males) liver, lung, gonads, brain; (females) lung, adrenal, brain

Gross pathology: stomach, area depressed: m 0-0-0-1; f 0-1-1-3

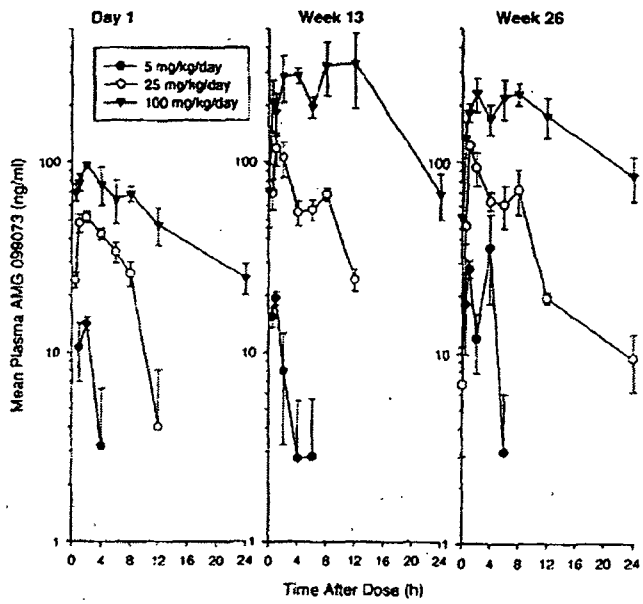
Histopathology

		Males	Females
TREATMENT PERIOD		Incidence out of (15,15,15,15)	Incidence out of (15,15,15,15)
Eye, lenticular degeneration (clusters of swollen/vacuolated/ruptured/liquefied, i.e., abnormal fibers in anterior cortex of lens)	Slight to mild	0,0,1,9	0,1,0,9
Kidney, mineralization, pelvis diverticulum (clusters of crystalloid material, free or beneath pelvis epithelium or in lumen of distal collecting tubules, sometimes with thickening of pelvis epithelium)		0,0,0,7	6,4,10,10
Cecum, hyperplasia, mucosal (increased cellular basophilia and mitotic figures, goblet cell loss, inflammatory cell increase)		0,3,2,2	0,1,2,7
Heart, infiltration, mononuclear cell		8,9,9,10	4,3,2,10
Heart, degeneration and/or necrosis, myocardial (small collections of mononuclear cells in left ventricle and ventricular papillary muscle, sometimes associated with fibrosis and/or degenerated and/or necrotic myocardial fibers)		1,3,5,8	0,0,0,4
Lung, alveolar histiocytosis		3,0,1,5	1,0,0,6
Thymus, lymphoid atrophy		0,0,2,1	0,0,0,2
Stomach, erosion		0,0,0,1	0,1,0,2
RECOVERY		Incidence out of (5,0,0,5)	Incidence out of (5,0,0,5)
Eye, lenticular degeneration		1,-,-,3	0,-,-,2
Kidney, mineralization, pelvis diverticulum		0,-,-,5	1,-,-,4
Heart, degeneration and/or necrosis, myocardial		1,-,-,0	0,-,-,0
Lung, histiocytosis		0,-,-,0	0,-,-,1
Thymus, lymphoid atrophy		1,-,-,0	0,-,-,1

- Eye and kidney findings were not reversed. The lens finding was probably related to hypocalcemia (ref) and an irreversible effect. The kidney finding of mineralization (material in lumen) and associated epithelial changes may have been related to serum calcium and phosphate changes (CaxP product increased) or increased urine calcium concentration, more likely the latter since Ca (urine) in m and f correlated to microscopic change. It is unclear why this was not reversed.
- Myocardial degeneration and/or necrosis (reversible) finding was interpreted by study pathologist as a background change (i.e., spontaneous cardiomyopathy) although dose-related increase in incidence in females was recognized. The cause of this finding was unclear, but the incidence suggests it was drug-related.
- Cecum finding of hyperplasia/inflammation was observed only in treated animals and was dose-related in females. The effect was reversible. Effect appeared to be drug-related.

Toxicokinetics -

Analysis of parent drug AMG-073 (males and female average)



Rat TK data (males and females, average)

	Dose (mg/kg)	Group	Cmax (ng/ml)	AUC(0-24h) (ng.h/ml)	Tmax (h)	CL/F (L/h/kg)	Accum. Ratio
Day 1	5	LD		36	2(m,f)	Nd	Na
	25	MD		390	2(m,f)	Nd	Na
	100	HD		1250	2(m,f)	Nd	Na
Week 13	5	LD		45	1(m,f)	111	1.3
	25	MD		905	2(m) 1(f)	27.6	2.3
	100	HD		5710	4(m) 12(f)	17.5	4.6
Week 26	5	LD		126	1(m) 4(f)	40	3.5
	25	MD		938	1(m,f)	27	2.4
	100	HD		3970	8(m) 6(f)	25	3.2

Nd not determined
Na not applicable

Rat TK data (males and females separate)

	Dose (mg/kg)	Group	Cmax (ng/ml)		$AUC_{(0\text{-}24h)}$ (ng.h/ml)	

			males	females	Males	females
Week 13	5	LD			54	36
	25	MD			837	981
	100	HD			3840	9010
Week 26	5	LD			116	85*
	25	MD			853	1020
	100	HD			3060	5170

*estimated

Metabolites were not measured in this study. Metabolite AMG102664 (M7) was measured in the carcinogenicity study. Exposure(AUC, ngxh/mL) to M7 was on average 50 times higher than to parent drug.

TK conclusions

- AUC was linear with dose in m and f over dose range of 5-100 mg/kg/day. Cmax was less than proportional. This suggests decreased rate of absorption at higher doses
- Cmax, AUC (f) > Cmax, AUC (m) at 100 mkd (Wks 13, 26, but not Day 1)
- Accumulation varied from 2.5-3.5 (AUC Day 180/AUC Day 1)

Summary and evaluation –

Effects of AMG-073 in a 6-month toxicity study in albino rats

	TREATMENT			RECOVERY PERIOD
		Males	Females	
Signs	Abnormal breathing, dehydration, salivation, broken teeth, thin, fur staining, fur wet, fur thin	MD, HD	MD, HD	Abnormal breathing in HD m and f
Body weight	BW and BW gain reduced	HD*	HD*	BW gains similar in HD and controls
Food consumption	FC reduced	HD (-20%)	HD (-20%)	FC in HD similar as in controls
Ocular effects	Early cataract formation (small, multi-focal opacities in anterior cortex, similar in Wks 13, 26, 31)	HD (all)	HD (all)	Effect persistent
Hematology	PT elevated	MD*, HD*	HD*	All parameters recovered
	APTT elevated		HD*	
	WBC increase (neutrophils)	HD	HD*	
Clinical chemistry	Ca (total or ionized) decrease	LD, MD*, HD*	LD, MD*, HD*	Recovered in m,f
(@Wk 27)	P increase	LD*, MD*, HD*	MD*, HD*	Recovered in m
	CK decrease	MD, HD*	MD*, HD*	Partially recovered
	Creatinine increase	HD*		Recovered in m,f
	BUN	HD*	HD*	Partially recovered
	ALT, Alk Phos increases	HD*	HD*	Recovered in m
	Albumin decrease (slight), beta and gamma-globulin increase (slight-moderate)	HD*	HD*	Recovered in m
	Glucose decrease	HD*	HD*	Recovered in m
	Slight decreases in: Cl, K, increase in Na	MD, HD*	MD*, HD*	Recovered in m
Endocrinology	Serum PTH decrease (predose)	LD, MD, HD	LD, MD, HD	Recovered in m,f
Urinalysis	Increase in urinary Ca content (up to 3-5x)	LD*, MD*, HD*	MD*, HD*	Normalized in m,f
	Decrease in urine Na, K (down to 0.5x)	HD*	MD*, HD*	Normalized in m, reversed in f
Gross pathology	Unremarkable			
Organ weight change	Absolute organ weight decreases (liver, spleen, heart, lungs, kidney, gonads, brain, thyroids, adrenals, thymus) Relative-to-body organ weight increases (liver, lung, adrenal, brain)	MD, HD*	MD, HD*	

*statistically significant effects

Human plasma level multiples (based on Wk 26 data)

Group	Rat dose (mg/kg)	Cmax (ng/ml)	AUC (ng.h/ml)	Multiple of human Cmax (180 mg dose)	Multiple of human AUC (180 mg dose)
LD	5	[redacted]	126	[redacted]	**0.2x**
MD	25	[redacted]	938	[redacted]	**1.4x**
HD	100	[redacted]	3970	[redacted]	**6.1x**

Human Cmax (175 mg dose) = [redacted] AUC = 648 ng.h/ml (median, Study 20000187, 7-day dosing, renal impaired patients)

NOTE: The multiples of the human plasma levels were calculated on the basis of parent drug. The parent drug constitutes <1% of the circulating compound-related material, and the remainder is metabolites.

The effect on (ionized) calcium (transient or persistent decrease) is related to the expected effect of the drug to suppress PTH secretion from the parathyroid. PTH increases tubular reabsorption of calcium in the distal convoluted tubule and low levels cause reduced calcium reabsorption with increased urinary levels. An increase in calcitonin release from thyroid C-cells may also have contributed to hypocalcemia due to a suppression of osteoclastic calcium resorption. The effect on phosphorus is probably due to an increase of P reabsorption due to low PTH levels, since PTH normally suppresses this reabsorption in the proximal tubule.

The hypocalcemia probably caused the clinical signs of salivation and abnormal breathing through effects on CNS or smooth muscle function. Hyperphosphatemia is generally thought to have no significant effects.

The cataracts were probably caused by hypocalcemia as described for rodents

Clotting parameters (PT, APTT) may have been elevated due to decrease in serum calcium and inhibition of calcium-dependent clotting factors

Cause and significance of other hematology findings (WBC increase), and clinical chemistry changes (CK decrease, ALT and ALP increase, serum protein changes) is unclear

Effects on BUN and creatinine may have been related to kidney mineralization and urinary tract obstruction, or dehydration.

The effect on serum glucose (decrease) at the high dose may be related to an inhibition by AMG-073 of the KATP channel (ATP-sensitive potassium channel) in pancreatic beta-cells. This channel is instrumental in the stimulation of insulin release from B-cells, and blockers of this channel can cause hypoglycemia.

The urine changes (increased Ca, decreased Na, K) were due to the effect of decreased PTH on tubular calcium reabsorption, and possibly direct effects of AMG-073 on Na and K excretion/reabsorption.

At the HD femoral length was reduced in both sexes, possibly secondary to FC and BW effects. At the MD (females) and at the HD (both sexes), total bone area, cortical bone area and thickness, and endocortical circumference were slightly increased in distal femur metaphysis and mid femur diaphysis. There were no clear BMD effects. Thus, at 100 mg/kg/day, bone was shorter and had a slightly thicker cortex. Effects were not reversed in 4 weeks. The effect on bone (BMD and size) was probably the combined result of the reduction in serum PTH, increase in calcitonin, and other potential effects of AMG-073 on bone, including direct effects on bone cells.

The effect on serum P (increase) is due to increased renal reabsorption.

The changes in absolute and relative organ weight were probably due to decreases in body weight.

The toxicological relevance of the cecum hyperplasia and cardiac findings is unclear.

