

CENTER FOR DRUG EVALUATION AND RESEARCH

APPROVAL PACKAGE FOR:

APPLICATION NUMBER

NDA 21-839

Pharmacology Review(s)

MEMORANDUM

Aug. 24, 2005

TO: File

FROM: Kenneth L. Hastings, Dr.P.H., D.A.B.T.

SUBJECT: NDA 21-839

I concur with the primary pharmacology/toxicology reviewer, Dr. Shen Xiao, and Dr. Jeri El-Hage, the pharmacology/toxicology supervisor, that this application may be approved. The proposed label for INRELEX® (mecasermin) should be changed to delete the warning concerning  since this is inconsistent with Pregnancy Category C, which is appropriate for this product based on nonclinical data.

Kenneth L. Hastings, Dr.P.H., D.A.B.T.

Associate Director for Pharmacology and Toxicology
Office of Drug Evaluations II & III

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Kenneth Hastings
8/24/2005 01:54:39 PM
PHARMACOLOGIST



DEPARTMENT OF HEALTH AND HUMAN SERVICES
PUBLIC HEALTH SERVICE
FOOD AND DRUG ADMINISTRATION
CENTER FOR DRUG EVALUATION AND RESEARCH

PHARMACOLOGY/TOXICOLOGY REVIEW AND EVALUATION

NDA NUMBER:	21-839
SERIAL NUMBER:	000
DATE RECEIVED BY CENTER:	2/24/05
PRODUCT:	INCRELEX (mecasermin [rDNA origin] injection) = rhIGF-1
INDICATION:	Long term treatment of growth failure in children with primary IGF-1 deficiency
SPONSOR:	Tercica Medica
DOCUMENTS REVIEWED:	Electronic
REVIEW DIVISION:	Division of Metabolic and Endocrine Drug Products (HFD-510)
PHARM/TOX REVIEWER:	Shen Xiao, Ph.D
PHARM/TOX SUPERVISOR:	Jeri El-Hage
DIVISION DIRECTOR:	David G Orloff
PROJECT MANAGER:	Enid M Galliers

Date of review submission to Division File System (DFS): July 20, 2005

TABLE OF CONTENTS

EXECUTIVE SUMMARY	3
2.6 PHARMACOLOGY/TOXICOLOGY REVIEW	7
2.6.1 INTRODUCTION AND DRUG HISTORY	7
2.6.2 PHARMACOLOGY.....	9
2.6.2.1 Brief summary.....	9
2.6.2.2 Primary pharmacodynamics.....	10
2.6.2.3 Secondary pharmacodynamics	12
2.6.2.4 Safety pharmacology	28
2.6.2.5 Pharmacodynamic drug interactions.....	28
2.6.3 PHARMACOLOGY TABULATED SUMMARY	31
2.6.4 PHARMACOKINETICS/TOXICOKINETICS.....	32
2.6.4.1 Brief summary.....	32
2.6.4.2 Methods of Analysis	33
2.6.4.3 Absorption.....	34
2.6.4.4 Distribution	43
2.6.4.5 Metabolism	49
2.6.4.6 Excretion.....	50
2.6.4.7 Pharmacokinetic drug interactions.....	50
2.6.4.8 Other Pharmacokinetic Studies	50
2.6.4.9 Discussion and Conclusions.....	50
2.6.4.10 Tables and figures to include comparative TK summary	51
2.6.5 PHARMACOKINETICS TABULATED SUMMARY.....	51
2.6.6 TOXICOLOGY	53
2.6.6.1 Overall toxicology summary.....	53
2.6.6.2 Single-dose toxicity	56
2.6.6.3 Repeat-dose toxicity	57
2.6.6.4 Genetic toxicology.....	108
2.6.6.5 Carcinogenicity	116
2.6.6.6 Reproductive and developmental toxicology	132
2.6.6.7 Local tolerance	148
2.6.6.8 Special toxicology studies.....	164
2.6.6.9 Discussion and Conclusions.....	166
2.6.6.10 Tables and Figures	169
2.6.7 TOXICOLOGY TABULATED SUMMARY.....	169
OVERALL CONCLUSIONS AND RECOMMENDATIONS.....	171
APPENDIX/ATTACHMENTS	176

EXECUTIVE SUMMARY

I. Recommendations

- A. Recommendation on approvability: Approval (AP)
- B. Recommendation for nonclinical studies: The non-clinical studies reviewed are sufficient to support the safety of subcutaneous administration of mecasermine (IGF-1) for the treatment of growth failure in children with primary IGF-1 deficiency. No further studies are required.
- C. Recommendations on labeling:

Mutagenesis: INCRELEX™ was not clastogenic in the in vitro chromosome aberration assay and the in vivo mouse micronucleus assay.

Impairment of fertility: INCRELEX™ was administered intravenously to rats at doses of 0.25, 1 and 4mg/day to conduct the fertility study. ☐

1

Pregnancy Category C: Embryo-fetal toxicity studies were conducted in SD rats with doses of 1, 4, and 16mg/kg/day, and in New Zealand White rabbits with doses of 0.125, 0.5 and 2.0mg/kg/day administered intravenously. No embryo-fetal developmental abnormalities were observed in rats with doses up to 16 mg/kg/day (20 times the MRHD based on body surface area [BSA] comparison). In the rabbit study, the NOAEL for maternal toxicity was 2.0mg/kg (8 times the MRHD based on BSA) and the NOAEL for fetal toxicity was 0.5mg/kg (2 times the MRHD based on BSA). INCRELEX™ displayed no teratogenicity at doses up to 2.0mg/kg (8 times the MRHD based on BSA).

The effects of INCRELEX™ on an unborn child have not been — studied. Therefore, there is insufficient medical information to determine whether there are significant risks to a fetus. ☐

1

Nursing Mothers: There have been no studies conducted with INCRELEX™ in nursing mothers. INCRELEX™ should not be administered to a nursing woman.

II. Summary of nonclinical findings

A. Brief overview of nonclinical findings: The bone growth was the primary endpoint for the evaluation of the effect of the IGF-1. Dose-dependent increases in tibial epiphyseal plate width, tibial length, or longitudinal bone growth of the tibia were observed following 7 days of SC infusions with mecasermin in the hypophysectomized rat and the dwarf (dw/dw) rat. These growth-deficient nonclinical pharmacology models are considered to be relevant to and predictive of the bone growth response observed in response to mecasermin in human clinical trials in children with primary IGFD. Drug-related hypoglycemic effects were observed in normal Sprague Dawley rats, hypophysectomized rats, aged Fischer rats, New Zealand rabbits, and rhesus monkeys following SC and IV bolus doses and SC infusion. These effects were noted after relatively higher SC and IV doses of mecasermin than those required for its growth promoting activity. In addition, dose-dependent increases in body weight were observed. The effects of mecasermin on enhancement of immune function were confirmed in studies in young and aged Balb/c and C57 black mice and aged Fischer rats. Greater increases in the width of the epiphyseal plate and/or longitudinal bone growth were noted in rats following mecasermin co-administered with rhGH, compared to treatment with mecasermin alone. No additional change was seen in blood glucose in rats following the administration of the two hormones compared to treatment with mecasermin alone. Greater increases in weight gain were seen following the co-administration of the two hormones for 28 days in dwarf rats and hypophysectomized rats compared to mecasermin treatment alone. In general, the co-administration of the two hormones produced similar enhancement of immune parameters as the administration of mecasermin alone in Balb/c mice. Mecasermin reversed some of the side effects seen with AZT dosing in Balb/c mice.

In toxicology studies, the single lethal doses for rats and dogs were 10mg/kg and 5mg/kg, respectively. The major cause of animal death was hypoglycemia. In the chronic toxicological studies, administration of mecasermin to both rats and dogs subcutaneously (sc) up to 26 weeks and intravenously (iv) up to 13 weeks were performed. Overall, hypoglycemia is the major effect in the toxicology studies, which was consistent with the expected physiological or exaggerated pharmacological effects of IGF-1. Other significant side effects included increases in body weight/body weight gain and/or food consumption, mild changes of clinical chemistry and hematological parameters, and increases in some organ weights including liver, kidney, heart, thymus, and/or spleen that correlated to the body weight changes were likely related to the anabolic activities of IGF-1. Hematology changes seen in one or both species consisted of higher hemoglobin and hematocrit, lower white blood cell and absolute lymphocyte counts, and/or lower eosinophil counts. Clinical chemistry changes consisted of transient alterations in serum electrolytes (sodium, chloride, potassium, phosphorus, and/or calcium), protein, albumin, and/or triglycerides. In most instances, the clinical pathology changes noted in the repeat dose studies were reversible. There are few histopathological changes associated with increases in organ weight. Histological changes identified in the 26-week study in the rat were confined to an increase in thymic cortical area at the end of the treatment period following sc doses of 4.0mg/kg. This increase is correlated with the increase in thymic weight and cellularity. In the 26-week dog study, histological changes consisted of adrenal hyperplasia with fibrosis which was noted at sc doses of 0.3mg/kg/day and above. Adrenal medullary fibrosis but not hyperplasia was still present in dogs treated with

0.6mg/kg/day after a 5 week recovery period. In the 13-week iv repeat dose toxicology studies, no drug-related changes in organ weights or histopathological findings were noted in rats. In dog, there was a significant shortening of the APTT in males at dose of 0.5mg/kg/day and a significant lengthening of the prothrombin time in females at dose of 0.5mg/kg. Histopathological findings at the end of treatment period were observed in cerebrum, cerebellum, medulla oblongata, heart, lungs, stomach, cecum, and liver in animals at dose of 0.5mg/kg/day. Most of these findings are related to slight to moderate hemorrhage and/or slight lymphocyte infiltration. The NOAELs in the 26-week sc repeated dose toxicology studies was 4mg/kg in rats and 0.15mg/kg in dogs. The systemic exposure of these doses was 15X and 3X the clinical exposure (AUC) with the maximum recommended human dose in rats and dogs, respectively. In the 13-week iv repeated dose toxicology studies, the NOAEL was 4mg/kg in rats and 0.125mg/kg in dogs similar to the NOAELs in the subcutaneous toxicity studies.

In the genotoxicology studies, mecasermin tested negative for genotoxic potential in both the in vitro chromosome aberration assay and the in vivo mouse micronucleus assay.

A two year carcinogenicity study of rhIGF-1 was conducted in Sprague Dawley rats. Drug was administered subcutaneously at doses of 0.25, 1, 4, and 10mg/kg/day. rhIGF-1 induced pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels. Increased keratoacanthoma in the skin in males were observed at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day. Mammary gland carcinomas were observed with increased frequency in both males and females at 10mg/kg/day. Compared with the historical data, the only positive findings in this carcinogenicity study were the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and the keratoacanthoma in males at doses of 4 and 10mg/kg. The NOEL in the carcinogenicity with rhIGF-1 was 1mg/kg/day in males and 4mg/kg for females. Doses of 4 and 10mg/kg/day exceeded the MTD based on mortality secondary to drug-induced hypoglycemia.

No embryo fetal developmental abnormalities were observed in rats treated with doses up to 16 mg/kg/day (20 times the MRHD based on BSA). In rabbit embryo-fetal toxicity study, the NOAEL for maternal toxicity was 2.0mg/kg and the NOAEL for fetuses was 0.5mg/kg. There was no teratogenicity of IGF-1 at doses up to 2.0mg/kg (2.5 times MRHD based on BSA). There were many resorbed embryos (early) and the index of dead embryos/fetuses increased in dams at dose of 2.0mg/kg. The NOAEL for fertility was 4mg/kg/day (5 times the MRHD based on BSA).

In the local tolerance studies, either subcutaneous or intravenous administration did not result in irritation that could be directly associated with the test material when compared with results of the effects of saline injection. In addition, mecasermin does not cause hemolysis of human or canine erythrocytes and is compatible with human and canine serum and plasma.

B. Pharmacologic activity: The pharmacologic activity following the SC administration of mecasermin in the nonclinical pharmacodynamic studies were increases in bone growth in GH and IGF-1 deficient animal models, acute decreases in blood glucose,

increases in body weight, and enhancement of immune function. The relevance of the nonclinical pharmacodynamic studies to human therapeutic use of mecasermin is supported by the demonstration of dose related pharmacological activity in all species tested and the apparent lack of species specificity. The data from these nonclinical pharmacodynamic studies support the use of mecasermin for long-term treatment of growth failure in children with primary IGFD.

C. Nonclinical safety issues relevant to clinical use: There were no toxicological findings other than those anticipated from exaggerated pharmacological effects of high doses of IGF-1.

Appears This Way
On Original

Appears This Way
On Original

Relevant INDs/NDAs/DMFs: IND 39,679 (Tercica)

Drug class: Growth hormones

Intended clinical population: Growth failure in children with primary IGF-1 deficiency

Clinical formulation: The dosage form is a sterile liquid solution containing 10mg/ml rhIGF-1, 50mM acetate, 5.84 mg/ml sodium chloride, 9mg/ml benzyl alcohol, and 2mg/ml polysorbate 20, at pH 5.4. Fill volume will be 4ml in a 5ml vial.

Route of administration: Subcutaneous injection

Disclaimer: Tabular and graphical information are constructed by the reviewer unless cited otherwise.

For (b)(2) applications: N/A

Data reliance: N/A

Studies reviewed within this submission:

	Brief Title Summary	Page#
Single dose toxicity studies	An IV study in mouse	56
	An IV study in rat	56
	An SC study in rat	56
	An IV study in rabbit	56
	An IV study in dog	57
	An SC study in dog	57
	An SC study in monkey	57
Repeat dose toxicity studies	4-week SC toxicity study in rats	57
	4-week SC toxicity study in dogs	57
	13-week IV toxicity study in rats	58
	13-week IV toxicity study in dogs	66
	26-week SC toxicity study in rats	81
	26-week SC toxicity study in dogs	94
Genetic toxicology studies	Chromosome aberration assay in Chinese Hamster Lung Fibroblasts	108
	In vivo micronucleus assay	112
Carcinogenicity study	2-year carcinogenicity study in rats	116
Reproductive toxicology studies	Fertility and general reproductive toxicology study in rats	132
	Embryo-fetal development study in rats	138
	Embryo-fetal development study in rabbits	143
Special toxicology studies	Local tolerance study after SC with different formulation in rabbit	148
	Local tolerance study after IV in rabbits	153
	Local tolerance study after SC in rabbits	158
	Hemolytic potential on human and dog blood in vitro	164

Studies not reviewed within this submission:

Repeat dose toxicity study	10- day IV toxicity study in rats
	4-week IV toxicity study in rats
	10- day IV toxicity study in dogs
	4-week IV toxicity study in dogs

2.6.2 PHARMACOLOGY

2.6.2.1 Brief summary:

The primary pharmacodynamic endpoint, bone growth, and secondary pharmacodynamic endpoints including hypoglycemic effects, body weight gain, and effects on immune function were evaluated in the pharmacology studies. In addition, drug interaction studies with mecasermin and rhGH, AZT, and dexamethasone were conducted during early development programs for other metabolic indications.

The bone growth was evaluated in two different growth-hormone and IGF-1 deficient rat models; the hypophysectomized rat and the dwarf (dw/dw) rat. Dose-dependent increases in tibial epiphyseal plate width, tibial length, or longitudinal bone growth of the tibia were observed following 7 days of continuous SC infusions with mecasermin (0.25 to 3.38 mg/kg/day). The hypophysectomized rat has been used as the bioassay or activity assay for the growth promoting activity of rhGH. These growth-deficient nonclinical pharmacology models are considered to be relevant to and predictive of the bone growth response observed in response to mecasermin in human clinical trials in children with primary IGFD. Increased bone growth was also observed in ovariectomized rats following 14 days of continuous SC infusion of mecasermin at doses of 0.66 to 2.0 mg/kg/day.

The drug-related hypoglycemic effects were studied in normal Sprague Dawley rats, hypophysectomized rats, aged Fischer rats, New Zealand rabbits, and rhesus monkeys following SC and IV bolus doses and SC infusion. These effects are observed after relatively higher SC and IV doses of mecasermin than those required for its growth promoting activity. Weight gain was measured in Balb/c mice, normal and ovariectomized Sprague Dawley rats, hypophysectomized and dwarf rats, and aged Fischer rats following SC infusion of mecasermin. In general, dose-dependent increases in body weight were seen compared to control animals. The effects of mecasermin on enhancement of immune function were confirmed in studies in young and aged Balb/c and C57 black mice and aged Fischer rats. It is likely that the demonstrated effects of mecasermin on the enhancement of these immune parameters are an extension of its anabolic activity on cellular proliferation and organ growth. Drug interaction studies with mecasermin and rhGH were conducted in Balb/c mice, normal and hypophysectomized Sprague Dawley rats, dwarf rats, and aged Fischer rats. Greater increases in the width of the epiphyseal plate and/or longitudinal bone growth were seen in rats following mecasermin co-administered with rhGH, compared to treatment with mecasermin alone. Although no additional change was seen in blood glucose in rats following the administration of the two hormones compared to treatment with mecasermin alone, greater increases in weight gain were seen following the co-administration of the two hormones for 28 days in dwarf rats and hypophysectomized rats compared to mecasermin treatment alone. In general, the co-administration of the two hormones produced similar enhancement of immune parameters as the administration of mecasermin alone in Balb/c mice. Mecasermin reversed some of the side effects seen with AZT dosing in Balb/c mice.

2.6.2.2 Primary pharmacodynamics

Mechanism of action: Human insulin like growth factor-1 (IGF-1) is a 7.649 kDa non-glycosylated protein of 70 amino acids with three intra-molecular disulfide bridges and is structurally homologous to proinsulin. Recombinant human (rh)IGF-1 or mecasermin is produced by recombinant DNA technology in *E. coli* and is identical in structure to endogenous human IGF-1. The actions of IGF-1 as a “somatomedin” are obligate for growth hormone (GH) to be able to stimulate bone and body growth. Therefore, a deficiency in IGF-1 leads to reduced body size in animals and humans. In addition to mediating many of the activities of GH, IGF-1 also has “insulin-like” metabolic activities that support statural growth. The hormonal, cellular and molecular mechanisms mediating the actions of rhIGF-1 on growth and metabolism are well characterized. In cell membranes of target tissues the Type 1 IGF-1 receptor, which is homologous to the insulin receptor, is activated by the binding of rhIGF-1 leading to intracellular signaling which stimulates multiple processes leading to statural growth. In animal models of IGF-1 deficiency, replacement with rhIGF-1 can improve or restore bone and body growth.

Drug activity related to proposed indication: The primary pharmacodynamic endpoint, bone growth, was evaluated in hypophysectomized rats, dwarf (dw/dw) rats, and ovariectomized Sprague Dawley rats.

Two studies were conducted in hypophysectomized rats. In study A, rats were administered excipient or mecasermin at 0.25, 0.71, or 2.2 mg/kg/day (n = 8 rats/group) for 7 days by continuous SC infusion using osmotic minipumps. In study B, the animals received excipient or mecasermin at 1.02 or 3.38 mg/kg/day (n = 3 to 5 rats/group) for 7 days by continuous SC infusion. Bone growth was determined in all surviving animals. Three of six animals in the 3.38 mg/kg/day dose group died on day one, presumably due to profound hypoglycemia. Dose-related increases in bone growth were seen in both studies in hypophysectomized rats after 7 days of mecasermin treatment. In study A, bone growth, as measured by new bone formed, increased 122, 198 and 267%, and the epiphyseal width increased 106, 121, and 149%, compared to excipient, following mecasermin treatment with 0.25, 0.71 or 2.2 mg/kg/day, respectively. No apparent change was seen in tibia length possibly due to the coarseness of this measurement. In study B a similar dose-relationship was observed. Bone growth increased 192 and 486% and epiphyseal width increased 138 and 168%, compared to excipient, following mecasermin treatment with 1.02 and 3.38 mg/kg/day, respectively. Tibia length was not measured in study B animals. Data were summarized in the following table.

Tibia Bone Parameters After 7 Days of Continuous SC Infusion of Mecasermin in Hypophysectomized Rats

Study A	Results			
Dose (mg/kg/day)	0	0.25	0.71	2.2
Tibia Length (mm)	27.5 ± 0.4	27.7 ± 0.9	28.0 ± 0.9	27.8 ± 0.5
Epiphyseal Width (µm)	211 ± 16	225 ± 20	256 ± 13**	314 ± 36***
Bone Growth (µm/day)	20.1 ± 5.7	24.6 ± 10	39.8 ± 14.4**	53.7 ± 19.7***
Study B				
Dose (mg/kg/day)	0	NA	1.02	3.38
Epiphyseal Width (µm)	184 ± 7	NA	254 ± 22***	310 ± 36**
Bone Growth (µm/day)	12.0 ± 2.3	NA	23.0 ± 5.8*	58.3 ± 4.2***

NA = not applicable

The groups were compared by an analysis of variance: * P<0.05, ** P<0.01, ***P<0.001 vs. excipient control

In the study of co-treatment with IGF-1 and growth hormone (GH) in female hypophysectomized rats, the growth plate of the tibia was significantly wider and there was significantly more longitudinal bone growth after administration of rhGH and the rhIGF-1 than there was in response to either hormone alone. Data were summarized in the following table.

Effects of rhGH, rhIGF-1, and des(1-3)rhIGF-1 on Parameters of Body Growth in Hypophysectomized Rats¹

Group (Doses mg/kg/day)	Weight Gain (g)	Serum IGF-1 (ng/ml)	Plate Width (µm)	Bone Growth (µm/day)
Study C				
Excipient	2.9 ± 3.5	122 ± 52	199 ± 15	ND
rhGH (0.015)	8.6 ± 2.3**	106 ± 18	247 ± 18**	ND
rhGH (0.06)	12.9 ± 1.2***	124 ± 35	294 ± 44***	ND
rhGH (0.24)	19.4 ± 1.7***	79 ± 13*	353 ± 27***	ND
rhIGF-1 (1.2)	16.6 ± 2.1***	62 ± 9**	275 ± 13***	ND
rhIGF-1 + rhGH	22.2 ± 2.7#	73 ± 6*	345 ± 33#	ND
des-IGF-1 (0.5)	19.9 ± 2.6***	195 ± 52**	288 ± 21***	ND
des-IGF-1 + rhGH	24.7 ± 1.3#	231 ± 32#	353 ± 32#	ND
Study D				
Excipient	0.0 ± 2.4	166 ± 109	235 ± 36	20 ± 4
rhGH (0.06)	9.0 ± 0.9***	103 ± 23	315 ± 35***	53 ± 8***
rhIGF-1 (1.2)	10.8 ± 3.7***	78 ± 31	284 ± 20**	44 ± 11**
rhIGF-1 + rhGH	19.3 ± 1.6#	85 ± 25	351 ± 29#	70 ± 7#
des-IGF-1 (0.5)	15.9 ± 2.5***	300 ± 113*	300 ± 17**	52 ± 13***
des-IGF-1 + rhGH	20.0 ± 2.4#	288 ± 163*	364 ± 31#	67 ± 14#

¹The Table shows the Mean ± SD (7-9 rats/group) for Studies C and D. In Studies C and D rhGH at 0.06 mg/kg/day was given in combination with rhIGF-1 or des-IGF-1. The asterisk (*) denotes different than excipient, with *, **, and ***, being p<0.05, p<0.01 and p<0.001, respectively. For the combination groups, # denotes different from either hormone given alone, p<0.05.

ND = Not Determined

In the studies of rhIGF-1 with or without rhGH in GH-deficient mutant dwarf rat, Mecasermin was administered at a dose of approximately 2.4 mg/kg/day for 7 days by continuous SC infusion using osmotic minipumps (n = 6 rats/group). The mean tibia epiphyseal plate width increased from 193 ± 26 µm in the control group to 223 ± 21 µm (P< 0.001) in the mecasermin treated animals. The longitudinal bone growth increased from 31 ± 16 µm/day in the control group to 46 ± 11 µm/day (P< 0.003) in the mecasermin treated animals. The width of the growth plate and the longitudinal bone growth of the tibia were significantly increased after co-administration of rhGH plus rhIGF-1 compared to rhGH alone. Data were summarized in the following table.

Study E: Body Growth Parameters in Dwarf Rats

Group (Dose mg/kg/day)	Body Weight Gain (g)	Plate Width (μ m)	Bone Growth (μ m/day)	Serum IGF-I (ng/mL)
Excipient	3.9 $\pm$ 3.6	193 $\pm$ 26	31 $\pm$ 16	151 $\pm$ 64
rhGH (0.125)	9.6 $\pm$ 2.4	201 $\pm$ 29	33 $\pm$ 17	88 $\pm$ 16
rhGH (0.5)	13.6 $\pm$ 5.0	237 $\pm$ 21	58 $\pm$ 19	90 $\pm$ 49
rhGH (2.0)	19.9 $\pm$ 4.5	244 $\pm$ 20	51 $\pm$ 14	80 $\pm$ 17
rhIGF-I (2.4)	12.1 $\pm$ 3.8	223 $\pm$ 21	46 $\pm$ 11	291 $\pm$ 63
rhGH (0.125) + rhIGF-I	21.4 $\pm$ 2.9	257 $\pm$ 15	63 $\pm$ 17	242 $\pm$ 67
rhGH (0.5) + rhIGF-I	24.6 $\pm$ 5.6	261 $\pm$ 31	61 $\pm$ 23	213 $\pm$ 37
rhGH (2.0) + rhIGF-I	28.4 $\pm$ 6.0	256 $\pm$ 12	66 $\pm$ 15	226 $\pm$ 21

P Values

Effect of rhIGF-I	0.001	0.001	0.003	0.001
Effect of rhGH	0.001	0.001	0.014	0.002
Interaction	0.684	0.157	0.298	0.869

The upper portion of the Table shows the means and standard deviations (n=6/group) for the 8 treatment groups. The lower portion of the Table shows the P values for each variable (column), for the two main effects, rhIGF-I or rhGH treatment or their interaction, from the 2 way ANOVA of these data.

In a study of the effect of the rhIGF-1 in ovariectomized rats, rhIGF-1 was delivered to SD rats by using implanted subcutaneously osmotic minipumps administering doses of 0.66 and 2mg/kg/day for 14 days. A dose related increase in bone growth was observed as follows: Group 1, ovariectomized-excipient (45.1 $\pm$ 8.2 μ m/day); Group 2, mecaseimerin low dose (50.0 $\pm$ 11.3 μ m/day); Group 3, mecaseimerin high dose (67.4 $\pm$ 7.6 μ m/day); Group 4, intact-excipient (39.1 $\pm$ 5.5 μ m/day). In another study with ovariectomized rats, rhIGF-1 was delivered to SD rats by using implanted subcutaneously osmotic minipumps delivering doses of 0.2mg/kg/day for 7 days and 0.24mg/kg/day for additional 28 days or 0.4mg/kg/day for 7 days and 0.48mg/kg/day for additional 28 days. The ovariectomized, excipient-treated animals showed a decrease in femur density compared to the excipient-treated sham operated animals. No change in femur density was observed with either mecaseimerin dose group when compared to the ovariectomized control group.

2.6.2.3 Secondary pharmacodynamics

Studies on secondary pharmacodynamics are summarized by mecaseimerin effects on blood glucose, body weight gain and immune function. Following administration of mecaseimerin, changes in blood glucose were studied in the Sprague Dawley rat, aged Fischer rat, hypophysectomized rat, New Zealand rabbit, and rhesus monkey. Changes in body weight were evaluated in the Balb/c mouse, normal and ovariectomized Sprague Dawley rat, aged Fischer rat, and hypophysectomized and dwarf (dw/dw) rat. Changes in the weight of various organs were monitored in several pharmacology studies. Immune function was evaluated in vitro with murine lymphocytes and in vivo in young and aged Balb/c mice, C57 black mice, and aged Fischer rats.

Hypoglycemia study: In the study of mecaseimerin effects on blood glucose in non-fasted male SD rats, subcutaneous bolus administration of mecaseimerin at 10.0 mg/kg resulted in a decrease in plasma glucose from 152 $\pm$ 19.2 mg/dL at 2 minutes postdose to a low of 70 $\pm$ 25.7 mg/dL (46% of 2 minutes postdose) 20 minutes postdose. By 180 minutes postdose, the plasma glucose had risen to 129 $\pm$ 38.1 mg/dL (85% of 2 minutes postdose) and remained above that level for the duration of the study. Subcutaneous bolus administration of

mecasermin at 1.0 mg/kg resulted in a decrease in plasma glucose from 137 ± 10.2 mg/dL at 2 minutes postdose to a low of 112 ± 11.7 mg/dL (82% of 2 minutes postdose) 30 minutes postdose. By 60 minutes postdose, the plasma glucose has risen to 149 ± 15.5 mg/dL (109% of 2 minutes postdose) and remained approximately at or above that level for the duration of the study. Subcutaneous bolus administration of mecasermin at 1.0 mg/kg resulted in a decrease in plasma glucose from 145 ± 10.4 mg/dL at 2 minutes postdose to a low of 132 ± 14.6 mg/dL (91% of 2 minutes postdose) 30 minutes postdose. By 60 minutes postdose, the plasma glucose has risen to 156 ± 13.1 mg/dL (108% of 2 minutes postdose) and remained approximately at or above that level for the duration of the study. Subcutaneous bolus administration of mecasermin at 0.1 or 0.3 mg/kg did not result in any significant decreases in plasma glucose. Intravenous bolus administration of mecasermin at 1.0 mg/kg resulted in a decrease in plasma glucose from 147 ± 31.2 mg/dL to 65 ± 14.6 mg/dL (44% of 2 minutes postdose) at 30 minutes postdose, returning to 129 ± 6.2 mg/dL (88% of 2 minutes postdose) plasma glucose by 60 minutes postdose and remaining above that level for the duration of the study. In fasted male SD rat, mecasermin was administered 1mg/kg IV bolus or 2.0mg/kg SC bolus. The mean plasma glucose concentration in the placebo group at 15 minutes was 155 mg/dL compared to 70 mg/dL (45% of placebo) in the group receiving mecasermin as a SC bolus. The plasma glucose concentration remained below 70 mg/dL until increasing to 143 mg/dL (92% of placebo) at 360 minutes. Intravenous administration of mecasermin resulted in a plasma glucose concentration at 15 minutes of 33 mg/dL (21% of placebo). At 120 minutes, the last time point taken in the IV group, the plasma glucose concentration was 70 mg/dL, (45% of placebo) compared to 55 mg/dL (35% of placebo) for the SC group. The IV group had a larger decrease in plasma glucose than the SC group, but the durations of the effect could not be compared because samples were not taken beyond 120 minutes in the IV group.

In study with aged male Fischer rats, continuous SC infusions using osmotic minipumps of mecasermin at doses of either at 1.0 mg/kg/day for 28 days ($n = 7$) or at 2.4 mg/kg/day for 14 days ($n = 7$) has no effect on blood glucose compared with the control. In hypophysectomized rats, received continuous SC infusions of mecasermin at doses of 0.25, 0.71 or 2.2mg/kg/day for 7 days using osmotic minipumps, all animals were euglycemic at sacrifice. In another study with hypophysectomized rats, mecasermin at 3.38 mg/kg/day caused three of six animals died within the first day. The blood glucose levels in this study were excipient group, 119 ± 17 mg/dL; 1.02 mg/kg/day mecasermin, 115 ± 29 mg/dL; 3.38 mg/kg/day mecasermin, 72 ± 31 mg/dL. Blood glucose at sacrifice was not measured in study B.

In male New Zealand White rabbits received a single bolus dose (2 to 4 animals/group) of mecasermin by either the IV (0.5 mg/kg) or SC (1.0 mg/kg), all animals treated with mecasermin experienced a hypoglycemic response, with glucose levels dropping below 90 mg/dL. Rabbits that received IV or SC mecasermin injections and normal diets had similar decreases in plasma glucose, to approximately 50% of baseline. The lowest glucose values for the IV groups occurred 30 to 50 minutes postdose and about 140 minutes postdose for the SC treatment group. The animals that received a liquid diet and IV mecasermin had the greatest decrease in glucose, to approximately 21% of baseline, at about 50 minutes postdose. Data were summarized in the following table.

Table 3: Summary of glucose concentrations for individual animals.

Group/ Treatment	Rabbit ID	Cpre (mg/dl)	Cnadir (mg/dl)	Change from Baseline (%)	Tnadir (Min)
Group 1/IV 0.5 mg/kg rhIGF-I Normal Diet	A	128	69	-46.09	20
	M	162	64	-60.49	40
	mean	145	66.5	-53.29	30
Group 2/IV 0.5 mg/kg rhIGF-I Liquid Diet	Ja	128	30	-76.56	60
	Jb	118	23	-80.51	40
	mean	123	26.5	-78.54	50
Group 3/SC 1.0 mg/kg rhIGF-I Normal Diet	C	124	88	-46.77	120
	D	122	67	-45.08	180
	E	130	32	-75.38	180
	F	124	81	-34.68	90
	mean	125	61.5	-50.48	142.5
	sd	3.46	20.82	17.44	45
Group 4/SC Vehicle Normal Diet	G	96	96	0	0
	H	139	137	-1.44	180
	mean	117.5	116.5	-0.72	90
Group 5/SC Vehicle Liquid Diet	K	121	116	-4.13	120
	L	139	121	-12.95	480
	mean	130	118.5	-8.54	300

^aAnimal I was given a 5% dextrose solution at 75 minutes.

^bAnimal J was given a 5% dextrose solution at 43, 62, and 75 minutes.

In the monkey study, six fasted adult male rhesus monkeys received 0.5 mg/kg mecasermin as a SC bolus dose. Administration of mecasermin produced an expected decrease in glucose at times varying from 1.25 to 4 hours postdose with three of six monkeys showing a substantial decrease (>25%) as shown in the following table.

**Glucose Parameters Following a
SC Bolus Dose of 0.5 mg/kg of GN0320A or GN0320B**

Monkey ID	Cpre ^a (mg/dL)		Cnadir (mg/dL)		Tnadir (hr)		AUC ₀₋₄ ^b (mg/dL-hr)	
	A ^c	B ^d	A	B	A	B	A	B
H104	65	64	41	37	1.75	2.00	69.1	70.6
M86	69	73	52	47	3.00	4.00	35.5	63.0
N97	97	81	ND ^e	ND	ND	ND	ND	ND
E891	90	103	79	99	2.00	4.00	9.9	4.0
N88	87	76	77	72	2.50	3.50	18.9	4.76
H116	77	76	61	43	1.25	2.00	25.1	87.6
Mean	81	79	62	60	2.10	3.10	31.7	46.0
SD	±13	±13	±16	±26	±0.68	±1.02	±22.9	±39.0

^a -0.17hr pre-dose concentrations

^b Only net negative concentrations values (reduction in glucose) were used in calculations. Calculations limited to 0-4hrs. Monkeys fed after 4-hr sample.

^c GN0320A="A"

^d GN0320B="B"

^e ND=not determined. Glucose did not decrease below Cpre value at any timepoint from 0-4hours

Body and organ weight changes: Dose-related effects on weight gain were seen in male Balb/c breeders (25 to 35 g) following a continuous SC infusion of mecasermin for 7 days and 14 days. Mecasermin was administered using osmotic mini pumps at doses of 7.5, 30 or 120 µg/day (approximately 0.25, 1.0, or 4.0 mg/kg/day) for 7 days or at a dose of 120 µg/day (4.0

mg/kg/day) for 14 days. There was a dose-related effect of mecasecmin on body weight gain in the 7 day treatment group (excipient 0.75 ± 0.75 g; low dose 0.86 ± 0.63 g; medium dose 1.31 ± 1.03 g; and high dose 2.43 ± 1.24 g), with the high dose being significantly greater compared to all other groups ($P < 0.001$). There was a large gain in body weight after 14 days of mecasecmin treatment (excipient 1.49 ± 0.46 g; mecasecmin 3.87 ± 0.45 g, $P < 0.001$).

To study the effect of rhIGF-1 in ovariectomized rats, rhIGF-1 was delivered to SD rats by using subcutaneously implanted osmotic minipumps administering doses of 0.66 and 2mg/kg/day for 14 days. At the end of study, body weights increased in both the low (23.2 ± 7.9 g) and high (49 ± 11.1 g) compared to control ovariectomized animals (18.1 ± 6.7 g) and control sham operated (3.24 ± 14.5 g) groups. Drug-related increases of organ weight were observed in liver and spleen were observed. Data were summarized in the following tables.

THE EFFECT OF 14 DAYS OF CONTINUOUS IGF-I ON ORGAN WET WEIGHTS IN RATS

	ovex excipient	ovex - low dose IGF-I	ovex - high dose IGF-I	intact excipient
Pituitary (mg)	11.4 ± 3.2	12.5 ± 3.3	12.5 ± 3.7	10.9 ± 2.9
Uterus (mg)	208 ± 66	193 ± 79	190 ± 49	$541 \pm 112^*$
Kidney (g)	$2.17 \pm 0.17\#$	2.50 ± 0.20	2.66 ± 0.30	$1.99 \pm 0.17\#$
Liver (g)	13.0 ± 0.99	13.7 ± 0.72	$14.8 \pm 1.2^*$	$11.3 \pm 1.2^*$
Spleen (mg)	$859 \pm 107^*$	$1020 \pm 97^*$	$1330 \pm 160^*$	$684 \pm 77^*$

mean $\pm$ SD (8 rats per group)

Statistics: * different from all other groups
excipient groups differ from IGF-I groups

TABLE 2

THE EFFECT OF 14 DAYS OF CONTINUOUS IGF-I ON ORGAN TO BODY WEIGHT RATIO IN RATS ($\times 10^3$)

	ovex excipient	ovex - low dose IGF-I	ovex - high dose IGF-I	intact excipient
Pituitary	0.035 ± 0.010	0.038 ± 0.010	0.035 ± 0.010	0.042 ± 0.011
Uterus	0.647 ± 0.210	0.589 ± 0.243	0.538 ± 0.142	$2.08 \pm 0.50^*$
Kidney	$6.71 \pm 0.35^*$	7.62 ± 0.60	7.51 ± 0.91	7.62 ± 0.67
Liver	40.2 ± 2.8	41.7 ± 1.9	41.9 ± 3.6	43.2 ± 2.8
Spleen	2.65 ± 0.29	$3.09 \pm 0.25^*$	$3.74 \pm 0.45^*$	2.62 ± 0.28

mean $\pm$ SD (8 rats per group)

Statistics: * different from all other groups

In another study with Sprague Dawley ovariectomized rats, mecasecmin was delivered by osmotic pump implants at doses of 0.2mg/kg/day for 7 days and 0.24mg/kg/day for an additional 28 days or 0.4mg/kg/day for 7 days and 0.48mg/kg/day for an additional 28 days.

Dose related increases in body weights were seen with mecasermin compared to excipient treated animals (sham and ovariectomized) beginning at 7 days post dose and continuing until day 35. At day 35, average body weight for the mecasermin low dose group of animals was 277 ± 5 g and 294 ± 7 g for the high dose group. At day 35, the average body weights of the excipient groups were 257 ± 5 for the sham group and 270 ± 6 g for the ovariectomized group.

In young female dwarf rats (dw/dw), a constant IV or SC infusion of mecasermin at dose of 1.0 mg/kg/day was administered for 10 days. After IV administration, the body weight compared with the control groups was unchanged. In hypophysectomized rats, after IGF-1 was delivered from osmotic minipumps as a continuous subcutaneous infusion for one week at doses of 0.25, 0.71 or 2.2mg/kg/day, dose-dependent significant increases of weight gains were observed. The average increase of weight gains was 5.4%, 12.9% and 16.1% in low, middle and high dose groups, respectively, as compared with the excipient control group. Increased organ weights were observed in the heart, thymus, spleen, kidneys and liver. Chemical analysis of the eviscerated body for water, protein, fat and ash indicated that IGF-treatment increased the water content of both the carcass and skin. Data were summarized in the following tables.

THE EFFECT OF IGF-1 ON BODY AND ORGAN WEIGHTS
AFTER SEVEN DAYS OF DOSING†

IGF-I Group	Excipient	Body and Organ Weights		
		Low	Medium	High
Study A				
Body wt (g)	97.5 ± 7.0	103 ± 6	110 ± 8***	113 ± 8***
Heart (mg)	357 ± 60	410 ± 55	406 ± 58	451 ± 67**
Thymus (mg)	246 ± 27	262 ± 42	306 ± 47*	442 ± 59***
Spleen (mg)	193 ± 30	211 ± 60	277 ± 47**	390 ± 90***
Kidneys (mg)	642 ± 64	641 ± 61	793 ± 77***	947 ± 99***
Brain (mg)	1,550 ± 300	1,610 ± 190	1,580 ± 170	1,540 ± 180
Liver (mg)	4,390 ± 310	4,600 ± 315	4,840 ± 460*	5,030 ± 440**
Study B				
Body wt (g)	89.6 ± 1.8	—	97.9 ± 1.2***	109 ± 15**
Heart (mg)	308 ± 20	—	332 ± 35	390 ± 42**
Thymus (mg)	210 ± 60	—	278 ± 42	476 ± 106***
Spleen (mg)	173 ± 24	—	280 ± 58**	438 ± 59***
Kidneys (mg)	588 ± 43	—	753 ± 83**	897 ± 122***
Brain (mg)	1,430 ± 110	—	1,420 ± 230	1,600 ± 50
Liver (mg)	3,470 ± 150	—	3,970 ± 370	4,300 ± 360**

† The mean $\pm$ SD for 8 rats per group in Study A and 3-5 rats per group in Study B.

Statistics: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ versus excipient using an analysis of variance with a Student's t-test for the level of significance.

**THE EFFECT OF IGF-I ON ORGAN/BODY WEIGHT RATIO
AFTER SEVEN DAYS OF DOSING†**

Organ to Body Weight Ratio ($\times 10^3$)

IGF-I Group	Excipient	Low	Medium	High
Study A				
Heart (mg)	3.7 $\pm$ 0.7	4.0 $\pm$ 0.6	3.7 $\pm$ 0.5	4.0 $\pm$ 0.5
Thymus (mg)	2.5 $\pm$ 0.3	2.6 $\pm$ 0.4	2.8 $\pm$ 0.4	3.9 $\pm$ 0.6***
Spleen (mg)	2.0 $\pm$ 0.3	2.1 $\pm$ 0.6	2.5 $\pm$ 0.3*	3.4 $\pm$ 0.7***
Kidneys (mg)	6.6 $\pm$ 0.4	6.2 $\pm$ 0.4	7.2 $\pm$ 0.4*	8.4 $\pm$ 0.6***
Brain (mg)	16 $\pm$ 3	16 $\pm$ 2	14 $\pm$ 2	14 $\pm$ 2
Liver (mg)	45 $\pm$ 4	45 $\pm$ 3	44 $\pm$ 2	44 $\pm$ 2
Study B				
Heart (mg)	3.4 $\pm$ 0.3	—	3.4 $\pm$ 0.4	3.6 $\pm$ 0.3
Thymus (mg)	2.3 $\pm$ 0.6	—	2.8 $\pm$ 0.4	4.3 $\pm$ 0.4***
Spleen (mg)	1.9 $\pm$ 0.2	—	2.9 $\pm$ 0.6**	4.0 $\pm$ 0.1***
Kidneys (mg)	6.6 $\pm$ 0.4	—	7.7 $\pm$ 0.8*	8.3 $\pm$ 0.3**
Brain (mg)	16 $\pm$ 1	—	14 $\pm$ 2	15 $\pm$ 2
Liver (mg)	39 $\pm$ 2	—	41 $\pm$ 4	40 $\pm$ 5

† The mean $\pm$ SD for 8 rats per group in Study A and 3-5 rats per group in Study B is listed.

Statistics: * P < 0.05, ** P < 0.01, *** P < 0.001 versus excipient using an analysis of variance with a Student's t-test for level of significance.

**THE EFFECT OF IGF-I ON BODY COMPOSITION IN MASS
AFTER SEVEN DAYS OF INFUSION†**

	IGF-I Dose Group			
	Excipient	Low	Medium	High
STUDY A				
Carcass				
Dry matter (g)	16.7 $\pm$ 1.6	17.9 $\pm$ 1.6	18.3 $\pm$ 1.4	17.8 $\pm$ 2.3
Fat (g)	3.66 $\pm$ 0.69	4.28 $\pm$ 1.00	4.22 $\pm$ 0.86	3.82 $\pm$ 1.38
Protein (g)	9.71 $\pm$ 1.01	10.1 $\pm$ 0.9	10.5 $\pm$ 0.7	10.3 $\pm$ 0.5
Ash (g)	3.22 $\pm$ 0.28	3.25 $\pm$ 0.31	3.43 $\pm$ 0.32	3.37 $\pm$ 0.2
Water (g)	33.5 $\pm$ 3.0	35.4 $\pm$ 2.9	36.3 $\pm$ 2.7	38.4 $\pm$ 2.8**
Skin				
Dry matter (g)	5.40 $\pm$ 1.09	5.23 $\pm$ 0.77	6.65 $\pm$ 1.01*	5.87 $\pm$ 1.06
Fat (g)	2.95 $\pm$ 0.84	2.91 $\pm$ 0.58	3.84 $\pm$ 0.89	3.14 $\pm$ 0.73
Protein (g)	2.37 $\pm$ 0.24	2.31 $\pm$ 0.33	2.77 $\pm$ 0.42*	2.68 $\pm$ 0.68
Ash (g)	0.077 $\pm$ 0.019	0.077 $\pm$ 0.016	0.098 $\pm$ 0.018*	0.103 $\pm$ 0.009**
Water (g)	7.77 $\pm$ 1.11	7.60 $\pm$ 1.21	9.01 $\pm$ 1.43	10.0 $\pm$ 1.2*
STUDY B				
Carcass				
Dry Matter (g)	15.6 $\pm$ 0.9	—	16.0 $\pm$ 0.9	16.2 $\pm$ 3.0
Fat (g)	3.43 $\pm$ 0.68	—	3.32 $\pm$ 0.56	3.07 $\pm$ 1.41
Protein (g)	9.02 $\pm$ 0.29	—	9.28 $\pm$ 0.21	9.72 $\pm$ 0.94
Ash (g)	2.92 $\pm$ 0.17	—	3.07 $\pm$ 0.16	3.21 $\pm$ 0.35
Water (g)	30.8 $\pm$ 0.5	—	32.5 $\pm$ 1.7	36.7 $\pm$ 5.7
Skin				
Dry Matter (g)	4.84 $\pm$ 0.44	—	5.27 $\pm$ 1.08	5.78 $\pm$ 1.13
Fat (g)	2.51 $\pm$ 0.39	—	2.75 $\pm$ 0.70	2.87 $\pm$ 0.88
Protein (g)	2.29 $\pm$ 0.29	—	2.37 $\pm$ 0.48	2.92 $\pm$ 0.41
Ash (g)	0.091 $\pm$ 0.042	—	0.092 $\pm$ 0.019	0.117 $\pm$ 0.020
Water (g)	7.01 $\pm$ 0.82	—	8.41 $\pm$ 1.58	10.6 $\pm$ 2.6

† The mean $\pm$ SD for 8 rats per group is listed for Study A and 3-5 rats per group for Study B. * P < 0.05, ** P < 0.01 versus excipient as assessed by analysis of variance and Student's t-tests.

**THE EFFECT OF IGF-I ON CARCASS AND SKIN COMPOSITION
EXPRESSED AS A PERCENT WEIGHT†**

		IGF-I Dose Group			
		Excipient	Low	Intermediate	High
STUDY A					
Carcass					
<u>Dry matter</u> (% carcass)	33.2 ± 0.8	33.5 ± 1.4	33.5 ± 0.8	31.6 ± 1.4**	
Fat (% dry)	21.8 ± 2.4	23.9 ± 4.5	23.0 ± 3.5	20.9 ± 5.0	
Protein (% dry)	58.1 ± 1.8	56.5 ± 3.0	57.8 ± 2.7	58.4 ± 5.4	
Ash (% dry)	19.3 ± 0.8	18.2 ± 1.5	18.8 ± 1.7	19.0 ± 1.1	
<u>Water</u> (% carcass)	66.8 ± 0.8	66.5 ± 1.4	66.5 ± 0.8	68.4 ± 1.4**	
Skin					
<u>Dry matter</u> (% carcass)	40.9 ± 3.8	40.9 ± 2.9	42.5 ± 3.2	36.8 ± 3.0*	
Fat (% dry)	54.1 ± 5.1	55.6 ± 4.4	57.3 ± 6.7	53.1 ± 4.1	
Protein (% dry)	44.8 ± 5.9	44.5 ± 3.9	42.0 ± 6.5	46.2 ± 3.7	
Ash (% dry)	1.4 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.8 ± 0.3*	
<u>Water</u> (% carcass)	59.1 ± 3.8	59.1 ± 2.8	57.5 ± 3.2	63.2 ± 3.0*	
STUDY B					
Carcass					
<u>Dry Matter</u> (% carcass)	33.6 ± 1.1	-	33.0 ± 1.0	30.6 ± 2.4	
Fat (% dry)	21.9 ± 3.4	-	20.7 ± 2.9	18.4 ± 5.1	
Protein (% dry)	57.9 ± 3.5	-	58.0 ± 3.6	60.6 ± 5.1	
Ash (% dry)	18.8 ± 1.2	-	19.3 ± 1.0	20.0 ± 2.1	
<u>Water</u> (% carcass)	66.4 ± 1.1	-	67.0 ± 1.0	69.4 ± 2.4	
Skin					
<u>Dry Matter</u> (% carcass)	40.9 ± 1.8	-	38.4 ± 2.6	35.5 ± 4.0	
Fat (% dry)	51.8 ± 5.1	-	51.9 ± 3.9	49.0 ± 6.1	
Protein (% dry)	47.5 ± 5.0	-	45.5 ± 5.8	50.9 ± 0.2	
Ash (% dry)	1.93 ± 1.05	-	1.76 ± 0.17	2.02 ± 0.18	
<u>Water</u> (% carcass)	59.1 ± 1.8	-	61.6 ± 2.6	64.5 ± 4.0	

† The mean ± SD for 8 rats per group is listed for Study A and 3-5 rats per group for Study B. * P < 0.05, ** P < 0.01 versus excipient as assessed by analysis of variance and Student's t-tests.

To study the effect of rhIGF-1 with or without rhGH on the catabolic state in male rats induced by glucocorticoid dexamethasone (DEX), rhIGF-1 was partially counteracted the steroid effect on body weight gain, whereas rhGH exacerbated the catabolism induced by DEX. rhIGF-1 plus rhGH had an intermediate effect on body weight gain. The organ wet weights indicated that rhIGF-1 partially reversed the splenic but not the thymic involution induced by Dex. rhGH treatment had no effect on the Dex-induced organ weight reductions. Serum proteins and triglycerides were elevated in the Dex plus rhGH groups. It seems that rhIGF-1 has anabolic activity by counteracting some of the catabolic effects of glucocorticoids-especially to protect the spleen from this catabolism. Data were shown in the following tables.

Table 1

Body Weight Increment (g) after IGF-I and GH in Dexamethasone (Dex)
Treated Rats

Day	Control			Dexamethasone 0.5 mg/L		
	Excipient#	IGF-I	GH	Excipient#	IGF-I	GH
		0.98 mg/kg	1.2 mg/kg		0.98 mg/kg	1.2 mg/kg
1	2.12 ± 4.7 ^a	7.24 ± 5.64	10.3 ± 3.9 ^a	0.03 ± 2.33	4.55 ± 2.53	6.52 ± 4.16
2	12.5 ± 5.0 ^{a,b}	20.1 ± 6.3 ^a	19.7 ± 2.7 ^b	7.55 ± 2.97 ^{c,d}	13.5 ± 4.4 ^c	12.9 ± 4.5 ^d
3	17.0 ± 4.0	22.6 ± 5.0	22.3 ± 3.6	4.99 ± 3.35	9.70 ± 4.27	6.81 ± 4.90
4	21.7 ± 5.9	27.6 ± 7.1	27.0 ± 3.6	4.09 ± 3.87	8.22 ± 5.73	1.58 ± 5.13
5	29.0 ± 5.1	34.0 ± 6.0	32.3 ± 3.2	6.38 ± 5.70	10.6 ± 6.4	0.14 ± 5.65
6	34.2 ± 6.3	40.4 ± 8.4	36.6 ± 3.7	6.27 ± 5.93	9.29 ± 9.27	-1.59 ± 5.79
7	39.9 ± 6.4	48.1 ± 8.9	45.4 ± 4.2	10.0 ± 6.5 ^a	12.1 ± 10.2	1.01 ± 6.66 ^a

Statistics:

All differences are at p < 0.05 by an analysis of variance. The # indicates that the dexamethasone/excipient treated group differed from the control/excipient group on all days. Identical letter superscripts for a given day indicate that the two groups are different from each other.

Table 2
The Effect of Dexamethasone and the Combination of GH and IGF-I on
Body Weight Increment (g)

Days	Control	Dexamethasone** 1.0 mg/L	
	Excipient	Excipient	IGF-I + GH
1	7.1 ± 1.7	2.6 ± 2.2	2.3 ± 1.6
2	17.0 ± 1.1	4.8 ± 1.9	5.3 ± 2.6
3	25.0 ± 1.6	6.6 ± 2.1	5.9 ± 3.0
4	33.0 ± 2.1	8.3 ± 2.8	3.9 ± 3.3
5	39.9 ± 4.3	6.9 ± 2.6	4.6 ± 2.3
6	47.0 ± 3.7	6.4 ± 2.4	3.9 ± 2.9
7	53.9 ± 3.3	9.4 ± 2.9	6.4 ± 2.1
8	60.7 ± 6.0	8.2 ± 1.5	5.6 ± 2.3
9	68.0 ± 7.4	10.7 ± 1.9	7.9 ± 2.5
10	74.7 ± 7.2	10.8 ± 1.8	8.8 ± 4.0
11	83.8 ± 8.7	14.6 ± 2.7	14.6 ± 4.5
12	90.2 ± 8.5	17.1 ± 4.0	17.3 ± 6.0
13	92.9 ± 10.1	16.4 ± 2.9	18.5 ± 6.4
14	103.0 ± 9.0	16.2 ± 2.4	19.9 ± 5.2

Statistics

- From Day 1, Dex suppressed weight gain in both the excipient and IGF-I + GH groups.
 --- P < 0.05 versus Dex - Excipient group.

Table 3

The Effect of Dexamethasone, IGF-I and GH on Rat Organ Weights

STUDY A: Seven Days

	Control			Dexamethasone 0.5 mg/L		
	Excipient	IGF-I 0.98 mg/kg	GH 1.2 mg/kg	Excipient	IGF-I 0.98 mg/kg	GH 1.2 mg/kg
Brain (mg)	1740 ± 100	1750 ± 150	1820 ± 100 ^a	1720 ± 90	1680 ± 12 ^a	1720 ± 80
Liver (g)	12.7 ± 1.0	12.4 ± 1.4	13.4 ± 0.9	11.4 ± 1.0 ^a	10.1 ± 0.9 ^{a,*}	11.3 ± 2.0
Kidneys (mg)	2290 ± 320	2440 ± 180 ^{a,b,c}	2270 ± 230	2030 ± 160 ^a	2090 ± 120 ^b	1960 ± 22 ^c
Spleen (mg)	720 ± 89	860 ± 152 [#]	667 ± 107	374 ± 47 [*]	543 ± 108 [#]	397 ± 74 [*]
Adrenal (mg)	44.9 ± 9.1	49.0 ± 5.2 ^a	45.6 ± 7.4	28.8 ± 6.8 [*]	37.4 ± 9.9 ^a	30.3 ± 4.9 [*]
Heart (mg)	959 ± 68 ^a	947 ± 65	936 ± 88	886 ± 50 ^a	899 ± 76	939 ± 126
Thymus (mg)	560 ± 103	545 ± 117	626 ± 110	276 ± 39 [*]	251 ± 45 [*]	240 ± 28 [*]

STUDY B: 14 Days

	Control*	Dexamethasone 1.0 mg/L				
	Excipient	Excipient	IGF-I 0.8 mg/kg	IGF-I 2.4 mg/kg	GH 0.8 mg/kg	GH 2.4 mg/kg
Liver (g)	11.4 ± 0.4	8.03 ± 0.99 [*]	8.60 ± 0.77 [*]	8.44 ± 1.09 [*]	7.05 ± 0.77 [#]	8.29 ± 0.75 [*]
Kidney (mg)	1060 ± 101 ^{a,b,c}	833 ± 90 ^a	1010 ± 50	1050 ± 80	812 ± 35 ^b	780 ± 50 ^c
Spleen (mg)	742 ± 63	309 ± 57 [*]	406 ± 63 [#]	515 ± 63 [#]	289 ± 64 [*]	336 ± 94 [*]
Heart (mg)	853 ± 52	697 ± 77 [*]	696 ± 46 [*]	729 ± 58 ^{a*}	677 ± 21 ^{a*}	694 ± 51 [*]
Thymus (mg)	487 ± 90	ND	ND	ND	ND	ND

Statistics: All differences are at p < 0.05 by an analysis of variance.

- a identical letters in a row indicate two groups differ
 # different from all other groups
 * different from control group
 ND not detectable
 Mean ± SD (n = 8 for Study A & 6 for Study B)

In an aged rodent growth model, mecaseimerin at 1.0, or 2.4 mg/kg/day was administered to 18 month old male Fischer rats (average initial weights 450 to 480 g) as a continuous SC infusion using osmotic mini pumps. The low dose (1.0 mg/kg/day, n = 7 animals) was delivered continuously for 28 days and the high dose (2.4 mg/kg/day, n = 7 animals) continuously for 14 days. A greater increase in body weight was seen following the higher dose for 14 days (34.5 ± 9.4 g ($P < 0.001$)) compared to an increase of 17.8 ± 13.6 g ($P < 0.05$) for the low dose administered for 28 days. The body weight increases of the animals in the excipient group ranged from 0.3 ± 9.4 to 1.0 ± 8.7 at 14 and 28 days, respectively.

In the combination study of rhIGF-1 with recombinant human growth hormone (rhGH) in hypophysectomized rats for one week, the increase in body weight induced by the both rhGH and the rhIGF-1 individually was further enhanced when rhGH and the rhIGF-1 were administered together. Absolute weights of the heart, kidneys, liver, spleen, and thymus were increased by rhIGF-1 with the combination of rhIGF-1 plus rhGH having greater effects than rhIGF-1 alone. However, there was no consistent additive effect of rhGH and rhIGF-1 on total serum IGF-1 concentrations. These data suggest a complex and additive interaction between rhIGF-1 and rhGH on these indices of growth. Data were summarized in the following tables.

**Effects of rhGH, rhIGF-I, and des(1-3)rhIGF-I on
Parameters of Body Growth in Hypophysectomized Rats¹**

Group (Doses mg/kg/day)	Weight Gain (g)	Serum IGF-I (ng/ml)	Plate Width (μ m)	Bone Growth (μ m/day)
Study C				
Excipient	2.9 ± 3.5	122 ± 52	199 ± 15	ND
rhGH (0.015)	$8.6 \pm 2.3^{**}$	106 ± 18	$247 \pm 18^{**}$	ND
rhGH (0.06)	$12.9 \pm 1.2^{***}$	124 ± 35	$294 \pm 44^{***}$	ND
rhGH (0.24)	$19.4 \pm 1.7^{***}$	$79 \pm 13^*$	$353 \pm 27^{***}$	ND
rhIGF-I (1.2)	$16.6 \pm 2.1^{***}$	$62 \pm 9^{**}$	$275 \pm 13^{***}$	ND
rhIGF-I + rhGH	$22.2 \pm 2.7^{\#}$	$73 \pm 6^*$	$345 \pm 33^{\#}$	ND
des-IGF-I (0.5)	$19.9 \pm 2.6^{***}$	$195 \pm 52^{**}$	$288 \pm 21^{***}$	ND
des-IGF-I + rhGH	$24.7 \pm 1.3^{\#}$	$231 \pm 32^{\#}$	$353 \pm 32^{\#}$	ND
Study D				
Excipient	0.0 ± 2.4	166 ± 109	235 ± 36	20 ± 4
rhGH (0.06)	$9.0 \pm 0.9^{***}$	103 ± 23	$315 \pm 35^{***}$	$53 \pm 8^{***}$
rhIGF-I (1.2)	$10.8 \pm 3.7^{***}$	78 ± 31	$284 \pm 20^{**}$	$44 \pm 11^{**}$
rhIGF-I + rhGH	$19.3 \pm 1.6^{\#}$	85 ± 25	$351 \pm 29^{\#}$	$70 \pm 7^{\#}$
des-IGF-I (0.5)	$15.9 \pm 2.5^{***}$	$300 \pm 113^*$	$300 \pm 17^{**}$	$52 \pm 13^{***}$
des-IGF-I + rhGH	$20.0 \pm 2.4^{\#}$	$288 \pm 163^*$	$364 \pm 31^{\#}$	$67 \pm 14^{\#}$

¹ The Table shows the Mean $\pm$ SD (7-9 rats/group) for Studies C and D. In Studies C and D rhGH at 0.06 mg/kg/day was given in combination with rhIGF-I or des-IGF-I. The asterisk (*) denotes different than excipient, with *, **, and ***, being $p < 0.05$, $p < 0.01$ and $p < 0.001$, respectively. For the combination groups, # denotes different from either hormone given alone, $p < 0.05$.

