Dose (mg/kg/day)		0	5	20	50/35
N examined		120	60	60	60
Mass(es)		90	34	39	27
Cervix	Enlarged	6	4	4	13
Liver	Prominent lobulation	27	2	3	3
Thymus	Reddened	0	0	0	3 all decedents
Uterus	Dilated	3	1	3	7
	Reddened	1	3	4	5
	Cyst	1	1	3	2

Histopathology:

Non-neoplastic:

MALES

Group		1+2	3	4	5	
Dose (mg/kg/day)		0	5	15	35	
N examined		120	60	60	60	
Lung	Vascular mineralization	32	32***	40***	34***	↑
	Minimal	24	23	20	17	
	Mild-Moderate	8	9	20	17	
	Bronchio-alveolar hyperplasia	0	1	4*	0	↑
Heart	Vascular mineralization	4	2	17***	38***	↑
	Minimal-Mild	3	1	14*	30***	
	Mod-Marked	1	1	3*	8***	
	Ossification	5	3	8*	9*	↑
	Minimal-mild	5	2	6	8	
	Mod-marked	0	1	2	1	
	Progressive cardiomyopathy	41	53**	49	45	↑
Thyroid gland	C-cell hyperplasia, focal	13	2	8	2	↓
Parathyroid gland	Hyperplasia, focal	18	5	6	5	↓
Pituitary gland	Cholesterol clefts	9	1	1	0*	↓
Testis	Tubular mineralization	6	3	11*	5	
	Vascular mineralization	7	14**	35***	30***	↑
	Sperm granuloma	0	0	1	2	↑
Epididymis	Vascular mineralization	0	0	5**	18***	↑
	Sperm granuloma	1	1	0	8***	↑
Kidney	Transitional cell hyperplasia, focal	22	10	20*	17	↑
	Chronic progressive nephropathy	97	51	54	43	≡
	Minimal	23	12	14	37***	↑
	Mild-Mod	70	36	42	6***	↓
	Marked-Severe	4	3	1	0	↓
	Tubular mineralization	5	1	5	8*	↑
	Vascular mineralization	2	3	11***	28***	↑
	Minimal-Mild	2	3	11***	20***	
	Mod-Marked	0	0	0	8***	

	Pyelitis	23	5	16	19	↑
Stomach	Mineralization, muscularis	0	1	1	7*	↑
Liver	Tension lipidosis	29	11	17	6*	↓
	Sclerosis, portal	11	8	8	11	↑
Eye	Cataract	3	0	1	4	↑
Harderian gland	Lobular hyperplasia	0	1	0	3*	↑
Spinal cord	Mineralization, meninges	0	0	3*	2	↑
	Compression	0	0	3*	0	
Sciatic nerve	Mineralization	0	0	0	3*	↑
Sternum	Mucinous degeneration, cartilage	0	0	3*	0	

FEMALES

Group		1+2	3	4	5	
Dose (mg/kg/day)		0	5	15	35	
N examined		120	60	60	60	
Lung	Vascular mineralization	25	33***	39***	33***	↑
	Minimal	20	26***	25***	15	
	Mild	5	7	14***	18***	
	Alveolar foamy macrophage accumulation	10	2	8	13*	↑
Heart	Vascular mineralization	2	6*	8**	15***	↑
	Minimal-mild	2	6*	8*	12*	
	Mod-marked	0	0	0	3*	
	Ossification	2	2	0	4	↑
	Progressive cardiomyopathy	51	30	38*	35*	↑
Thyroid gland	C-cell hyperplasia, focal	14	9	7	2	↓
Parathyroid gland	Hyperplasia, focal	5	3	0	0	↓
Adrenal gland	Medullary cell hyperplasia, focal	15	7	8	2	↓
Uterus	Squamous cyst	2	1	1	4	↑
	Cystic endometrial hyperplasia	14	11	15*	13	↑
	Cervical hypertrophy	19	10	13	11	≡
	Mild	15	6	10	2	↓
	Mod-severe	4	4	3	9	↑
	Dilation	0	1	2	3*	↑
Vagina	Squamous cell hyperplasia	0	1	1	4*	↑
Kidney	Chronic progressive nephropathy	80	34	44	52*	≡
	Minimal	20	4	15	25***	↑
	Mild-Mod	58	29	28	27	≡
	Marked-Sev	1	1	1	0	↓
	Fatal	1				↓
	Tubular mineralization	18	7	11	11	≡
	Vascular mineralization	1	0	3	8***	↑
	Minimal	1	0	2	4*	
	Mild	0	0	1	4*	
Liver	Tension lipidosis	35	18	11	5**	↓
Pancreas	Lipomatosis	1	0	0	4*	↑
Mammary gland	Periductal fibrosis	23	9	11	19	↑

	Minimal	21	9	10	11	
	Mild-mod	2	0	1	8**	
Eye	Cataract	3	0	1	3	↑
Spinal cord	Mineralisation, meninges	2	7**	**7	36***	↑
Sternum	Mucinous degeneration, cartilage	1	0	0	12***	↑

Small incidences of vascular mineralization were also observed in HD groups (males and/or females) in several organs (lung, lymph node, pancreas, brain, Zymbal's gland)

Neoplastic:

MALES

Group		1+2	3	4	5	p-value* (trend test, exact)	Historical control incidence range (%)
Dose (mg/kg/day)		0	5	15	35		
N examined		120	60	60	60		
BW gain		100%	98%	94%	74%		
Lung	Bronchio-alveolar carcinoma (M)	0	0	0	2 (3.3%)	0.030	0-1.7%
	Bronchio-alveolar adenoma	1	1	0	0	>0.05	0-4%
Hematopoietic system	Histiocytic sarcoma (M)	0	2	1	2	>0.05	0-8%
	Lymphoma (M)	0	1	1	1	>0.05	-
	Lymphoma, granular cell (M)	1	0	0	1	>0.05	-
	Lymphoma, lymphocytic (M)	0	0	0	1	0.2170	-
Combined	Malignant lymphomas	1	1	1	3 (5%)	0.0462	0-8%
Brain	Malignant astrocytoma (M)	0	1	0	1	>0.05	0-3.3%
	Ependymoma (B)	0	0	0	1	0.2099	0-0%
Combined	Glia cell tumors	0	1	0	2	0.0586	0-4.1%
Thyroid gland	C-cell adenoma (B)	25	6	2**	1***	↓	
Parathyroid gland	Adenoma (B)	8	2	1	1	↓	
Adrenal gland	Phaeochromocytoma (B)	14	8	4	0**	↓	
	Phaeochromocytoma (M)	4	0	2	0	↓	
Pituitary gland	Adenoma, anterior lobe (B)	29	16	16	11	↓	

FEMALES

Group		1+2	3	4	5	p-value* (trend test, exact)	Historical control incidence range (%)
Dose (mg/kg/day)		0	5	20	50/35		
N examined		120	60	60	60		
BW gain		100%	99+%	77%	64%		
Hematopoietic	Lymphoma (M)	0	1	0	2	0.0587	-

system							
	Lymphoma, lymphocytic (M)	0	0	0	1	0.2203	-
Combined	Malignant lymphomas	0	1	0	3 (5%)	**0.0163***	0-4%
Thyroid gland	C-cell adenoma	10	5	2	3	↓	
Parathyroid gland	Adenoma (B)	3	0	0	0	↓	
Adrenal gland	Phaeochromocytoma (B)	6	2	1	2	↓	
	Phaeochromocytoma (M)	2	0	1	0	↓	
Pituitary gland	Adenoma, anterior lobe (B)	70	38	33	25	↓	
Bone (other)	Osteosarcoma (M)	0	1	1	1		

*finding not significant if p >0.025 (rare tumor), or p>0.005 (common tumor)
Historical control incidences from 17 experiments (1994-2001)

AMG 099073-01
104 Week Carcinogenicity Study of AMG 099073-01 in Rats with Administration by Diet
Table 33 ***Summary of Tumour Findings: All Animals Combined***

	TUMOUR TABLE							
	Males				Females			
GROUP / DOSE	Cont 0 mg/kg /day	Grp 3 5 mg/kg /day	Grp 4 15 mg/kg /day	Grp 5 35 mg/kg /day	Cont 0 mg/kg /day	Grp 3 5 mg/kg /day	Grp 4 20 mg/kg /day	Grp 5 50/35 mg/kg /day
NUMBER OF ANIMALS	120	60	60	50	120	60	60	60
NUMBER OF ANIMALS WITH TUMOURS	92	44	37	35	112	54	50	51
NUMBER OF ANIMALS WITH SINGLE TUMOURS	39	23	19	26	26	15	16	23
NUMBER OF ANIMALS WITH MULTIPLE TUMOURS	53	21	18	9	86	39	34	28
NUMBER OF ANIMALS WITH BENIGN TUMOURS	74	38	32	26	106	52	45	44
NUMBER OF ANIMALS WITH MALIGNANT TUMOURS	38	11	12	11	45	15	22	19
NUMBER OF ANIMALS WITH METASTASISING TUMOURS	5		4		5	3	2	3
TOTAL NUMBER OF TUMOURS	199	71	68	46	301	155	120	105
TOTAL NUMBER BENIGN TUMOURS	153	60	53	33	236	136	97	81
TOTAL NUMBER OF MALIGNANT TUMOURS	46	11	15	13	65	19	23	24
TOTAL NUMBER OF METASTASISING TUMOURS	5		4		5	3	2	3
% ANIMALS WITH TUMOURS	77	73	62	58	93	90	83	85
% ANIMALS WITH SINGLE TUMOURS	33	38	32	43	22	25	27	38
% ANIMALS WITH MULTIPLE TUMOURS	44	35	30	15	72	65	57	47
% ANIMALS WITH BENIGN TUMOURS	62	63	53	43	88	87	75	73
% ANIMALS WITH MALIGNANT TUMOURS	32	18	20	18	38	25	37	32
% ANIMALS WITH METASTASISING TUMOURS	4		7		4	5	3	5

Control Groups (1 and 2) are combined

NOTES:

- There were no effects on # or % of animals with tumors, or on # of tumors
- There were no increases in incidence of tumors of the same histomorphogenic type (e.g. haemangiosarcoma), when combined for different anatomic sites.
- Sponsor submitted historical control data from control groups of CD-1 mice from 17 two-year studies (1994-2001). The current study was included in the historical database.