In conclusion, the main potential target organs for toxicity of AMG-073 appear to be:
Eye, kidney, liver, pancreas, bone, heart

Similar results from 3-month rodent dietary toxicity studies

Decreased body weight gain (rat, mouse)
Serum Ca decrease, P increase (rat, mouse)
Kidney weight decrease (rat)
Kidney pelvic dilation (rat, mouse)
Kidney transitional cell hyperplasia (male rat)

Histopathology Inventory

Sponsor Study # 100020 Species: Monkey	Organ weight	Pathology
Adrenals	X	X*
Aorta		X
Bone Marrow smear (sternum)		X
Bone marrow (rib)		X
Bone (femur)		X
Bone (sternum)		X
Brain	X	X*
Cecum		X
Colon		X
Duodenum		X
Epididymis	X	X*
Esophagus		X
Eye		X
Fallopian tube		
Gall bladder		X
Gross lesions		X
Harderian gland		
Heart	X	X*
Hyphophysis		
Ileum		X
Injection site		
Jejunum		X
Kidneys	X	X*
Lacrimal gland		X
Larynx		
Liver	X	X*
Lungs with bronchi	X	X*
Lymph nodes, cervical		
Lymph nodes mandibular		X
Lymph nodes, mesenteric		X
Mammary Gland		X
Nasal cavity		
Nerve (brachial) + muscle		X
Optic nerves		X
Ovaries	X	X*
Pancreas		X
Parathyroid		X
Peripheral nerve (brachial)		X
Pharynx		
Pituitary	X	X*
Prostate	X	X*
Rectum		X
Salivary gland		X
Sciatic nerve		
Seminal vesicles		X
Skeletal muscle		X
Skin		X
Spinal cord		X
Spleen	X	X*
Stomach		X
Testes	X	X*

Thymus	X	X*
Thyroid	X	X*
Tongue		X
Tonsils		X
Trachea		X
Ureter		X
Urethra		X
Urinary bladder		X
Uterus		X
Vagina		X
Zymbal gland		

* organ weight obtained

THREE-MONTH ORAL TOXICITY STUDY WITH AMG 099073-01 IN CYNOMOLGUS MONKEYS WITH A 2-WEEK RECOVERY PERIOD

Sponsor Study Nr. 100020
Performing Laboratory: [redacted]
Laboratory study identification: [redacted] Study Nr. 6271-165
Study period January-June 1998
GLP and QA statements included, not signed.
Lot Nr. 709001 , [redacted]
Purity: Assumed to be 100%
Assay of test compound (in vehicle-prepared dosing solution): HPLC
Vehicle/control: 0.5% methylcellulose in distilled water.

METHODS

Cynomolgus monkeys (*Macaca fascicularis*) (4 or 6/sex/dose group) from [redacted] stock colony, age at start 3-6 years, body weight range 2.3-2.7 kg (m), 2.0-3.2 kg (f), were dosed orally, by nasal gastric intubation, **with 0, 5, 50, 100, 150/100** (Day1-15)/(Day 16-end) **mg/kg/day**, for at least 13 weeks (5 ml/kg/day). Groups 1-4 received dose once daily. Group 5 was dosed bid with 2x75mg/kg/day on Days 1-15, and dosed bid with 2x50mg/kg/day from Day 16 through termination. Each dose was flushed with 5 ml tap water. Food consumption and feeding schedule were not given. In Group 1 and Group 4, 2 recovery animals were taken off treatment at ca. 13 weeks, and continued in study for 2 weeks.

Dose groups

Group	1	2	3	4	5
	control	LD	MD	HD1	HD2
N/sex/group (main study)	6	4	4	6	4
N/sex (recovery)	2	0	0	2	0
Dose (mg/kg/day):					
Day 1-end	0	5	50	100	
Day 1-15					150
Day 16-end					100

Observation times:

Clinical signs	Twice daily
Body weights	Day 1, then weekly
Food consumption	Not recorded
Ophthalmoscopy	Predosing, and prior to necropsy
EKG	Predosing, and prior to necropsy
Hematology	Weeks -2, 5, 8, 12, 15
Clinical chemistry	Weeks -2, 5, 8, 12, 15
PTH	Day 1, and Weeks 4, 8, 12. Samples predose and 2h postdose
Ionized Ca	Assayed in samples for toxicokinetic analysis
Urinalysis	Weeks -2, 5, 8, 12, 15
Gross pathology	At sacrifice (see Histopathology Inventory in Appendix I)
Organs weighed	Listed in histopathology inventory (Appendix I)
Histopathology	At sacrifice (see Appendix I)
Toxicokinetics	Day 1 and end of Weeks 4, 12: (A) Groups 1-4:prior to dose,, 0.5,1,2,4,8,12,24h postdose; (B) Group 5: prior to dose, 0.5,1,2,4,6h post dose (2x/day). Samples used for assay of drug and ionized Ca.

RESULTS

Clinical signs
No deaths

On Day 16, HD2 was reduced from 75 mg/kg twice daily to 50 mg/kg twice daily based on emesis, poor appetite, soft feces (HD1 was given 100 mg/kg daily).

Signs

-poor to fair appetite in most or all MD,HD_1,HD_2 m,f
-emesis in 1 to 4 MD,HD_1,HD_2 m,f
-soft/liquid feces in 1 to 4 LD, MD, HD_1,HD_2 m, and in 1 to 3 MD, HD_1,HD_2 f
-excessive salivation (particularly during and post dosing) in 2 to 5 MD,HD_1,HD_2 m,f
-all signs reversible

Body weights

Wk 1-2: Weight loss or reduced weight gain in $HD_{1,2}$
Wk1-13, and Wk 14-16: See Tables

Body weight data

Body weight (kg)	control	LD	MD	HD_1	HD_2
Week 13					
males	3.6	3.1	3.0	3.1	2.9
females	2.8	2.4	2.4	2.5	2.5
Week 16 (Recovery)					
males	4.2			3.7	
females	3.0			2.9	

*statistically significantly different from control

Body weight gain data

Body weight gain (kg)	control	LD	MD	HD_1	HD_2
Week 1-13					
males	0.5	0.3	0.2	0.1*	0.1*
females	0.4	0.1*	0.2*	0.0*	0.1*
Week 14-16 (Recovery)					
males	0.1			0.0	
females	0.2			0.0	

*statistically significantly different from control

Food consumption

No data

Ophthalmoscopy

No test article related findings

EKG

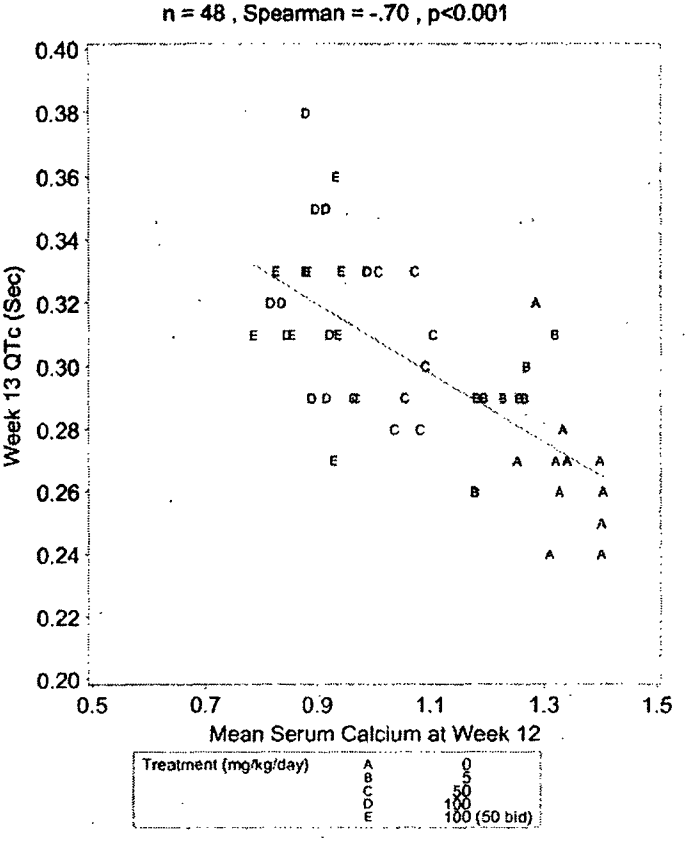
Prolonged QT intervals in HD_1 m,f and HD_2 m,f (probably due to hypocalcemia)

QT interval prolongation in Week 13

Dose group	sex	N/sex	Incidence of animals with increased QT interval (>20 msec above upper range of QT interval normal for particular Heart Rate (HR) Interval)
Group 1 (control)	m,f	6	0/4
Group 2 (LD)	m,f	4	0/4
Group 3 (MD)	m,f	4	0/4
Group 4 (HD1)	males	6	5/6
	females	6	4/6
Group 5 (HD2)	males	4	2/4
	females	4	4/4

Determined: Heart rate, Heart rhythm, P-QRS-T complexes and intervals, Mean electrical axis

Subsequently, Sponsor performed a statistical analysis of QT data and established a correlation between serum Ca levels and QTc (rr –0.7)



Hematology

Hematology data

			control	LD	MD	HD_1	HD_2		control	HD_1
	N		6	4	4	6	4		2	2
RBC	males	Week 12	6.75	6.27	6,33	6.41	6.21	Week 16	6.34	6.10
(mi/ul)	females	Week 12	6.94	6.3	6.49	5.97	5.99	Week 16	6.57	5.59
Hb (g/dl)	males	Week 12	12.4	11.9	11.4	11.6	11.6	Week 16	11.9	11.3
	females		12.8	11.5	11.3	**11.1***	**11.0***		11.6	10.4
Hct (%)	males	Week 12	43.1	40.5	39.5	39.4	39.0	Week 16	40:3	37.3
	females		44.3	39.8	**38.8***	**38.3***	**37.3***		40.4	36.9
PT (sec)	males	Week 5	10.1	10.2	10.4	**10.7***	**11.1***			
	females		10.4	10.1	10.6	10.8	10.9			
	males	Week 12	10.9	10.7	10.8	11.1	11.3	Week 16	10.9	10.9
	females		11.3	10.5	11.0	11.4	11.1		10.8	10.9
WBC AB	males	Week 8	10.2	10.5	10.9	9.1	8.8			
	females		12.8	11.1	8.4	**6.7***	**7.0***			
	males	Week 12	11.0 (7.8-15.6)	10.3 (7.6-16.2)	12.0 (8.0-15.4)	9.7 (7.0-12.8)	9.7 (5.9-14.9)	Week 16	9.0	12.8
	females		11.4 (7.2-15)	11.4 (6.6-20)	8.9 (7.7-10.9)	7.5 (5.2-11.9)	12.6** (6.5-25.5)		10.9	5.5
Mono AB	males	Week 8	0.6	0.3	0.6	0.5	0.5			

	females		0.7	0.7	0.3	**0.3***	**0.3***			
	males	Week 12	0.5	0.4	0.7	0.5	0.5	Week 16	0.3	0.5
	females		0.6	0.7	0.3	0.3	0.7**		0.5	0.4
Eosin AB	males	Week 8	0.3	0.1	0.2	0.4	0.1			
	females		0.5	0.3	0.2	**0.1***	**0.1***			
	males	Week 12	0.3	0.1	0.3	0.4	0.1	Week 16	0.3	0.6
	females		0.5	0.3	0.4	**0.1***	0.2		0.3	0.1

* statistically significantly different from control
** extremely high WBC and Monocyte counts in 1 out of the 4 HD_2 females

No changes in MCV, MCH, MCHC, platelet count, reticulocyte count, neutrophil, lymphocyte counts.

Summary Results Hematology (Week 12)
(Note: Changes listed in table are based on evaluation of average + individual animal data)

	Males				Fermales			
	LD	MD	HD1	HD2	LD	MD	HD1	HD2
RBC				↓			↓	↓
Hb		↓	↓	↓	↓	↓	↓**	↓**
Hct	↓	↓	↓	↓	↓	↓**	↓**	↓**
PT			↑	↑				
WBC			↓	↓		↓	↓	↓
Mono						↓	↓	?
Eosin							↓**	↓

** statistically significantly different from control

Reversibility of hematology effects
(control and HD_1, 2 animals/group, 2 week recovery):

	males	Females
RBC	-	No
Hb	no	No
Hct	no	No
PT	yes	-
WBC	yes	?
Mono	-	Yes
Eosin	-	?