ND = Not Determined

rhGH and rhIGF-I Elicit Different Organ Weight Responses

	Excipient	rhGH	rhIGF-I	rhGH + rhIGF-I
Wet Weights				
Study C				
Heart (mg)	291 ± 20	324 ± 13*	341 ± 24*	344 ± 16*
Kidneys (mg)	650 ± 46	686 ± 60 ^{ab}	849 ± 50 ^{*a}	869 ± 31 ^{*b}
Liver (g)	3.8 ± 0.2	4.0 ± 0.2 ^{ab}	4.4 ± 0.3 ^{*a}	4.4 ± 0.4 ^{*b}
Spleen (mg)	234 ± 56	244 ± 26 ^{ab}	369 ± 50 ^{*a}	389 ± 54 ^{*b}
Thymus (mg)	233 ± 24	317 ± 82 ^{*a}	391 ± 49*	414 ± 110 ^{*a}
Study D				
Heart (mg)	355 ± 22	374 ± 43 ^a	375 ± 22 ^b	440 ± 65 ^{*ab}
Kidneys (mg)	688 ± 37	736 ± 44 ^{ab}	856 ± 69 ^{*ab}	973 ± 45 ^{*ab}
Liver (g)	3.8 ± 0.3	4.0 ± 0.3 ^a	4.4 ± 0.4*	4.6 ± 0.1 ^{*a}
Spleen (mg)	197 ± 16	260 ± 24 ^{*a}	290 ± 32 ^{*b}	342 ± 23 ^{*ab}
Thymus (mg)	257 ± 42	336 ± 50	436 ± 140*	450 ± 113*
Organ to Body Weight Ratio (x 10⁻³)				
Study C				
Heart/BW	3.0 ± 0.2	3.1 ± 0.2	3.1 ± 0.2	3.0 ± 0.2
Kidneys/BW	6.7 ± 0.5	6.5 ± 0.6 ^{ab}	7.7 ± 0.4 ^{*a}	7.5 ± 0.4 ^{*b}
Liver/BW	39.2 ± 1.8	37.9 ± 1.7	40.2 ± 2.1	38.0 ± 2.2
Spleen/BW	2.4 ± 0.7	2.3 ± 0.3 ^{ab}	3.4 ± 0.5 ^{*a}	3.3 ± 0.4 ^{*b}
Thymus/BW	2.4 ± 0.3	3.0 ± 0.7	3.6 ± 1.0*	3.6 ± 1.0*
Study D				
Heart/BW	3.9 ± 0.2	3.7 ± 0.3	3.7 ± 0.2	4.0 ± 0.5
Kidneys/BW	7.6 ± 0.3	7.3 ± 0.3 ^{ab}	8.4 ± 0.7 ^{*a}	8.8 ± 0.4 ^{*b}
Liver/BW	41.4 ± 2.2	40.2 ± 1.9	42.7 ± 4.2	41.4 ± 1.0
Spleen/BW	2.2 ± 0.1	2.7 ± 0.5 ^{*a}	2.9 ± 0.3*	3.1 ± 0.2 ^{*a}
Thymus/BW	2.8 ± 0.4	3.4 ± 0.5	4.3 ± 1.4*	4.1 ± 1.0*

¹The same doses of rhIGF-I (1.2 mg/kg/day) and rhGH (0.06 mg/kg/day) were given alone and together. Mean ± SD (7-9 rats/group): the asterisk (*) denotes different than excipient, and similar letter superscripts denote group differences at p < 0.05.

BW = Body weight

In the studies of rhIGF-1 with or without rhGH in two animal models including female hypophysectomized rat and a GH-deficient mutant female dwarf rat, the body weight of the dwarf rats increased 3.9 ± 3.6 g in the excipient group compared to 12.1 ± 3.8 g ($P < 0.001$) in the mecasermin treated group. The excipient group of hypophysectomized rats increased in body weight 4.5 ± 1.7 g compared to 18.2 ± 2.0 g in the mecasermin group.

IGF-1 increased the absolute weight of the kidney, liver, spleen, and thymus in both animal models. rhGH increased the absolute weight of kidney and liver in both models and increased thymus, spleen and heart only in the hypophysectomized rat. The relationships between spleen and thymus weight and the logarithm of rhGH dose were parallel for the rhGH alone and rhGH plus rhIGF-1 groups. However, the responses of the spleen and thymus to rhIGF-1 were much greater than to rhGH. Data were summarized in the following tables.

Table 1
Study E: Body Growth Parameters in Dwarf Rats

Group (Dose mg/kg/day)	Body Weight Gain (g)	Plate Width (μ m)	Bone Growth (μ m/day)	Serum IGF-I (ng/mL)
Excipient	3.9 $\pm$ 3.6	193 $\pm$ 26	31 $\pm$ 16	151 $\pm$ 64
rhGH (0.125)	9.6 $\pm$ 2.4	201 $\pm$ 29	33 $\pm$ 17	88 $\pm$ 16
rhGH (0.5)	13.6 $\pm$ 5.0	237 $\pm$ 21	58 $\pm$ 19	90 $\pm$ 49
rhGH (2.0)	19.9 $\pm$ 4.5	244 $\pm$ 20	51 $\pm$ 14	80 $\pm$ 17
rhIGF-I (2.4)	12.1 $\pm$ 3.8	223 $\pm$ 21	46 $\pm$ 11	291 $\pm$ 63
rhGH (0.125) + rhIGF-I	21.4 $\pm$ 2.9	257 $\pm$ 15	63 $\pm$ 17	242 $\pm$ 67
rhGH (0.5) + rhIGF-I	24.6 $\pm$ 5.6	261 $\pm$ 31	61 $\pm$ 23	213 $\pm$ 37
rhGH (2.0) + rhIGF-I	28.4 $\pm$ 6.0	256 $\pm$ 12	66 $\pm$ 15	226 $\pm$ 21

P Values

Effect of rhIGF-I	0.001	0.001	0.003	0.001
Effect of rhGH	0.001	0.001	0.014	0.002
Interaction	0.684	0.157	0.298	0.869

The upper portion of the Table shows the means and standard deviations (n=6/group) for the 8 treatment groups. The lower portion of the Table shows the P values for each variable (column), for the two main effects, rhIGF-I or rhGH treatment or their interaction, from the 2 way ANOVA of these data.

Table 2
Study E: Absolute and Relative Organ Weights in Dwarf Rats

Absolute Weights

Group (Dose mg/kg/day)	Spleen (mg)	Thymus (mg)	Kidneys (g)	Heart (mg)	Liver (g)
Excipient	305 $\pm$ 24	181 $\pm$ 55	1.03 $\pm$ 0.10	532 $\pm$ 92	4.84 $\pm$ 0.53
rhGH (0.125)	345 $\pm$ 43	206 $\pm$ 65	1.10 $\pm$ 0.05	619 $\pm$ 156	5.26 $\pm$ 0.40
rhGH (0.5)	369 $\pm$ 64	246 $\pm$ 40	1.14 $\pm$ 0.12	615 $\pm$ 132	5.46 $\pm$ 0.82
rhGH (2.0)	395 $\pm$ 64	208 $\pm$ 46	1.12 $\pm$ 0.04	586 $\pm$ 64	6.04 $\pm$ 0.77
rhIGF-I (2.4)	665 $\pm$ 197	255 $\pm$ 51	1.40 $\pm$ 0.12	664 $\pm$ 89	5.34 $\pm$ 0.53
rhGH (0.125) + rhIGF-I	612 $\pm$ 80	280 $\pm$ 46	1.42 $\pm$ 0.05	588 $\pm$ 58	6.18 $\pm$ 0.30
rhGH (0.5) + rhIGF-I	684 $\pm$ 39	292 $\pm$ 79	1.54 $\pm$ 0.05	750 $\pm$ 255	6.13 $\pm$ 0.22
rhGH (2.0) + rhIGF-I	662 $\pm$ 80	278 $\pm$ 67	1.48 $\pm$ 0.07	633 $\pm$ 37	6.66 $\pm$ 0.65

P Values

Effect of rhIGF-I	0.001	0.001	0.001	0.065	0.001
Effect of rhGH	0.379	0.202	0.010	0.343	0.001
Interaction	0.526	0.918	0.715	0.348	0.827

Relative Weights (% Bwt × 10)

Group (Dose mg/kg/day)	Spleen (% Bwt)	Thymus (% Bwt)	Kidneys (% Bwt)	Heart (% Bwt)	Liver (% Bwt)
Excipient	2.3 ± 0.1	1.4 ± 0.4	7.9 ± 0.5	4.0 ± 0.4	37 ± 2
rhGH (0.125)	2.5 ± 0.3	1.5 ± 0.5	8.1 ± 0.6	4.5 ± 1.1	39 ± 2
rhGH (0.5)	2.6 ± 0.2	1.8 ± 0.3	8.1 ± 0.4	4.3 ± 0.6	39 ± 3
rhGH (2.0)	2.7 ± 0.2	1.4 ± 0.3	7.7 ± 0.9	4.0 ± 0.1	41 ± 3
rhIGF-I (2.4)	4.7 ± 1.2	1.8 ± 0.4	10.0 ± 0.9	4.8 ± 0.9	38 ± 1
rhGH (0.125) + rhIGF-I	4.2 ± 0.3	1.9 ± 0.4	9.7 ± 0.7	4.0 ± 0.5	42 ± 2
rhGH (0.5) + rhIGF-I	4.6 ± 0.4	2.0 ± 0.6	10.3 ± 0.8	5.0 ± 1.6	41 ± 3
rhGH (2.0) + rhIGF-I	4.3 ± 0.4	1.8 ± 0.5	9.6 ± 0.9	4.1 ± 0.4	43 ± 3

P Values

Effect of rhIGF-I	0.001	0.005	0.001	0.321	0.003
Effect of rhGH	0.699	0.423	0.375	0.351	0.001
Interaction	0.204	0.893	0.730	0.251	0.713

The upper sections of each Table show means ± standard deviations (n=6/group), the lower sections show the P values for each variable (column), for the two main effects (rhIGF-I or rhGH treatment) and their interaction from the 2 way ANOVA of these data.

Bwt=body weight

Table 3**Study G: Absolute Organ Weights in Hypophysectomized Rats**

Group (Dose mg/kg/day)	Spleen (mg)	Thymus (mg)	Kidneys (mg)	Heart (mg)	Liver (g)
Excipient	206 ± 24	296 ± 61	704 ± 58	384 ± 44	4.42 ± 0.41
rhGH (0.08)	238 ± 25	313 ± 105	777 ± 69	416 ± 34	4.46 ± 0.34
rhGH (0.40)	278 ± 13	409 ± 75	847 ± 57	428 ± 70	4.91 ± 0.24
rhGH (2.0)	320 ± 22	404 ± 64	847 ± 47	414 ± 28	5.28 ± 0.25
rhGH (10.0)	346 ± 66	468 ± 79	913 ± 69	499 ± 70	5.47 ± 0.37
rhGH (50.0)	385 ± 49	541 ± 133	984 ± 40	440 ± 19	5.90 ± 0.52
rhIGF-I (2.4)	441 ± 68	494 ± 67	1029 ± 71	454 ± 49	5.00 ± 0.59
rhGH (0.08) + IGF-I	479 ± 55	617 ± 66	1107 ± 52	474 ± 42	5.30 ± 0.29
rhGH (0.40) + IGF-I	442 ± 35	522 ± 80	1083 ± 40	449 ± 34	5.14 ± 0.67
rhGH (2.0) + IGF-I	480 ± 20	573 ± 73	1201 ± 97	501 ± 104	5.57 ± 0.70
rhGH (10.0) + IGF-I	612 ± 95	677 ± 110	1276 ± 111	582 ± 94	5.91 ± 0.57
rhGH (50.0) + IGF-I	619 ± 128	746 ± 184	1311 ± 114	523 ± 60	6.65 ± 0.85

P Values

Effect of rhIGF-I	0.001	0.001	0.001	0.001	0.001
Effect of rhGH	0.001	0.001	0.001	0.001	0.001
Interaction	0.185	0.325	0.352	0.756	0.649

The upper part of the Table shows the means and standard deviations (n=6/group) for the 12 treatment groups. The lower part of the Table shows the P values for each variable (column), for the two main effects, rhIGF-I or rhGH treatment, and their interaction from the 2 way ANOVA of these data.

Table 4
Study G: Relative Organ Weights in Hypophysectomized Rats

Group (Dose mg/kg/day)	Spleen (% Bwt)	Thymus (% Bwt)	Kidney (% Bwt)	Heart (% Bwt)	Liver (% Bwt)
Excipient	2.1±0.2	3.1±0.6	7.3±0.4	4.0±0.4	46±3
rhGH (0.08)	2.2±0.2	2.9±0.9	7.3±0.5	3.9±0.3	42±2
rhGH (0.40)	2.5±0.1	3.6±0.7	7.5±0.4	3.8±0.7	44±2
rhGH (2.0)	2.7±0.2	3.4±0.5	7.2±0.3	3.5±0.2	45±1
rhGH (10.0)	2.8±0.5	3.8±0.6	7.3±0.5	4.0±0.6	44±2
rhGH (50.0)	3.0±0.3	4.2±1.0	7.7±0.3	3.4±0.2	46±4
rhIGF-I (2.4)	4.0±0.5	4.5±0.7	9.3±0.4	4.1±0.4	45±4
rhGH (0.08) + IGF-I	4.0±0.3	5.1±0.6	9.2±0.5	4.0±0.4	44±3
rhGH (0.40) + IGF-I	3.6±0.3	4.3±0.7	8.8±0.5	3.7±0.3	42±5
rhGH (2.0) + IGF-I	3.8±0.3	4.5±0.5	9.4±0.6	3.9±0.8	44±3
rhGH (10.0) + IGF-I	4.6±0.6	5.1±0.6	9.6±0.6	4.4±0.6	44±4
rhGH (50.0) + IGF-I	4.4±0.7	5.3±1.0	9.5±0.7	3.8±0.5	48±5

P Values

Effect of rhIGF-I	0.001	0.001	0.001	0.098	0.765
Effect of rhGH	0.001	0.015	0.460	0.052	0.031
Interaction	0.064	0.193	0.204	0.677	0.588

The Table shows the relative organ weights, calculated as a percentage of body weight (Bwt) multiplied by 10: (organ weight/body weight) × 1000. The upper part of the Table shows the means and standard deviations (n=6/group) for the 12 treatment groups. The lower part of the Table shows the P values for each variable (column), for the two main effects, rhIGF-I or rhGH treatment, and their interaction from the 2 way ANOVA of these data.

Table 5
Study G: Relative Organ Weights in Hypophysectomized Rats

Group (Dose mg/kg/day)	Glucose (mg/dL)	Calcium (mg/dL)	Creatinine (mg/dL)	Phosphate (mg/dL)	BUN (mg/dL)	Total Protein (g/dL)
Excipient	124±14	10.9±1.5	0.70±0.07	7.5±1.0	35.0±8.6	5.5±0.1
rhGH (0.08)	129±18	10.4±1.2	0.66±0.11	7.7±0.9	32.6±3.4	5.7±0.7
rhGH (0.40)	132±19	10.5±0.6	0.67±0.05	7.6±0.7	27.7±3.1	5.6±0.4
rhGH (2.0)	124±7	11.0±1.0	0.67±0.05	8.5±0.8	29.4±3.4	5.0±0.2
rhGH (10.0)	135±14	11.1±1.0	0.65±0.05	8.9±1.0	25.8±3.6	5.2±0.3
rhGH (50.0)	134±10	11.7±0.8	0.63±0.05	9.2±0.6	22.8±1.7	5.3±0.5
rhIGF-I (2.4)	131±23	10.8±0.7	0.55±0.05	8.9±0.7	15.2±2.2	5.3±0.3
rhGH (0.08) + IGF-I	130±15	12.1±1.9	0.53±0.05	10.7±1.7	16.8±2.9	4.5±0.1
rhGH (0.40) + IGF-I	132±25	10.8±1.0	0.53±0.08	9.9±1.0	15.5±2.9	4.6±0.5
rhGH (2.0) + IGF-I	130±16	10.5±1.0	0.57±0.08	9.0±1.0	12.8±2.6	4.7±1.1
rhGH (10.0) + IGF-I	128±11	10.7±0.6	0.52±0.04	9.6±0.9	13.8±2.3	4.6±0.3
rhGH (50.0) + IGF-I	128±13	10.9±1.2	0.58±0.08	10.1±0.9	14.0±2.3	4.9±0.9

P Values

Effect of IGF-I	0.928	0.992	0.001	0.001	0.001	0.001
Effect of rhGH	0.946	0.669	0.719	0.009	0.001	0.212
Interaction	0.870	0.084	0.524	0.018	0.016	0.184

The upper part of the Table shows means and standard deviations (n=6/group) for the 12 treatment groups. The lower part of the Table shows the P values for each variable (column), for the two main effects, rhIGF-I or rhGH treatment, and their interaction from the 2 way ANOVA of these data.

Table 4

The Effect of Dexamethasone, IGF-I and GH on Rat Organ to Body Weight Ratio ($\times 10^{-3}$)

STUDY A: Seven Days

	Control			Dexamethasone 0.5 mg/L		
	Excipient	IGF-I 0.98 mg/kg	GH 1.2 mg/kg	Excipient	IGF-I 0.98 mg/kg	GH 1.2 mg/kg
Brain/BW	5.93 $\pm$ 0.32 [*]	5.98 $\pm$ 0.74 [*]	6.18 $\pm$ 0.46	6.67 $\pm$ 0.46	6.68 $\pm$ 0.53	6.70 $\pm$ 0.31
Liver/BW	43.1 $\pm$ 3.1	42.0 $\pm$ 2.8	45.5 $\pm$ 2.3	43.7 $\pm$ 4.4	40.1 $\pm$ 3.9	43.8 $\pm$ 6.0
Kidneys /BW	7.79 $\pm$ 1.07	8.30 $\pm$ 0.67	7.71 $\pm$ 0.75	7.81 $\pm$ 0.68	8.29 $\pm$ 0.58	7.64 $\pm$ 0.71
Spleen/BW	2.45 $\pm$ 0.26 ^{a,b}	2.93 $\pm$ 0.51 [*]	2.27 $\pm$ 0.35 ^{c,d}	1.44 $\pm$ 0.16 ^{a,c,e}	2.15 $\pm$ 0.44 ^{e,f}	1.54 $\pm$ 0.29 ^{b,d,f}
Adrenal/BW	0.152 $\pm$ 0.02	0.167 $\pm$ 0.022 ^{a,b}	0.155 $\pm$ 0.024	0.111 $\pm$ 0.024 ^a	0.148 $\pm$ 0.040	0.119 $\pm$ 0.019 ^b
Heart/BW	3.27 $\pm$ 0.20 ^a	3.23 $\pm$ 0.29 ^{b,c}	3.18 $\pm$ 0.31 ^d	3.41 $\pm$ 0.28	3.56 $\pm$ 0.30 ^b	3.65 $\pm$ 0.41 ^{a,c,d}
Thymus/BW	1.91 $\pm$ 0.36 [*]	1.84 $\pm$ 0.30 [*]	2.13 $\pm$ 0.38 [*]	1.06 $\pm$ 0.14	0.99 $\pm$ 0.16	0.94 $\pm$ 0.12

STUDY B: 14 Days

	Control	Dexamethasone 1.0 mg/L				
	Excipient	Excipient	IGF-I 0.8 mg/kg	IGF-I 2.4 mg/kg	GH 0.8 mg/kg	GH 2.4 mg/kg
Liver/BW	41.3 $\pm$ 1.1 ^a	43.0 $\pm$ 4.6	43.8 $\pm$ 3.4	40.9 $\pm$ 5.2 ^b	39.8 $\pm$ 4.1 ^c	47.6 $\pm$ 2.9 ^{a,b,c}
Kidney/BW	3.85 $\pm$ 0.28 [*]	4.46 $\pm$ 0.51 ^{a,b}	5.16 $\pm$ 0.23 ^{a,c}	5.09 $\pm$ 0.38 ^{b,e,f}	4.48 $\pm$ 0.29 ^{c,e}	4.59 $\pm$ 0.20 ^{d,f}
Spleen/BW	2.70 $\pm$ 0.24 ^{a,b,c,d}	1.66 $\pm$ 0.28 ^{a,e}	2.06 $\pm$ 0.32 ^{ab}	2.50 $\pm$ 0.35 ^{e,f,g}	1.63 $\pm$ 0.34 ^{c,f}	1.94 $\pm$ 0.63 ^{d,f,g}
Heart/BW	3.11 $\pm$ 0.16 [*]	3.73 $\pm$ 0.32	3.54 $\pm$ 0.27 ^a	3.53 $\pm$ 0.27 ^b	3.82 $\pm$ 0.14	3.99 $\pm$ 0.19 ^{a,b}
Thymus/BW	1.78 $\pm$ 0.32	ND	ND	ND	ND	ND

Statistics: All differences are at $p < 0.05$ by an analysis of variance.

a identical letters in a row indicate two groups differ

different from all other groups

* all Dex values differ from control

ND not detectable

Mean $\pm$ SD ($n = 8$ for Study A & 6 for Study B)

Table 5

The Effect of Dexamethasone, IGF-I and GH on Serum Glucose, Protein and Triglycerides

STUDY A: Seven Days

	Control			Dexamethasone		
	Excipient	IGF-I 0.98 mg/kg	GH 1.2 mg/kg	Excipient	IGF-I 0.98 mg/kg	GH 1.2 mg/kg
Total IGF-I (ng/ml)	730 ± 82 ^{a,b}	837 ± 118 ^c	541 ± 89 ^{a,c,e,f}	700 ± 106 ^{d,e}	875 ± 139 ^{b,f,g}	412 ± 123 [#]
Insulin (ng/ml)	2.32 ± 1.64 [*]	1.89 ± 1.50 [*]	2.61 ± 1.36 [*]	7.77 ± 0.60 ^a	4.27 ± 2.46 ^{a,b}	7.32 ± 1.19 ^b
Glucose (mg/dL)	155 ± 43	152 ± 16	143 ± 21	183 ± 27	159 ± 26	164 ± 25
Total Protein (gm/dL)	6.74 ± 0.52 ^{a,c}	6.82 ± 0.98 ^{b,d}	7.59 ± 0.56 ^{a,b}	7.55 ± 0.29 ^{c,d}	7.03 ± 0.41	9.46 ± 0.68 [#]
Triglycerides (mg/dL)	125 ± 34	110 ± 35 ^{a,b}	163 ± 45 ^a	166 ± 49 ^b	133 ± 30	213 ± 57 [#]

STUDY B: 14 Days

	Control	Dexamethasone 1.0 mg/L				
	Excipient	Excipient	IGF-I 0.8 mg/kg	IGF-I 2.4 mg/kg	GH 0.8 mg/kg	GH 2.4 mg/kg
Total IGF-I (ng/ml)	569 ± 131 [°]	341 ± 96	493 ± 83 [°]	552 ± 224 [°]	302 ± 21	290 ± 45
Total Protein (gm/dL)	7.43 ± 1.01 ^{a,b}	8.65 ± 0.88	8.37 ± 1.19 ^c	8.32 ± 1.34 ^d	10.1 ± 2.7 ^a	11.1 ± 1.7 ^{b,c,d}
Triglycerides (mg/dL)	173 ± 56 ^a	219 ± 67 ^b	224 ± 103	161 ± 71 ^c	505 ± 209 [#]	401 ± 127 ^{a,b,c}

Statistics: All differences are at p < 0.05 by an analysis of variance.

- a identical letters in a row indicate two groups differ
- ° different from Dex-Excipient and both GH groups
- # different from all other groups
- * all Dex values differ from control

Mean ± SD (n = 8 for Study A & 6 for Study B)

Immune function: Immunologic effects were evaluated in retired male Balb/c breeders following a continuous SC infusion of mecasermin at doses of 7.5, 30 or 120 µg/day (approximately 0.25, 1.0, or 4.0 mg/kg/day) for 7 days using osmotic minipumps or at a dose of 120 µg/day (4.0 mg/kg/day) for 14 days. After 7 days of mecasermin treatment at 4.0 mg/kg/day, viable spleen cell number were increased 2.5-fold. This increase was due to both B and T cells. When B and T cell numbers were quantitated by [] and Thy1+ cells, B cell numbers increased threefold relative to excipient, while T cell numbers increased only slightly. In the thymus, Thy1+ thymocytes increased 2.4-fold relative to excipient, suggesting that mecasermin has a mitogenic effect on lymphocyte subpopulations.

In contrast to this increase in lymphocyte number, the in vitro responses from mecasermin treated mice to stimulation by LPS (B cells) and Con A (T cells) were decreased and responses to Pokeweed mitogen were unchanged compared to excipient treated mice. These depressed mitogen responses suggest a functional immaturity in the expanded lymphocyte populations following 7 days of mecasermin treatment. After 14 days of mecasermin treatment at 4.0 mg/kg/day, thymocytes increased significantly in the thymus and viable spleen cell number increased 2-fold relative to excipient. There was an increase in both the T cell and B cell numbers in the spleen. In contrast to the decreased mitogenic response seen after 7 days of mecasermin treatment, the mitogenic response of splenocytes was significantly elevated compared to excipient. Seven days post administration of 4.0 mg/kg mecasermin (day 21) total splenocytes, B cell numbers, T cells (CD4+ and CD8+) and peripheral lymph node cell numbers were elevated compared to excipient controls. By 21 days post-treatment (Day 35) both mecasermin and excipient treated mice had equivalent splenocyte and thymocyte numbers. Responses to mitogens were still elevated in the mecasermin treated group.

Lymphocyte recruitment and maturation effects were studied in bone marrow reconstituted young Balb/c male mice (19 to 26 g, 6 to 7 weeks old) following continuous SC infusion of 1.0 or 4.0 mg/kg/day of mecasermin for 14 or 21 days with an implanted minipump. The animals were irradiated 24 hours after pumps were implanted and then received injections with syngenic bone marrow cells approximately 25 hours after the beginning of mecasermin administration. The animals were divided in 2 groups and sacrificed at either 14 or 21 days following bone marrow reconstitution. At 14 days post irradiation, animals receiving 4.0 mg/kg/day mecasermin had increased numbers of CD4+ T cells in the peripheral blood compared to excipient controls. The CD4 +/CD8+ ratio increased from 2 to 4 compared to controls. No increase was seen in peripheral B cell number. Splenic T cells, both CD4+ and CD 8+, were significantly increased in mice receiving 4.0 mg/kg mecasermin compared to excipient. No increase was seen in mitogenic responsiveness of these splenocytes relative to excipient. At 21 days post irradiation, mecasermin treatment increased the peripheral blood CD4+ T cell population and increased the CD4+/CD8+ ratio. The total splenocyte numbers in the mecasermin treated animals returned to normal values. ConA stimulation of splenic T cells tripled in the animals treated with 4.0 mg/kg/day mecasermin. B cell responses to LPS were unaffected by mecasermin treatment. Thymic lymphocyte numbers were increased relative to excipient in the 1.0 and 4.0 mg/kg/day mecasermin treated groups.

To evaluate the effect of mecasermin on antibody response following antigen challenge, (C57BL/6XDBA/2)F1(BDF1) mice received a single bolus dose of 1 µg dinitrophenyl ovalbumin adsorbed to 1 mg of aluminum hydroxide gel (alum). Twenty-one days post immunization, mice were given a continuous infusion of excipient or 120 µg/day mecasermin (about 4 mg/kg/day) for 14 days. The mice were also given a booster injection of 1 µg dinitrophenyl ovalbumin adsorbed alum. Serum was collected weekly for 13 weeks for IgG determinations. Mecasermin treatment significantly increased the secondary IgG response to antigen at all sampling times.

In aged Fisher rats (18 months) used in a rodent model of aging to determine the effects of mecasermin on the cells and tissues of the immune system, a continuous SC infusions using

osmotic minipumps of mecasermin at 2.4 mg/kg/day for 14 days caused a 5-fold increase in the number of thymic cells compared to excipient. The number of cells in the spleen was not significantly increased. Thymic cells from mecasermin treated animals showed an increased response to mitogens (PHA and IL-1) in vitro.

2.6.2.4 Safety pharmacology

Formal safety pharmacology studies were not performed regarding possible activity of mecasermin or formulation components on known pharmacologic receptors (e.g., beta adrenergic, serotonin). In addition, no formal in vivo safety pharmacology studies were performed. Specific evaluations of cardiovascular and respiratory parameters (electrocardiography, blood pressure, respiration rate) were performed in Beagle dogs. The summary of this data is provided in the toxicology section of this review.

2.6.2.5 Pharmacodynamic drug interactions

Pharmacodynamic drug interaction studies were performed with mecasermin and rhGH in mice, and normal, aged, hypophysectomized, and dwarf rats. Effects on bone growth, changes in blood glucose, changes in body weight, and immune function were evaluated when these two hormones were co-administered. In studies investigating the use of mecasermin to treat AIDS related wasting, pharmacodynamic drug interactions were studied in mice treated with mecasermin following pretreatment with AZT for 10 days. The pharmacodynamic drug interaction studies addressed multiple endpoints, including primary and secondary pharmacodynamic endpoints. The pharmacodynamic drug interaction studies were not conducted under GLP conditions.

The effect of co-treatment with mecasermin and recombinant rhGH on bone growth was studied in female hypophysectomized rats. Mecasermin was administered at a dose of approximately 1.2 mg/kg/day for 7 days by continuous SC infusion using osmotic minipumps (n = 7 to 9 rats/group). The rhGH was delivered by a daily SC bolus injection of 0.06 mg/kg/day. The mean tibia epiphyseal plate width increased from $199 \pm 15 \mu\text{m}$ in the excipient group to $275 \pm 13 \mu\text{m}$ in the mecasermin treated animals and to 345 ± 33 in the mecasermin plus rhGH treated animals. The bone growth increase in the mecasermin treated animals was significantly greater than excipient ($P < 0.001$) and the combination of mecasermin and rhGH resulted in an increase of bone growth greater than either hormone given alone ($P < 0.05$). The longitudinal tibia growth increased from $20 \pm 4 \mu\text{m/day}$ in the excipient group to $44 \pm 11 \mu\text{m}$ in the mecasermin treated animals and to $70 \pm 7 \mu\text{m}$ in the animals treated with the combination of mecasermin and rhGH. The longitudinal bone growth increase in the mecasermin treated animals was significantly greater than excipient ($P < 0.01$) and the combination of mecasermin and rhGH resulted in an increase of bone growth greater than either hormone given alone ($P < 0.05$).

In the study of the effect of mecasermin and rhGH co-administration on bone growth in GH-deficient mutant female dwarf rats (100 to 140 g). Mecasermin was administered at a dose of approximately 2.4 mg/kg/day for 7 days by continuous SC infusion using osmotic minipumps (n= 6 rats/group). The rhGH was given as daily SC injections of 0.125, 0.5 or 2.0 mg/kg/day. The mean tibia epiphyseal plate width increased from $193 \pm 26 \mu\text{m}$ in the control group to $223 \pm 21 \mu\text{m}$ ($P < 0.001$) in the mecasermin treated animals and to $257 \pm 15 \mu\text{m}$, $261 \pm 31 \mu\text{m}$, and

256 ± 12 µm in the animals treated with mecasermin plus 0.125, 0.5, and 2.0 mg/kg/day rhGH, respectively ($P < 0.001$ for all rhGH doses). The longitudinal bone growth increased from 31 ± 16 µm/day in the control group to 46 ± 11 µm/day ($P < 0.003$) in the mecasermin treated animals and to 63 ± 17 µm/day, 61 ± 23 µm/day, and 66 ± 15 µm/day in the animals treated with mecasermin plus 0.125, 0.5, and 2.0 mg/kg/day rhGH, respectively ($P < 0.014$ for all rhGH doses).

In study of the effect of co-administration of mecasermin and rhGH on hypoglycemia, Hypophysectomized rats (80 to 105 g) received continuous SC infusions using osmotic minipumps of mecasermin at a dose of 2.4 mg/kg/day ($n = 6$ rats/group) for 7 days alone or co-administered with 0.08, 0.40, 2.0, 10.0, or 50.0 mg/kg/day of rhGH given as daily SC injections. Rats in all treatment groups were euglycemic at sacrifice. In male Fischer rats 18 month of age, a continuous SC infusions using osmotic minipumps of mecasermin at 2.4 mg/kg/day for 14 days ($n = 7$) alone or mecasermin plus daily injections of 2.2 mg/kg/day rhGH did not cause a significant difference from excipient treated animals at sacrifice.

In the study of the effect of co-administration of mecasermin and rhGH on weight gain, Administration of mecasermin induced and maintained an increase in body weight in female hypophysectomized rats that was further enhanced when mecasermin and rhGH were given together. Mecasermin was administered as a continuous SC infusion at 1.2 mg/kg/day to female hypophysectomized rats (85 to 105 g, $n = 7$ to 9 rats /group) for 7 days using osmotic minipumps. The rhGH was given as daily SC injections of 0.06 mg/kg/day. Mecasermin increased the body weights 16.6 ± 2.1 g ($P < 0.001$) and the combination of mecasermin and rhGH increased body weights 22.2 ± 2.7 g ($P < 0.05$, different than either hormone alone) compared to an increase of 2.9 ± 3.5 g in the excipient treated controls. In two animal models of growth retardation due to GH deficiency including GH-deficient mutant female dwarf rats (100 to 140 g) and female hypophysectomized rats (85 to 105 g), mecasermin given alone or in combination with rhGH, produced significant increases in body weight gain compared to excipient. A 2.4 mg/kg/day dose of mecasermin administrated for 7 days by continuous SC infusion using osmotic mini pumps ($n = 6$ rats/group) with rhGH as single daily SC injections at doses of 0.125, 0.5, and 2.0 mg/kg/day to dwarf rats. The body weight of the dwarf rats increased 3.9 ± 3.6 g in the excipient group compared to 12.1 ± 3.8 g ($P < 0.001$) in the mecasermin alone treated group and 21.4 ± 2.9 g ($P < 0.001$), 24.6 ± 5.6 g ($P < 0.001$), 28.4 ± 6.0 g ($P < 0.001$) for the co-administered doses of 0.125, 0.5, and 2.0 mg/kg/day rhGH, respectively. In dwarf rats the combination of mecasermin plus rhGH yielded greater body weight gains than either hormone alone and appeared to be additive over a broad range of doses of rhGH. In hypophysectomized rats, 2.4 mg/kg/day dose of mecasermin administrated for 7 days by continuous SC infusion using osmotic mini pumps ($n = 6$ rats/group) with rhGH as single daily SC injections at doses of 0.08, 0.4, 2.0, 10.0, and 50.0 mg/kg/day. The body weight of the hypophysectomized rats increased 4.5 ± 1.7 g in the excipient group compared to 18.2 ± 2.0 g in the mecasermin alone treated group and mean weight gains of 28.2, 30.7, 34.8, 41.6 and 46.3 g for the co-administered doses of 0.08, 0.4, 2.0, 10.0, and 50.0 mg/kg/day rhGH, respectively. In hypophysectomized rats the combination of mecasermin plus rhGH yielded greater body weight gains than either hormone alone and appeared to be additive over a broad range of doses of rhGH.

In an aged rodent growth model, a greater increase in body weight gain was seen at sacrifice following the co-administration of mecasermin and rhGH compared to animals receiving mecasermin alone. Mecasermin was administered to 18 month old male Fischer rats (average initial weight 450 g) as a continuous SC infusion using osmotic minipumps, delivered continuously for 14 days at 2.4 mg/kg/day (n = 7 animals). The rhGH was given as daily SC injections of 2.2 mg/kg/day. Co-administration of mecasermin and rhGH resulted in mean body weight increases of 45.5 ± 9.9 g ($P < 0.05$ different from all groups), 34.5 ± 9.4 g ($P < 0.001$ compared to excipient) for mecasermin alone and 0.3 ± 9.4 g for excipient treated animals.

Immunologic effects of co-administration of mecasermin and rhGH were evaluated in retired male Balb/c breeders (25 to 35 g) following a continuous SC infusion of mecasermin or a combination of an infusion of mecasermin and daily SC injections of rhGH for 14 days. After 14 days of mecasermin treatment at 4.0 mg/kg/day, thymocytes increased significantly in the thymus and viable spleen cell number increased 2-fold relative to excipient. There was an increase in both the T cell and B cell numbers in the spleen. The mitogenic response of splenocytes in mecasermin treated animals was significantly elevated compared to excipient. Whether alone, or in combination with rhGH, mecasermin treatment increased the total lymphocyte number in the spleen, largely due to an increase in B-cell number. The combination of mecasermin and rhGH had a pronounced effect on thymocyte number, exceeding the effects of mecasermin or rhGH given separately. Seven days post-administration (Day 21) the mecasermin or the mecasermin plus rhGH treated mice still had significantly elevated splenocytes and B-cell numbers. An increase in T-cell number was significant in the mecasermin treated group but not in the mecasermin plus rhGH group. Both CD4+ and CD8+ T-cell populations were elevated in the group receiving mecasermin alone compared to excipient controls. In addition, mecasermin alone or in combination with rhGH produced an increase in peripheral lymph node cell numbers. By 21 days post treatment (Day 35) all groups of mice had equivalent splenocyte and thymocyte numbers. Responses to Con A and Pokeweed mitogen were still elevated in the mecasermin treated group, relative to excipient controls, and responses to Con A and LPS were elevated in the mecasermin plus rhGH treated group.

In the study of the interaction of mecasermin with AZT, male BALB/c mice, retired breeders between 27 and 33 grams and 9 to 10 months of age, were pretreated with approximately 40, 100 and 130 mg/kg/day (low, moderate and high doses) of Zidovudine (AZT; Retrovir; 3'-Azido-3'-deoxythymidine) administered in drinking water for 10 days. After 10 days of AZT treatment the mice were divided into two weight balanced groups and implanted with osmotic minipumps that administered excipient or approximately 3.5 mg/kg/day of mecasermin) for 14 days. The red blood cell count, total hematocrit and hemoglobin concentration were decreased following high dose AZT treatment. In all cases this effect was reversed to normal values by mecasermin treatment. High dose AZT caused a decrease in white blood cell count that was not reversed by mecasermin treatment. Moderate and high dose AZT treatment also produced an increase in platelet counts which was significantly reduced by administration of mecasermin in the high AZT dose group only. During the post-implant period, animals receiving high, moderate, or low dose AZT lost 0.6 ± 0.9 g, 1.1 ± 0.6 g, 1.5 ± 0.8 g of body weight, respectively. Treatment with mecasermin prevented this weight loss. Animals

receiving AZT plus mecasermin showed significant stimulation of body weight gain in all groups compared to their respective AZT-only group or excipient controls. High dose AZT resulted in a decrease of the splenic CD4+ T-cell population. The decrease in CD4+ cells was reversed by mecasermin treatment.

2.6.3 PHARMACOLOGY TABULATED SUMMARY

(pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

Tabulated summary

Test Article:	mecasermin	
Type of Study	Test System	Method of administration
Primary Pharmacodynamics^a		
Bone growth ^b	Hypophysectomized rats	SC infusion
Bone growth ^c	Hypophysectomized rats	SC infusion
Bone growth ^d	Dwarf rats	SC infusion
Bone growth ^e	Ovariectomized Sprague Dawley rats	SC infusion
Bone growth ^e	Ovariectomized Sprague Dawley rats	SC infusion
Secondary Pharmacodynamics^a		
Hypoglycemia ^g	Sprague Dawley rats	SC bolus
Hypoglycemia	Sprague Dawley rats	SC and IV bolus
Hypoglycemia	Sprague Dawley rats	SC and IV bolus
Hypoglycemia	Sprague Dawley rats	SC and IV bolus
Hypoglycemia ^h	Aged Fischer rats	SC infusion
Hypoglycemia ⁱ	Sprague Dawley rats	SC infusion
Hypoglycemia	New Zealand rabbits	SC and IV bolus
Hypoglycemia	Rhesus monkey	SC bolus
Weight gain ⁱ	Balb/c mice	SC infusion
Weight gain	Dwarf dw/dw rats	SC, IV infusion
Safety Pharmacology^j	NA	NA
Pharmacodynamic Drug Interactions^a		
Interaction with AZT	Balb/c mice, murine lymphocytes	SC infusion

^a Studies listed according to main pharmacology endpoint. If additional information was included in the secondary pharmacology or drug interaction sections, a footnote will be added to the study in this overview table that describes where the additional information can be found. ^b Also contains additional information on secondary pharmacology endpoints hypoglycemia and weight gain. ^c Also contains additional information on secondary pharmacology endpoints weight gain and drug interaction information with rhGH on bone growth and weight gain. ^d Also contains additional information on secondary pharmacology endpoints hypoglycemia and weight gain in hypophysectomized rats and drug interaction information with rhGH on bone growth, hypoglycemia, and weight gain. ^e Also contains additional information on secondary pharmacology endpoint of weight gain. ^f Also contains additional information on secondary pharmacology endpoint of weight gain and drug interactions with co-administration of rhGH on weight gain. ^g Also contains additional information on drug interactions with co-administration of rhGH on hypoglycemia. ^h Also contains additional information on secondary pharmacology endpoint of weight gain and immune function and drug interactions with co-administration of rhGH on hypoglycemia and weight gain. ⁱ Also contains additional information on secondary pharmacology endpoint of immune function in C57 black mice and murine lymphocytes and drug interactions with co-administration of rhGH on immune function. ^j No formal safety pharmacology studies were conducted, specific evaluations of cardiovascular and respiratory parameters (electrocardiography, blood pressure, respiration rate) were performed as part of the pivotal GLP studies in Beagle dogs and are listed in the toxicology section of this document.

In summary and conclusion: rhGH and mecasermin have most similar actions on the bone growth, weight gain and immune function. However, a major difference between mecasermin and rhGH is that mecasermin (like insulin) has hypoglycemic activity whereas rhGH opposes insulin action and is diabetogenic. The principal findings following the SC administration of mecasermin in the nonclinical pharmacodynamic studies were increases in bone growth in GH and IGF-1 deficient animal models, acute decreases in blood glucose, increases in body weight, and enhancement of immune function. The relevance of the nonclinical pharmacodynamic studies to human therapeutic use of mecasermin is supported by the demonstration of dose related pharmacological activity in all species tested and the apparent lack of species specificity. The data from these nonclinical pharmacodynamic studies support the use of mecasermin for long-term treatment of growth failure in children with primary IGFD.

2.6.4 PHARMACOKINETICS/TOXICOKINETICS

2.6.4.1 Brief summary

The ADME/toxicokinetic studies were conducted in adult normal Sprague Dawley rats, New Zealand White rabbits (males), Beagle dogs (males and females), and rhesus monkeys (males). The SC and IV routes of administration were investigated. The mecasermin used in the nonclinical and clinical studies was derived from — manufacturing processes: ㄣ

Appropriate physical-chemical and in vitro and in vivo nonclinical and/or clinical studies were conducted to demonstrate that the material made using each new process was comparable to the material made using the previous process.

In the intravenous studies, the mean total IGF-1 AUC overall increased with less than dose proportionally in SD rats. Total IGF-1 was relatively rapidly eliminated after IV administration, with a mean elimination $t_{1/2}$ of 43 to 96 minutes in the majority of cases and a mean clearance of 3.7 to 12.3 mL/min/kg in the majority of the cases. The V_1 and V_{ss} were 19 to 165 and 50 to 825 mL/kg, respectively. Free IGF-1 showed a mean $t_{1/2}$ of 20 minutes and a mean clearance of 15.1 mL/min/kg. The V_1 and V_{ss} were 149 and 203 mL/kg, respectively. The half-life and clearance did not appear to change consistently with increasing dose. There were no substantial, consistent differences in pharmacokinetic parameters between male and female animals. In the subcutaneous studies, T_{max} of mecasermin was 15 to 180 minutes in SD rats, 50 minutes in New Zealand White Rabbits, 48 to 198 minutes in beagle dogs, and 103 to 238 minutes in male Rhesus monkeys. The mean bioavailability was 38 to 57% in SD rats, and 47% in New Zealand White Rabbits. In SD rat studies, the total mean C_{max} and AUC increased with increasing dose, less than dose proportionally. The mean free IGF-1 C_{max} and AUC increased with increasing dose, greater than or equal to dose proportionally. The mean total IGF-1 $t_{1/2}$ overall appeared to decrease with increasing dose (155 to 604 minutes at 0.25 mg/kg/day and 41 to 573 minutes at 10.0 mg/kg/day in the majority of cases). The mean free IGF-1 $t_{1/2}$ overall did not show consistent changes with increasing dose (23 to 140 minutes). In the majority of cases male rats showed higher mean total and free IGF-1 C_{max} and AUC than female rats. There were no consistent gender-related differences in total or free IGF-1 $t_{1/2}$ values. Total and free IGF-1 predose concentrations showed minimal

changes over time, suggesting accumulation did not occur on multiple dosing. The mean total and free IGF-1 $t_{1/2}$ values increased on multiple dosing. Male and female SD rats in the pivotal long term toxicology studies were adequately exposed to total and free IGF-1 plasma concentrations and demonstrated pharmacological and/or toxicological responses to mecasermin over the entire duration of the studies. In Beagle dog studies with SC administration for up to 26 weeks, overall the mean total IGF-1 C_{max} and AUC increased with increasing dose, less than dose proportionally. The mean free IGF-1 C_{max} and AUC increased with increasing dose greater than or equal to dose proportionally. The mean total and free IGF-1 $t_{1/2}$ values did not change consistently with increasing dose (198 to 558 and 48 to 78 minutes, respectively). There were no substantial, consistent, male/female differences in pharmacokinetic parameters for total or free IGF-1. Mean total and free IGF-1 C_{max} and AUC values generally did not change consistently or substantially on multiple dosing. The 24-hour postdose concentrations of total and free IGF-1 were approximately equivalent to the predose concentrations, suggesting accumulation did not occur on multiple dosing. The mean total IGF-1 $t_{1/2}$ showed slight decreases over the time course of the study in the majority of cases. The mean free IGF-1 $t_{1/2}$ values generally showed no consistent changes over the time course of the study. No antibodies to IGF-1 were detectable in rats and dogs. In the male Rhesus monkey study with single dose SC administration (0.5mg/kg), the mean total IGF-1 C_{max} and AUC(0-16hr) values were 1.71 to 2.08 $\mu\text{g/mL}$ and 1039 to 1139 $\mu\text{g}\cdot\text{min/mL}$, respectively, and the mean free IGF-1 C_{max} and AUC(0-16hr) values were 0.513 to 0.692 $\mu\text{g/mL}$ and 62.4 to 82.1 $\mu\text{g}\cdot\text{min/mL}$, respectively.

In the tissue distribution studies, after single dose IV administration of ^{125}I - rhIGF-1 to male Sprague Dawley rats, radioactivity was distributed primarily to highly perfused organs, and/or possible organs of elimination/action (kidney and liver) for up to 200 minutes postdose. Organ to blood radioactivity ratios were greater than one only for the kidney. At 400 minutes postdose, the reproductive tract, liver and kidney showed the highest remaining percentages of the radioactive dose (1.4 to 2.8%). The brain showed negligible accumulation of radioactivity. In the protein binding assay, after IV administration of tracer doses of ^{125}I -mecasermin to male and female rats, four peaks of radioactivity were observed in plasma; these are believed to correspond to ~150 to 160 kDa and ~20 to 30 kDa binding protein complexes, free ^{125}I -mecasermin, and free ^{125}I . Unbound ^{125}I -mecasermin accounted for only a small percentage of the plasma radioactivity. Clearance of radioactivity in the ~150 to 160 kDa complex was 0.5 to 10 mL/min/kg, radioactivity in the ~20 to 30 kDa complex was intermediate in clearance (15 to 48 mL/min/kg), and free radiolabeled mecasermin was cleared most rapidly (168 to 204 mL/min/kg). In the toxicokinetic support studies, the percent of total IGF-1 that was free IGF-1 increased with increasing dose of mecasermin in rats (3.1 to 10% at 0.1 mg/kg/day vs. 54 to 73% at 10.0 mg/kg/day) and Beagle dogs (7.1 to 22% at 0.15 mg/kg/day vs. 37 to 44% at 0.6 mg/kg/day). In the metabolism study, after IV administration of ^{125}I - mecasermin in male and female rats, 80% of the plasma radioactivity was TCA precipitable for up to 210 minutes postdose. No in-house studies of the excretion of mecasermin were conducted.

2.6.4.2 Methods of Analysis

The bioanalytical method used to determine the free and total IGF-1 in the nonclinical studies was a radioimmunoassay (RIA). The RIA was used for detection of both endogenous IGF-1 and exogenous rhIGF-1. Prior to RIA, two different methods were used for sample

preparation including [to determine total IGF-1 or [to determine free IGF-1. The potential for formation of antibodies to IGF-1 was investigated in many of the toxicology studies using enzyme-linked immunosorbent assay methodology (ELISA). Mecasermin was radiolabeled with ¹²⁵I for nonclinical studies conducted in Sprague Dawley rats to characterize the tissue distribution, protein binding, and metabolism of mecasermin.

2.6.4.3 Absorption

In rat studies, three single dose pharmacokinetic studies were conducted in male Sprague Dawley rats after SC administration of mecasermin over the dose range of 0.1 to 10.0 mg/kg and six single dose pharmacokinetic studies were conducted in male and female Sprague Dawley rats after IV administration of mecasermin. Absorption was generally rapid after SC administration. The mean T_{max} for total IGF-1 occurred between 15 to 180 minutes post dose. Overall, the mean total IGF-1 C_{max} and AUC increased with increasing dose, but less than dose proportionally. The mean T_{max} for free IGF-1 occurred between 15 to 60 minutes. The mean free IGF-1 C_{max} and AUC overall increased with increasing dose approximately dose proportionally. In the single IV administration, total IGF-1 was rapidly eliminated after IV administration of mecasermin, with a mean elimination half-life of 42.9 to 96 minutes in the majority of cases (5.2 to 402 min at the extremes). The half-life did not appear to change consistently with increasing dose. Mean clearance was 3.7 to 12.3 mL/min/kg and did not appear to change systematically with increasing dose. The mean V_{ss} was 50 to 825 mL/kg, consistent with distribution in the extracellular fluid to whole body fluid compartments. The mean elimination half-life for free IGF-1 was 20 minutes and the mean clearance was 15.1 mL/min/kg. The mean volume of the central compartment was 149 mL/kg, consistent with distribution primarily in the vascular to extracellular fluid compartments. The mean volume of distribution at steady state was 203 mL/kg, consistent with distribution in the extracellular fluid to whole body fluid compartments. Data were summarized in the following tables.

Mean Total IGF-1 Pharmacokinetic Parameters Following Single SC Doses of Mecasermin in Male Sprague Dawley Rats ([]

Study	93-240-0320PK ^a			94-103-0320 ^b	93-240-0320PK ^a
Dose (mg/kg)	0.1	0.3	1.0	3.0	10.0
C _{max} (µg/mL)	0.496	0.837	0.865	2.208	6.132
T _{max} (min)	180	15	15	20	20
AUC (µg·min/mL)	198	311	301	419	1070
F (%)	NC	NC	NC	57	NC

NC = not calculated; Data were not corrected for endogenous IGF-1

a: AUC(2-480min); n = 3/dose group

b: AUC(0-360min); F based on AUC(0-180min); n = 4

Mean Total IGF-1 Pharmacokinetic Parameters Following Single SC Doses of Mecasermin in Male Sprague Dawley Rats ([7])

Study	93-240-0320PK _a	90-168-0320 _b	94-103-0320 _c
Dose (mg/kg)	1.0	2.0	3.0
C _{max} (µg/mL)	0.888	0.959	0.975
T _{max} (min)	120	22.5 _d	30
AUC (µg·min/mL)	312	NC	263
F (%)	NC	NC	38

NC = not calculated

Data were not corrected for endogenous IGF-1

_a: AUC(2-480min); n = 3

_b: AUC(0-480min); n = 4

_c: AUC(0-360min); F based on AUC(0-180min); n = 4

_d: Median

Mean Free IGF-1 Pharmacokinetic Parameters Following Single SC Doses of Mecasermin in Male Sprague Dawley Rats ([7])

Study	93-240-0320PK		
Dose (mg/kg)	0.1	0.3	1.0
C _{max} (µg/mL)	0.048	0.116	0.329
T _{max} (min)	30	60	15
AUC (µg·min/mL)	7.69	16.3	33.0

NC = not calculated

Data were not corrected for endogenous IGF-1

AUC(2-480min); n = 3 per dose group

Mean Free IGF-1 Pharmacokinetic Parameters Following Single SC Doses of Mecasermin in Male Sprague Dawley Rats ([7])

Study	93-240-0320PK _a	90-168-0320 _b
Dose (mg/kg)	1.0	2.0
C _{max} (µg/mL)	0.264	0.375
T _{max} (min)	30	22.5 _c
AUC (µg·min/mL)	28.6	63
F (%)	NC	42

Data were not corrected for endogenous IGF-1

_a: AUC(2-480min); n = 3

_b: AUC(0-480min); n = 4

_c: Median

Mean Total IGF-1 Pharmacokinetic Parameters Following Single IV Doses of Mecasermin in Male and Female Sprague Dawley Rats

Study	89-004-0320-569	93-240-0320PK	94-103-0320		90-168-0320	89-193-0320-569		
Process	L		J					
Gender	M	M	M	M	M	F	M	M
Dose (mg/kg)	0.719	1.0	1.0	1.0	1.0	1.0	1.0	10.0
C(0) (µg/mL)	19.4-37.4	16.182	7.227	7.703	7.112 _f	6.06-19.6 _g	13.1-28.4 _g	179 _g
AUC (µg·min/mL)	72.3-106.1	NR	139.7	153	NC	191-232	132-271	1060
CL(mL/min/kg)	6.8-9.9	4.5	12.3	11.1	NC	4.3-5.2	3.7-7.6	9.4
t1/2(min)	5.2-6.5	96	47.3	69.0	NC	42.9-64.7	7.2-402	68.1
V1 (mL/kg)	19-37	62	132	137	NC	51.0-165	35.2-76.3	55.9
Vss (mL/kg)	50-63	510	604	825	NC	312-516	161-507	295

F = female, M = male, NC = not calculated, NR = not reported

Data corrected for endogenous IGF-1 except 90-168-0320 and for AUC in study 94-103-0320

Mean Free IGF-1 Pharmacokinetic Parameters Following Single IV Doses of Mecasermin in Male Sprague Dawley Rats (I J

Study	90-168-0320
Dose (mg/kg)	1.0
C(0) (µg/mL)	17.384
AUC(0-∞) (µg·min/mL)	75
CL(mL/min/kg)	15.1
t _{1/2} (min)	20
V ₁ (mL/kg)	149
V _{ss} (mL/kg)	203

In single dose pharmacokinetic study in male New Zealand White rabbits after SC administration of mecasermin at 1.0 mg/kg, the mean C_{max} was 0.8116 µg/mL, the median T_{max} was 50 minutes and the mean AUC was 484.0 µg·min/mL (time period not specified). The mean SC bioavailability was 47.39%. Mecasermin was rapidly absorbed after SC administration. In single dose pharmacokinetic study in male New Zealand White rabbits after IV administration of mecasermin at 0.5 mg/kg, the pharmacokinetic parameters for the normal diet vs. the liquid diet were: mean C(1 min) = 8.00954 vs. 7.20505 µg/mL and mean AUC = 515.0 vs. 289.2 µg·min/mL (time period not specified). Animals fed a normal diet showed higher mean total IGF-1 C(1 min) and AUC than those fed a liquid diet (approximately 1- to 2-fold higher). Data were shown in the following table.

Species/Strain	New Zealand White Rabbit		
Gender/No.	Male/4	Male/2	Male/2
Feeding Condition	Fed normal diet	Fed normal diet	Fed liquid diet
Vehicle/Formulation; Lot/Process	[]		
Method of Administration	SC	IV	IV
Dose (mg/kg)	1.0	0.5	0.5
Sample	Plasma		
Analyte	Total IGF-1		
Assay	RIA []	] sample preparation for total IGF-1	
Cmax/C(t) (µg/mL)	0.8116 ± 0.0589 (Cmax)	8.00954 [C(1 min)]	7.20505 [C(1 min)]
Tmax (min) (median)	50	1	1
AUC (µg·min/mL)	484.0 ± 82.0	515.0	289.2
F (%)	47.39	NA	NA
Additional Information:	AUC interval not defined. Not corrected for endogenous IGF-1. Mean endogenous IGF-1 was 0.17399, 0.23019, and 0.14130 µg/mL, respectively. * Assumed		

In single dose pharmacokinetic study in male rhesus monkeys after SC administration of mecasermin at a dose of 0.5 mg/kg, the mean Cmax was 1.8 and 0.6 µg/mL, the median Tmax was 200 and 110 minutes and the mean AUC was 1089 and 72 µg·min/mL for total IGF-1 and free IGF-1, respectively. Data were summarized in the following table.

Mean Total and Free IGF-1 Pharmacokinetic Parameters Following Single SC Doses of Mecasermin in Male Rhesus Monkeys

Study	93-241-0320			
Analyte	Total IGF-1	Total IGF-1	Free IGF-1	Free IGF-1
Process	[]			
Dose (mg/kg)	0.5	0.5	0.5	0.5
Cmax (µg/mL)	2.0777	1.710	0.692	0.513
Tmax (min)	237.6	160.2	115.2	102.6
AUC(0-16hr) (µg·min/mL)	1139.4	1039.32	82.08	62.4

Data were not corrected for endogenous IGF-1

In multiple dose studies in rats, mecasermin was administered SC at doses of 0.1 to 10.0 mg/kg/day for approximately 4 weeks to 2 years. In the 4-week study, male and female Sprague Dawley rats received mecasermin SC once daily at doses of 0.1, 0.3, and 1.0 mg/kg/day. The 30-minute postdose concentrations of free and total IGF-1 increased with increasing dose, less than dose proportionally for total IGF-1 C(30min) and approximately dose proportionally for free IGF-1. Male animals showed higher total and free IGF-1 C(30 min) values than females in the majority of the cases. Data were shown in the following table.

Mean Plasma Concentrations of Total and Free IGF-1 Following Four Weeks of Daily SC Doses of Mecasermin in Male and Female Sprague Dawley Rats

Study	93-120-0320PK			
Analyte	Total IGF-1	Total IGF-1	Free IGF-1	Free IGF-1
Study Day	Day 1	Day 29	Day 1	Day 29
Gender	Male			
Dose (mg/kg/day)	0.1			
C(30 min) (µg/mL)	0.408 - 0.411	0.541 - 0.558	0.017 - 0.021	0.018 - 0.019
Dose (mg/kg/day)	0.3			
C(30 min) (µg/mL)	0.513 - 0.678	0.565 - 0.734	0.061 - 0.064	0.112 - 0.118
Dose (mg/kg/day)	1.0			
C(30 min) (µg/mL)	0.532 - 1.129	0.849 - 1.150	0.155 - 0.205	0.239
Gender	Female			
Dose (mg/kg/day)	0.1			
C(30 min)(µg/mL)	0.453 - 0.476	0.396 - 0.477	0.014 - 0.015	0.013 - 0.018
Dose (mg/kg/day)	0.3			
C(30 min) (µg/mL)	0.481 - 0.483	0.407 - 0.478	0.029 - 0.036	0.046 - 0.063
Dose (mg/kg/day)	1.0			
C(30 min) (µg/mL)	0.806 - 1.023	0.232 - 0.919	0.186 - 0.234	0.187 - 0.278

In the 26-week TK study, male and female Sprague Dawley rats received mecasermin SC once daily at 0.25, 1.0, and 4.0 mg/kg/day as part of the 26-week toxicology study. Mean concentrations of total and free IGF-1 increased with increasing dose on Day 1 and during Week 26, less than dose proportionally for total IGF-1 C(30 min) and approximately dose proportionally for free IGF-1. Male animals showed slightly higher plasma concentrations at 30min post dose (C/30 min) for total and free IGF-1 than females in the majority of cases. There were no substantial, consistent changes in the mean total IGF-1 C(30 min) on multiple dosing; however, free IGF-1 showed slight increases in mean Cmax for Week 26 values compared to Day 1 values. Data were summarized in the following table.

Mean Plasma Concentrations of Total and Free IGF-1 Following Twenty-Six Weeks of Daily SC Doses of Mecasermin in Male and Female Sprague Dawley Rats

Study	96-267-0320			
Analyte	Total IGF-1	Total IGF-1	Free IGF-1	Free IGF-1
Sample Interval	Day 1	Week 26	Day 1	Week 26
Gender	Male			
Dose (mg/kg/day)	0.25			
C(30 min) (µg/mL)	1.480	1.445	0.1029	0.1255
Dose (mg/kg/day)	1.0			
C(30 min) (µg/mL)	2.275	2.293	0.424	0.644
Dose (mg/kg/day)	4.0			
C(30 min) (µg/mL)	4.345	4.708	1.790	2.168
Gender	Female			
Dose (mg/kg/day)	0.25			
C(30 min) (µg/mL)	1.351	1.107	0.0738	0.0981
Dose (mg/kg/day)	1.0			
C(30 min) (µg/mL)	2.053	1.653	0.360	0.504
Dose (mg/kg/day)	4.0			
C(30 min) (µg/mL)	4.230	4.500	1.763	2.298

In the toxicokinetic support portion of the rat carcinogenicity study male and female Sprague Dawley rats received doses of 0.25, 1.0, 4.0, and 10.0 mg/kg/day of mecasermin SC once daily for approximately 2 years, the mean Tmax for free and total IGF-1 occurred from 30 to 180 minutes post dose and did not appear to change with increasing dose or on multiple dosing or to differ between male and female animals. The mean Cmax and AUC for total and free IGF-1 overall increased with increasing dose, less than dose proportionally for total IGF-1 and greater than or approximately equal to dose proportionally for free IGF-1, respectively. The mean half-life values for total IGF-1 decreased with increasing dose for male and female animals on Day 1 and during Week 79. The mean t_{1/2} values were 154.8 to 603.6 min at 0.25 mg/kg/day and 40.8 to 573 min at 10.0 mg/kg/day for total IGF-1. The mean free IGF-1 t_{1/2} overall did not show consistent changes with increasing dose (23.4 to 140.4 minutes). Male rats in the majority of cases had higher systemic exposure for both free and total IGF-1 than female rats based on their mean Cmax and AUC values at the same dose level on Day 1 and during Week 79. Data were summarized in the following tables.