Summary of findings:

Group		1+2	3	4	5
Dose (mg/kg/day)		0	5	15	35

Agitation	M				
	F				
Body weight gain decrease	M				
	F				
Food consumption decrease	M				
	F				
Increase in neutrophils, Wk 52 and 104	M				
Decrease in Hct, Wk 104	M				
Decrease in serum PTH	M,F				
Decrease in serum Ca/ionized Ca	M,F				
Increase in serum P	M,F				
Decrease in triglycerides	M				
	F				
Increase in bilirubin	F				
Kidney weight decrease	M,F				
Uterus dilation, reddening	F				
Uterine cyst	F				
Cervical enlargement	F				

Comment:

Histopathology

Non-neoplastic findings:

- Increased incidence of vascular mineralization in lung (all doses; m,f), heart (all doses; m,f), kidney (all doses; m,f), testis (all doses; m), epididymis (MD, HD; m)
- Increased incidence of ossification in heart (MD, HD; m,f)
- Increased incidence of mineralization in kidney (tubular), stomach and sciatic nerve (HDm). Increased mineralization in meninges, spinal cord (MD HDm; all doses; f)
- Eye, increased incidence of cataracts (HD; m,f)
- Sternum, mucinous degeneration (MDm, HDf)
- Kidney, reduced degree of chronic progressive nephropathy (HD; m,f)
- Mammary gland, increased incidence and degree of periductal fibrosis (HD, f)
- Thyroid, decreased incidence of C-cell hyperplasia (HD; m,f) possibly due to reduction in stimulation of calcitonin release from C-cells secondary to PTH reduction
- Parathyroid, decreased incidence of hyperplasia (MD, HD; m,f) due to redcution in stimulation of PTH release.

Neoplastic findings:

- Reviewer evaluated tumor findings in light of the notion that body weight was decreased in the HD groups with potential decrease in tumor rates.
- For rare tumors (control incidence <1%) the p-value cut off is 0.025, for common tumors (>1%) the p-value cut off is 0.005.
- Incidences given below are for (combined controls, LD, MD, HD)

Tumor findings:

- Lung, bronchio-alveolar adenocarcinoma, in males (0-0-0-2, i.e., 0-0-0-3.3%) was increased in the HD group above the historical control range (range 0-1.7%, average 0.1%, 1 incidence in 17 experiments). However, the finding was not statistically significant (p=0.0307, trend test). Hyperplasia incidence at this site was 0-1-4*-0, i.e., 0-1.7-6.7-0% (historical control range 0-4.2%; average 0.7%), with a significant effect in the MD group. The hyperplasia incidence was not dose-dependent, although this may have been because tumors were formed in HD rats. The size of the tumor effect in the HD group (2/60) was 2x the highest incidence of 1/60 in one control experiment. The lung tumors in the HD may have been treatment-related.
- Lymphoma, combined, in females (0-1-0-3, i.e., 0-1.7%-0-5%) was increased in the HD group above the historical control range (range 0-4%, average 1.4%). The highest incidence was 2/50 (4%) in a historical control group. This finding was statistically significant (p=0.0163, trend test) when tested using combined control groups, and assuming lymphoma is a rare tumor (<1% control rate). When tested using separate control groups, the finding in females was not statistically significant (p=0.0391 and p=0.0396). Based on low incidences (HD incidence 1.25 x highest control rate), the fact that the tumor is probably a common tumor based on average historical control incidence of 1.4%, and the absence of tumors in the MD group, Reviewer feels the finding is not biologically significant.
- Lymphoma, combined, in males (1-1-1-3, i.e. 0.8-1.7-1.7-5%) was within historical control range for males in all dose groups (range 0-8%, average 2%). The effect was not significantly significant (p=0.046). The finding in males is not biologically significant.
- Ependymoma, in males (0-0-0-1, i.e., 0-0-0-1.7%) was increased in the HD group above the historical control range (range 0-0%, average 0%). Finding was not statistically significant (p=0.21). Due to the single incidence, Reviewer feels it can not be concluded that this finding is biologically significant.
- Combined glia cell tumors, in males (0-1-0-2, i.e., 0%-1.7%-0%-3.3%) were within historical control range (0-4.1%) in all groups. Finding was also not statistically significant (p=0.059), trend test)
- Histiocytic sarcoma, in males (0-2-1-2 i.e., 0%-3.3%-1.7%-3.3%) was within historical control range (0-8%) in all groups. Finding was also not statistically significant (p>0.05), trend test)

Tumors with drug-related decrease in incidence:

- Thyroid, C-cell adenoma, markedly reduced particularly in males (all doses). The cause of this is unclear. Calcimimetics can increase calcitonin secretion from the thyroid C-cell. However, hypocalcemia due to low PTH levels would probably suppress C-cell stimulation and calcitonin release.

- Parathyroid gland, adenoma, reduced incidence in all dose groups, probably related to reduced parathyroid cell stimulation and PTH secretion.
- Adrenal, pheochromocytoma, reduced incidence, possibly related to reduced serum Ca levels, since hypercalcemia can lead to increases in this tumor rate (observed with Vitamin D analogue)
- Pituitary, adenoma, small reduction in HD (m,f)

Conclusion

Potentially biologically significant tumor findings:

- Lung, bronchio-alveolar adenocarcinoma, in males

NOTE: Upon discussion of the tumor findings with the Exec CAC (Dec 16, 2003), the Committee concluded that the lung carcinoma (males) and combined lymphoma (females) in the rat may have been biologically significant and treatment-related findings, but need not be included in the label based on low incidences and/or lack of statistical significance.

Histopathology inventory

	Organ weight	Histology
Adrenals	X	X
Aorta		X
Bone Marrow smear		X
Bone (femur)		X
Brain	X	X
Cecum		X
Cervix		
Colon		X
Duodenum		X
Epididymis	X	X
Esophagus		X
Eye		X
Fallopian tube		
Gall bladder		X
Gross lesions		X
Harderian gland		X
Heart	X	X
Ileum		X
Injection site		
Jejunum		X
Kidneys	X	X
Lachrymal gland		
Larynx		
Liver	X	X

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

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3.4.5. Reproductive and developmental toxicology

Summary

Reproductive studies were carried out in rats and rabbits by the oral route. The effect of cinacalcet on fertility was studies in rats, on development of the fetus in rat and rabbit, and on delivery and pre- and postnatal development in rats. Exposure to parent drug and serum ionized calcium levels during pregnancy were determined in Segment 2 dose range finding studies in the rat and rabbit. Fetal exposure was determined in the pregnant rabbit and excretion in the milk was studies in the lactating rat. The high doses in the reprotoxicity studies were limited by maternal toxicity evidenced by clinical signs (post dose salivation, rales, labored breathing) and reductions in food consumption and body weight gain. As a result, exposure (AUC) at the high dose in the definitive studies was usually a small multiple of the expected human exposure at the maximum dose of 180 mg/day.

Table 2.6.7.1. Toxicology Overview (Continued)
Test Article: Cinacalcet Hydrochloride (AMG 073)

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg*)	GLP	Testing Facility	Study No.
Reproduction Toxicity							
	Sprague-Dawley Rat	Oral Gavage	M: 4 weeks prior to mating, during 21-day mating period, and 2-week post-mating period F: 2 weeks prior to mating, during mating, gestation days 0-7	0, 5, 25,75	Yes		100468
	Sprague-Dawley Rat	Oral Gavage	Gestation days 6-17	0, 5, 25, 50, 75	Yes		100202
	Sprague-Dawley Rat	Oral Gavage	Gestation days 6-17	0, 1, 5, 25, 100	Yes		100022
	Sprague-Dawley Rat	Oral Gavage	Gestation days 6-17	0, 2, 25, 50	Yes		100341
	New Zealand White Rabbit	Oral Gavage	Gestation days 6-18 for the range finding and toxicokinetics; gestation days 6-21 for the placental transfer	0.1, 5, 25, 100, 200	Yes		100021
	New Zealand White Rabbit	Oral Gavage	Gestation days 6-18	0, 2, 12, 25	Yes		100219
	Sprague-Dawley Rat	Oral Gavage	Gestation day 6 to lactation day 7	0, 5, 15, 25, 50	No		100584
	Sprague-Dawley Rat	Oral Gavage	Gestation day 6 to lactation day 20	0, 5, 15, 25	Yes		100734
Juvenile							
	Sprague Dawley Rat	Gavage	28 days	0, 0.5, 1.5, 5	Yes		101939
	Beagle Dog	Gavage	28 days	0, 0.5, 1.5, 5	Yes		101938
Local Tolerance (in vitro)							
	Sprague-Dawley Rat, cynomolgus monkey and human blood	In vitro direct contact	N/A	1.33 mg/mL AMG 073 HCl 5 mg/mL – all mesylate test articles	No		100359

(Continued)

* Unless otherwise specified

In the oral Segment 1 study in the rat, the number of corpora lutea, implantation sites and live fetuses were slightly reduced at the HD of 75 mg/kg/day. At this dose there was maternal toxicity during the 2-week premating period reflected by salivation, alopecia and rales, and reduced food consumption and body weight. There were no other effects on male or female fertility or reproductive performance.

In the oral Segment 2 study in the rabbit, there was no fetal toxicity (mortality, fetal weight), and there were no effects on fetal external, visceral, skeletal malformations (i.e., no teratogenicity) or variations at doses up to 25 mg/kg/day. Maternal toxicity evident as clinical signs, decreased body weight gain and food consumption was observed at 12 and 25 mg/kg/day.
In the dose-range finding Segment 2 study, there was maternal mortality at 200 mg/kg/day. Reduced maternal food consumption and body weight or body weight gain were seen at doses ≥25 mg/kg/day and clinical signs at 100 and 200 mg/kg/day. There were no external fetal anomalies at doses up to 100 mg/kg/day. Reductions in serum ionized calcium concentrations were observed at doses ≥5 mg/kg/day and were maximal at 2-4h post dosing. The parent compound was detected in fetal plasma at concentrations approximately 10-fold lower than maternal plasma concentrations.

In the oral Segment 2 study in rats, there were no effects on fetal external, visceral or skeletal malformations or variations at doses up to 50 mg/kg/day. Fetal body weight was slightly reduced at 2, 25, 50 mg/kg/day in parallel with decreases in maternal food consumption and body weight gain. Maternal toxicity as also evident as clinical signs at 25 and 50 mg/kg/day. In two dose range finding Segment 2 study in the rat at doses up to 75 mg/kg/day and 100 mg/kg/day maternal and fetal body weight effects were also observed at doses ≥ 25 mg/kg/day. There were no external fetal malformations or variations in any of these two studies at any dose. Toxicokinetic data from these studies showed that exposure to parent drug was similar in pregnant rats as compared to non-pregnant animals. Serum ionized calcium was reduced at doses ≥2 mg/kg/day and were maximally reduced at 4-8hours post dosing.

In the oral Segment 3 study in rats, one dam dosed with 15 mg/kg/day was found dead with a prolapsed uterus and delivery complications on the day of delivery. In F0 dams, clinical signs (rales and lethargy) were seen at 25 mg/kg/day. Maternal food consumption and body weight gain were decreased at 15 and 25 mg/kg/day on gestation days 6-12. One dam in each dose group (5, 15, 25 mg/kg/day) had total litter loss by lactation days 1, 2 and 5, respectively. The number of pups dying, missing or cannibalized on PPD0-4 was increased at 25 mg/kg/day. Reductions in maternal food consumption and body weight and F1 pup body weight were observed on PPD 10-14 and PPD 10-17, respectively, at 25 mg/kg/day. A minimal reduction in F1 pup body weight gain unaccompanied by maternal effects was observed at 15 mg/kg/day on PPD 10-17. There were no effects on F1 pre- or postweaning development. In F1 male parental animals, there were treatment-related clinical observations (incisor abnormalities and chromodacryorrhea) at 5, 15, 25 mg/kg/day. There were no effects on F1 fertility and reproductive performance, F1 gestation or lactation, and F2 pup viability or weight.
In the oral dose range finding study, one dam dosed with 50 mg/kg/day was sacrificed at delivery with a prolapsed uterus. Pup body weight in the 50 mg/kg/day group was decreased

at delivery and was associated with decreased maternal gestational food consumption and body weight gain. Pup viability index was reduced at 50 mg/kg/day. Pup body weight gain was unaffected.