Clinical chemistry

Serum chemistry data

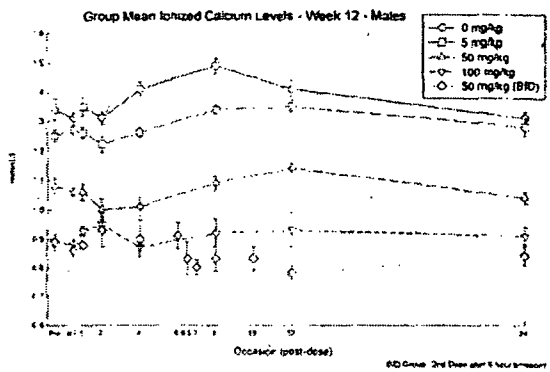
			control	LD	MD	HD_1	HD_2		control	HD_1
	N		6	4	4	6	4		2	2
T Chol (mg/dl)	males	Week 12	170	146	**116***	**131***	**110***	Week 16	193	131
	females		167	169	**121***	**101***	**113***		173	131
Triglyc	males		44	44	66	57	**99***	Week 16	51	42
	females		55	50	68	62	57		53	34
AST (u/l)	males	Week 12	35	32	36	47	55	Week 16	20	32
	females		38	33	38	54	38		31	29
ALT (u/l)	males	Week 12	49	39	64	93	**142***	Week 16	32	28
	females		70	58	38	**159***	**105***		50	71
Ca (mg/dl)	males	Week 8	10.8	9.8	**8.0***	**7.1***	**7.1***			
	females		9.8	9.4	**7.8***	**6.9***	**6.8***			
	males	Week 12	11.8	10.9	**9.9***	**8.4***	**8.5***	Week 16	11.1	11.3
	females		11.3	10.9	**9.6***	**8.5***	**8.5***		11.0	10.7
P (mg/dl)	males	Week 12	6.8	6.4	8.3	**9.4***	**10***	Week 16	6.7	7.6

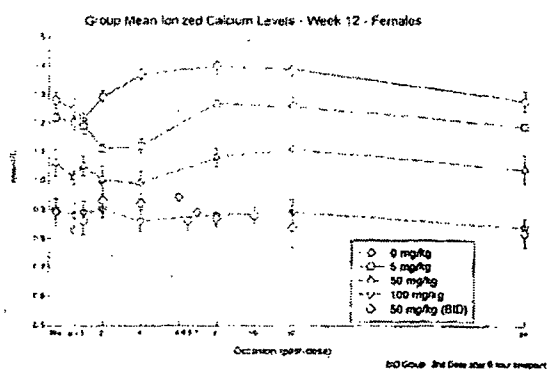
	females		6.0	5.2	8.1*	7.9*	8.9*		5.3	5.4
Creat Kinase	males	Week 12	182	212	217	262*	473*	Week 16	109	86
	females		241	205	306	323	315		327	86

No significant effects on: urea, ALK P, creatinine, total protein, total bilirubin, GGT, serum electrolytes, glucose.
Reversibility: All parameters affected were reversed, except cholesterol which was still lower after 2 weeks recovery.

Serum PTH
No conclusive data obtained (serum levels below LLQ)

Serum ionized Ca
Decrease in serum ionized Ca:
LD (m,f) groups:
Day 1: from 2-4h to 8-12h postdosing
Week 4: from 0.5-2h to 8-12h postdosing
Week 12: from 2-4h to 8h postdosing
MD,HD (m,f) groups:
Day 1: from 2h to 24h postdosing
Week 4: at all time points (pre and postdosing)
Week 12: at all time points (pre and postdosing)





Serum pH:
Increase in serum pH in MD,HD (m only) groups:
Day 1: at 4h postdosing
Weeks 4, 12: no effects

Urinalysis
No treatment related findings

Gross pathology

Gross pathology data

		MALES						FEMALES					
Term Sacr			control	LD	MD	HD_1	HD_2		control	LD	MD	HD_1	HD_2
N			4	4	4	4	4		4	4	4	4	4
Thymus	Small		0	0	0	1	0		0	0	0	1	0
Recovery			control			HD1			control			HD1	
N			2			2			2			2	
Thymus	Small		0			0			0			0	

Organ weight (Organ list: see Appendix)

Body weight data

	MALES					FEMALES				
	control	LD	MD	HD	HD	control	LD	MD	HD	HD
Body weight (kg)	3.18	3.13	3.05	2.85	2.93	2.75	2.43	2.48	2.28	2.55

Organ weights: Change in absolute (relative-to-body) weight

	MALES					FEMALES					
		LD	MD	HD_1	HD_2		LD	MD	HD_1	HD_2	Extent of Change
		4	4	4	4		4	4	4	4	
Kidney									↑ (↑*)	↑ (↑)	small-moderate (DR)
Thymus		↓ (↓)	↓ (↓)	↓ (↓)	↓ (↓)		↓* (↓*)	↓ (↓)	↓* (↓*)	↓ (↓)	moderate-marked (not DR)
Pituitary[?]								↓* (↓)[?]	(↑*)		moderate
Testis			↓ (↓)	↓* (↓*)	↓ (↓)						moderate-marked (DR?)
Epididymis				↓* (↓)	↓ (↓)						moderate
Liver/gallbladder			↑ (↑)	↑ (↑*)	↑ (↑*)				(↑*)	↑* (↑*)	small-moderate (DR)
Spleen										↑	moderate
Thyroid/parathyroid			↓ (↓)	↓(↓)	↓ (↓)						small-moderate (not DR)

Bolded arrows represent statistically significant changes
DR = dose-related
[?] Odd result (pituitary)

Histopathology (Organ list: see Appendix)

Histopathology data

	MALES						FEMALES					
Terminal Sacrifice		control	LD	MD	HD_1	HD_2		control	LD	MD	HD_1	HD_2
N		4	4	4	4	4		4	4	4	4	4
Lung	Macrophage infiltrate	0	0	0	2	1		0	0	0	1	0
Kidney	Polykaryocytosis, urothelium, pelvis	0	0	0	1	1		0	0	1	1	2
	Tubule regeneration	1	0	0	0	2		0	1	0	2	0
Skeletal muscle	Degeneration	0	0	1	2	0		1	0	0	1	0
Liver	Necrosis	0	0	0	0	0		0	0	0	0	1
Thymus	Involution	0	1	1	1	2		2	2	0	2	1
Spleen	Congestion	0	0	0	0	0		0	0	1	0	2
Testis	Juvenile	0	0	1	1	0						
Recovery Sacrifice		control	LD	MD	HD_1	HD_2		control	LD	MD	HD_1	HD_2
N		2			2			2			2	
Kidney	Tubule regeneration	0			1			0			1	
Skeletal muscle	Degeneration										1	

Other findings:
One MD male had pneumonia, diagnosed as tuberculosis.
Parasitic lesions were seen in multiple animals, usually in one animal/sex of one or two dose groups (including controls). Parasites were present in stomach, intestine, esophagus, lung, skeletal muscle and tongue.
In multiple tissues, lymphohistiocytic infiltrates were observed (liver, kidney, tongue, trachea, urethra, vagina). Treatment-relatedness, mechanism and relevance of this effect were inconsistent and/or unclear.

Toxicokinetics
Determined:

- Cmax, Tmax, AUC (0-24h), CL/F, T1/2
- Day 1, 27, 83

Toxicokinetic data (average values)

			LD	MD	HD_1	HD_2
Cmax (ng/ml)	males	Day 1				
		27				
		83				
	females	Day 1				
		27				
		83				
Tmax (h)	males	Day 1	3	4	6.3	12.5
		27	3	5	7.3	12
		83	2.3	4	7.3	9
	females	Day 1	1.7	4	5.8	14.5
		27	2.5	4	4.7	9
		83	3	4	7.3	9
AUC 0-24h (ng*h/ml)	males	Day 1*	34.3	1330	1740	2040
		27	58	1200	1500	2590
		83	61	599	977	1380
	females	Day 1*	72	651	1290	1550
		27	52	643	1180	1270
		83	96	283	1020	1280
CL/F (l/h/kg)	males	Day 1*	-	-	-	-
		27	118	50	71	56
		83	58	98	127	73
	females	Day 1*	-	-	-	-
		27	134	85	91	80
		83	91	184	128	99
T1/2 (h)	males	Day 1*	-	-	-	Nd
		27	2.5	6.5	6.8	Nd
		83	3.3	6.7	9.4	Nd
	females	Day 1*	-	-	-	Nd
		27	2.0	4.9	5.8	Nd
		83	2.5	5.7	8.6	Nd

* Values for Day 1 (AUC) may be underestimated (most values BLQ)
**Nd = Not determined: T1/2 not determined for HD2 group (bid dosing schedule)

Number of animals for which TK parameters were determined

N	Males					Females			
	LD	MD	HD1	HD2		LD	MD	HD1	HD2
Day 1	2	4	6	4		3	4	6	4
Day 27	4	4	6	4		4	4	6	4
Day 83	3	4	6	4		4	4	6	4

Summary toxicokinetics:

- Large variability in Cmax and AUC data, ie, inconsistent changes with dose and over time
- T1/2 varied from 5-10h
- Tmax varied from 2-15h
- Dosing b.i.d. increased AUC as compared to dosing q.d. (compare HD1 and HD2)
- Values suggest that AUC and $T_{1/2}$ values in LD groups may be inaccurate and/or underestimated
- CL/F was not significantly related to dose
- Cmax and AUC non-linearly increased with dose, possibly due to relative decrease in absorption at higher doses
- Sponsor concluded that AUC (f) < AUC (m). Although statistically CL/F was overall significantly higher in females than in males, examination of AUC values does not support consistent differences (eg AUC values on Day 83 are similar in HD males and HD females)
- Sponsor concluded that CL/F (Day83)>CL/F(Day27). The increase in CL/F over time suggests induction of clearance (metabolism). Reviewer concurs with this conclusion.
- There was no evidence of accumulation of parent drug over time.

SUMMARY AND EVALUATION

Introduction:

The pharmacologic action of the calcimimetic AMG073 consists of a lowering of parathyroid gland PTH secretion and reduction of serum calcium levels. PTH levels are elevated in patients with hyperparathyroidism (HPT). In primary HPT this is due to excessive PTH secretion, and in secondary HPT it is usually due to renal failure with chronic hypocalcemia and an adaptive increase in PTH secretion. The compound AMG-073 is being developed for the treatment of primary and secondary HPT.

Summary:

Effects of AMG-073 in a 3-month toxicity study in cynomolgus monkeys

		Males	Females
Signs	Soft/liquid feces	LD, MD, HD	LD, MD, HD
Body weight	BW gain reduced	LD, MD, HD*	LD*, MD*, HD*
EKG	QT interval prolonged	HD	HD
Hematology	Hb, Hct decrease	MD, HD	LD, MD*, HD*
	PT increase	HD*	
	WBC decrease	HD	MD, HD*
	Monocyte/eosinophil decrease		MD, HD*
Clinical chemistry	Total cholesterol decrease	MD*, HD*	MD*, HD*
	Triglyceride increase	HD2*	
	AST increase	HD2	HD1
	ALT increase	HD1, HD2*	HD*
	Ca (total or ionized) decrease	LD, MD*, HD*	LD, MD*, HD*
	P increase	LD, MD, HD*	LD, MD*, HD*
	Creatine kinase increase	HD*	HD
Gross pathology	Thymus small	HD1	HD1
Organ weight change (absolute and/or relative)	Kidney increase		HD1*, HD2
	Thymus decrease	LD, MD, HD	LD*, MD, HD1*, HD2
	Testis decrease	MD, HD1*, HD2	
	Epididymis decrease	HD1*, HD2	
	Liver/gallbladder increase	MD, HD*	HD*
	Thyr/Parathyroid decrease	MD, HD	
	Spleen increase		HD2
Histopathology	Kidney polykaryocytosis, pelvic urothelium	HD	MD, HD

	Spleen congestion		MD, HD2
	Liver necrosis		HD2
	Thymus involution	LD, MD, HD	

*statistically significant effects

NOAEL **<5 mg/kg/day**
LOAEL **5 mg/kg/day**

The effects first observed at the LOAEL (LD 5 mg/kg/day) were (in both sexes):
Abnormal clinical signs (m,f)
Body weight gain reduction (f>m)
Hemoglobin and hematocrit decrease (f>m)
Serum Ca decrease and P increase (m,f)
Thymus weight decrease (m,f)
Thymus involution (m)

The effects first observed at the next higher dose (MD 50 mg/kg/day) were:
WBC decrease (f>m)
Cholesterol decrease (m,f)
Testis weight decrease (m)
Liver/gallbladder weight increase (m>f)
Thyroid/parathyroid weight decrease (m)
Kidney pelvic polykaryocytosis (f>m)
Spleen congestion (f)

The effects first observed at the next higher dose (HD 100 mg/kg/day) were:
Prolonged QT interval (f>m)
PT increase (m)
Triglyceride increase (m)
ALT increase (f>m)
Creatine kinase increase (m>f)
Kidney weight increase (f)
Epididymis weigth decrease (m)
Spleen weight increase (f)
Thymus small (m,f)
Thymus involution (m)
Liver necrosis (f)

Safety Evaluation:

The currently reviewed study indicates that AMG-073, as expected, lowers serum calcium in healthy normocalcemic animals. Although data on PTH were not provided it is likely that this is the result of lowering PTH secretion by the parathyroid gland. The hypocalcemia was seen at all doses applied (5-50-100 mg/kg/day).

The hypocalcemia is expected to cause a number of secondary changes in clinical and histo-pathology. The findings of emesis, soft feces, reduced body weight gain, extended PT time, and a prolonged cardiac QT interval, seen in this study, are probably (or at least partly) hypocalcemia-related.