Mean Pharmacokinetic Parameters for Total IGF-1 during 2 Years of Daily SC Doses of Mecasermin in Male and Female Sprague Dawley Rats

Study	96-177-0320			
Sample Interval	C _{max} (µg/mL)	T _{max} (min)	AUC(0-24hr) (µg·min/mL)	t _{1/2} (min)
Gender	MALE			
Dose (mg/kg/day)	0.25			
Day 1	0.567	30	94.86	434.4
Week 79	0.723	60	215.22	NC
Dose (mg/kg/day)	1.0			
Day 1	1.050	60	276.36	138
Week 79	1.150	60	375.18	604.8
Dose (mg/kg/day)	4.0			
Day 1	2.810	30	414.6	64.8
Week 79	3.090	60	1067.1	299.4
Dose (mg/kg/day)	10.0			
Day 1	7.060	30	700.98	47.4
Week 79	5.280	90	1997.46	79.8
Gender	FEMALE			
Dose (mg/kg/day)	0.25			
Day 1	0.624	60	71.16	154.8
Week 79	0.473	180	86.88	603.6
Dose (mg/kg/day)	1.0			
Day 1	0.826	60	158.58	130.8
Week 79	0.705	30	216.42	779.4
Dose (mg/kg/day)	4.0			
Day 1	2.620	30	273.72	59.4
Week 79	2.230	30	526.38	304.8
Dose (mg/kg/day)	10.0			
Day 1	6.830	30	502.44	40.8
Week 79	4.620	30	1375.2	573

Mean Pharmacokinetic Parameters for Free IGF-1 during 2 Years of Daily SC Doses of Mecasermin in Male and Female Sprague Dawley Rats

Study	96-177-0320			
Sample Interval	C _{max} (µg/mL)	T _{max} (min)	AUC(0-24hr) (µg·min/mL)	t _{1/2} (min)
Gender	MALE			
Dose (mg/kg/day)	0.25			
Day 1	0.0521	30	3.54	60
Week 79	0.0467	60	7.14	116.4
Dose (mg/kg/day)	1.0			
Day 1	0.250	30	21.18	40.2
Week 79	0.420	30	40.38	66
Dose (mg/kg/day)	4.0			
Day 1	0.825	60	87.06	28.8
Week 79	2.000	60	269.82	42.6
Dose (mg/kg/day)	10.0			
Day 1	3.830	30	331.74	23.4
Week 79	3.590	90	1895.58	140.4
Gender	FEMALE			
Dose (mg/kg/day)	0.25			
Day 1	0.0388	30	3.24	34.8
Week 79	0.0591	30	3.72	52.2
Dose (mg/kg/day)	1.0			
Day 1	0.157	30	12.06	39.6
Week 79	0.288	30	30.12	63.6
Dose (mg/kg/day)	4.0			
Day 1	1.230	30	84.54	25.8
Week 79	1.530	30	210.24	55.8
Dose (mg/kg/day)	10.0			
Day 1	3.810	30	285.12	24.6
Week 79	3.350	60	707.28	105.6

A TK study was conducted in male and female Beagle dogs after SC administration of mecasermin in conjunction with the 26-week toxicology study. The mean total IGF-1 T_{max} occurred from 78 to 198 minutes and the mean free IGF-1 T_{max} occurred from 48 to 78 minutes. These parameters showed no consistent changes with increasing dose, on multiple dosing, or between male and female animals. The mean total IGF-1 C_{max} and AUC increased with increasing dose, less than dose proportionally. By 24 hours postdose, the concentrations of total IGF-1 were approximately equivalent to the predose concentrations. Mean total IGF-1 t_{1/2} did not change consistently with increasing dose (366 to 558 minutes for

males and females collectively on Day 1 and 198 to 468 minutes for males and females collectively during Month 6). The mean free IGF-1 C_{max} and AUC increased with increasing dose, approximately equal to or greater than dose proportionally. By 24 hours postdose, the concentrations of free IGF-1 were approximately equivalent to the predose concentrations. The mean free IGF-1 t_{1/2} did not change consistently with increasing dose (48 to 78 minutes on Day 1 and during Month 6). Data were summarized in the following tables.

Mean Pharmacokinetic Parameters for Total IGF-1 Following Twenty-Six Weeks of Daily SC Doses of Mecasermin in Beagle Dogs

Study	96-268-0320					
	Male			Female		
Day 1						
Dose (mg/kg/day)	0.15	0.3	0.6	0.15	0.3	0.6
Cmax (µg/mL)	0.469	0.443	0.670	0.558	0.480	0.671
Tmax (min)	162	120	90	120	198	78
AUC(0-t) (µg·min/mL)	352.32	275.34	435.6	370.86	324.54	402.18
t1/2(min)	462	558	444	444	366	414
Month 6						
Dose (mg/kg/day)	0.15	0.3	0.6	0.15	0.3	0.6
Cmax(µg/mL)	0.437	0.534	0.877	0.537	0.735	0.841
Tmax (min)	120	102	102	120	108	120
AUC(0-t) (µg·min/mL)	195.06	187.98	481.08	210.24	497.58	351.84
t1/2(min)	198	222	348	318	468	216

Mean Pharmacokinetic Parameters for Free IGF-1 Following Twenty-Six Weeks of Daily SC Doses of Mecasermin in Beagle Dogs

Study	96-268-0320					
	Male			Female		
Day 1						
Dose (mg/kg)	0.15	0.3	0.6	0.15	0.3	0.6
Cmax/ (µg/mL)	0.1049	0.1493	0.2868	0.0732	0.1034	0.2983
Tmax (min)	60	60	54	60	60	48
AUC(0-t) (µg·min/mL)	14.4	20.7	53.34	7.62	15.6	45.24
t1/2(min)	66	60	60	48	60	60
Month 6						
Dose (mg/kg)	0.15	0.3	0.6	0.15	0.3	0.6
Cmax (µg/mL)	0.0538	0.1333	0.3437	0.0379	0.1873	0.3152
Tmax (min)	48	78	60	72	72	60
AUC(0-t) (µg·min/mL)	7.98	23.16	72.06	4.86	23.82	61.74
t1/2(min)	78	66	66	66	60	66

2.6.4.4 Distribution

A single dose tissue distribution study was conducted in male rats after IV administration of ^{125}I -labeled rhIGF-1 formulated in PBS. The tissue distribution at 10, 60, 200, and 400 minutes postdose for tracer only and at 60 minutes for tracer plus excess unlabeled rhIGF-1 was examined after a radiolabeled tracer-only dose ($\sim 0.00006 \text{ mg/kg}/8.7 \text{ } \mu\text{Ci/kg}$) and after a radiolabeled tracer dose plus an excess of unlabeled mecasermin ($\sim 0.0002 \text{ mg/kg}/25.8 \text{ } \mu\text{Ci/kg}$ plus 1.2 mg/kg unlabeled mecasermin). After the tracer-only dose, the rank order of accumulation of radioactivity over 10 to 200 minutes postdose was kidney > liver > small intestine > reproductive tract > large intestine > stomach (excluding blood, plasma, and injection site). Organ to blood radioactivity ratios at 10 minutes were greater than 1 for kidney only. At 400 minutes postdose the rank order was reproductive tract > liver > kidney > small intestine. Co-administration of excess unlabeled mecasermin decreased the percentage of the radioactive dose that accumulated in liver and blood at 60 minutes postdose by approximately 2-fold. The excess unlabeled mecasermin increased by $\sim 35\%$ the percentage of the radioactive dose in the kidney and the kidney organ to blood ratios at 60 minutes postdose; the radioactivity in other organs was relatively unaffected. Co-administration of excess unlabeled mecasermin yielded organ/blood ratios greater than 1 for kidney and bladder. Displacement of radioactivity in liver by excess unlabeled mecasermin may suggest this is a site of specific, saturable mecasermin uptake. Kidney accumulation of radioactivity did not appear to be saturable. Therefore, after IV administration of ^{125}I -rhIGF-1 in male Sprague Dawley rats, radioactivity was distributed primarily to highly perfused organs, and/or presumed organs of elimination (liver and kidney). Data were summarized in the following tables.

Species/Strain	Sprague Dawley Rat, strain not specified				
Gender/No.	Male/5 (1 per time point)			Male/1	
Feeding Condition	Fasted				
Vehicle/Formulation; Lot/Process	Phosphate Buffered Saline; rhIGF-1 , 07-0104, lot 88-G4				
Method of Administration	IV single dose				
Dose (mg/kg)	~0.00006/8.7 $\mu\text{Ci/kg}$, tracer dose			~0.0002/25.8 $\mu\text{Ci/kg}$; tracer dose plus 1.2 mg/kg unlabeled rhIGF-1 (excess)	
Radionuclide	^{125}I (Iodogen method)			^{125}I (Iodogen method)	
Specific Activity	150 $\mu\text{Ci}/\mu\text{g}$ assumed			150 $\mu\text{Ci}/\mu\text{g}$ assumed	
Organ/Tissue containing > 1% of the radioactive dose	Sampling Time (% radioactive dose/organ or tissue)				
	10 min	60 min	200 min	400 min	60 min (with excess unlabeled rhIGF-1)
Blood	47.60 (1)	39.69 (1)	26.29 (1)	27.49 (1)	21.16 (1)
Plasma	44.60 (1.47)	34.50 (1.37)	25.00 (1.50)	20.30 (1.16)	14.40 (1.07)
Kidney	21.78 (3.52)	3.74 (0.78)	1.75 (0.56)	1.40 (0.41)	5.01 (1.99)
Liver	5.15 (0.17)	4.16 (0.18)	1.75 (0.12)	1.52 (0.09)	1.70 (0.16)
Stomach	0.77 (0.19)	1.01 (0.30)	0.88 (0.44)	0.53 (0.26)	0.63 (0.34)
Small intestine	4.25 (0.19)	2.85 (0.17)	1.70 (0.15)	1.24 (0.10)	3.11 (0.35)
Large intestine	1.18 (0.18)	1.19 (0.19)	0.41 (0.13)	0.25 (0.06)	0.72 (0.29)
Reproductive tract	1.98 (0.11)	1.97 (0.14)	1.46 (0.14)	2.81 (0.27)	1.77 (0.23)
Injection site	1.01 (0.61)	0.75 (0.68)	1.69 (2.35)	0.38 (0.26)	0.66 (0.61)
Total (minus plasma)	86.08 (NA)	57.73 (NA)	37.30 (NA)	37.01 (NA)	36.64 (NA)

Species/Strain	Sprague Dawley Rat; strain not specified				
Gender/No.	Male/5 (1 per time point)			Male/1	
Feeding Condition	Fasted				
Vehicle/Formulation; Lot/Process	Phosphate Buffered Saline; rhIGF-1, 07-0104, lot 88-G4				
Method of Administration	IV single dose				
Dose (mg/kg)	~0.00006/8.7 µCi/kg, tracer dose			~0.0002/25.8 µCi/kg; tracer dose plus 1.2 mg/kg unlabeled rhIGF-1 (excess)	
Radionuclide	¹²⁵ I (Iodogen method)			¹²⁵ I (Iodogen method)	
Specific Activity	150 µCi/µg assumed			150 µCi/µg assumed	
Organ/Tissue	Sampling Time (cpm/g tissue)				
	10 min	60 min	200 min	400 min	60 min (with excess unlabeled rhIGF-1)
Blood (1 mL)	169000	134400	92900	97950	239400
Plasma (1 mL)	249000	184000	139000	114000	255900
Kidney	592303	104741	52071	40084	475694
Liver	29379	24165	11010	9046	37757
Adrenals	152720	25840	7200	5186	76551
Small intestine	32600	22361	14300	10226	83561
Pituitary	39800	3525	14500	4810	91500
Reproductive tract	18386	18179	12791	26766	34230
Injection site	102793	90735	218464	25175	146577
Stomach	31738	40156	40986	25523	80399
Large intestine	30231	25255	11901	6206	70167
Heart	29016	22848	12543	11543	35443
Lung	26303	27205	18397	16040	66394
Thyroid	25131	20050	13318	21275	69257
Bone	19389	14715	10057	21555	64956
Spleen	17007	14353	17370	13155	56708
Skin	16745	26927	21883	18466	106111
Snout	15952	19668	10604	9597	73570
Bladder	15604	43408	26942	82923	339600
Thymus	10960	12330	9086	7640	41966
Muscle	10121	13979	5421	4969	31737
Fat	6703	4002	4935	5087	46604
Brain	6249	3491	2034	1666	7506
Additional Information:	Animals were pretreated with NaI. Control animal received no treatment.				

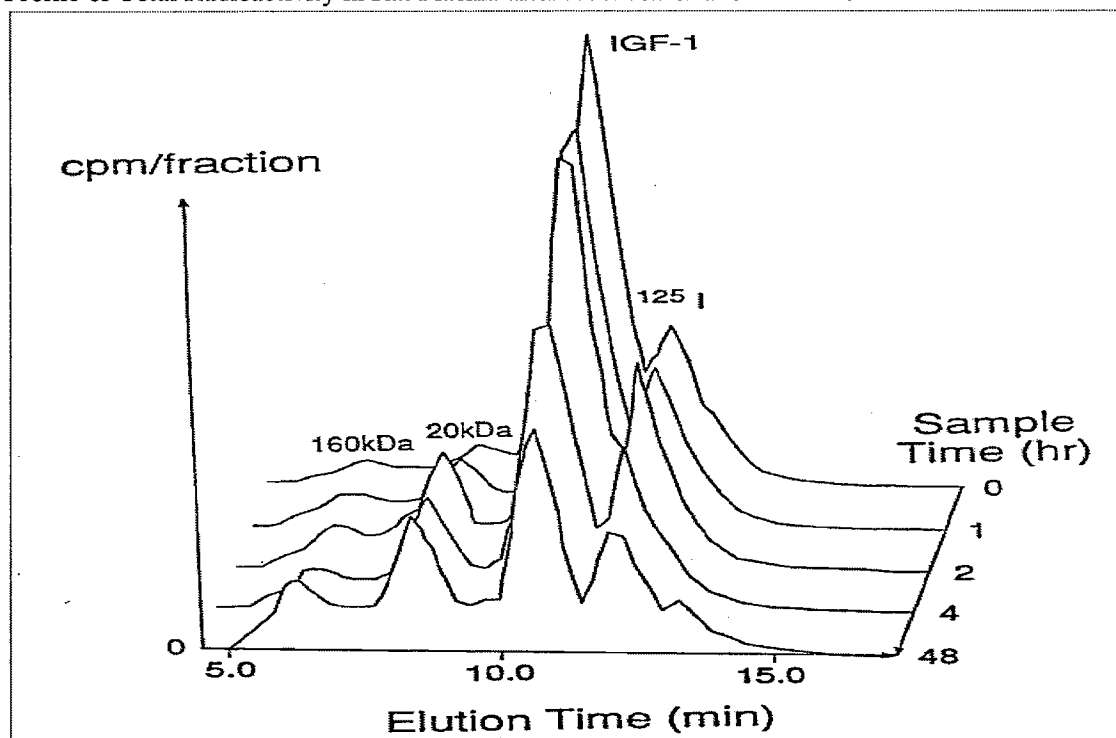
In the in vitro protein binding study, ¹²⁵I-Mecasermin plus increasing concentrations of unlabeled α 1 : mecasermin (0.001 to 10.0 µg/mL) were incubated with a fixed concentration of IGFBP-1 and IGFBP-2 (0.150 µg/mL) or IGFBP-3 (0.1 µg/mL) overnight at 4°C in 50 mM Tris buffer, 0.5% BSA, pH 7.5. Data were shown in the following table.

In Vitro Binding of ¹²⁵I-Labeled Mecasermin to IGF-1 Binding Proteins

Study	96-275-0320	
	Mean Kd (nM)	
Binding Protein	Mecasermin —	Mecasermin —
IGFBP-1	1.98 ± 0.78	1.56 ± 0.26
IGFBP-2	1.25 ± 0.68	0.72 ± 0.09
IGFBP-3	1.53 ± 1.25	1.23 ± 0.98

In another in vitro binding study, male Sprague-Dawley rat blood was used to determine the in vitro protein binding characteristics of rhIGF-1. ^{125}I -labeled $[\text{ }^{125}\text{I}]\text{rhIGF-1}$ was used in this study (specific activity = $46 \mu\text{Ci}/\mu\text{g}$; $0.24 \mu\text{g}/\text{mL}$, $11.4 \mu\text{Ci}$). Four peaks of radioactivity were observed in rat plasma samples. These radioactive peaks were believed to correspond to ^{125}I -rhIGF-1 bound in $\sim 160 \text{ kDa}$ and $\sim 20 \text{ kDa}$ binding protein complexes, free ^{125}I -rhIGF-1 ($\sim 7 \text{ kDa}$), and free ^{125}I . The majority of the plasma radioactivity was free ^{125}I -rhIGF-1 at all time points. This in vitro result was not in agreement with the in vivo protein binding results, where bound mecaseimerin predominated at all time points. A 25% decrease in free ^{125}I -rhIGF-1 was observed with a concomitant increase in radioactivity in the $\sim 20 \text{ kDa}$ binding protein complex (18%) and the $\sim 160 \text{ kDa}$ binding protein complex (7%) over the 48-hour time course. The majority of this redistribution of radioactivity occurred within 4 hours. Data were described in the following figure.

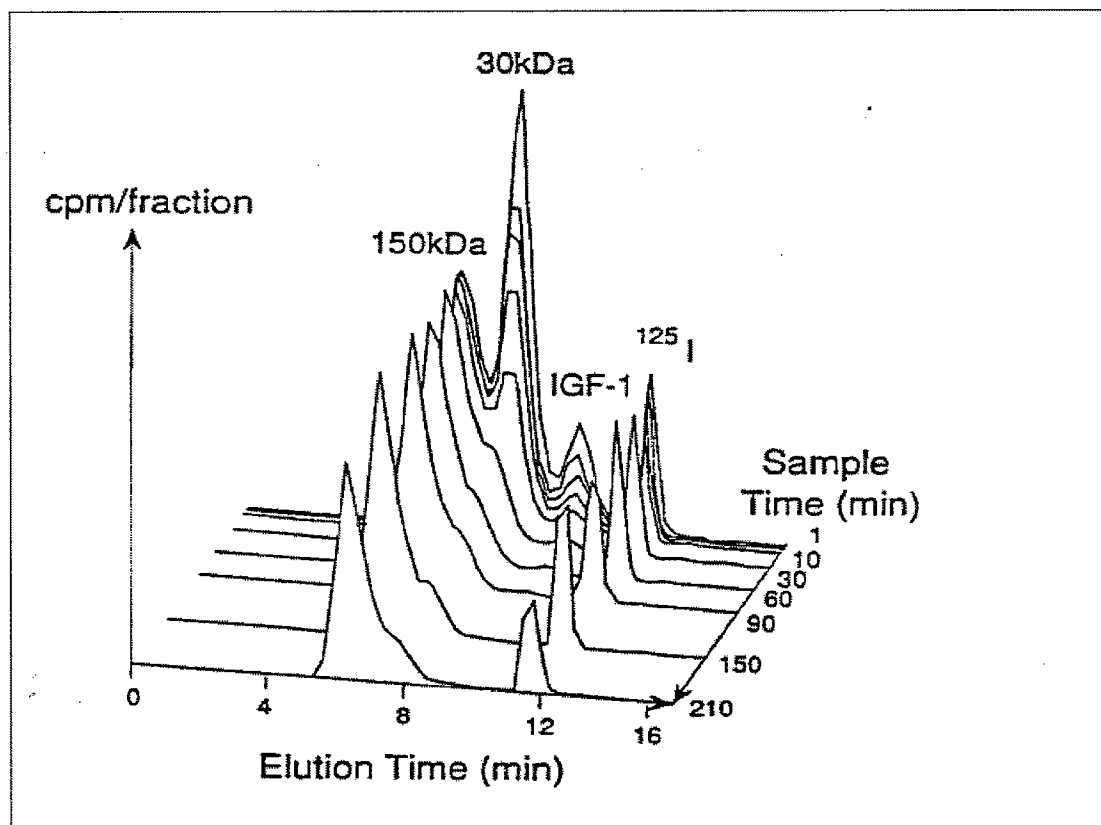
Profile of Total Radioactivity in Rat Plasma after Addition of ^{125}I - $[\text{ }^{125}\text{I}]\text{rhIGF-1}$ In Vitro



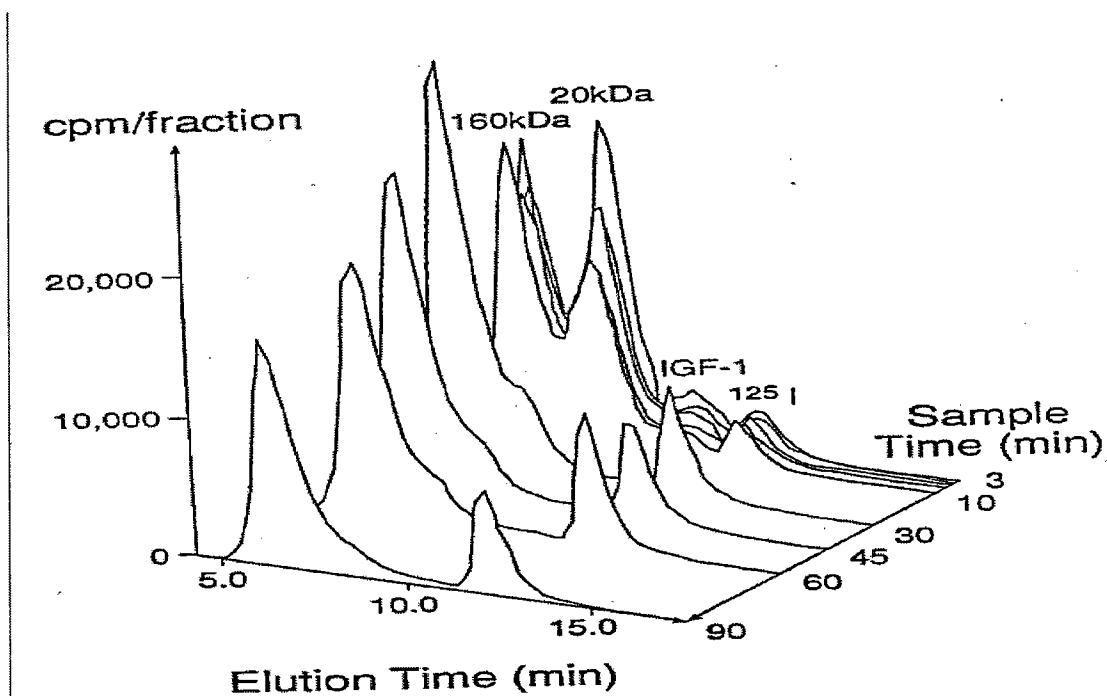
In the in vivo protein binding studies, five single dose IV studies were conducted in male and female Sprague-Dawley rats to determine the in vivo protein binding characteristics of mecaseimerin. These protein binding studies used mecaseimerin $[\text{ }^{125}\text{I}]\text{mcaseimerin}$ and commercially available $[\text{ }^{125}\text{I}]\text{rhIGF-1}$. Mecasermin or rhIGF-1 were radiolabeled with ^{125}I by the Iodogen method for these studies (specific activities = 94 to $180 \mu\text{Ci}/\mu\text{g}$). Mecasermin was administered as a tracer dose alone or with excess unlabeled mecaseimerin. Four peaks of radioactivity were observed in rat plasma samples in all experiments. These radioactive peaks were believed to correspond to ^{125}I -mecasermin or rhIGF-1 bound in ~ 150 to 160 kDa and ~ 20 to 30 kDa binding protein complexes, free ^{125}I -mecasermin or rhIGF-1, and

free ^{125}I . For the experiments where only tracer doses were administered, there was an increase in the proportion of radioactivity associated with the ~150 to 160 kDa complex over 210 minutes or up to 30 minutes, depending on the experiment, followed by a slow decline. The radioactivity associated with the ~20 to 30 kDa complex showed a rapid decline over less than or equal to 90 minutes. Free radiolabeled mecasermin or rhIGF-1 accounted for a small percentage of the radioactivity initially; after approximately 10 minutes, only minimally detectable amounts remained. Free ^{125}I showed an overall increase over 210 minutes (up to 13%). Increasing doses of unlabeled mecasermin resulted in increasingly smaller amounts of radioactivity associated with the binding protein complexes. At an unlabeled mecasermin dose of 1 mg/kg, essentially all the radioactivity was free ^{125}I -mecasermin or free ^{125}I . These results suggest excess unlabeled mecasermin prevented binding of the labeled compound to its binding proteins. Data were described in the following figures.

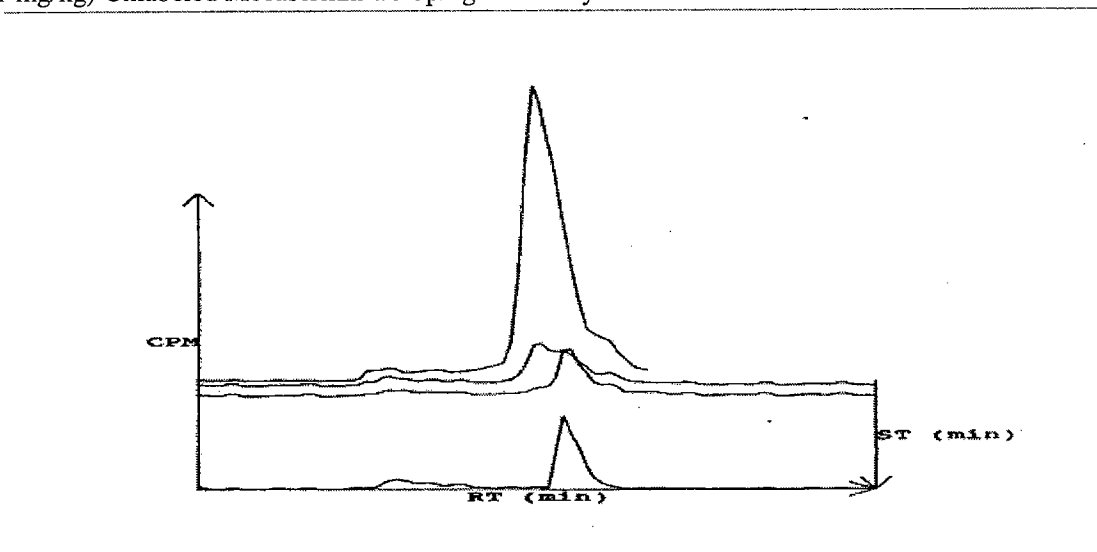
Profile of Total Radioactivity in Rat Plasma after IV Administration of ^{125}I -Mecasermin To Sprague Dawley Rats



Profile of Total Radioactivity in Rat Plasma after IV Administration of ^{125}I -rhIGF-1 To Sprague Dawley Rats



Profile of Total Radioactivity in Rat Plasma after IV Administration of ^{125}I - Mecermin Plus Excess (1 mg/kg) Unlabeled Mecermin To Sprague Dawley Rats



Data Source: Table 2.6.5.6.5, rat protein binding/89-194-0320-569

RT = retention time = 0 to 22.5 min

CPM = 0 to 12272

ST = sampling time; upper curve = 1 min, then 10, 30, 210 min

Relative clearance differed for free radiolabeled mecermin and radioactivity associated with the binding protein complexes. Radioactivity associated with the ~150 to 160 kDa complex

cleared slowly (0.5 to 10 mL/min/kg), radioactivity associated with the ~20 to 30 kDa complex was intermediate in clearance (16 to 48 mL/min/kg), and free radiolabeled mecasecmin was cleared most rapidly (168 to 204 mL/min/kg).

The percent free IGF-1 calculated based on the mean C_{max} ratios for free and total IGF-1 (x100) across all of the SC ADME/toxicokinetic studies done in rats and dogs with unlabeled mecasecmin is shown in the following tables. Generally, the percentage of free IGF-1 increased with increasing dose in male and female Sprague Dawley rats and Beagle dogs in both the single dose and multiple dose studies.

Percent Free IGF-1 Based on Mean C_{max} Ratios for Total and Free IGF-1 Across All SC Nonclinical Studies (Single Doses)

Dose (mg/kg/day)	Percent Free IGF-1 Males	Percent Free IGF-1 Females	Study Reference
Single SC Dose Rat			
0.1	10	NA	93-240-0320PK ^a
0.1	4.6	3.1	93-120-0320PK Day 1 ^b
0.25	7.0	5.5	96-267-0320 Day 1 ^c
0.25	9.2	6.2	96-177-0320 Day 1 ^d
0.3	14	NA	93-240-0320PK ^a
0.3	10	6.7	93-120-0320PK Day 1 ^b
1.0	30	NA	93-240-0320PK ^a
1.0	38	NA	93-240-0320PK ^a
1.0	22	23	93-120-0320PK Day 1 ^b
1.0	19	18	96-267-0320 Day 1 ^c
1.0	24	19	96-177-0320 Day 1 ^d
2.0	39	NA	90-168-0320 ^e
4.0	41	42	96-267-0320 Day 1 ^c
4.0	29	47	96-177-0320 Day 1 ^d
10.0	54	56	96-177-0320 Day 1 ^d
Single SC Dose Dog			
0.15	22	13	96-268-0320 Day 1 ^f
0.3	34	22	96-268-0320 Day 1 ^f
0.6	43	44	96-268-0320 Day 1 ^f

Data Source:

^a Table 2.6.5.3.2, rat single dose/93-240-0320PK

^b Table 2.6.5.4.1, rat 4-week/93-120-0320PK; midpoint of ranges of values compared

^c Table 2.6.5.4.2, rat 26-week/96-267-0320

^d Table 2.6.5.4.3, rat carcinogenicity/96-177-0320

^e Table 2.6.5.3.1, rat single dose/90-168-0320

^f Table 2.6.5.4.5, dog 26-week/96-268-0320

Percent Free IGF-1 Based on Mean Cmax Ratios for Total and Free IGF-1 Across All SC Nonclinical Studies (Multiple Doses at Steady State)

Dose (mg/kg/day)	Percent Free IGF-1 Males	Percent Free IGF-1 Females	Study Reference
Multiple SC Dose Rat			
0.1	3.4	3.6	93-120-0320PK Day 29 ^a
0.25	8.7	8.9	96-267-0320 Week 26 ^b
0.25	6.5	12	96-177-0320 Week 79 ^c
0.3	18	12	93-120-0320PK Day 29 ^a
1.0	24	40	93-120-0320PK Day 29 ^a
1.0	28	30	96-267-0320 Week 26 ^b
1.0	37	41	96-177-0320 Week 79 ^c
4.0	46	51	96-267-0320 Week 26 ^b
4.0	65	69	96-177-0320 Week 79 ^c
10.0	68	73	96-177-0320 Week 79 ^c
Multiple SC Dose Dog			
0.15	12	7.1	96-268-0320 Month 6 ^d
0.3	25	25	96-268-0320 Month 6 ^d
0.6	39	37	96-268-0320 Month 6 ^d

Data Source:

^a Table 2.6.5.4.1, rat 4-week/93-120-0320PK, Day 29

^b Table 2.6.5.4.2, rat 26-week/96-267-0320, Week 26

^c Table 2.6.5.4.3, rat carcinogenicity/96-177-0320, Week 79

^d Table 2.6.5.4.5, dog 26-week/96-268-0320, Month 6

No studies of the distribution of mecasermin into blood cells and the placental transfer were conducted.

2.6.4.5 Metabolism

Metabolism studies were conducted in male and female Sprague Dawley rats after IV administration with mecasermin [] and commercially available [] rhIGF-1. The mecasermin or rhIGF-1 was radiolabeled with ¹²⁵I by the Iodogen method (specific activities = 94 to 180 µCi/µg). Four peaks of radioactivity were observed in the rat plasma samples in all experiments. These radioactive peaks were believed to correspond to ¹²⁵I-mecasermin or rhIGF-1 bound in high molecular weight and low molecular weight binding protein complexes, free ¹²⁵I-mecasermin or rhIGF-1, and free ¹²⁵I. Relative clearance (total radioactive dose divided by the area under the HPLC peak height vs. time curve) differed for free ¹²⁵I-mecasermin or rhIGF-1 and radioactivity associated with the binding protein complexes. Radioactivity associated with the high molecular weight binding protein complex cleared slowly (0.5 to 10 mL/min/kg), radioactivity associated with the low molecular weight binding protein complex was intermediate in clearance (16 to 48 mL/min/kg), and free ¹²⁵I-mecasermin or rhIGF-1 was cleared most rapidly (168 to 204 mL/min/kg). The expected consequence of metabolism of therapeutic proteins is the degradation to small peptides and individual amino acids. Since the administered mecasermin is indistinguishable from the naturally occurring endogenous human IGF-1, and is proposed to be administered as

replacement therapy, its metabolism should be the same as the endogenous hormone. Enzyme induction/inhibition studies were not conducted for mecasermin.

2.6.4.6 Excretion

Excretion studies for mecasermin were not conducted.

2.6.4.7 Pharmacokinetic drug interactions

No in vitro or in vivo pharmacokinetic or metabolism drug interaction studies were conducted for mecasermin.

2.6.4.8 Other Pharmacokinetic Studies

No other pharmacokinetic studies were conducted for mecasermin.

2.6.4.9 Discussion and Conclusions

ADME/Toxicokinetic studies of mecasermin were conducted in adult Sprague Dawley rat, New Zealand White rabbit, Beagle dog, and rhesus monkey. The routes, and doses used in these studies were the same as those used in the pharmacological (rat, rabbit, monkey) and pivotal toxicological (rat, rabbit, dog) evaluations done to support the safe use of mecasermin in humans. RIA, ELISA, radiolabeled, and associated laboratory methods of sufficient quality for the intended purposes were used in the ADME/toxicokinetic evaluations.

Mecasermin was relatively quickly absorbed after SC administration in rats, rabbits, monkeys and dogs. The mean total and free IGF-1 T_{max} values were 15 to 238 minutes. After SC administration in rats and dogs, C_{max} and AUC for total and free IGF-1 increased with increasing doses, less than dose proportionally for total IGF-1 and equal to or greater than dose proportionally for free IGF-1. The SC bioavailability values were 38 to 57% in rats and rabbits. Mean total and free IGF-1 C_{max} and AUC values were unchanged or higher at steady state on multiple (once daily) SC dosing than after single doses in rats but did not show consistent changes on multiple dosing in dogs. The 24-hour total and free IGF-1 concentrations approximated predose concentrations; therefore, no significant accumulation appeared to be occurred upon multiple dosing. Gender-related differences in mean total and free IGF-1 C_{max} and AUC values were observed in rats (males greater than females), but not in dogs. The toxicokinetic studies in the long term toxicology studies in male and female Sprague Dawley rats and Beagle dogs demonstrated adequate exposure of the toxicology species to IGF-1 plasma concentrations throughout the entire duration of the long term toxicology studies. No antibodies to IGF-1 were detected in the long term toxicology studies in rats and dogs; however, anti-IGF-1 antibodies were detected in one 4-week IV toxicology study in Beagle dogs.

The kidney and liver appeared to be the major organs of distribution for ¹²⁵I-rhIGF-1 after IV administration in male rats. After IV administration of a tracer dose of ¹²⁵I-mecasermin to male and female rats, radioactivity was observed in ~150 to 160 kDa and ~20 to 30 kDa plasma binding protein complexes, as free ¹²⁵I-mecasermin, and as free ¹²⁵I. Clearance of radioactivity in the binding protein complexes was considerably slower than the clearance of free ¹²⁵I-mecasermin. In the toxicokinetic support studies in rats and dogs, the percent of total IGF-1 that was free IGF-1 generally increased with increasing dose of mecasermin (from a low of approximately 3.1% at 0.1 mg/kg/day to a high of approximately 73% at 10.0 mg/kg/day).

After IV administration of ^{125} I-mecasermin in male and female rats, the majority (80%) of the injected mecasermin remained intact up to 210 minutes postdose. No in vivo metabolism and excretion studies were conducted.

2.6.4.10 Tables and figures to include comparative TK summary

Tables and figures have been incorporated into the body documents.

2.6.5 PHARMACOKINETICS TABULATED SUMMARY

Overview				
Test Article:	Mecasermin			Page 1 of 4
Type of Study	Test System	Method of Administration	Testing Facility	Study No.
Absorption				
Absorption	Sprague Dawley Rat, male	SC single dose 2.0 mg/kg	Genentech, Inc. South San Francisco CA	90-168-0320 ^a
		IV single dose 1.0 mg/kg		
Absorption and toxicokinetic support for 93-119-0320	Sprague Dawley Rat, male	SC single dose 0.1, 0.3, 1.0, 10.0 mg/kg	[]	93-240-0320PK ^b
		IV single dose 1.0 mg/kg		
Absorption	Sprague Dawley Rat, male	SC single dose 3.0 mg/kg	Genentech, Inc. South San Francisco CA	94-103-0320 ^a
		IV single dose 1.0 mg/kg		
Absorption	Sprague Dawley Rat, male	IV single dose 0.719 mg/kg	Genentech, Inc. South San Francisco CA	89-004-0320-569 ^a
Absorption	Sprague Dawley Rat, male and female	IV single dose 0.0035, 0.01, 0.1, 1.0, 10.0 mg/kg	[]	89-193-0320-569 ^b
Absorption	Sprague Dawley Rat, male	IV single dose 0.0021 mg/kg	Genentech, Inc. South San Francisco CA	89-163-0320-569 ^a
Absorption	New Zealand White Rabbit, male	SC, single dose 1.0 mg/kg	Genentech, Inc. South San Francisco CA	93-157-0320 ^a
		IV, single dose 0.5 mg/kg		
Absorption	Rhesus Monkey, male	SC, single dose 0.5 mg/kg	[]	93-241-0320 ^b
Absorption and toxicokinetic support for 93-120-0320	Sprague Dawley Rat, male and female	SC, QD x 4 weeks 0.1, 0.3, 1.0 mg/kg/day	[]	93-120-0320PK ^b
Absorption and toxicokinetics within toxicology report	Sprague Dawley Rat, male and female	SC, QD x 26 weeks 0.25, 1.0, 4.0 mg/kg/day	[]	96-267-0320 ^b
Absorption and toxicokinetics within toxicology report	Sprague Dawley Rat, male and female	SC, QD x 2 years 0.25, 1.0, 4.0, 10.0 mg/kg/day	[]	96-177-0320 ^b
Absorption	Sprague Dawley Rat, male and female	IV, QD x 5 days 0.03, 0.09, 0.3, 1.5 mg/kg/day	Genentech, Inc. South San Francisco CA	88-132-0320-569 ^a
Absorption and toxicokinetics within toxicology report	Beagle Dog, male and female	SC, QD x 26 weeks 0.15, 0.3, 0.6 mg/kg/day	[]	96-268-0320 ^b

^aNot GLP, ^bGLP

Overview (Continued)

Test Article:	Mercasermin			Page 3 of 4
Type of Study	Test System	Method of Administration	Testing Facility	Study No.
Distribution				
Organ/Tissue distribution	Sprague Dawley Rat, male	IV, single dose ~0.00006, ~0.0002/1.2 mg/kg	Genentech, Inc. South San Francisco CA	88-125-0320- 569 ^a
Protein Binding				
In vitro protein binding	Human binding proteins 1, 2, 3	In vitro	Genentech, Inc. South San Francisco CA	96-275-0320 ^a
In vitro and in vivo protein binding	Sprague Dawley Rat, male	IV, single dose 0.0021 mg/kg	Genentech, Inc. South San Francisco CA	88-404-0320- 569 ^a
In vivo protein binding	Sprague Dawley Rat, male and female	IV, single dose 0.0035 mg/kg	[]	89-062-0320- 569 ^a
In vivo protein binding	Sprague Dawley Rat, male	IV, single dose 0.01, 0.1, 1.0 mg/kg	Genentech, Inc. South San Francisco CA	89-194-0320- 569 ^a
In vivo protein binding	Sprague Dawley Rat, male and female	IV single dose 0.0035, 0.01, 0.1, 1.0, 10.0 mg/kg	[]	89-193-0320- 569 ^b
In vivo protein binding	Sprague Dawley Rat, male	IV single dose 0.0021 mg/kg	Genentech, Inc. South San Francisco CA	89-163-0320- 569 ^a
Metabolism				
In vivo metabolism	Sprague Dawley Rat, male	IV single dose 0.0021 mg/kg	Genentech, Inc. South San Francisco CA	89-163-0320- 569 ^a
In vivo metabolism	Sprague Dawley Rat, male and female	IV single dose 0.0035, 0.01, 0.1, 1.0, 10.0 mg/kg	[]	89-193-0320- 569 ^b
Excretion				
	NA	NA	NA	NA
Pharmacokinetic Drug Interactions				
	NA	NA	NA	NA
Other				
	NA	NA	NA	NA

^aNot GLP, ^bGLP

2.6.6 TOXICOLOGY

2.6.6.1 Overall toxicology summary

Nonclinical toxicology of mecasermin was evaluated in a series of single and repeat dose studies in CD-1 mice, Sprague Dawley rats, New Zealand White rabbits, Beagle dogs, and Rhesus monkeys. Rats and dogs were selected as the test species for the single and repeat dose toxicology studies. Rabbits were used for local tolerance studies, and rats and rabbits were used for reproductive toxicology studies. The repeated dose toxicology studies were up to 6 months in duration. *In vitro* and *in vivo* genotoxicity assays were performed in addition to a 2-year rat carcinogenicity study.

In conjunction with the single and repeat dose studies, blood was collected for toxicokinetic, serum glucose, and immunogenicity (anti-IGF-1) analyses. The hypoglycemic effects of mecasermin were maintained throughout the repeat dose studies indicating that adequate exposure to mecasermin was maintained throughout the course of each experiment. Anti-IGF-1 antibody responses were rare. The single and repeat dose studies used both the IV and SC routes of administration, the latter being the clinical route of administration. The high doses utilized in the definitive repeat dose toxicology studies evaluated a range of multiples of the highest anticipated clinical dose and reached maximum tolerated doses based on hypoglycemia and mortalities seen in rat and dog repeat dose studies. The mecasermin was derived from manufacturing processes:

Two formulations, based on either citrate or acetate, were also used.

General toxicology: In single dose studies, a series of single dose SC and IV toxicology studies were performed in CD-1 mice, Sprague Dawley rats, New Zealand White rabbits, Beagle dogs, and Rhesus monkeys. The primary findings from these studies included the reductions in serum glucose and associated clinical manifestations associated with profound hypoglycemia seen at higher dose levels. In the definitive rat and dog GLP single dose toxicology studies, hypoglycemic responses were exacerbated by the animals being fasted overnight prior to dosing and deaths occurring. No evidence of anti-IGF-1 antibody formation was noted in any of the single dose studies.

In multiple dose toxicity studies, mecasermin was administered by SC or IV to rats or dogs up to 26 weeks. The majority of effects noted in these toxicology studies are consistent with the expected physiological or exaggerated pharmacological effects of this molecule. The most dramatic effect was hypoglycemia. Other significant adverse side effects included increases of body weight gain and food consumption, mild changes of clinical chemistry (increases of serum total protein, albumin, sodium, and chloride and decreases of serum glucose, triglycerides, phosphorus, and potassium) and hematological parameters (transient increases in hemoglobin and hematocrit and reductions in white blood cells and lymphocytes), and increases of some organ weights (heart, lung, liver, kidneys, thymus, adrenal gland in rats, and adrenal gland, thymus, heart, submandibular gland, and pituitary tissues in dogs) with a few histopathological changes (like increased incidence of extramedullary hematopoiesis in the liver, spleen, increased cortical area of the thymus in rats, and adrenal medullary hyperplasia

and fibrosis in dogs). There was no detectable antibody formation to IGF-1 in both the rat and dog toxicology studies.

Genetic toxicology: No evidence of genotoxicity was observed with mecasermin in an *in vitro* chromosomal aberration tested conducted in Chinese hamster lung fibroblasts. No evidence of genotoxicity was observed in an *in vivo* mouse micronucleus test employing IV doses.

Carcinogenicity: In a 2-year carcinogenicity study performed with mecasermin derived from *L. 3*, rats were dosed daily SC at doses of 0 (vehicle), 0.25, 1.0, 4.0, or 10.0 mg/kg. Mortality was increased in a statistically significant manner in males and in females at doses $\geq$ 1.0 and 4.0 mg/kg/day, respectively. Hypoglycemia appeared to be a major contributor to drug-related deaths. Because of low survival, males and females in the high dose group were terminated during Week 84 and the remaining male and female groups were terminated during Weeks 99 and 105, respectively. In addition, because of low survival, dose administration was discontinued for approximately 4 weeks prior to termination for males given 1.0 mg/kg/day and for approximately 7 and 13 weeks for males and females given 4.0 mg/kg/day. Aside from dose-related decreases in plasma glucose at doses $\geq$ 1.0 mg/kg/day, dose-related alterations in selected clinical pathology parameters (decreased red blood cell count, and serum albumin and increased white blood cell count, serum phosphorus, and potassium) and elevations in food consumption and body weight were observed.

Drug-related macroscopic findings included the increased incidence rate of mass in skin and mammary gland in male rats at doses of 4 and 10mg/kg, and the increased incidence rate of mass in skin in female rats in all dose levels. Non-neoplastic microscopic findings included lung and thymus congestion at doses of 4 and 10mg/kg/day; decreased hematopoiesis in spleen, hyperplasia in pituitary, congestion of submaxillary lymph nodes, and hyperplasia of leydig cell in testis at dose of 10mg/kg/day in males, increased necrosis of gray matter and hippocampus in brain, hyperplasia of transitional cell in kidney, thymus congestion, hyperplasia in pituitary, stomach congestion at doses of 4 and 10mg/kg/day, and dose-dependent increases of hyperplasia of adrenal medulla in all treated groups in females. Drug-related neoplastic findings included pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels; keratoacanthoma in the skin in males at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day; and mammary gland carcinoma in both males and females at 10mg/kg/day. Regarding the skin neoplasms, the sponsor suggested that the tumors may be related to the mechanical trauma due to repeated subcutaneous injections and/or potentially high local concentrations of rhIGF-1 may contribute to the development of skin neoplasms. However, based on the local tolerance toxicity study, there was no difference between the control and treated groups. Therefore, there was no evidence to support that the tumors in the skin were the secondary to drug irritation. Compared with the historical data, the only positive findings in this carcinogenicity study were the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg. The No-Observed-Effect-Level for these lesions was 1.0 mg/kg/day which represents a 3 to 6-fold multiple of the clinical exposure on an AUC basis.

Reproductive toxicology: Reproductive and developmental toxicology studies were performed in rats and rabbits. In the rat reproductive toxicological study, mecasermin was administered by IV bolus injection to rats at doses of 0.25, 1.0, and 4.0mg/kg/day. There were dose-dependent increases of body weight, body weight gain, and food consumption in both male and female parent rats at doses of 1 and 4mg/kg/day. There were no significant differences between control and treatment groups in estrus count and estrous cycle during the pre-mating administration period. No significant difference was observed between control and treatment groups in copulation index, indices of insemination and fertility. For the embryos and fetuses, a dose-dependent increase of early resorptions was observed. There were no significant differences between control and treatment groups in the number of corpora lutea, the number of implantations, and implantation index or the number of live fetuses. No external malformations were noted. Based on the sponsor, the NOEL is 4mg/kg/day. However, a slight dose-dependent increase in the total number of dead/resorbed fetuses at doses of 0.25mg/kg/day or higher were observed. With the maximum recommended human dose (MRHD) of 120µg/kg, the systemic exposures in rats are 8 times for 4mg/kg and 0.5times MRHD 0.25mg/kg based on BSA.

In the developmental toxicological study with rats, mecasermin was administered by iv bolus at doses of 1, 4 and 16mg/kg/day. There was a transient decrease in spontaneous movement at dose of 16mg/kg during the administration period in the dams. No drug-related changes of body weight, food consumption, or gross pathology were noted. In addition, there were no drug-related abnormal findings in the maintenance of pregnancy, cesarean section, parturition or lactation indices. In F1 fetuses, there were no drug-related changes of sex ratio, body weight, rate of incidence of pups exhibiting external, visceral or skeletal abnormalities or skeletal variations. No drug-related effects on growth and development of pups were noted. In live born F1 pups, the differentiation index for opening of the eyelid on day 14 after birth was significantly delayed at dose of 16mg/kg (47.2% vs 71.3% in control). However, this differentiation index on day 17 after birth was 100%. There were no other drug-related changes of body weight after birth, viability, external differentiation, reflexes, behavioral tests and reproductive performance tests. The non-toxic effect dose level was 16mg/kg which is 32 times the maximum recommended human dose (120µg/kg) based on body surface area (BSA).

In the rabbit study, mecasermin was administered by iv bolus to rabbits at doses of 0.125, 0.5 and 2.0mg/kg/day. No drug-related animal deaths or drug-related findings of clinical signs, body weight change or food consumption were noted. However, the food consumption of animals in which a decrease in the amount of feces was noted in the general condition was remarkably lower than the other animals. There were many early resorbed embryos and the index of dead embryos/fetuses showed significantly high values in dams at dose of 2.0mg/kg. No significant differences between the control group and each test article treatment group in the number of corpora lutea, number of implantations, number of live fetuses and placental weights were observed. There were no drug-related effects on sex ratio, body weight, external, visceral or skeletal examinations of live fetuses. The NOAEL for dam reproductive ability was 2.0mg/kg and the NOAEL for fetuses was 0.5mg/kg which is 8 and 2 times the MRHD, respectively, based on the BSA. No teratogenicity of MKC-031 (mecasermin) was observed at doses up to 2.0mg/kg.

Special toxicology: Special toxicology studies included the local tolerance study, and the in vitro hemolytic compatibility study with the mecasermin.

Local tolerance studies utilizing intravenous and subcutaneous injection with different formulation were performed in rabbits. In a study with various IGF-1 formulation buffers injected subcutaneously in rabbits, subcutaneous injection of acetate-containing buffers resulted in mild to moderately severe inflammatory changes in the muscular and hypodermal layers in the rabbit. The acetate plus mannitol preparation induced the mildest reaction. Subcutaneous injections of citrate buffers did not induce similar inflammatory changes. In the acute subcutaneous tolerance study, subcutaneous administration of 0.5ml of IGF-1 solution at concentration of 10mg/ml produced no local irritation in rabbits. In the intravenous injection study, administration of IGF-1 solution intravenously via the marginal ear vein for 1 or 5 days did not result in irritation that could be directly associated with the test material when compared with results of the effects of saline injection.

In the hemolytic and compatibility in vitro studies, mecasermin in a vehicle solution at a concentration of 5mg/ml does not cause hemolysis of human or canine erythrocytes and is compatible with human and canine serum and plasma.

2.6.6.2 Single-dose toxicity

Test Article: Mecasermin						
Species/ Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings
Mouse — CD1 (ICR)BR/VAF Plus®	Intravenous	0, 0.01, 0.1, 1.0, 10.0	5 M 5 F	10.0	NA	10.0 mg/kg: NOAEL
Rat/ — CD® BR/VAF Plus®	Subcutaneous	0, 0.1, 0.3, 1.0, 10.0	5 M 5 F	1.0	10.0	Doses ≥ 1.0 mg/kg: Mild to marked decrease in serum glucose Dose of 10.0 mg/kg: Marked decrease in serum glucose. One out of five males died.
Rat/ — CD® BR/VAF Plus®	Subcutaneous	0, 0.1, 0.3, 1.0, 10.0	5 M 5 F	1.0	10.0	Doses ≥ 0.3 mg/kg: Dose-related moderate to marked reductions in serum glucose (15 to 30 minutes postdose) Doses ≥ 1.0mg/kg: Hypoactivity Dose of 10.0 mg/kg: Death (50% of animals) by Day 2; irregular/labored respiration; recumbency; coldness to touch; excessive salivation; red haircoat; red nasal discharge; few or no feces; convulsions; tremors; barely noticeable breathing; gasping; hypersensitivity to touch and sound; arching of back; paddling of hindlimbs; uncoordinated movements; vocalization Most signs of toxicity not evident by Day 2.
Rat/ ● CD® BR/VAF Plus®	Intravenous	0, 0.03, 0.1, 0.3, 1.0	3 M 3 F	1.0	NA	Doses ≥ 0.3 mg/kg: Decreased serum glucose
Rat/Sim(SD)FBR (Albino)	Intravenous	0, 0.01, 0.1, 1.0, 10.0	5 M	1.0	10.0	Doses ≥ 1.0 mg/kg: Decreased activity; decreased serum glucose Dose of 10.0 mg/kg: Deaths (3 out of 15 males), convulsions. No signs of toxicity evident by Day 2.

Best Possible Copy

Single Dose Toxicity (Continued)

Test Article: Mecasermin						
Species/ Strain	Method of Administration	Doses (mg/kg)	Gender and No. per Group	Observed Maximum Nonlethal Dose (mg/kg)	Approximate Lethal Dose (mg/kg)	Noteworthy Findings
Rat/ CD-1 BR/VAF Plus®	Intravenous	0, 0.01, 0.1, 1.0, 10.0	10 M 10 F	1.0	10.0	Doses $\geq$ 0.1 mg/kg: Transient mild-to-marked glucose reductions Doses $\geq$ 1.0 mg/kg: Languidness. Dose of 10.0 mg/kg: Death (3/20 animals), convulsions, salivation
Rat/ CD-1 BR/VAF Plus®	Intravenous	0, 0.001, 0.1	10 M 10 F	0.1	NA	Dose of 0.001 mg/kg: NOEL Dose of 0.1 mg/kg: Decrease in serum glucose. Both test articles produced statistically equivalent effects.
Rabbit NZW	Intravenous	1.0	3 F	1.0	NA	No clinical signs observed
Dog/ Beagle	Subcutaneous	0, 0.1, 0.3, 1.0	3 M 3 F	1.0	NA	All doses well-tolerated. Dose of 0.3 mg/kg: NOEL Dose of 1.0 mg/kg: Transient moderate decrease in serum glucose
Dog/ Beagle	Intravenous	5.0	1 F	NA	5.0	Severe hypoglycemia, semi-rigidity, hypoactivity, prostration, vomiting, convulsions, recumbency, excessive ptialism, injected sclera, moribund sacrifice 6 hours post-dose
Dog/ Beagle	Intravenous	0, 0.005, 0.05, 0.5, 2.5	3 M 3 F	2.5	NA	Doses $\geq$ 0.005 mg/kg: Mild, dose-related clinical signs including injected sclera and red mucous membranes Doses $\geq$ 0.05 mg/kg: Reversible dose-related reductions in serum glucose Dose of 2.5 mg/kg: Hypoactivity All changes observed were reversible by Day 2
Monkey/ Rhesus	Subcutaneous	0.5	3 M	0.5	NA	Hypoglycemia and clinical signs consisting of weak and pale appearance (1 out of 6 monkeys) and vomiting (1 out of 6 monkeys)

2.6.6.3 Repeat-dose toxicity**Four-week repeat dose toxicity study in rats and dogs**

Rats, — CD 10- 15/sex/group (5 for recovery)	4-week	SC	0, 0.2, 0.6, 2.0	• $\uparrow$bw gain in F, $\uparrow$Hb and Ht, Na, and $\downarrow$leukocyte counts and lymphocyte at $\geq$ 0.6mg/kg. • $\uparrow$total protein, A and Cl, and $\downarrow$ glucose, TG and PO₄ at 2.0mg/kg. • Mild extramedullary hematopoiesis in liver and spleen. • All findings were reversible except $\uparrow$ Hb and Ht at 2.0mg/kg. • No antibody titers to rhIGF-1
Rats, — CD 20- 27/sex/group (7 for recovery)	4-week	SC	0, 0.1, 0.3, 1.0	• $\uparrow$ Cl and $\downarrow$ leukocyte and lymphocyte at $\geq$ 0.1mg/kg • $\uparrow$ bw gain, T, A, Ca and $\downarrow$ glucose, TG and PO₄ in M and F at $\geq$0.3mg/kg • $\uparrow$bw, Hb, Ht and $\downarrow$ K at 1.0mg/kg • All changes were reversible • No rhIGF-1 related gross or microscopic organ/tissue changes • No antibody titers to rhIGF-1
Dogs Beagle 6/sex/group	4-week	SC	0, 0.1, 0.3, 1.0	• $\uparrow$Cl, $\downarrow$ TG, glucose, Ht and eosinophils at $\geq$ 0.3mg/kg • All animals were moribund due to hypoglycemic-induced convulsion at 1mg/kg • Depletion of pancreatic zymogen granules in acini, stress-related lymphoid necrosis in thymus, mesenteric lymph nodes and prescapular lymph nodes at 1mg/kg • No antibody titers to rhIGF-1

Study title: A 13-week intravenous toxicity study of MKC-031(mecasermin, IGF-1) in rats with a 5-week recovery period.

Key study findings:

- Increased body weight and food consumption were noted in all treated groups
- Increased inorganic phosphate was noted in males at dose of 4mg/kg/day at the end of treatment period and not noted at the end of recovery period.
- Increased urine volume and urine excretion of sodium, potassium and chloride in both male and female rats at dose of 4mg/kg/day were observed in Week 13 of administration. No drug-related urinalysis changes were noted in Week 5 of administration or Week 5 of recovery period.
- No drug-related changes of organ weight, gross or histopathological findings were observed either in treatment period or recovery period. The NOAEL was 4mg/kg/day.

Study no.: 96-266-0320 (2T002 (-2153)

Volume #, and page #: E-file

Conducting laboratory and location: ☐ 1

Date of study initiation: January 30, 1992

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: MKC-031, Lot# M6-24 (contains MKC-031 in the aqueous solution at a concentration of 4.87mg/ml), and purity — %.

Methods

Test Groups	Dose Levels (mg/kg)	Concentration of Test Solution (mg/ml)	Sex	Immediate Group		Recovery Group	
				No. of Animals	Animal No.	No. of Animals	Animal No.
Control	0	0	m	10	1001-1010	5	1011-1015
			f	10	1101-1110	5	1111-1115
Low	0.25	0.1	m	10	2001-2010	—	—
			f	10	2101-2110	—	—
Intermediate	1	0.4	m	10	3001-3010	—	—
			f	10	3101-3110	—	—
High	4	1.6	m	10	4001-4010	5	4011-4015
			f	10	4101-4110	5	4111-4115

Doses: 0, 0.25, 1, and 4mg/kg/day for 13 weeks.

Species/strain: Rats/ — CD(SD) SPF from ☐ 1

Number/sex/group or time point (main study): 10/sex/group. 5/sex/group in both control and high dose groups in recovery period.

Route, formulation, volume, and infusion rate: Intravenous, solution, 2.5ml/kg with the rate of 2ml/minute.

Satellite groups used for toxicokinetics or recovery: Not performed

Age: 6 weeks

Weight: Male 186 to 225g, female 138 to 184g

Sampling times: Animals sacrificed at the end of 13-week and at the end of 5-week recovery.

Unique study design or methodology (if any): None

Mortality: Once daily

Clinical signs: Three times a day.

Body weights: Twice a week.

Food consumption: Twice a week.

Ophthalmoscopy: 5 animals in each group were examined in the week 13 of administration and week 5 of recovery period.

EKG: Not performed

Hematology: Samples collected at the end of treatment period and recovery period.

Parameters included RBC, Hb, Hct, MCV, MCH, MCHC, reticulocyte ratio, platelet count, WBC with differential count, PT, APTT and fibrogen.

Clinical chemistry: Samples collected at the end of treatment period and recovery period.

Parameters included GOT, GPT, LDH, ALP, total cholesterol, triglyceride, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, inorganic phosphorus, total protein, albumin, A/G ratio and protein fractions.

Urinalysis: Urine samples were collected on Weeks 5 and 13 of administration and Week 5 of recovery period. Parameters included pH, protein, ketone, glucose, occult blood, bilirubin, urobilinogen, urine color, urine sediments, urine volume (24-hour), specific gravity, sodium, potassium, chloride.

Gross pathology: After blood sample collection, the animals were sacrificed by exsanguinations from the abdominal aorta, after examining for external abnormalities, the major organs and tissues in the cephalic, thoracic and abdominal cavities were examined.

Organ weights (specify organs weighed if not in histopath table): Brain, pituitary, thyroid gland (including parathyroid), salivary glands (submandibular/sublingual), thymus, heart, lung (including bronchial tubes), liver, spleen, kidneys, adrenals, testes, ovaries, prostate, seminal vesicles and uterus.

Histopathology: Adequate Battery: yes (x), no ()—explain

Peer review: yes (x), no ()

Histopathology study was performed in the following tissues and organs:

brain, spinal cord, sciatic nerve, abdominal aorta, heart, trachea, lungs (including bronchial tubes), tongue, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, salivary glands (submandibular/sublingual), liver, pancreas, pituitary, thyroid (including parathyroids), adrenals, thymus, spleen, mesenteric lymph nodes, cervical lymph nodes, kidneys, urinary bladder, testes, epididymis, seminal vesicles, prostate, ovaries, uterus, vagina, mammary glands, skin, eyes, optic nerve, Harderian glands, bone and bone marrow (sternum, femur), femoral muscle, injection site (tail) and any macroscopic lesions.

Results

Mortality: None

Clinical signs: No drug-related clinical signs were noted.

Body weights: Increased body weight and body weight gain were noted in both males and females in all treated groups. Data were summarized in the following tables.

Table 2-1

A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks

Body weight (Administration period)

Male

Dose mg/kg	Day of administration											
	0	3	7	10	14	17	21	24	28	31	35	38
0	No.	16	16	16	16	16	16	16	16	16	16	16
	Mean	206.1	229.5	262.4	288.8	324.3	346.5	376.2	391.6	417.3	430.0	448.1
	S.D.	9.8	11.8	14.9	15.9	18.5	21.9	27.1	29.6	36.9	39.2	41.9
0.25	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	204.7	226.5	262.3	289.6	324.7	348.1	376.9	394.8	420.0	434.6	452.8
	S.D.	12.1	14.2	17.4	19.1	22.6	24.6	26.9	30.6	30.7	30.9	32.7
1	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	206.8	230.1	265.1	292.4	328.5	351.7	380.1	400.2	426.9	438.2	458.1
	S.D.	9.3	11.8	13.0	16.7	17.6	20.1	21.3	23.6	23.8	23.1	24.8
4	No.	15	15	15	15	15	15	15	15	15	15	15
	Mean	206.2	231.0	266.2	291.2	327.5	351.9	380.1	398.3	424.9	437.9	459.3
	S.D.	9.3	12.2	14.4	17.2	19.4	18.7	20.8	23.2	23.1	23.6	26.1

Dose mg/kg	Day of administration											
	42	45	49	52	56	59	63	66	70	73	77	80
0	No.	16	16	16	16	16	16	16	16	16	16	16
	Mean	479.7	493.2	504.1	515.9	525.7	535.4	544.5	551.3	557.9	564.3	573.2
	S.D.	45.8	49.6	52.1	53.6	55.9	58.2	61.7	61.0	63.1	65.9	68.9
0.25	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	482.5	496.7	510.1	519.0	533.1	541.9	554.2	560.9	573.7	579.1	590.0
	S.D.	37.7	40.1	46.2	41.2	43.2	45.6	46.7	47.9	46.0	49.4	50.2
1	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	492.2	507.1	514.8	522.3	541.6	560.7	566.4	574.4	587.7	592.0	604.2
	S.D.	30.8	31.6	43.2	52.4	49.8	46.0	48.6	47.1	48.4	50.9	53.0
4	No.	15	15	15	15	15	15	15	15	15	15	15
	Mean	489.1	502.2	517.3	527.5	541.1	561.6	564.6	571.7	583.2	592.4	603.5
	S.D.	30.2	30.0	29.6	31.0	31.8	33.6	36.3	36.4	39.6	41.8	46.5

Best Possible Copy

Dose mg/kg	Day of administration			Gain 0-91
	84	87	91	
0	No.	15	15	15
	Mean	584.0	588.2	592.9
	S.D.	71.3	73.0	74.7
0.25	No.	10	10	10
	Mean	607.2	618.8	619.8
	S.D.	51.3	51.9	53.5
1	No.	10	10	10
	Mean	621.3	631.4	635.9
	S.D.	56.8	60.3	61.7
4	No.	15	15	15
	Mean	623.9	622.6	638.0
	S.D.	48.2	48.3	60.0

Table 2-4 A 13-week intravenous toxicity study of MRC-031 in rats with a recovery period of 5 weeks
Body weight (Administration period)
Female

Dose mg/kg	Day of administration											
	0	3	7	10	14	17	21	24	28	31	36	39
0	No.	15	15	15	15	15	15	15	15	15	15	15
	Mean	158.1	164.0	176.1	185.1	200.7	212.6	225.1	235.9	244.9	246.8	255.6
	S.D.	12.5	12.4	14.5	15.3	19.5	21.9	23.9	23.5	27.5	25.2	26.9
0.25	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	155.1	167.9	182.8	193.1	210.6	225.1	238.0	248.5	259.9	265.0	275.0
	S.D.	10.6	11.4	13.8	14.2	16.1	18.4	19.8	22.0	22.9	22.3	27.0
1	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	156.0	168.3	183.0	194.9	209.3	222.4	235.2	245.6	257.3	268.6	273.4
	S.D.	9.8	8.9	10.2	14.3	16.2	15.9	17.7	22.1	21.6	19.9	23.6
4	No.	15	15	15	15	15	15	15	15	15	15	15
	Mean	158.3	170.5	185.6	197.4	214.0	224.7	240.3	260.0	264.3	274.5*	284.9*
	S.D.	9.3	11.2	11.7	16.2	17.3	19.5	21.4	23.9	26.1	23.0	24.9

Dose mg/kg	Day of administration											
	42	45	49	52	56	59	63	66	70	73	77	80
0	No.	15	15	15	15	15	15	15	15	15	15	15
	Mean	258.9	275.9	281.6	284.8	290.5	293.6	297.2	300.1	304.9	307.0	311.7
	S.D.	28.9	31.1	32.3	33.4	33.9	34.1	35.4	37.1	36.9	37.5	38.5
0.25	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	294.6	299.2	307.2	311.9	318.2	325.5	329.1	332.5	339.2	342.5	348.9
	S.D.	27.2	29.0	31.6	32.2	29.6	38.1	38.4	38.4	37.8	42.3	40.6
1	No.	10	10	10	10	10	10	10	10	10	10	10
	Mean	288.3	295.4	301.6	307.3	311.7	315.6	322.5	325.6	331.0	334.2	340.6
	S.D.	25.7	25.9	27.8	27.6	24.9	30.7	32.7	30.2	36.6	34.7	35.4
4	No.	15	15	15	15	15	15	15	15	15	15	15
	Mean	302.0*	308.4	314.1	320.2*	330.6*	335.1*	344.1**	348.4*	353.7*	355.2*	361.1*
	S.D.	28.2	32.1	31.8	34.2	36.3	34.0	35.9	37.8	39.0	42.5	42.9

Dose mg/kg	Day of administration			Gain 0-91
	84	87	91	
0	No.	15	15	15
	Mean	319.1	318.7	319.8
	S.D.	41.0	41.7	41.0
0.25	No.	10	10	10
	Mean	355.5	355.3	361.0
	S.D.	43.7	42.1	44.6
1	No.	10	10	10
	Mean	352.1	352.2	364.9
	S.D.	36.5	37.5	38.9
4	No.	15	15	15
	Mean	374.9**	377.1**	380.9**
	S.D.	45.9	42.4	43.5

Unit : g

* : p(0.05) ; ** : p(0.01) (Significant difference from control)

After the 5-week recovery period, increased body weight was noted in both males and females at dose of 4mg/kg compared to the control. However, the body weight gain was lower than

Best Possible Copy

that of the control suggesting a recovery tendency. Data were summarized in the following table.