In a lacteal excretion study in rats, parent and metabolites were excreted into the milk. Parent was excreted into milk to larger extent than metabolites.

Exposure data

RABBIT (oral Segment 2 study)

Study	Study Nr.	Doses (mg/kg/day)	Cmax (ng/mL)	AUC parent (ngxh/mL)	**AUC multiple RABBIT:HUMAN* (parent)**
Segment 2	# 100021	1		Blq	-
		5		Blq	-
		25		258*	**0.4x**
		100		1820	**2.8x**

*underestimated since plasma levels Blq after 12h

RAT (oral Segment 2 studies)

Study	Study Nr.	Doses (mg/kg/day)	Cmax (ng/mL)	AUC parent (ngxh/mL)	**AUC multiple RAT:HUMAN* (parent)**
Segment 2	# 100202	5		187	0.3x
drf		25		1920	3.0x
		50		2850	4.4x
		75		3610	5.6x
Segment 2	100022	5		197	0.30x
drf		25		2120	3.3x
		100		7900	12.2x

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Fertility and early embryonic development

Study title: Study of the effects of oral administration of AMG 099073-01 on fertility and early embryonic development to implantation in rats

Key study findings: Male rats were treated over a 4-week premating period, a 3-week mating period, and 2 weeks postmating. Females were treated over a 2-week premating period, during mating, until GD 7, with 0, 5, 25, 75 mg/kg/day. Females were sacrificed on GD 15, and males were sacrificed after females. Toxicity in males and females was seen at 75 mg/kg/day as decreased body weight gain and food consumption mainly during first week of dosing. Clinical signs including post dose salivation was seen in males and females at 25 and 75 mg/kg/day. # Corpora lutea, implantation sites and live fetuses were reduced at 75 mg/kg/day, a dose at which maternal toxicity was observed. There was no effect on female or male fertility indices, or on estrous cycling or sperm parameters. Maternal NOAEL was 5 mg/kg/day.

Study no.: 99-4167.
Sponsor Study No.: 100468
Conducting laboratory:
Date of study initiation: February 11, 1999
GLP compliance: Yes
QA reports: Yes
Drug, lot #, and % purity: #709001 (99.7%)

Methods

Doses: 0, 5, 25, 75 mg/kg/day
Species/strain: Crl:CD(SD) BR albino rats
Number/sex/group: 26/sex/grp
Dosing period: Males: at least 4 weeks prior to mating, during mating (3 weeks), and post-mating (2 weeks)
Females: at least 2 weeks prior to mating, during mating (max 3 weeks), and through GD7
Route, formulation: Oral gavage, suspension in 0.5% (w/v) aqueous methylcellulose
Volume, and infusion rate: 5 ml/kg
Satellite groups for TK: None
Study design:

Group	Group Designation	Doses			Treatment Schedule Pre-mating Period		Number of Animals	
		Dose (mg/kg)	Volume (mL/kg)	Concentration (mg/mL)	Males[b]	Females[c]	Males	Females
I	Control[a]	0	5	0	4 weeks	2 weeks	26	26
II	Low	5	5	1	4 weeks	2 weeks	26	26
III	Mid	25	5	5	4 weeks	2 weeks	26	26
IV	High	75	5	15	4 weeks	2 weeks	26	26

[a] Control animals were treated with control article only with the same treatment regimen as the treated groups.

[b] Males were dosed once daily, seven days/week for at least four weeks prior to mating initiation. Treatment continued during the

APPEARS THIS WAY
ON ORIGINAL

Parameters measured: Viability, signs, body weight, food consumption, estrous cycling, macrosopic examination of reproductive organs (males: testes and epididimes, sperm count and morphology, sperm motility; females: uteri)

Dose selection: Based on 6-month rat toxicity study (No. 100082; doses 0, 5, 25, 100 mg/kg/day).

Results

Pregnancy and survival (females)

FEMALES					
Grp		ctrl	LD	MD	HD
Dose (mkd)		0	5	25	75
N mated		26	26	26	26
N pregnant		26	26	26	25
N died		0	0	0	0
N examined at C-section		24	26	26	25
N with viable fetuses		24	26	26	25
N evaluated (BW, FC)*		24	26	26	24

* 2 controls and 1 HD animal had no confirmed mating date

Mating and survival (males)

1LD, 1MD, 1HD died due to dosing errors. 2 MD males died, one with autolysis, the other with no clear findings

MALES				
Grp	ctrl	LD	MD	HD
Dose (mkd)	0	5	25	75
N evaluated	26	26	26	26
N died or sacrificed	0	1	3	1
N mated	26	26	25	26
N with females pregnant	26	26	25	25

No effect on female fertility index, male mating index, male fertility index

Clinical signs:

MALES (65 day dosing period, 30 premating+22 mating+14postmating)
Number of animals affected/26

Grp	ctrl	LD	MD	HD
Dose (mkd)	0	5	25	75
Clinical signs				
Salivation post-dose	0	2	24	25
Lacrimation	0	2	3	2
Chromodacryorrhea	0	2	3	2
Incisors broken/missing	0	2	1	3
Rales	0	0	0	1
Decreased fecal volume	0	0	1	7

FEMALES (>29 (14+15) days of dosing)
Number of animals affected/26

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	5	25	75
PREMATING					
Salivation post-dose		0	0	3	14
Alopecia		1	0	0	13
Rales		0	0	0	1
GESTATION					
Salivation post-dose		0	0	23	14
Alopecia		2	2	2	16
Incisors broken/missing		0	0	1	2
Rales		0	0	1	5

Body weight:

Body weight males

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	5	25	75
PREMATING (Day 0-26)					
BW Day 0 (gr)		328	328	325	327
BW Day 26 (gr)		428	419	429	401*
BW gain Days 0-9 (gr)		44	37	45	15**
BW gain Days 9-26 (gr)		58	52	59	60
BW gain Days 0-26 (gr)		100	91	104	75**

*statistically significant

Body weight females

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	5	25	75
PREMATING (PMD12-26)					
BW PMD 12 (gr)		238	239	239	240
BW PMD 26 (gr)		253	253	257	246
BW gain PMD 12-19 (gr)		5	1	4	-9**

BW gain PMD 19-26 (gr)	10	11	14	14
BW gain PMD 12-26 (gr)	15	13	17	6**
GESTATION (GD0-15)				
BW GD 0	260	260	266	254
BW GD15	337	338	342	332
BW gain GD 0-15	77	78	76	78

Food consumption:

Food consumption males

Grp	ctrl	LD	MD	HD
Dose (mkd)	0	5	25	75
PREMATING (Day 0-26)				
FC Day 0-5 (gr/d)	27	28	28	22**
FC Day 5-26 (gr/d)	27	27	28	26

*statistically significant

Food consumption females

Grp	ctrl	LD	MD	HD
Dose (mkd)	0	5	25	75
PREMATING (Day 14-26)				
FC Day 14-26 (gr/d)	18	19	19	15**
GESTATION (GD0-15)				
FC GD 0-7	23	24	25*	23
FC GD 7-15	25	25	27**	28**
FC GD 0-15	24	25	26**	25

Effect mainly in first 5-7 days of premating

No effect on female estrus (# with estrus), or estrus cycle days

Fertility parameters (C-section data: corpora lutea, preimplantation loss):

Evaluated in N=26,26,26,25 pregnant dams. No effects on preimplantation loss (# or % per animal), live fetuses (m or f) (# per animal), postimplantation loss (# or % per animal), early or late resorptions (# or % per animal)

Grp	ctrl	LD	MD	HD
Dose (mkd)	0	5	25	75
N pregnant	26	26	26	25
Corpora lutea (N/animal)	16.8	16.9	16.3	15.1*
Implantation sites (N/animal)	15	14	14.5	13**
Live fetuses (N/animal)	14.1	13.3	14.0	11.8**

Sponsor argues that decreases in CL and IS were not related to treatment, based on historical control data (range #CL/animal 13.6-18.2, 7 studies, no time period given). Reviewer feels the data suggest impaired female fertility in the HD group. This may have been due to maternal toxicity evidenced by decreased FC and BWG during premating dosing period.

Necropsy:

Males: no clear dose-related findings
Organ weights: slight non-significant increases in testes, epididymis absolute weight
Sperm assessment: No effects on sperm count, morphology, motility

Females
Alopecia, incisors broken/missing (see signs)

Toxicokinetics were not performed in this study. However, data from the 6-month toxicity study (#100082) or the developmental toxicity dose range finding studies (# 100202) can be used to estimate plasma levels in this Segment 1 study.

Dose	AUC (parent) (0-24h) (ngxh/mL)		Multiple of human AUC @ 180 mg/day (648 ngxh/mL)	
	(A) Extrapolated from data from Study #100082 (Wks 13 and 26)	(B) Extrapolated from Study #100202	(A)	(B)
5	51	187	0.1x	0.3x
25	1001	1920	1.5x	3.0x
75	Appr. 5000	3610	Appr. 7.7x	5.6x

Embryofetal development

The Effects on Embryo-Fetal Development of Oral Administration of AMG 099073-01 to Pregnant Rabbits

Key study findings:
Pregnant rabbits were dosed from GD6-18 with 0, 2, 12, 25 mg/kg/day, and C-sectioned on GD29. Maternal toxicity evident as clinical signs, decreased body weight gain and food consumption was seen in at 12 and 25 mg/kg/day. There was no fetal toxicity (mortality, body weight, implantation, resorption), and there were no effects on fetal external, visceral, skeletal malformations (i.e., no teratogenicity) or variations at any dose. Maternal NOAEL was 2 mkd.

Study No.: 98-4153
Sponsor Study No.: 100219
Conducting laboratory: [redacted]
Date of study initiation: Nov 30, 1998
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, lot #, and % purity: 709001; 99.7%

Methods

Doses: 0, 2, 12, 25 mg/kg/day (free base)
Species/strain: New Zealand White Rabbits (— (NZW)SPF)
Number/sex/group: 20/grp (time-mated females)
Dosing period: Days 6-18 of gestation
Route, formulation: Oral gavage, suspension in 0.5% (w/v) aqueous methylcellulose
Volume, and infusion rate: 2 ml/kg,
Satellite groups for TK: None
Study design:

MATERIALS AND METHODS

3. Experimental Design

		Daily Doses[a]				Number of Animals				
							Euthanized	Proportion of Gestation Day 29 Fetus/Litter Malformation/Variation Evaluations		
Group	Group Designation	Dose mg/kg	Concentration mg/mL	Volume mL/kg	Treatment Schedule	Mated Females	Gestation Day 29	External	Soft Tissue	Skeletal
I	Control	0	0	2	Gestation Days 6-18	20	All	All	All	All
II	Low	2	1.0	2	Gestation Days 6-18	20	All	All	All	All
III	Mid	12	6.0	2	Gestation Days 6-18	20	All	All	All	All
IV	High	25	12.5	2	Gestation Days 6-18	20	All	All	All	All

[a]Doses given represent doses of test article as the free base. A factor of 1.1 was used to adjust for HCl content in the formulation.