However, various other findings have an unclear relationship to the hypocalcemia. These include hematology findings, serum chemistry changes, gross pathology and histopathology changes and organ weight changes.

The decrease in Hb and Hct values in males and females, and the reduced RBC in females was small but significant, and appeared not to be reversed after 2 weeks. The WBC decrease which was more prominent in females appeared to be partially transient over time, and reversible upon recovery. The mechanism of these hematology changes is unclear.

The cholesterol decrease may have been related to some effect of the test compound on hepatic lipid metabolism, and the ALT increase in the HD groups suggests possible liver cell injury. Cholesterol increase was partially and ALT increase was completely reversed in 2 weeks.

The creatine kinase increase at high doses suggests muscle injury, and may be related to hypocalcemia.

The thymus involution in drug-treated males, and the incidence of small thymus and the (non dose-related) thymus weight decrease in both sexes, particularly females, is unexplained. The (non-dose related) thyroid/parathyroid weight decrease in males may be a study artifact or may be related to the pharmacologic effect of the test compound to inhibit parathyroid PTH release. The kidney weight increase seen in high dose females is unexplained. The mechanism of the testicular and epididymal weight decrease in males is also unclear.

Regarding the increased liver weight, particularly in males, the Sponsor has stated in a telephone conversation with this Reviewer (March 8, 1999) that the compound induces microsomal enzyme activity and that this is the underlying cause of the liver weight and serum enzyme increases. Reviewer feels that the toxicokinetic data on CL/F may support this conclusion.

Histopathologically, there were findings in kidney, spleen and liver. Accompanying this were weight increases of all three organs in HD groups. The kidney findings included polykaryocytosis of pelvic urothelium in MD and HD males and females. The toxicological relevance of this finding and its relation to the increased kidney weight observed in females is unclear. Spleen congestion was seen in 1 MD and 2 HD females and its cause is unclear. Liver necrosis was observed in 1 out of 4 females monkeys in the HD_2 group. The relevance of this finding is unsettled. The animal with focal necrosis had slightly elevated ALT values. However, other animals e.g. in the HD_1 group had much higher ALT values while no observed liver histopathology abnormalities.

Drug-related histopathology findings for testis/epididymis, or thyroid/parathyroid, i.e., organs whose weight was reduced by drug treatment, were not observed.

In conclusion, the main potential target organs for toxicity of AMG-073 appear to be:
kidney, spleen, liver, testis and epididymis, thymus, thyroid/parathyroid.

Comparison with results from rodent toxicity studies
Findings from previously reported rat and mouse three-month toxicity studies with AMG-073 (Submission date: August 14, 1998, Review date: September 17, 2998) that were similar to findings in the 3-month monkey study were:
Decreased body weight gain (rat, mouse)
Serum Ca decrease, P increase (rat, mouse)
Kidney pelvic dilation (rat, mouse)
Kidney transitional cell hyperplasia (male rat)

Other findings in the rodent studies were not seen or were not consistent with the present findings in the monkey. For example, except for the anticipated serum Ca and P changes, there were no significant hematology or serum chemistry findings, and several organ weights, including liver and kidney, were decreased rather than increased. The species specificity of the test compound's effects may be related to species-specific drug metabolism.

CONCLUSION
In the current study report, except for the calcium lowering effect of the test compound, the Sponsor did either not comment on the apparently treatment-related effects, or stated that the effects were not considered toxicologically adverse, or that they were possibly due to nonspecific stress of test article administration. Reviewer feels there is no satisfactory explanation for the treatment-related findings in monkeys that were not clearly related to the hypocalcemic action of the drug, nor do we know their clinical relevance. These findings included Hb, Hct, RBC and WBC decreases, serum cholesterol decrease and ALT increase, testis and epididymes weight decrease, kidney weight increase, thymus weight decrease and involution, liver weight increase and necrosis, spleen weight increase and congestion.

Histopathology Inventory

Sponsor Study # 100020		
Species: Monkey	Organ weight	Pathology
Adrenals	X	X*
Aorta		X
Bone Marrow smear (sternum)		X
Bone marrow (rib)		X
Bone (femur)		X
Bone (sternum)		X
Brain	X	X*
Cecum		X
Colon		X
Duodenum		X
Epididymis	X	X*
Esophagus		X
Eye		X
Fallopian tube		
Gall bladder		X
Gross lesions		X
Harderian gland		
Heart	X	X*
Hyphophysis		
Ileum		X
Injection site		
Jejunum		X
Kidneys	X	X*
Lacrimal gland		X
Larynx		
Liver	X	X*
Lungs with bronchi	X	X*
Lymph nodes, cervical		
Lymph nodes mandibular		X
Lymph nodes, mesenteric		X
Mammary Gland		X
Nasal cavity		
Nerve (brachial) + muscle		X
Optic nerves		X
Ovaries	X	X*
Pancreas		X
Parathyroid		X
Peripheral nerve (brachial)		X
Pharynx		
Pituitary	X	X*
Prostate	X	X*
Rectum		X
Salivary gland		X
Sciatic nerve		
Seminal vesicles		X
Skeletal muscle		X
Skin		X
Spinal cord		X
Spleen	X	X*
Stomach		X
Testes	X	X*
Thymus	X	X*
Thyroid	X	X*
Tongue		X
Tonsils		X
Trachea		X
Ureter		X

Urethra		X
Urinary bladder		X
Uterus		X
Vagina		X
Zymbal gland		

* organ weight obtained

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ONE-YEAR ORAL TOXICITY STUDY WITH AMG 099073-01 IN CYNOMOLGUS MONKEYS WITH A 6-MONTH INTERIM SACRIFICE AND A 4-WEEK RECOVERY PERIOD

Sponsor Study Nr. 100188
Performing Laboratory: [redacted]
Laboratory study identification: [redacted] Study Nr. 6271-183
Study period June 1998-June 1999
GLP and QA statements included
Lot Nr. 709001 [redacted]
Purity: Assumed to be 100%
Assay of test compound (in vehicle-prepared dosing solution): HPLC
Vehicle/control: 0.5% methylcellulose in distilled water.

METHODS

Cynomolgus monkeys (*Macaca fascicularis*) (8/sex/dose group in Grps 1 and 4, and 6/sex/group in Grps 2 and 3) from [redacted] stock colony, age at start 2-4 years, body weight range 1.9-3.2kg (m), 1.8-2.7kg (f), were dosed orally, once daily, by nasal gastric intubation, with **0, 5, 50, 100 mg/kg/day**, for 26 or 52 weeks (5 ml/kg/day). Test article was suspended in 0.5% methyl cellulose vehicle in water. Each dose was flushed with 5 ml tap water. Food consumption and feeding schedule were not given. In Groups 1 and 4, 2/sex were taken off treatment after 52 weeks, and retained for a 4-week recovery period. In all groups, 3/sex were sacrificed after 26 weeks. Samples were taken for determination of ionized calcium on Day 1, and at the end of Wk26 and Wk52. These samples were used for TK analysis of parent compound by HPLC-[redacted]

Dose groups

Group	1	2	3	4
	Control	LD	MD	HD
N/sex (total)	8	6	6	8
N/sex (26-wk interim sacrifice)	3	3	3	3
N/sex (52 wk terminal sacrifice)	3	3	3	3
N/sex (52-wk treatment + 4-wk recovery)	2	0	0	2
Dose (mg/kg/day):	0	5	50	100

Observation times:

Clinical signs	Twice daily
Bleeding time	Wks 17, 25, 52, 56 (external observation)
Body weights	Day 1, then weekly
Food consumption	Not recorded
Ophthalmoscopy	Pre-treatment, Wks 13, 26, 39, 52, and after recovery
EKG	Pre-treatment, Wks 13, 26, 39, 52, and after recovery
Hematology	Wk –1 (pretest), Wks 4, 13, 26, 39, 52 (dosing), and Wk 57 (recovery)
Clinical chemistry	Wk –1 (pretest), Wks 4, 13, 26, 39, 52 (dosing), and Wk 57 (recovery)
Urinalysis	Wk –1 (pretest), Wks 4, 13, 26, 39, 52 (dosing), and Wk 57 (recovery)
Endocrinology	ACTH: Day 1, and Wks 13, 26, 39, 52 (pre- and 4h postdose) PTH: Day 1, and Wks 13, 26, 39, 52 (pre- and 2,4,6,12h postdose) TSH, T3, T4: Wk –1 (pretest), and Wks 4, 13, 26, 39, 52, and 57 VitD, reverse T3, calcitonin: Wk 53 (4h postdose) Testosterone: Wks 26, 52 (pre- and 4h postdose)
Ionized Ca/pH	Day 1, and Wks 13, 26, 39, 52 (predose, and 0.5, 1, 2, 4, 8, 12, 24h postdose)
Toxicokinetics	Day 1 and end of Weeks 26, 52 (predose, and 0.5,1,2,4,8,12,24h postdose.
Organs weighed	Listed in Histopathology Inventory (Appendix I)
Gross pathology	At sacrifice (Histopathology Inventory in Appendix I)
Histopathology	At sacrifice (Histopathology Inventory in Appendix I)
Hepatic P450	Frozen liver samples were shipped to Sponsor for microsome analysis

RESULTS

Mortality

No drug-related deaths

Clinical signs

Fair/poor appetite, emesis, soft/liquid feces in HD in Wks1-3

Decreased appetite in MD, HD and abnormal feces throughout study in LD, MD, HD

One HDf not dosed during 8 days (in 2nd month of study) due to thinness/poor appetite

No signs during recovery (signs reversible)

I. Bleeding time

No treatment-related effects

Body weights

Wk 1-2: Weight loss or reduced weight gain in MD, HD

Body weight data

Body weight (kg)		control	LD	MD	HD
Week 27	Males	2.8	2.8	2.5	2.4
	Females	2.4	2.3	2.2	2.2
Week 53	Males	3.4	3.4	3.2	2.8
	Females	2.6	2.4	2.3	2.4

*statistically significantly different from control

Body weight gain data

Body weight gain (kg)		control	LD	MD	HD
Week 1-27	Males	0.3	0.5	0.3	0.1
	Females	0.2	0.2	0.1	0.0*
Week 28-53	Males	0.3	0.5	0.5	0.3
	Females	0.1	0.0	0.1	0.2
Week 54-57 (Recovery)	Males	0.2	-	-	0.4
	Females	0.2	-	-	0.4

*statistically significantly different from control

Figure 2. Body Weight in Male Monkeys Administered Cinacalcet HCl for 52 Weeks

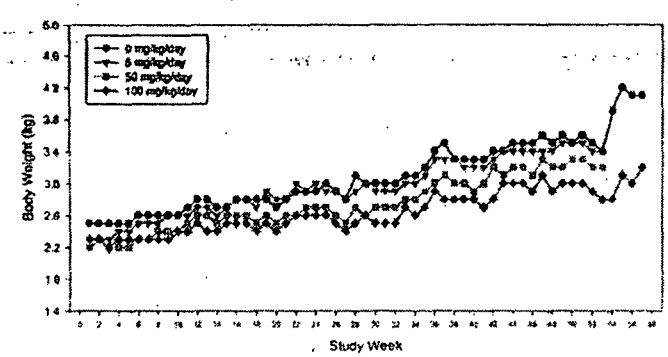
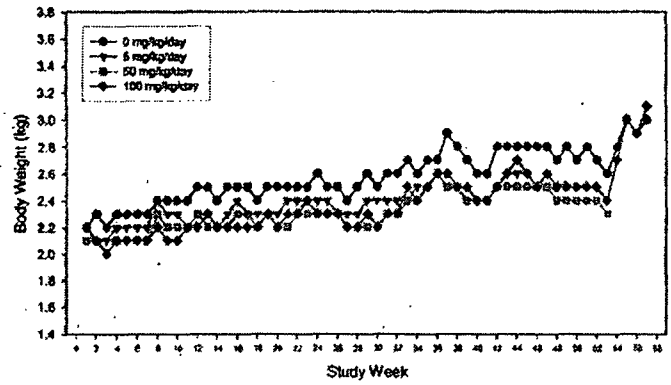


Figure 3. Body Weight in Female Monkeys Administered Cinacalcet HCl for 52 Weeks



Food consumption
No data

Ophthalmoscopy
No treatment-related findings

EKG
All EKG's were " within normal limits". It is unclear at what time after dosing the EKG's were recorded.