Table 2-7 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Body weight (Recovery period)

Dose mg/kg		Day of recovery											Gain 1-25
		1	3	7	10	14	17	21	24	28	31	35	
0	No.	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	880.6	863.6	872.6	881.2	888.8	897.2	902.2	898.6	897.2	898.2	924.0	83.4
	S.D.	83.8	86.6	87.2	87.2	90.4	91.6	95.1	96.6	97.2	96.6	100.2	23.7
4	No.	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	533.6	538.4	642.8	647.0	652.2	660.2	662.0	669.0	674.8	675.4	675.4	41.8
	S.D.	80.7	80.3	65.6	66.7	70.5	70.1	71.2	71.5	73.1	70.8	67.4	10.8

Table 2-8 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Body weight (Recovery period)

Dose mg/kg		Day of recovery											Gain 1-25
		1	3	7	10	14	17	21	24	28	31	35	
0	No.	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	309.4	308.0	309.6	314.2	317.2	324.0	322.8	323.0	327.8	328.4	330.6	20.6
	S.D.	27.8	30.4	33.9	33.4	35.6	36.6	38.6	37.4	37.9	40.6	37.6	11.6
4	No.	5	5	5	5	5	5	5	5	5	5	5	5
	Mean	374.6**	376.2**	371.8**	370.0*	372.2*	377.4*	379.6*	382.8*	387.4*	386.0*	390.6*	16.0
	S.D.	26.7	23.5	19.3	17.5	17.8	20.1	22.2	25.8	27.9	25.5	26.8	12.3

Unit: g

*: p<0.05; **: p<0.01 (Significant difference from control)

Food consumption: During the treatment period, the food consumptions in all treated groups tend to be higher than the controls during the treatment period. In the recovery period, food consumption was still higher in females at dose of 4mg/kg/day compared to the controls. Data were summarized in the following tables.

Table 3-1 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Food consumption (Administration period)

Dose mg/kg		Day of administration											
		0	3	7	10	14	17	21	24	28	31	35	38
0	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	26.0	26.1	27.0	28.8	31.2	31.3	32.6	32.2	32.8	31.4	31.7	32.4
	S.D.	2.0	2.2	2.6	2.5	2.5	3.0	3.4	3.7	4.0	3.8	3.7	3.7
0.25	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	25.8	25.4	27.3	28.3	31.8	32.2	33.3	33.4	32.8	31.6	32.4	31.6
	S.D.	2.7	1.6	2.5	2.3	2.4	2.0	1.9	2.7	2.0	1.9	2.7	2.2
1	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	26.0	25.8	28.2	29.6	32.6	32.3	33.9	34.4	33.8	32.6	33.2	34.0
	S.D.	2.0	1.6	1.7	2.8	2.3	2.5	2.5	2.4	2.2	2.6	2.1	2.0
4	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	28.0	25.5	28.0	28.5	31.2	31.7	32.5	32.5	32.9	32.2	33.2	32.9
	S.D.	1.8	1.6	1.4	2.1	2.1	1.8	1.7	1.7	2.3	2.3	2.4	2.5

Unit: g/rat/day

Best Possible Copy

Dose mg/kg	Day of administration												
		42	45	49	52	56	59	63	66	70	73	77	80
0	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	32.5	32.2	31.6	32.1	32.1	31.8	31.4	30.3	30.1	30.4	32.1	31.7
	S.D.	3.9	2.8	3.6	3.4	4.0	4.2	6.0	4.1	3.8	4.2	4.6	4.3
0.25	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	33.3	32.3	31.4	32.3	32.0	32.2	32.4	31.4	31.8	31.7	32.5	32.6
	S.D.	2.6	2.7	2.1	2.6	2.6	2.6	1.9	2.4	2.5	3.3	2.8	2.1
1	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	35.7	33.7	32.6	30.8	34.9	33.0	33.8	32.3	33.9*	32.5	35.1	34.8
	S.D.	2.6	2.5	5.6	10.0	3.8	2.2	3.0	2.4	2.8	3.2	3.0	2.6
4	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	33.8	32.2	32.4	32.6	34.0	32.2	33.0	31.7	32.6	32.2	34.0	33.3
	S.D.	2.1	2.4	2.5	2.8	2.8	2.7	2.7	2.6	2.6	2.4	3.1	3.2

Unit : g/rat/day

* : p<0.05 (Significant difference from control)

Dose mg/kg		Day of administration		
		84	87	91
0	No.	15	15	15
	Mean	29.7	28.7	28.0
	S.D.	7.4	9.0	6.1
0.25	No.	10	10	10
	Mean	32.2	32.4	29.4
	S.D.	2.7	3.2	2.9
1	No.	10	10	10
	Mean	33.3	33.7	31.2
	S.D.	3.0	3.6	2.3
4	No.	15	15	15
	Mean	32.1	32.5	31.0
	S.D.	3.0	3.0	2.7

Table 3-4

A 13-week intravenous toxicity study of MEC-031 in rats with a recovery period of 5 weeks

Food consumption (Administration period)

Female

Dose mg/kg		Day of administration											
		0	3	7	10	14	17	21	24	28	31	35	38
0	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	18.4	18.0	19.4	20.2	21.4	22.3	23.2	23.4	23.2	23.8	23.1	23.3
	S.D.	3.2	2.3	1.5	2.4	3.3	3.0	3.2	2.4	2.9	2.7	3.0	3.4
0.25	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	17.6	19.3	20.0	21.2	22.6	24.0	23.9	23.6	24.3	22.3	24.9	24.9
	S.D.	1.5	2.1	2.0	2.1	2.6	2.2	2.6	2.3	2.5	2.6	2.7	2.9
1	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	18.2	19.4	20.2	21.3	21.6	22.8	23.2	22.9	23.2	21.2	24.3	24.2
	S.D.	2.3	1.3	1.9	2.9	2.3	2.5	2.7	2.9	2.5	2.5	3.0	2.8
4	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	19.3	19.6	20.0	20.8	21.9	23.3	24.1	23.9	24.9	23.6	24.3	24.6
	S.D.	3.1	1.6	2.0	2.6	2.0	2.6	2.9	3.1	2.9	2.6	2.8	3.0

Dose mg/kg		Day of administration											
		42	45	49	52	56	59	63	66	70	73	77	80
0	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	24.9	23.4	22.6	22.5	23.2	22.7	22.7	22.3	22.4	23.4	22.6	22.7
	S.D.	4.1	3.3	5.0	2.0	2.6	2.8	3.1	2.9	2.6	2.6	3.1	2.7
0.25	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	25.7	26.0	24.7	24.1	24.9	25.1	24.1	23.3	23.9	23.4	24.9	24.3
	S.D.	3.0	3.6	3.6	3.0	3.1	4.3	3.1	3.1	3.2	3.4	3.0	3.7
1	No.	10	10	10	10	10	10	10	10	10	10	10	10
	Mean	24.7	23.9	24.1	23.6	24.4	23.7	23.5	23.8	23.0	23.4	24.0	23.4
	S.D.	2.9	3.3	2.7	2.9	2.6	3.2	3.0	2.7	3.1	2.5	2.7	3.1
4	No.	15	15	15	15	15	15	15	15	15	15	15	15
	Mean	25.9	26.0	25.0	25.0	26.2	24.6	25.3	24.4	25.7	24.8*	26.3*	25.8
	S.D.	2.9	3.1	3.2	4.2	3.1	2.5	2.4	3.1	2.6	3.3	3.1	2.8

Unit : g/rat/day

* : p<0.05 (Significant difference from control)

Best Possible Copy

Dose mg/kg		Day of administration		
		54	57	91
0	No.	15	15	15
	Mean	22.1	20.1	19.5
	S.D.	3.1	2.5	2.1
0.25	No.	10	10	10
	Mean	23.5	22.6	21.8
	S.D.	2.9	3.1	2.8
1	No.	10	10	10
	Mean	23.8	22.2	22.2
	S.D.	2.4	2.3	2.6
4	No.	15	15	15
	Mean	25.9*	24.3**	23.4**
	S.D.	3.3	2.9	2.7

Unit : g/rat/day

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Table 3-7 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Food consumption (Recovery period)
Male

Dose mg/kg		Day of recovery									
		3	7	10	14	17	21	24	28	31	35
0	No.	5	5	5	5	5	5	5	5	5	5
	Mean	28.7	30.2	30.9	31.3	32.5	32.3	32.8	31.7	29.7	31.1
	S.D.	5.8	4.7	4.4	4.8	5.8	4.9	8.6	5.3	5.1	5.3
4	No.	5	5	5	5	5	5	5	5	5	5
	Mean	31.1	33.5	33.7	31.0	33.3	32.6	32.5	31.9	29.0	31.1
	S.D.	3.4	2.4	2.5	2.3	2.5	3.2	2.6	2.6	2.1	2.3

Unit : g/rat/day

Table 3-8 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Food consumption (Recovery period)
Female

Dose mg/kg		Day of recovery									
		3	7	10	14	17	21	24	28	31	35
0	No.	5	5	5	5	5	5	5	5	5	5
	Mean	18.3	21.1	30.3	21.5	21.3	22.9	21.3	21.8	20.4	20.0
	S.D.	2.7	1.4	1.0	2.3	3.2	2.4	3.5	2.0	2.0	1.5
4	No.	5	5	5	5	5	5	5	5	5	5
	Mean	22.5	24.8**	23.7*	24.2	24.7	24.6	24.1	24.9*	22.6	23.8**
	S.D.	4.0	1.8	2.8	2.4	1.9	1.3	1.9	2.1	2.0	1.9

Unit : g/rat/day

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Ophthalmoscopy: No drug-related findings either in treatment period or recovery period were noted.

EKG: Not performed

Hematology: There were no drug-related changes of hematological parameters.

Clinical chemistry: Increased inorganic phosphate was noted in males at dose of 4mg/kg/day at the end of treatment period and not noted at the end of recovery period. Data were summarized in the following table.

Best Possible Copy

Table 7-2 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Blood chemistry (13 weeks)
Male

Dose mg/kg	No.		BUN mg/dl	Crea- tinine mg/dl	Na mEq/l	K mEq/l	Cl mEq/l	Ca mg/dl	P mg/dl
0	10	Mean	15.0	0.66	143.1	4.6	118.1	9.2	6.2
		S.D.	2.1	0.04	1.0	0.3	1.6	0.9	0.6
0.25	10	Mean	13.8	0.64	142.9	4.8	117.4	9.2	5.6
		S.D.	2.0	0.06	1.1	0.3	1.5	0.3	0.8
1	10	Mean	14.6	0.65	143.5	4.6	119.0	9.2	6.8
		S.D.	0.7	0.04	1.0	0.2	1.8	0.4	0.4
4	9 ^{a)}	Mean	14.9	0.69	143.9	4.5	118.8	9.2	7.4**
		S.D.	1.0	0.05	1.2	0.2	1.4	0.2	0.6

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

a) : Number of rats examined (data from one male was not available, because overnight fasting prior to necropsy was not completed)

Urinalysis: Increased urine volume and urine excretion of sodium, potassium and chloride in both male and female rats at dose of 4mg/kg/day were observed in Week 13 of administration. No drug-related urinalysis changes were noted in Week 5 of administration or Week 5 of recovery period. Drug-related changed data were summarized in the following tables.

Table 8-12 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Urinalysis (13 weeks)
Male

Dose mg/kg	No.		Urine volume ml/24hrs	S.G.	Na mEq/24hrs	K mEq/24hrs	Cl mEq/24hrs
0	20	Mean	10.6	1.055	1.06	3.46	5.46
		S.D.	7.8	0.013	0.90	1.43	1.96
0.25	20	Mean	20.3	1.053	2.32	4.16	2.96
		S.D.	8.4	0.008	0.45	0.97	0.73
1	20	Mean	31.2	1.058	4.53	4.78	3.94
		S.D.	6.7	0.015	0.99	0.95	0.37
4	20	Mean	24.3	1.053	2.83	5.28*	3.22
		S.D.	4.6	0.005	0.53	0.96	0.65

* : p<0.05 (Significant difference from control)

Table 8-13 A 13-week intravenous toxicity study of MKC-031 in rats with a recovery period of 5 weeks
Urinalysis (13 weeks)
Female

Dose mg/kg	No.		Urine volume ml/24hrs	S.G.	Na mEq/24hrs	K mEq/24hrs	Cl mEq/24hrs
0	10	Mean	10.8	1.053	1.86	2.86	1.89
		S.D.	0.4	0.014	0.42	0.87	0.59
0.25	10	Mean	9.4	1.066	1.33	2.12	1.54
		S.D.	2.3	0.013	0.39	0.81	0.41
1	10	Mean	32.3	1.066	1.32	2.36	2.56
		S.D.	5.7	0.013	0.60	0.93	0.95
4	10	Mean	10.4	1.069	1.36	2.39	2.39
		S.D.	7.3	0.014	0.30	1.05	0.72

Gross pathology: No drug-related macroscopic findings were noted in either at the end treatment period or recovery period.

Organ weights (specify organs weighed if not in histopath table): No drug-related changes of organ weight including brain, pituitary, thyroid gland (including parathyroid), salivary glands (submandibular/sublingual), thymus, heart, lung (including bronchial tubes), liver, spleen, kidneys, adrenals, testes, ovaries, prostate, seminal vesicles and uterus were noted in either at the end of treatment period or recovery period.

Best Possible Copy

Histopathology: There were no drug-related histopathological findings were noted either in treatment period or recovery period.

Toxicokinetics: Not performed

Study title: A 13-week intravenous toxicity study of MKC-031(mecasermin, IGF-1) in dogs with a 5-week recovery period.

Key study findings:

- Two females died at dose of 0.5mg/kg/day and the exact reasons were not clear may be related to hemorrhage in central nervous system, heart, lung and many other organs. Clinical symptoms and signs included a decrease in spontaneous movement, sitting, staggering gait, salivation, redness of the conjunctive and oral mucosa, prone or lateral position and xeransis of the muzzle, as well as cephalic tremor, tachypnea, and clonic or tonic convulsion. Pathological findings were included retention of foam in the trachea and partial dark red change in the thymus, lung, and heart under macroscopic observation and hemorrhagic changes in a number of organs and tissues including lung, heart, GI tracts and central nervous system under the microscopic observations.
- In surviving animals, drug-related clinical signs included decrease in spontaneous movement in Week 5 of administration in one female (#4105) and a single incidence of conjunctival redness in Week 12 of administration in one male (#4003) at dose of 0.5mg/kg/day. In addition, a similar clinical symptom and sign to that observed in the animals that died was noted in 1 female (#4102) at dose of 0.5mg/kg/day on 2 days before necropsy in Week 13 of administration. For all of other animals throughout the administration and recovery periods, a dose-dependent increase of vomiting of feed and foam, loose stools, mucous stools or bloody stools were observed in all animals included the controls.
- There was a tendency toward to increase in body weight and body weight gain in both males and females at dose of 0.5mg/kg/day. No drug-related changes of food and water consumption, urinalysis, ophthalmolog, EKG, blood pressure or body temperature were noted.
- There was a significant shortening of the APTT in males at dose of 0.5mg/kg/day and a significant lengthening of the prothrombin time in females at dose of 0.5mg/kg in Week 13 of administration in comparison to the control group. In the clinical chemistry, no drug-related changes of clinical chemistry parameters in either treatment period before administration or recovery period. However, in the examination conducted 30 minutes after administration, significantly low values or a tendency toward low values for glucose, potassium and inorganic phosphorus were observed in both males and females in all treated groups.
- In pathology study, a dark red change in all the lobes of the lung was noted in one female (#4102) at dose of 0.5mg/kg/day, and a tendency of dose-dependent increase of the adrenal gland weight was noted in treated males and females during the treatment period. Histopathological findings in the surviving animals at the end of treatment period included cerebrum, cerebellum, medulla oblongata, heart, lungs, stomach, cecum, and liver in animals at dose of 0.5mg/kg/day. Most of these findings are related to slight to moderate hemorrhage and/ or slight lymphocyte infiltration.
- The NOAEL was 0.125mg/kg in this 13-week study based on the histopathological findings.

Study no.: 95-354-0320

Volume #, and page #: E-file

Conducting laboratory and location: L

J

Date of study initiation: February 23, 1993

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: MKC-031, Lot# M6-29 (contains MKC-031 in the aqueous solution at a concentration of 6.0mg/ml), and purity: — %.

Methods

Test Group	Dose Level (mg/kg)	Sex	13-Week Administration Group		5-Week Recovery Group	
			No. of Animals	Animal No. (2417-)	No. of Animals	Animal No. (2417-)
Control	0	m	3	1001-1003	2	1004-1005
		f	5	1101-1105		
Low	0.025	m	3	2001-2003		
		f	3	2101-2103		
Intermediate	0.125	m	3	3001-3003		
		f	3	3101-3103		
High	0.5	m	3	4001-4003	2	4004-4005
		f	5	4101-4105		

Doses: 0, 0.025, 0.125, and 0.5mg/kg/day for 13 weeks.

Species/strain: Beagle dogs L J from L

J

Number/sex/group or time point (main study): 3/sex/group in low and middle dose groups and in male dogs in high dose group; 5/sex/group in female dogs in high dose group in treatment period (as shown above table).

Route, formulation, volume, and infusion rate: Intravenous, solution, 1ml/kg with the rate of 20 to 30ml/minute.

Satellite groups used for recovery: 2/sex/group in both control and high dose groups in male dogs in recovery period (as shown in above table).

Age: 7 months

Weight: Male 7.9-11.4kg, female 7.3 to 11.3kg

Sampling times: Animals sacrificed at the end of 13-week and at the end of 5-week recovery.

Unique study design or methodology (if any): None

Mortality: 5 times daily

Clinical signs: 5 times daily

Body weights: Weekly

Food consumption: Daily

Water consumption: Weekly

Ophthalmoscopy: 1 week before the start of administration, in weeks 4 and 13 of administration (before dosing) and in week 5 of recovery (morning).

EKG: 2 weeks before the start of administration, in weeks 4 and 12 of administration (before dosing) and in week 5 of recovery (morning).

Blood pressure and Body temperature: Blood pressure and rectal temperature measured in 2 weeks before the start of administration, in weeks 2, 4, 8, and 12 of administration (before dosing) and in weeks 5 of recovery (morning)

Hematology: Samples collected at 2 weeks and 1 week before the start of administration, in Weeks 4 and 13 of administration and Week 5 of recovery period. Parameters included RBC, Hb, Hct, MCV, MCH, MCHC, reticulocyte ratio, platelet count, WBC with differential count, PT, APTT and fibrogen.

Clinical chemistry: Samples collected at 2 weeks and 1 week before the start of administration, in Weeks 4 and 13 of administration and Week 5 of recovery period. Parameters included GOT, GPT, ALP, total cholesterol, triglyceride, phospholipids, total bilirubin, glucose, blood urea nitrogen, creatinine, sodium, potassium, chloride, calcium, magnesium, inorganic phosphorus, total protein, albumin, A/G ratio and protein fractions.

Urinalysis: Samples collected at 2 weeks and 1 week before the start of administration, in Weeks 4 and 13 of administration and Week 5 of recovery period. Parameters included pH, protein, ketone, glucose, occult blood, bilirubin, urobilinogen, urine color, urine sediments, urine volume (24-hour), specific gravity, osmotic pressure, sodium, potassium, chloride.

Gross pathology: Organs and tissues in the cephalic, thoracic and abdominal cavities were examined for signs of toxicity. Dead animals were subjected to necropsy immediately after discovery.

Organ weights (specify organs weighed if not in histopath table): Brain, pituitary, thyroid gland (including parathyroid), salivary glands (submandibular/sublingual), thymus, heart, lung (including bronchial tubes), liver, spleen, kidneys, pancreas, adrenals, testes, epididymides, ovaries, prostate, and uterus.

Histopathology: Adequate Battery: yes (x), no ()—explain
Peer review: yes (x), no ()

Histopathology study was performed in the following tissues and organs:

cerebrum, cerebellum, medulla oblongata, pituitary, thyroid gland, parathyroid, thymus, trachea, lungs (including bronchi), heart, liver, gallbladder, kidneys, pancreas, adrenals, testes, epididymides, ovaries, esophagus, stomach, duodenum, jejunum, ileum, cecum, colon, rectum, prostate, uterus, vagina, submandibular gland, sublingual gland, parotid gland, spleen, spinal cord, eyeball, optic nerve, lacrimal gland, urinary bladder, mesenteric lymph node, submandibular lymph node, ribs, sternum (including bone marrow), femur (including bone marrow), skeletal muscle (femoral region), sciatic nerve, aorta (aortic arch), mammary gland, skin (hypogastrium), straight abdominal muscle, tongue, larynx, auricle (including tattooed number) and the administration site (subcutaneous tissue and veins)

Toxicokinetics: Samples collected before dosing and at 2, 10, 15 and 30 minutes, 1, 3, 6, and 24 hours after dosing on the starting day of administration and in week 12 of administration via cephalic vein.

Others: Samples for antibody production test were collected from the cephalic vein at the time points of before the start of administration, in weeks 1, 2, 4, 8 and 13 after the start of administration (before dosing) and in week 5 of recovery (morning).

Results

Mortality: Two females (#4101 and 4103) at dose of 0.5mg/kg died 3 hours after dosing on day 49 of administration and before dosing on day 82 of administration, respectively. The clinical signs in these two animals before death included a decrease in spontaneous movement, sitting, staggering gait, salivation, redness of the conjunctive and oral mucosa, prone or lateral position and xeransis of the muzzle after each dosing from week 3-4 of administration, as well as cephalic tremor, tachypnea, and clonic or tonic convulsion. These symptoms and signs were mainly transient changes that disappeared within 4-5 hours after dosing. In the condition of animal #4101 on the day it died, salivation, vomiting, tachypnea or oligopnea, prone position, conjunctival redness and tonic convulsions were seen around 1 hour after dosing, and the animal then exhibited clonic or tonic convulsions and died 3 hours after administration.

Clinical signs: Of the surviving animals, decrease in spontaneous movement in Week 5 of administration was observed in one female (#4105) and a single incidence of conjunctival redness in Week 12 of administration was noted in one male (#4003) at dose of 0.5mg/kg/day. In addition, a similar clinical symptom and sign to that observed in the animals that died was noted in 1 female (#4102) at dose of 0.5mg/kg/day on 2 days before necropsy in Week 13 of administration. For all of other animals throughout the administration and recovery periods, a dose-dependent increase of vomiting of feed and foam, loose stools, mucous stools or bloody

stools were observed in all animals included the controls. Data were summarized in the following tables.

Table 1 A 13-week intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

2417

General condition

Sex : Male

Dose (mg/kg)	Animal number	-1*	Dosing period (weeks)											Recovery period (weeks)						
			1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5
0	1001	-	-	-	-	-	-	-	-	-	-	-	B	-	-					
	1002	-	-	A	-	-	-	-	-	-	-	-	-	-	-					
	1003	-	-	-	A	-	-	-	-	-	-	-	-	-	-					
	1004	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	1005	-	-	-	-	-	-	-	-	-	-	-	B	-	-					
0.025	2001	A	-	-	-	-	-	-	-	-	-	-	-	-	A					
	2002	A	-	-	A	-	-	-	-	-	-	-	-	-	-					
	2003	-	-	A	-	-	-	-	-	-	-	-	-	-	-					
0.125	3001	B	A	-	-	AB	B	-	-	B	-	-	B	-	BC					
	3002	-	-	-	-	-	-	-	-	-	-	-	-	-	-					
	3003	-	-	-	-	-	-	-	B	B	B	-	-	-	-					
0.5	4001	-	-	-	-	A	-	-	-	-	-	-	A	-	-					
	4002	-	-	-	-	A	-	-	-	-	-	-	-	-	-					
	4003	-	A	-	-	-	-	-	-	-	-	-	-	E	-					
	4004	-	A	-	-	-	-	-	-	A	-	-	-	A	-		E	-	-	-
	4005	-	A	-	-	-	-	-	-	-	C	-	A	-	-		-	-	-	-

* : 1 week before dosing

- : Normal

A : Vomiting

B : Loose stool or mucous stool

C : Bloody stool

E : Conjunctival redness

Table 2 A 13-week intravenous toxicity study of MRN-021 in beagle dogs with a recovery period of 5 weeks

2417

General condition

Sex : Female

Dose (mg/kg)	Animal number	-1*	Dosing period (weeks)													Recovery period (weeks)				
			1	2	3	4	5	6	7	8	9	10	11	12	13	1	2	3	4	5
0	1101	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-
	1102	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-
	1103	-	-	-	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-
	1104	-	-	-	-	-	B	-	-	-	-	AQ	B	-	-	-	-	-	-	-
	1105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.025	2101	-	-	-	-	-	B	B	-	-	-	-	-	-	A	-	-	-	-	-
	2102	-	-	-	-	-	-	-	Q	Q	-	Q	Q	-	-	-	-	-	-	-
	2103	-	-	-	-	-	-	B	-	-	-	-	-	-	-	-	-	-	-	-
0.125	3101	-	-	-	-	-	-	-	C	-	-	Q	Q	Q	-	-	-	-	-	-
	3102	-	-	A	A	-	-	-	A	-	-	-	-	A	-	-	-	-	-	-
	3103	-	-	-	-	-	-	B	A	-	-	-	-	-	-	-	-	-	-	-
0.5	4101a)	-	-	-	-	-	DEFG	EHJJ	EGHI	AEFGH	ABGJ	-	-	-	-	-	-	-	-	-
	4102	-	-	-	-	-	A	-	-	-	-	-	-	-	-	-	-	GHJJ	LOQ	-
	4103b)	-	-	-	A	AGN	EHJK	EGHI	AEFGH	EFQHI	EGHI	EHJJ	AEMI	AEMI	DEGH	-	-	-	-	-
	4104	-	-	-	-	-	L	JK	JKLNO	JLNO	JKL	L	JL	JL	JLNO	-	-	-	-	-
	4105	-	-	-	-	-	-	I	-	-	-	A	-	-	-	-	-	-	-	-

* : 1 week before dosing

- : Normal

A : Vomiting

B : Loose stool or mucous stool

C : Bloody stool

D : No defecation

E : Conjunctival redness

F : Redness of oral mucosa

G : Salivation

H : Xerostomia of muzzle

I : Decrease in spontaneous movement or sitting

J : Prone position or lateral position

K : Staggering gait

L : Cephalic tremor

M : Clonic convulsion

N : Tonic convulsion

O : Tachypnea

P : Oligopnea

Q : Estrus hemorrhage

a) : Died on day 49

b) : Died on day 82

Body weights: There was a tendency toward to increase in body weight and body weight gain in both males and females at dose of 0.5mg/kg/day. Data were summarized in the following tables.

Best Possible Copy

Table 3 A 13-week intravenous toxicity study of MMN-031 in beagle dogs with a recovery period of 5 weeks

P-2417

Body weight (kg)

Sex : Male

Dose (mg/kg)	Animal number	Dosing period (weeks)											
		-1	0	1 day	1	2	3	4	5	6	7	8	9
0	1001	11.5	11.3	11.1	11.6	11.8	11.8	11.9	12.5	12.6	12.6	13.1	13.1
	1002	10.7	11.0	10.7	10.9	11.1	11.0	10.9	11.1	11.1	11.5	11.6	11.5
	1003	7.9	7.9	8.2	8.3	8.2	8.5	8.3	8.6	8.6	8.7	9.1	9.2
	1004	11.0	11.1	11.0	11.1	11.2	11.4	11.2	11.7	11.9	11.2	11.7	11.9
	1005	9.8	9.7	9.7	10.0	10.1	10.6	10.4	10.8	10.9	11.0	11.3	11.0
	Mean	10.2	10.2	10.1	10.4	10.5	10.6	10.5	10.9	11.0	11.0	11.4	11.4
	S.D.	1.4	1.4	1.2	1.3	1.4	1.2	1.4	1.5	1.5	1.4	1.4	1.4
0.025	2001	11.2	11.4	11.3	11.7	12.0	12.4	11.6	12.2	12.6	12.4	12.7	12.5
	2002	9.8	9.7	9.7	10.2	10.2	10.4	10.4	10.8	11.0	11.2	11.2	11.3
	2003	10.2	10.1	10.1	10.6	10.7	11.2	11.0	11.6	11.8	11.3	11.6	11.8
	Mean	10.4	10.4	10.4	10.8	11.0	11.3	11.0	11.5	11.8	11.6	11.8	11.9
	S.D.	0.7	0.9	0.8	0.8	0.9	1.0	0.6	0.7	0.8	0.7	0.8	0.6
0.125	3001	8.8	8.9	8.8	9.0	9.4	9.4	9.4	9.8	9.8	9.9	9.8	10.1
	3002	10.6	10.8	10.5	11.4	11.4	11.5	11.5	11.8	12.1	12.2	12.2	12.3
	3003	11.0	10.9	10.8	11.5	11.6	11.9	11.9	12.2	12.3	12.7	12.7	12.7
	Mean	10.2	10.2	10.1	10.6	10.8	10.9	10.9	11.3	11.4	11.5	11.6	11.7
	S.D.	1.1	1.1	1.0	1.4	1.2	1.3	1.3	1.3	1.4	1.5	1.6	1.4
0.5	4001	10.6	10.7	10.7	11.3	11.5	11.8	12.0	12.0	12.4	12.6	13.0	12.8
	4002	9.5	9.2	9.3	9.4	9.9	10.2	10.4	10.5	10.8	11.0	11.0	10.8
	4003	11.0	11.0	11.1	11.6	11.9	11.9	12.1	12.4	12.5	12.5	12.0	12.5
	4004	9.8	10.1	10.3	11.0	11.4	11.6	11.8	12.3	12.9	12.7	12.8	13.1
	4005	10.4	10.5	10.5	11.2	11.2	11.5	11.5	11.7	12.0	12.0	11.5	11.9
	Mean	10.3	10.3	10.4	10.9	11.2	11.4	11.5	11.8	12.1	12.2	12.1	12.2
	S.D.	0.5	0.7	0.7	0.9	0.8	0.7	0.7	0.7	0.8	0.7	0.8	0.9

Dose (mg/kg)	Animal number	Dosing period (weeks)					Gain 0-13	Recovery period (weeks)					Gain 1-5
		10	11	12	13	1		2	3	4	5		
0	1001	13.4	13.5	13.4	13.6	2.3							
	1002	11.6	11.8	11.5	11.7	0.7							
	1003	9.1	9.4	9.3	9.7	1.8							
	1004	12.2	12.5	12.2	12.3	1.2	12.5	12.5	13.0	12.9	13.0	0.8	
	1005	11.3	10.9	11.0	11.2	1.5	10.8	10.8	10.9	10.9	10.9	0.6	
	Mean	11.6	11.6	11.5	11.7	1.5	11.7	11.7	12.0	11.9	12.0	0.3	
	S.D.	1.6	1.6	1.5	1.4	0.6	1.1	1.2	1.5	1.4	1.5	0.4	
0.025	2001	13.0	13.0	12.9	13.0	1.6							
	2002	11.4	11.1	10.8	11.3	1.6							
	2003	11.8	11.8	11.9	12.0	1.9							
	Mean	12.1	12.0	11.9	12.1	1.7							
	S.D.	0.8	1.0	1.1	0.9	0.2							
0.125	3001	10.2	10.3	10.1	10.0	1.1							
	3002	12.5	12.6	12.7	13.0	2.2							
	3003	12.7	12.3	12.7	13.1	2.2							
	Mean	11.8	12.1	11.8	12.0	1.8							
	S.D.	1.4	1.6	1.5	1.8	0.5							
0.5	4001	12.8	13.0	13.1	13.4	2.7							
	4002	11.0	11.3	11.3	11.4	2.2							
	4003	13.0	13.6	13.4	13.8	2.8							
	4004	13.3	13.4	13.5	13.7	3.6	13.0	13.0	12.5	12.6	12.9	-0.1	
	4005	12.3	12.4	12.5	12.7	2.2	12.4	12.3	12.4	12.6	13.8	0.4	
	Mean	12.5	12.7	12.8	13.0	2.7*	12.7	12.7	12.5	12.6	12.9	0.2	
	S.D.	0.8	0.9	0.9	1.0	0.5	0.4	0.5	0.1	0.0	0.1	0.4	

* : p<0.05 (Significant difference from control)

Best Possible Copy

Table 3 A 13-week intravenous toxicity study of MNX-031 in beagle dogs with a recovery period of 5 weeks

S-2417

Body weight (kg)

Sex : Female

Dose (mg/kg)	Animal number	Dosing period (weeks)										
		-1	0	1 day	1	2	3	4	5	6	7	8
0	1101	11.3	11.3	11.2	11.2	11.4	11.8	11.8	11.9	12.3	12.5	12.5
	1102	7.3	7.3	7.4	7.4	7.5	7.6	7.7	7.8	7.8	8.1	8.2
	1103	8.5	8.2	8.1	8.4	8.3	8.8	9.6	9.0	9.0	9.0	9.2
	1104	8.8	8.8	8.7	9.0	9.0	9.9	9.1	9.2	9.4	9.7	9.9
	1105	7.6	7.8	7.7	7.6	8.1	8.5	8.3	8.4	8.2	8.5	8.7
	Mean	8.7	8.7	8.6	8.8	8.9	9.1	9.1	9.2	9.3	9.6	9.7
	S.D.	1.8	1.6	1.5	1.5	1.5	1.6	1.6	1.8	1.8	1.7	1.8
0.025	2101	9.8	9.5	9.2	8.9	9.8	9.8	9.3	9.8	10.0	9.9	10.0
	2102	7.1	7.5	7.2	7.7	7.9	7.5	7.5	8.1	8.5	8.7	8.5
	2103	8.9	9.0	8.9	9.1	9.3	9.3	8.9	9.4	9.5	9.6	9.7
	Mean	8.5	8.7	8.4	8.9	9.0	8.9	8.6	9.1	9.3	9.4	9.5
	S.D.	1.3	1.0	1.1	1.1	1.0	1.2	0.9	0.9	0.8	0.6	0.8
0.125	3101	9.4	9.4	9.9	9.8	9.9	10.0	10.0	9.8	9.8	10.5	10.4
	3102	7.9	8.3	7.9	8.3	8.6	8.7	8.7	8.8	9.1	9.4	9.5
	3103	8.1	8.1	8.1	8.4	8.6	8.6	8.6	9.1	9.3	9.4	9.3
	Mean	8.5	8.6	8.4	8.8	9.0	9.1	9.2	9.3	9.4	9.8	9.7
	S.D.	0.8	0.7	0.8	0.8	0.8	0.8	1.0	0.6	0.4	0.6	0.6
0.5	4101a)	8.0	8.2	8.1	8.4	8.9	9.3	8.7	9.3	9.3	9.9	11.6
	4102	9.0	8.9	9.0	9.2	10.1	10.3	10.3	10.5	10.9	11.1	11.2
	4103b)	7.9	8.3	8.2	8.9	9.0	9.2	8.9	9.5	9.0	9.8	10.0
	4104	8.7	8.7	8.8	9.1	9.2	9.5	9.4	9.6	9.5	9.4	10.0
	4105	8.8	8.9	8.8	8.2	9.5	9.7	9.9	10.2	10.3	10.4	10.4
	Mean	8.5	8.6	8.6	9.1	9.4	9.6	9.4	9.6	9.8	9.9	10.4
	S.D.	0.5	0.3	0.4	0.5	0.5	0.4	0.7	0.5	0.8	0.6	0.8

a) : Died on day 49

b) : Died on day 82

Dose (mg/kg)	Animal number	Dosing period (weeks)					Gain 0-13	Recovery period (weeks)					Gain 1-5
		10	11	12	13			1	2	3	4	5	
0	1101	12.6	12.7	12.8	12.0	1.7							
	1102	8.1	8.1	7.9	5.7	0.4							
	1103	9.2	9.4	9.5	9.6	1.4							
	1104	9.9	10.0	9.7	10.0	1.2							
	1105	8.7	8.5	8.4	8.5	0.7							
	Mean	9.7	9.7	9.6	9.8	1.1							
	S.D.	1.8	1.8	1.8	2.0	0.5							
0.025	2101	10.1	10.1	10.0	10.3	0.8							
	2102	8.6	8.9	8.9	8.7	1.2							
	2103	9.8	10.0	9.8	9.6	0.6							
	Mean	9.4	9.7	9.6	9.5	0.9							
	S.D.	0.8	0.7	0.6	0.8	0.3							
0.125	3101	11.0	10.8	10.8	10.8	1.4							
	3102	9.4	9.6	9.5	9.6	1.3							
	3103	9.4	9.4	9.2	9.4	1.3							
	Mean	9.9	9.9	9.6	9.9	1.3							
	S.D.	0.9	0.8	0.9	0.8	0.1							
0.5	4101a)	11.9	11.8	12.1	12.5	3.6							
	4102	10.3	10.5										
	4103b)	9.7	9.8	9.8	9.9	1.2							
	4104	10.4	10.6	10.5	10.7	1.8							
	Mean	10.6	10.7	10.8	11.0	2.2							
	S.D.	0.9	0.8	1.2	1.3	1.2							

a) : Died on day 19

b) : Died on day 32

After the 5-week recovery period, no drug-related change of body weight was noted in high dose group vs control group.

Food consumption: No drug-related changes of food consumption were observed.

Water consumption: No drug-related changes of water consumption were observed.

Ophthalmoscopy: No drug-related findings either in treatment period or recovery period were noted.

EKG: No drug-related findings either in treatment period or recovery period were noted.

Best Possible Copy

Blood pressure and body temperature: No drug-related findings either in treatment period or recovery period were noted.

Hematology: There was a significant shortening of the APTT in males at dose of 0.5mg/kg/day and a significant lengthening of the prothrombin time in females at dose of 0.5mg/kg in Week 13 of administration in comparison to the control group. No other drug-related changes were noted. Data were summarized in the following tables.

Table 4: A 13-week intravenous toxicity study of MNM-031 in beagle dogs with a recovery period of 5 weeks

9-2417

Hematology
Sex : Male

Dose (mg/kg)	Animal number	Prothrombin time (sec.)					Activated partial thromboplastin time (sec.)				
		Dosing period		Recovery period			Dosing period		Recovery period		
		-2 week	-1 week	4 week	13 week		-2 week	-1 week	4 week	13 week	
0	1001	6.1	6.4	6.2	6.3		15.3	14.2	15.9	13.8	
	1002	6.4	6.5	6.2	6.3		15.0	16.2	17.0	18.3	
	1003	6.2	6.6	6.3	6.4		15.0	16.2	17.0	17.2	
	1004	6.2	6.3	6.1	6.2	6.1	15.2	13.9	14.0	14.4	12.4
	1005	6.2	6.3	6.2	6.3	6.6	17.1	17.8	17.6	17.5	16.3
	Mean	6.2	6.4	6.2	6.3	6.5	15.9	15.1	16.7	16.6	14.5
	S.D.	0.1	0.1	0.1	0.1	0.5	2.4	2.2	1.5	1.6	2.5
0.025	2001	6.8	6.6	6.4	6.7		17.9	14.7	16.4	15.4	
	2002	6.3	6.3	6.3	6.5		18.2	15.9	16.7	16.5	
	2003	6.4	6.3	6.4	6.3		17.7	16.4	15.8	17.7	
	Mean	6.4	6.4	6.4	6.5		17.9	15.0	17.0	16.2	
	S.D.	0.1	0.2	0.1	0.2		0.3	1.3	1.6	1.3	
0.125	3001	6.4	6.4	6.2	6.3		14.0	12.6	14.3	11.9	
	3002	6.2	6.3	6.5	6.5		11.9	13.6	16.1	17.2	
	3003	6.2	6.2	6.3	6.3		14.4	14.6	15.9	14.9	
	Mean	6.3	6.4	6.3	6.4		13.4	14.2	16.4	14.7	
	S.D.	0.1	0.2	0.2	0.1		2.3	1.5	1.0	2.7	
0.5	4001	6.2	6.4	6.2	6.4		13.7	11.5	11.9	10.7	
	4002	6.0	6.7	6.5	6.6		10.7	15.3	18.7	13.1	
	4003	6.5	6.4	6.2	6.6		15.5	13.8	11.8	12.4	
	4004	6.3	6.7	6.3	6.5	6.2	13.4	15.8	18.8	12.9	16.4
	4005	6.6	6.4	6.6	6.6	6.5	12.9	13.7	13.3	11.1	
	Mean	6.4	6.6	6.4	6.5	6.4	13.3	14.0	14.2	12.0**	15.2
	S.D.	0.2	0.2	0.2	0.1	0.2	1.7	1.7	3.1	1.1	1.8

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

Table 5: A 13-week intravenous toxicity study of MNM-031 in beagle dogs with a recovery period of 5 weeks

9-2417

Hematology
Sex : Female

Dose (mg/kg)	Animal number	Prothrombin time (sec.)					Activated partial thromboplastin time (sec.)				
		Dosing period		Recovery period			Dosing period		Recovery period		
		-2 week	-1 week	4 week	13 week		-2 week	-1 week	4 week	13 week	
0	1101	6.6	6.2	6.3	6.2		12.9	8.5	12.8	13.3	
	1102	6.3	6.0	6.2	6.3		17.4	17.2	18.3	19.9	
	1103	6.4	6.4	6.4	6.2		15.3	16.1	15.3	19.6	
	1104	6.1	6.4	6.4	6.3		15.2	16.4	17.3	16.7	
	1105	6.1	6.3	6.2	6.4		14.7	14.0	17.0	14.2	
	Mean	6.3	6.3	6.3	6.3		15.1	14.3	16.3	16.7	
	S.D.	0.2	0.1	0.1	0.1		1.6	3.4	2.3	2.0	
0.025	2101	6.1	6.3	6.0	6.6		14.6	14.4	14.7	14.4	
	2102	6.3	7.0	5.9	7.0		13.7	18.2	11.9	10.2	
	2103	6.5	6.4	6.2	6.4		16.0	15.7	12.9	15.4	
	Mean	6.3	6.6	6.4	6.7		14.8	16.1	12.8	13.3	
	S.D.	0.2	0.4	0.5	0.3		1.7	0.7	1.4	0.9	
0.125	3101	6.3	6.5	6.3	6.3		15.2	15.9	16.6	19.1	
	3102	6.6	6.5	6.8	6.6		17.9	19.0	19.0	17.4	
	3103	6.6	6.3	6.3	6.5		10.7	11.4	11.7	10.8	
	Mean	6.5	6.5	6.5	6.5		14.6	15.4	16.8	15.8	
	S.D.	0.1	0.0	0.0	0.2		2.6	3.8	3.7	4.4	
0.5	4101a)	6.4	6.3	6.0			17.9	18.7	16.1		
	4101	6.3	6.3	6.3	6.1		12.1	14.1	12.1	17.0	
	4102b)	6.7	6.8	6.3			14.6	15.7	16.5		
	4104	6.4	6.4	6.2	6.5		16.3	16.0	14.8	22.4	
	4105	6.1	6.7	6.2	6.7		15.3	14.5	13.6	13.2	
	Mean	6.3	6.4	6.3	6.4		16.2	16.8	14.8	17.5	
	S.D.	0.9	1.2	1.5	0.9		2.1	1.8	1.5	4.6	

* : p<0.05 (Significant difference from control)

a) : Died on day 49

b) : Died on day 52

Best Possible Copy

Clinical chemistry: No drug-related changes of clinical chemistry parameters in either treatment period before administration or recovery period. In the examination conducted 30 minutes after administration, significantly low values or a tendency toward low values for glucose, potassium and inorganic phosphorus were observed in both males and females in all treated groups. In addition, a significant increase of chloride was observed in both males and females at doses of 0.125mg/kg and above. Data were summarized in the following tables.

Table 58 A 13-week intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

2417

Blood chemistry

Sex : Male

Dose (mg/kg)	Animal number	ALP (U/l)					Glucose (mg/dl)				
		Dosing period					Dosing period				
		-2 week	-1 week	4 week	13 1) week	13 2) week	-2 week	-1 week	4 week	13 1) week	13 2) week
0	1001	185.0	189.0	208.0	102.0	93.0	101	112	97	100	89
	1002	278.0	203.0	196.0	121.0	111.0	100	101	91	91	89
	1003	267.0	205.0	193.0	177.0	162.0	94	97	88	99	88
	1004	258.0	260.0	237.0	174.0	170.0	82	99	93	87	88
	1005	236.0	242.0	199.0	126.0	111.0	106	103	92	97	97
Mean		245.0	232.0	203.2	142.0	129.6	99	102	92	95	92
S.D.		36.4	33.3	21.3	32.5	34.6	6	6	3	6	5
0.025	2001	225.0	195.0	175.0	116.0	113.0	102	106	89	100	75
	2002	275.0	240.0	212.0	125.0	113.0	111	99	92	91	73
	2003	272.0	259.0	217.0	148.0	140.0	106	99	97	99	83
	Mean	257.3	231.3	201.3	129.7	122.0	106	101	93	97	77*
	S.D.	28.0	32.9	22.9	16.5	15.6	5	4	4	5	5
0.125	3001	213.0	200.0	161.0	140.0	122.0	90	89	86	93	30
	3002	302.0	278.0	263.0	178.0	169.0	94	100	101	100	50
	3003	267.0	252.0	199.0	153.0	141.0	99	108	101	101	43
	Mean	260.7	243.2	207.7	157.0	144.0	94	99	97	97	41**
	S.D.	44.8	39.7	51.5	19.3	22.6	5	10	8	6	10
0.5	4001	189.0	175.0	149.0	106.0	92.0	91	106	89	97	36
	4002	207.0	184.0	177.0	130.0	116.0	103	96	95	92	33
	4003	238.0	230.0	229.0	150.0	149.0	109	109	96	99	27
	4004	209.0	196.0	203.0	149.0	149.0	104	111	95	100	37
	4005	294.0	272.0	243.0	172.0	168.0	97	92	82	94	41
Mean		230.2	211.6	200.0	143.2	134.8	101	103	92	96	35**
S.D.		44.2	40.2	38.5	26.4	30.4	7	8	7	3	5

1) : Before dosing
2) : After dosing
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Table 59 A 13-week intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

2417

Blood chemistry

Sex : Male

Dose (mg/kg)	Animal number	Sodium (mEq/l)					Potassium (mEq/l)				
		Dosing period					Dosing period				
		-2 week	-1 week	4 week	13 1) week	13 2) week	-2 week	-1 week	4 week	13 1) week	13 2) week
0	1001	147	147	149	150	151	4.5	4.1	4.5	4.4	4.4
	1002	148	148	147	150	149	4.4	4.9	4.4	4.5	4.7
	1003	149	147	149	147	149	4.4	4.3	4.0	4.4	4.3
	1004	147	148	149	149	150	4.5	4.3	4.1	4.5	4.5
	1005	146	148	148	149	149	4.7	4.3	4.2	4.4	4.1
Mean		147	148	148	149	150	4.5	4.4	4.2	4.5	4.4
S.D.		1	1	1	1	1	0.1	0.3	0.2	0.1	0.2
0.025	2001	147	151	150	148	150	4.8	4.5	4.4	4.5	4.1
	2002	149	149	151	149	149	4.8	4.6	4.1	4.1	3.4
	2003	148	148	146	148	148	3.9	3.7	3.5	3.9	3.3
	Mean	148	149	149	148	149	4.5	4.3	4.0	4.2	3.6
	S.D.	1	2	3	1	1	3.5	0.5	0.5	0.3	0.4
0.125	3001	146	149	150	146	148	4.1	3.9	3.9	4.0	3.1
	3002	149	148	147	149	151	4.7	4.3	4.3	4.3	3.1
	3003	150	149	150	148	150	4.2	3.8	4.0	4.1	3.2
	Mean	148	148	149	148	150	4.3	4.0	4.0	4.1	3.1**
	S.D.	2	1	2	2	2	0.3	0.3	0.2	0.2	0.1
0.5	4001	149	150	149	147	151	4.4	4.4	4.4	4.4	3.2
	4002	147	147	142	151	152	4.4	4.2	4.5	4.4	3.1
	4003	147	146	149	146	150	4.9	4.5	4.7	5.0	3.3
	4004	149	146	149	150	148	4.4	4.2	4.5	4.6	3.2
	4005	149	147	146	149	148	4.4	4.4	4.8	4.6	3.3
Mean		148	147	147	149	150	4.5	4.3	4.6	4.6	3.2*
S.D.		1	2	2	2	1	0.2	0.1	0.2	0.2	0.2

1) : Before dosing
2) : After dosing
* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Best Possible Copy

Table 84 A 13-week intravenous toxicity study of NKN-031 in beagle dogs with a recovery period of 5 weeks

W-2417

Blood chemistry

Sex : Male

Dose (mg/kg)	Animal number	Chloride (mEq/l)						Calcium (mg/dl)					
		-2 week	Dosing period			Recovery period		-2 week	Dosing period			Recovery period	
			-1 week	4 week	13 (1) week	13 (2) week	5 week		-1 week	4 week	13 (1) week	13 (2) week	5 week
0	1001	115	115	119	115	113		10.4	10.4	10.2	10.4	10.1	
	1002	115	115	120	113	113		9.6	9.8	9.4	9.9	9.8	
	1003	117	116	120	117	114		10.1	10.1	9.3	9.9	10.1	
	1004	118	115	120	115	115	117	10.1	10.4	10.0	9.9	10.1	10.0
	1005	117	115	117	115	113	112	10.5	10.2	9.8	10.1	9.9	10.4
	Mean	117	115	119	115	114	115	10.1	10.2	9.7	10.1	10.0	10.2
0.025	2001	115	117	120	118	120		9.9	9.5	9.5	10.3	9.7	
	2002	118	115	120	110	115		10.2	10.0	10.1	10.3	9.7	
	2003	112	114	115	110	115		10.4	10.2	9.5	10.4	10.1	
	Mean	116	115	118	117	117		10.2	10.1	9.7	10.3	9.8	
	S.D.	2	2	3	4	3		0.3	0.2	0.3	0.1	0.2	
0.125	3001	115	115	122	118	121		9.9	10.1	9.8	9.7	9.6	
	3002	121	121	122	120	121		10.0	9.8	9.7	9.8	10.0	
	3003	115	118	121	117	120		10.6	10.4	10.1	10.2	10.0	
	Mean	115	119	122	118	121**		10.2	10.1	9.8	9.9	9.8	
	S.D.	3	2	1	2	1		0.4	0.3	0.3	0.3	0.3	
0.5	4001	117	118	121	117	119		10.4	10.4	9.8	10.0	10.2	
	4002	116	115	117	117	120		9.9	10.0	9.0	10.1	9.7	
	4003	119	119	119	115	120		10.0	10.3	9.5	9.8	9.9	
	4004	119	117	119	115	121	113	9.9	10.1	9.5	10.3	10.2	10.0
	4005	117	115	115	117	119	116	10.2	10.6	9.7	10.2	10.2	10.2
	Mean	118	117	118	117	120**	115	10.1	10.3	9.5	10.2	10.0	10.1
	S.D.	1	2	2	1	1	2	0.2	0.1	0.3	0.3	0.2	0.1

1) : Before dosing

2) : After dosing

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

Table 85 A 13-week intravenous toxicity study of NKN-031 in beagle dogs with a recovery period of 5 weeks

W-2417

Blood chemistry

Sex : Male

Dose (mg/kg)	Animal number	Phosphorus (mg/dl)						Magnesium (mg/dl)					
		-2 week	Dosing period			Recovery period		-2 week	Dosing period			Recovery period	
			-1 week	4 week	13 (1) week	13 (2) week	5 week		-1 week	4 week	13 (1) week	13 (2) week	5 week
0	1001	7.7	7.5	8.9	8.5	8.6		1.9	1.9	1.9	2.0	1.8	
	1002	7.3	7.4	8.8	8.2	8.4		1.8	1.9	1.8	2.0	1.8	
	1003	7.3	6.9	8.7	8.1	8.0		1.8	2.0	1.7	1.7	1.7	
	1004	7.0	7.3	8.5	8.1	8.0	8.4	1.8	1.8	1.9	1.9	1.8	1.8
	1005	7.3	7.4	8.5	8.7	8.4	8.3	1.6	1.8	1.9	1.9	1.9	2.1
	Mean	7.3	7.3	8.7	8.1	8.1	8.4	1.8	1.9	1.8	1.9	1.8	2.0
0.025	2001	7.0	6.7	8.2	8.0	8.3		1.8	1.9	2.0	2.0	1.8	
	2002	6.5	6.6	8.9	8.6	8.2		1.9	2.2	2.0	2.1	1.8	
	2003	6.6	6.9	8.7	8.2	8.2		2.0	1.9	1.9	2.0	1.9	
	Mean	6.7*	6.4**	8.2	8.6	8.3**		1.9	2.0	2.0	2.0	1.8	
	S.D.	0.3	0.4	0.3	0.4	0.5		0.1	0.2	0.1	0.1	0.1	
0.125	3001	7.7	7.5	8.2	8.7	8.5		1.9	1.7	1.8	1.8	1.7	
	3002	7.5	7.2	7.2	8.3	8.2		1.8	1.7	1.8	1.8	1.7	
	3003	7.3	7.2	6.9	8.2	8.7		2.0	1.8	1.8	1.8	1.7	
	Mean	7.5	7.3	8.0	8.1	8.3**		1.9	1.7	1.8	1.8	1.7	
	S.D.	0.2	0.2	0.2	0.3	0.4		0.1	0.1	0.1	0.1	0.1	
2.5	4001	7.1	7.3	7.6	8.5	8.0		2.1	2.1	2.1	2.1	2.0	
	4002	7.2	6.9	7.2	7.1	8.1		1.8	1.8	1.8	1.8	1.8	
	4003	7.5	6.8	7.3	8.5	8.5		1.8	1.8	2.0	1.9	1.7	
	4004	6.9	6.8	7.3	8.1	8.5	5.2	1.6	1.6	1.7	1.9	1.7	1.9
	4005	6.9	6.8	8.3	8.3	8.8	4.9	1.8	1.7	1.8	1.8	1.6	2.0
	Mean	7.1	6.9	7.0	8.4	8.6**	5.1	1.8	1.8	1.9	2.0	1.7	2.0
	S.D.	0.2	0.3	0.4	0.3	0.7	0.2	0.2	0.2	0.2	0.2	0.2	0.1

1) : Before dosing

2) : After dosing

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

Best Possible Copy

Table 7: A 13-week intravenous toxicity study of MN-031 in beagle dogs with a recovery period of 5 weeks

W-2417

Blood chemistry

Sex: Female

Dose (mg/kg)	Animal number	A/P (U/l)						Glucose (mg/dl)					
		-2 week	Dosing period			Recovery period		-2 week	Dosing period			Recovery period	
			-1 week	4 week	13 1)	13 2)	5 week		-1 week	4 week	13 1)	13 2)	5 week
0	1101	200.0	179.0	152.0	110.0	105.0		97	100	92	90	90	
	1102	259.0	258.0	204.0	127.0	119.0		89	93	83	83	71	
	1103	350.0	328.0	277.0	196.0	183.0		92	100	89	90	82	
	1104	289.0	243.0	206.0	142.0	133.0		94	106	87	98	89	
	1105	186.0	164.0	137.0	112.0	111.0		111	116	103	105	105	
	Mean	256.8	236.4	197.2	137.4	130.6		97	103	91	93	87	
0.025	2101	207.0	177.0	148.0	90.0	100.0		94	94	86	100	68	
	2102	277.0	273.0	307.0	159.0	141.0		84	84	74	84	76	
	2103	281.0	209.0	191.0	166.0	149.0		108	114	102	98	69	
	Mean	255.0	229.7	218.3	135.0	130.0		95	97	86	94	71	
	S.D.	41.6	48.7	82.2	39.0	26.3		12	15	14	9	4	
0.125	3101	230.0	233.0	212.0	134.0	119.0		101	110	98	102	40	
	3102	245.0	273.0	206.0	177.0	156.0		98	98	98	100	45	
	3103	290.0	264.0	239.0	139.0	133.0		99	102	87	95	44	
	Mean	255.3	256.7	219.0	150.0	136.0		99	103	94	99	43	
	S.D.	31.1	21.0	17.8	23.5	18.7		2	6	6	4	3	
0.5	4101a)	171.0	168.0	158.0				87	98	87			
	4102	171.0	171.0	150.0	99.0	96.0		101	103	89	95	29	
	4103b)	285.0	268.0	239.0				107	108	101			
	4104	391.0	350.0	360.0	264.0	266.0		90	95	87	92	25	
	4105	298.0	268.0	222.0	185.0	146.0		88	96	88	99	28	
	Mean	282.2	245.0	226.4	172.7	166.0		95	100	90	95	28**	
	S.D.	83.8	76.6	83.8	83.8	81.9		9	5	5	4	2	

1) : Before dosing

2) : After dosing

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

a) : Died on day 49

b) : Died on day 82

Table 76: A 13-week intravenous toxicity study of MN-031 in beagle dogs with a recovery period of 5 weeks

W-2417

Blood chemistry

Sex: Female

Dose (mg/kg)	Animal number	Sodium (mEq/l)						Potassium (mEq/l)					
		-2 week	Dosing period			Recovery period		-2 week	Dosing period			Recovery period	
			-1 week	4 week	13 1)	13 2)	5 week		-1 week	4 week	13 1)	13 2)	5 week
0	1101	147	148	153	150	150		4.8	4.6	4.3	4.4	4.5	
	1102	147	148	148	147	149		4.8	4.1	4.0	4.6	4.5	
	1103	147	150	151	147	150		4.8	4.2	4.0	4.7	4.4	
	1104	148	148	149	148	148		4.9	4.3	4.2	4.4	4.5	
	1105	149	147	147	147	149		4.9	4.5	4.3	4.4	4.3	
	Mean	148	148	150	148	149		4.8	4.3	4.2	4.5	4.4	
0.025	2101	149	149	146	145	151		4.3	4.2	4.0	4.1	3.7	
	2102	147	144	145	147	147		3.8	3.8	3.9	4.0	3.8	
	2103	152	148	150	150	151		4.4	4.1	4.1	4.1	3.5	
	Mean	149	147	147	149	150		4.2*	4.0	4.0	4.1	3.6**	
	S.D.	3	3	3	2	2		0.3	0.3	0.1	0.1	0.1	
0.125	3101	145	148	149	147	149		4.8	4.4	4.3	4.5	3.5	
	3102	148	149	150	152	153		4.6	4.5	4.7	4.6	3.6	
	3103	148	149	146	148	150		4.5	4.2	4.2	4.3	3.3	
	Mean	147	148	148	149	151		4.6	4.4	4.4	4.5	3.5**	
	S.D.	2	2	2	3	2		0.2	0.2	0.2	0.2	0.2	
0.5	4101a)	150	148	150				4.9	4.5	4.7			
	4102	149	147	150	149	149		4.7	4.5	5.0	4.9	3.5	
	4103b)	147	150	150				4.7	4.5	4.2			
	4104	149	148	152	147	149		4.2	3.9	4.2	4.6	3.4	
	4105	150	152	150	148	150		4.4	4.4	4.3	4.2	3.5	
	Mean	149	149	150	148	149		4.6	4.4	4.5	4.6	3.5**	
	S.D.	1	2	1	1	1		0.3	0.3	0.4	0.3	0.1	

1) : Before dosing

2) : After dosing

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

a) : Died on day 49

b) : Died on day 82

Best Possible Copy

Table 77 A 13-week intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

2417

Blood chemistry

Sex : Female

Dose (mg/kg)	Animal number	Chloride (mEq/l)						Calcium (mg/dl)					
		Dosing period					Recovery period	Dosing period					Recovery period
		-2 week	-1 week	4 week	13 (1) week	13 (2) week		-2 week	-1 week	4 week	13 (1) week	13 (2) week	
0	1101	114	117	118	117	114		10.5	10.3	9.5	10.0	9.9	
	1102	113	113	119	115	113		10.7	10.9	9.7	10.3	10.1	
	1103	114	118	122	117	116		10.1	10.0	9.2	9.9	9.9	
	1104	116	116	119	116	115		10.4	10.5	9.9	10.4	10.4	
	1105	118	118	120	118	115		10.2	10.1	9.5	10.1	9.7	
	Mean	115	116	120	116	114		10.4	10.4	9.6	10.1	10.0	
	S.D.	2	2	2	1	1		0.2	0.4	0.3	0.2	0.3	
0.025	2101	117	114	119	118	117		10.3	10.5	9.8	10.2	9.8	
	2102	119	117	120	112	115		10.2	10.0	9.4	10.0	9.8	
	2103	121	121	121	119	119		10.5	10.5	9.9	10.3	9.9	
	Mean	119	117	120	116	117		10.3	10.3	9.7	10.2	9.8	
	S.D.	2	4	1	4	2		0.2	0.3	0.3	0.2	0.1	
0.125	3101	117	118	120	116	118		10.4	10.6	9.8	10.6	10.3	
	3102	116	116	121	118	120		10.3	10.7	10.1	10.3	10.0	
	3103	114	119	120	119	120		10.6	10.5	9.8	10.2	10.0	
	Mean	116	117	120	118	119**		10.4	10.6	9.9	10.4	10.1	
	S.D.	2	2	1	2	1		0.2	0.1	0.2	0.2	0.2	
0.5	4101a)	117	115	119				10.1	10.3	9.8			
	4102	112	116	119	114	117		10.4	10.3	9.4	10.4	10.1	
	4103b)	116	115	118				10.6	10.7	10.2			
	4104	115	118	119	116	119		10.3	9.9	9.6	10.0	9.9	
	4105	118	115	121	117	121		10.5	10.9	10.4	10.2	10.4	
	Mean	116	116	119	116	119*		10.4	10.4	9.9	10.2	10.1	
	S.D.	2	1	1	2	2		0.2	0.4	0.4	0.2	0.3	

1) : Before dosing

2) : After dosing

* : p<0.05 ; ** : p<0.01 (Significant difference from control)

a) : Died on day 49

b) : Died on day 82

Table 78 A 13-week intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

2417

Blood chemistry

Sex : Female

Dose (mg/kg)	Animal number	Phosphorus (mg/dl)					Magnesium (mg/dl)				
		Dosing period				Recovery period	Dosing period				Recovery period
		-2 week	-1 week	4 week	13 (1) week		-2 week	-1 week	4 week	13 (1) week	
0	1101	7.9	7.3	6.4	5.7	5.6	1.9	1.8	1.9	1.9	1.8
	1102	7.7	7.3	6.4	6.0	5.8	2.0	2.1	2.1	2.2	2.0
	1103	6.6	6.3	5.7	4.8	4.7	2.1	1.9	2.0	2.2	2.2
	1104	7.3	7.1	5.9	5.9	5.8	2.1	2.0	1.9	2.9	2.1
	1105	8.1	7.7	6.5	5.2	5.1	2.1	1.9	1.9	1.9	2.0
	Mean	7.5	7.1	6.2	5.5	5.4	2.0	1.9	2.0	2.0	2.0
	S.D.	0.6	0.5	0.4	0.5	0.5	0.1	0.1	0.1	0.2	0.1
0.025	2101	6.9	7.3	6.0	5.4	4.8	1.9	1.9	1.9	2.0	1.9
	2102	7.4	7.2	6.2	5.9	5.7	2.0	1.9	2.0	1.9	1.8
	2103	7.3	6.7	6.0	5.5	4.0	2.0	1.7	1.9	2.0	1.8
	Mean	7.2	7.1	6.1	5.6	4.8	2.0	1.8	1.9	2.0	1.8
	S.D.	0.3	0.3	0.1	0.3	0.9	0.1	0.1	0.1	0.1	0.1
0.125	3101	7.1	7.0	7.2	6.5	4.5	1.9	1.9	2.0	2.0	1.9
	3102	7.6	7.0	6.5	5.9	3.8	2.1	2.0	2.0	2.0	1.9
	3103	7.2	6.9	5.6	5.4	3.3	2.2	2.0	2.0	2.1	1.9
	Mean	7.3	7.0	6.4	5.9	3.9*	2.1	1.9	2.0	2.0	1.9
	S.D.	0.3	0.1	0.8	0.6	0.6	0.2	0.1	0.0	0.1	0.0
0.5	4101a)	6.9	7.2	7.1			2.1	2.0	2.2		
	4102	7.9	7.8	7.6	6.8	4.8	2.0	2.0	2.0	2.2	2.0
	4103b)	7.2	7.2	6.7			2.0	1.9	1.9		
	4104	6.4	5.9	5.6	5.5	3.8	2.1	2.0	2.1	2.1	1.9
	4105	5.4	6.3	6.4	5.5	3.8	2.1	2.0	2.0	1.9	1.8
	Mean	7.0	6.9	5.7	6.0	4.1	2.1	2.0	2.0	2.1	1.9
	S.D.	0.6	0.8	0.8	0.7	0.6	0.1	0.0	0.1	0.2	0.1

1) : Before dosing

2) : After dosing

* : p<0.05 (Significant difference from control)

a) : Died on day 49

b) : Died on day 82

Urinalysis: A significant increase of Chloride was noted in males at dose of 0.5mg/kg./day as compared with control group. No other drug-related changes were noted.