Parameters measured: Signs, BW, FC, necropsy, maternal reproductive system, fetal weight, feral abnormalities (external, viscera, skeletal)

Dose selection: The high dose caused ca. 32% decrease in BW gain in a range finding study (Study Nr. 100021)

Definitions:
Preimplantation loss = no. of corpora lutea – no. of implantation sites
Postimplantation loss = Total no. of early and late resorptions and dead fetuses

Results

Mortality (dams): 1 MD dam found dead on GD16, cause unknown

Pregnancy rate: 2/20, 2/20, 0/20, 1/20 non-pregnant in ctrl, LD, MD, HD

Clinical signs (dams):

Clinical signs in dams (N animals affected)

Grp		ctrl	LD	MD	HD
Dose (mkd)		1	2	12	25
Ano-genital staining (dark)		1	3	3	4
Rales		0	0	1	2
Decreased fecal volume		4	6	7	11
Salivation, post-dose		0	0	0	2

Body weight (dams):

Body weight dams

Grp		ctrl	LD	MD	HD
Dose (mkd)		1	2	12	25
BW Day 0 (gr)		3572	3598	3581	3602
BW Day 29 (gr)		4076	4027	4082	4052
BW gain Days 0-6 (gr)		102	42	91	40
BW gain Days 6-18 (gr)		222	209	174	129*
BW gain Days 19-29 (gr)		179	199	226	281

*statistically significant

Food consumption (dams):

Food consumption dams

Grp		ctrl	LD	MD	HD
Dose (mkd)		1	2	12	25
FC Day 6-18 (gr/kg/d)		47	45	42*	35*
FC Day 19-29 (gr/kg/d)		36	38	38	42*

*statistically significant

Increase in FC (gr/kg/day) in HD animals on D19-29 was partly due to decreased BW gain during dosing period (D6-18).

Terminal and necroscopic evaluations:

None remarkable

C-section data (implantation sites, pre- and post-implantation loss, etc.):
Evaluated in N=18,17,20,19 pregnant dams. No effects on corpora lutea (# per animal), implantation sites (# per animal), preimplantation loss (# or % per animal), live fetuses (m or f) (# per animal), postimplantation loss (# or % per animal), early or late resorptions (# or % per animal), fetal body weight (m or f) (gr)

Fetal data:

Fetal body weight:
No treatment effects

Fetal observations:

Malformations

Grp		ctrl	LD	MD	HD
Dose (mkd)		1	2	12	25
Litters examined		18	17	20	19
Fetuses examined		158	152	173	161
External malformations					
Exencephaly	Litter incidence	0	0	0	1 (5%)
	Fetal incidence	0	0	0	1 (0.6%)
Skeletal malformations					
Sternebrae fused	Litter incidence	2 (11%)	1 (6%)	3 (15%)	3 (16%)
	Fetal incidence	2 (1.3%)	3 (2.0%)	4 (2.3%)	3 (1.9%)
Total malformations (external, visceral, skeletal)					
	Litter incidence	3	1	3	4
	Fetal incidence	3	3	4	4

*statistically significant

Variations

Grp		ctrl	LD	MD	HD
Dose (mkd)		1	2	12	25
Litters examined		18	17	20	19
Fetuses examined		158	152	173	161
External, visceral, skeletal variations		No external variations; No treatment effects on visceral and skeletal variations			

Historical control data were given for New Zealand rabbits from the conducting laboratory for 1988-1993 (10 definitive +7 pilot studies). Individual study data were not presented.

Historical control incidence

			Number of studies	N	Avg %
External	Exencephaly	Litter incidence	17	0/199	0%

		Fetal incidence	17	0/1667	0%
Skeletal	Sternebrae fused	Litter incidence	10	14/162	8.6%
		Fetal incidence	10	20/1373	1.5%

The incidence of fused sternebrae in MD and HD was higher than the concurrent and average historical control incidence. However, based on the absence of clear dose-dependence and likely variability between groups Reviewer feels the finding was not biologically significant.
The significance of 1 fetus (male) with exencephaly in the HD group was unclear. A web search for data on spontaneous incidence of exencephaly in the rabbit provided a little information. The CRL database does not offer spontaneous incidences of malformations for rabbits yet. According to one website, exencephaly (defect of calvarium withh extrusion of brain, condition proceeding anencephaly) is seen in lagomorphs at a rate more frequent than anencephaly (partial or total absence of forebrain) (www.netvet.wustl.edu). In hamsters, exencephaly was seen in a study on the reprotoxicity of alpha-chaconine and alpha-solanine, in 1 out of 393 fetuses in the control group. Reviewer concluded there was insufficient evidence that this finding was drug-related.

Toxicokinetics:
Toxicokinetics were not performed in this study. However, data from the developmental toxicity dose range finding study in rabbits (# 100021) can be used to estimate plasma levels in this Segment 2 study.

Dose	AUC (parent) (0-24h) (ngxh/mL)	Multiple of human AUC @ 180 mg/day (648 ngxh/mL)
	Extrapolated from data from Study #100021	
2	21	0.03x
12	125	0.2x
25	258*	0.4x*

* maybe underestimated

Dose-range finding studies
Study title: A combined range-finding developmental, toxicity and toxicokinetic/placental transfer study in rabbits with AMG 099073-01 via oral administration (Study # 100021) [redacted] (Lot Nr. 709001).

A dose-range finding developmental toxicity and toxicokinetic/placental transfer study was carried out in rabbits (Study No. 100202) to obtain preliminary data on maternal and fetal toxicity (RF), to obtain TK data of AMG-073 in the pregnant rabbit (TK) and to determine if the drug was transferred to the fetus upon maternal exposure (PT). Pregnant NZW rabbits (12/group), were dosed with 0, 1, 5, 25, 100, 200 mg/kg/day daily by oral gavage (5 mL/kg/d) on GD6-18 (RF, TK), or GD6-21 (PT). Dose-range phase (RF) was carried out in N=6/grp, TK phase in 3/grp, PT phase in 3/grp. Body weight and food consumption were measured in RF phase only. RF animals were C-sectioned on GD29. In TK phase, blood samples for TK and ionized Ca were collected on GD18 at predose, 0.5, 1, 2, 4, 8, 12, 24h postdosing (N=3/grp/time). In PT phase, does were anesthesized and amniotic fluid and fetal blood samples were collected from all fetuses in the litter on GD21 at 2h postdosing. Fetal samples

were pooled for analysis. Samples were assayed for AMG-073 (parent drug) by LC/MS/MS. PTH was measured in predose and 2h samples.

		Daily Doses[a]				Number of Animals				
						Sacrificed Gestation				Fetal Examination[e]
Group	Group Designation	Dose (mg/kg)	Conc. mg/mL	Vol. (mL/kg)	Treatment Schedule	Mated Females[b]	Day 21 Placental Transfer	Day 19 Toxicokinetic	Day 29 Fetal Exam	External
I	control	0	0	5	GD 6-18	12	3	3	6	All fetuses (RF)
II	low-	1	0.2	5	GD 6-18	12	3	3	6	All fetuses (RF)
III	low-mid-	5	1	5	GD 6-18	12	3	3	6	All fetuses (RF)
IV	mid-	25	5	5	GD 6-18	12	3	3	6	All fetuses (RF)
V	mid-high-	100	20	5	GD 6-18	12	3	3	6	All fetuses (RF)
VI[d]	high-	200	40	5	GD 6-15	12	3	3	6	c

Key: Vol.=Volume; Conc.=Concentration; GD=Gestation Day; RF=Range-Finding.

[a]Doses given represent doses of test article as the free base. A factor of 1.1 was used to adjust for the HCl in the formulation and the free base content. The animals designated for the placental transfer component were treated from Gestation Day 6-21.

[b]The first six animals per group were used for the developmental range-finding phase, the next 3 animals per group were used for the Placental Transfer phase and the last 3 animals per group were used for Toxicokinetic, Ionized calcium/pH and Parathyroid hormone evaluations. In addition, blood was also collected from the three extra animals retained but not used on study for Ionized calcium/pH analysis. Analyses of these extra animals not used on study served as controls for the Group VI animals.

[c]Pertains only to the examination of fetuses from day 29 of gestation maternal sacrifices (range-finding component).

[d]Due to toxicity seen in Group VI, the Sponsor decided to terminate this group on 8 May 1998 (day 15 of gestation).

[e]Group VI fetuses were not examined due to early termination of the group.

Findings:
Mortality at 200 mg/kg/day. In RF phase (N=6/grp), 2 HD females died with signs on GD 14-15, and 1 died due to dosing accident on GD 13. One 5 mkd animal died due to dosing accident. In TK phase (N=3/grp), 1 female died (treatment-related) and 1 was sacrificed (dosing accident) on GD 14-15.All animals in 200 mkd group were terminated early on GD15.

Pregnancy rate: Not affected. In RF phase 2 controls were not pregnant, in TK phase one 1 mkd, and one 200 mkd animal were not pregnant

Clinical signs: rales, labored breathing, decreased feces at 100 and 200 mkd

Body weight: Dose-related decrease (body weight loss) on GD6-9 and GD9-12 at 25, 100, 200 mkd.

Food consumption: Dose-related decrease on GD 6-9 and GD9-12 at 25, 100, 200 mkd.

Uterine data: No effects on # corpora lutea, implantation sites, live fetuses, pre- or postimplantation loss up to 100 mkd.

Fetal body weight: No clear dose-related effects

Fetal anomalies (external examination only) (# fetusus examined: 31-61-50-65-55):
1 fetus in a 100 mkd litter had gastroschisis. This external malformation was not seen in historical controls (199 litters, 1667 fetuses, 17 studies).

Gastroschisis is an abnormality (defect or hole) in the abdominal wall that allows the abdominal contents to protrude outside the body. The spontaneous incidence in rabbits is unclear. It has been observed in developmental studies, in both treated and untreated groups (Kelich et al, 1995; Thorpe et al, 1972). Reviewer concluded there was insufficient evidence that this finding was drug-related.

There were no fetal variations in any group.

	Report Title: A Combined Range-Finding Developmental Toxicity and Toxicokinetic/Placental Transfer Study in Rabbits with AMG 099073-01 via Oral Administration					
	Duration of Dosing: RF & TK – G6-18 PT – G6-21				Study No. 100021	
Species/ Strain: New Zealand White Rabbits Initial Age: 5-6 months	Day of Mating: Day 0 Day of C-Section: RF – G29[a], TK – G19[a] PT – G21[a]					
Date of First Dose: RF & TK - 29 Apr 98 PT – 28 Apr 98	Method of Administration: Gavage					
Special Features: PTH and ionized calcium, placental transfer	Vehicle/Formulation: 0.5% aqueous methylcellulose				GLP Compliance: Yes	
No Observed Effect Level: F_0 Females: 5 mg/kg/day F_1 Litters: 5 mg/kg/day						
Daily Dose (mg/kg)	0 (Control)	1	5	25	100	200
Dams/ Does: Toxicokinetics: (G18)						
AUC (0-24hr) (ng*hr/mL)	BLQ	BLQ	BLQ	258[b]	1820	n/a[a]
Cmax (ng/mL)						

No statistical analysis performed; RF – Range Finding, TK – Toxicokinetic, PT – placental transfer, G – Gestation Day, BLQ – below the level of quantification

[a] Treatment-related mortality/morbidity observed in 3 animals treated with 200 mg/kg prompted early sacrifice of the surviving females in this dose group on Day 15 of gestation.