EKG: AMENDMENT TO FINAL REPORT

Individual animal data on heart rate and P,Q,S duration, PR, QRS, QT and QTc, ST interval, and P,Q,R,S,T amplitude were submitted. QTc was calculated as QT – (0.087x[60/HR-1]).

QTc interval (sec) (males)

Group	Pre-treatment	Wk13	Wk26	Wk39	Wk52	Wk57 (rec)
Control	0.31	0.27	**0.24**	0.26	0.26	0.28
5	0.30	0.27	**0.29**	0.27	0.27	
50	0.32	0.29	**0.31**	0.28	0.28	
100	0.29	0.30	**0.32**	0.28	0.28	0.30
Serum Ca_i (100 mkd) @4h postdose (mM)	*1.4*	*0.92*	***0.84***	-	*0.76*	-

QTc interval (sec) (females)

Group	Pre-treatment	Wk13	Wk26	Wk39	Wk52	Wk57 (rec)
Control	0.30	0.25	**0.27**	0.27	0.25	0.27
5	0.30	0.27	**0.28**	0.26	0.26	
50	0.30	0.29	**0.31**	0.27	0.26	
100	0.30	0.29	**0.33**	0.27	0.27	0.26
Serum Ca_i (100 mkd) @4h postdose (mM)	*1.4*	*0.90*	***0.83***	-	*0.79*	-

QT, QTc and ST interval were increased in all dose groups in dose-related manner. An effect on QRS interval was not resolved (0.04sec in most animals). The QT effect was more pronounced at Wk 13 and especially Wk26, with little effect in Wk 39 or 52. This did not correlate with serum ionized calcium levels, which were decreased at particularly at the later time points (Wk52>26>13). The QT effect was similar in males and females.

Hematology

Hematology data

			control	LD	MD	HD		control	HD
	N		8	6	6	8		2	2
RBC	males	Week 52	7.51	7.36	7.20	6.66	Week 57	6.95	6.66
(mi/ul)	females	Week 52	7.27	6.77	6.87	**6.35***	Week 57	7.07	6.59
Hb (g/dl)	males	Week 52	13.2	12.7	12.0	**11.4***	Week 57	13.0	11.9
	females	Week 52	12.3	11.3	11.3	11.0	Week 57	12.1	11.9
Hct (%)	males	Week 52	48.9	44.6	**42.1***	**40.3***	Week 57	47.2	**43.8***
	females	Week 52	44.0	43.3	40.5	**39.6***	Week 57	45.2	**43.1***
WBC AB	males	Week 26	10.6	13.8	7.8	**6.8***			
	females		13.7	14.1	11.8	**7.2***			
	males	Week 52	14.7	18.1	14.8	10.8	Week 57	11.3	11.2
	females		17.9	15.6	12.8	**9.4***		10.9	8.3

* statistically significantly different from control

Comments:

RBC decrease was paralleled by decrease in reticulocytes (Absolute Count and %RBC)

WBC decrease consisted of decreases in neutrophil (mainly), lymphocyte and monocyte/eosinophil counts

Other effects:

Transient slight increase in PT (sec) in m and f at Week 26 but not Week 52

No significant changes in MCV, MCH, MCHC, platelet count

Reversibility:

Effects were partially or completely reversed in 4 weeks

Clinical chemistry

Serum chemistry data

			control	LD	MD	HD		control	HD
	N	Week 13	8	6	6	8			
		Week 52	5	3	3	5		2	2
Ca (mg/dl)	males	Week 13	10.2	**9.1***	**7.5***	**7.2***			
	females		9.9	9.7	**7.6**	**7.2**			
	males	Week 52	10.4	9.6	**7.5***	**6.5***	Week 57	10.2	9.7
	females		9.9	10.1	**7.1***	**6.4***		10.8	9.0
P (mg/dl)	males	Week 52	6.3	6.6	**9.8***	**9.8***	Week 57	5.7	6.8*
	females		5.2	5.8	**8.3***	**7.2***		4.8	5.9
Glucose	males	Week 26	98	**83***	**77***	**65***			
	females		86	77	81	**65***			
	males	Week 52	94	77	86	74	Week 57	63	67
	females		65	85	84	77		73	70
Triglyc	males	Week 13	36	45	**68***	**58***			
	females		38	42	**63***	**51***			
	males	Week 52	50	35	54	52	Week 57	61	68
	females		44	49	59	55		65	83
T.Chol	males	Week 52	130	155	113	123	Week 57	126	146
	females		147	150	132	110		149	152
AST (u/l)	males	Week 13	42	41	46	**61***			
	females		34	31	39	**56***			
	males	Week 52	49	34	42	67	Week 57	67	131
	females		43	36	35	72		89	72
ALT (u/l)	males	Week 13	64	60	60	**108***			
	females		59	62	75	**152***			

	males	Week 52	80	55	44*	139	Week 57	77	70
	females		57	63	74	**252***		60	**101***
ALK P	males	Week 13	833	844	572	**482***			
	females		369	354	381	327			
	males	Week 52	690	646	530	542	Week 57	463	587
	females		317	278	370	274		257	255
Creat Kinase	males	Week 13	186	281	232	**465***			
(U/L)	females		202	137	465	**429***			
	males	Week 52	207	155	247	1208 (due to 1 animal)	Week 57	851	1554
	females		236	177	326	444		1728	1728

No consistent/significant effects on:

Urea, creatinine, total protein, albumin, globulin, total bilirubin, GGT, serum electrolytes.

Slight serum glucose decrease was observed in Weeks 26 and 39.

Reversibility:

All parameters affected were partially or completely reversed towards control values

Serum ionized Ca

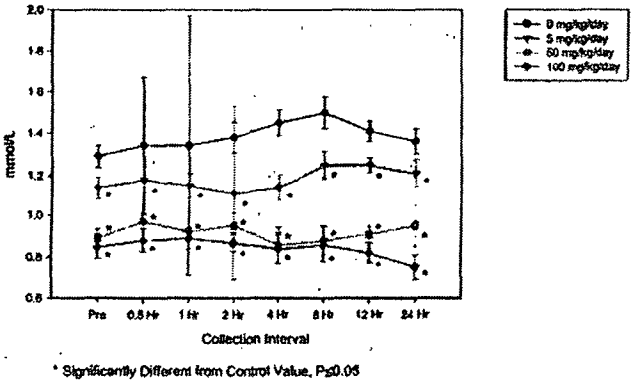
Day 1:

Decreased from 2h to 24h postdosing

Weeks 13, 26, 39, 52:

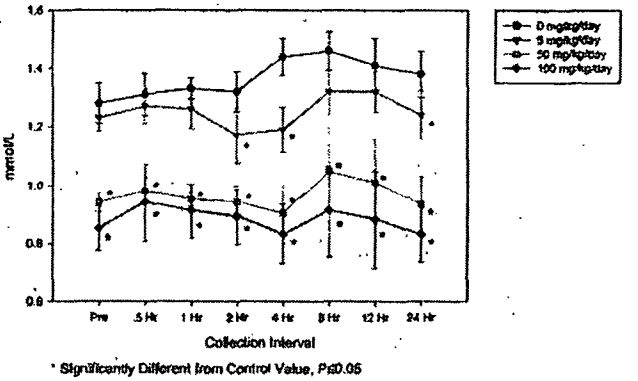
Decreased at all time points (pre and postdosing). Decrease was dose-dependent, amounted to 10%(LD) - 40%(HD) as compared to control values, and was generally constant over the 24h post-dose period throughout treatment. The Ca decrease was more pronounced at Wk52>26>13. This was in contrast to the QTc prolongation, which was most pronounced at Wk26.

Figure 4. Ionized Calcium Levels in Male Monkeys Administered Cinacalcet HCl at 6 Months



* Significantly Different from Control Value, P≤0.05

Figure 5. Ionized Calcium Levels in Female Monkeys Administered Cinacalcet HCl at 6 Months



* Significantly Different from Control Value, P≤0.05

Serum pH:

No significant effects

Serum PTH

Control animals:

Levels varied from 50-100 pg/mL at predose, were generally lower at 2h post dosing (with vehicle), and lowest from 4-12h post dosing (10-20 pg/mL) with several control animals in this period having levels BDL (below-detectable-limit). This may be a circadian pattern.

Treatment effect:
Predose:
Levels decreased as compared to controls (1/2x or 1/3x) in MD,HD in Wks 13, 26, 39; and in LD,MD,HD in Wk52
Postdose:
Levels decreased to BDL at most time points in most animals of LD, MD, HD groups on Day 1, and Wks 13, 26, 39, 52.

Serum VitD
Significantly decreased in MDf and HDm,f (Wk 53)

Thyroid hormones
Serum T3 decreased in MD,HDm and HDf in Wks 4, 13, 26, 39, 52
Serum T4 slightly increased in MD, HD in Wks 4, 13, 26, 39, 52
TSH: No treatment effects
Comment:
T3, T4 changes in MD,HD consistent with poor appetite and lower body weights
Reversibility:
Effect was no longer observed in Wk 57.

ACTH
No treatment effects throughout study period

Testosterone
Decrease in HDm @predose in both Wks 26,52.
Significant and marked dose-dependent decrease (of ca. 70-90%) in LD,MD,HDm @4h postdose in Wks 26,52.

Table 3. Mean Testosterone Levels (ng/mL) Pre-dose and 4 Hours Post-dose at 26 and 52 Weeks in Monkeys

Group (mg/kg/day)	26 wk - pre[b]	n	26 wk - 4 hr[b]	n	52 wk - pre	n	52 wk - 4 hr	n
0	2.35 (1.965)	8	0.72 (0.810)	8	5.77 (3.420)	5	1.33 (0.607)	5
5	0.82 (0.641)	6	0.08 (0.066)[a]	6	2.49 (0.953)	3	0.33 (0.172)[a]	3
50	1.13 (1.756)	5	0.15 (0.211)	5	3.82 (1.645)	3	0.39 (0.142)[a]	3
100	0.18 (0.142)[a]	8	0.04 (0.093)[a]	8	1.70 (2.340)	5	0.14 (0.178)[a]	5

Testosterone (ng/mL) Mean (SD)
[a] Significantly different from controls P<0.05
[b] RT - data analyzed following rank transformation

Reverse T3, calcitonin
No treatment effects

Urinalysis

			control	LD	MD	HD		control	HD
	N	Week 13	8	6	6	8			
		Week 52	5	3	3	5		2	2
Volume (ml)	males	Week 13	43	54	57	63			
	females		31	43*	65*	71*			
	males	Week 52	84	104	115	62	Week 57	42	43
	females		47	87*	104*	36		34	58

Comment:

Higher urine volumes were accompanied by lower urine analyte (Na, K, Cl, Ca, creatinine) concentrations.

Other findings:

Urine specific gravity was decreased in Wks 4,13 in MD and/or HD

Ketonuria incidence was increased in Wks 13,26,39 and/or 52 in MD and/or HD

Gross pathology

A few MD and HD females had macroscopic lung findings (raised, dark or pale area) associated with histologic inflammation or fibrosis in interim sacrifice animals. This finding was of unclear significance.