Gross pathology: In the two females that died at dose of 0.5mg/kg/day, retention of whitish foam in the trachea, dark red changes of part of the inside wall of the left ventricular of the heart, milky white change of part of the membrane in the spleen and dark red change at the injection site were observed in 1 animal (#4101) and dark red change in the thymus and lungs, retention of reddish foam in the trachea and paleness in color of the spleen were observed in the other (#4103), but no fatal change were observed.

In the surviving animals, a dark red change in all the lobes of the lung was noted in one female (#4102) at dose of 0.5mg/kg/day in the treatment period. There were no abnormal findings at the end of recovery period. Data were summarized in the following table.

Table 37 A 13-week intravenous toxicity study of NKX-021 in beagle dogs with a recovery period of 5 weeks

Gross pathological finding

Organ	Sex	Dose (mg/kg)	Male				Female			
			0	0.025	0.125	0.5	0	0.025	0.125	0.5
-findings	animal		1 1 1 1	2 2 2	3 3 3	4 4 4 4	1 1 1 1	2 2 2	3 3 3	4 4 4 4
	number		0 0 0 0	0 0 0	0 0 0	0 0 0 0	0 0 0 0	0 0 0	0 0 0	0 0 0 0
			1 2 3 4 5	1 2 3	1 2 3	1 2 3 4 5	1 2 3 4 5	1 2 3	1 2 3	1 2 3 4 5
Thymus										
-dark red change			-	-	-	-	-	-	-	-
Trachea										
-retention of whitish foam			-	-	-	-	-	-	-	-
-retention of reddish foam			-	-	-	-	-	-	-	-
Lung										
-red spot (2mm in diameter)			-	-	-	-	-	-	-	-
-red spot (8mm in diameter)			-	-	-	-	-	-	-	-
-dark red change in all lobes			-	-	-	-	-	-	-	-
-dark red change			-	-	-	-	-	-	-	-
Heart										
-dark red change of left ventricular at the inside wall in part			-	-	-	-	-	-	-	-
Spleen										
-milky white change of membrane in part			-	-	-	-	-	-	-	-
-pale in color			-	-	-	-	-	-	-	-
Injection site										
-dark red change			-	-	-	-	-	-	-	-

- : Negative + : Positive
 R): Recovery group
 a): Died on day 49
 b): Died on day 52

Best Possible Copy

Organ weights (specify organs weighed if not in histopath table): A tendency of dose-dependent increase of the adrenal gland weight was noted in treated males and females during the treatment period. No drug-related changes of organ weight were noted in recovery period.

Histopathology: In the two females (# 4101, 4103) that died at dose of 0.5mg/kg/day, the histopathology findings included in cerebrum, medulla oblongata, heart, lungs, stomach, duodenum, rectum, liver, thymus, spleen, submandibular lymph node, mesenteric lymph node, ovaries, uterus, and injection site. In cerebrum, mild ischemic change of the nerve cells and focal hemorrhage in the cortex were observed in #4103 and slight perivascular cellular infiltration (lymphocyte and neutrophil) was observed in both animals. In medulla oblongata, slight petechial hemorrhage was observed in both animals. In the heart, slight or mild subendocardial hemorrhage was observed in both animals and mild myocardial degeneration in the papillary muscle and slight hemorrhage in the arterial wall were observed in #4101. In the lungs, slight or mild edema and slight hemorrhage were observed in both animals. In stomach, hemorrhage in the submucosa and muscle layer was observed in both animals and mild necrosis of the mucosal epithelium was observed in #4101 and slight hemorrhage in the arterial wall in #4103. In the duodenum and rectum, moderate necrosis of the mucosal epithelium was observed in #4101. In the liver, moderate congestion and mild hemorrhage were observed in #4101. In the thymus, slight or moderate hemorrhage was observed in both

animals. In the spleen, moderate necrosis of the follicular lymphocytes and mild fibrous thickening of the capsule were observed in #4101. In the submandibular lymph node, moderate blood absorption was observed in #4103. In the mesenteric lymph node, moderate necrosis of the lymphocytes was observed in #4101. In ovaries, slight focal hemorrhage was observed in #4101. In uterus, slight endometrial hemorrhage was observed in both animals. In the injection site, slight hemorrhage was observed in both animals.

In the surviving animals at the end of treatment period, histopathological findings included cerebrum, cerebellum, medulla oblongata, heart, lungs, stomach, cecum, and liver in animals at dose of 0.5mg/kg/day. Most of these findings are related to slight to moderate hemorrhage and or slight lymphocyte infiltration. Data were summarized in the following tables. There were no drug-related histopathological findings in the recovery period.

Table 117 A 13-week intravenous toxicity study of HEN-032 in beagle dogs with a recovery period of 5 weeks
Histopathological finding (13 weeks group)
Sex: MALE

Organs	Dose (mg/kg)	0			0.025			0.125			0.5		
		1	2	3	1	2	3	1	2	3	1	2	3
Findings	Animal number	1	2	3	1	2	3	1	2	3	1	2	3
Liver		0	0	0	0	0	0	0	0	0	0	0	0
Gallbladder		0	0	0	0	0	0	0	0	0	0	0	0
Pancreas		0	0	0	0	0	0	0	0	0	0	0	0
Salivary		0	0	0	0	0	0	0	0	0	0	0	0
-cyst		0	0	0	0	0	0	0	0	0	0	0	0
Thyroid		0	0	0	0	0	0	0	0	0	0	0	0
Parathyroid		0	0	0	0	0	0	0	0	0	0	0	0
Adrenal		0	0	0	0	0	0	0	0	0	0	0	0
Thymus		0	0	0	0	0	0	0	0	0	0	0	0
Spleen		0	0	0	0	0	0	0	0	0	0	0	0
Submandibular lymph node		0	0	0	0	0	0	0	0	0	0	0	0
Mesenteric lymph node		0	0	0	0	0	0	0	0	0	0	0	0
Kidney		0	0	0	0	0	0	0	0	0	0	0	0
-pyelonephritis		0	0	0	0	0	0	0	0	0	0	0	0
-scar		0	0	0	0	0	0	0	0	0	0	0	0
Urinary bladder		0	0	0	0	0	0	0	0	0	0	0	0
-cystitis		0	0	0	0	0	0	0	0	0	0	0	0
Testis		0	0	0	0	0	0	0	0	0	0	0	0
Epididymis		0	0	0	0	0	0	0	0	0	0	0	0
-cellular infiltration in interstitium		0	0	0	0	0	0	0	0	0	0	0	0
-arteritis		0	0	0	0	0	0	0	0	0	0	0	0
Prostate		0	0	0	0	0	0	0	0	0	0	0	0
-cellular infiltration in interstitium		0	0	0	0	0	0	0	0	0	0	0	0
Mammary gland		0	0	0	0	0	0	0	0	0	0	0	0
Skin		0	0	0	0	0	0	0	0	0	0	0	0
Eye		0	0	0	0	0	0	0	0	0	0	0	0
Optic nerve		0	0	0	0	0	0	0	0	0	0	0	0
Lacrimal gland		0	0	0	0	0	0	0	0	0	0	0	0
Stomach (Bone marrow)		0	0	0	0	0	0	0	0	0	0	0	0
Femur (Bone marrow)		0	0	0	0	0	0	0	0	0	0	0	0
Musculus rectus abdominis		0	0	0	0	0	0	0	0	0	0	0	0
-focal necrosis of muscle fiber		0	0	0	0	0	0	0	0	0	0	0	0
Skeletal muscle		0	0	0	0	0	0	0	0	0	0	0	0
Injection site		0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0
-fibrosis		0	0	0	0	0	0	0	0	0	0	0	0

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

Best Possible Copy

Appears This Way
On Original

Table 118 A 13-week Intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks B-2417
 Histopathological finding (13 weeks group)
 Sex : Female

Organs	Dose (mg/kg)	0				0.025			0.125			0.5			
		1	1	1	1	2	2	2	3	3	3	4	4	4	4
-findings	Animal number	1	2	3	4	1	2	3	1	2	3	1	2	3	4
Brain															
-ischemic change of nerve cells		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-local hemorrhage in cortex		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-perivascular cellular infiltration		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerebellum															
-petechial hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla oblongata															
-perivascular cellular infiltration		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-petechial hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spinal cord															
-Cervical nerve		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aorta															
Heart															
-subendocardial hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-myocardial degeneration in papillary muscle		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage in arterial wall		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung															
-fracture		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Liver															
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-local pneumonia		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tongue															
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach															
-hemorrhage in submucosa and muscle layer		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage in arterial wall		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esophagus															
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jejunum															
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ileum															
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cecum															
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-cellular infiltration in submucosa		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colon															
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rectum															
-necrosis of mucosal epithelium		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Submandibular gland															
Salivary gland															
Parotid gland															

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

Table 119 A 13-week Intravenous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks B-2417
 Histopathological finding (13 weeks group)
 Sex : Female

Organs	Dose (mg/kg)	0				0.025			0.125			0.5			
		1	1	1	1	2	2	2	3	3	3	4	4	4	4
-findings	Animal number	1	2	3	4	1	2	3	1	2	3	1	2	3	4
Liver															
-congestion		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-degeneration and hemorrhage in arterial wall		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gallbladder															
-congestion		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pancreas															
-congestion		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid															
-hyperplasia		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal															
-hyperplasia in medulla		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymus															
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spleen															
-necrosis of follicular lymphocytes		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-fibrous thickening of capsule		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Submandibular lymph node															
-blood absorption		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesenteric lymph node															
-necrosis of lymphocytes		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney															
-necrosis of lymphocytes		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urinary bladder															
-local hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Micro															
-subcutaneous hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-agitation		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Salivary gland															
Skin															
Eye															
Optic nerve															
Lacrimal gland															
Ear															
Stomach (bone marrow)															
Femur (bone marrow)															
Paracervical muscle															
Abdominal muscle															
Injection site															
-fibrosis		1	0	0	0	1	0	0	0	1	0	0	0	0	0

0 : No remarkable changes 1 : Slight 2 : Mild 3 : Moderate 4 : Severe
 A : Autopsy L : Local specimens
 A1: Died on day 49
 A2: Died on day 82

Toxicokinetics: Data were not provided.

Others:

Samples for antibody production test: Data were not provided.

Best Possible Copy

Study title: 26-week subcutaneous toxicity study with mecasermin (MKN-031) in rats with a 5-week recovery period

Key finding:

- There were no drug-related clinical observations in any of the animals during the administration or recovery period.
- Dose-dependent increases of body weight gain and food consumption were observed in both males and females at all doses and did not reverse after 5-week recovery.
- Increased MCV, MCH in administration period, and fibrinogen in recovery period in males at dose of 4mg/kg were observed.
- In urinalysis, there were dose-dependent increases of urine volume, sodium, potassium, and chloride excretion in both sexes as compared to the control group after 3 months of administration. In week 5 of recovery, significantly increased sodium and chloride excretion were observed in males at dose 4mg/kg.
- The drug-related changes of organ weights included the increases of thymus, liver and kidney at dose of 4mg/kg in males, and increases of pituitary, salivary gland, liver, and uterus weights at doses of 1 and 4mg/kg and thyroid weight at dose of 4mg/kg in females at the end of treatment period. The increased liver weight in both males and females, and increased pituitary and uterus weights in females were not completely reversible in the recovery period. The changes of thyroid and salivary gland weights were reversible.
- There were no drug-related macroscopic findings. In histopathological study, a dose-dependent increase of the incidence rate of the increased cortex area in thymus was observed in male rats at doses of 1 and 4mg/kg. After 5-week recovery, a slightly decreased cortex area was observed in 1 male at dose of 4mg/kg. No changes in liver, kidney, pituitary, salivary gland or uterine pathology were observed which correlated with the organ weight changes observed.
- The NOAEL was 4mg/kg (assumed the changes of thymus cortex was the pharmacological effect rather than a toxicological effect) based on pathology examination. The systemic exposure was 8X for the maximum recommended dose (120 µg/kg) based on BSA and about 15X based on the Cmax (since the TK was not performed in this study but the plasma concentrations of IGF-1 were measured at pre, post 30 minutes and 24 hours).

Study no: 96-267-0329 (12937)

Volume #, and page #: Volume 1 and Page 1.

Conducting laboratory and location: C

Date of study initiation: February 10, 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity: MKN-031, Lot # M6-46, and — % purity

Formulation/vehicle: Test article was dissolved in acetate buffered solution (pH 5.4) containing — mg sodium acetate, — mg acetate, — mg sodium chloride, and — mg benzyl alcohol per 1ml of water for injection.

Methods (unique aspects):**Main and recovery groups:**

Group	Dose Level (mg/kg)	Concentration (mg/ml)	Sex	Main Group No. of Animals	Main Group Animal No.	Recovery Group No. of Animals	Recovery Group Animal No.
Control	0	0	M	12	1001~1012	6	1013~1018
			F	12	1101~1112	6	1113~1118
Low	0.25	0.25	M	12	2001~2012	—	—
			F	12	2101~2112	—	—
Middle	1	1	M	12	3001~3012	—	—
			F	12	3101~3112	—	—
High	4	4	M	12	4001~4012	6	4013~4018
			F	12	4101~4112	6	4113~4118

Satellite group:

Group	Dose Level (mg/kg)	Concentration (mg/ml)	Sex	Satellite Group No. of Animals	Satellite Group Animal No.
Control	0	0	M	8	1019~1026
			F	8	1119~1126
Low	0.25	0.25	M	8	2013~2020
			F	8	2113~2120
Middle	1	1	M	8	3013~3020
			F	8	3113~3120
High	4	4	M	8	4019~4026
			F	8	4119~4126

Dosing:

Species/strain: Rats/ ~ CD (SD) SPF rats L

J

#/sex/group or time point (main study): 12/sex/group

Satellite groups used for determination of plasma concentration: 8/sex/group

Age: 6 weeks

Weight: 185 to 213 g for male rats, and 147 to 179g for female rats

Doses in administered units: 0, 0.25, 1, 4 mg/kg

Route, form, volume, and infusion rate: Subcutaneous route, solution, 0.01ml/kg

Observations and times:

Clinical signs: 2-3 times a day during administration period and once during recovery period

Body weights: Twice a week for first 13 weeks and then once a week up to the end of the administration period. Day 1, 3 and then twice a week during recovery period.

Food consumption: Once a week

Ophthalmoscopy: During the quarantine/acclimatization period, in months 3, 6 of administration, and in week 5 of recovery.

EKG: Not measured

Hematology: At the end of necropsy. Parameters including RBC, Hb, Hct, MCV, MCH, MCHC, Ret, platelet, WBC counts with differential, PT, APTT, fibrinogen.

Clinical chemistry: At the end of necropsy. Parameters including GOT, GPT, LDH, AKP, total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, BUN, Cr, Na, K, Cl, Ca, P, total protein, albumin, A/G ratio.

Urinalysis: In months 3 and 6 of administration and week 5 of recovery. Parameters including pH, protein, ketone bodies, glucose, occult blood, bilirubin, urobilinogen, urine color, urine sediments, urine volume, specific gravity (S.G), sodium, potassium, chloride, water intake.

Gross pathology: Examinations consisted of an external examination including identification of all clinically recorded lesions as well as a detailed internal examination. A complete necropsy was conducted on all animals and included gross examination of organs and tissues listed in the following table.

Tissues for Gross Evaluation.

Adrenal glands	Ovaries
Aorta	Pancreas
Bone marrow	Parathyroid
Brain	Pituitary
Cervix	Prostate
Clitoral gland	Rectum
Colon	Salivary glands
Cecum	Sciatic nerve
Duodenum	Seminal vesicles
Epididymis	Skeletal muscle
Esophagus	Skin
Eye and optic nerve	Spinal cord
Heart	Spleen
Ileum	Stomach
Jejunum	Testes
Kidneys	Thymus
Larynx	Thyroid
Liver	Tongue
Lungs	Trachea
Lymph nodes	Urinary bladder
Mammary gland	Uterus
Ocular accessory gland	Vagina

Organs weighed: Brain, pituitary, thyroids with parathyroids, salivary glands (submandibular and sublingual glands), thymus, heart, lungs, liver, spleen, kidney, adrenals, testes, ovaries, prostate, seminal vehicle and uterus.

Histopathology: Organs and tissues from animals sacrificed at the end of administration and recovery periods in the control group and high dose group, thymus from all groups, and all of the gross lesions and all the organs and tissues of the dead animal in any of the test groups.

Toxicokinetics: Not performed. Plasma concentrations of test article and blood glucose were measured at before dosing, 30 minutes and 24 hours after dosing on the starting day of administration, and in week 26 of administration.

Other: Determination of antibody titer at the end of administration and recovery period.

Results:

Mortality: One male rat at dose 0.25mg/kg (No. 2005) died on day 140 before dosing with no abnormalities in the clinical observation

Clinical signs: No drug-related clinical signs were observed.

Body weights: There were dose-dependent increases of body weight at doses of 0.25mg/kg or higher. The statistically increased body weight was observed on days 119, 133 of administration for males and on day 112 for females at dose of 0.25mg/kg, from day 56 of administration for males and on days 119, and 126 for females at dose of 1mg/kg, and from day 21 for males and 17 for females at dose of 4mg/kg. The significant increase of body weight gain at the end of administration were observed in males at doses of 0.25 and 1mg/kg (increases of 13.4% and 15.1% vs control) and in both male and females at dose of 4mg/kg (increases of 24.1% in male and 32.7% in female vs control) in administration period. In the recovery period, the body weight gain in males at dose of 4mg/kg was lower than that of control group. The trend of body weight changes were described in the following figure. Body weight gains were summarized in the following table.

Summary of Body Weight Gain in Both Male and Female Rats (g)

Dose (mg/kg)	Administration Period at the end of 26 weeks		Recovery Period at the end of 5-weeks	
	Male	Female	Male	Female
0	506±64	220±43	23±13	12±20
0.25	574±49*	266±46		
1	596±52**	272±65		
4	667±68**	292±52**	-11±10**	4±20

*: $p < 0.05$; **: $p < 0.01$

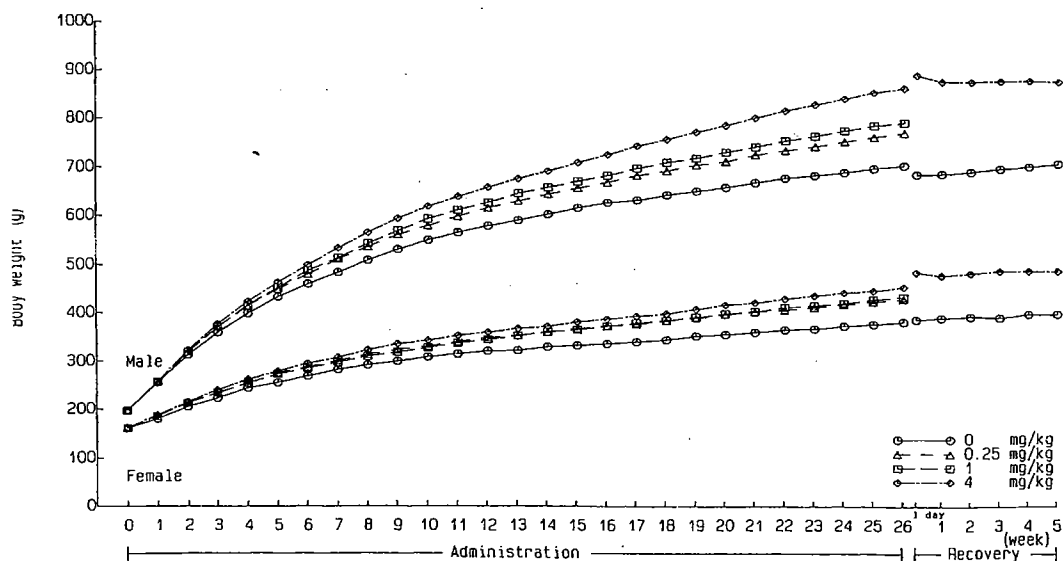


Fig. 1 A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks

Body weight

Food consumption: In males, significantly increased food consumption was observed from day 63 of administration at dose of 1mg/kg and day 28 at dose of 4mg/kg/. In females,

significantly increased food consumption was observed from day 91 at dose of 1mg/kg and day 63 at dose of 4mg/kg. During recovery period, food consumption at 4mg/kg was still higher than that of the control group. However, food consumption tended to decrease gradually. The changes of food consumption were described in the following figure.

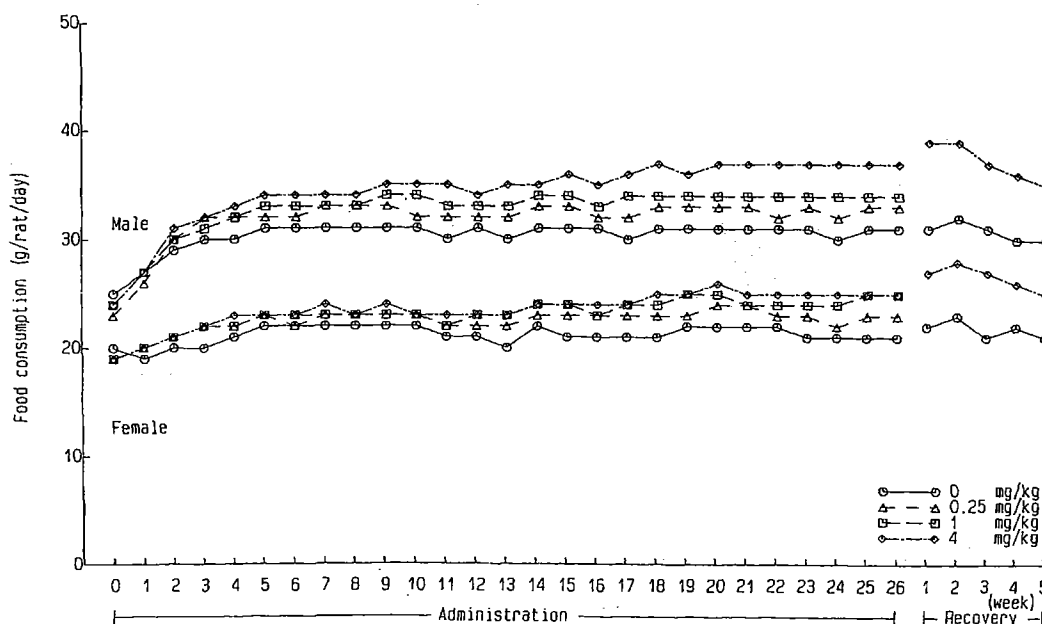


Fig.2 A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks

Ophthalmoscopy: No drug-related changes were noted.

Electrocardiography: Not measured

Hematology: At the end of administration period, a slight increases of MCV (5.3% vs control) and MCH (4.8% vs control) was observed in male rats at dose of 4mg/kg. At the end of recovery period, there was a significantly increased fibrinogen in males at dose of 4mg/kg (increase of 25% vs control).

Clinical chemistry: An 11.6% increase of glucose was observed in female rats at dose of 4mg/kg at the end of administration period. At the end of recovery period, significant increases of total cholesterol, triglyceride and phospholipids, and decreases of alkaline phosphatase (AKP) activity, and A/G ratio due to a low value of albumin ratio and a high value of α 1-globulin ratio were observed in male rats at dose of 4mg/kg. Data were summarized in the following table.

Summary of Clinical Chemistry Parameters at the End of Recovery Period

Dose (mg/kg)		No.	Albumin g/dl	A/G	α 1 G %	T cho mg/dl	TG mg/dl	PL mg/dl	AKP IU/l
M	0	6	2.7 $\pm$ 0.1	0.72 $\pm$ 0.05	24.2 $\pm$ 1.4	85 $\pm$ 22	101 $\pm$ 22	132 $\pm$ 22	173 $\pm$ 46
	4	6	2.4 $\pm$ 0.2*	0.60 $\pm$ 0.07**	28.4 $\pm$ 3.0*	124 $\pm$ 29*	192 $\pm$ 73*	182 $\pm$ 40*	120 $\pm$ 27*
F	0	6	3.5 $\pm$ 0.2	0.91 $\pm$ 0.07	19.4 $\pm$ 1.9	91 $\pm$ 36	117 $\pm$ 60	198 $\pm$ 50	70 $\pm$ 20
	4	6	3.7 $\pm$ 0.3	0.87 $\pm$ 0.06	20.6 $\pm$ 2.5	106 $\pm$ 22	133 $\pm$ 61	221 $\pm$ 36	84 $\pm$ 20

*: p<0.05 vs control; **:p<0.01 vs control

Urinalysis: In the month 3 of administration, there were dose-dependent increases of urine volume, sodium, potassium and chloride excretion in both sexes as compared to the control group. Statistical differences were found at dose of 4mg/kg in male rats. In the examination of urinary sediments, there were tendencies toward increase in squamous epithelial cells and small round epithelial cell in females at doses of 1 and 4mg/kg, and in small round epithelial cells in males at dose of 4mg/kg. In the month 6 of administration, same pattern were noted. Statistically significant changes included the increase of potassium excretion at 1mg/kg, sodium, potassium, and chloride excretion at 4mg/kg and in urinary volume for males. In week 5 of recovery, significantly increased sodium and chloride excretion were observed in males at dose 4mg/kg. Data were summarized in the following tables.

A 26-week subcutaneous toxicity study of HKN-031 in rats with a recovery period of 6 weeks
Urinalysis (3 months)

		URINE SEDIMENT																													
		CRYSTALLIZATION																													
Sex	Dose mg/kg	No.	RBC				WBC				SEC				SREC				Cast		PS				CO						
			-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	-	+	++	+++	-	+	++	+++			
Female	0	12	12	0	0	0	0	12	0	0	0	0	0	12	0	0	0	0	12	0	0	6	6	0	0	0	12	0	0	0	0
	0.25	12	12	0	0	0	0	11	1	0	0	0	0	12	0	0	0	0	12	0	0	3	8	1	0	0	12	0	0	0	0
	1	12	12	0	0	0	0	11	1	0	0	0	0	12	0	0	0	0	12	0	0	4	8	0	0	0	12	0	0	0	0
	4	12	12	0	0	0	0	10	2	0	0	0	0	12	0	0	0	0	6	3	2	0	1	12	0	0	9	3	0	0	0
Male	0	12	12	0	0	0	0	12	0	0	0	0	0	12	0	0	0	0	12	0	0	6	5	1	0	0	12	0	0	0	0
	0.25	12	12	0	0	0	0	11	1	0	0	0	0	12	0	0	0	0	12	0	0	5	6	1	0	0	12	0	0	0	0
	1	12	12	0	0	0	0	10	2	0	0	0	0	11	0	1	0	0	8	2	2	0	0	12	0	0	5	7	0	0	0
	4	12	12	0	0	0	0	9	3	0	0	0	0	9	3	0	0	0	6	4	2	0	0	12	0	0	6	6	1	0	0
			: : Squamous Epithelial Cell				- : Negative																								
			SC : Small Round Epithelial Cell				+- : Slight																								
			: Phosphate Salts				+ : Mild																								
			: Calcium Oxalate				++ : Moderate																								
							+++ : Severe																								

Best Possible Copy

Appears This Way
On Original

A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks
Water intake and urinalysis (3 months)

Sex	Dose mg/kg	No.		Water intake ml/rat/24hrs	Urine volume ml/24hrs	S.G.	Na		K	Cl
							mEq/24hrs		mEq/24hrs	mEq/24hrs
Male	0	12	Mean	43	13.5	1.068	1.97	3.29	2.43	
			S.D.	6	5.4	0.010	0.79	0.78	0.76	
	0.25	12	Mean	43	16.0	1.058	2.29	3.73	2.74	
			S.D.	10	5.0	0.012	0.44	0.68	0.51	
	1	12	Mean	46	17.4	1.059	2.48	4.23	2.96	
			S.D.	7	4.6	0.013	0.48	0.73	0.49	
	4	12	Mean	56	23.6**	1.056	2.86**	5.16**	3.72**	
			S.D.	16	8.7	0.014	0.75	1.42	1.10	
Female	0	12	Mean	33	8.9	1.052	0.92	1.58	1.16	
			S.D.	8	7.1	0.015	0.58	0.97	0.69	
	0.25	12	Mean	37	9.5	1.061	1.12	1.83	1.38	
			S.D.	8	4.1	0.014	0.32	0.48	0.43	
	1	12	Mean	38	9.9	1.051	1.14	1.75	1.29	
			S.D.	12	5.6	0.012	0.54	0.80	0.64	
	4	12	Mean	38	11.1	1.057	1.38	2.47	1.80	
			S.D.	9	4.6	0.010	0.50	0.96	0.72	

* p<0.01 (Significant difference from control group)

A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks
Urinalysis (6 months)

			URINE SEDIMENT												CRYSTALLIZATION												
Sex	Dose mg/kg	No.	RBC				WBC				SEC				SREC				Cast	PS				CO			
			-	+	++	+++	-	+	++	+++	-	+	++	+++	-	+	++	+++		-	+	++	+++	-	+	++	+++
Male	0	12	10	2	0	0	0	11	1	0	0	0	0	12	0	0	0	12	0	0	0	0	12	0	0	0	0
	0.25	11	10	1	0	0	0	9	2	0	0	0	0	11	0	0	0	11	0	0	0	0	11	0	0	0	0
	1	12	11	1	0	0	0	10	1	1	0	0	0	11	1	0	0	9	3	0	0	0	12	0	0	0	0
	4	12	12	0	0	0	0	11	1	0	0	0	0	12	0	0	0	11	1	0	0	0	12	0	0	0	0
Female	0	12	11	1	0	0	0	12	0	0	0	0	0	12	0	0	0	12	0	0	0	0	8	3	1	0	0
	0.25	12	11	1	0	0	0	11	0	1	0	0	0	10	2	0	0	10	2	0	0	0	12	0	0	0	0
	1	12	12	0	0	0	0	11	1	0	0	0	0	8	4	0	0	12	0	0	0	0	9	3	0	0	0
	4	12	12	0	0	0	0	12	0	0	0	0	0	8	3	1	0	12	0	0	10	2	0	0	0	0	0

: Squamous Epithelial Cell
 C : Small Round Epithelial Cell
 : Phosphate Salts
 : Calcium Oxalate
 - : Negative
 +- : Slight
 + : Mild
 ++ : Moderate
 +++ : Severe

Best Possible Copy

A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks
Water intake and urinalysis (6 months)

Sex	Dose mg/kg	No.		Water intake ml/rat/24hrs	Urine volume ml/24hrs	S.G.	Na mEq/24hrs	K mEq/24hrs	Cl mEq/24hrs
female	0	12	Mean	39	17.1	1.055	2.41	3.56	2.83
			S.D.	6	4.7	0.014	0.52	0.65	0.61
	0.25	11	Mean	44	20.6	1.055	2.64	4.32	3.21
			S.D.	13	7.2	0.013	0.57	0.59	0.56
	1	12	Mean	46	23.0	1.056	2.88	4.44*	3.38
			S.D.	11	9.9	0.017	0.62	0.92	0.79
	4	12	Mean	57**	29.3**	1.061	3.52**	6.23**	4.67**
			S.D.	12	7.9	0.013	0.90	1.50	1.20
	0	12	Mean	35	12.2	1.047	1.13	1.87	1.38
			S.D.	12	9.0	0.014	0.49	0.75	0.56
	0.25	12	Mean	38	11.3	1.050	1.32	2.07	1.64
			S.D.	7	5.2	0.010	0.44	0.57	0.51

* : p<0.05 ; ** : p<0.01 (Significant difference from control group)

A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks
Water intake and urinalysis (Recovery)

Sex	Dose mg/kg	No.		Water intake ml/rat/24hrs	Urine volume ml/24hrs	S.G.	Na mEq/24hrs	K mEq/24hrs	Cl mEq/24hrs
female	0	6	Mean	38	14.2	1.067	1.75	3.41	2.27
			S.D.	6	9.1	0.020	0.58	0.81	0.68
	4	6	Mean	48	21.5	1.057	2.52*	4.40	3.14*
			S.D.	10	9.9	0.012	0.54	0.76	0.63
	0	6	Mean	42	19.2	1.039	1.50	2.84	1.91
			S.D.	7	12.1	0.007	0.73	1.51	0.99
male	4	6	Mean	54	19.7	1.036	1.50	2.67	1.81
			S.D.	14	7.2	0.008	0.47	0.65	0.59

p<0.05 (Significant difference from control group)

Organ weights: Drug-related changes of organ weights included increases for the thymus, liver and kidney at dose of 4mg/kg in males, and increases of pituitary, salivary gland, liver, uterus at doses of 1 and 4mg/kg and thyroid at dose of 4mg/kg in females. Data were summarized in the following tables.

Appears This Way
On Original

Best Possible Copy

Nale 1 2

Nale

Best Possible Copy

89

A 26-week subcutaneous toxicity study of NKX-031 in rats with a recovery period of 5 weeks
Absolute and relative organ weight (6 months)

Female

	Dose mg/kg		Body weight g	Brain g/g%	Pituitary mg/mg%	Thyroid (R) mg/mg%	Thyroid (L) mg/mg%	Thyroid (R+L) mg/mg%	Salivary gl.(R) mg/mg%	Salivary gl.(L) mg/mg%	Salivary gl.(R+L) mg/mg%	Thymus mg/mg%
soluble	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	355	2.01	17.8	10.5	10.5	21.1	241	234	475	157
		S.D.	42	0.08	4.3	1.9	2.5	4.1	26	22	46	28
	0.25	No.	12	12	12	12	12	12	12	12	12	12
		Mean	404	2.08	20.0	11.0	10.5	22.2	261	262**	522*	175
		S.D.	44	0.06	4.9	1.4	2.1	2.8	18	15	32	44
	1	No.	12	12	12	12	12	12	12	12	12	12
		Mean	409*	2.06	25.5**	12.4	10.7	23.0	292**	287**	579**	175
		S.D.	65	0.08	5.0	2.2	2.8	4.5	24	21	43	44
	4	No.	12	12	12	12	12	12	12	12	12	12
		Mean	418**	2.10	26.3**	13.9**	12.5	26.5**	295**	293**	587**	179
		S.D.	40	0.09	4.2	2.2	2.5	4.1	28	24	51	53
soluble	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.57	5.0	3.0	3.0	6.0	69	67	135	45	8
		S.D.	0.07	0.9	0.5	0.8	1.1	8	8	16	8	8
	0.25	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.52	5.0	2.9	2.7	5.6	66	66	130	44	11
		S.D.	0.05	1.1	0.5	0.6	0.9	7	8	15	11	11
	1	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.52	6.3**	3.1	2.7	5.8	73	72	145	43	9
		S.D.	0.07	0.9	0.7	0.9	1.5	13	13	26	9	9
	4	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.61	6.3**	3.4	3.0	6.4	71	71	141	43	11
		S.D.	0.06	1.1	0.6	0.5	1.0	7	5	12	11	11
soluble	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	1.12	1.21	8.62	0.57	1.07	1.08	2.15	38	41	80
		S.D.	0.12	0.08	1.48	0.07	0.10	0.11	0.20	5	8	11
	0.25	No.	12	12	12	12	12	12	12	12	12	12
		Mean	1.21	1.35**	10.04	0.62	1.18	1.20	2.38	38	41	79
		S.D.	0.12	0.11	1.63	0.14	0.08	0.08	0.10	3	5	7
	1	No.	12	12	12	12	12	12	12	12	12	12
		Mean	1.29**	1.38**	11.01**	0.63	1.27**	1.27**	2.54**	44*	51**	96**
		S.D.	0.14	0.11	1.79	0.08	0.14	0.16	0.30	7	5	14
	4	No.	12	12	12	12	12	12	12	12	12	12
		Mean	1.30**	1.40**	11.07**	0.68	1.28**	1.29**	2.57**	48**	50**	96**
		S.D.	0.11	0.09	1.09	0.11	0.14	0.16	0.29	6	5	12
soluble	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.32	0.34	2.42	0.16	0.30	0.31	0.61	11	12	23
		S.D.	0.03	0.03	0.20	0.02	0.03	0.02	0.06	2	2	4
	0.25	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.30	0.34	2.48	0.15	0.30	0.30	0.59	9	10	20
		S.D.	0.03	0.04	0.23	0.03	0.02	0.03	0.06	1	1	2
	1	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.32	0.34	2.70**	0.16	0.31	0.31	0.63	11	13	24
		S.D.	0.03	0.04	0.22	0.02	0.04	0.03	0.07	2	2	4
	4	No.	12	12	12	12	12	12	12	12	12	12
		Mean	0.31	0.34	2.66*	0.16	0.31	0.31	0.62	11	12	23
		S.D.	0.03	0.03	0.24	0.02	0.03	0.03	0.06	2	2	4
soluble	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	36.9	34.4	71.3	764	764	764	764	764	764	764
		S.D.	9.9	9.2	18.1	150	150	150	150	150	150	150
	0.25	No.	12	12	12	12	12	12	12	12	12	12
		Mean	39.0	43.9	82.8	709	709	709	709	709	709	709
		S.D.	10.7	11.6	21.3	120	120	120	120	120	120	120
	1	No.	12	12	12	12	12	12	12	12	12	12
		Mean	28.5	28.0	56.5	956**	956**	956**	956**	956**	956**	956**
		S.D.	8.5	9.7	17.0	141	141	141	141	141	141	141
	4	No.	12	12	12	12	12	12	12	12	12	12
		Mean	35.0	37.2	73.1	918*	918*	918*	918*	918*	918*	918*
		S.D.	9.8	12.4	20.2	182	182	182	182	182	182	182
soluble	0	No.	12	12	12	12	12	12	12	12	12	12
		Mean	10.6	9.8	20.4	218	218	218	218	218	218	218
		S.D.	3.3	2.8	5.8	60	60	60	60	60	60	60
	0.25	No.	12	12	12	12	12	12	12	12	12	12
		Mean	9.6	10.8	20.4	177	177	177	177	177	177	177
		S.D.	2.3	2.5	4.6	34	34	34	34	34	34	34
	1	No.	12	12	12	12	12	12	12	12	12	12
		Mean	7.0**	5.9*	13.9**	240	240	240	240	240	240	240
		S.D.	1.7	2.3	3.7	44	44	44	44	44	44	44
	4	No.	12	12	12	12	12	12	12	12	12	12
		Mean	8.6	8.9	17.5	224	224	224	224	224	224	224
		S.D.	2.1	2.6	4.2	58	58	58	58	58	58	58

*:P<0.05; **:P<0.01 vs Control

Best Possible Copy

At the end of recovery period, drug-related organ weight changes included decrease of thymus and increase of liver at dose of 4mg/kg in males, and increases of pituitary, liver, uterus at dose of 4mg/kg in females. Compare with the treatment period, the increased liver weight in both males and females, and increased pituitary weight, uterus in females were not completely reversible in the recovery period. The changes of thyroid and salivary weight were reversible. Data were summarized in the following tables.

A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks

Absolute and relative organ weight (Recovery)

Male

Dose mg/kg		Body weight g	Brain g/g%	Pitui- lary mg/mg%	Thyroid (R) mg/mg%	Thyroid (L) mg/mg%	Thyroid (R+L) mg/mg%	Salivary gl.(R) mg/mg%	Salivary gl.(L) mg/mg%	Salivary gl.(R+L) mg/mg%	Thymus mg/mg%
solute 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean	673	2.27	14.2	14.4	12.6	26.9	387	386	773	148
	S.D.	21	0.09	2.5	3.1	2.0	4.9	54	52	105	10
	No.	6	6	6	6	6	6	6	6	6	6
	Mean	841**	2.38	15.6	16.2	16.9*	33.1	403	404	807	98**
	S.D.	58	0.08	1.9	3.3	4.0	6.9	35	27	59	13
relative 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean		0.34	2.1	2.2	1.9	4.0	57	57	115	22
	S.D.		0.01	0.4	0.5	0.3	0.8	7	7	14	2
	No.	6	6	6	6	6	6	6	6	6	6
	Mean		0.29**	1.9	1.9	2.0	3.9	48*	48*	96*	12**
	S.D.		0.02	0.3	0.3	0.4	0.7	4	4	7	1
Dose mg/kg		Heart g/g%	Lung g/g%	Liver g/g%	Spleen g/g%	Kidney (R) g/g%	Kidney (L) g/g%	Kidney (R+L) g/g%	Adrenal (R) mg/mg%	Adrenal (L) mg/mg%	Adrenal (R+L) mg/mg%
solute 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean	1.70	1.69	18.60	0.92	1.83	1.88	3.71	32	35	67
	S.D.	0.11	0.06	1.59	0.06	0.12	0.13	0.23	4	3	7
	No.	6	6	6	6	6	6	6	6	6	6
	Mean	1.97**	1.98**	24.18*	1.04	2.37**	2.39**	4.76**	35	39	74
	S.D.	0.09	0.13	5.74	0.19	0.19	0.29	0.40	5	6	10
relative 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean	0.26	0.25	2.76	0.14	0.27	0.28	0.55	5	5	10
	S.D.	0.02	0.01	0.24	0.01	0.02	0.02	0.04	1	1	1
	No.	6	6	6	6	6	6	6	6	6	6
	Mean	0.24	0.24	2.85	0.12	0.28	0.29	0.57	4	5	9
	S.D.	0.02	0.02	0.46	0.02	0.04	0.02	0.04	1	1	1
Dose mg/kg		Testis (R) g/g%	Testis (L) g/g%	Testis (R+L) g/g%	Seminal vesicle g/g%	Prostate g/g%					
solute 0	No.	6	6	6	6	6					
	Mean	1.85	1.84	3.69	1.74	1.29					
	S.D.	0.15	0.10	0.25	0.39	0.23					
	No.	6	6	6	6	6					
	Mean	1.77	1.79	3.56	1.72	1.08					
	S.D.	0.11	0.11	0.22	1.14	0.10					
relative 0	No.	6	6	6	6	6					
	Mean	0.28	0.27	0.55	0.26	0.19					
	S.D.	0.03	0.02	0.05	0.06	0.03					
	No.	6	6	6	6	6					
	Mean	0.21**	0.21**	0.43**	0.21	0.13**					
	S.D.	0.02	0.02	0.04	0.16	0.02					

*:p<0.05; **:p<0.01

Best Possible Copy

A 26-week subcutaneous toxicity study of MKN-031 in rats with a recovery period of 5 weeks

Absolute and relative organ weight (Recovery)

Female

Dose mg/kg		Body weight g	Brain g/g%	Pituitary mg/mg%	Thyroid (R) mg/mg%	Thyroid (L) mg/mg%	Thyroid (R+L) mg/mg%	Salivary gl.(R) mg/mg%	Salivary gl.(L) mg/mg%	Salivary gl.(R+L) mg/mg%	Thymus mg/mg%
solute 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean	375	2.03	18.2	10.4	10.0	20.4	235	235	471	119
	S.D.	56	0.07	3.2	2.7	2.4	4.8	13	13	21	17
4	No.	6	6	6	6	6	6	6	6	6	6
	Mean	454*	2.10	28.9	11.4	10.4	21.8	260	267	527	116
	S.D.	71	0.14	14.6	1.1	1.7	2.4	28	30	57	52
relative 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean		0.55	4.9	2.7	2.6	5.4	64	64	128	33
	S.D.		0.07	0.8	0.4	0.4	0.5	10	10	19	9
4	No.	6	6	6	6	6	6	6	6	6	6
	Mean		0.46	6.2	2.5	2.3	4.8	67	59	116	26
	S.D.		0.08	3.2	0.6	0.7	1.2	11	12	22	8
Dose mg/kg		Heart g/g%	Lung g/g%	Liver g/g%	Spleen g/g%	Kidney (R) g/g%	Kidney (L) g/g%	Kidney (R+L) g/g%	Adrenal (R) mg/mg%	Adrenal (L) mg/mg%	Adrenal (R+L) mg/mg%
solute 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean	1.05	1.15	9.31	0.54	1.09	1.09	2.18	36	37	72
	S.D.	0.08	0.06	1.68	0.08	0.12	0.08	0.20	4	10	14
4	No.	6	6	6	6	6	6	6	6	6	6
	Mean	1.30**	1.35**	11.86*	0.69	1.27*	1.27*	2.54*	44*	49	93*
	S.D.	0.07	0.08	1.71	0.10	0.11	0.12	0.23	8	9	17
relative 0	No.	6	6	6	6	6	6	6	6	6	6
	Mean	0.28	0.32	2.48	0.14	0.29	0.29	0.58	10	10	20
	S.D.	0.03	0.05	0.29	0.02	0.03	0.03	0.06	2	3	5
4	No.	6	6	6	6	6	6	6	6	6	6
	Mean	0.29	0.30	2.57	0.13	0.28	0.28	0.56	10	11	21
	S.D.	0.04	0.04	0.18	0.03	0.04	0.03	0.07	3	3	5
Dose mg/kg		Ovary (R) mg/mg%	Ovary (L) mg/mg%	Ovary (R+L) mg/mg%	Uterus mg/mg%						
solute 0	No.	6	6	6	6						
	Mean	33.9	36.6	70.5	715						
	S.D.	11.5	11.2	22.1	140						
4	No.	6	6	6	6						
	Mean	34.1	31.8	65.9	917						
	S.D.	8.5	16.3	23.6	202						
relative 0	No.	6	6	6	6						
	Mean	9.0	8.9	18.9	195						
	S.D.	2.7	3.3	5.8	55						
4	No.	6	6	6	6						
	Mean	7.5	7.0	14.5	201						
	S.D.	2.0	3.6	5.3	50						

*:P<0.05; **:P<0.01

Gross pathology: No drug-related macroscopic findings were noted.

Histopathology: A dose-dependent increase of the incidence rate of the increased cortex area in thymus was observed in male rats. The slightly increased cortex area was found in 1 male each in control and at dose of 0.25mg/kg, 2 males at dose 1mg/kg and 7 males at dose of 4mg/kg at the end of the administration period. After 5-week recovery, a slightly decreased cortex area was observed in 1 male at dose of 4mg/kg. No other drug-related findings were noted.

Toxicokinetics: Not performed. However, the plasma concentrations of test article and blood glucose were measured. The plasma concentrations of IGF-1, both of total and free form were shown a dose-dependent increase in both sexes. After 24 hours of dosing, plasma

Best Possible Copy

concentrations of total and free form IGF-1 were similar to the pre-dosing and control levels. Data were summarized in the following tables.

Summary of Plasma Concentrations of IGF-1 in Both Male and Female Rats (n=4, ng/ml)

Times	Gender	Dose mg/kg	Time after administration					
			Pre		30min		24 hour	
			Total	Free	Total	Free	Total	Free
Day 1	Male	0	1133±71	23.6±6.9	1063±92	16.0±2.9	1140±156	12.6±0.9
		0.25	1096±143	22.5±2.4	1480±243	102.9±19.3	1043±136	16.0±4.3
		1	1073±86	19.3±3.4	2275±341	424±29	1123±87	17.7±5.1
		4	1205±87	20.6±3.3	4345±522	1790±309	1110±161	32.4±3.8
	Female	0	1153±101	15.7±1.8	1063±156	13.4±2.0	1117±141	17.4±2.2
		0.25	1120±88	16.8±2.6	1351±308	73.8±8.5	1128±87	16.5±4.8
		1	1203±102	16.4±2.1	2053±294	360±87	1079±170	18.2±1.4
		4	1076±140	15.5±3.4	4230±446	1763±381	1087±161	29.0±6.3
Week 26	Male	0	1133±104	7.90±0.16	1181±133	9.15±0.93	1067±200	6.49±2.22
		0.25	1068±205	7.41±1.08	1445±194	125.5±13.1	1107±199	7.83±1.07
		1	1148±179	8.77±0.79	2293±515	644±86	1089±105	11.06±2.07
		4	1330±91	9.19±0.92	4708±101	2168±378	1044±190	22.5±5.7
	Female	0	695±126	7.90±1.16	774±121	6.86±4.58	764±79	4.23±1.68
		0.25	799±99	7.69±1.61	1107±127	98.1±29.3	774±82	8.11±1.71
		1	787±109	6.50±4.44	1653±263	504±46	767±80	10.12±1.06
		4	863±118	7.16±1.26	4500±357	2298±239	816±84	23.5±3.2

In analysis of plasma glucose level, the plasma glucose concentration decreased significantly in females at dose of 1mg/kg and in both sexes at dose of 4mg/kg after 30min of dosing in the first dosing. After 24 hours, the plasma levels of glucose were back to pre-dosing and control. In week 26 administration, significant decrease of glucose level was found in both sexes at dose of 4mg/kg, and return to pre-dose after 24 hours. Data were summarized in the following table.

Summary of Plasma Concentrations of Glucose in Both male and Female Rats (n=4, mg/dl)

Times	Gender	Dose mg/kg	Time after administration		
			Pre	30min	24 hour
Day 1	Male	0	158±5	139±13	147±8
		0.25	143±30	140±30	148±8
		1	135±8	112±8	158±12
		4	134±12	61±15**	145±6
	Female	0	137±6	143±9	149±13
		0.25	143±6	151±7	146±11
		1	140±6	112±11**	144±7
		4	144±14	59±16**	150±7
Week 26	Male	0	133±6	132±11	128±5
		0.25	136±10	179±45	132±14
		1	143±9	129±10	141±7
		4	135±7	50±6**	149±28
	Female	0	120±4	128±18	124±8
		0.25	125±9	152±6	136±5
		1	129±10	127±5	141±14
		4	126±5	68±19**	142±9

** : P<0.01 vs control

Others: Determination of antibody titer was done on all surviving males and females at the end of the administration and recovery periods; anti-MKN-031 antibody was not detected in any of the animals.

Study title: A 26-week subcutaneous toxicity study of MKN-031(mecasermin) with 5 weeks follow-up observation after drug withdrawal in beagle dogs

Key findings:

- Three dogs died due to hypoglycemia at dose of 0.6mg/kg/day.
- In the clinical chemistry, drug-related changes included significant decrease of glucose, potassium, and inorganic phosphorus in both male and female dogs at doses of 0.15 and 0.13mg/kg. In addition, the trend of increase of phosphorus was observed in both male and female dogs at dose of 0.6mg/kg.
- In the test for antibody formation, no antibodies against mecasermin were detected in any dosed animals.
- Drug-related organ weight changes included the increases of absolute weight of adrenal in males at dose of 0.6mg/kg, absolute weight of the heart in females at doses of 0.3 and 0.6mg/kg, both absolute and relative weight of the thymus in females at doses of 0.15 and 0.3mg/kg. For some organs, though statistically insignificant, increase of absolute or relative weight of the adrenals in both sexes at dose 0.6mg/kg, absolute weight of the heart in males at doses of 0.3 and 0.6mg/kg, and both absolute and relative weights of the thymus in males and females at dose of 0.6mg/kg were noted. In addition, increases of absolute weight of the pituitary glands in male at dose of 0.6mg/kg, absolute weight of the submandibular glands in females at dose of 0.6mg/kg, absolute weight of spleen in males at dose of 0.6mg/kg, and decrease of relative weight of the testis at dose of 0.6mg/kg were noted. After the recovery period, a tendency of increase of absolute and relative weights of the thymus in all females, and absolute and relative weights of the adrenals in one female at dose of 0.6mg/kg were observed.
- In histopathological study, drug-related microscopic changes included mild hyperplasia of medullary cells associated with fibrosis in adrenals at doses of 0.3 and 0.6mg/kg. After the recovery period, increase of the adrenal weight and medullary fibrosis still remained.
- Based on the pathological study, the NOEL was 0.15mg/kg. The systemic exposure was 1X based on the AUC data (MRHD 120µg/kg, bid with AUC of 3780ng.h/ml).

Study no: 96-268-0320 (12956)

Volume #, and page #: Volume 1 and Page 1.

Conducting laboratory and location: 1

Date of study initiation: February 24, 1995

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, radiolabel, and % purity: MKN-031, Lot # M6-46, and — , purity

Formulation/vehicle: Test article was dissolved in acetate buffered solution (pH 5.4) containing — mg sodium acetate, — mg acetate, — mg sodium chloride, and — mg benzyl alcohol per 1ml of water for injection.

Methods (unique aspects):

The experiment was comprised of 4 groups as shown in the following table.

Test Groups	Dosage (mg/kg)	Sex	Main group		Recovery group	
			No. of Animals (~ 2956-)	Animal Number (~ 2956-)	No. of Animals (~ 2956-)	Animal Number (~ 2956-)
Control	0	M	5	1001 - 1005**	2	1104 - 1105
		F	3	1101 - 1103		
Low	0.15	M	3	2001 - 2003		
		F	3	2101 - 2103		
Medium	0.3	M	3	3001 - 3003		
		F	3	3101 - 3103		
High	0.6	M	6	4001 - 4006*	3	4104 - 4106
		F	3	4101 - 4103		

*: Includes animals which had been assigned to the recovery group but found dead or sacrificed in extremis during treatment

**: All control males were sacrificed with the main group, because all recovery males of the 0.6 mg/kg took fatal course.

Dosing:

Species/strain: Dogs, $\mathcal{L}$ — — — — — J

#/sex/group or time point (main study): 3/sex/group

Satellite groups used recovery: 2/sex/group in control and 3/sex/group in high dose.

However, no recovery observation could be conducted for males because all males (3/3) were found dead or sacrificed in extremis during treatment.

Age: 6 months

Weight: 7.7 to 9.6kg for males and 5.6 to 9.3 kg for females

Doses in administered units: 0, 0.15, 0.3, and 0.6mg/kg/day

Route, form, volume, and infusion rate: Subcutaneous, solution, 0.2ml/kg

Observations and times:

Clinical signs: Twice a day

Body weights: Weekly

Food consumption: Daily

Ophthalmoscopy: 1 week prior to the start of administration (morning), in months 3 and 6 of the administration period (morning before dosing of the day) and in week 5 of the recovery period (morning).

EKG and blood pressure: 2 weeks prior to the start of administration (morning), in months 3 and 6 of the administration period (morning before dosing of the day) and in week 5 of the recovery period (morning).

Body temperature: Body (rectal) temperature were measured in 2 weeks prior to the start of administration (morning), in months 3 and 6 of the administration period (morning before dosing of the day) and in week 5 of the recovery period (morning).

Hematology: In weeks 2 and 1 prior to the start and months 1, 3 and 6 of the treatment period and in week 5 of the recovery period. The samples were taken from the cephalic vein after deprivation of food for 16 hours in the morning or before dosing of the day. Parameters including RBC, Hb, Hct, MCV, MCH, MCHC, Ret, platelet, WBC counts with differential, PT, ATPP, fibrinogen.

Clinical chemistry: Blood samples collected at the same time as for the hematological examination were subjected to blood chemical examination. In addition, blood samples were collected on the males and females of the control group and 0.15 and 0.3mg/kg groups in months 3 and 6 of the treatment period 1 hour after treatment of the day. 0.6mg/kg group was excluded from the post dosing examination because the blood sampling after dosing under fasting condition was considered to give rise serous untoward effect in high dose animals based on the sponsor. Parameters including GOT, GPT, AKP, total cholesterol, triglycerides, phospholipids, total bilirubin, glucose, BUN, Cr, Ua, Na, K, Cl, Ca, P, Mg, total protein, protein fractions, albumin, A/G ratio.

Urinalysis: In weeks 2 and 1 prior to the start and months 1, 3 and 6 of the treatment period and in week 5 of the recovery period. Parameters including pH, protein, ketone bodies, glucose, occult blood, bilirubin, urobilinogen, urine color, urine sediments, urine volume, specific gravity (S.G), sodium, potassium, chloride, and osmotic pressure.

Gross pathology: Examinations consisted of an external examination including identification of all clinically recorded lesions as well as a detailed internal examination. A complete necropsy was conducted on all animals and included gross and histopathological examination of organs and tissues listed in the following table.

Tissues for Gross Evaluation.

Adrenal glands	Ocular accessory gland
Aorta	Ovaries
Auricle (including tattooed numbers)	Pancreas
Bone marrow	Parathyroid
Brain: Cerebrum, cerebellum, medulla oblongata	Pituitary
Cervix	Prostate
Colon	Rectum
Cecum	Salivary glands
Duodenum	Sciatic nerve
Epididymis	Seminal vesicles
Esophagus	Skeletal muscle
Eye and optic nerve	Skin
Heart	Spinal cord
Injection site (subcutaneous tissues)	Spleen
Ileum	Stomach
Jejunum	Testes
Kidneys	Thymus
Larynx	Thyroid
Liver	Tongue
Lungs	Trachea
Lymph nodes	Urinary bladder
Mammary gland	Uterus
Muscle rectus abdominis	Vagina

Organs weighed: Brain, pituitary, thyroid glands (including parathyroids), submandibular glands, sublingual glands, thymus, heart, lungs (including bronchial tubes), liver, pancreas, kidneys, adrenal, spleen, testes, epididymides, prostate, ovaries, and uterus.

Histopathology: Organs and tissues in above table from all the animals including the deceased cases were fixed and conducted in histopathological examination.

Toxicokinetics: Samples were collected on the day of first treatment and in months 3 and 6 of the dosing period, immediately before, 15 and 30 minutes, 1, 2, 4, 6, 8 and 24 hours after dosing of the day. Samples were also carried out on day 10 of the dosing period 1 and 2 hours after dosing of the day.

Other:

Antibody formation: Predosing period, weeks 2 and 4 and months 3 and 6 of the treatment period (before dosing of the day), and in week 5 of the recovery period (morning).

Plasma glucose: Plasma glucose concentration was measured with the TK studies.