[b] Concentrations fell below detectible limits after 12 hours, therefore, AUC may be underestimated.

Daily Dose (mg/kg)	0 (Control)	1	5	25	100	200
Dams/ Does: Mean Serum PTH (pg/mL) G18						
Predose	6.60	17.38	6.09	7.79	17.67	12.53[b]
2 hr postdose	4.35	9.34	17.67	4.34	2.79	n/a[b]
Normalized ionized calcium (mmol/L)						
Predose	1.85	1.82	1.80	1.66	1.32	n/a[a]
4 hr	1.76	1.78	1.65	1.31	1.08	n/a[a]
No. Mated	6	6	6	6	6	6
No. Pregnant	4	6	6	6	6	6
No. Died or Sacrificed Moribund	0	0	1[c]	0	0	3[b]
No. Elective Sacrifice	0	0	0	0	0	3[a]
No. Aborted or with Total Resorption of Litter	0	0	0	0	0	n/a[a]

(Continued)

No statistical analysis performed; RF – Range Finding, TK – Toxicokinetic, PT – placental transfer, G – Gestation Day, BDL – below detectable limits

[a] Treatment-related mortality/morbidity observed in 3 animals treated with 200 mg/kg prompted early sacrifice of the surviving females in this dose group on Day 15 of gestation.

[b] Samples taken G15 prior to sacrifice

[c] One animal from each of the 5 mg/kg/day and 200 mg/kg/day dosing groups died immediately after dosing and upon necropsy were determined to have died of a dosing injury.

Daily Dose (mg/kg)	0 (Control)	1	5	25	100	200
Dams/ Does: Clinical Observations (frequency/animals)						
Rales - Moist	0/0	0/0	0/0	0/0	8/2	3/1
Rales – Moist (post dose)	0/0	0/0	0/0	0/0	6/2	0/0
Rales – Dry	0/0	0/0	0/0	0/0	14/3	20/5
Rales – Dry (post dose)	0/0	0/0	0/0	0/0	1/1	2/1
Labored Breathing	0/0	0/0	0/0	0/0	7/2	4/1
Labored Breathing (post dose)	0/0	0/0	0/0	0/0	4/1	0/0
Unformed Stool	0/0	0/0	0/0	0/0	0/0	1/1
Decreased Fecal Volume	2/1	3/1	0/0	3/2	15/4	0/0
Urine discolored red	0/0	0/0	0/0	0/0	0/0	1/1
Necropsy Observations	-	-	-	-	-	-
Body Weight Gain (grams)						
Gestation Days 6-9	20	18	18	-3	-147	-289
Gestation Days 9-12	33	32	34	4	-116	-180
Food Consumption (g/animal/day)						
Gestation Days 6-9	189	181	183	158	85	46
Gestation Days 9-12	190	179	151	121	44	11
Mean No. Corpora Lutea	11.0	13.0	12.0	13.2	10.8	n/a[a]
Mean No. Implantations	8.0	11.2	10.2	11.5	9.7	n/a[a]
Mean % Preimplantation Loss / animal	27.6	12.9	13.4	12.0	11.4	n/a[a]

(Continued)

No statistical analysis performed due to small group size

[a] Treatment-related mortality/morbidity observed in 3 animals treated with 200 mg/kg prompted early sacrifice of the surviving females in this dose group on Day 15 of gestation.

Study No. 100021 (Continued)						
Daily Dose (mg/kg)		0 (Control)	1	5	25	100
Litters:	No. Litters Evaluated	4	6	5	6	6
	Mean No. Live Fetuses per animal	7.8	10.0	10.0	10.3	9.0
	Mean No. Resorptions – Early	0.3	0.7	0.0	0.0	0.0
	Mean No. Resorptions – Late	0.0	0.3	0.2	0.7	0.5
	Mean No. of Dead Fetuses per animal	0.0	0.2	0.0	0.5	0.2
	Mean % Postimplantation Loss / animal	5.0	10.3	1.7	8.8	5.2
	Mean Fetal Body Weight (g)	47.2	39.2	43.8	39.1	37.9
	Fetal Sex Ratios [a]					
	Fetal Anomalies					
	Gross External					
	Gastroschisis	0	0	0	0	1
	Total Affected Fetuses (Litters)	0	0	0	0	1
	Placental transfer - 2h post dose G21 (ng/mL) Mean±SD	BLQ	BLQ	2.62±2.16	6.27±5.63	21.4±18.7

No statistical analysis performed; BLQ – Below the limit of quantification
[a] No fetal sex ratios were reported
[b] Gastroschisis has not been seen in historical control data, but is known to occur spontaneously in rabbits.

TK: Plasma levels in pregnant rabbits on GD 18

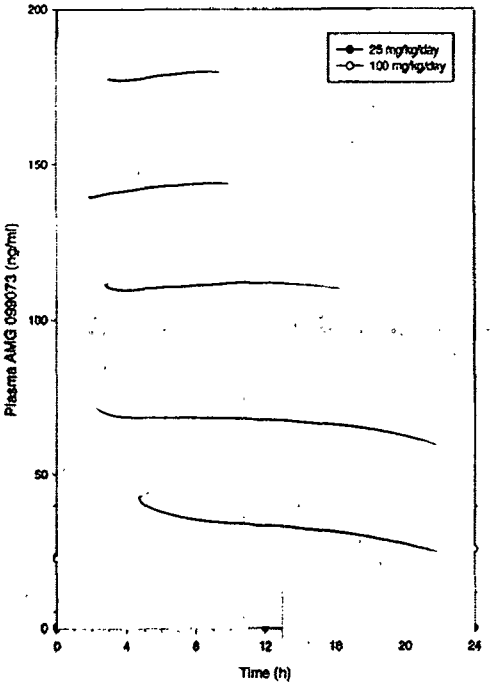
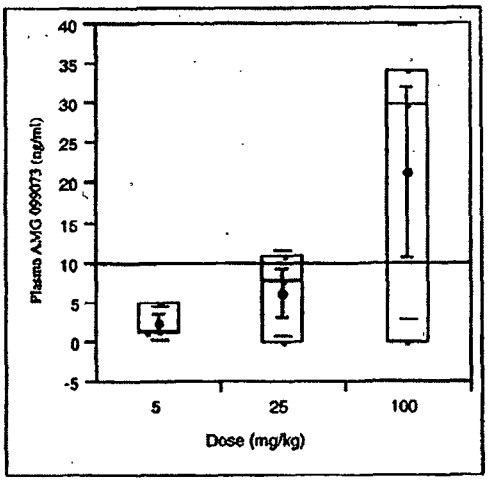


Table B. Toxicokinetic Parameters for AMG 099073-01 in Maternal Rabbit Plasma

Dose[a] mg/kg/day	N	Cmax ng/ml (Stdev)	Tmax hours (Stdev)	AUC (0-24h) ng*h/ml (Stdev)	CL/F L/h/kg (Stdev)	t1/2 hours (Stdev)
25	3		1.3 (0.58)	258 (83.5)	106 (41.8)	3.71 (1.76)
100	3		2.0 (0.0)	1820 (224)	55.5 (7.33)	9.56 (6.92)

[a] All samples for the 1 and 5 mg/kg/day dose groups were below the limits of quantification

Plasma levels in pooled fetal plasma (2h post-dose).



Means and Std Deviations

Level	Number	Mean	Std Dev	Std Err Mean
5	3	2.5167	2.1543	1.244
25	3	6.2700	5.6320	3.252
100	3	21.4333	18.6859	10.788

Maternal plasma levels were highest at 1-2 h post dosing.
Ratio of maternal: fetal plasma levels at 2h postdose was approximately 10x at 25 mkd, and 7x at 100 mkd.

Dose (mg/kg/day)	Maternal plasma (Cmax)	Fetal plasma (2h post dose)	Ratio maternal: fetal
5	[redacted]	2.52*	-
25		6.27	9.6x
100		21.4	7.3x

* LLQ in fetal plasma assay was [redacted]

Amniotic fluid levels were not determined due to lack of assay

Calcium and PTH levels in maternal serum:

GD 18: Ca decreased post dose at 5, 25, 100 mkd with lowest levels 2- 4h post dose. On Predose levels (i.e., 24 post dose) were decreased at 25 and 100 mkd, due to previous 12 days of dosing. Serum pH was not affected.

PTH levels were erratic. A clear decrease post dose was only seen at 100 mkd. Other levels were variable.

Conclusions

- Maternal toxicity at 25 mkd (BW, FC), 100(signs, BW,FC) and 200 mkd (signs, mortality, BW, FC)
- Serum Ca levels decreased post dosing at 5, 25, 100 mkd
- No clear effect on fetal external malformations at maternal doses up to 100 mkd on GD6-GD18
- No effect on resorptions or postimplantation loss up to 100 mkd
- Compound is transferred to some extent from maternal to fetal plasma via placenta.

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ON ORIGINAL

The Effects on Embryo-Fetal Development of Oral Administration of AMG 099073-01 to Pregnant Rats

Key study findings:
Pregnant rats were dosed from GD6-17 with 0, 2, 25, 50 mg/kg/day, and C-sectioned on GD20. Maternal toxicity evident as clinical signs and statistically significantly decreased body weight gain and food consumption was seen at 25 and 50 mg/kg/day. At 2 mg/kg/day, maternal body weight was minimally (not statistically significantly) decreased. Fetal body weight was minimally (but statistically significantly) decreased in females at 2 mg/kg/day, and slightly reduced in males and females at 25 and 50 mg/kg/day. There were no significant external, visceral or skeletal malformations or variations. Maternal NOAEL was 2 mkd.

Study No.: 98-4152
Sponsor Study No.: 100341
Conducting laboratory: [redacted]
Date of study initiation: Nov 6, 1998
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, lot #, and % purity: 709001; 99.7%

Methods

Doses: 0, 2, 25, 50 mg/kg/day (free base)
Species/strain: Albino rats [Crl:CD®(SD)[redacted]BR]
Number/sex/group: 25/grp (mated females)
Dosing period: Days 6-17 of gestation
Route, formulation: Oral gavage, suspension in 0.5% (w/v) aqueous methylcellulose
Volume, and infusion rate: 5 ml/kg
Satellite groups for TK: None
Study design:

3. **Experimental Design**

		Daily Doses*				Number of Animals				
							Euthanized	Proportion of Gestation Day 20 Fetuses/Litter Malformation/Variation Evaluations		
Group	Group Designation	Dose mg/kg	Concentration mg/mL	Volume mL/kg	Treatment Schedule	Mated Females	Gestation Day 20	External	Soft Tissue	Skeletal
I	Control	0	0	5	Gestation Days 6-17	25	All	All	½	½
II	Low	2	0.4	5	Gestation Days 6-17	25	All	All	½	½
III	Mid	25	5	5	Gestation Days 6-17	25	All	All	½	½
IV	High	50	10	5	Gestation Days 6-17	25	All	All	½	½

*Doses given represent doses of test article as the free base. A factor of 1.1 was used to adjust for the HCl content in the formulation.