Organ weight (Organ list: see Appendix)

Body weight (kg)

SACRIFICE		males					females			
		control	LD	MD	HD		control	LD	MD	HD
INTERIM		2.37	2.70	2.27	2.13		2.03	2.20	2.03	1.93
TERMINAL		3.07	3.40	3.20	2.83		2.50	2.37	2.33	2.17
RECOVERY		4.15	-	-	3.20		2.95	-	-	3.05

Organ weights (relative-to-body)

SACRIFICE		males					Females			
		control	LD	MD	HD		Control	LD	MD	HD
INTERIM										
Kidney		0.39	0.43	**0.55***	**0.54***		0.48	0.45	0.53	0.51
Testis		0.16	0.11	0.30	0.05					
Epididymides		0.05	0.05	0.07	0.04					
Thyroid/parath		0.013	0.010	0.010	0.009		0.008	0.011	0.011	0.011
Liver/gallbladd		1.7	1.9	**2.2***	**2.4***		2.0	2.2	2.5	**2.9***
Thymus		0.14	0.19	0.08	0.10		0.12	0.13	0.09	0.06
TERMINAL										
Kidney		0.41	0.38	0.45	0.47		0.43	0.47	0.56	0.53
Testis		0.27	0.29	0.23	0.16					
Epididymides		0.06	0.07	0.06	0.05					
Thyroid/parath		0.012	0.010	0.012	0.010		0.014	0.012	0.014	0.013
Liver/gallbladd		1.6	1.6	1.9	2.0		2.2	2.1	2.4	2.7
Thymus		0.09	0.11	0.07	0.10		0.11	0.13	0.13	0.06
RECOVERY										
Kidney		0.34	-	-	0.40		0.38	-	-	0.43
Testis		0.48	-	-	0.12		-	-	-	-
Epididymides		0.09			0.05					
Liver/gallbladd		1.6	-	-	1.8		1.9	-	-	1.8
Thymus		0.04	-	-	0.12		0.17	-	-	0.12

* statistically significant changes

Comments:

- Relative liver and kidney weight increases in mid and high dose interim and terminal sacrifice groups were partly due to increased organ weights and partly to decreased body weights.
- Liver weight increase may have been correlated with the histologic finding of periportal vacuolation. This may have been associated with an increase in P450 enzyme activity (induction) particularly at the interim sacrifice time point (D180: 76% increase in hepatic P450 content in high dose relative to controls)
- Kidney weight increase in interim and terminal animals may have been associated with tubule mineralization, but the association is not convincing.
- Thyroid/parathyroid weight decrease (absolute and relative) possibly resulting from pharmacologic drug effect was only seen in high dose males

- Decreased (absolute and relative) testicular and epididymidal weight in high dose interim/terminal/recovery animals was of unclear significance. The finding may have been drug-related in parallel with decreased testosterone levels.
- Lower (absolute and relative) thymus weight in mid and/or high dose interim/terminal sacrifice animals was associated with a higher incidence of histologic involution, the higher weight in the recovery animals with a lower degree of involution.

Histopathology (Organ list: see Appendix)

Histopathology findings: Incidence (numbers in parentheses represent mean incidence x severity, where relevant)

SACRIFICE		Males					Females			
		control	LD	MD	HD		control	LD	MD	HD
INTERIM		(3)	(3)	(3)	(3)		(3)	(3)	(3)	(3)
Adrenal cortex	Mineralization		1	2						
Heart	Myofiber degeneration						2 (0.7)	1 (0.3)		
Liver	Vacuolation, hepatocellular, periportal (MS)	1 (0.3)								2 (1.7)
	Eosinophilia, cytoplasmic, increased				1 (0.7)				1 (0.3)	
	Necrosis						1 (0.3)		1 (0.3)	
Kidney										
	Tubule, mineralization	2 (0.7)	2 (0.7)	2 (0.7)	3 (1.3)		3 (1.0)		1 (0.3)	3 (1.0)
	Tubule, degeneration		1 (0.3)		1 (0.3)					
	Inflammation, suppurative				1					
	Polykaryocytosis, ducts				1					
Lymph node, mesenteric	Macrophages, pigmented		1	1	1				1	2
Thymus	Involution	2 (0.7)		2 (0.7)	3 (1.7)		2 (1.0)	2 (0.7)	3 (1.0)	3 (2.0)
Testis	Juvenile	2	1	1	3					
Vagina	Infiltrate, lymphohistiocytic						1 (0.7)	2 (1.0)	3 (1.3)	3 (1.7)
Marrow (sternum)	Lymphoid germinal center			1 (0.3)	1 (0.3)			1 (0.3)	2 (0.7)	
TERMINAL		(3)	(3)	(3)	(3)		(3)	(3)	(3)	(3)
Adrenal cortex	Mineralization								1	
Heart	Myofiber degeneration									1 (0.3)
Liver	Vacuolation, hepatocellular, periportal			1 (0.3)						
	Necrosis			1 (0.3)						1 (0.7)
Kidney	Tubule, mineralization	2 (0.7)	1 (0.3)	2 (1.0)	2 (0.7)				2 (0.7)	1 (0.3)
	Tubule, degeneration				1 (0.3)			1 (0.3)		
Lymph node, mesenteric	Macrophages, pigmented	0	2 (0.7)	1 (0.7)	3 (1.3)				1 (0.3)	3 (1.7)
Thymus	Involution	2 (0.7)	2 (0.7)	3 (1.3)	3 (1.3)		2 (0.7)	1 (0.3)	2 (0.7)	3 (2.0)
	Hemorrhage				2				1	
Muscle	Necrosis			1 (0.7)						
	Hemorrhage			1 (0.7)						
	Inflammation, suppurative							1 (0.7)		
Testis	Juvenile	2			2					
Vagina	Infiltrate, lymphohystiocytic						1 (0.3)	3 (1.3)	3 (1.7)	2 (0.7)
Marrow (sternum)	Lymphoid germinal			1 (0.7)	1 (0.7)		1 (0.3)	1 (0.3)	1 (0.3)	2 (1.0)

	center									
Marrow (rib)	Lymphoid germinal center				1				1	1
RECOVERY		(2)			(2)		(2)			(2)
Liver	Vacuolation, hepatocellular, periportal									1 (0.5)
Kidney	Tubule, mineralization	0			1 (1.0)		1 (0.5)			2 (1.0)
Thymus	Involution	2 (2.5)			2 (1.0)					1 (0.5)

Comments:

- In determining possibly drug-related toxicities findings in MD and HD group can be considered together since AUC values in these groups were similar
- For most findings the number of animals was too small to conclude unequivocally whether the effect was drug-related
- The adrenal cortical mineralization was possible drug-related
- The cardiac and muscle histopathology findings in HD and MD groups, respectively, were not associated with increased serum creatine kinase levels in the affected animals
- The liver finding of periportal vacuolation in the interim sacrificed high dose females and the increased eosinophilia may have been drug-related and associated with the increased liver weight and hepatic P450 enzyme induction but the evidence is not conclusive
- The liver necrosis in the terminal HD female was possible drug-related
- The kidney finding of tubule mineralization may have been drug-related and associated with the increased kidney weights in mid and high dose animals (particularly interim sacrificed males) but the evidence is not conclusive
- The kidney tubule degeneration was possible drug-related
- The lymph node finding in interim and terminal animals (pigmented macrophages) appeared to be treatment-related but the significance of this finding is unclear.
- The thymus finding of involution had a drug-related increase in incidence and severity
- The vagina finding in interim and terminal animals (infiltrate) appeared to be treatment-related but the significance of this finding is unclear.
- The bone marrow finding in interim and terminal animals (lymphoid germinal center) appeared to be treatment-related.
-

Other findings:

- Parasitic lesions were seen in multiple animals and most frequently these were granulomas in cecum and colon. Parasites were also present in stomach, skeletal muscle and tongue.
- In multiple tissues, lymphohistiocytic infiltrates were observed (liver, kidney, brain, tongue, lacrimal gland, salivary gland, urinary bladder, urethra and vagina). The one tissue were this finding appeared treatment/dose-related was the vagina but this may have been a coincidental result. The mechanism and relevance of this lesion is unclear.

Toxicokinetics

TK parameters for parent drug compound (average values, m and f) (N=6-8/sex on D1-180, and 3-5/sex on D358)

Group		control	LD	MD	HD
Mg/kg/day			5	50	100
Cmax (ng/ml)	Day 1	NDL*	[redacted]		
	180	NDL			
	358	NDL			
Tmax (h)	Day 1	NDL	3.1	5.8	7.8
	180	NDL	2.5	5.3	6.4
	358	NDL	2.7	8.0	8.2
AUC 0-24h	Day 1	NDL	68	1080	1440

(ng*h/ml)	180	NDL	101	1030	1180
	358	NDL	97	1080	1180
CL/F (l/h/kg)	Day 1	NDL	79	44	69
	180	NDL	57	52	94
	358	NDL	55	50	94
Acc. Ratio	Day 1	NDL	-	-	-
	180	NDL	1.5	1.1	1.2
	358	NDL	1.6	0.95	0.95

*NDL = no detectable level (<5 ng/ml)
Acc.Ratio = AUC(Day 180 or Day358) divided by AUC(Day 1)

AMENDMENT to FINAL REPORT

Toxicokinetics of metabolite M7 (AMG102664)

TK parameters for M7 (average values, m and f) (N=6-8/sex on D1-180, and 3-5/sex on D358)

Group		LD	MD	HD
Mg/kg/day		5	50	100
AUC (ngxh/mL)	Day 1	3770	32500	64800
	180	5700	67600	131000
	358	4840	61200	65900

These additional data show high exposure to M7, which is increased on Day180 as compared to D1 (in accordance with induction of hepatic P450 metabolism, and/or long T1/2 for metabolites). AUC was increased linearly with increasing dose.

AUC Ratio M7:parent (ngxh/mL:ngxh/mL)

Group		LD	MD	HD
Mg/kg/day		5	50	100
AUC Ratio M7:parent	Day 1	55x	30x	45x
	180	56x	66x	111x
	358	50x	57x	59x

This indicates a ratio of approximately 50x for M7 (as compared to parent). This agrees with single dose PK and plasma metabolite data for monkey. This ratio confirmed the validity of ratios assumed by Reviewer for calculation of exposure multiples for parent and metabolites (ADME section of NDA review). M7 is not present in humans (M5 is). M5 and M7 are present in monkeys at similar levels. Thus, it is expected that both M5 and M7 AUC levels in monkey are about 50x higher than parent AUC.

Hepatic cytochrome P450

Hepatic cytochrome P450 content (average values, m and f) (N=6/sex, D180, and N=3/sex, D358)

		control	LD	MD	HD
Nmoles/mg pretein	Day 180	1.53	-	-	2.69 (1.76x)
	358	1.43	1.40	-	1.95 (1.36x)

Summary toxicokinetics:

- Large variability in Cmax and AUC data (SD up to 50%)
- Tmax varied from 2-12h and was increased with dose
- AUC and Cmax values in LD group may be slightly underestimated (several samples NDL)
- CL/F not significantly different in females than in males,
- AUC and Cmax increased much less than proportionally between 50 and 100 mg/kg/day on all days particularly on Days 180 and 358. Sponsor suggests that this may have been due to a decrease in rate and

extent of absorption at the HD related to lipophilicity of molecule. This Reviewer concurs with this possibility.

- On Days 180 and 358 this less-than-proportional increase in Cmax and AUC in HD was more prominent than on Day 1 and this was paralleled by a decrease in CL/F in HD on Days 180 and 358 as compared to Day 1. Thus, there was a possible induction of clearance in the HD group that was revealed at the later times in the study (autoinduction).
- Metabolite M7 was present in serum at levels of ca. 50x those for parent drug
- Hepatic cytochrome P450 content was increased in the HD as compared to control (Day 180, 1.8x control value) or as compared to control and LD (Day 358, 1.4x control value). Content was same for males and females. This indicates induction of P450 enzymes and increased hepatic clearance at 100 mkd (HD) as suggested by the CL/F data.
- There was no significant accumulation of test compound (as in 3-month study)

SUMMARY AND EVALUATION

Background

Calcimimetics are small molecules that act as allosteric modulators of calcium sensing receptors present in the parathyroid gland, thyroid C-cells, kidney and other cells in the body. The main pharmacologic action of the calcimimetic AMG073 consists of a lowering of parathyroid gland PTH secretion due to a drug-induced increase in the sensitivity of the parathyroid gland to extracellular calcium. Calcimimetics also stimulate thyroid C-cell calcitonin secretion. These actions result in a reduction of serum calcium levels. In patients with hyperparathyroidism (HPT) PTH levels are elevated. In primary HPT this is due to excessive PTH secretion, and in secondary HPT it is usually due to renal failure with chronic hypocalcemia and an adaptive increase in PTH secretion. The compound AMG-073 is being developed for the treatment of primary and secondary HPT.