Results:

Mortality: Three male dogs at dose of 0.6mg/kg were found dead or sacrificed in extremis during treatment. One dog (4005) was found dead after an overnight fasting for blood sampling and one (4004) was sacrificed in extremis due to a severely deteriorated condition on day 80. Another one (4006) was found dead on day 135 of treatment. All of three animals exhibited decrease in spontaneous movement, lateral position and reddening of the conjunctiva 4 hours after dosing of the day before day of death or sacrifice. Some of them also exhibited tremors of the head and extremities (4004 and 4005), hypothermia (4004 and 4006) and clonic convulsion (4006). The causes of deaths in these animals were due to the hypoglycemia based on the clinical signs and the fasting plasma level of glucose.

Clinical signs: One male (4001) at dose of 0.6mg/kg exhibited similar signs to those fatal cases once in week 12 of treatment (after an overnight fasting) and 1 to 3 similar episodes per week in week 21 of treatment and thereafter. The animal recovered to its usual condition on the next day after each episode. Both male and female animals at dose of 0.6mg/kg except one male (4003) and one female (4104) at dose exhibited transient reddening of the auricles or conjunctiva in week 12 of treatment and thereafter. Reddening of the conjunctiva was also seen in 1 female (3103) at dose of 0.3mg/kg once in week 13 of treatment. Other symptoms and signs including vomiting of ingestion or foamy fluid and changes of feces (soft, mucous or watery) as well as estrus hemorrhage were found in all groups and were not considered as treatment –related. Data were summarized in the following tables.

Appears This Way
On Original

A 26-week subcutaneous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

General condition

Sex : Male

Dose mg/kg	Animal number	Dosing period (weeks)																	
		-1+	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
0	1001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	1002	-	-	-	-	B(1)	-	-	-	-	-	-	-	-	-	-	-		
	1003	-	B(1)	-	-	-	-	-	-	-	C(1)	-	-	-	-	-	-		
	1004	-	-	-	-	-	-	-	-	-	-	-	A(1)	-	-	A(1)	-		
	1005	-	A(1)	-	-	C(1)	-	-	-	-	A(1)	-	-	-	-	-	-		
0.15	2001	-	A(1)	-	-	-	-	-	-	-	-	-	-	-	A(1)	-	-		
	2002	-	B(1)	-	-	-	-	-	-	-	A(1)	-	-	-	-	-	-		
	2003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
0.3	3001	-	-	C(1)	-	-	-	-	-	-	-	-	-	-	-	-	-		
	3002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
	3003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		
0.6	4001	-	A(1)	A(1)	-	-	-	-	A(1)	A(1)	A(1)	-	A(1)	-	A(1)	E(2)	A(2) E(1)	E(4)	
																E(1)			
																E(1)			
																E(1)			
																E(1)			
																E(1)			
																E(1)			
																E(1)			
																E(1)			
																E(1)			
															E(1)				
	4002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	4003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	4004a)	-	A(1)	-	-	A(1)	-	-	-	-	-	-	-	-	-	A(1)	C(1)	E(2)	
	4005b)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	
	4006c)	-	-	-	A(1)	A(1)	-	-	-	C(1)	-	-	-	-	-	-	E(3)	A(1) E(4)	E(1)

Dose mg/kg	Animal number	Dosing period (weeks)														
		16	17	18	19	20	21	22	23	24	25	26				
0	1001	-	-	-	A(1)	-	-	-	-	A(1)	-	-	-	-	-	-
	1002	-	-	-	-	D(1)	-	-	-	-	-	-	-	-	-	-
	1003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1004	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1005	-	-	-	-	-	A(1)	-	-	-	-	-	-	-	-	-
0.15	2001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2002	-	-	-	-	-	-	-	-	-	A(1)	-	-	-	-	-
	2003	-	-	-	-	-	-	-	-	-	C(1)	-	-	-	-	-
0.3	3001	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3003	-	-	-	-	-	-	-	-	-	A(1)	-	-	-	-	-
0.6	4001	-	A(1)	E(1)	E(2)	E(1)	E(4)	A(1)	A(1)	E(2)	A(1)	E(1)	E(1)	E(1)	E(1)	E(1)
	4002	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4003	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4004a)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4005b)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	4006c)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Numeral in parenthesis means incidence of respective signs in the week

a) : Sacrificed in a moribund state on day 80.

b) : Died on day 80.

c) : Died on day 135.

- : No abnormality

A : Vomiting

C : Loose stool

D : Watery stool

E : Flush (conjunctiva or auricle)

F : Decrease in locomotor activity

G : Sitting position

H : Prone position

I : Lateral position

L : Clonic convulsion

M : Hypothermia

N : Salivation

Best Possible Copy

A 26-week subcutaneous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

General condition

Sex : Female

Dose mg/kg	Animal number	Dosing period (weeks)															
		-1*	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
0	1101	C(1)	-	-	C(1)	A(1) C(2)	C(2)	C(1)	B(1) C(2)	C(6)	C(3)	-	C(1)	C(1)	A(1) C(1)	C(2)	C(2) O(5)
	1102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1105	-	A(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.15	2101	A(1)	A(2)	-	-	A(2)	-	-	A(1)	-	-	-	-	-	A(2)	-	A(1)
	2102	-	A(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2103	-	-	-	-	-	-	A(1)	-	A(2)	-	-	-	-	-	-	-
0.3	3101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	3102	B(1)	-	-	-	-	-	-	O(6) C(1)	O(7)	O(5)	-	-	-	-	-	-
	3103	-	-	-	-	-	-	-	-	-	-	-	-	-	E(1)	-	-
0.6	4101	-	-	-	-	-	-	-	-	-	-	-	-	-	E(2)	E(5)	E(1)
	4102	-	-	-	C(1)	-	C(1)	-	-	-	B(1)	C(1)	-	-	B(1)	-	B(1)
	4103	-	-	A(2)	A(1)	-	C(2) D(1)	-	-	-	-	C(1)	-	-	-	A(1)	E(2)
	4104	-	-	-	-	-	-	-	-	-	-	-	-	-	C(1)	-	-
	4105	-	-	A(1)	-	-	A(1) C(1)	-	-	-	-	-	-	-	A(1)	-	-
	4106	-	A(1) D(1)	-	C(1)	-	-	-	B(1)	-	B(1)	-	-	-	C(2)	B(1)	B(2) C(2) E(5)

Dose mg/kg	Animal number	Dosing period (weeks)										Recovery period (weeks)					
		16	17	18	19	20	21	22	23	24	25	26	1	2	3	4	5
0	1101	A(1) C(3) O(7)	O(7)	-	A(1) C(1)	-	-	-	-	-	-	-	-	-	-	-	-
	1102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1104	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	1105	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.15	2101	A(1)	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2102	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	2103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.3	3101	-	-	-	-	O(1)	O(7)	O(5)	-	-	-	-	-	-	-	-	-
	3102	-	-	-	-	-	-	-	-	-	-	O(5) O(7)	O(9)	O(9)	-	-	-
	3103	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.6	4101	-	O(5)	O(7)	-	E(4)	-	E(1)	E(1)	-	-	-	E(1)	E(2)	-	-	-
	4102	-	O(5)	C(1)	A(1)	B(1)	-	-	-	-	-	-	-	-	-	-	-
	4103	E(2)	E(1)	O(7) A(1)	O(7) E(1)	E(4)	E(1)	E(3)	E(1)	E(2)	E(1)	A(1) O(7)	A(1) E(1) O(9)	-	-	-	-
	4104	-	-	C(1)	-	-	-	-	-	-	-	-	-	O(5)	O(7)	O(3)	-
	4105	-	-	A(1)	-	E(3)	O(4)	E(2) O(7)	O(7)	A(1) O(7)	O(7)	O(7)	E(1) O(4)	-	-	-	-
	4106	E(1)	B(2) E(3)	B(2) E(1)	B(1) E(3)	C(1) E(5)	E(4)	E(5) E(1) O(6)	O(7)	E(5) O(7)	E(5) O(7)	B(1) E(4) O(4)	B(1) B(1)	B(1)	A(1) B(1)	B(1)	-

Numeral in parenthesis means incidence of respective signs in the week

- : No abnormality

A : Vomiting

B : Mucous stool

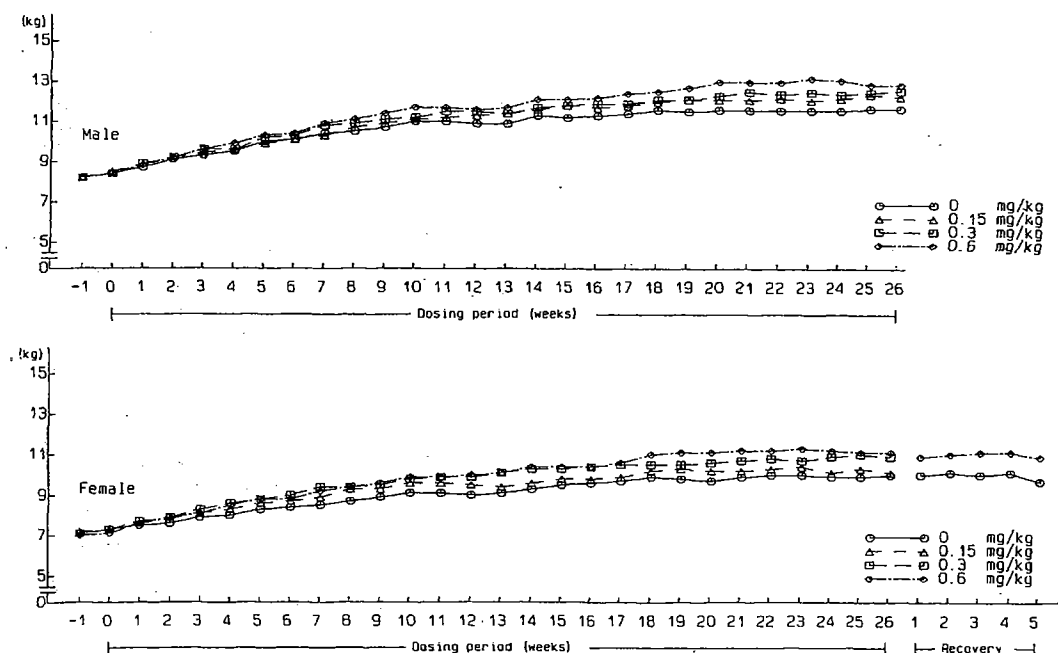
C : Loose stool

E : Flush (conjunctiva or auricle)

O : Estrus hemorrhage

Body weights: There were no significant differences between any treated group and control group. However, body weight of all treated groups except the female group at 0.15mg/kg tended to exceed that of the control and the weight gain during 26-week treatment period in males and females of the groups at 0.3mg/kg or higher tended to exceed that of the control. The change of body weight is shown in the following figures.

Best Possible Copy



Food consumption: Food consumption was comparable between all treated groups and control group.

Ophthalmoscopy: No drug-related changes were noted.

Electrocardiography: No drug-related changes were noted.

Blood pressure and body temperature: No drug-related changes were noted.

Hematology: There were no drug-related changes in animals sacrificed as scheduled.

In animals that were found dead or sacrificed in extremis during treatment, high values for red blood cell count, Hb content, Hct, fibrinogen level, white blood cell count and ratio of stab and segmented neutrophils, prolongation of APTT and low lymphocyte ratio were found in one dying animal (4004, before dosing on day 80 of treatment), and high values for white blood cell count and ratio of stab neutrophils and low lymphocyte ratio in one animal (4006, 1 day before death on day 134). Data was reported in the following table.

A 26-week subcutaneous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

Hematology (The animals found dead or sacrificed in a moribund state)

Sex : Male

Item	Dose (mg/kg)	0.6	
		Animal No.	4004a) 4006c)
Erythrocyte	($\times 10^9/\text{mm}^3$)		972 729
Hemoglobin	(g/dl)		21.6 17.5
Hematocrit	(%)		63 49
MCV	(μ^3)		65.2 57.8
MCH	(pg)		22.2 24.0
MCHC	(%)		34.1 35.4
Reticulocyte	(%)		5 5
Platelet	($\times 10^9/\text{mm}^3$)		33.7 23.3
Prothrombin time	(sec.)		6.2 6.1
APTT	(sec.)		16.3 11.3
Fibrinogen	(mg/dl)		565 130
Leukocyte	($\times 10^9/\text{mm}^3$)		376 236
Lymphocyte	(%)		6.0 9.0
Stab-neutrophile	(%)		3.0 7.5
Segment-neutrophile	(%)		88.5 77.5
Eosinophile	(%)		0.0 0.5
Basophile	(%)		0.0 0.0
Monocyte	(%)		2.5 5.5
Others leukocyte	(%)		0.0 0.0

a) : Sacrificed in a moribund state on day 80, blood sample was collected on day 80.

c) : Died on day 135, blood sample was collected on day 134.

Best Possible Copy

Clinical chemistry: Drug-related changes included significant decrease of glucose, potassium and inorganic phosphorus in both male and female dogs at doses of 0.15 and 0.3mg/kg. The post dose immediate effects in high dose group were not measured (see the methods for collecting the samples). In addition, the trend of increase of phosphorus was observed in both male and female dogs at dose of 0.6mg/kg. Data were summarized in the following table.

Summary of Drug-related Changes of Clinical Chemical Parameters in Male and Female Dogs

Parameters		Time ^a	0	0.15mg/kg	0.3mg/kg	0.6mg/kg
Male	Glucose	Month 3,1	110±5	96±11	103±3	106±6
		Month 3, 2	99±5	45±4**	37±5**	
		Month 6, 1	99±4	92±3	95±8	103±3
		Month 6, 2	84±7	39±6**	35±6**	
	Potassium	Month 3,1	4.3±0.4	4.4±0.2	4.1±0.5	4.6±0.3
		Month 3, 2	4.4±0.2	3.4±0.1**	3.0±0.3**	
		Month 6, 1	4.3±0.2	4.2±0.1	4.3±0.5	4.8±0.6
		Month 6, 2	4.3±0.2	3.6±0.3*	3.1±0.3**	
	Phosphorus	Month 3,1	5.9±0.3	5.9±0.6	6.3±0.9	7.1±0.5*
		Month 3, 2	6.0±0.2	4.2±0.3**	3.9±0.9**	
		Month 6, 1	4.4±0.4	4.8±0.5	5.3±0.9	5.8±0.6
		Month 6, 2	5.3±0.4	3.3±0.6**	3.0±0.6**	
Female	Glucose	Month 3,1	95±6	103±5	104±5	98±5
		Month 3, 2	96±7	49±7**	39±4**	
		Month 6, 1	104±12	115±4	112±5	104±7
		Month 6, 2	79±6	42±15**	31±3**	
	Potassium	Month 3,1	4.0±0.1	4.5±0.3*	4.4±0.1	4.4±0.3
		Month 3, 2	4.0±0.2	3.4±0.2**	3.0±0.1**	
		Month 6, 1	4.2±0.3	4.6±0.2	4.2±0.2	4.5±0.2
		Month 6, 2	4.1±0.3	3.6±0.1*	3.3±0.2	
	Phosphorus	Month 1	6.1±0.5	7.6±0.4**	7.0±0.2*	7.4±0.3**
		Month 3,1	5.2±0.6	6.4±0.1	6.5±0.4	6.1±1.1
		Month 3, 2	5.3±0.2	4.5±0.3**	4.0±0.3**	
		Month 6, 1	4.1±0.2	4.6±0.3	5.3±0.2	5.4±0.8*
		Month 6, 2	4.5±0.2	3.5±0.2**	3.4±0.6**	

a: blood samples were not collected from animals at dose of 0.6mg/kg 1 hour after administration.

1: before dosing; 2: after dosing

*: P<0.05 vs control; **P<0.01 vs control.

In animals that were found dead or sacrificed in extremis during treatment, abnormal values for almost all test items except GPT activity, glucose level, potassium level and ratio of serum protein fractions in the animal (4004) in a dying state before dosing on day 80. In the animal (4006) found dead 7 hours after dosing on day 134 of treatment, low values for glucose and potassium levels were noted in the examination conducted on the day before death. Data were shown in the following table.

A 26-week subcutaneous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks
 Blood chemistry (The animals found dead or sacrificed in a moribund state)
 Sex : Male

Item	Dose (mg/kg)	0.6	
		4004a)	4006c)
GOT	(IU/l)	111	36
GPT	(IU/l)	60	52
ALP	(IU/l)	513	154
Total protein	(g/dl)	8.2	6.3
Total cholesterol	(mg/dl)	225	102
Triglyceride	(mg/dl)	68	55
Phospholipid	(mg/dl)	499	278
Total bilirubin	(mg/dl)	0.23	0.09
Glucose	(mg/dl)	112	33
Urea nitrogen	(mg/dl)	48	10
Creatinine	(mg/dl)	1.25	0.58
Uric acid	(mg/dl)	0.81	0.47
Sodium	(mEq/l)	157	147
Potassium	(mEq/l)	4.0	2.9
Chloride	(mEq/l)	127	118
Calcium	(mg/dl)	11.1	10.0
Phosphorus	(mg/dl)	9.6	6.1
Magnesium	(mg/dl)	3.1	1.7
A/G		0.80	0.96
Albumin	(%)	44.31	49.01
α_1 globulin	(%)	4.04	4.58
α_2 globulin	(%)	9.27	7.73
α_3 globulin	(%)	9.04	11.01
β_1 globulin	(%)	12.49	6.19
β_2 globulin	(%)	11.49	13.02
γ globulin	(%)	9.35	8.45

a) : Sacrificed in a moribund state on day 80, blood sample was collected on day 80.
 c) : Died on day 135, blood sample was collected on day 134.

Urinalysis: No drug-related changes were noted.

Organ weights: Drug-related organ weight changes were observed in the adrenal gland, heart, thymus, pituitary glands, submandibular glands, spleen and testis. In male animals, organ weight changes included the increases of adrenal gland, pituitary, spleen weights and decrease of testis weights at dose of 0.6mg/kg. There is also a tendency of dose-dependent increased heart weight in all treated male dogs. In female animals, organ weight changes include the increase of thymus weight at doses of 0.15mg/kg and more, and increase of submandibular gland weight at dose of 0.6mg/kg. After the recovery period, a tendency of increase of the thymus weight in females at dose of 0.6mg/kg was observed. Data were summarized in the following tables.

Summary of Drug-related Absolute and Relative (Body Weight) Organ Weight Changes in Male Dogs

Organs		0		0.15 mg/kg		0.3mg/kg		0.6mg/kg	
		Weight	g%	Weight	g%	Weight	g%	Weight	g%
Body Weight kg	Mean	11.4		12.3		12.2		12.6	
	SD	1.9		1.0		0.9		0.8	
Adrenal gland ^a mg	Mean	497	4.42	557	4.54	575	4.72	721*	5.79
	SD	76	0.72	86	0.55	52	0.64	142	1.43
Heart g	Mean	83.5	0.74	97.4	0.80	100.3	0.82	107.8	0.86
	SD	13.4	0.13	1.7	0.08	8.9	0.01	8.4	0.05
Thymus g	Mean	4.9	0.045	8.27	0.069	5.83	0.048	9.75	0.077
	SD	0.62	0.009	2.20	0.024	1.27	0.013	4.37	0.033
Pituitary ^a mg	Mean	63	0.56	74	0.60	73	0.60	83*	0.66
	SD	6	0.09	10	0.07	7	0.02	3	0.02
Submandibular g	Mean	5.13	0.047	6.50	0.052	7.55	0.059	7.47	0.0580.
	SD	1.02	0.012	1.12	0.012	1.66	0.005	2.36	0.019
Spleen g	Mean	24.2	0.22	37.5*	0.31	30.5	0.25	35.9*	0.29
	SD	2.6	0.04	5.5	0.07	6.1	0.06	2.5	0.02
Testis g	Mean	8.05	0.07	7.87	0.07	7.74	0.06	6.35	0.05*
	SD	1.10	0.01	0.71	0.01	0.69	0.01	0.78	0.00

a: unit for relative weight is mg%; *: P<0.05 vs control; **P<0.01 vs control

Best Possible Copy

Summary of Drug-related Absolute and Relative (Body Weight) Organ Weight Changes in Female Dogs

Organs		0		0.15 mg/kg		0.3mg/kg		0.6mg/kg	
		Weight	g%	Weight	g%	Weight	g%	Weight	g%
Body Weight (kg)	Mean	9.5		9.9		10.7		10.5	
	SD	2.8		0.8		1.3		1.4	
Adrenal gland ^a (mg)	Mean	465	5.01	541	5.48	533	5.03	622	6.00
	SD	95	0.86	50	0.63	92	0.92	44	0.96
Heart (g)	Mean	67.7	0.71	80.5	0.77	93.1**	0.71	87.3*	0.70
	SD	10.9	0.10	5.7	0.07	7.5	0.14	4.0	0.10
Thymus (g)	Mean	3.91	0.041	8.83**	0.090*	12.28**	0.117**	6.07	0.058
	SD	1.64	0.006	1.28	0.018	1.01	0.024	1.67	0.017
Pituitary ^a (mg)	Mean	70	0.76	72	0.73	68	0.64	74	0.73
	SD	7	0.14	12	0.11	8	0.07	15	0.23
Submandibular (g)	Mean	4.69	0.050	5.82	0.059	5.91	0.056	7.31**	0.070
	SD	0.88	0.008	1.15	0.013	0.24	0.006	0.57	0.005
Spleen (g)	Mean	26.2	0.28	30.4	0.30	31.1	0.29	24.7	0.24
	SD	6.8	0.02	12.9	0.11	3.4	0.03	1.5	0.02
Recovery period									
Adrenal gland ^a (mg)	Mean	497	5.10	-				634	6.07
	SD	1	0.10					104	0.74
Thymus (g)	Mean	3.76	0.039	-				6.76**	0.065
	SD	0.08	0.001					1.24	0.011

a: unit for relative weight is mg%; *: P<0.05 vs control; **P<0.01 vs control

In animals sacrificed in extremis or found dead, all 3 animals (4004, 4005, 4006) exhibited an increase of adrenal weights. In addition, increase of the thymus, heart and lungs was observed in 2 deceased animals (4005, 4006). The increased adrenal weights were probably associated with the stress secondary to drug-induced hypoglycemia.

Gross pathology: In animals sacrifice as scheduled, partial dark reddening of left caudal lobe and caudal cranial lobe of the lung was noted in one male (4003) at dose of 0.6mg/kg, dark red spot (diameter 2-3mm) in the right atrioventricular valve of the heart was noted in one male (2002) at dose of 0.15mg/kg, one male (3003) at dose of 0.3mg/kg, and one female (4101) at dose of 0.6mg/kg. There were no other abnormalities after recovery period.

In animals sacrificed in extremis or found dead at dose of 0.6mg/kg, partial reddening of the lungs in one male (4004), total dark reddish discoloration of the lungs and liver, and paleness of the spleen in two males (4005, 4006), and dark reddish spot in the thymus in one of them (4005) and dark reddish discoloration of the mandibular lymph nodes, thymus and kidney and ulceration (1x10mm) at the pyloric portions of the stomach in another (4006) were observed.

Histopathology: In animals sacrificed at the termination of dosing, drug-related microscopic changes included slight perivascular cell infiltration in cerebrum in one male (4001) at 0.6mg/kg, slight perivascular cell infiltration in medulla oblongata in one male (4003), slight gliosis in medulla oblongata in one male (4002) at dose of 0.6mg/kg, mild vascular engorgement in the heart in one case each (2002, 3003, 4101) in each treated group, and slight (4001) or mild (3002, 3102, 4003, 4101-4103) hyperplasia of medullary cells associated with fibrosis in adrenals at doses of 0.3 and 0.6mg/kg. After the recovery period, slight and localized calcification in the lamina propria mucosae, mild vascular degeneration of

muscle cells and mild arteritis in stomach, mild fibrosis in the medulla in adrenals, and slight lipidosis in the glomerulus were observed in female animals at dose of 0.6mg/kg. No significant drug-related pathology was observed in the low dose or middle dose groups.

In three fatal cases, the major abnormal findings included slight or mild perivascular cell infiltration and mild spongy state of neuropils, and moderate or slight ischemic change of neurons in cerebrum; slight petechial bleeding in medulla oblongata; mild interstitial bleeding, and slight bleeding in arterial wall in the heart; mild cell infiltration in laryngeal region; mild and localized pneumonia, mild congestion, and or moderate bleeding in lung; mild erosion and slight bleeding in arterial wall in stomach; moderate or slight atrophy of acinar cells in submandibular glands; mild or slight atrophy of acinar cells in parotid glands; mild congestion in liver; moderate decrease in zymogen granules in pancreas; slight bleeding in posterior lobe in hypophysis; mild hyperplasia of medullary cells associated with fibrosis and moderate cell infiltration in the cortex in adrenals; moderate atrophy and mild or moderate bleeding in thymus; mild bleeding in submandibular lymph nodes; mild iridic bleeding in eyes; and slight atrophy of acinar cells in lacrimal glands. The total histological data were summarized in the following tables.

A 26-week subcutaneous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

Histopathological finding (Main group)

Sex : Male

Organs	Dose (mg/kg)					0.15			0.3			0.6					
	0					0.15			0.3			0.6					
-findings	Animal number	1	2	3	4	5	1	2	3	1	2	3	1	2	3	4	5
Cerebrum																	
-perivascular cellular infiltration		0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	2
-ischemic change of nerve cell		0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	1
-spongy state		0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Cerebellum																	
Medulla oblongata																	
-perivascular cellular infiltration		0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0
-gliosis		0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
-petechial hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0
Spinal cord		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sciatic nerve		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aorta		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart																	
-telangiectasis		0	0	0	0	0	0	2	0	0	2	0	0	0	0	0	0
-hemorrhage in interstitium		0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
-hemorrhage in arterial wall		0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0
Larynx																	
-cellular infiltration		0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0
Trachea		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung (Bronchus)																	
-focal pneumonia		0	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0
-congestion		0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	2
Tongue		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esophagus		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach																	
-focal calcification in lamina propria		0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0
-erosion		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2
-hemorrhage in arterial wall		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
Duodenum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Jejunum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ileum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cecum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colon		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rectum		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Submandibular gland																	
-atrophy of acinar cell		0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	1
Sublingual gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parotid gland																	
-atrophy of acinar cell		0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	1

Best Possible Copy

Organs	Dose (mg/kg)	0				0.15			0.3			0.6			
		1	2	3	4	1	2	3	1	2	3	1	2	3	4
-findings	Animal number	1	2	3	4	1	2	3	1	2	3	1	2	3	4
Liver															
-interlobar adhesion		0	2	0	0	0	0	0	0	0	0	0	0	0	0
-congestion		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gallbladder		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pancreas		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-decreased zymogen granule		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pituitary		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage in posterior lobe		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parathyroid		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hyperplasia of medullary cell with fibrosis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-cellular infiltration in cortex		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymus		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-atrophy		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spleen		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-follicular atrophy		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Submandibular lymph node		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesenteric lymph node		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urinary bladder		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Testis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Epididymis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-arteritis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Prostate		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mammary gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eye		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hemorrhage in iris		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Optic nerve		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimal gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-atrophy of acinar cell		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sternum (Bone marrow)		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Femur (Bone marrow)		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rib		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Musculus rectus abdominis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skeletal muscle		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Injection site		0	0	0	0	0	0	0	0	0	0	0	0	0	0

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

A : Autolysis

B : Sacrificed in a moribund state on day 80.

C : Died on day 80.

D : Died on day 135.

A 26-week subcutaneous toxicity study of MKN-031 in beagle dogs with a recovery period of 5 weeks

Histopathological finding (Main group)

Sex : Female

Organs	Dose (mg/kg)	0				0.15			0.3			0.6			
		1	2	3	4	1	2	3	1	2	3	1	2	3	4
-findings	Animal number	1	2	3	4	1	2	3	1	2	3	1	2	3	4
Cerebrum		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cerebellum		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Medulla oblongata		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spinal cord		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sciatic nerve		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Aorta		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Heart		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-telangiectasis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Larynx		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Trachea		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lung (Bronchus)		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-granuloma		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Tongue		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Esophagus		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Stomach		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Duodenum		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ileum		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Cecum		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Colon		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rectum		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Submandibular gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sublingual gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parotid gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0

Organs	Dose (mg/kg)	0				0.15			0.3			0.6			
		1	2	3	4	1	2	3	1	2	3	1	2	3	4
-findings	Animal number	1	2	3	4	1	2	3	1	2	3	1	2	3	4
Liver		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Gallbladder		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pancreas		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Pituitary		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thyroid		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Parathyroid		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Adrenal		0	0	0	0	0	0	0	0	0	0	0	0	0	0
-hyperplasia of medullary cell with fibrosis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Thymus		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Spleen		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Submandibular lymph node		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mesenteric lymph node		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Kidney		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Urinary bladder		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Ovary		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Uterus		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Vagina		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Mammary gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skin		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Eye		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Optic nerve		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Lacrimal gland		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Sternum (Bone marrow)		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Femur (Bone marrow)		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Rib		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Musculus rectus abdominis		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Skeletal muscle		0	0	0	0	0	0	0	0	0	0	0	0	0	0
Injection site		0	0	0	0	0	0	0	0	0	0	0	0	0	0

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

Best Possible Copy

A 26-week subcutaneous toxicity study of MXN-031 in beagle dogs with a recovery period of 5 weeks

Histopathological finding (Recovery group)

Sex : Female

Organs	Dose (mg/kg)	0			0.5		
		1	1	4	4	4	4
-findings	Animal number	4	0	4	0	0	0
Cerebrum		0	0	0	0	0	0
-perivascular cellular infiltration		0	0	0	0	0	0
Cerebellum		0	0	0	0	0	0
Medulla oblongata		0	0	0	0	0	0
Spinal cord		0	0	0	0	0	0
Sciatic nerve		0	0	0	0	0	0
Aorta		0	0	0	0	0	0
Heart		0	0	0	0	0	0
Larynx		0	0	0	0	0	0
Trachea		0	0	0	0	0	0
Lung (Bronchus)		0	0	0	0	0	0
Tongue		0	0	0	0	0	0
Esophagus		0	0	0	0	0	0
Stomach		0	0	0	0	0	0
-focal calcification in lamina propria		0	0	2	0	0	0
-focal vacuolar degeneration of muscle fiber		0	0	0	0	0	0
-arteritis		0	0	0	0	0	0
Duodenum		0	0	0	0	0	0
Jejunum		0	0	0	0	0	0
Ileum		0	0	0	0	0	0
Cecum		0	0	0	0	0	0
Colon		0	0	0	0	0	0
Rectum		0	0	0	0	0	0
Submandibular gland		0	0	0	0	0	0
Sublingual gland		0	0	0	0	0	0
Parotid gland		0	0	0	0	0	0

Organs	Dose (mg/kg)	0			0.6		
		1	1	4	4	4	4
-findings	Animal number	4	0	4	0	0	0
Liver		0	0	0	0	0	0
Gallbladder		0	0	0	0	0	0
Pancreas		0	0	0	0	0	0
Pyilary		0	0	0	0	0	0
Thyroid		0	0	0	0	0	0
Parathyroid		0	0	0	0	0	0
Adrenal		0	0	0	0	0	0
-fibrosis in medulla		0	0	0	0	0	0
Thymus		0	0	0	0	0	0
Spleen		0	0	0	0	0	0
Submandibular lymph node		0	0	0	0	0	0
Mesenteric lymph node		0	0	0	0	0	0
Kidney		0	0	0	0	0	0
-glomerular lipidosis		0	0	0	0	0	0
Urinary bladder		0	0	0	0	0	0
Ovary		0	0	0	0	0	0
Uterus		0	0	0	0	0	0
Vagina		0	0	0	0	0	0
Mammary gland		0	0	0	0	0	0
Skin		0	0	0	0	0	0
Eye		0	0	0	0	0	0
Optic nerve		0	0	0	0	0	0
Lacrimal gland		0	0	0	0	0	0
Sternum (Bone marrow)		0	0	0	0	0	0
Femur (Bone marrow)		0	0	0	0	0	0
Rib		0	0	0	0	0	0
Musculus rectus abdominis		0	0	0	0	0	0
Skeletal muscle		0	0	0	0	0	0
Injection site		0	0	0	0	0	0

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

Best Possible Copy

Toxicokinetics: The TK parameters of both free and total IGF-1 data were summarized in the following tables.

Toxicokinetic Parameters of IGF-1 in Male Dogs

Time	Dose mg/kg	Total IGF-1				Free IGF-1			
		Tmax h	Cmax ng/ml	AUC _{0-t} ng.h/ml	T1/2 h	Tmax h	Cmax ng/ml	AUC _{0-t} ng.h/ml	T1/2 h
Day 1	0.15	2.7±1.2	469±63	5872±885	7.7±2.0	1.0±0.0	104.9±17.2	240±34	1.1±0.1
	0.3	2.0±1.7	443±48	4589±1103	9.3±1.1	1.0±0.0	149.3±6.7	345±82	1.0±0.2
	0.6	1.5±0.5	670±116	7260±1068	7.4±1.2	0.9±0.2	286.8±55.3	889±142	1.0±0.1
Month 3	0.15	1.7±0.6	405±68	3242±1121	5.0±2.2	1.3±0.6	55.6±48.0	116±105	1.4±0.3
	0.3	2.0±0.0	616±214	3852±2446	3.3±1.8	0.7±0.3	133.3±12.9	334±16	1.2±0.1
	0.6	1.8±0.5	935±98	8846±2353	6.4±2.3	1.5±0.6	329.0±126.8	1142±312	1.0±0.1
Month 6	0.15	2.0±0.0	437±28	3251±1078	3.3±1.0	0.8±0.3	53.8±29.2	133±58	1.3±0.2
	0.3	1.7±0.6	534±114	3133±778	3.7±0.3	1.3±0.6	133.3±22.9	386±67	1.1±0.1
	0.6	1.7±0.6	877±105	8018±3384	5.8±1.0	1.0±0.0	343.7±73	1201±316	1.1±0.1

Toxicokinetic Parameters of IGF-1 in Female Dogs

Time	Dose mg/kg	Total IGF-1				Free IGF-1			
		Tmax h	Cmax ng/ml	AUC _{0-t} ng.h/ml	T1/2 h	Tmax h	Cmax ng/ml	AUC _{0-t} ng.h/ml	T1/2 h
Day 1	0.15	2.0±0.0	558±116	6181±323	7.4±1.7	1.0±0.0	73.2±2.3	127±15	0.8±0.1
	0.3	3.3±1.2	480±9	5409±743	6.1±3.4	1.0±0.0	103.4±7.0	260±14	1.0±0.2
	0.6	1.3±0.5	671±84	6703±864	6.9±1.3	0.8±0.3	298.3±77.1	754±143	1.0±0.1
Month 3	0.15	2.0±0.0	496±63	3286±646	3.9±1.7	1.0±0.0	29.4±7.7	60±22	1.2±0.2
	0.3	1.7±0.6	666±143	6352±1867	6.5±0.9	1.0±0.0	107.5±13.9	274±48	1.1±0.2
	0.6	1.5±0.5	850±64	5090±1489	3.5±0.7	1.0±0.0	337.5±34.4	958±183	1.0±0.1
Month 6	0.15	2.0±0.0	537±36	3504±1527	5.3±2.8	1.2±0.8	37.9±15.8	81±20	1.1±0.2
	0.3	1.8±1.9	735±174	8293±4456	7.8±3.6	1.2±0.8	187.3±114.0	397±102	1.0±0.2
	0.6	2.0±1.1	841±139	5864±1751	3.6±1.1	1.0±0.5	315.2±56.8	1029±215	1.1±0.2

Plasma level of free IGF-1 showed a dose-dependent increase in both sexes in day 1, month 3 and month 6 treatment. The total IGF-1 concentrations were not clear. The peak plasma levels for free IGF-1 and total IGF-1 were detected 1 hour and about 2 hours after the treatment. Twenty four hours after administration, levels of total and free IGF-1 in the plasma were almost the same with predosing values. After 3 months and 6 months treatment, no significant drug accumulation was observed.

In determination of blood glucose level, significant decrease of blood glucose level was noted. The blood glucose levels began to decrease as early as 15 to 30 minutes after dosing and reached to the lowest level 30 to 60 minutes after dosing, and then returned to the level comparable with predosing 4 hours after dosing at dose of 0.15mg/kg, 4 to 6 hours after dosing at 0.3mg/kg, and 6 to 8 hours after dosing at dose of 0.6mg/kg. Data were summarized in the following tables.

Summary of Blood Glucose Level in Male Dogs

Time	Dose mg/kg	Time after dosing								
		Pre	15 min	30 min	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Day1	0	96±4	80±9	80±8	85±6	87±9	95±6	90±4	86±4	93±8
	0.15	97±9	69±10	27±5**	36±8**	69±6	108±6	103±7*	95±6	97±6
	0.3	94±1	75±8	34±4**	45±14**	44±7**	78±13	90±3	94±3	102±4
	0.6	95±5	68±18	31±8**	35±7**	42±11**	75±22	89±6	95±8	106±8
Month 3	0	99±6	103±6	102±8	95±5	97±6	104±9	105±6	100±5	111±10
	0.15	101±6	98±11	70±9**	60±26*	84±11	104±4	94±2	103±14	116±11
	0.3	113±4	102±7	68±6**	49±10**	56±10**	97±10	105±6	111±7	115±12
	0.6	105±7	96±8	52±6**	35±8**	42±14**	58±3**	92±20	113±5	112±7
Month 6	0	82±8	85±8	81±9	83±10	86±7	92±8	98±7	88±9	92±9
	0.15	81±8	82±13	43±13**	35±16**	62±20	90±3	103±10	92±7	94±7
	0.3	80±10	89±9	63±8	39±10**	47±6*	77±8	99±8	96±6	94±4
	0.6	82±5	68±10	26±7**	26±4**	42±14**	45±11**	66±33	79±45	91±5

*: P<0.05 vs control; **: P<0.01 vs control.

Summary of Blood Glucose Level in Female Dogs

Time	Dose mg/kg	Time after dosing								
		Pre	15 min	30 min	1 hr	2 hr	4 hr	6 hr	8 hr	24 hr
Day1	0	95±5	81±5	78±7	82±5	84±5	95±8	93±5	91±6	94±6
	0.15	97±10	71±22	34±14**	37±6	70±15	98±2	97±5	97±11	104±7
	0.3	96±8	84±8	44±17**	36±3**	46±11**	83±26	96±9	96±10	95±15
	0.6	95±10	60±16	30±5**	37±16*	63±8*	79±17	93±8	100±11	99±4
Month 3	0	102±6	100±3	94±8	91±7	97±10	105±11	99±5	101±1	113±8
	0.15	107±10	105±7	78±15	65±23	69±20	120±9	106±1	107±14	113±12
	0.3	1106	952	5317**	409**	7322	8710	1115	1096	10510
	0.6	104±6	77±15*	36±14**	36±7**	38±7**	65±14**	95±11	108±8	101±5
Month 6	0	79±10	91±16	83±7	78±10	86±8	87±12	96±13	87±9	87±11
	0.15	84±12	85±33	60±37	47±22	75±12	92±22	101±7	90±2	89±5
	0.3	78±1	86±14	53±25	46±19	42±12**	93±5	104±3	101±9	90±7
	0.6	83±9	60±20	28±12*	23±9**	35±9**	41±10**	83±24	114±25	88±12

*: P<0.05 vs control; **: P<0.01 vs control.

Others: In the test for antibody formation, no antibodies against MKN-031 were detected in any dosed animals throughout the treatment and recovery periods.

2.6.6.4 Genetic toxicology

Study title: In vitro chromosome aberration test of MKC-031

Key findings: MKC-031 tested negative in the in vitro chromosome aberration assay in either direct method or metabolic method with or without S9 mixture.

Study no.: 2P122

Volume #, and page #: E-file

Conducting laboratory and location: ㄥ

Date of study initiation: June 2, 1992

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: MKC-031 (IGF-1), Lot# M6-24, and purity — %.

Methods

Strains/species/cell line: The CHL/IU fibroblast cell line derived from Chinese hamster lung tissue was obtained from ㄥ] at June 10, 1986. The cells at passage No. ㄥ] were used in the cell growth inhibition test and the chromosome aberration test, respectively.

Doses used in definitive study: 0, 122, 244, 487μg/ml.

Basis of dose selection: The basis of dose selection was based on the preliminary test. The maximum volume of citrate buffer with no influence on cell growth was found to be 10% of the culture medium, MKC-031 test substance solution (vehicle: citrate buffer) was added at final 10% in the culture for each treatment. Total 8 serial two-fold dilutions of test substance were prepared in the preliminary test, in which the highest final concentration was set as 487µg/ml by treating with undiluted MKC-031 itself (concentrations: 4.87mg/ml, the maximal solubility).

Negative controls: The citrate buffer was used as a vehicle control.

Positive controls: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) in a final concentration of 2µg/ml in the absence of S9 mixture and Benzo(a)pyrene (B(a)P) in a final concentration of 10 µg/ml in the presence of S9 mixture.

Incubation and sampling times: In the direct method, the cells were seeded at 2×10^4 cells/5ml of culture medium in 60mm Petri dish and cultivated for 3 days. After 0.5ml of the medium was removed from the culture, equal amount of either test substance solution or negative control or 0.025ml of the positive control was added to each dish and cultivated for 24 and 48 hours.

In the metabolic activation method, the cells were seeded at 2×10^4 cells/5ml of culture medium in 60mm Petri dish and cultivated for 3 days. After the medium was discarded, 2.2ml of fresh medium and 0.5 ml of S9 mixture were added with the addition of 0.3 ml of either test substance solution or negative control. In the treatment of positive control, after the medium was discarded, 2.5ml of fresh medium, 0.5 ml of S9 mixture and positive control were added to the dish. The cells were treated for 6 hours and cultured in 5ml of fresh medium for further 18 hours followed by slide preparation.

In the absence of S9 mixture, the cells were seeded at 2×10^4 cells/5ml of culture medium in 60mm Petri dish and cultivated for 3 days. After the medium was discarded, 2.7ml of fresh medium and 0.3ml of either test substance or negative control was added to each dish. In the treatment with positive control, 3ml of fresh medium and 0.015ml of positive control solution were added to the dish after the medium was added. The cells were treated for 6 hours and cultured in 5ml of fresh medium for further 18hours, followed by slide preparation.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): MKC-031 did not inhibit cell growth more than 50% in the dose range from 3.80 to 487µg/ml both in direct method and in the metabolic activation method. Therefore, the highest three concentrations of 122, 244, and 487µg/ml were selected in the definitive study. Based on that the maximum volume of citrate buffer as a vehicle with no influence on cell growth was found to be 10% of the culture medium and the undiluted MKC-031 concentration is 4.87mg/ml, the dose selection was adequate. The positive control response was appropriate in the number of the mutant frequency compared to the negative control in the direct method and activation method in the conditions of the presence or absence of the activation system.

Table 1 Cytotoxicity Tests of MEC-031 in CHL/IO Cells

Conc. ^{a)} (μ g/ml)	Relative growth rate (%)					
	direct method			metabolic activation method		
	24hrs ^{b)}		48hrs	6hrs		
Nontreatment	107, 103	(104) ^{c)}	89, 94 (98)	97, 97	(96)	
0 ^{d)}	100, 101	(100)	100, 87 (100)	100, 102	(100)	
3.80	99, 95	(96)	88, 100 (100)	113, 105	(108)	
7.61	95, 107	(100)	99, 88 (100)	105, 113	(108)	
15.2	97, 101	(98)	99, 90 (101)	113, 108	(110)	
30.4	105, 107	(106)	89, 100 (101)	102, 118	(109)	
60.8	99, 101	(99)	99, 89 (100)	105, 112	(108)	
122	111, 107	(108)	106, 86 (102)	102, 108	(104)	
244	105, 97	(100)	93, 101 (103)	99, 108	(103)	
487	109, 103	(105)	102, 92 (103)	102, 108	(104)	

a) concentration; b) treatment time; c) average (relative rate to control)
d) vehicle

Study outcome: There was no significant difference between the test articles and negative control in the percentages of cells with chromosome aberrations in the in the direct method and activation method in the conditions of the presence or absence of the activation system. The averages of the percentage of cells with aberrations scored were summarized in the follow tables. Therefore, MKC-031 has no potential to induce chromosomal aberration under the experimental conditions performed in this study.

Table 4. Characteristics observed in the study of HIV-101 in CML-200 cells. Values are means $\pm$ standard deviation.

[illegible][illegible]

Table 3 Chromosome Aberration Tests of MEC-001 in CHL/TH Cells (Genotoxic activities method without IF media)

Exposure	Conc. (µg/mL)	No. of cells	No. of polyploid cells (%)	No. of cells with structural chromosome aberrations						TPO (%)	TA (%)
				gap	dic	dic	dic	dic	other		
CONTROL		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
MEC-001	0 ⁺	100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0	0	0	0	0	0	0
		100	0	0	0						

Study title: In vivo micronucleus test of MKC-031

Key findings: MKC-031 was negative in the micronucleus assay of mouse bone marrow cells

Study no.: 2P097

Volume #, and page #: E-file

Conducting laboratory and location: ☐

Date of study initiation: May 18, 1992

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: MKC-031 (IGF-1), Lot# M6-24, and purity — %.

Methods

Strains/species/cell line: Male ICR mice (— Cd-1, SPF) weight range of 34.7 to 45.2g at the age of 9-10 weeks old obtained from ☐

Doses used in definitive study: 24.4, 48.7 and 97.4 mg/kg (97.4mg/kg is the maximum dose that can be iv practically, the undiluted concentration is 4.87mg/ml).

Basis of dose selection: The final dose selection was based on the range finding study and preliminary micronucleus test.

Negative controls: Citrate buffer (vehicle)

Positive controls: Mitomycin C (MMC)

Incubation and sampling times: Six animals per group were treated with MKC-031 at doses of 24.4, 48.7 and 97.4mg/kg. Citrate buffered solution (negative control) was administered to animals at the volume of 20ml/kg, and MMC (positive control) was administered at the dose of 2mg/kg. Animals were then sacrificed 24 hours after the iv injection.

For each animal, one thousand polychromatic erythrocytes were scored for the presence of micronuclei at a magnifying power of 1000. The frequency of reticulocytes in 500 erythrocytes for each animal was also recorded.

Results

Study validity (comment on replicates, counting method, criteria for positive results, etc.): There were no mortality, clinical signs or body weight change in the range finding study at doses of 24.4, 48.7 and 97.4mg/ml. So 97.4mg/kg, the maximum dose that can be iv practically was selected as the highest dose.

Regarding the harvest time, there were no significant effects on micronucleated polychromatic erythrocytes and reticulocytes due to MKC-031 compared to the negative control at any harvest times in the preliminary study as shown in the following table. Therefore, the sampling time in the definitive micronucleus test was determined to be 24 hours after injection.

Table 2 Results of Preliminary Micronucleus Test

Test Substance	Dose level (mg/kg)	Sampling time (hr)	Animal number	PCE scored	MNPCE		Frequency of RET (%)
					Number	Frequency (%)	
Negative Cont. (Citrate buffer)	0	24	1	1000	1	0.1	51.2
			2	1000	2	0.2	47.8
			3	1000	1	0.1	52.2
			Total	3000	4	0.13±0.06 ^b	50.40±2.31 ^b
MKC-031	97.4	24	1	1000	1	0.1	40.4
			2	1000	0	0	53.8
			3	1000	0	0	54.4
			Total	3000	1	0.03±0.06	49.58±7.92
MKC-031	97.4	30	1	1000	1	0.1	37.6
			2	1000	0	0	43.8
			3	1000	0	0	55.0
			Total	3000	1	0.03±0.06	47.13±8.82
MKC-031	97.4	48	1	1000	3	0.3	69.2
			2	1000	3	0.3	48.6
			3	1000	5	0.5	46.6
			Total	3000	11	0.37±0.12	51.47±6.77
MKC-031	97.4	72	1	1000	1	0.1	67.8
			2	1000	0	0	44.0
			3	1000	2	0.2	36.4
			Total	3000	3	0.10±0.10	49.40±16.38

PCE : polychromatic erythrocytes, MNPCE : micronucleated polychromatic erythrocytes, RET : reticulocytes, 1) : mean ± S.D.

The positive control, MMC induced a significant increase in micronucleated polychromatic erythrocytes without showing any effect on reticulocytes.

Study outcome: There was no significant effect on micronucleated polychromatic erythrocytes due to MKC-031 compared to the negative control. Therefore, MKC-031 was considered negative in the micronucleus assay of male ICR mouse bone marrow cells. Data were summarized in the following tables.

Table 4 Results of Definitive Micronucleus Test

Test Substance	Dose level (mg/kg)	Sampling time (hr)	Animal number	PCE scored	MNPCE		Frequency of RET (%)
					Number	Frequency (%)	
Negative Cont. (Citrate buffer)	0	24	1	1000	0	0	53.4
			2	1000	0	0	47.0
			3	1000	0	0	50.8
			4	1000	4	0.4	39.4
			5	1000	2	0.2	46.0
			6	1000	1	0.1	47.6
			Total	6000	7	0.12±0.16 ⁰	47.37±4.77 ⁰
MKC-031	24.4	24	1	1000	4	0.4	47.8
			2	1000	0	0	50.4
			3	1000	3	0.3	66.0
			4	1000	0	0	60.2
			5	1000	2	0.2	59.4
			6	1000	0	0	56.8
			Total	6000	9	0.15±0.18	56.77±6.71 [*]
MKC-031	48.7	24	1	1000	1	0.1	41.4
			2	1000	0	0	53.2
			3	1000	1	0.1	58.0
			4	1000	1	0.1	47.2
			5	1000	2	0.2	49.8
			6	1000	1	0.1	56.0
			Total	6000	6	0.10±0.06	50.93±6.11
MKC-031	97.4	24	1	1000	1	0.1	39.0
			2	1000	0	0	53.2
			3	1000	2	0.2	35.2
			4	1000	1	0.1	47.0
			5	1000	2	0.2	50.8
			6	1000	0	0	53.6
			Total	6000	6	0.10±0.09	47.80±8.79
MMC	2	24	1	1000	81	8.1	59.0
			2	1000	106	10.6	38.0
			3	1000	63	6.3	35.6
			4	1000	85	8.5	36.4
			5	1000	118	11.8	44.2
			6	1000	42	4.2	40.6
			Total	6000	495 [#]	8.25±2.77	42.80±8.76

PCE : polychromatic erythrocytes, MNPCE : micronucleated polychromatic erythrocytes, RET : reticulocytes, 1) : mean±S.D., * : p<0.05 (Student's t-test), # : p<0.05 (method of Kastenbaum & Bowman)

2.6.6.5 Carcinogenicity

Study title: Effects of rhIGF-1 on the incidence and development of DMBA-induced mammary tumors in SD female rats.

The effect of mecasermin (rhIGF-1) on the growth of mammary tumors induced by the chemical initiating agent dimethylbenzanthracene (DMBA) was assessed. Female rats were treated with mecasermin at doses of 0.25, 1.0, 4.0 or 10mg/kg/day subcutaneously following a single dose of DMBA administered by oral gavage. Following approximately 5 months treatment, preliminary data shown that the increased incidence of mammary findings (adenomas, carcinomas-low grad, fibroadenoma, and hyperplasia) were observed at doses of 4 and 10mg/kg. A marginal increase in the incidence of mammary hyperplasia was seen at dose of 1mg/kg. Data were summarized in the following table. Since the study was terminated early and final study report was not submitted except the following table, definitive conclusions regarding the experimental results can not be made.

2.6.7.10.1 Carcinogenicity- Initiation/Promotion: Study 96-453-0320

Report Title: Effect of Mecasermin upon the Incidence and Development of DMBA induced mammary Tumors in SD Female Rats

Test Article: Mecasermin

Study No. : 96-453-0320^b

Species/Strain: Rat — CD®(SD)BR

VAF/Plus®

GLP Compliance: No

Duration of Dosing: ~5 months

Method of Administration: Subcutaneous

Vehicle/Formulation: Acetate —

Treatment Controls: Vehicle

Page 1 of 1

Basis for High-Dose Selection: Anticipated maximum tolerated dose based upon 6-month study and hypoglycemic response at 10.0 mg/kg. Same dose levels as used in carcinogenicity study in this species.

Special Features:

Daily Dose (mg/kg)	0 (Control)		0.25		1.0		4.0		10.0	
	M	F	M	F	M	F	M	F	M	F
No. of Animals										
At Start		30		20		20		20		20
Died or Sacrificed Moribund		0		0		0		1		5
No. of Animals Evaluated		30		20		20		19		15
Mammary Findings ^a										
Adenoma		1(2)		1(1)		2(2)		3(8)		6(8)
Carcinoma – Low Grade		0		1(1)		1(2)		5(21)		11(26)
Carcinoma – Invasive		2(2)		0		0		1(1)		1(3)
Fibroadenoma		0		0		0		2(2)		2(2)
Hyperplasia		2(2)		1(1)		4(4)		5(3)		5(5)

^a First number reflects number of animals with finding. Number in parentheses indicates number of times finding recorded in treatment group across all animals.

^b Study stopped early and only preliminary results available. No final study report. Data available on request.

Study title: 104-Week subcutaneous carcinogenicity study with rhIGF-1 in rats

Key study findings: rhIGF-1 induced pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels; keratoacanthoma in the skin in males at doses of 4 and 10mg/kg/day; mammary gland carcinoma in males at 10mg/kg/day. However, as comparing with the historical data, the only positive findings in this carcinogenicity study was the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg.

Study number: 96-177-0320, 16281-377

Volume #, and page #: Volume 1, and page 1.

Conducting laboratory and location: J

Date of study initiation: June 28, 1996

GLP compliance: Yes

QA report: yes (x) no ()

Drug, lot #, and % purity: rhIGF-1, Lot# D9820AX, D9818AX, D9809AX, D9834AX, D9849AX, D9871AX, purity 100%

CAC concurrence: In the cover letter, sponsor described that this study has concurrence with FDA CAC but no record was found.

Study Type: 2-year bioassay

Species/strain: Rats/ CD@ (SD)BR VAF/Plus®

Number/sex/group: 75/sex/group in main study; 15/sex/group in satellite study

Age at start of study: 41-44 days old at initiation of treatment

Animal housing: Animals were housed individually in stainless-steel, screen-bottom cages. Certified rodent diet 1 was provided. Water was provided *ad libitum*. Animal rooms were set to maintain 19° to 25° C, a relative humidity of 30 to 70%, and a 12-hour light/12-hour dark cycle; light from 09:30 to 21:30 hours.

Formulation/vehicle: Solution/ 1 mg/ml sodium chloride, 1 mg/ml benzyl alcohol, 1 mg/ml polysorbate 80 sodium acetate, pH 5.4

Drug stability/homogeneity: Drug was stable in 1 in a refrigerator set to maintain 2° to 8° C. Dose preparations were stable for 1 in a refrigerator set to maintain 2° to 8° C and for at least 1 when stored at room temperature. A separate homogeneity analysis was not performed since the rhIGF-1 and its vehicle dose preparations were solutions and confirmation of dose concentration was performed.

Methods:

Groups		Dose level (mg/kg/day)	Dose concentration (mg/ml)	Number of Animals	
				Male	Female
Carcinogenicity Animals	1. Vehicle	0	0	75	75
	2. Low	0.25	0.25	75	75
	3. Mid 1	1.0	1.0	75	75
	4. Mid 2	4.0	4.0	75	75
	5. High	10.0	10.0	75	75
Satellite ^a animals	6. Vehicle	0	0	15	15
	7. Low	0.25	0.25	15	15
	8. Mid 1	1.0	1.0	15	15
	9. Mid 2	4.0	4.0	15	15
	10. High	10.0	10.0	15	15

a: The satellite animals were used for collection of samples to measure clinical pathology, PK, glucose response, serum binding, and antibody.

Doses:

Basis of dose selection: Dose selection was based on the hypoglycemic response to IGF-1. In the previous studies, a single dose of 10mg/kg administered subcutaneously to fasting rats resulted in 50% mortality. In non-fasted rats, doses of up to 64mg/kg are not lethal but do result in severe prolonged hypoglycemia. As rats age, there will be periodic episodes in which the rats will not eat sufficiently overnight, 10mg/kg was chosen as the high dose to avoid an unacceptable deaths during the latter half of this 2-year study. Based on the 26 weeks general toxicity study, 4mg/kg was chosen as the middle dose which induced limited toxicity including increase in body weight, food consumption and organ weights, and thymic pathology. The low dose of 0.25mg/kg was chosen in an attempt to obtain a no-effect level.

Restriction paradigm for dietary restriction studies: Not restricted

Route of administration: Subcutaneous injection

Frequency of drug administration: Daily rotating between four different sites on the dorsal surface.

Dual controls employed: None

Interim sacrifices: None

Satellite PK or special study group(s): 15/sex/group

Deviations from original study protocol: As a result of decreased survival related to test material administration, the duration of dose administration was modified as follows: Dosing given 10mg/kg/day was discontinued during Week 84, at which time these groups were terminated; Dosing given 4mg/kg/day was discontinued during Week 92. Dosing given 1mg/kg/day for males was discontinued during Week 95. Dosing continued for control males and males given 0.25mg/kg/day through the scheduled early termination of all remaining male groups during Week 99.

Statistical methods: Levene's test was done to test variance homogeneity. In the case of heterogeneity of variance at $P < 0.05$, transformations were used to stabilize the variance. ANOVA was used to analyze body weight, body weight changes, food consumption, and continuous clinical pathology values. If the ANOVA was significant, Dunnett's t-test was used for pairwise comparisons between treated and control groups. Group comparisons were evaluated at the 5.0% two-tailed probability level.

For the survival and tumor analysis, statistical analyses were performed separately for each sex. Evaluation of trend and heterogeneity of survival data were analyzed by life table techniques consisting of Kaplan-Meier product limit estimates and Cox-Tarone binary regression test. The dose-response relationship of mortality was tested by the logistic regression method at the 5%, two-tailed probability level. Tests were run with equally spaced dose group scores. Each tumor was classified as “fatal,” “mortality-independent or observable,” or “incidental” according to the context of the observations described in the IARC document-type test. The number and percentage of animals with tumors were summarized by group and sex. Fatal and observable tumors were analyzed using the same methods as used for analysis of survival.

Observations and times:

Clinical signs: Twice daily

Body weights: Weekly for 26 weeks and every other week thereafter

Food consumption: Weekly for 26 weeks and every other week thereafter

Hematology: RBC, Hb, Hct, MCV, MCH, MCHC, Platelet, WBC counts and differential, segmented neutrophil, lymphocyte, monocyte, eosinophil, basophil, blood cell morphology, PT, and APTT.

Clinical chemistry: Blood samples were collected from Groups 6 through 10 (all satellite animals) during Weeks 31 and 57 not from main study groups (Groups 1 through 5). Parameters included glucose, BUN, Cr, total protein, albumin, globulin, A/G ratio, cholesterol, total bilirubin, AST, ALT, AKP, GGT, calcium, P, Na, K, Cl, and triglycerides.

Organ weights: Not measured

Gross pathology: Necropsy was done on each animal in groups 1 through 5 that died or was euthanized at unscheduled intervals. Macroscopic examination of the external surface of the body; all orifices; the cranial cavity; the brain and spinal cord; the nasal cavity and paranasal sinuses; and the thoracic, abdominal, and pelvic cavities and viscera.

Histopathology: Organs and tissues were collected as following for histopathology study.

Adrenal glands	Ovaries
Aorta	Pancreas
Brain	Parathyroid
Cecum	Pituitary
Colon and rectum	Prostate
Duodenum	Salivary gland
Epididymis	Sciatic nerve
Esophagus	Seminal vesicles
Eye	Skeletal muscle (thigh)
Femur with bone marrow	Skin
Heart	Spinal cord
Ileum	Spleen
Injection site	Sternum with bone marrow
Jejunum	Stomach
Kidneys	Testes

Lacrimal gland (exorbital)	Thymus
Lesions	Thyroid
Liver	Tongue
Lymph node (submaxillary, mesenteric)	Trachea
Lungs with mainstem bronchi	Urinary bladder
Mammary gland	Uterus
Masses and associated tissues	Vagina

Toxicokinetics: Blood samples were collected in predose and at approximately 0.5, 1, 1.5, 3, and 24 hours postdose on Day 1 and once during Weeks 13, 26, 39, 52, and 65. During Week 79, samples were collected in predose and at approximately 0.5, 1, 1.5, 3, 4, 8, and 24 hours postdose. During Week 84, samples were collected from Group 10 in predose and at approximately 0.5, 1, 1.5, and 3 hours postdose. During Week 91, samples were collected from Groups 6 through 9 predose and at approximately 0.5, 1, 1.5, 3, 4, 8 and 24 hours postdose. During Week 99, samples were collected males in group 6 and 7 predose and at approximately 0.5, 1, 1.5, 3, 4, 8 and 24 hours postdose. During Week 104, samples were collected from females in group 6, 7, and 8 predose and at approximately 0.5, 1, 1.5, 3, 4, 8 and 24 hours postdose.

Others:

Antibody analysis: blood was collected in groups 6 through 10 only within two hours before dosing from up to 5 animals/sex/group/time point predose on Day 1 and once during Weeks 13, 26, 39, 52, 65, 79, 84 (group10 only), and 91(groups 6 through 10).

Serum binding protein analysis: blood was collected in groups 6 through 10 once during Week 52 approximately 1 hour postdose from up to 5 animals/sex/group designated for antibody analysis.