Parameters measured: Signs, BW, FC, necropsy, maternal reproductive system, fetal abnormalities (external, viscera, skeletal), resorptions

Dose selection: A high dose of 50 mkd was selected because it caused a small effect on fetal BW in the range finding study (Study Nr. 100202).

Definitions:
Preimplantation loss = no. of corpora lutea – no. of implantation sites
Postimplantation loss = Total no. of early and late resorptions and dead fetuses

Results

Mortality (dams): None

Pregnancy rate: 100%-96%-100%-100% in ctrl, LD, MD, HD

Clinical signs (dams):

Clinical signs in dams (N/25 animals affected)

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	2	25	50
Alopecia-extremities/snout		2	0	0	4
Scabs		0	0	0	2
Alopecia-general		1	0	0	3
Decreased fecal volume		1	0	2	3
Rales		0	0	0	4
Salivation, post-dose		1	0	12	21

Body weight (dams):

Body weight dams

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	2	25	50
BW Day 0 (gr)		243	241	239	242
BW Day 20 (gr)		392	381	379	368*
BW gain Days 0-6 (gr)		25	28	29	27
BW gain Days 6-7 (gr)		5	3	-2**	-6**
BW gain Days 6-9 (gr)		15	13	7	-5**
BW gain Days 6-18 (gr)		91	81	78*	64*
BW gain Days 18-20 (gr)		34	32	33	35

*statistically significant

BW gain decreased slightly-moderately in MD and HD (sign), and slightly in LD (non-sign) on GD6-18. Effect most pronounced on first days of dosing.

Food consumption (dams):

Food consumption dams

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	2	12	25
FC Day 6-7 (gr/kg/d)		86	83	70**	60**
FC Day 6-9 (gr/kg/d)		85	83	72*	57**
FC Day 6-18 (gr/kg/d)		83	82	78*	71**
FC Day 18-20 (gr/kg/d)		76	76	80	87**

*statistically significant

Decrease in FC particularly in first 3 days of dosing, diminishing effect thereafter. In the LD group there was a very minimal decrease in FC (D6-9). Increase in FC (gr/kg/day) in HD animals on D18-20 was due to decreased BW.

Toxicokinetics:

No data

Terminal and necroscopic evaluations:

Maternal necropsy

Clinical signs in dams (N/25 animals affected)

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	2	25	50
Alopecia-extremities/snout		1	0	0	3
Alopecia-general		1	0	0	2
Diverticulum		0	0	2	0

C-section data:

Evaluated in N=25,24,25,25 pregnant dams. No effects on corpora lutea (# per animal), implantation sites (# per animal), preimplantation loss (# or % per animal), live fetuses (m or f) (# per animal), postimplantation loss (# or % per animal), dead fetuses (# or %), early or late resorptions (# or % per animal)

Fetal body weight (m or f) (gr)

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	2	25	50
Fetal body weight		4.3	4.1*	4.1**	3.9**
males		4.4	4.3	4.2*	4.0**
females		4.2	4.0*	3.9**	3.8**

Fetal data:

Malformations

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	2	12	25
Visceral malformations					
Litters examined		25	24	25	25
Fetuses examined		163	141	155	164
Interventricular septal defect	Litter incidence	0	0	1 (4%)	0
	Fetal incidence	0	0	1 (0.7%)	0
Lung agenesis	Litter incidence	0	0	1 (4%)	0
	Fetal incidence		0	1 (0.7%)	0

Total malformations (external, visceral, skeletal)					
	Litter incidence	0	0	1/25	0
	Fetal incidence	0	0	1/155	0

*statistically significant

There was one fetus in the MD group with lung agenesis and the interventricular septal defect.

Variations

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	2	25	50
Skeletal variations					
Litters examined		25	24	25	25
Fetuses examined		170	140	158	160
Cervical arches reduced ossification	Litter incidence	0	0	1	3 (12%)
	Fetal incidence	0	0	1	3 (2%)
Sternebra(e) 5 and/or 6 unossified	Litter incidence	6	15* (63%)	11	15 (60%)
	Fetal incidence	9	18	23	31 (19%)
External, visceral variations		No treatment effects			

Historical control data were given for CD rats from the conducting laboratory for 1989-1994 (18 studies).

Historical control incidence

Malformations				
Visceral				
	Interventricular septal defect	Litter incidence	0/441	0%
		Fetal incidence	0/3197	0%
	Lung agenesis	Litter incidence	0/441	0%
		Fetal incidence	0/3197	0%
Variations				
Skeletal	Cervical vertebrae (transverse process) incomplete ossification	Litter incidence	X/440	9-44%
		Fetal incidence	X/3204	1.2-14%
	5th or 6th sternebrae unossified	Litter incidence	X/440	10-96%
		Fetal incidence	X/3204	2-64%

X: number affected not mentioned, only number examined, and % affected

The ossification variations in 5th and/or 6th sternebrae and cervical vertebrae appeared to be dose-related findings. However, they were seen in historical control groups at % incidences exceeding the ones in the treated groups of the current study (*note*: control data for cervical vertebrae are from different anatomical location). The prevalence in historical controls indicates that reduced ossification in the cervical vertebrae and the 5th or 6th sternebrae is a fairly common spontaneous variation.

Historical control data (Crl: CD®(SD)BR rat; [redacted] 1992-1994)

Malformations			No.	Avg %	Max %
Visceral					
	Ventricular septal defect, muscular	Litter incidence	4 out of 4,935	0.13%	10%

		Fetal incidence	4 out of 24,340	0.018%	1.3%
	Lung agenesis	Litter incidence	1-2 out of 4,935	0.03-0.07%	4.2-5.0%
		Fetal incidence	1-2 out of 24,340	0.002-0.009%	0.26-0.63%

In the lab's historical control database there were no findings of interventricular septal defect or lung agenesis. The [redacted] database shows that the interventricular and lung agenesis have very low spontaneous incidences in the CD rat. Based on the data, this did not appear to be a significant drug-related finding.

Conclusions

- Maternal NOAEL 2 mg/kg/day (LD)
- Maternal toxicity (signs, BW, FC) at 25 (MD) and 50 (HD) mkd. At 2 mkd there was a small decrease in maternal BW gain on GD6-18, but no effect on FC.
- Slight fetal body weight reduction in LD (f, significant) and MD, HD (m and f, significant).
- In MD and HD fetal body weight effects were probably related to maternal toxicity as evidenced by significantly decreased maternal body weight gain and food consumption during dosing period (GD6-18).
- No significant external, visceral or skeletal malformations or variations in the absence of maternal toxicity.

Dose-range finding studies

Study title: A range-finding developmental toxicity study in rats with AMG 099073-01 via oral administration (Study # 100202) [redacted] (Lot Nr. 709001).

A dose-range finding developmental rat toxicity study (Study No. 100202) was conducted to obtain preliminary data on maternal and fetal toxicity and to obtain TK data of AMG-073 in the pregnant rat. Pregnant Crl: CD ® (SD) [redacted] BR rats (20/group), [redacted] were dosed with 0, 5, 25, 50, 75 mg/kg/day daily by oral gavage (5 mL/kg/d) on GD6-17. Dose-range phase was carried out in N=8/grp, TK phase in 12/grp. Blood samples for TK and ionized Ca were collected on GD17 at predose, 0.5, 1, 2, 4, 8, 12, 24h postdosing (N=3/grp/time). Samples were assayed for AMG-073 (parent drug) by validated LC/MS/MS method (LLQ [redacted]. No tests of significance were done. Fetuses were examined for external malformations and variations.

Group	Group Designation	Daily Doses[a]			Treatment Schedule	Number of Animals		
						Sacrificed		
		Dose (mg/kg)	Conc. mg/mL	Vol. (mL/kg)		Mated Females[b]	Gestation Day 20[c]	External Fetal Examination
I	control	0	0	5	GD 6-17	20	8	All fetuses[d]
II	low-	5	1	5	GD 6-17	20	8	All fetuses[d]
III	low-mid-	25	5	5	GD 6-17	20	8	All fetuses[d]
IV	mid-	50	10	5	GD 6-17	20	8	All fetuses[d]
V	high-	75	15	5	GD 6-17	20	8	All fetuses[d]

Key: Vol.=Volume; Conc.=Concentration; GD=Gestation Day.

[a]Doses given represent doses of test article as the free base. A factor of 1.1 was used to adjust for the HCl in the formulation and the free base content.

[b]The last twelve animals per group were used for toxicokinetic and ionized calcium/pH evaluations.

[c]Females in the range-finding component were sacrificed on day 20 of gestation. Females in the toxicokinetic component were sacrificed on day 18 of gestation.

[d]Day 20 gestation fetuses from the range-finding study.

Results

(A) Range-finding phase

No mortality.

Signs: Increased incidence of rales and alopecia in LMD, MD, HD (25, 50, 75 mkd) (N=8/grp), emaciation in MD, HD

Body weight gain (dams, N=7,8,8,8,6)

BWG reduced at 25, 50, 75 mkd

Grp		ctrl	LD	LMD	MD	HD
Dose (mkd)		1	5	25	50	75
BW gain Days 3-6 (gr)		26	29	23	31	25
BW gain Days 6-18 (gr)		92	100	80 ↓	61 ↓	44 ↓
BW gain Days 18-20 (gr)		34	33	37	42	39

Food consumption (dams, N=7,8,8,8,6):

Decrease in FC particularly in first 3 days of dosing, diminishing effect thereafter. Increase in FC (gr/kg/day) in HD animals on D18-20 was due to decreased BW

Grp		Ctrl	LD	LMD	MD	HD
Dose (mkd)		1	5	25	50	75
FC Day 6-9 (gr/kg/d)		86	86	46	39	39
FC Day 9-18 (gr/kg/d)		83	84	80 ↓	71 ↓	63 ↓
FC Day 18-20 (gr/kg/d)		77	78	84	100	104

Terminal and necroscopic evaluations (N=8/grp):

Maternal necropsy

Clinical signs in dams (N/8 animals affected)

Grp		ctrl	LD	LMD	MD	HD
Dose (mkd)		1	5	25	50	75
Alopecia-extremities/snout		0	1	1	3	1
Alopecia-general		1	0	1	2	0

C-section data (GD20):

Evaluated in N=7,8,8,8,6 pregnant dams.