Summary of findings in one-year monkey toxicity study

LD, MD, HD: 5, 50, 100 mg/kg/day

		Males	Females
Signs	Non-formed feces	LD, MD, HD	LD, MD, HD
	Poor appetite	MD, HD	MD, HD
Body weight	BW gain reduced (Wk1-26)	MD, HD	MD, HD*
Hematology	RBC decrease	HD	HD*
	Hb, Hct decrease	MD*, HD*	MD, HD*
	WBC decrease	HD	MD, HD*
	Reticulocyte count decrease	HD*	HD*
	PT increase (@ Wk26 only)	HD*	HD*
Electrocardiography	Increase in QT and QTc (Wk 26)	LD, MD, HD	LD, MD, HD
Clinical chemistry	Ca (total or ionized) decrease	LD, MD*, HD*	LD, MD*, HD*
	P increase	MD, HD*	MD*, HD*
	Glucose decrease	HD*	HD
	Triglyceride increase (@ Wk13)	MD, HD*	MD*, HD*
	Cholesterol decrease		HD
	AST increase (@ Wk13)	HD*	HD*
	ALT increase	HD* (2x)	HD* (4x)
	Creatine kinase increase	HD (6x, due to 1 animal)	HD (2x)
Endocrinology	Serum PTH decrease	LD, MD, HD	LD, MD, HD
	Vit D decrease	HD	MD, HD
	T3 decrease	MD, HD	HD
	T4 increase	MD, HD	MD, HD
	Testosterone decrease (marked)	LD, MD, HD	
	TSH, ACTH	No effects	

Organ weight	Kidney increase (@ 6mo)	MD*, HD*	
change (absolute	Kidney increase (@ 12mo)	HD	MD
and/or relative)	Liver/gallbladder increase (@6mo)	MD*, HD*	MD, HD*
	Liver/gallbladder increase (@12mo)	MD, HD	HD
	Testis decrease (@6,12 mo)	HD	
	Epididymides decrease (@6,12 mo)	HD	
	Thymus decrease (@6 mo)	MD	HD
Histopathology	Adrenal cortex, mineralization	LD, MD	
@6mo	Liver, hepatocellular vacuolation		HD
	Liver, increased eosinophilia	HD	MD
	Kidney, tubule mineralization	HD	
	Kidney, tubule degeneration	LD, HD	
	Lymph node, pigmented macrophages	LD, MD, HD	MD, HD
	Thymus, involution	HD	MD, HD
	Vagina, infiltrate		LD, MD, HD
	Bone marrow, lymphoid germinal center	MD, HD	LD, MD
Histopathology	Adrenal cortex, mineralization		MD
@12mo	Liver, necrosis		HD
	Kidney, tubule degeneration	HD	LD
	Lymph node, pigmented macrophages	LD, MD, HD	MD, HD
	Vagina, infiltrate		LD, MD, HD
	Thymus, involution	MD, HD	HD
	Bone marrow, lymphoid germinal center	MD, HD	MD, HD

*statistically significant effects

Findings by dose group (MD and HD combined due to similar AUC values)

Dose group	Dose (mg/kg/d)	Human AUC multiple*	Findings
LD	5	0.3x	Abnormal feces, testosterone decrease, kidney tubule degeneration, lymph node macrophages, bone marrow lymphoid center Ca decrease, PTH decrease (pharmacologic effects)
MD HD	50 100	3x 4x	Appetite suppression, body weight reduction, hematology changes, triglyceride increase, ALT/AST increase, CK increase, endocrinology changes, kidney and liver weight effects and pathology changes, testicular and thymus weight effects, thymus involution P increase (pharmacologic effect)

*based on human 100 mg dose (AUC 300 ngxh/ml)

NOAEL <5 mg/kg/day
LOAEL 5 mg/kg/day (LD)

Comments

The results of this study indicate that AMG-073, as expected, markedly and dose-dependently suppresses PTH secretion and lowers serum calcium and increases serum phosphorus in healthy normocalcemic animals. The hypocalcemia and hyperphosphatemia was mainly seen at mid and high doses (50-100 mg/kg/day). The decreased serum Ca levels are due to the lowering of serum vitamin D resulting in decreased Ca absorption and to inhibition of renal Ca reabsorption. Hyperphosphatemia resulted from elevated reabsorption of phosphate in the renal proximal tubule due to lowered PTH levels.

Hypocalcemia generally results in immediate physiological effects while hyperphosphatemia is generally thought to have no significant effects. Most findings in this study however have an unclear (if any) relationship to the hypocalcemia. These included clinical findings of emesis, soft feces, and reduced body weight gain, and hematology findings, serum chemistry changes, endocrinology changes (other than PTH and VitD), histopathology and organ weight changes.

The extent and severity of the toxicologic effects observed in this study was small to moderate and it could be argued that the doses used were too low. However, in a previous 3-month monkey toxicity study the high dose of 150 mg/kg/day was lowered to 100 mg/kg/day due to marked body weight and food consumption effects.

The mechanism underlying the adverse gastrointestinal and body weight effect is unclear. The mechanism of the drug-related hematology changes (decrease in RBC, Hb and Hct and WBC) is also unclear. The triglyceride increase may have been related to some effect of the test compound on hepatic lipid metabolism. The transient decrease in serum glucose in HD groups in Wk26 was small but the significance unclear. The AST and ALT increases in the HD groups are of unclear origin. The creatine kinase increase at high doses could be due to the hypocalcemia affecting the integrity of the muscle cell membrane or due to hypocalcemia-induced neuromuscular irrritability. Muscle or cardiac histopathology in animals with elevated CK levels was not observed.

The effects on thyroid hormones and testosterone are unexplained. The decrease in serum testosterone accompanied the decrease in testicular (and epididymidal) weight in the HD group. However, the hormone effect also occurred in the lower dose groups. There was no male reproductive organ pathology.

The kidney weight increase in mid and high dose males at interim sacrifice was also seen although less pronounced in females and at terminal sacrifice. There was some indication that this was associated with an increased in kidney tubular mineralization but the evidence was not conclusive. Kidney tubule degeneration was also seen in some treated animals but incidence and severity were very small.

An increase in liver weight was observed in MD and HD males and females particularly at interim sacrifice. This finding was not clearly associated with liver histopathology findings although in interim females there was an increased incidence and severity of periportal vacuolation and in males there was some increase in eosinophilia and in a terminal female there was an increased degree of hepatocellular necrosis. The increased weight may simply have been related to the induction of microsomal P450 enzyme activity demonstrated in HD males and females.

The thyroid/parathyroid weight decrease in males particularly in the 6-month interim animals may have been related to the pharmacologic effect of the test compound to inhibit parathyroid PTH release. However there were no parathyroid histology findings.

The increased incidence and severity of thymus involution in drug-treated animals and the associated decrease in thymus weight in both sexes indicated that this finding was clearly drug-related. The etiology of this finding however is unclear but may be related to the decreased WBC (lymphocyte) count.

The presence of pigmented macrophages (histiocytes) in the lymph node of treated animals appeared to be drug-dose-related but the mechanism underlying this effect is unclear. The cells may have been involved in some form of phagocytosis. The drug-related finding of increased incidence and/or severity of lymphohystiocytic infiltrate in the vagina and other tissues is also unexplained. The presence of lymphoid germinal centers (immature lymphocyte clusters) in the treated animals in sternum and rib bone marrow was of unclear significance but may have been related to the presence of tissue lymphohistiocytic infiltrates. The lymphohistiocytic findings may be related to the parasites.

Calcium-sensing receptor-like sequences have been found in brain, intestine, testis, and skin and this may explain some of the findings such as the body weight and GI effects and the testicular effects observed in this study.

In conclusion, the main potential target organs for toxicity of AMG-073 appear to be GI tract, hematopoietic system, liver, kidney, thyroid, testis, epididymis, thymus, lymph node, bone marrow, muscle/heart, adrenal

ADME and Comparative PK

Absorption of AMG073 is extensive (80%) but bioavailability of absorbed radioactivity is limited (25%) due to first pass metabolism. There is extensive hepatic metabolism in animals and humans via oxidation and conjugation prior to eliminiation. After a radioactive dose, <0.3% of the circulating radioactivity is due to unchanged AMG (parent drug). Monkey and human metabolite profiles are most similar. Main metabolites (M1-M8) are carboxylic acid derivatives, and dihydrodiol (through oxidation of naphthalene ring) and are thought to be inactive. The metabolites are conjugated to glycine or glucuronide in mice, monkeys and humans. PK in humans is linear in the range of [redacted]. AMG073 is extensively bound to protein in mouse, rat, dog, monkey and human plasma in the range of 92-99%. Elimination is predominantly renal.

Human PK data (from 8-day and 18-week studies).

Study	Dose	Cmax (ng/ml)	AUC (ng.h/ml)	Tmax (h)
8 days	25 mg		36	2-4h (single dose)
8 days	50 mg		128	
??	100 mg		184	
18 weeks	100 mg		300*	
#20000187 (ascending dose stud, 7 days on each dose)	175 mg		648 (median)	2h

*extrapolated value on basis of Cmax measured

Human AUC multiples attained in 1-year monkey study*

Dose group	Dose (mg/kg/day)	Monkey AUC values @Wk 26 (ngxh/ml)	Human AUC multiple (based on 180 mg human dose)*
LD	5	101	**0.16x**
MD	50	1030	**1.6x**
HD	100	1180	**1.8x**

*Based on maximum (ceiling) human AUC of 648 ngxh/mL @ 175 mg dose (Study # 20000187)

Notes:

- The calculated human AUC multiples are based on parent drug compound, which constitutes <1% of the circulating drug pool. However, since the metabolite profiles appear to be similar in monkey and man (and the metabolites appear to be pharmacologically inactive) the multiples based on parent drug are relevant.
- M5 and M7 levels in monkey are 50x those of parent, and M2-Glu levels are 100x those of parent. In humans, M5 levels approximately 100x times those of parent and M2-Glu levels 15x parent (in ng/mL units). This suggests that exposure multiples in monkey based on the sum of (metabolites + parent) would be increased as compared to those based on parent only. However, since M2-Glu and M5/M7 metabolites are inactive at the CaR, parent multiples are most relevant.
- In various subsequent clinical PK studies Cmax values or ranges attained at the doses mentioned in the Table above were larger than those mentioned in this table (cf. Submission October 5, 2001, #140, pre-meeting briefing package). This means that the human AUC values in the Table above may be underestimated and the calculated human AUC multiples may be overestimated.

Comparison with results from previous toxicity studies

In the 3-month monkey study similar effects to those seen in the 1-year study were body weight reduction, hematology effects, Ca, P, trig, AST, ALT, CK increases, thymus small, kidney and liver weight increases, testis and epididymides weight decreases, liver necrosis, thymus involution.

In 3-month and 6-month rat and mouse studies the effects of the test compound that were similar to the effects in the monkey studies were: body weight reduction, Ca decrease and P increase, ALT increase, triglyceride increase, serum PTH decrease, kidney mineralization (pelvis) (rat), thymus lymphoid atrophy

Safety Evaluation

Apart from the expected pharmacologic effects of the test compound several findings were seen in multiple organ systems that appeared to be drug-related. This Reviewer concludes that the NOAEL was <5 mg/kg/day, and the LOAEL 5 mg/kg/day. The Sponsor concluded that the NOAEL was 5 mg/kg/day. Sponsor also concluded that there were no test-article related microscopic alterations and that the predominant treatment effects were clinical signs, clinical pathology changes and organ weight effects. Although this Reviewer generally concurs with the Sponsor there were microscopic effects that may have been associated with clinical pathology or organ weight findings. However, the number of animals was too small to come to a definite conclusion regarding most of these microscopic effects.

The effects observed at the LOAEL of 5 mg/kg/day (human AUC multiple 0.3x) included GI effects (abnormal feces), testosterone decrease, kidney tubule degeneration, lymph node macrophages, bone marrow lymphoid germinal center.

At doses above the LOAEL (50 and 100 mg/kg/day; human AUC multiples 3-4x) effects included appetite suppression, body weight reduction, hematology changes, triglyceride increase, ALT/AST increase, CK increase, endocrinology changes, kidney and liver weight increases and pathology changes, liver enzyme induction, testicular, epididymidal and thymus weight reduction (in addition to the effects seen at the LOAEL).
For most findings there is no explanation based on the pharmacologic action of the drug and the clinical relevance of these findings is unclear.
Thus, the target organs identified in this study are GI tract, hematopoietic system, liver, kidney, testis, thymus, neuromuscular apparatus.

AMENDMENT at NDA submission (Sept 5, 2003)

Toxicokinetics: AMENDMENT to Final Report:
Analysis of metabolites AMG102664 (M7) showed exposure at all dose levels, at least 30-fold greater than to parent AMG099073-01. There was no sex difference in exposure (AUC) to M7 metabolite. The metabolite M7 is monkeys is present at similar levels as metabolite AMG501345 (M5). Based on single dose plasma metabolite analysis, both metabolites together constitute approximately 1/3 of total drug-related material. About 2/3 of total material is glucuronidated AMG-073, or metabolite M2. Parent AUC in monkey is ca. 0.3% of total drug-related material. The results and implications of the single dose data as interpreted by Reviewer (NDA review) were confirmed by the data on M7 acquired in the current 1-year study.