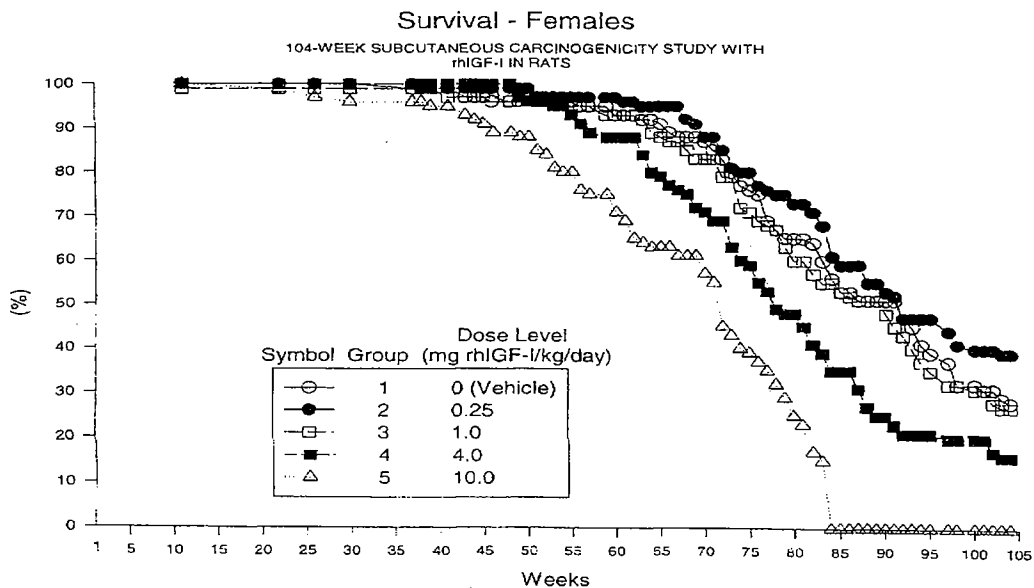
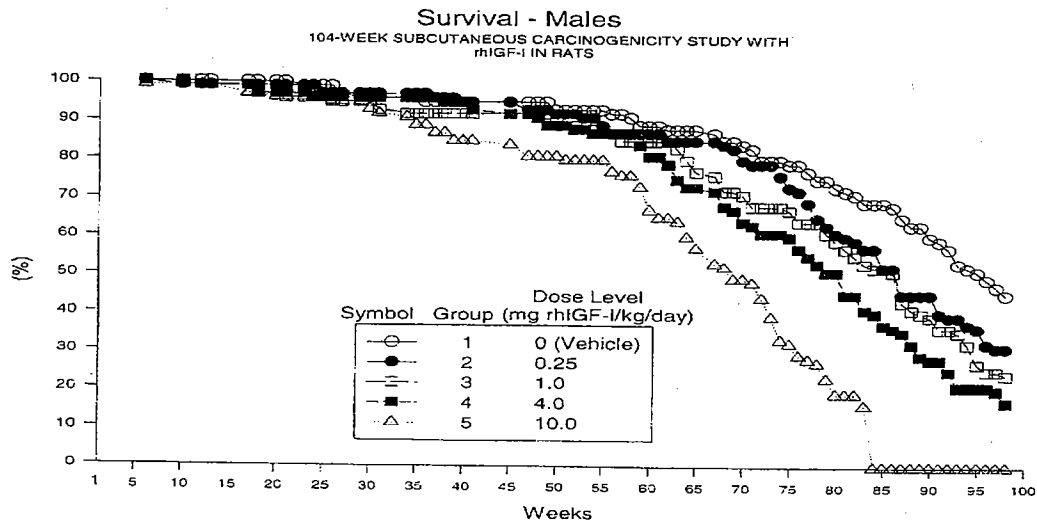
Results:

Mortality: There were significant dose-related decreases in survival for males at all dose levels and in females at doses of 4 and 10mg/kg/day. As a result of drug-related decreased survival, animals at dose of 10mg/kg were euthanized during Week 84 and the remainder of the male groups during Week 99. The remaining female groups were euthanized at the scheduled study termination during Week 105. In addition, because of low survival, dose administration was discontinued for approximately 4 week prior to termination for males at dose of 1.0mg/kg/day and for approximately 7 and 13 weeks for males and females at dose of 4mg/kg/day, respectively. No cause could be found to attribute to the early death of animals based on the histological examination of tissues. Marked hypoglycemia, a pharmacologic effect of the IGF-1, and an increased incidence of palpable masses were found in some animals at higher dose groups, which were euthanized due to poor health. The data were summarized in the following table and described in the following figures.

Summary of Survival Rate of Male and Female Rats at the End of Study

Groups		1	2	3	4	5*
Dose (mg/kg/day)		0	0.25	1	4	10
Number of male animals	Start of study	75	75	75	75	75
	End of study	34	23	18	13	12
	Survival rate#	45%	31%	24%	17%	16%
Number of female animals	Start of study	75	75	75	75	75
	End of study	21	29	20	12	11
	Survival rate	28%	39%	27%	16%	15%

*: Rats in this group were euthanized on Week 84. #: Male rats at groups 1 through 4 were euthanized on Week 99.



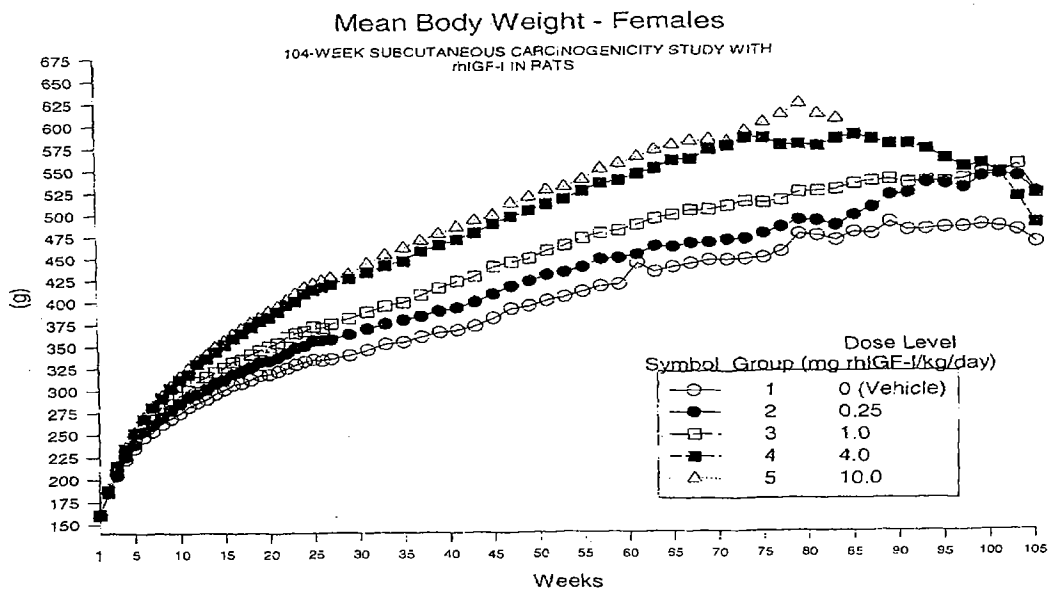
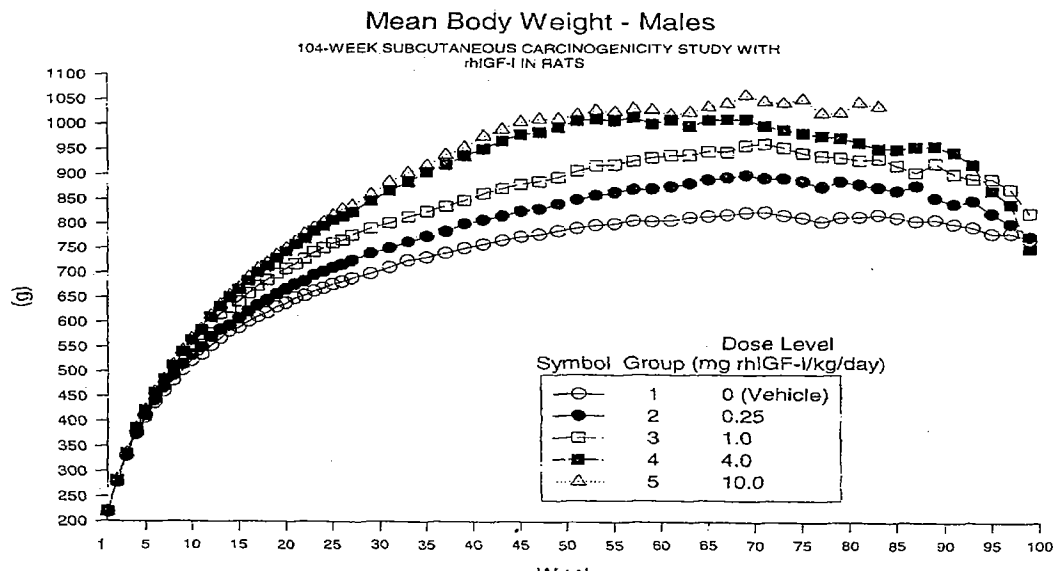
Clinical signs: Drug-related increases in the incidence of palpable masses and wart-like lesions in multiple sites in both males and females at doses of 4 and 10mg/kg were noted. These findings were consistent with the increased incidence of skin and mammary gland neoplasms in these groups (see pathology findings). In addition, the onset of palpable masses was earlier in females at doses of 4 and 10 mg/kg/day compared with controls. In ophthalmology study, there were no drug-related ophthalmic findings.

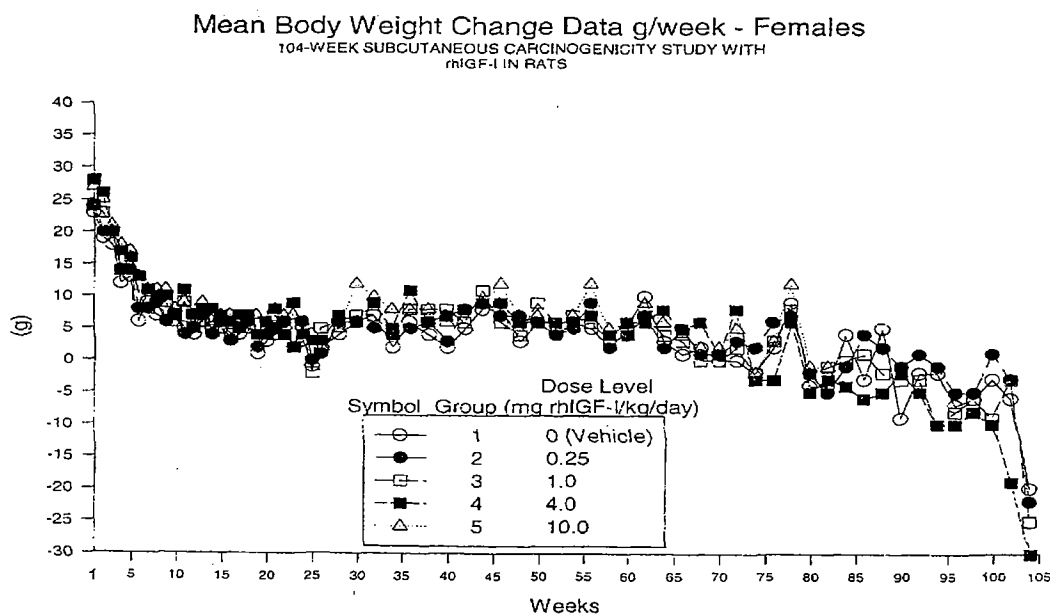
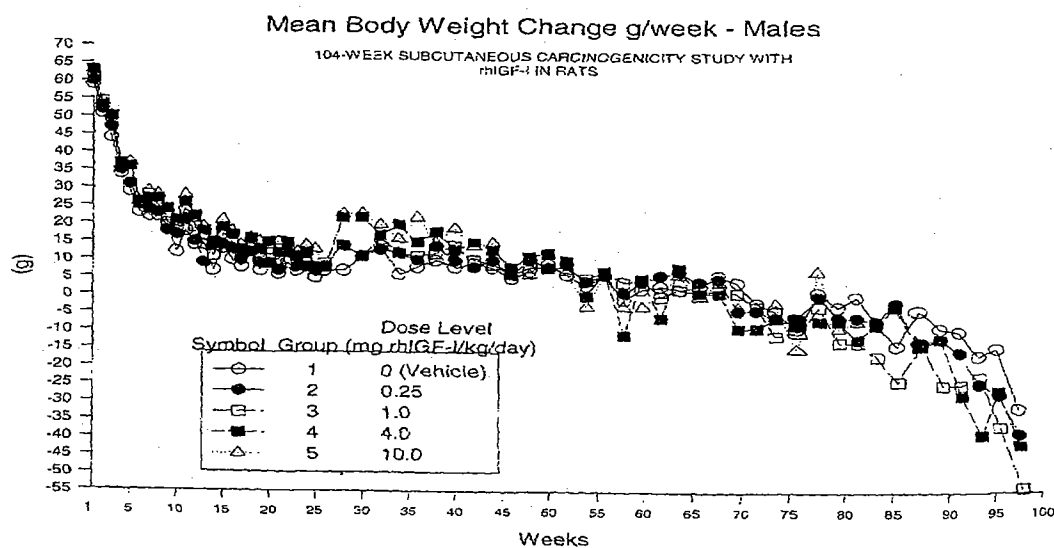
Body weights: There were dose-related increases of body weight gains in both males and females at all dose levels. In males, the mean body weight was generally statistically significant from Week 6 throughout the majority of the study at doses of 1, 4, and 10mg/kg/day, and from Week 22 at dose of 0.25mg/kg/day. In females, the mean body weight increased from Week 3 at doses of 4 and 10mg/kg/day, Week 6 at dose of 1.0mg/kg/day, and from Week 20 to 63 at dose of 0.25mg/kg/day. At the beginning of Week 69, a time when survival rate of both males and females at high dose was at least 50%, mean body weight for males were 109%, 116%, 123%, and 129% of control value at doses of 0.25, 1.0, 4.0, and 10mg/kg, respectively; for females this was 104%, 113%, 128, and 131% of control values, respectively. Results were summarized in the following table and described in the following figures.

Summary of Body Weight Data at the End of Study

	Time (Week)		Control	0.25 (mg/kg/day)	1 (mg/kg/day)	4 (mg/kg/day)	10 (mg/kg/day)
Male	1	N	75	75	75	75	75
		Mean±SD	220±20.4	218±19.8	220±20.2	220±20.0	219±20.2
	69	N	64	63	54	51	39
		Mean±SD	823±97.8	899±105.5*	958±101.8*	1010±142.6*	1059±135.1*
	83	N	53	43	41	34	14
		Mean±SD	819±116.8	874±118.8	932±125.6*	952±147.4*	1036±131.9*
	99	N	34	23	17	12	0
		Mean±SD	767±109.3	773±134.2	821±152.7	748±148.3	
Female	1	N	75	75	75	75	75
		Mean±SD	161±14.6	161±15.4	161±14.5	160±14.0	162±16.1
	69	N	66	69	64	56	46
		Mean±SD	445±67.9	465±68.6	503±83.7*	571±100.3*	582±85.7*
	83	N	48	53	43	31	13
		Mean±SD	467±66.8	484±88.3	525±85.5*	582±103.6*	604±98.1*
	105	N	21	29	20	12	0
		Mean±SD	463±75.8	520±106.8	519±76.8	485±124.9	

*: Statistically significant at $P \leq 0.05$





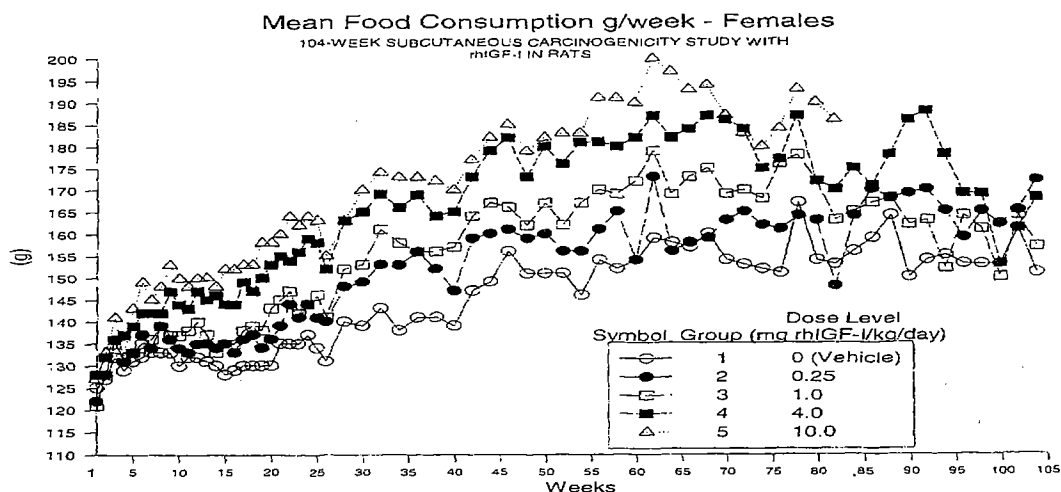
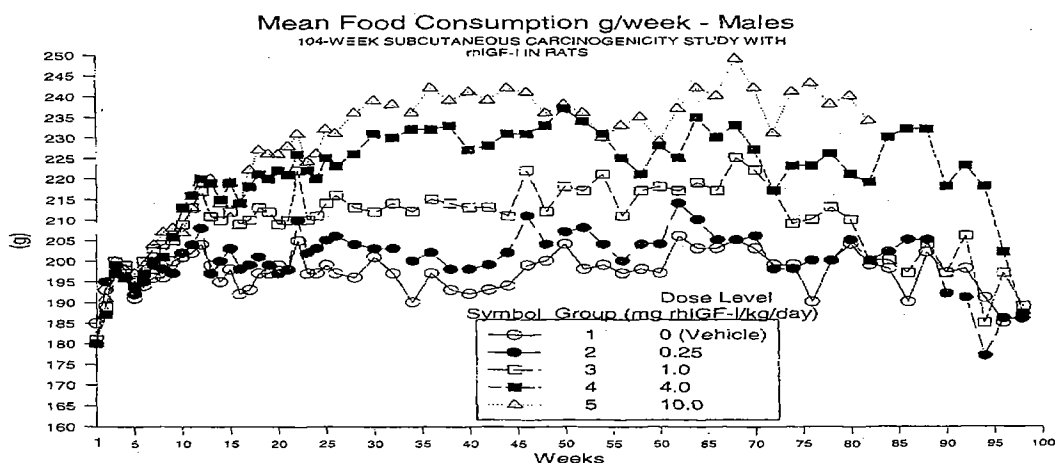
Food consumption: There were dose-related increases of food consumption in both males and females at all dose levels. The statistically significant increases of food consumption in males at doses of 1.0, 4.0, and 10mg/kg/day were generally from Week 11 and continued throughout most of the study. In females, the differences were beginning at Weeks 2, 5, and 10, at doses of 10, 4, and 1mg/kg/day, respectively, and continued throughout most of the study. Through Week 68, a time when survival rate for both males and females at high dose was at least 50, mean cumulative food consumption (total of Weeks 1 through 68, assessed for animals with no missing values over this period) for males at doses of 0.25, 1, 4, and 10mg/kg was 103%, 108%, 111%, and 113% of control values, respectively; for females

this was 104%, 109%, 113%, and 116% of control values, respectively. The increase food consumption was consistent with the increased body weight. Data were summarized in the following table and described in the following figures.

Mean Cumulative Food Consumption at Week 68 for Males and Females* (unit:g)

	Time (Week)		Control	0.25 (mg/kg/day)	1 (mg/kg/day)	4 (mg/kg/day)	10 (mg/kg/day)
Male Rats	1-68	N	31	38	28	30	17
		Mean±SD	9041±747.8	9318±700.9	9798±742.3	9990±902.3	10213±798.2
Female Rats	1-68	N	29	27	21	12	20
		Mean±SD	6373±610.9	6619±678.2	6926±604.6	7170±565.3	7396±658.6

* Animals with no missing values over this period were analyzed.



Hematology: Drug-related findings included mildly higher white blood cell counts (primarily due to the higher absolute lymphocyte counts) in males at doses of 1.0mg/kg/day

and higher, and in females at doses of 4mg/kg/day and higher; and mildly decreased red blood cell counts in females at doses of 4mg/kg/day and higher. Data were shown in the following table.

Summary of Drug-related Changes of Hematology Parameters

Time (Week)		Parameters		Control	0.25 mg/kg	1 mg/kg	4 mg/kg	10 mg/kg
Week 53	M	RBC 10 ⁶ /mcL	M±SD N	8.01±0.728 70	8.18±0.450 68	7.91±0.905 67	8.07±0.578 66	8.10±0.529 60
		WBC E3/UL	M±SD N	8.8±2.47 70	9.4±2.05 69	9.7±2.32 67	10.9±2.57* 66	10.9±2.67* 60
	F	RBC 10 ⁶ /mcL	M±SD N	7.28±0.374 72	7.19±0.428 73	7.13±0.513 72	6.90±0.803* 71	6.85±0.831* 63
		WBC E3/UL	M±SD N	5.3±1.51 72	5.2±1.37 73	5.6±1.16 72	6.8±2.45* 71	7.7±2.06* 63
	Week 79	RBC 10 ⁶ /mcL	M±SD N	7.50±0.572 56	7.37±0.821 47	7.26±0.850 46	7.44±0.664 39	7.63±0.687 18
		WBC E3/UL	M±SD N	8.9±2.40 56	9.6±2.46 47	10.3±3.25* 46	10.9±2.62* 39	11.2±2.55* 18
Week 99	M	RBC 10 ⁶ /mcL	M±SD N	6.87±0.753 49	6.73±0.699 56	6.54±0.846 49	6.48±0.914 37	6.35±0.782* 23
		WBC E3/UL	M±SD N	5.5±1.64 49	5.6±2.17 56	6.3±1.94 49	7.2±2.02* 37	9.0±3.15* 23
	F	RBC 10 ⁶ /mcL	M±SD N	6.87±1.167 34	7.03±0.735 23	6.92±0.899 18	6.78±1.255 13	-
		WBC E3/UL	M±SD N	8.3±2.92 34	9.2±2.51 23	8.9±2.50 18	11.3±2.73* 13	-
	Week 105	RBC 10 ⁶ /mcL	M±SD N	6.79±0.887 21	6.16±1.298 29	6.25±1.303 20	6.64±1.365 12	-
		WBC E3/UL	M±SD N	7.2±2.80 21	6.4±2.82 29	6.9±2.64 20	7.1±3.26 12	-

*: Statistically significant at $P \leq 0.05$

Clinical chemistry: Drug-related findings included mildly higher glucose in males at doses of 4mg/kg/day or higher, in females at doses of 10mg/kg/day; mildly lower albumin and mildly higher inorganic phosphorus at 10mg/kg/day, and mildly to moderately higher potassium in males at 10.0mg/kg/day. According to the sponsor, the findings for glucose and potassium may represent rebound effects secondary to the pharmacologic activity of IGF-1 since several animals in the high dose group that were euthanized at unscheduled intervals because of poor health exhibited marked hypoglycemia. Data were summarized in the following table.

Appears This Way
On Original

Summary of Drug-related Changes of Clinical Chemistry Parameters

Time (Week)		Parameters		Control	0.25 mg/kg	1 mg/kg	4 mg/kg	10 mg/kg
Week 31	M	Glucose mg/dl	M±SD N	125±12.1 15	125±13.2 13	125±9.5 14	130±7.8 14	140±8.9 14
		Albumin g/dl	M±SD N	4.6±0.34 15	4.6±0.23 13	4.6±0.23 14	4.5±0.19 14	4.4±0.21 14
		Phosphorus mg/dl	M±SD N	6.1±0.39 15	6.1±0.42 13	6.4±0.43 14	6.7±0.40 14	7.1±0.54 14
		Potassium Mmol/L	M±SD N	5.6±0.27 15	5.7±0.51 13	5.8±0.36 14	5.8±0.33 14	6.0±0.41 14
	F	Glucose mg/dl	M±SD N	112±9.2 15	109±10.3 15	113±6.6 15	125±9.8 14	125±10.6 13
		Albumin g/dl	M±SD N	6.0±0.44 15	6.0±0.45 15	5.9±0.40 15	5.9±0.40 14	5.6±0.36 13
		Phosphorus mg/dl	M±SD N	4.8±0.52 15	4.9±0.47 15	5.1±0.63 15	5.4±0.70 14	5.9±0.60 13
		Potassium Mmol/L	M±SD N	5.4±0.43 15	5.3±0.39 15	5.3±0.48 15	5.5±0.49 14	5.5±0.33 13
Week 57	M	Glucose mg/dl	M±SD N	129±12.8 15	126±7.6 13	132±9.1 12	145±13.1 11	157±11.0 11
		Albumin g/dl	M±SD N	4.4±0.27 15	4.4±0.13 13	4.5±0.32 11	4.3±0.22 11	4.1±0.23 11
		Phosphorus mg/dl	M±SD N	5.7±0.30 15	5.7±0.49 13	6.2±0.69 12	6.0±0.32 11	6.7±0.29 11
		Potassium Mmol/L	M±SD N	5.4±0.44 15	5.3±0.43 13	5.9±0.56 12	5.8±0.43 11	6.3±0.48 11
	F	Glucose mg/dl	M±SD N	108±15.6 14	107±11.2 14	111±10.5 14	118±12.9 14	128±10.8 10
		Albumin g/dl	M±SD N	5.8±0.46 14	5.8±0.37 14	5.6±0.50 14	5.7±0.30 14	5.1±0.46 10
		Phosphorus mg/dl	M±SD N	5.2±0.82 14	5.3±0.43 14	5.4±0.44 14	5.8±0.52 14	6.3±0.53 10
		Potassium Mmol/L	M±SD N	5.1±0.45 14	5.1±0.34 14	5.1±0.44 14	5.4±0.59 14	5.5±0.35 10

Organ weights: Not measured

Gross pathology: Drug-related macroscopic findings included the increased incidence rate of mass in multiple sites in skin and mammary gland in male rats at doses of 4 and 10mg/kg, and the increased incidence rate of mass in skin in female rats in all dose levels. In addition, increased incidence rate of enlarged lymphatic node in submaxillary gland in all dose levels, red focus in thymus, enlarged adrenal cortex, dark focus in stomach at doses of 4 and 10mg/kg in male rats and enlarged spleen in all dose levels, enlarged pituitary, dark focus in stomach at doses of 4 and 10mg/kg in females were noted in the macroscopic examination. Data were shown in the following table.

Summary of Macroscopic Observation in Both Male and Female Rats

	Organs	Macroscopic findings	Number of affected animals				
			0	0.25 mg/kg	1 mg/kg	4 mg/kg	10 mg/kg
Male	Thymus	Red focus(i)/area(s)	1	1	0	4	6
	Adrenal cortex	Large	3	2	2	12	7
	Stomach	Dark focus(i)/area(s)	0	8	8	26	32
	Skin	Mass	4	7	3	9	19
	Submaxillary, LN	Large	3	5	7	14	29
	Mammary	Mass	5	10	5	11	12
Female	Spleen	Large	1	6	6	6	9
	Pituitary	Large	9	8	5	14	15
	Stomach	Dark focus(i)/area(s)	5	6	5	12	35
	Skin	Mass	0	2	1	2	4

Histopathology:

Non-neoplastic: Compared to the control group, the higher incidence rate of non-neoplastic findings in male rats included lung and thymus congestion at doses of 4 and 10 mg/kg/day; decreased hematopoiesis in spleen, hyperplasia in pituitary, congestion of submaxillary lymphatic node, and hyperplasia of leydig cell in testis at dose of 10 mg/kg/day. In female rats, the increased non-neoplastic findings in treated groups included necrosis of gray matter and hippocampus in brain, hyperplasia of transitional cell in kidney, thymus congestion, hyperplasia in pituitary, stomach congestion at doses of 4 and 10 mg/kg/day, and dose-dependent increases of hyperplasia of adrenal medulla in all treated groups. Data were summarized in the following table.

Summary of Non-neoplastic Microscopic Observation in Both Male and Female Rats

	Organs	Microscopic findings	Number of affected animals				
			0	0.25 mg/kg	1 mg/kg	4 mg/kg	10 mg/kg
Male	Lung	Congestion	2	5	3	5	10
	Thymus	Congestion	1	3	1	4	8
	Spleen	Decreased hematopoiesis	0	1	0	1	3
	Pituitary	Hyperplasia	17	12	8	7	28
	Submaxillary	Lymphatic congestion	8	10	6	6	19
	Testis	Hyperplasia, leydig cell	1	3	2	1	5
Female	Brain	Gray matter necrosis	0	0	0	4	6
		Hippocampus necrosis	0	0	0	3	6
	Kidney	Transitional cell hyperplasia	7	7	5	10	11
	Thymus	Congestion	0	0	1	2	5
	Adrenal	Medullary hyperplasia	1	3	11	19	15
	Pituitary	Hyperplasia	6	5	4	9	13
	Stomach	Congestion	4	2	3	6	14

Neoplastic: Drug-related neoplasms were noted in adrenal medulla, skin, and mammary gland in both male and female rats. In the adrenal medulla, pheochromocytoma occurred in males at doses of 1.0 mg/kg/day and higher, and in females at all dose levels. In

the skin, there was dose-related increase in benign epithelial neoplasms including keratoacanthoma and squamous cell papilloma in males at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day. There was a marginal increase in the incidence of mammary gland carcinoma in males at 10mg/kg/day. In females, there was a dose-related increase in the number of palpable skin (primarily mammary gland) masses but by the end of the study, the overall incidence, multiplicity, and type of mammary gland neoplasms including fibroadenoma and carcinoma were similar among control and dosed groups. However, when adjusted for survival, there was a statistically positive response for fibroadenoma at doses of 4 and 10mg/kg/day and for carcinoma at dose of 10mg/kg/day. As comparing with the historical data, the only positive findings in this carcinogenicity study was the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg. Since the tumors were only observed with doses which exceeded the MTD, these tumor findings would not be labeled. Data were summarized in the following tables.

Summary of Drug-related Neoplastic Findings in Both Male and Female Rats

Gender		Male					Female				
Group		1	2	3	4	5	1	2	3	4	5
Dose (mg/kg/day)		0	0.25	1.0	4.0	10.0	0	0.25	1.0	4.0	10.0
Adrenal medulla	Number examined	75	75	75	74	75	73	74	74	73	74
	Pheochromocytoma (%)	10 (13.3)	12 (16.0)	16 (21.3)	25 (33.8)	18 (24.0)	1 (1.3)	6 (8.1)	6 (8.1)	8 (10.9)	13 (17.6)
		1.67 to 21.67% in historical range					6.28 to 11.67% in historical range				
Skin	Number examined	75	75	75	75	75	75	75	75	74	75
	Keratoacanthoma (%)	1 (1.3)	4 (5.3)	3 (4.0)	11 (14.7)	19 (25.3)	0 (0)	1 (1.3)	1 (1.3)	0 (0)	1 (1.3)
		1.28 to 8.47% in historical range					1.67 to 3.33% in historical range				
	Squamous cell papilloma (%)	1 (1.3)	1 (1.3)	3 (4.0)	1 (1.3)	4 (5.3)	0 (0)	0 (0)	0 (0)	0 (0)	1 (1.3)
Mammary gland		0.79 to 6.90% in historical range					0.79 to 2.00% in historical range				
	Number examined	75	75	75	75	75	75	75	75	75	75
	Fibroadenoma (%)	1 (1.3)	2 (2.6)	3 (4.0)	2 (2.6)	1 (1.3)	22 (29.3)	35 (46.7)	29 (38.7)	33 (44.0)	26 (34.7)
		1.28 to 4.44% in historical range					17.95 to 52.54% in historical range				
	Carcinoma (%)	0 (0)	1 (1.3)	0 (0)	0 (0)	4 (5.1)	32 (42.7)	26 (34.7)	29 (38.7)	27 (36.0)	26 (34.7)
		0.79 to 2.0% in historical range					6.67 to 43.33% in historical range				

Historical range data were from Charles River Laboratory.

Toxicokinetics: After the subcutaneous administration of rhIGF-1, the systemic exposures of free and total IGF-1 were evaluated on Day 1, Weeks 13, 26, 52, 65, 79, and 91. Both the C_{max} and AUC values were dose proportional across all dose groups. Compared to Day1, the AUC values of both the free and total IGF-1 were higher on Week 79. These changes were noted as early as Week 13. It has also been shown that rhIGF-1 is eliminated faster in younger rats. Male rats generally had higher systemic exposure for both free and total IGF-1 than female rats based on the AUC values. Data were summarized in the following table.

Summary of TK Parameters of Free and Total IGF-1 in Male and Female Rats

Time	Dose (mg/kg)	rhIGF-1	AUC ₀₋₂₄ (ng/ml.hr)		Cmax (ng/ml)		T _{1/2} (hr)	
			Male	Female	Male	Female	Male	Female
Day 1	0.25	Free	59	54	52.1	38.8	1.00	0.58
		Total	1581	1186	567	624	7.24	2.58
	1.0	Free	353	201	250	157	0.67	0.66
		Total	4606	2643	1050	826	2.30	2.18
	4.0	Free	1451	1409	825	1230	0.48	0.43
		Total	6910	4562	2810	2620	1.08	0.99
	10.0	Free	5529	4752	3830	3810	0.39	0.41
		Total	11683	8374	7060	6830	0.79	0.68
Week 13	0.25	Free	203	39	67.1	53.9	1.32	0.66
		Total	NA	1554	662	426	8.45	NA
	1.0	Free	1317	575	430	319	0.98	0.61
		Total	3065	2561	1070	655	4.10	4.27
	4.0	Free	NA	3509	1790	1800	1.14	0.63
		Total	27440	5008	4690	2180	2.30	1.20
	10.0	Free	27524	21448	4920	5580	1.73	1.17
		Total	35089	42662	4320	6600	5.18	2.23
Week 26	0.25	Free	213	72	30.0	31.9	13.09	0.80
		Total	3865	791	720	417	NA	5.65
	1.0	Free	1270	465	238	159	5.18	0.83
		Total	7105	2671	906	652	11.74	14.51
	4.0	Free	6719	3279	1510	937	3.30	0.90
		Total	15455	7525	2920	2550	2.47	1.38
	10.0	Free	22482	17525	3350	2220	2.68	2.24
		Total	33827	18266	5040	3600	2.92	1.74
Week 52	0.25	Free	253	67	51.9	47.8	11.62	0.51
		Total	2644	2514	583	484	89.27	2.00
	1.0	Free	1702	486	321	390	5.29	0.43
		Total	4670	3773	953	741	24.25	1.60
	4.0	Free	17617	3525	1640	1480	2.92	0.65
		Total	22861	8487	2750	2190	11.69	1.31
	10.0	Free	36519	10900	3970	3290	2.52	0.93
		Total	50978	19770	5060	4510	7.18	1.36
Week 79	0.25	Free	119	62	46.7	59.1	1.94	0.87
		Total	3587	1448	723	473	NA	10.06
	1.0	Free	673	502	420	288	1.10	1.06
		Total	6253	3607	1150	705	10.08	12.99
	4.0	Free	4497	3504	2000	1530	0.71	0.93
		Total	17785	8773	3090	2230	4.99	5.08
	10.0	Free	31593	11788	3590	3350	2.34	1.76
		Total	33291	22920	5280	4620	1.33	9.55
Week 91	0.25	Free	102	85	64.3	55.9	1.60	0.62
		Total	2373	198	627	498	9.56	14.72
	1.0	Free	1542	472	377	290	3.24	0.75
		Total	7024	2587	1110	729	2.61	14.19
	4.0	Free	19811	6454	3050	1480	1.72	1.93
		Total	19894	7293	2920	2150	3.06	1.61

Other studies: Antibody analysis reported that none of the rats showed a positive response in the anti-IGF-1 antibody ELISA.

Summary of individual study findings: There were dose-related increases of mortality rate in both male and female rats. The high dose groups were terminated during Week 84 and the remaining male groups during Week 99. The remaining female groups were terminated during Week 105. Drug-related clinical signs included the increases in the incidence of palpable masses and wart-like lesions in both males and females at doses of 4 and 10mg/kg. These findings were consistent with the increased incidence of skin and mammary gland neoplasms in these groups. There were dose-related increases of body weight gain and food consumption in both males and females at all dose levels. Hematological parameters showed mildly higher white blood cell counts (primarily due to the higher absolute lymphocyte counts) in males at doses of 1.0mg/kg/day and higher, and in females at doses of 4mg/kg/day and higher; and mildly decreased red blood cell counts in females at doses of 4mg/kg/day and higher. In clinical chemistry analysis, drug-related changes included mildly higher glucose in males at doses of 4mg/kg/day or higher, in females at doses of 10mg/kg/day; mildly lower albumin and mildly higher inorganic phosphorus at 10mg/kg/day, and mildly to moderately higher potassium in males at 10.0mg/kg/day. In TK study, Both the C_{max} and AUC values were dose proportional across all dose groups. Compared to Day 1, the AUC values of both the free and total IGF-1 were higher on Week 79. These changes were noted as early as Week 13. It has also been shown that rhIGF-1 is eliminated faster in younger rats. Male rats generally had higher systemic exposure for both free and total IGF-1 than female rats based on the AUC values. Antibody analysis reported that none of the animals showed a positive response in the anti-IGF-1 antibody ELISA.

In pathological study, drug-related macroscopic findings included the increased incidence rate of mass in skin and mammary gland in male rats at doses of 4 and 10mg/kg, and the increased incidence rate of mass in skin in female rats in all dose levels. Non-neoplastic microscopic findings included lung and thymus congestion at doses of 4 and 10mg/kg/day; decreased hematopoiesis in spleen, hyperplasia in pituitary, congestion of submaxillary lymphatic node, and hyperplasia of leydig cell in testis at dose of 10mg/kg/day in males, increased necrosis of gray matter and hippocampus in brain, hyperplasia of transitional cell in kidney, thymus congestion, hyperplasia in pituitary, stomach congestion at doses of 4 and 10mg/kg/day, and dose-dependent increases of hyperplasia of adrenal medulla in all treated groups in females. Drug-related neoplasms findings included pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels; keratoacanthoma in the skin in males at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day; and mammary gland carcinoma in both males and females at 10mg/kg/day. Regarding the skin neoplasms, the sponsor suggested that the tumors may be related to the mechanical trauma due to repeated subcutaneous injections and/or potentially high local concentrations of rhIGF-1 may contribute to the development of skin neoplasms. However, based on the local tolerance toxicity study, there was no difference between the control and treated groups. Therefore, there was not enough evidence to support that the tumors in the skin are the secondary to the irritation. Compared with the historical data, the only positive findings in this carcinogenicity study was the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg.

Adequacy of the carcinogenicity study and appropriateness of the test model: The test species appears to be appropriate and the high dose at 10mg/kg/day was adequate for this carcinogenicity study based on the increased mortality in both male and female rats.

Evaluation of tumor findings: Drug-related neoplasms findings included pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels; keratoacanthoma in the skin in males at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day. There was a marginal increase in the incidence of mammary gland carcinoma in males at 10mg/kg/day. When comparing with the historical control data, the only positive findings in this carcinogenicity study was the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg.

Carcinogenicity summary: Two year carcinogenicity study of rhIGF-1 was conducted in rats. Drug was administered subcutaneously at doses of 0.25, 1, 4, and 10mg/kg/day. These doses are 0.3, 1.3, 3.6, and 7.4 times the human highest dose (0.12mg/kg twice daily) based on the total IGF-1 AUC. Because of low survival, the high dose groups were terminated during Week 84 and the remaining male group during Week 99, and the remaining female groups were terminated during Week 105. In addition, because of low survival, dose administration was discontinued for approximately 4 weeks prior to termination for males at dose of 1.0mg/kg/day and for approximately 7 and 13 week for males and females at dose of 4mg/kg/day, respectively. Based on the data reviewed by Dr. Karl Lin and Moh-Jee Ng from Biometrics Division 2 in this agency, the statistically significant tumor findings included an increase rate of pheochromocytoma occurred in males at doses of 1mg/kg/day and higher, and in females at all dose levels, keratoacanthoma in the skin at doses of 4 and 10mg/kg/day, a marginal increase in the incidence of mammary gland carcinoma in males and females at 10mg/kg/day.

Carcinogenicity conclusions: In the two-year carcinogenicity study, rhIGF-1 induced pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels; keratoacanthoma in the skin in males at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day; and mammary gland carcinoma in both males and females at 10mg/kg/day. Compare with the historical data, the only positive findings in this carcinogenicity study was the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg. Therefore, the NOEL for the carcinogenicity of rhIGF-1 was 1mg/kg/day in males and 4mg/kg for females. Doses of 4 and 10mg/kg/day exceeded the MTD based on mortality secondary to drug-induced hypoglycemia.

Recommendations for further analysis: None

Labeling Recommendations: The pheochromocytomas in both males and females, keratoacanthomas in the skin of males, and mammary gland carcinoma in males were drug related. These tumor findings were only observed at doses that exceeded the MTD, based on excess mortality secondary to IGF-1 induced hypoglycemia.

2.6.6.6 Reproductive and developmental toxicology

Fertility and early embryonic development

Study title: A fertility study in rats treated intravenously with MKC-031

Key study findings:

- Dose-dependent increased body weight gain at doses of 1 and 4mg/kg/day in both male (11% and 15% vs control) and female rats (45% and 56% vs control).
- Dose-dependent increased food consumption in male rats at doses of 0.25mg/kg/day or higher, and in female rats at doses of 1mg/kg/day or higher
- A slight dose-dependent increase of the index of dead/resorbed fetuses at doses of 0.25mg/kg/day or higher. No significant differences between control and treatment groups in the number of corpora lutea, the number of implantations, and implantation index and the number of live fetuses.
- Based on the AUC, the high dose of 4mg/kg/day gives about 4 times the MRHD and no significant toxicity was observed. Therefore, the tested doses were too low.

Study no.: 96-269-0320 (R432)

Volume #, and page #: Volume 1 of 1, and Page 1

Conducting laboratory and location: []

Date of study initiation: April 23, 1992

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: MKC-031 supplied by []

lot M6-28, and — % purity.

Formulation/vehicle: MKC-031 was dissolved in citric acid buffer solution (pH 6.0±0.5) containing — mg citric acid monohydrate, — mg trisodium citrate dehydrate, and — mg sodium chloride per 1ml of water.

Methods:

Test Groups	Dose Levels (mg/kg)	Concentration of Test Solutions (mg/ml)	Sex	No. of Animals	Animal No.
Control	0	0	m	22	1001 ~ 1022
			f	22	1101 ~ 1122
Low Dose	0.25	0.1	m	22	2001 ~ 2022
			f	22	2101 ~ 2122
Intermediate Dose	1	0.4	m	22	3001 ~ 3022
			f	22	3101 ~ 3122
High Dose	4	1.6	m	22	4001 ~ 4022
			f	22	4101 ~ 4122

Species/strain: — CD (SD) SPF rats from []

Doses employed: Control, 0.25, 1, 4mg/kg. The high dose of 4mg/kg is 8 times exposure of MRHD (120 µg/kg) based on BSA.

Route of administration: Intravenous administration

Study design: MKC-031 was administered intravenously at doses of 0.25, 1, and 4mg/kg/day with vehicle control to male rats for 9 weeks before mating, during mating and after copulation until the day before autopsy, and to female rats for 2 weeks before mating, during mating and after copulation until day 7 of gestation. This study design included treated males with treated females, untreated females, and treated females with untreated males. Drug-related general toxicological effects on parental animals, the effects on the reproductive ability of parental animals and the effects on embryos and fetuses were examined.

Number/sex/group: 22/sex/group

Parameters and endpoints evaluated:

Maternal toxicity: Clinical appearance, effects on body weight and food consumption, effects on estrous cycles and mating, gross necropsy and histopathological findings, and reproductive outcome including the implantation index (number of implantations/number of corpora lutea x 100) and index of dead/resorbed fetuses (number of dead/resorbed fetuses/number of implantations x100).

Fetal toxicity: Live fetuses were examined for external malformations after determining fetal sex, body weights.

Results:

Mortality: None

Clinical signs: No drug-related clinical signs were noted in either male or female F0 dams.

Body weight: There were dose-dependent increases of body weight and body weight gains in both male and female F0 dams.

In male rats, the body weight begin to statistically increase from the day 49 after the start of administration at dose of 1mg/kg/day and day 21 at dose of 4mg/kg/day. There was significantly increased body weight gain at doses of 1 and 4mg/kg/day compared with control groups (11% and 15% vs control) as shown in the following table.

Fertility study in rats treated intravenously with MKC-031

Body weights of F0 male rats during the pre-mating period

Dose		Administration											
mg/kg		0	3	7	10	14	17	21	24	28	31	35	38a)
0	No.	22	22	22	22	22	22	22	22	22	22	22	22
	Mean	206.2	230.5	263.5	289.2	317.4	335.2	358.9	372.8	391.5	404.7	419.6	430.1
	S.D.	6.6	8.3	11.2	11.6	15.5	18.0	19.8	22.4	24.8	26.0	28.5	28.5
0.25	No.	22	22	22	22	22	22	22	22	22	22	22	22
	Mean	206.3	232.1	267.0	292.6	324.0	343.7	367.7	382.5	403.7	417.8	436.5	446.2
	S.D.	6.4	9.2	9.9	11.2	12.0	13.1	15.1	16.1	17.4	19.8	21.9	22.4
1	No.	22	22	22	22	22	22	22	22	22	22	22	22
	Mean	206.2	231.5	265.4	293.5	324.5	345.0	370.9	386.9	407.9	422.3	439.5	450.7
	S.D.	6.5	7.8	10.3	11.1	14.0	15.3	18.1	19.9	21.9	22.6	24.6	26.6
4	No.	22	22	22	22	22	22	22	22	22	22	22	22
	Mean	206.1	231.7	269.2	295.5	325.3	348.0	375.0*	390.4	412.6*	427.2*	445.0*	457.4*
	S.D.	6.5	8.8	10.9	12.0	15.2	18.2	20.2	23.4	25.7	28.4	32.3	33.7

Dose mg/kg	Administration							Body weight gains g - 63
		42	45	49	52	55	59	
0	No.	22	22	22	22	22	22	22
	Mean	441.2	449.5	460.6	467.1	475.0	482.5	489.4
	S.D.	30.3	31.0	31.7	33.2	34.5	35.6	36.2
0.25	No.	22	22	22	22	22	22	22
	Mean	456.6	467.2	478.8	487.0	497.5	505.8	513.8
	S.D.	24.1	25.1	26.6	29.1	28.5	29.9	32.5
1	No.	22	22	22	22	22	22	22
	Mean	465.9	474.1	490.6*	498.9*	508.0*	516.3*	525.9*
	S.D.	27.3	30.3	30.4	32.7	35.0	36.2	35.8
4	No.	22	22	22	22	22	22	22
	Mean	472.5**	482.3**	497.6**	507.1**	518.2**	527.8**	539.8**
	S.D.	35.5	36.3	38.2	40.9	43.1	46.0	46.3

Unit : g.

No. : No. of animals.

a) : Day of administration.

* : p<0.05 ; ** : p<0.01 (Significant difference from control).

In female rats, the body weight gain was significantly higher at doses of 1 and 4mg/kg/day compared with control (45% and 56% vs control) during the pre-mating administration period and at 4mg/kg from day 0 to day 7 of gestation period. Data were summarized in the following tables.

Fertility study in rats treated intravenously with MKC-031
Body weights of F0 female rats during the pre-mating period

Dose mg/kg	Administration					Body weight gains 0-14
		0	3	7	14a)	
0	No.	22	22	22	22	22
	Mean	291.2	293.6	293.8	299.7	299.7
	S.D.	21.4	21.7	20.7	21.5	22.1
0.25	No.	22	22	22	22	22
	Mean	291.2	293.2	297.2	301.6	302.2
	S.D.	21.3	20.9	21.0	21.2	21.3
1	No.	22	22	22	22	22
	Mean	291.2	294.7	297.1	303.9	307.4
	S.D.	20.5	20.3	20.8	22.3	23.7
4	No.	22	22	22	22	22
	Mean	291.3	295.2	299.0	307.8	310.6
	S.D.	20.7	18.7	20.0	20.0	19.6

Unit : g.

No. : No. of animals.

a) : Day of administration.

* : p<0.05 ; ** : p<0.01 (Significant difference from control).

Best Possible Copy

Fertility study in rats treated intravenously with MKC-031
Body weights of F0 dams during the gestation period

Dose mg/kg		Administration							Body weight gains	
		0	4	7	11	14	17	20a)	0-7	7-20
0	No.	21	21	21	21	21	21	21	21	21
	Mean	305.1	322.3	329.1	347.4	363.6	391.9	443.6	24.0	114.5
	S.D.	20.7	22.7	22.8	24.7	22.8	23.0	26.0	5.5	17.2
0.25	No.	20	20	20	20	20	20	20	20	20
	Mean	309.6	327.0	333.8	363.1	368.2	398.2	451.3	24.2	117.5
	S.D.	24.8	27.6	27.3	29.7	30.0	32.0	37.9	6.4	21.5
1	No.	20	20	20	20	20	20	20	20	20
	Mean	318.8	336.1	344.6	362.3	377.0	408.1	459.1	25.8	114.5
	S.D.	23.9	23.2	23.9	25.3	22.8	25.1	27.6	4.9	12.9
4	No.	21	21	21	21	21	21	21	21	21
	Mean	314.6	335.8	347.0	365.4	384.0	417.6	471.1	32.6	124.1
	S.D.	17.2	18.6	19.0	20.7	22.0	26.0	27.8	8.4	14.5

Unit: g

No.: No. of dams.

a): Day of gestation.

*: p<0.05; **: p<0.01 (Significant difference from control).

Food consumption: As correlated with the increases of body weights, there were dose-dependent increases of food consumption in both male and female F0 dams.

In male rats, significantly increased food consumption was observed on day 35 after the start of administration at doses of 1 and 4mg/kg/day. In addition, there was temporarily increased food consumption on day 59 after the start of administration at dose of 0.25mg/kg/day. Data were show in the following tables.

Fertility study in rats treated intravenously with MKC-031
Food consumption of F0 male rats during the pre-mating period

Dose mg/kg		Administration									
		3	7	10	14	17	21	24	28	31	35
0	No.	11	11	11	11	11	11	11	11	11	11
	Mean	28.3	28.0	29.5	30.5	30.0	30.3	30.5	30.4	31.2	30.1
	S.D.	1.9	2.0	1.8	2.0	2.2	1.6	1.9	2.0	2.6	1.6
0.25	No.	11	11	11	11	11	11	11	11	11	11
	Mean	27.0	28.0	29.3	31.1	30.1	30.5	31.2	31.0	31.6	31.0
	S.D.	1.8	1.6	2.1	2.1	2.2	2.1	1.8	1.9	2.0	2.4
1	No.	11	11	11	11	11	11	11	11	11	11
	Mean	27.0	28.1	30.3	32.2	31.6	32.1	32.6	32.1	32.2	32.9*
	S.D.	1.4	1.2	1.8	1.6	1.6	1.8	1.9	1.6	1.4	1.2
4	No.	11	11	11	11	11	11	11	11	11	11
	Mean	27.0	28.5	29.8	31.1	31.5	31.8	31.5	32.3	32.5	33.0*
	S.D.	1.3	1.2	1.3	1.7	1.2	1.3	1.4	1.3	1.6	2.2

Dose mg/kg		Administration							Mean 3-59b)
		42	45	49	52	56	59	63a)	
0	No.	11	11	11	11	11	11	11	11
	Mean	31.2	31.8	31.1	30.8	29.0	29.5	28.3	29.9
	S.D.	1.6	1.8	2.4	2.3	2.7	1.8	3.0	1.4
0.25	No.	11	11	11	11	11	11	11	11
	Mean	31.5	32.9	31.8	32.9	31.5	32.0*	30.5	31.0
	S.D.	2.9	2.1	2.9	2.2	1.6	1.9	2.7	1.9
1	No.	11	11	11	11	11	11	11	11
	Mean	33.8*	33.8	34.4*	34.6**	32.2*	32.6**	32.3**	32.2**
	S.D.	1.6	2.0	1.9	1.9	2.5	1.7	1.3	1.3
4	No.	11	11	11	11	11	11	11	11
	Mean	33.5	34.2*	33.2	34.6**	33.5**	33.0**	32.7**	32.1*
	S.D.	2.0	1.4	1.6	1.4	1.0	1.5	2.1	1.1

Unit: g/rat/day.

No.: No. of cages.

a): Day of administration.

b): Mean food consumption of F0 male rats during the pre-mating period.

*: p<0.05; **: p<0.01 (Significant difference from control).

Best Possible Copy

In female rats, there was no difference for food consumption during pre-mating period. During the gestation period, a dose-dependent increase of food consumption was observed at dose of 1 and 4mg/kg/day.

Fertility study in rats treated intravenously with MKC-031
Food consumption of F0 dams during the gestation period

Dose mg/kg	Administration										Mean	
	1	4	7	11	14	17	20a)	21	21	21	1-7	11-20b)
0	No.	21	21	21	21	21	21	21	21	21	21	21
	Mean	22.0	24.6	25.7	28.0	26.1	28.6	29.7	24.4	28.2		
	S.D.	2.8	3.0	2.5	3.5	2.4	3.0	2.9	2.2	2.5		
0.25	No.	20	20	20	20	20	19 ^{c)}	20	20	19 ^{c)}		
	Mean	22.3	25.6	26.1	29.3	27.6	29.9	31.6	24.7	29.7		
	S.D.	3.0	3.8	3.1	4.0	4.3	3.0	3.0	2.6	3.1		
1	No.	20	20	20	20	20	20	20	20	20		
	Mean	22.3	26.7	27.8	29.9	28.4*	30.8	31.4	25.6	30.2		
	S.D.	3.1	2.8	2.9	2.7	2.2	1.9	2.1	2.6	1.6		
4	No.	21	21	21	21	21	21	21	21	21		
	Mean	23.6	26.8	28.4*	31.1	30.7**	32.6**	33.4**	26.3	32.0**		
	S.D.	3.3	2.7	2.4	3.9	2.6	3.4	3.3	2.3	2.6		

Unit: g/rat/day.

No.: No. of dams.

a): Day of gestation.

b): Mean food consumption of F0 dams during the gestation period.

c): One dam was excluded because of measurement error.

*: p<0.05; **: p<0.01 (Significant difference from control).

Toxicokinetics: Not performed

For fertility studies:

In-life observations: There were no drug-related abnormal findings except the increases of body weight gain and food consumption in both male and female animals as summarized above.

Terminal and necropsic evaluations: In male rats, macropathological findings included uni- or bilateral atrophy of the testis and epididymis in two animals at dose of 1mg/kg and increases of absolute testis and epididymis weights at dose of 4mg/kg. However, these increases of organ weights were due to the increases of body weight since there was no difference of relative organ weights between control and treatment groups as shown in the following tables.

Fertility study in rats treated intravenously with MKC-031
Macropathological findings and testis and epididymis weights of F0 male rats

Dose (mg/kg)			0	0.25	1	4
No. of animals examined			22	22	22	22
No. of animals with abnormal findings (X)			0	0	2 (9.1)	0
Testis						
Atrophy (unilateral or bilateral)			0	0	2 (9.1)	0
Epididymis						
Atrophy (unilateral or bilateral)			0	0	2 (9.1)	0
Testis (g)	Right	Mean	1.50	1.61	1.60	1.70*
		S.D.	0.12	0.15	0.30	0.17
	Left	Mean	1.60	1.62	1.57	1.70
		S.D.	0.11	0.15	0.36	0.11
Epididymis (mg)	Right	Mean	536.3	562.3	567.1	574.8
		S.D.	63.1	50.8	89.5	51.5
	Left	Mean	539.0	545.9	550.5	560.9
		S.D.	41.3	37.0	97.5	41.4

*: p<0.05 (Significant difference from control).

Best Possible Copy

Fertility study in rats treated intravenously with MKC-031
Relative testis and epididymis weights of P0 male rats

Dose mg/kg	No. of animals		Testis		Epididymis	
			(R) mg%	(L) mg%	(R) mg%	(L) mg%
0	22	Mean S.D.	0.32 0.03	0.32 0.03	107.2 14.4	107.8 10.7
0.25	22	Mean S.D.	0.31 0.03	0.31 0.03	104.6 11.2	103.4 9.3
1	22	Mean S.D.	0.29 0.06	0.29 0.07	104.6 17.1	101.5 18.2
4	22	Mean S.D.	0.31 0.03	0.31 0.03	103.6 10.9	101.2 10.5

In female rats, no drug-related abnormalities were observed in all groups.

For the reproductive performance, there were no significant difference between control and treatment groups in estrus count and estrous cycle during pre-mating administration period. No significant difference was observed between control and treatment groups in copulation index, indices of insemination and fertility. In the embryos and fetuses analysis after cesarean section, there was a dose-dependent increase in early resorptions. There were no significant differences between control and treatment groups in the number of corpora lutea, the number of implantations, and implantation index and the number of live fetuses. Data were summarized in the following table. For the external examination of fetuses, no drug-related abnormalities were observed.

Fertility study in rats treated intravenously with MKC-031
Cesarean section data on P0 dams

Dose mg/kg	No. of dams		No. of corpora lutea	No. of implan- tations	Implan- tation index % a)	No. of resorbed or dead fetuses			No. of live fetuses
						Total (x) b)	Early c)	Late d)	
0	22	Total Mean S.D.	346 16.5 2.6	324 15.4 3.4	93.6	10 (4.9)	16	0	308 14.7 3.6
0.25	20	Total Mean S.D.	333 16.7 2.8	312 15.6 3.9	93.7	21 (6.7)	20	1	291 14.6 4.2
1	20	Total Mean S.D.	342 17.1 2.4	315 15.8 2.9	92.1	22 (7.0)	22	0	293 14.7 2.7
4	21	Total Mean S.D.	353 18.9 1.7	341 16.2 1.8	96.1	30 (8.8)	30	0	311 14.8 1.9

a): (No. of implantations / No. of corpora lutea) X 100.
b): (No. of resorbed or dead fetuses / No. of implantations) X 100.
c): Implantation trace, resorbed embryo and placental remnant.
d): Early macerated fetus, late macerated fetus and dead fetus.

Summary of individual study findings: There are dose-dependent increases of body weight, body weight gain, and food consumption in both male and female parent rats at doses of 1 and 4mg/kg/day. The abnormal findings of testis and epididymis were not considered drug-related since they were only found in middle dose level and no difference of organ weights after calculation with body weight.

In the reproductive performance, there were no significant difference between control and treatment groups in estrus count and estrous cycle during pre-mating administration period. No significant difference was observed between control and treatment groups in copulation index, indices of insemination and fertility.

For the embryos and fetuses, a dose-dependent increase of early resorptions was observed. There were no significant differences between control and treatment groups in the number of corpora lutea, the number of implantations, and implantation index and the number of live fetuses. No external malformations were noted.

Based on the sponsor, the NOEL is 4mg/kg/day. However, a slight dose-dependent increase of the total number of dead/resorbed fetuses at doses of 0.25mg/kg/day or higher were observed. According to the maximum recommended human dose is 120 µg/kg, bid, the systemic exposure is 5 times for 4mg/kg and 0.3 times for 0.25mg/kg based on BSA.

Embryofetal development

Study title: A Study of MKN-031 by Intravenous Administration in Rats During the Period of Fetal Organogenesis

Key study findings:

- There was a transient decrease in spontaneous movement at dose of 16mg/kg during the administration period in dams
- In liveborn F1 pups, the differentiation index for opening of the eyelid on day 14 after birth was significantly delayed at dose of 16mg/kg (47.2% vs 71.3% in control). However, this differentiation index on day 17 after birth was 100%.
- No other drug-related findings in either dams, F1 fetuses, or liveborn F1 pups were noted.
- The NOEL was 4mg/kg, which is 5 times MRHD based on BSA.

Study no.: R-471

Volume #, and page #: Volume 1 of 1, and Page 1

Conducting laboratory and location: ζ

Date of study initiation: July 30, 1993

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, radiolabel, and % purity: MKN-031 supplied by ζ lot M6-29, and $\bar{}$ purity.

Formulation/vehicle: MKN-031 was dissolved in citric acid buffer solution (pH 6.0±0.5) containing $\bar{}$ mg citric acid monohydrate, $\bar{}$ mg trisodium citrate dehydrate, and $\bar{}$ mg sodium chloride per 1ml of water.

Methods:

Test Group	Dose Level (mg/kg)	Concentration of Test Solution (mg/ml)	Concentration of Test Solution (%)	Cesarean Section Group		Spontaneous Delivery Group	
				No. of Copulated Females	Animal No.	No. of Copulated Females	Animal No.
Control	0	0	0	24	1101-1124	12	1125-1136
Low	1	0.33	0.03	24	2101-2124	12	2125-2136
Inter-mediate	4	1.33	0.13	24	3101-3124	12	3125-3136
High	16	5.33	0.53	24	4101-4124	12	4125-4136

Species/strain: — CD(SD) SPF rats from C

1

Doses employed: 0, 1, 4, and 16 mg/kg

Route of administration: Intravenous administration

Study design: Vaginal plugs- or sperm-positive female rats treated with vehicle or test article at 1, 4, or 16 mg/kg/day in a volume of 3 ml/kg/day on GD 7 through 17, which corresponds to the period of fetal organogenesis. The effects of test article on dams and next generation pups were examined by either cesarean section (observation of fetuses at late gestation) in 24 rats on GD 20 or spontaneous delivery (observation of parturition and lactation) in 12 rats. Dams that delivered were allowed to nurse their pups for 21 days post partum and their lactation condition was observed. They were subjected to necropsy on day 21 post partum.

Number/sex/group: 36/female/group

Parameters and endpoints evaluated:

Maternal toxicity: Clinical appearance, effects on body weight and food consumption, gross necropsy findings, and reproductive outcome.

Developmental toxicity: Effects on embryo/fetal survival (pre- and post-implantation loss), litter size, fetal body weight, placental weight and appearance, fetal sex distribution, external, visceral, and skeletal variations and malformations.

Functional and behavioral test on F1 pups: All surviving pups from each dam were tested for righting reflex, papillary reflex, pinna reflex, corneal reflex and Preyer's (auditory) reflex on day 21 after birth. One male and female from each dam were subjected to an open field test at 5 weeks of age to examine the amount of spontaneous movement and emotional response, and to a water filled multiple T-maze test at 7-8 weeks of age to examine learning ability.

Reproductive performance tests on F1 pups: Reproductive performance was assessed by observing the reproductive performance of one male and female pup from each dam at 10-11 weeks of age. Females subjected to cesarean-sectioning on Day 20 of gestation and males were sacrificed at approximately the same time.

Various maternal and reproductive parameters were calculated for each dam and litter and were statistically compared at the 5% and 1% level of significance between control and treated groups.

Several indices were used in this study:

Implantation index = (No. of implantations/No. of corpora lutea) x 100 and Index of dead embryos/fetuses = (No. of dead/resorbed fetuses/No. of implantations) x 100 for observation of fetuses at late gestation (cesarean section).

Delivery index = (No. of females with liveborn pups/ No. of pregnant females) x 100 and Still birth index = (No. of stillborn pups/No. of pups born) x 100 for observation of parturition and lactation (spontaneous delivery).

Birth index = (No. of liveborn pups/No. of implantation sites) x 100, Viability index on day 4 after birth = (No. of pups alive on day 4 after birth/ No. of liveborn pups) x 100, and Weaning index on day 21 after birth = (No. of pups alive on day 21/ No. of pups alive on day 4 after culling) x 100 for observation of F1 pups.

Copulation index = (No. of successfully copulated animals/No. of mated animals) x 100, insemination index = (No. of males that impregnated females/ No. of successfully copulated males) x 100, and fertility index = (No. of pregnant females/No. of successfully copulated females) x 100 for the reproductive performance tests on F1 pups.

Results:

Mortality: No animals died before scheduled termination

Clinical signs: A transient decrease in spontaneous movement was observed in some animals at dose of 16mg/kg at 1 hour after dosing during the administration period. This symptom was not observed at the end of the administration period.