No effects on corpora lutea (# per animal), implantation sites (# per animal), preimplantation loss (# or % per animal), live fetuses (m or f) (# per animal), postimplantation loss (# or % per animal), dead fetuses (# or %), early or late resorptions (# or % per animal)

Fetal body weight (m or f) (N=7,8,8,8,6 dams)

Reduction in MD (f), HD (m,f)

Grp		ctrl	LD	LMD	MD	HD
Dose (mkd)		1	5	25	50	75

Fetal body weight		3.9	3.9	4.0	3.7 ↓	3.5 ↓
males		3.9	4.0	4.0	3.9	3.6 ↓
females		3.8	3.8	3.9	3.6 ↓	3.4 ↓

Fetal data:
No external malformations or variations in any fetus (N=7,8,8,8,6 dams; N=90,104,99,101,76 fetuses)

(B) Toxicokinetic phase:
N=12/grp.

No mortality.

Signs: rales at 25, 50, 75 mkd, emaciation at 50, 75 mkd, tremors at 50, 75 mkd

Pregnancy rate: 11-11-11-11-12

No effect at maternal necropsy

C-section data (GD18): No effect on #live fetuses or #, % postimplantation loss

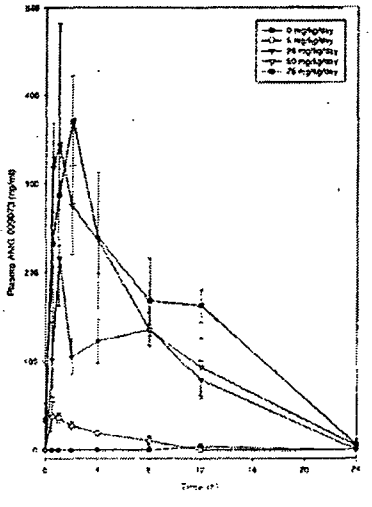
No fetal examination performed in TK phase

Ionized Calcium
Decrease upon dosing. Lowest Ca values at 4-8h post dosing. Dose-dependent effect in all dose groups that persisted up to 24h after dosing. No effect on serum pH.

(pH-)normalized Ca (N=2 or 3/time/grp)

Grp		Ctrl	LD	LMD	MD	HD
Dose (mkd)		0	2	25	50	75
nCa (nmol/L)						
Predose		1.39	1.37	1.28	1.28	1.02
8h postdose		1.39	1.21	0.92	0.89	0.76

Plasma drug concentrations
TK data were analyzed for all animals (N=12 controls, N=48 dosed), since there was no obvious difference between dosed pregnant (N=45) and non-pregnant rats (N=3).



TK parameters for pregnant rats (GD17) (Dosing GD6-17, oral, daily)

Dose mg/kg/day	Cmax ng/ml	Tmax h	AUC (0-24h) ng*h/ml	CL/F L/h/kg	t1/2 h
5	[redacted]	0.50	187	26.8	4.3
25	[redacted]	1.0	1920	13.1	5.2
50	[redacted]	1.0	2850	17.5	2.8
75	[redacted]	2.0	3610	20.8	2.5

At doses >25 mg/kg/day, Cmax and AUC increased less than proportionally with dose

- Comparison with TK data from non-pregnant rats (Study #100082, 26-week repeat dose oral rat toxicity study) shows average Cmax and AUC are somewhat higher in pregnant than non-pregnant animals, while ranges are similar

Conclusions

- NOAEL for maternal toxicity 5 mg/kg/day
- Maternal body weight gain reduced at 25, 50, 75 mkd
- NOAEL for fetal toxicity (BW decrease) 25 mg/kg/day
- Fetal body weight reduced at 50, 75 mkd
- No external fetal malformations or variations in any dose group (5, 25, 50, 75 mkd)

Study title: A range-finding developmental toxicity study in rats with AMG 099073-01 via oral administration (Study # 100022) ([redacted]) (Lot Nr. 709001).

In another dose-range-finding study (Study # 100022) with TK evaluation CD rats were dosed with 0, 1, 5, 25, 100 mkd (N=20/grp) on GD6-17. Dose-range-finding (RF) phase was carried out in N=8/grp, TK phase in 12/grp. Pregnancy rates were low in some groups (N=8/8, 7/8, 7/8, 3/7, 5/7 in RF phase, N=11/12, 8/12, 10/11, 9/11, 5/11 in TK phase). BW gain was reduced slightly/moderately to severely at 25 and 100 mkd (up to 75%). Implantation sites slightly reduced at 100 mkd. Fetal BW was slightly decreased (12%) in the 100 mkd group. There were no external fetal malformations or variations. Because of the low pregnancy rates the body weight effects Sponsor decided to repeat this study with other doses, including a lower high dose (Study Nr. 100202; 0, 5, 25, 50, 75 mg/kg/day).

Study design:

		Daily Doses[a]				Number of Animals		
Group	Group Designation	Dose (mg/kg)	Conc. (mg/mL)	Vol. (mL/kg)	Treatment Schedule	Mated Females[b]	Sacrificed Gestation Day 20[c]	External Fetal Examination[d]
I	Control	0	0	5	GD 6-17	20	8	All fetuses
II	low-	1	0.2	5	GD 6-17	20	8	All fetuses
III	low-mid-	5	1	5	GD 6-17	20	8	All fetuses
IV	mid-	25	5	5	GD 6-17	20	8	All fetuses
V	high-	100	20	5	GD 6-17	20	8	All fetuses

Key: Vol.=Volume; Conc.=Concentration; GD=Gestation Day

[a]Doses given represent doses of test article as the free base. A factor of 1.1 was used to adjust for the HCl in the formulation and the free base content.

[b]The last twelve animals per group were used for toxicokinetic and ionized calcium/pH evaluations.

[c]Surviving females in the range-finding component were sacrificed on day 20 of gestation. Surviving females in the toxicokinetic component were sacrificed on day 18 of gestation.

[d]Performed only on fetuses of dams which were part of range-finding component

TK phase data (GD17)

Ionized Calcium

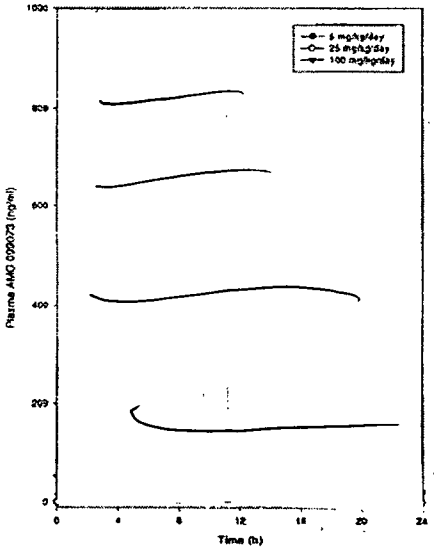
Decrease upon dosing. Lowest Ca values at 4-8h post dosing. Dose-dependent effect in all dose groups that persisted up to 24h after dosing. No effect on serum pH.

(pH-)normalized Ca (N=2 or 3/time/grp)

Grp		Ctrl	LD	LMD	MD	HD
Dose (mkd)		0	1	5	25	100
nCa (nmol/L)						
Predose		1.37	1.38	1.38	1.33	1.09
8h postdose		1.39	1.37	1.21	0.99	0.71

Plasma drug concentrations

TK data were analyzed for all animals



TK parameters for pregnant rats (GD17) (Dosing GD6-17, oral, daily)

Table B. Toxicokinetic Parameters for AMG 099073-01 in Rats

Dose mg/kg/day	Cmax ng/ml	Tmax h	AUC (0-24h) ng*h/ml	CL/F L/h/kg	t1/2 h
5		1.0	197	25.4	2.3
25		8.0	2120	11.8	2.7
100		1.0	7900	12.7	2.6

- Proportional increases in Cmax and AUC
- TK data very similar to data from the other dose range finding study (No. 100202)

- Comparison with TK data from non-pregnant rats (Study #100082, 26-week repeat dose oral rat toxicity study) shows average Cmax and AUC are somewhat higher in pregnant than non-pregnant animals, but ranges are similar

Conclusions

- NOAEL for maternal toxicity 5 mg/kg/day
- Maternal body weight gain reduced at 25, 100 mkd
- NOAEL for fetal toxicity (BW decrease) 25 mg/kg/day
- Fetal body weight reduced at 100 mkd
- No external fetal malformations or variations in any dose group (5, 25, 100 mkd)

APPEARS THIS WAY
ON ORIGINAL

Prenatal and postnatal development

Study title: Study of effects of oral administration of AMG 099073-01 on pre- and postnatal development, including maternal function in rats

Key study findings:
Pregnant rats were dosed from GD6 through Lactation Day 20 with 0, 5, 15, 25 mg/kg/day. One dam dosed with 15 mg/kg/day was found dead with a prolapsed uterus and delivery complications on the day of delivery. In F0 dams, clinical signs (rales and lethargy) were seen at 25 mg/kg/day. Maternal food consumption and body weight gain were decreased at 15 and 25 mg/kg/day on gestation days 6-12. One dam in each dose group (5, 15, 25 mg/kg/day) had total litter loss by lactation days 1, 2 and 5, respectively. The number of pups dying, missing or cannibalized on PPD0-4 was increased at 25 mg/kg/day. Reductions in maternal food consumption and body weight (or BW gain) and reductions in F1 pup body weight (or BW gain) were observed on PPD 10-14 and PPD 10-17, respectively, at 25 mg/kg/day. A minimal reduction in F1 pup body weight gain unaccompanied by maternal effects was observed at 15 mg/kg/day on PPD 10-17. There were no effects on F1 pre- or postweaning development. In F1 male parental animals, there were treatment-related clinical observations (incisor abnormalities and chromodacryorrhea) at 5, 15, 25 mg/kg/day. There were no effects on F1 fertility and reproductive performance, F1 gestation or lactation, and F2 pup viability or weight.

Study No.: 99-4175
Sponsor Study No.: 100734
Conducting laboratory: [redacted]
Date of study initiation: Oct 6, 1999
GLP compliance: Yes
QA reports: Yes (X) No ( )
Drug, lot #, and % purity: 709001; 99.7%

Methods

Doses: 0, 5, 15, 25 mg/kg/day (free base)
Species/strain: Albino rats [Crl:CDR(SD)— BR] rats
Number/sex/group: 25/group (time mated females)
Dosing period: GD6-LD21
Route, formulation: Oral gavage, suspension in 0.5% (w/v) aqueous methylcellulose
Volume: 5 ml/kg,
Satellite groups for TK: None
Study design:

Experimental Design

		Daily Dosages				Number of Animals	
Group	Group Designation	Dose Level (mg/kg/day)	Dose Volume (mL/kg/day)	Concentration (mg/mL)	F_0 Treatment Schedule	F_0 Mated Female Rats	F_1 Reproductive Assessment
I	Control[a]	0	5	0	GD6-LD20	25	25/sex
II	Low	5	5	1	GD6-LD20	25	25/sex
III	Mid	15	5	3	GD6-LD20	25	25/sex
IV	High	25	5	5	GD6-LD20	25,	25/sex

[a] Control animals were treated with vehicle (0.5% methyl cellulose (400 CPS) in distilled water) only at the same volume administered at the high dose level.
Key: D = Day; G = Gestation; L = Lactation.