EKG: AMENDMENT to Final Report
EKG analysis showed increases in QT, QTc and ST interval in all dose groups in dose-related manner, at Wk13 and Wk26, as was observed in the 3-month study at Wk13. The effect was less pronounced at Wk39 and minimal at Wk52. Effect was similar in males and females. The QT prolongation may have been related to hypocalcemia. However, serum calcium levels were lower in Wk 52 than in Wk 26. Sponsor argued that the attenuation of the QT(c) effect over time in the 12-month study is an "adaptive effect" to chronic hypocalcemia. There are no data in the literature suggesting such a phenomenon.

Histopathology Inventory

Sponsor Study # 100188	
Species: Monkey	Examined
Adrenals	X*
Aorta	X
Bone Marrow smear (sternum)	X
Bone marrow (rib)	X
Bone (femur)	X
Bone (sternum)	X
Brain	X*
Cecum	X
Colon	X
Duodenum	X
Epididymis	X*
Esophagus	X
Eye	X
Fallopian tube	
Gall bladder	X
Gross lesions	X
Harderian gland	
Heart	X*
Hyphophysis	
Ileum	X
Injection site	
Jejunum	X
Kidneys	X*
Lacrimal gland	X

Larynx	
Liver	X*
Lungs with bronchi	X*
Lymph nodes, cervical	
Lymph nodes mandibular	X
Lymph nodes, mesenteric	X
Mammary Gland	X
Nasal cavity	
Nerve (brachial) + muscle	X
Optic nerves	X
Ovaries	X*
Pancreas	X
Parathyroid	X
Peripheral nerve (brachial)	X
Pharynx	
Pituitary	X*
Prostate	X*
Rectum	X
Salivary gland	X
Sciatic nerve	
Seminal vesicles	X
Skeletal muscle	X
Skin	X
Spinal cord	X
Spleen	X*
Stomach	X
Testes	X*
Thymus	X*
Thyroid	X*
Tongue	X
Tonsils	X
Trachea	X
Ureter	X
Urethra	X
Urinary bladder	X
Uterus	X
Vagina	X
Zymbal gland	

* organ weight obtained

APPEARS THIS WAY ON ORIGINAL

SUMMARY TABLES OF TOXICITY FINDINGS IN MOUSE, RAT AND MONKEY

MOUSE, acute studies (LOAEL values)

	Acute oral (#970153)	Acute i.p. (#970154)
Doses (mg/kg/day)	0, 10, 100, 500	0, 1, 5, 20
N/sex/group	5	5
Mortality	10 (1f), 100 (1f), 500 (1m)	20 (1m,1f)
Clinical signs	500 (decreased activity, abnormal gait, discolored urine, respiratory signs, followed by death in m)	20 (hypoactivity, abnormal gait, flaccid body tone, body quiver/prostation)
Body weight reduction	500 (m)	>20
Food consumption reduction	500 (m)	>20
Organ weight change	None	None
Pathology findings	10 (GI distension, in m and f that died)	20 (GI distension and/or dark discoloration, in m and f that died)
LD_{50}		
NOAEL	<<10	5
LOAEL	<10	20

RAT, acute studies (LOAEL values)

	Acute oral (#970151)	Acute ip. (#970152)	Acute oral (#970012) non-GLP*	Acute oral (#100326)
Doses (mg/kg/day)	0, 10, 100, 500	0, 1, 5, 20	1000, 2000	1000, 1500
N/sex/group	5	5	5 (f only)	5
Mortality	500 (1f) unclear cause	None	None	1500 (f)
Clinical signs	No signs	No signs	1000 (salivation, ↓activity, rales, ptosis, staining eye and nose)	1000 (thin, hypoactivity, hypersensitivity, red face staining, squinted eyes, liquid feces, tremor) 1500 (f that died): abnormal gait+posture, lacrimation, urogenital staining)
Body weight reduction	500	20 (m)	nd	No sign effect
Food consumption reduction	500	>20	nd	Nd
Organ weight change	None	None	nd	Nd
Pathology findings	None	5 (spleen and liver adhesion, discoloration, fibrosis, capsulitis, discoloration due to drug-induced local irritation)	nd	None
LD_{50}				>1500
NOAEL	100	1	<1000	<1000
LOAEL	500	5	<1000	<1000

Nd= not determined

* lack of effect possibly due to heating of test article solution; results not reliable

RAT, repeat dose oral studies (LOAEL values)

Study	14-day, oral gavage (#970018)	1-month, oral gavage (#970070)	6-month, oral gavage (#100082)
Doses (mg/kg/day)	0, 50, 250, 500	0, 5, 50, 125	0, 5, 25, 100
N/sex/grp	5	10+6	20+6, 15+6, 15+6, 20+6
N/sex/grp (recovery)			5, 0, 0, 5 (4 wks)
Mortality	250	No drug-related	No drug-related
Clinical signs	50 (stained fur, abnormal breathing, dehydration 250/500 (salivation, sneezing, eyes closed, pallor, weakness, tremors, cold to touch, thin, hunched posture, distention of abdomen, soft feces, reduced feces, reduced activity, tremors) 500 (convulsions, in 1/5m, 2/5f)	50 (stained fur, abnormal breathing, dehydration 125 (pallor, hunched posture, decreased activity, cold to touch, weak, salivation, thinness)	25 (abnormal breathing , dehydration, salivation)
Body weight reduction	50	50	25 (m), 100 (f)
Food consumption reduction	50	50	25 (m), 100 (f0
Early cataract formation	250 (all animals @250, 500)	50	100
Reduced RBC, Hb, Hct	50	-	-
Increased RBC distrib width	50	-	-
Reduced WBC	250	-	-
Increased neutrophil count	-	-	100
Increased PT	250	50 (f), 125 (m)	25 (m), 100 (f)
Increased APTT	-	-	100 (f)
Decrease in serum Ca	50 dd	5 dd	5 dd
Increase in serum P	50 dd	50 dd	5 dd
Glucose decrease	-	-	100
ALT increase	250	50	100
BUN increase	250	50	25 (Wk 13), 100 (Wk 26)
Creatinine increase	500(m)	125 (m)	100
Cholesterol increase	50	50	100
Triglyceride decrease			100
Albumin decrease	250	50(f)	100
G-globulin increase			100
Total protein decrease	500(m)	125(f)	100
Serum Na, Cl, and/or K decrease	250	50	100
Alk Phos increase	-	-	100
Creatine kinase decrease	nd	nd	25(f), 100(m)
Decrease in serum PTH @ 2h post dose	50 (non dd, 2h post dose)	5 (non dd, 2h post dose)	5 (non-dd, 2h post dose)
WBC in urine	250	-	-
Urine volume	nd	nd	nd
Urine Ca (mg/dL) increase	nd	50 (marked)	5(m), 25(f)
Urine Na and/or K (meq/L) decrease	250	50	25(f), 100(m)
Liver weight increase (rel)	500	50	100
Uterus weight decrease (abs, rel)	250	125	-
Thymus weight decrease (abs, rel)	250	-	-
Prostate weight decrease (abs, rel)	250	-	-
Thyr/parathyr weight increase (rel)			100
Emacation	250	125(f)	-
Thymus small	250	-	100
GI dilatation, thickening	500	-	-
Prostate, small	250	-	-
Eye, lenticular degeneration	-	125	100
Adrenal, degeneration/necrosis, zona fasciculate	250	125(f)	-
Heart, necrosis and/or myocardial	250	125(f)	25(m), 100(f)

degeneration			
Heart, myocardial inflammation	-	125	100(f)
Kidney, necrosis/regeneration of cortical tubules	250	-	-
Kidney, mineralization, pelvis diverticulum	-	-	25(f), 100(m)
Lung, histiocytosis	-	-	100
Thymus, lymph node, spleen, lymphoid atrophy/necrosis	250	125 (f)	25(m), 100(f)
Stomach, erosion	-	-	100(f)
Salivary gland, acinar hypertrophy	250	-	-
Uterus, atrophy	250	125	-
Ovary, degeneration	125	125	-
Bone marrow, hypocellularity	250	125	-
Prostate, atrophy	500	-	-
Testis, tubular atrophy	-	50	100
Intestine, atrophy	500	-	-
Colon/rectum, hyperplasia, mucosal	500	125	-
Cecum, hyperplasia, mucosal/inflammation	-	50(f), 125(m)	5

- = no effects
dd dose-dependent
nd not determined

Reversibility of findings at 100 mkd HD in 6-month study:

Reversed or partially reversed were: Body weight and food consumption effects, neutrophil change, APTT increase, PT increase (partial), serum Ca, serum P (partial), serum BUN/creatinine (partial), ALT, CK changes, urine electrolyte changes, liver weight increase (partial), cecum mucosal hyperplasia, myocardial degeneration/necrosis, thymus lymphoid atrophy

Not reversed were: Cataract formation, femoral bone changes (area increase), kidney mineralization, pelvis diverticulum (crystalloid material near/in thickened pelvis epithelium or in distal collecting tubule lumen), serum protein/albumin decrease and G-globulin increase (f, 6-mo study)

MONKEY, repeat dose oral studies (LOAEL values)

Study	3-month nasal gastric intubation (#100020)	1-year nasal gastric intubation (#100188)
Doses (mg/kg/day)	0, 5, 50, 100, 150(D1→16)/100	0, 5, 50, 100
N/sex/grp	6 , 4, 4, 6	8, 6, 6, 8 3/s/g sacrificed at 26wks and 52 wks
N/sex/grp (recovery)	2, 0, 0, 2 (2 wks)	2, 0, 0, 2 (4 wks)
Mortality	None	none
Signs	5 (soft, liquid feces) 50 (emesis, poor-to-fair appetite, excessive salivation)	5 (abnormal or non-formed feces) 50 (poor appetite) 100 (emesis)
Body weight loss	100 (first 2 wks)	50 (first 2 wks)
Body weight gain reduced	5 (f), 50 (m) (13 wks)	100 (m), 50 (f) (first 26 wks)
QT interval prolongation	5 (m,f) (Wk 13)	5 (m,f) (Wks 13, 26, 39, 52)
RBC decrease	100	100 (wk 13-52)
Hb, Hct decrease	50	50 (wk 13-52)
Reticulocyte decrease	100	100 (wk 13-52)
PT increase	100	50 (wk 13-26)
APTT increase	150/100	-
WBC decrease (neutrophil, lymphocyte)	100	100 (wk 26-52)
Monocyte/eosinophil decrease	50 (f)	100
Ca (total or ionized) decrease	5	5 (wk 4-52)
P increase	50	50 (wk 13-52)
Glucose decrease	-	50 (wk 26-39)
Total cholesterol decrease	50	100 (f) (wk 4-52)
Triglyceride increase	100 (m)	50 (wk 13-39)
AST increase	100	100 (wk 13-52)
ALT increase	100	100 (wk 4-52)
ALKP decrease	-	50(m) (wk 4-52)
Albumin decrease	150/100(f)	-
Creatine kinase increase	100	100 (wk 13-52)
Urine volume increase	-	5 (f) (wk 13-52, not clearly dd)
Urine Na, K, Cl (meQ/L and meQ/t) decrease	-	5 (f) (wk 13-52, sporadic)
Urine Ca increase (mg/dL)	-	-
Urine specific gravity decrease	nd	50 (Wk 4-13)
Serum PTH decrease	5 (2h post dose)	5 (2-12h post dose)
Vit D decrease	nd	50 (f), 100 (m)
Total T3 decrease	nd	50 (m), 100 (f)
Total T4 increase	nd	50(f), 100 (m)
Testosterone decrease (marked)	nd	5
TSH, ACTH	nd	No effects
Thymus weight decrease (abs, rel)	5-100 non-dd	50, 100 (f) (wk 26)
Testis weight decrease (abs, rel)	50	100 (wk 26-52)
Epididymis weight decrease (abs, rel)	100	100 (wk 26-52)
Thyr/parathyroid weight decrease (abs, rel)	50 (m)	100 (m) (wk 26-52)
Liver/gallbladder weight increase (abs, rel)	50 (m), 100 (f) (abs and rel)	50 (wk 26-52) (rel)
Kidney weight increase (abs, rel)	100 (f)	50 (wk 26-52)
Increase in hepatic P450 content	nd	100
Thymus small	100	-
Lung, macrophage infiltrate	100	-
Kidney polykaryocytosis, pelvic urothelium	50 (m), 100 (f)	-
Kidney, tubule degeneration	-	5
Kidney, tubule regeneration	100	-
Muscle, skeletal, degeneration	100(m)	-