Parameters measured:	Mortality and signs (F0), BW and FC (F0), parturition, litters (culling to 10/litter on PPD4), F1 pup physical condition and BW, F1 pre- and postweaning developmental landmarks, F1 behavioral assessments, F1 parental observation and BW and FC, F1 reproductive assessment, F1 parturition and lactation, F2 litters, unscheduled or scheduled euthanasia/terminal necropsy (F0, F1, F2).
Dose selection:	A high dose of 25 mkd was selected because it caused only a small effect on gestational BW and FC in the range finding study, but did not interfere with parturition or pup viability (Study Nr. 100584).

Definitions:
Live birth index: total number of liveborn pups / total number of pups born
Viability index: Number of pups alive on LD4 (precull) / number alive on LD0
Lactation index: Number of pups alive on LD21 / number alive on LD4 (postcull)

Results

F_0 in-life:

Mortality: 4 deaths. 1 MD found dead on PPD0 due to delivery complications (prolapsed uterus). This F0 dam also had discolored lungs at necropsy, and one male fetus in uterus. 1LD, 1MD, 1HD euthanized on Days 1, 2, 5 of Lactation, respectively, due to total litter loss.

Pregnancy rate: N=24-23-25-24

F0 Clinical signs:

Gestation

Grp		ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
N examined		25	25	25	25
Rales		0	0	0	1

Lactation

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
Euthanized		0	1	1	1
Prolapsed uterus		0	0	1	0
Lethargy		0	0	0	1
Rales		0	0	0	2

F0 Body Weight (N=24-23-25-24)

BW gain reduced at 15 and 25mkd during gestation, in first days of dosing, related to decreased food consumption. The decrease in body weight gain during PPD10-14 at 25 mkd (HD) was accompanied by FC reduction. The apparent BW gain increase in HD on PPD14-18 was related to a decrease in control values. However, FC was the same in all groups during this period. The cause of the anomalous BW effect in PPD14-18 is unclear. BW's were similar at end of lactation period (PPD 21).

F0 body weight

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
BW GD0		244	245	245	246
BW GD20		395	397	390	382
BW gain GD 6-9 (gr)		14	16	10*	8*
BW gain GD 9-12 (gr)		22	20	20	18
BW gain GD 0-20 (gr)		152	153	144	137*
BW PPD 1		299	301	299	297
BW PPD 14		362	364	355	339*
BW PPD 21		348	353	353	350
BW gain PPD 1-4 (gr)		15	17	12	8
BW gain PPD 4-7 (gr)		19	17	14	18
BW gain PPD 7-10 (gr)		15	14	13	15
BW gain PPD 10-14 (gr)		15	15	15	-1**
BW gain PPD 14-18 (gr)		-20	-10	-6	10**
BW gain PPD 18-21 (gr)		6	-1	4	1

F0 Food consumption (dams, N= 24,23,25,24)

Decrease in FC particularly during first 3 days of dosing. Also decrease in FC during lactation days 4-7, 10-14, and 10-14, significant during latter only.

F0 food consumption

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
FC GD 6-9 (gr/kg/d)		86	85	77**	72**
FC GD 9-12 (gr/kg/d)		86	87	81*	78**
FC PPD 1-4 (gr/kg/d)		104	110	111	103
FC PPD 4-7 (gr/kg/d)		140	143	141	136
FC PPD 7-10 (gr/kg/d)		160	161	164	154
FC PPD 10-14 (gr/kg/d)		188	190	193	169**
FC PPD 14-18 (gr/kg/d)		190	193	196	192

F0 Delivery and litter data

No effect on gestation duration, mating index, fertility index, gestation index

Total litter loss in I LD, MD, HD each

Increase in # pups dying, missing or cannibalized (statistically significant), and decrease in viability index in HD on PPD0-4.

Note: Litters were culled on PPD4 to 10/litter. At weaning (PPD21) F1 parental animals were selected (1 pup/sex/litter), with additional animals added to give 25 F1 pups/sex/grp. At a minimum age of 85 days, males were mated with females from same dose group (maximum 20 days cohabitation).

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
Females delivering		24	23	24	24
Females with stillborn pups		0	2	1	3
Litters with liveborn but no pups on PPD 21[&] (i.e.. total litter loss)		0	1	1	1
Pups delivered	N	303	312	310	295
	Mean	12.6	13.7	13.0	12.4
	Stillborn	0	2	1	3
Pups dying, missing, cannibalized	PPD 0-4	4	7	3	17*
	PPD5-21	2	1	1	4
Viability index (pups surviving to PPD4) (%)		98.0	94.2	99.0	94.2 ns
Live pups/litter	PPD0	12.6	13.6	12.9	12.3
	PPD4 (post cull)	9.7	9.5	9.6	9.0
Pups after culling on PPD4	N	239	237	233	234

[&]Total litter loss in 1 LD (14 pups), 1 MD (2 pups), and 1HD (14 pups) dam, on Days 1, 2, 0-5. Dams were euthanized after loss.

F1 observations:

F1 clinical observations: In HD, there was one litter in which there were 2 pups with hypothermia and no milk in stomach (none found in other dose groups)

F1 pup body weight (grams)

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
BW					
Day 1	M+F	7.2	6.9	7.2	7.1
Day 10	M+F	20.4	20.6	20.9	20.5
Day 14	M+F	30.0	30.2	29.7	28.3
Day 17	M+F	36.9	37.0	36.0	34.7
	Females	36.4	36.4	35.5	34.1*
	Males	37.4	37.6	36.6	35.2
BW gain					
Day 1-4	M+F	2.9	3.0	3.0	3.0
Day 4-7	M+F	4.6	4.7	4.8	4.5
Day 7-10	M+F	5.7	6.0	5.9	5.6
Day 10-14	M+F	9.6	9.6	8.7*	7.9**
Day 14-17	M+F	6.8	6.8	6.4	6.3
Day 17-21	M+F	11.3	10.9	12.1	11.7

Decreases in BW gain on Days 10-17 were similar in male and female pups

F_1 development and behavior:

No effect on preweaning pup developmental landmarks (day of eye opening, pinna unfolding, acoustic startle).
No effects on postweaning pup developmental landmarks (day of preputial separation, or vaginal opening).
No effects on behavior, i.e., open field evaluation (PPD 22 or 23), locomotor activity (photobeam activity system) on Days 28 or 55, learning and memory (— water maze performance, PPD 50)

F_1 reproduction:

F1 generation clinical signs (Week 7 to Week 23)

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
N examined		25	25	25	25
MALES - Week 15					
Chromodacryorrhea (unilateral)		0	2	1	2
Lacrimation (unilateral)		0	2	0	1
Incisors maloccluded		1	2	1	3
Incisors broken/missing		0	0	0	1
MALES - Week 23					
Chromodacryorrhea (unilateral)		0	1	2	2
Lacrimation (unilateral)		1	1	2	2
Incisors maloccluded		1	1	2	3
Incisors broken/missing		0	1	1	4
FEMALES – Week 15					
Chromodacryorrhea (unilateral)		0	1	0	0
Incisors maloccluded		1	1	0	0

No female data for Week 23 (female pups were mated before that)

No significant effects on F1 body weight (gain) or food consumption during Week 7-Week 22

No effects on F1 clinical signs during gestation and lactation
No effect on F1 gestational body weight (gain) (GD 0-20)
No effect on F1 lactational body weight (gain (Day 0-4)
No effect on F1 estrous cycle length

Grp		Ctrl	LD	MD	HD
Dose (mkd)		0	5	15	25
N examined		25	25	25	25
Females with estrus		25	25	23	22
Days of estrus		2.2.	2.1	2.1	2.2

1 MD and 3 HD females with no estrus became pregnant (total pregnant: 22-24-24-25)

No effect on F1 fertility, i.e. cohabitation, pregnancy and survival (mating index, fertility index, pregnancy rate, dams delivering, dams dying)

No effects on F1 delivery and litter data (gestation index, gestation duration, pups delivered, live birth index, F2 pups dying, F2 pup viability index, F2 live pups, F2 sex ratio)

F_2 findings:
No effects on F2 pup weight (Day 1-4)

No effects on clinical observations during lactation

F_0 necropsy:
1 MD dam with lungs discolored (dam died at delivery)

F_1 necropsy:
Pups: no findings
Parental animals: no findings

F_2 necropsy:
Pups: No effects

Dose-range finding study
Study title: A range-finding pre- and postnatal development study in rats via oral administration of AMG 099073-01 (Study # 100584) ——— (Lot Nr. 709001).

A dose-range finding study (Study No. 100584) was conducted to obtain preliminary data on maternal and fetal/pup toxicity, including parturition effects. Pregnant Crl: CD ® (SD) — BR rats (8/group), — were dosed with 0, 5, 15, 25, 50 mg/kg/day daily by oral gavage (5 mL/kg/d) on GD6-PPD7. Animals were sacrificed on PPD8. Day of parturition initiation was PPD0. Evaluated were mortality and signs (F0), BW and FC (F0), parturition, litters (F1), pup physical condition and BW (F1), necropsy (F0 and F1). No toxicokinetic data obtained.

F0 female data

Pregnancy rate: 8-8-8-7-8

Mortality: One dam at 50 mkd (HD) was sacrificed with prolapsed uterus after parturition. The prolapse may have been related to maternal hypocalcemia, interfering with smooth muscle contraction during delivery.

Signs: Alopecia in MD and HD, rales and decreased fecal volume in HD

Body weight gain (dams, N=8,8,8,7,8)

BWG reduced at 50 mkd during gestation, particularly in first days of dosing. Body weight gain increased during lactation, resulting in similar BW's at end of lactation.

Grp	Ctrl	LD	LMD	MD	HD
Dose (mkd)	0	5	15	25	50
BW gain GD 6-9 (gr)	17	14	13	2*	-12**
BW gain GD 6-20 (gr)	126	114	109	115	73**
BW gain PPD 1-7 (gr)	33	32	29	42	49*

Food consumption (dams; N=8,8,8,7,8):

Decrease in FC particularly in first 3 days of dosing, diminishing effect thereafter. Increase in FC (gr/kg/day) in HD animals on D18-20 was due to decreased BW

Grp	Ctrl	LD	LMD	MD	HD
Dose (mkd)	0	5	15	25	50
FC GD 6-9 (gr/kg/d)	95	92	82	70**	52**
FC GD 6-20 (gr/kg/d)	88	85	81	81	68**
FC PPD 1-7 (gr/kg/d)	149	137	139	160	162

Gestation rate and gestation period not affected.

F1 litter data
Pup viability index (#pups alive on PPD4/# live-born pups) decreased in HD (100-88-95-90-79** %).

Pup body weight (PPD 0) decreased in HD on PPD 0 (m,f), PPD 4 (m,f) and PPD 7 (f). Pup body weight gain unaffected.

F0 necropsy
Prolapsed uterus in a HD dam

F1 necropsy
Autolysis in 1 HD pup, associated with reduced viability index.

Conclusion
Prolapsed uterus in HD
Reduced gestational BW gain and FC in HD
Small transient decrease in gestational BW gain and FC in MD
Decreased viability index PPD0-4 in HD
Decreased pup weight (PPD0) in HD
No effect on pup weight gain during PPD0-7
Selection of MD (25 mkd) as high dose for definitive study was appropriate.