Body weight: All treatment groups showed similar changes to those of the control group throughout the gestation (day 7 to 20 of gestation) and lactation periods (day 0-21 after birth).

Food consumption: No drug-related changes were observed.

Toxicokinetics: Not conducted

Embryofetal development studies:

In-life observations:

Terminal and necroscopic evaluations:

Dams: In the cesarean section group, there was no significant difference between the control group and treatment groups in the number of corpora lutea, number of implantations (index), index of dead embryos/fetuses and number of live fetuses. There were no abnormal macropathological findings in control and treatment groups. For the non-pregnant animals subjected to necropsy on day 24 of gestation, no macropathological findings were noted either.

In parturition, the delivered live pups and the delivery index for all groups was 100% from all pregnant dams. There were no significant difference in the length of gestation, number of implantation sites, number of stillbirths (index), number of liveborn pups and the birth index compared to the control group. In the lactation condition, no abnormalities were noted in the indicators of lactation condition in all groups. In addition, there were no abnormal macropathological findings in any of groups from dams at weaning.

Offspring: In the live fetuses (F1) at late gestation, there were no significant increases of abnormal external, visceral, and skeletal findings in treatment groups as compared with control group. Data were summarized in the following tables.

*Appears This Way
On Original*

R-471

Table 7 Teratological study in rats treated intravenously with MKN-031
Cesarean section data on FO dams

Dose (mg/kg)	No. of dams		No. of corpora lutea	No. of implantations	Implantation index % a)	No. of resorbed or dead fetuses			No. of live fetuses
						Total (X)b)	Early c)	Late d)	
0	24	Total	391	378	96.7	36	36	0	342
		Mean	16.3	15.8	98.7	(10.1)			14.3
		S.D.	1.4	1.5	4.2	(19.7)			3.5
1	23	Total	385	347	90.8	19	18	1	338
		Mean	16.7	15.1	90.8	(4.9)			14.3
		S.D.	4.3	4.0	12.5	(5.8)			3.7
4	23	Total	359	349	97.3	31	31	0	318
		Mean	15.6	15.2	92.3	(8.7)			13.8
		S.D.	3.0	2.6	17.3	(7.9)			3.7
6	21	Total	342	330	96.7	18	17	1	312
		Mean	16.3	15.7	96.7	(8.6)			14.9
		S.D.	1.3	1.0	5.0	(5.5)			1.3

a) (No. of implantations / No. of corpora lutea) X 100.
b) (No. of resorbed or dead fetuses / No. of implantations) X 100.
c) Implantation trace, resorbed embryo and placental remnant.
d) Early macerated fetus, late macerated fetus and dead fetus.

Table 12 Teratological study in rats treated intravenously with MKN-031
Visceral examination of F1 live fetuses

Dose (mg/kg)	0	1	4	16
No. of fetuses examined	166	156	152	151
No. of fetuses with abnormality (%)	40 (24.1)	36 (23.1)	29 (19.1)	21 (13.9)
Abnormality (%)				
Bilateral anophthalmia	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Dilatation of lateral ventricle	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Dilatation of third ventricle	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)
Thymic remnant in neck	35 (21.1)	34 (21.8)	23 (15.1)	18 (11.9)
Anomaly of right subclavian artery	0 (0.0)	0 (0.0)	1 (0.7)	0 (0.0)
Ventricular septal defect	1 (0.6)	1 (0.6)	5 (3.3)	0 (0.0)
Abnormal lobation of liver	0 (0.0)	0 (0.0)	0 (0.0)	1 (0.7)
Dilatation of renal pelvis	4 (2.4)	3 (1.9)	2 (1.3)	1 (0.7)
Dilatation of ureter	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Left umbilical artery	2 (1.2)	1 (0.6)	3 (2.0)	1 (0.7)

Best Possible Copy

Table 13 Teratological study in rats treated intravenously with MKN-031
Skeletal examination of F1 live fetuses

Dose (mg/kg)	0	1	4	16
No. of fetuses examined	175	168	164	161
No. of fetuses with anomaly (%)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
No. of fetuses with variation (%)	17 (9.7)	20 (11.9)	14 (8.5)	20 (12.4)
Cervical rib	4 (2.3)	2 (1.2)	1 (0.6)	6 (3.7)
14th rib	7 (4.0)	9 (5.4)	6 (3.7)	5 (3.1)
Shortened 13th rib	0 (0.0)	3 (1.8)	1 (0.6)	0 (0.0)
Splitting of thoracic vertebral body	5 (2.9)	3 (1.8)	5 (3.0)	7 (4.3)
Sacroization of lumbar vertebra	2 (1.1)	3 (1.8)	3 (1.8)	2 (1.2)
Progress of ossification				
Reduced ossification of pubis	0 (0.0)	0 (0.0)	1 (0.6)	0 (0.0)
No. of ossified sternebrae (%)				
1st	175 (100.0)	168 (100.0)	163 (99.4)	161 (100.0)
2nd	175 (100.0)	167 (99.4)	163 (99.4)	161 (100.0)
3rd	175 (100.0)	168 (100.0)	163 (99.4)	161 (100.0)
4th	175 (100.0)	167 (99.4)	163 (99.4)	161 (100.0)
5th	155 (88.6)	132 (78.6)	151 (92.1)	145 (90.1)
6th	175 (100.0)	167 (99.4)	163 (99.4)	160 (99.4)
No. of ossified metacarpus (Mean±S.D.)				
Right	4.00±0.00	4.00±0.02	3.94±0.21	4.00±0.00
Left	4.00±0.00	4.00±0.02	3.94±0.21	4.00±0.00
No. of ossified metatarsus (Mean±S.D.)				
Right	4.38±0.25	4.53±0.29	4.40±0.24	4.53±0.26
Left	4.39±0.27	4.63±0.29	4.45±0.27	4.57±0.21
No. of ossified sacral and caudal vertebrae (Mean±S.D.)	8.04±0.47	7.95±0.57	7.93±0.80	8.13±0.45

In the liveborn (F1) pups, there were no drug-related changes in the sex ratio, body weight from day 0 to day 70 after birth, variability index on day 4 after birth and weaning index on day 21 after birth. In the observation of external development, the index for opening of the eyelid on day 14 after birth was significantly low at dose of 16mg/kg (47.2% vs 71.3% in control). However, this differentiation index on day 17 after birth was 100%. There were no changes of other differentiation index indicators (pinna detachment, appearance of abdominal hair, eruption of lower incisor, descent of testis and opening of vagina), body weight during lactation period, and there were no other changes indicating retardation of growth. There were no drug-related changes in the functional tests at weaning, open field tests and water filled multiple T-maze test.

In the reproductive ability tests, there were no abnormal findings of copulation, insemination and fertility from the male and female F1 pups, and no drug-related changes of body weight, and cesarean section data of F1 dams and F2 fetuses.

Summary of individual study findings:

In the dams, there was a transient decrease in spontaneous movement at dose of 16mg/kg during the administration period. No drug-related changes of body weights, food consumption, or gross pathology were noted. In addition, there were no drug-related abnormal findings in the maintenance of pregnancy, cesarean section, parturition or lactation condition.

In F1 fetuses, there were no drug-related changes of sex ratio, body weight, incidence of pups exhibiting external, visceral or skeletal abnormalities or skeletal variations. No drug-related effects on growth and development of pups were noted.

Best Possible Copy

In liveborn F1 pups, the differentiation index for opening of the eyelid on day 14 after birth was significantly low at dose of 16mg/kg (47.2% vs 71.3% in control). However, this differentiation index on day 17 after birth was 100%. There were no other drug-related changes of body weight after birth, viability, external differentiation, reflexes, behavioral tests and reproductive performance tests.

From above results, and under the conditions of this study, the non-toxic effect dose level was 16mg/kg which is 20 times of maximum recommended human dose (120 µg/kg, bid) based on body surface area.

Study title: A study of MKN-031 by intravenous administration in rabbits during the period of fetal organogenesis.

Key study findings:

- No drug-related animal death or drug-related findings of clinical signs, body weight change or food consumption were noted. However, the food consumption of animals in which a decrease in the amount of feces was noted during the observations of general condition was remarkably lower than the other animals.
- There were many early resorbed embryos and the index of dead embryos/fetuses showed significantly increased values in dams at dose of 2.0mg/kg. There were no significant differences between the control group and each test article treatment group in the number of corpora lutea, number of implantations, number of live fetuses and placenta weights.
- There were no drug-related findings on sex ratio, body weight, external, visceral or skeletal examinations of live fetuses.
- The NOAEL for dam reproductive ability was 2.0mg/kg and the NOAEL for fetuses was 0.5mg/kg. There was no teratogenicity of MKC-031 at doses up to 2.0mg/kg.

Study no.: 95-355-0320 (R-473)

Volume #, and page #: E-file

Conducting laboratory and location: J

Date of study initiation: September 16, 1993

GLP compliance: yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: MKN-031, lot # M6-29 and — purity

Methods

Test Group	Dose Level (mg/kg)	Concentration of Test Solutions (%)	No. of Copulated Animals	Animal No.
Control	0	0	16	1101 ~ 1116
Low	0.125	0.0125	16	2101 ~ 2116
Intermediate	0.5	0.05	16	3101 ~ 3116
High	2.0	0.2	16	4101 ~ 4116

Doses: MKN-031 at doses of 0, 0.125, 0.5 and 2.0mg/kg/day for 13 days.

Species/strain: New Zealand White (— NZW) SPF rabbits from 1

2, at the age of 29-30 weeks and the body weights between 3.16 to 4.32kg.

Number/sex/group: 16/female/group

Route, formulation, volume, and infusion rate: Intravenous route via auricular vein, solution, 1ml/kg with the infusion rate of 2ml/min.

Satellite groups used for toxicokinetics: Not performed

Study design: Females with purplish swollen vulvae that were judged to be in estrus were mated with 29-30 week old males, on a one-to-one basis in mating cages. When copulation was confirmed twice in females, they were considered to be copulated females and that day taken to be day 0 of gestation. The vehicle control or MKN-301 at doses of 0.125, 0.5 or 2mg/kg were then administered intravenously for 13 days, from day 6 through day 18 of gestation, which corresponds to the period of fetal organogenesis.

Parameters and endpoints evaluated: All surviving animals were sacrificed on day 28 of gestation. Parameters and endpoints included the maternal toxicity and developmental toxicity.

Maternal toxicity: Clinical appearance, effects on body weight and food consumption, gross necropsy findings, and reproductive outcome.

Developmental toxicity: Effects on embryo/fetal external examination, fetal body weight, fetal sex distribution, visceral, and skeletal variations and malformations.

Indices were used in this study:

Implantation index = (No. of implantations/No. of corpora lutea) x 100

Index of dead/resorbed embryos = (No. of dead/resorbed fetuses/No. of implantations) x 100

Results

Mortality (dams): No drug-related death was noted. One animal at dose of 2mg/kg (#4101) died of an accident on day 12 of gestation and 1 animal at the same dose (#4103) was sacrificed on day 20 of gestation because of dislocated lumbar vertebrae.

Clinical signs (dams): Non-dose dependent decreases of feces which may be related to the decrease of food consumption were noted in all treated groups. Spontaneous abortion was noted in one animal in low and middle dose groups each but not in high dose group. Data were summarized in the following table.

Table 1 Teratological study in rabbits treated intravenously with MEN-031
General condition in dams

Dose mg/kg	Symptoms	Administration																											
		0-5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28 a)				
0	No. of dams	11	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13	13		
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	No. of non-pregnant animals	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3		
	No. of non-pregnant animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
0.125	No. of dams	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16	16		
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	1	4	4	3	2	1	1	1	1	1	1	1	1	1	1	0		
	Decrease in the amount of feces	0	0	0	0	0	0	0	0	0	0	1	4	4	3	2	1	1	1	1	1	1	1	1	1	0	0		
	Abortion	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0		
0.5	No. of dams	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14	14		
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	1	1	3	0	0	0	0	0	1	1	1	1	1	1	1	1		
	Decrease in the amount of feces	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	1	1	1	1	1	1	1	1		
	Abortion	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0		
	No. of non-pregnant animals	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2		
	No. of non-pregnant animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		
	b)																												
	c)																												
2.0	No. of dams	14	14	14	14	14	14	14	13	13	13	13	13	13	13	13	13	13	12	12	12	12	12	12	12	12	12		
	No. of dams with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2	2	1	1	1	2	3	2	2		
	Decrease in the amount of feces	0	0	0	0	0	0	0	0	0	0	0	0	1	1	2	2	2	2	2	1	1	1	2	3	2	2		
	No. of non-pregnant animals	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2	2		
	No. of non-pregnant animals with abnormal findings	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0		

a) : Day of gestation

b) : One dam died on day 12 of gestation because of accident.

c) : One dam was sacrificed on day 20 of gestation because of fractured lumbar vertebrae.

Body weight (dams): No drug-related changes of body weight were noted.

Food consumption (dams): No drug-related changes of food consumption were noted. However, the food consumption of animals in which a decrease in the amount of feces was noted in the observations of general condition was remarkably lower than the other animals.

Toxicokinetics: Not performed

Terminal and necroscopic evaluations: No drug-related macroscopic findings were noted.
C-section data (implantation sites, pre- and post-implantation loss, etc.): In animals at dose of 2mg/kg, there were many early resorbed embryos and the index of dead embryos/fetuses showed significantly high values. There were no significant differences between the control group and each test article treatment group in the number of corpora lutea, number of implantations, number of live fetuses and placenta weights. Data were summarized in the following table.

Best Possible Copy

Table 5 Teratological study in rabbits treated intravenously with MKN-031
Cesarean section data on dams

Dose mg/kg	No. of dams	No. of corpora lutea	No. of implan- tations	Implan- tation index % a)	No. of resorbed or dead fetuses Total (2)b)	Early c)	Late d)	No. of live fetuses	Placental weight(g)	
0	13	Total Mean S.D.	142 10.9 2.1	100 8.4 9.4	77.7 31.1	1 0.9 3.1	0	1	108 8.3 3.4	3.47 0.99
0.125	15	Total Mean S.D.	177 11.8 2.3	152 10.1 2.9	85.3 13.0	23 12.4 19.1	9	14	120 8.6 2.2	3.49 0.63
0.5	13	Total Mean S.D.	151 11.6 1.7	123 9.6 3.1	82.0 26.0	12 8.5 9.2	7	5	111 8.5 2.2	3.57 1.01
2.0	12	Total Mean S.D.	125 10.4 1.6	110 9.2 1.9	88.1 12.9	24 22.3 23.8	19	5	86 7.2 2.7	3.41 0.45

a): (No. of implantations / No. of corpora lutea) × 100
b): (No. of resorbed or dead fetuses / No. of implantations) × 100
c): Resorbed embryo and placental remnant
d): Early macerated fetus, late macerated fetus and dead fetus
*: p < 0.05 (Significant difference from control)

Offspring (malformations, variations, etc.):

There were no drug-related effects on sex ratio, body weight or external examination as shown in the following table.

Table 6 Teratological study in rabbits treated intravenously with MKN-031
External examination of live fetuses

Dose mg/kg	No. of dams	No. of males	No. of females	Sex ratio (Male/Female)	Fetal body weight(g)		No. of fetuses with external malformations (%)a)	
					Male	Female		
0	13	Total Mean S.D.	53 4.1 2.3	55 4.2 2.0	0.96	35.55 8.43 6.97	34.44 6.97	0 (0.0) (0.0)
0.125	15	Total Mean S.D.	71 4.7 1.5	58 3.9 2.5	1.22	37.13 4.44 6.03	33.61 6.03	0 (0.0) (0.0)
0.5	13	Total Mean S.D.	53 4.1 1.6	58 4.5 2.4	0.91	36.14 5.21 4.74	34.35 4.74	0 (0.0) (0.0)
2.0	12	Total Mean S.D.	43 3.6 2.0	43 3.6 1.9	1.00	38.08 4.17 3.79	31.89 3.79	0 (0.0) (0.0)

a): (No. of live fetuses with external malformations / No. of live fetuses) × 100

In the visceral examination, the numbers of fetuses with visceral anomalies were 3 from 3 dams in control group, 3 from 3 dams at dose of 0.125mg/kg, 8 from 6 dams at dose of 0.5mg/kg and 5 from 5 dams at dose of 2mg/kg. There was no significant difference in the incidence rate between the control and any treated groups. Data were summarized in the following table.

Best Possible Copy

Table 7 Teratological study in rabbits treated intravenously with MKN-031
Visceral examination of live fetuses

Dose (mg/kg)	0	0.125	0.5	2.0
No. of fetuses examined	55	66	54	42
No. of fetuses with anomaly (%)	3(4.1)	3(5.2)	8(15.7)	5(9.2)
Thymic remnant in neck	1(1.3)	1(2.2)	1(1.7)	1(1.7)
Abnormal lobating of lung	1(1.3)	2(3.0)	1(1.7)	4(7.5)
Anomalous origin of artery from aortic arch	0(0.0)	0(0.0)	1(2.1)	0(0.0)
Aberrant right subclavian artery	0(0.0)	0(0.0)	5(10.3)*	0(0.0)
Anomalous of artery from left common carotid artery	1(1.5)	0(0.0)	0(0.0)	0(0.0)
No. of fetuses with variation (%)	42(75.0)	40(63.0)	26(49.3)	21(65.8)
Supernumerary right coronary orifice	3(11.2)	10(17.3)	7(12.8)	6(17.9)
Variation of the aortic arch a)				
Type-3	33(60.8)	28(42.0)	21(38.9)	19(56.5)
Type-4	8(12.3)	11(16.4)	2(3.5)	2(5.6)

a) : Type-3 ; Small artery arising from bifurcation site of left common carotid artery of brachiocephalic trunk
Type-4 ; Left common carotid artery arising from aorta
* : p<0.05 (Significant difference from control)

There were no drug-related findings in the skeletal examinations as shown in the following table.

Table 8 Teratological study in rabbits treated intravenously with MKN-031
Skeletal examination of live fetuses

Dose (mg/kg)	0	0.125	0.5	2.0
No. of fetuses examined	53	54	57	44
No. of fetuses with anomaly (%)	2(3.1)	1(1.3)	3(3.1)	0(0.0)
Multiple anomalies of cervical vertebrae	0(0.0)	0(0.0)	1(1.3)	0(0.0)
Fusion of sternbrae	2(3.1)	1(1.3)	1(1.3)	0(0.0)
No. of fetuses with variation (%)	0(0.0)	1(1.3)	0(0.0)	0(0.0)
Splitting of thoracic vertebral body	0(0.0)	1(1.3)	0(0.0)	0(0.0)
No. of fetuses with 13th rib (%)				
Right	4(7.5)	3(5.2)	4(6.5)	2(5.8)
Left	3(5.1)	3(5.2)	4(6.5)	4(7.5)
Bilateral	37(71.1)	50(75.2)	36(68.3)	23(53.2)
Total	44(83.3)	56(85.7)	46(81.3)	29(66.5)
Progress of ossification (Mean±S.D.)				
Sterebrae	5.92±0.18	5.85±0.22	5.65±0.38	5.78±0.28
Forelimbs (right)				
Metacarpal phalanges	4.87±0.23	4.89±0.14	4.88±0.24	4.83±0.31
Proximal phalanges	4.98±0.06	5.00±0.00	5.00±0.00	5.00±0.00
Middle phalanges	3.83±0.29	3.84±0.21	3.85±0.12	3.64±0.38
Distal phalanges	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Forelimbs (left)				
Metacarpal phalanges	4.87±0.23	4.88±0.14	4.88±0.24	4.81±0.32
Proximal phalanges	4.98±0.06	5.00±0.00	5.00±0.00	5.00±0.00
Middle phalanges	3.83±0.29	3.84±0.21	3.87±0.19	3.61±0.39
Distal phalanges	5.00±0.00	5.00±0.00	5.00±0.00	5.00±0.00
Hindlimbs (right)				
Metatarsal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Proximal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Middle phalanges	4.00±0.00	3.99±0.05	4.00±0.00	3.92±0.29
Distal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Hindlimbs (left)				
Metatarsal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Proximal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Middle phalanges	4.00±0.00	3.99±0.05	4.00±0.00	3.92±0.29
Distal phalanges	4.00±0.00	4.00±0.00	4.00±0.00	4.00±0.00
Sacral and caudal vertebrae	19.46±0.65	19.57±0.44	19.64±0.22	19.53±0.49

a) : Combined with asymmetry of sternbrae

Prenatal and postnatal development

A prenatal and postnatal development study was not performed

Best Possible Copy

2.6.6.7 Local tolerance

Study title: Pilot irritancy of various IGF-1 formulation buffers injected subcutaneously in rabbits

Key study findings:

1. Subcutaneous injection of acetate-containing buffers resulted in mild to moderately severe inflammatory changes in the muscular and hypodermal layers in the rabbit.
2. The acetate plus mannitol preparation induced the mildest reaction
3. Subcutaneous injections of citrate buffers did not induce similar inflammatory changes.
4. Citrate-based buffer or the acetate/mannitol buffer caused less local irritation than the acetate buffer formulations.

Study no.: 88-477-0320-565

Volume #, and page #: E-file

Conducting laboratory and location: Department of Pharmacological Sciences, Genetech, Inc. 460 Point San Bruno Blvd. South San Francisco, CA 94080

Date of study initiation: December 22, 1988

GLP compliance: No.

QA reports: yes (x) no ()

Methods

Animal No.	Vol. (ml)	Treatment		ID No.
		Right Side	Left Side	
1-3	0.5	10 mM acetate, pH 4	Saline	A
1-3	2.0	10 mM acetate, pH 4	Saline	B
1-3	0.5	10 mM acetate, pH 4 + 50 mg/ml mannitol	Saline	C
1-3	2.0	10 mM acetate, pH 4 + 50 mg/ml mannitol	Saline	D
1-3	0.5	10 mM acetate, pH 4 + 19 mg/ml glycine	Saline	E
1-3	2.0	10 mM acetate, pH 4 + 19 mg/ml glycine	Saline	F
4-6	0.5	10 mM citrate, pH 6 + 126 mM saline	Saline	A
4-6	2.0	10 mM citrate, pH 6 + 126 mM saline	Saline	B
4-6	0.5	10 mM citrate, pH 6 + 50 mg/ml mannitol	Saline	C
4-6	2.0	10 mM citrate, pH 6 + 50 mg/ml mannitol	Saline	D
4-6	0.5	10 mM citrate, pH 6 + 19 mg/ml glycine	Saline	E
4-6	2.0	10 mM citrate, pH 6 + 19 mg/ml glycine	Saline	F

Study design: 3/male/group rabbits were treated with subcutaneous injections of the test buffers at the volumes indicated in the above table. Prior to injections, the hair was removed from an approximately 6x10cm region on the dorsum and a grid of sex squares marked with a permanent marker. The upper half of each right side square received a single 0.5ml SC injection of a test buffer; the lower half received a 2.0ml SC injection of the same buffer as shown in the following figures. The contralateral square received identical volumes of saline

as a control. Injection sites and the injection “bleb” were marked and documented for each animal.

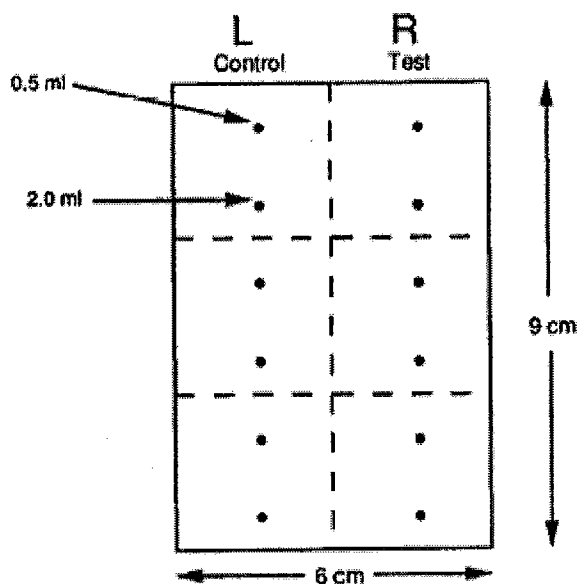


Figure 1: Injection grid (rabbit dorsum)

Results: The most significant lesions occurred in the subdermal muscle and hypodermis. Muscular lesions were generally multifocal except at one injection site that received 2ml of 10mM acetate not containing mannitol or glycine. At this site, the lesion was severe and diffuse. Lesions in the muscle consisted of individual cell necrosis and degeneration accompanied by mixed inflammatory cell accumulation. Hypodermal lesions were typically composed of scattered mononuclear inflammatory cell accumulations and diffuse edema.

Using muscular and hypodermal lesions as an indicator, animals in the acetate group (nos.1, 2, and 3) had substantially elevated numbers (8 of 18 positive incidences) of lesions at injection sites and had more server than the citrate-injected animals. Within the acetate group, the acetate plus mannitol treatment sites showed minimal evidence of treatment-related inflammatory changes. Data were summarized in the following tables.

Table 1: Incidence of Inflammatory Changes in Treated Animals^a

Site of Inflamm.	Acetate	Acetate + mannitol	Acetate + glycine	Citrate + NaCl	Citrate + mannitol	Citrate + glycine	Saline
AILb	5/6 ^c	1/6	2/6	0/6	1/6	0/6	4/36
Musc./Hypoderm.d	5/6	1/6	2/6	0/6	0/6	0/6	2/36

^a Animals received single subcutaneous injections of indicated buffer at a volume of 0.5 or 2.0 ml; data from the two injection volumes are combined for summary purposes. Three animals per treatment were utilized.

^b Lesions of severity greater than 1+ (see Methods) located in the mid- or deep-dermis, subdermal muscle and hypodermis.

^c Number of injection sites exhibiting inflammation at the indicated sites. Observations from all 3 animals are combined.

^d Lesions of severity greater than 1+ (see Methods) located in the subdermal muscle or hypodermis.

TABLE 2: INDIVIDUAL ANIMAL DATA

	0.5 saline	0.5 acet.	2.0 saline	2.0 acet.
Animal #1				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	2+
Necrosis	0	0	0	0
Animal #2				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	1+	2+	0	2+
Necrosis	0	0	0	0
Animal #3				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	3+
Necrosis	0	0	0	3+
Hypodermis				
Inflammation	0	3+	0	0
Necrosis	0	0	0	0
INCIDENCE >+1	0\3	2\3	0\3	3\3
(Musc./Hypo)	0\3	2\3	0\3	3\3

TABLE 2: (Cont'd)

	0.5 saline	0.5 citr./NaCl	2.0 saline	2.0 citr./NaCl
Animal #4				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #5				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #6				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
INCIDENCE _{>+1}	0/3	0/3	0/3	0/3
(Musc.,Hypo.)	0/3	0/3	0/3	0/3

TABLE 2: (Cont'd)

	0.5 saline	0.5 acet./man	2.0 saline	2.0 acet./man
Animal #1				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #2				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	2 +
Necrosis	0	0	0	0
Animal #3				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
INCIDENCE	0/3	0/3	0/3	1/3
(Musc.,Hypo.)	0/3	0/3	0/3	1/3

TABLE 2: (Cont'd)

	0.5 saline	0.5 citr./man	2.0 saline	2.0 citr./man
Animal #4				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	2+
Necrosis	0	0	0	0
Muscle				
Inflammation	1+	0	0	0
Necrosis	2+	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #5				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #6				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	2+	0	0	0
Necrosis	2+	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	1+
Necrosis	0	0	0	0
INCIDENCE >+1	2/3	0/3	0/3	1/3
(Musc., Hypo.)	1/3	0/3	0/3	0/3

TABLE 2: (Cont'd)

	0.5 saline	0.5 acet/gly	2.0 saline	2.0 acet/gly
Animal #1				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	1+	0	0	0
Necrosis	1+	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #2				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	2+
Necrosis	0	0	0	2+
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #3				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	2+	0
Necrosis	0	0	2+	0
Hypodermis				
Inflammation	0	0	0	2+
Necrosis	0	0	0	0
INCIDENCE >+1	1/3	0/3	1/3	2/3
(Musc., Hypo.)	0/3	0/3	1/3	2/3

*4+ hemorrhage, compatible with local traumatic vascular injury

TABLE 2: (Cont'd)

	0.5 saline	0.5 citr./gly	2.0 saline	2.0 citr./gly
Animal #4				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #5				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Animal #6				
Epidermis				
Necrosis	0	0	0	0
Dermis				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Muscle				
Inflammation	0	0	0	0
Necrosis	0	0	0	0
Hypodermis				
Inflammation	0	0	0	1 +
Necrosis	0	0	0	0
INCIDENCE >+1				
(Musc., Hypo.)				
	0\3	0\3	0\3	0\3
	0\3	0\3	0\3	0\3

In summary, subcutaneous injection of acetate-containing buffers resulted in mild to moderately severe inflammatory changes in the muscular and hypodermal layers in the rabbit. The acetate plus mannitol preparation induced the mildest reaction. Subcutaneous injections of citrate buffer did not induce similar inflammatory changes. Therefore, it is recommended that either citrate-based buffer or the acetate/mannitol buffer be utilized in the formulation of IGF-1 for parenteral administration.

Study title: One/Five-day intravenous tolerance study with GN0320 in Rabbits

Key study findings: 5mg/ml formulation of GN0320 injected intravenously at 0.1 or 1.0ml is well-tolerated locally.

Study no.: 89-016-0320

Volume #, and page #: E-file

Conducting laboratory and location: ☐
 ☒

Date of study initiation: April 13, 1989

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: GN0320, Lot # M3-RD1, purity — 3

Formulation/vehicle: Vehicle excipient of GN0320 was clear, colorless liquid, lot#M3-RD2 (a sterile solution in 100ml USP including — ng benzyl alcohol, — mg sodium chloride, — g polysorbate — mg sodium acetate/acetic acid in sterile water for injection).

Methods

Group	Number of Animals	Number of Dosing Days	Treatment (ml)		Animal Numbers
			Left Ear	Right Ear	
Phase I					
1	3	1	0.1 GN0320	0.1 Placebo	F27589-F27591
2	3	1	1.0 GN0320	1.0 Placebo	F27592-F27594
3	3	1	0.1 Saline	1.0 Saline	F27595-F27597
Phase II					
4	3	5	0.1 GN0320	0.1 Placebo	F27598-F27600
5	3	5	1.0 GN0320	1.0 Placebo	F27601-F27603
6	3	5	0.1 Saline	1.0 Saline	F27604-F27606

Doses: test article in 5mg/ml, placebo (vehicle) or saline

Species/strain: Male — NZW)SPF rabbits from 7

Number/sex/group or time point (main study): 3/sex/group

Route, formulation, volume, and infusion rate: Intravenous, solution, 0.1 or 1ml for 1 one day or 5 days.

Satellite groups used for recovery: None

Weight: 2.6 to 3.4kg

Injection site: Approximately 24 hours prior to injection, the hair was clipped from broad areas surrounding the injection sites. Just before dosing, the sites were swabbed with 70% isopropyl alcohol. The test material, placebo or saline was injected into the right and left marginal ear vein using sterile syringes and needles. The injection sites were marked with an indelible link pen for identification at necropsy.

Clinical general observations: Observed before dosing, 0.5, 1, 2, 4, and 6 to 7 hours post-dose (Day 0) and twice daily thereafter.

Body weight: Day 0 and the day of necropsy

Food consumption: Daily by visual inspections

Dermal irritation: Each injection site was scored for erythema and edema using the modified Draize Scoring Scale approximately 24 hours after each injection.

Pathology: At approximately 24 hours after the last dose (phase I. one day and phase II 5-day study), the injection sites from all animals were observed for macroscopic and histopathological changes.

Results:

Clinical general observations: No drug-related changes of general clinical signs through out the study were noted.

Body weight: No drug-related changes of body weight or body weight gain were noted.

Food consumption: No drug-related effects on food consumption were noted.

Dermal irritation: A very slight to well-defined erythema and very slight edema were observed for all groups. No drug-related irritation was noted as shown in the following table.

Table 2

Summary of Dermal Irritation Scores^a

Observation	Time of Observation (Day)										
	Intravenous - Left Ear						Intravenous - Right Ear				
	1	2	3	4	5	Average	1	2	3	4	5
Group 1											
Erythema						0.33					1.33
None	2	-	-	-	-		1	-	-	-	-
Very slight	1	-	-	-	-		0	-	-	-	-
Well-defined	0	-	-	-	-		2	-	-	-	-
Moderate	0	-	-	-	-		0	-	-	-	-
Severe	0	-	-	-	-		0	-	-	-	-
Edema						0.33					0.67
None	2	-	-	-	-		1	-	-	-	-
Very slight	1	-	-	-	-		2	-	-	-	-
Slight	0	-	-	-	-		0	-	-	-	-
Moderate	0	-	-	-	-		0	-	-	-	-
Severe	0	-	-	-	-		0	-	-	-	-

a Number of animals affected.

Table 2 (Continued)

Summary of Dermal Irritation Scores^a

Observation	Time of Observation (Day)										
	Intravenous - Left Ear						Intravenous - Right Ear				
	1	2	3	4	5	Average	1	2	3	4	5
Group 2											
Erythema						1.00					1.67
None	1	-	-	-	-		0	-	-	-	-
Very slight	1	-	-	-	-		1	-	-	-	-
Well-defined	1	-	-	-	-		2	-	-	-	-
Moderate	0	-	-	-	-		0	-	-	-	-
Severe	0	-	-	-	-		0	-	-	-	-
Edema						0.67					1.00
None	1	-	-	-	-		0	-	-	-	-
Very slight	2	-	-	-	-		3	-	-	-	-
Slight	0	-	-	-	-		0	-	-	-	-
Moderate	0	-	-	-	-		0	-	-	-	-
Severe	0	-	-	-	-		0	-	-	-	-

a Number of animals affected.

Best Possible Copy

Table 2 (Continued)
Summary of Dermal Irritation Scores^a

Observation	Time of Observation (Day)										
	Intravenous - Left Ear						Intravenous - Right Ear				
	1	2	3	4	5	Average	1	2	3	4	5
Group 3											
Erythema						1.00					1.33
None	1	-	-	-	-		0	-	-	-	-
Very slight	1	-	-	-	-		2	-	-	-	-
Well-defined	1	-	-	-	-		1	-	-	-	-
Moderate	0	-	-	-	-		0	-	-	-	-
Severe	0	-	-	-	-		0	-	-	-	-
Edema						0.67					1.00
None	1	-	-	-	-		0	-	-	-	-
Very slight	2	-	-	-	-		3	-	-	-	-
Slight	0	-	-	-	-		0	-	-	-	-
Moderate	0	-	-	-	-		0	-	-	-	-
Severe	0	-	-	-	-		0	-	-	-	-

a Number of animals affected.

Table 2 (Continued)
Summary of Dermal Irritation Scores^a

Observation	Time of Observation (Day)										
	Intravenous - Left Ear						Intravenous - Right Ear				
	1	2	3	4	5	Average	1	2	3	4	5
Group 4											
Erythema						0.93					1.07
None	0	3	1	0	2		0	0	0	1	0
Very slight	3	3	1	3	0		2	2	3	2	3
Well-defined	0	3	1	0	1		1	1	0	0	0
Moderate	0	3	0	0	0		0	0	0	0	0
Severe	0	3	0	0	0		0	0	0	0	0
Edema						0.67					0.87
None	0	3	1	2	2		0	0	0	2	0
Very slight	3	3	2	1	1		3	3	3	1	3
Slight	0	0	0	0	0		0	0	0	0	0
Moderate	0	0	0	0	0		0	0	0	0	0
Severe	0	0	0	0	0		0	0	0	0	0

a Number of animals affected.

Best Possible Copy

Table 2 (Continued)
Summary of Dermal Irritation Scores^a

Observation	Time of Observation (Day)											
	Intravenous - Left Ear						Intravenous - Right Ear					
	1	2	3	4	5	Average	1	2	3	4	5	Average
Group 5												
Erythema						1.40						0.87
None	0	0	0	1	0		0	0	1	3	0	
Very slight	1	1	3	1	1		2	2	2	0	3	
Well-defined	2	2	0	1	2		1	1	0	0	0	
Moderate	0	0	0	0	0		0	0	0	0	0	
Severe	0	0	0	0	0		0	0	0	0	0	
Edema						0.80						0.73
None	0	0	1	2	0		0	0	1	3	0	
Very slight	3	3	2	1	3		3	3	2	0	3	
Slight	0	0	0	0	0		0	0	0	0	0	
Moderate	0	0	0	0	0		0	0	0	0	0	
Severe	0	0	0	0	0		0	0	0	0	0	

a Number of animals affected.

Table 2 (Continued)
Summary of Dermal Irritation Scores^a

Observation	Time of Observation (Day)											
	Intravenous - Left Ear						Intravenous - Right Ear					
	1	2	3	4	5	Average	1	2	3	4	5	Average
Group 6												
Erythema						0.67						0.73
None	2	0	1	2	2		1	0	2	2	1	
Very slight	1	3	1	1	0		2	3	1	0	1	
Well-defined	0	0	1	0	1		0	0	0	1	1	
Moderate	0	0	0	0	0		0	0	0	0	0	
Severe	0	0	0	0	0		0	0	0	0	0	
Edema						0.33						0.40
None	2	1	2	3	2		2	1	3	2	1	
Very slight	1	2	1	0	1		1	2	0	1	2	
Slight	0	0	0	0	0		0	0	0	0	0	
Moderate	0	0	0	0	0		0	0	0	0	0	
Severe	0	0	0	0	0		0	0	0	0	0	

a Number of animals affected.

Pathology: No drug-related macroscopic findings or histopathological changes in the multiple injection sites were observed as shown in the following tables.

Best Possible Copy

MLA 6261-113
Genentech 85-016-0320TABLE 3
INCIDENCE OF MICROSCOPIC OBSERVATIONS

*** PATH/TE SYSTEM OUTPUT ***

PRINTED: 25-MAY-89
TABLE PAGE: 3

TABLE INCLUDES:		--- NUMBER - OF - ANIMALS - AFFECTED ---						
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; SUBSET=ALL		SEX: -----MALE-----						
		GROUP: -1- -2- -3- -4- -5- -6-						
ORGAN AND KEYWORD(S) OR PHRASE		NUMBER: 3 3 3 3 3 3						
** TOP OF LIST **		---						
LF (NO SITE) (150)		NUMBER EXAMINED: 3 3 3 3 3 3						
		NOT REMARKABLE: 3 3 1 3 1 2						
RED FOCUS(ES)/AREA(S)		1 1 2 0 1 1						
RT (NO SITE) (150)		NUMBER EXAMINED: 3 3 3 3 3 3						
		NOT REMARKABLE: 0 0 0 3 1 1						
RED FOCUS(ES)/AREA(S) MISSING/NOT IDENTIFIED		3 2 3 0 1 2						
		0 0 1 0 0 1						
GENERAL COMMENT (00)		NUMBER EXAMINED: 3 3 3 3 3 3						
		NOT REMARKABLE: 3 3 3 0 1 2						
** ALL TISSUES/ORGANS NORMAL		0 0 0 3 2 1						
** END OF LIST **								

TABLE 4
INCIDENCE OF MICROSCOPIC OBSERVATIONSMLA 6261-113
Genentech 85-016-0320

*** PATH/TE SYSTEM OUTPUT ***

PRINTED: 25-MAY-89
TABLE PAGE: 4

TABLE INCLUDES:		--- NUMBER - OF - ANIMALS - AFFECTED ---						
SEX=ALL; GROUP=ALL; WEEKS=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----						
		GROUP: -1- -2- -3- -4- -5- -6-						
ORGAN AND FINDING DESCRIPTION		NUMBER: 3 3 3 3 3 3						
** TOP OF LIST **		---						
LF (NO SITE) (150)		NUMBER EXAMINED: 3 3 3 3 3 3						
		NOT REMARKABLE: 1 1 2 2 1 1						
--HEMORRHAGE, PERIVASCULAR/SCURVING		2 1 1 0 2 0						
--THROMBOSIS, VENOUS		0 0 0 3 0 0						
--FIBROPLASIA, PERIVASCULAR		0 0 0 1 2 0						
RT (NO SITE) (150)		NUMBER EXAMINED: 3 3 2 1 2 4						
		NOT REMARKABLE: 0 1 0 1 2 3						
--HEMORRHAGE, PERIVASCULAR/SCURVING		3 1 2 0 1 0						
--THROMBOSIS, VENOUS		0 0 0 1 0 0						
--FIBROPLASIA, PERIVASCULAR		0 0 0 1 0 0						
** END OF LIST **								

Conclusion: Administration of GN0320 or the placebo intravenously via the marginal ear vein for 1 or 5 days did not result in irritation that could be directly associated with the test material when compared with results of the effects of saline injection.

Study title: Acute subcutaneous tolerance study with GN0320 in Rabbits

Key study findings: Subcutaneous administration of 0.5ml GN0320 at a dose concentration of 10mg/ml produced no local irritation in rabbits (this formulation comparable to the to-be-marketed formulation).

Best Possible Copy

Study no.: 93-1180-0320

Volume #, and page #: E-file

Conducting laboratory and location: L

J

Date of study initiation: April 29, 1993

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: GN0320, Lot# M3-RD251 and purity — %

Formulation/vehicle: Vehicle excipient of GN0320 was clear, colorless liquid, lot#M3-RD254 (a sterile solution in 100ml USP including —mg benzyl alcohol, — mg sodium chloride, — polysorbate L J ng sodium acetate/acetic acid in sterile water for injection).

Methods

Group	Treatment	Dose Level ^a (mg/kg)	Dose Concentration (mg/mL)	Dose Volume (mL/dose)	Number of Males ^b
1	GN0320 Vehicle	0	0	0.5	6
2	GN0320	2	10	0.5	6

a The calculated dose level (mg/kg) for Group 2 was determined based on actual average body weight data recorded on the day of dosing (Day 1).

b Three animals/group were sacrificed 1 or 7 days after dose administration (Days 2 and 8, respectively).

Doses: test article in 10mg/ml or placebo (vehicle)

Species/strain: Male — (NZW)SPF rabbits from L

J

Number/sex/group or time point (main study): 6/male/group

Route, formulation, volume, and infusion rate: Subcutaneous, solution, 0.5ml in single dose.

Satellite groups used for recovery: None

Age: Approximate 16 weeks

Weight: 2.2 to 2.7kg

Injection site: Approximately 24 hours prior to injection, the hair was clipped from broad areas surrounding the subcutaneous injection sites. Just before dosing, the sites were swabbed with 70% isopropyl alcohol. The test material or placebo was injected into the right dorsal region, lateral to the spinal column of each animal using sterile, 1-cc syringes and 25-L J 5/8-inch needles. The injection site of each animal was marked with an indelible link pen for identification at necropsy.

Clinical general observations: Observed before dosing, 0.5, 1, 2, and 4 hours post-dose (Day 0) and twice a day thereafter.

Body weight: Days 1 and 8.

Food consumption: Daily by visual inspections

Local irritation: Each injection site was observed for redness and swelling, and the area of irritation was measured. The observations and measurements were made before dosing, approximately 0.5, 6, and 24 hours post dose, and once daily thereafter according to a modified Draize Scoring scale.

Plasma glucose level: Samples were collected 30 minutes and 1 hour after post-dose.

Pathology: Three animals/group were sacrificed and necropsied on Days 2 (following the 24-hour post-dose observations) and 8. The necropsy included a macroscopic examination of the external surface of the body, injection site, and all orifices. The injection site was observed for macroscopic change. Histopathological examinations were performed for one section each from the dorsal periphery, central region, and ventral periphery of the SC injection area. Each section included skin, underlying subcutaneous tissues, and gross lesions (external).

Results:

General observations: All animals survived to scheduled sacrifice and appeared healthy throughout the study.

Body weight: No drug-related changes of body weight were noted.

Food consumption: No drug-related changes of food consumptions were noted.

Local irritation: No drug-related local injection site irritation was noted as shown in the following tables.

HWI 6201-209
Genentech 33-118-0320

Table 4.1
Summary of Local Irritation Data

PRINTED: 18-AUG-93
PAGE: 1

DAYS 1-8		NUMBER OF ANIMALS AFFECTED	
CATEGORY	SEX: --MALE--	GROUP: 1 2	DOSE: 0 10
KEYWORD:	NUMBER: 6 6		
QUALIFIER:			
*** TOP OF LIST ***			
LOCAL IRRITATION			
REDNESS			
SWELLING	6 6		
NONE	6 6		
*** END OF LIST ***			

HWI 6201-209
Genentech 33-118-0320

Table 4.2
Incidence of Local Irritation Data

PRINTED: 18-AUG-93
PAGE: 1

DAYS 1-8		NUMBER OF ANIMALS AFFECTED / NUMBER ALIVE AT BEGINNING OF DAY	
CATEGORY	SEX: --MALE--	GROUP: 1 2	DOSE: 0 10
KEYWORD:	NUMBER: 6 6		
QUALIFIER:			
*** TOP OF LIST ***			
LOCAL IRRITATION			
REDNESS			
SWELLING	6/6 6/6		
NONE	3/3 3/3		
*** END OF LIST ***			

Plasma level of glucose: A single 0.5ml subcutaneous injection of GN0320 at a dose concentration of 10mg/ml resulted in decreased mean serum glucose concentrations. The mean concentration for animals given GN0320 was approximately 53% lower (30 minutes post-dose) and 45% lower (1 hour post-dose) than that of the control animals. Data were summarized in the following tables.

Table 7

SUMMARY OF SERUM GLUCOSE DATA		
MALES DAY 1 30 MINUTE POSTDOSE		
DOSE MG/ML		GLU MG/DL
0	MEAN	121
	S.D.	2.9
	N	6
10	MEAN	57 *
	S.D.	5.1
	N	6

* Statistical significance at $p \leq 0.05$.

Table 8

SUMMARY OF SERUM GLUCOSE DATA		
MALES DAY 1 1 HOUR POSTDOSE		
DOSE MG/ML		GLU MG/DL
0	MEAN	131
	S.D.	3.8
	N	6
10	MEAN	72 *
	S.D.	9.3
	N	6

* Statistical significance at $p \leq 0.05$.

Pathology: There were no macroscopic or microscopic changes at the injection site attributed to the single 0.5ml subcutaneous injection of a 10mg/ml concentration of GN0320. Red areas on the skin between the shoulder blades, noted macroscopically and several microscopic findings including superficial dermal inflammation, hyperkeratosis, and hemorrhage, were attributed to clipping of the fur before treatment. These findings were observed primarily for animals necropsied on Day 2. Data were summarized in the following tables.

TABLE 9
INCIDENCE OF MACROSCOPIC OBSERVATIONS
DAY 2 SACRIFICE

HWI 6241-288
GENENTECH 93 118-6329

PRINTED: 09-JUN-93
TABLE PAGE: 9-1

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED	
SEX=ALL; GROUP=ALL; WEEKS=ALL	SEX: --MALE--		
DEATH=1; SUBSET=1	GROUP: -1- -2-		
ORGAN AND KEYWORD(S) OR PHRASE	NUMBER:	3	3
** TOP OF LIST **			
INJECTION SITE (IS)	NUMBER EXAMINED:	3	3
NO MACROSCOPIC LESION(S)		3	3
SKIN (SK)	NUMBER EXAMINED:	3	3
	NOT REMARKABLE:	0	0
RED FOCUS(1)/AREAS(S)		3	3
** END OF LIST **			

TABLE 10
INCIDENCE OF MACROSCOPIC OBSERVATIONS
DAY 8 SACRIFICE

NW1 6281-289
GENENTECH 93-118-0320

PRINTED: 08-JUN-93
TABLE PAGE: 10.1

TABLE INCLUDES:		SEX: --MALE--	
SEX=ALL;GROUP=ALL;WEEKS=ALL		GROUP: -1- -2-	
DEATH=T;SUBSET=ALL		NUMBER: 3 3	
ORGAN AND KEYWORD(S) OR PHASE		NUMBER EXAMINED:	
** TOP OF LIST **		3 3	
GENERAL COMMENT (GC)		3 3	
NO MACROSCOPIC LESIONS		3 2	
INJECTION SITE (IS)		3 3	
NO MACROSCOPIC LESIONS		3 3	
SKIN (SK)		3 3	
NOT REMARKABLE:		3 2	
RED FOCUS(ES)/AREA(S)		0 1	
** END OF LIST **			

TABLE 11
INCIDENCE OF MICROSCOPIC OBSERVATIONS
DAY 2 SACRIFICE

NW1 6281-289
GENENTECH 93-118-0320

PRINTED: 12-AUG-93
TABLE PAGE: 11.1

TABLE INCLUDES:		SEX: --MALE--	
SEX=ALL;GROUP=ALL;WEEKS=ALL		GROUP: -1- -2-	
DEATH=T;FIND=ALL;SUBSET=ALL		NUMBER: 3 3	
ORGAN AND FINDING DESCRIPTION		NUMBER EXAMINED:	
** TOP OF LIST **		3 3	
INJECTION SITE (IS)		NOT REMARKABLE: 0 0	
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.		1 2	
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.		3 3	
SKIN (SK)		NUMBER EXAMINED: 3 3	
NOT REMARKABLE:		0 0	
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.		3 3	
--HYPERKERATOSIS/ACANTHOSIS		2 3	
--ULCERATION		2 1	
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.		3 3	
--HEMORRHAGE		3 3	
** END OF LIST **			

TABLE 12
INCIDENCE OF MICROSCOPIC OBSERVATIONS
DAY 8 SACRIFICE

NW1 6281-289
GENENTECH 93-118-0320

PRINTED: 12-AUG-93
TABLE PAGE: 12.1

TABLE INCLUDES:		SEX: --MALE--	
SEX=ALL;GROUP=ALL;WEEKS=ALL		GROUP: -1- -2-	
DEATH=T;FIND=ALL;SUBSET=ALL		NUMBER: 3 3	
ORGAN AND FINDING DESCRIPTION		NUMBER EXAMINED:	
** TOP OF LIST **		3 3	
INJECTION SITE (IS)		NOT REMARKABLE: 0 0	
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.		0 1	
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.		2 3	
SKIN (SK)		NUMBER EXAMINED: 0 1	
NOT REMARKABLE:		0 0	
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS		0 1	
--HEMORRHAGE		2 1	
** END OF LIST **			

TABLE 13
INCIDENCE OF SEVERITY OF MICROSCOPIC OBSERVATIONS
DAY 2 SACRIFICE

HWI 6281-289
GENENTECH 93-118-0320

PRINTED: 12-AUG-93
TABLE PAGE: 13.1

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---	
SEX=ALL; GROUP=ALL; WEEKS=ALL	SEX: --MALE--		
DEATH=1; FIND=ALL; SUBSET=ALL	GROUP: -1- -2-		
ORGAN/TISSUE EXAMINED	NUMBER:	1	3
** TOP OF LIST **			
INJECTION SITE (IS)	NUMBER EXAMINED:	3	3
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS	1	2	1
CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH	2	1	2
NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.	TL	3	3
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS	1	3	3
CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR TL	2	3	3
NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.			
SKIN (SK)			
	NUMBER EXAMINED:	3	3
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS	1	0	1
CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH	2	2	2
NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.	3	1	0
	TL	3	3
--HYPERKERATOSIS/ACANTHOSIS	2	3	3
	TL	3	3
--ULCERATION	1	1	2
	2	3	1
	3	1	0
	TL	3	3
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS	2	1	3
CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR	3	2	0
NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.	TL	3	3
** CONTINUED ON NEXT PAGE **			

TABLE 13
INCIDENCE OF SEVERITY OF MICROSCOPIC OBSERVATIONS
DAY 2 SACRIFICE

HWI 6281-289
GENENTECH 93-118-0320

PRINTED: 12-AUG-93
TABLE PAGE: 13.2

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---	
SEX=ALL; GROUP=ALL; WEEKS=ALL	SEX: --MALE--		
DEATH=1; FIND=ALL; SUBSET=ALL	GROUP: -1- -2-		
ORGAN/TISSUE EXAMINED	NUMBER:	3	3
** FROM PREVIOUS PAGE **			
SKIN (SK)	NUMBER EXAMINED:	3	3
--HEMORRHAGE	1	2	3
	2	1	0
	TL	3	3
** END OF LIST **			

TABLE 14
INCIDENCE OF SEVERITY OF MICROSCOPIC OBSERVATIONS
DAY & SACRIFICE

HWI 6281-289
GENENTECH 93-118-0320

PRINTED: 12-AUG-93
TABLE PAGE: 14.1

TABLE INCLUDES:		SEX: --MALE--	
SEX=ALL; GROUP=ALL; WEEKS=ALL		GROUP: -1- -2-	
DEATH=T; FIND=ALL; SUBSET=ALL		NUMBER: 3 3	
ORGAN/TISSUE EXAMINED			
--- NUMBER OF ANIMALS AFFECTED ---			
** TOP OF LIST **			
INJECTION SITE (IS)	NUMBER EXAMINED:	3	3
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS	-->	3	2
CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH 1)	TL>	0	1
NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.		3	3
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS	1)	3	3
CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR TL>		3	3
NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.			
SKIN (SK)	NUMBER EXAMINED:	0	1
--EXUDATE, INFLAMMATORY, SUPERFICIAL EPIDERMIS	-->	0	1
CHARACTERIZED BY MULTIFOCAL AREAS OF PROTEINACEOUS EXUDATE WITH TL>		0	1
NECROTIC KERATINOCYTES AND INFLAMMATORY CELLS.			
--HYPERKERATOSIS/ACANTHOSIS	-->	0	1
	TL>	0	1
--ULCERATION	-->	0	1
	TL>	0	1
--INFLAMMATION, SUBACUTE, SUPERFICIAL DERMIS	2)	0	1
CHARACTERIZED BY HISTIOCYTES, EOSINOPHILS, LYMPHOCYTES, AND/OR TL>		0	1
NEUTROPHILS SCATTERED IN SUPERFICIAL DERMIS.			
--HEMORRHAGE	1)	0	1
	TL>	0	1
** END OF LIST **			

Conclusion: Based on the results, the subcutaneous administration of 0.5ml GN0320 at a concentration of 10mg/ml did not cause local irritation in rabbits.

2.6.6.8 Special toxicology studies

Study title: Hemolytic potential and compatibility testing with GN0320 (IGF-1)

Key study findings: GN0320 in a vehicle solution at a concentration of 5mg/ml does not cause hemolysis of human or canine erythrocytes and is compatible with human and canine serum and plasma.

Study no.: 89-015-0320

Volume #, and page #: E-file

Conducting laboratory and location: 1

1

Date of study initiation: February 6, 1989

GLP compliance: Yes

QA reports: yes (x) no ()

Drug, lot #, and % purity: GN0320, Lot# M3-RD1 and purity — %

Formulation/vehicle: Vehicle excipient of GN0320 was clear, colorless liquid, lot#M3-RD2 (ingredients were not characterized from the sponsor).

Methods

Doses: 5mg/ml of GN0320

Study design: The hemolytic potential and compatibility effects of GN0320 on fresh canine and human whole blood and plasma were tested in vitro.

Hemolytic Potential

Using the test material and fresh canine and human blood and plasma, test tubes were set up as follows:

Tube Number	Canine		Human		Test Material In Vehicle (5 mg/mL)	Vehicle	Saponin (1%)
	Blood	Plasma	Blood	Plasma			
1	+	-	-	-	+	-	-
2	+	-	-	-	-	+	-
3 ^a	+	+	-	-	-	-	-
4 ^b	+	-	-	-	-	-	+
5	-	-	+	-	+	-	-
6	-	-	+	-	-	+	-
7 ^a	-	-	+	+	-	-	-
8 ^b	-	-	+	-	-	-	+

+ 0.5 mL added to tube.

- Not added to tube.

a Negative control for hemolysis.

b Positive control for hemolysis. Saponin is a hemolytic agent used to lyse erythrocytes.

Compatibility

Using the test material and fresh canine and human serum and plasma, test tubes were set up as follows:

Tube Number	Canine		Human		Test Material In Vehicle (5 mg/mL)	Vehicle
	Serum	Plasma	Serum	Plasma		
1	+	-	-	-	+	-
2	-	+	-	-	+	-
3	-	-	+	-	+	-
4	-	-	-	+	+	-
5	+	-	-	-	-	+
6	-	+	-	-	-	+
7	-	-	+	-	-	+
8	-	-	-	+	-	+

+ 0.5 mL added to tube.

- Not added to tube.

Results: In the hemolytic potential study, neither the vehicle nor a solution of the test material in vehicle (5mg/ml) caused lysis of erythrocytes when mixed with equal volumes of human or canine whole blood.

In the compatibility test, neither the vehicle nor a solution of the test material in vehicle (5mg/ml) caused precipitation or coagulation when mixed with equal volumes of human or canine serum or plasma. Data were summarized in the following table.

TABLE 1

Hemolytic Potential/Compatibility Test Results

DATE February 9, 1989STUDY 6281-112

Hemolytic Potential/Compatibility Test Results

HEMOLYTIC POTENTIAL (Concentration of Drug Formulation = 5 mg/mL)Cyanmethemoglobin Reagent lot # 035-037 (Exp. 7/1/89)Cyanmethemoglobin Standard Lot # 615-107 (Exp. 3/1/89)

Standard curve for hemoglobin concentration:

<u>STANDARDS</u>	<u>ABSORBANCE (x)</u>	<u>CONCENTRATION (y) (g/dL)</u>
A	<u>0.133</u>	<u>5.2</u>
B	<u>0.263</u>	<u>10.2</u>
C	<u>0.514</u>	<u>20.1</u>

Equation for standard curve line: $y = \underline{39.1} (x) + (\underline{-0.043})$ Coefficient of linear correlation: 0.9999

Hemoglobin concentrations of the mixture supernatants:

<u>MIXTURE</u>	<u>ABSORBANCE</u>	<u>CONCENTRATION (g/dL)</u>	<u>TEST RESULT</u>
<u>ANIMAL BLOOD+</u>			
Drug/Vehicle	<u>0.000</u>	<u>-0.043</u>	<u>Negative</u>
Vehicle	<u>0.000</u>	<u>-0.043</u>	<u>Negative</u>
Animal Plasma	<u>0.001</u>	<u>-0.003</u>	<u>Negative</u>
1% Saponin	<u>0.146</u>	<u>5.673</u>	<u>Positive</u>
<u>HUMAN BLOOD+</u>			
Drug/Vehicle	<u>0.000</u>	<u>-0.043</u>	<u>Negative</u>
Vehicle	<u>0.000</u>	<u>-0.043</u>	<u>Negative</u>
Human Plasma	<u>0.000</u>	<u>-0.043</u>	<u>Negative</u>
1% Saponin	<u>0.060</u>	<u>2.306</u>	<u>Positive</u>

TABLE 1 (Continued)

Hemolytic Potential/Compatibility Test Results

COMPATIBILITY (Concentration of Drug Formulation = 5 mg/mL)MIXTURE TEST RESULTDRUG/VEHICLE+

Animal Serum Negative
 Animal Plasma Negative
 Human Serum Negative
 Human Plasma Negative

VEHICLE+

Animal Serum Negative
 Animal Plasma Negative
 Human Serum Negative
 Human Plasma Negative

Key: Negative = Compatible.
 Positive = Incompatible.

2.6.6.9 Discussion and Conclusions: Toxicological studies in this NDA application included single dose toxicological studies in mouse, rats, rabbit, dog, and monkey, multiple dose chronic toxicological studies up to 26 weeks in rats and dogs, a 2-year carcinogenicity study

in rats, reproductive toxicology studies in rats and rabbit, genotoxicology studies, local tolerance studies in rabbits.

In the single dose toxicology studies with mecasermin, there were no mice deaths at doses up to 10mg/kg i.v. No rats died at doses up to 1mg/kg iv or sc were noted. At dose of 10mg/kg iv or sc, rats died secondary to hypoglycemia. There were no rabbit deaths at dose up to 1mg/kg, iv. In dog study, animal died at dose of 5mg/kg, sc due to hypoglycemia. In monkey study after sc administration, 0.5mg/kg was well tolerated.

In the multiple dose chronic toxicology studies, administration of mecasermin to both rats and dogs, sc up to 26 weeks or iv up to 13 weeks were performed. Overall, hypoglycemia was the major effect noted in the toxicology studies, which was consistent with the expected physiological or exaggerated pharmacological effects of high doses of IGF-1. Other significant side effects included increases in body weight/body weight gain and/or food consumption, mild changes in clinical chemistry and hematological parameters, and increases in some organ weights including liver, kidney, heart, thymus, and/or spleen that correlated with the body weight changes and are likely related to the anabolic activities of IGF-1. Hematology changes seen in one or both species consisted of higher hemoglobin and hematocrit, lower white blood cell and absolute lymphocyte counts, and/or lower eosinophil counts. Clinical chemistry changes consisted of transient alterations in serum electrolytes (sodium, chloride, potassium, phosphorus, and/or calcium), protein, albumin, and/or triglycerides. In most instances, the clinical pathology changes noted in the repeat dose studies were reversible.

There are few histopathological changes associated with increases in organ weight and the organ weight increases were reversible. Histologic changes identified in the 26-week study in the rat were confined to an increase in thymic cortical area at the end of the treatment period following sc doses of 4.0mg/kg. This increase is correlated with the increase in thymic weight and cellularity. In dogs that survived until scheduled sacrifice in the 26-week dog study, histologic changes consisted of adrenal hyperplasia with fibrosis which was noted at sc doses at 0.3mg/kg/day and above. Adrenal medullary fibrosis but not hyperplasia was still present in dogs treated with 0.6mg/kg/day, 5 weeks following the cessation of treatment. In the 13-week iv repeated dose toxicology studies, no drug-related organ weight or histopathological findings were noted in rats. In dog, there was a significant shortening of the APTT in males at dose of 0.5mg/kg/day and a significant lengthening of the prothrombin time in females at dose of 0.5mg/kg. Histopathological findings at the end of treatment period were observed in the cerebrum, cerebellum, medulla oblongata, heart, lungs, stomach, cecum, and liver in animals at dose of 0.5mg/kg/day. Most of these findings are related to slight to moderate hemorrhage and/or slight lymphocyte infiltration. The NOAELs in the 26-week sc repeated dose toxicology studies was 4mg/kg in rats and 0.15mg/kg in dogs. The systemic exposure at the rat and dog NOAEL doses are 15X and 3X the MRHD, respectively. In the 13-week iv repeated dose toxicology studies, the NOAEL was 4mg/kg in rats and 0.125mg/kg in dogs. There were no effects noted with mecasermin in any of the repeat dose studies upon ophthalmology (dog and rat), EKG, blood pressure, heart rates, respiratory rates, or body temperatures.

In the genotoxicology studies, mecasermin tested negative in both in the in vitro chromosome aberration assay and the in vivo mouse micronucleus assay.

In the carcinogenicity study, A two year carcinogenicity study of rhIGF-1 was conducted in rats. Drug was administered subcutaneously at doses of 0.25, 1, 4, and 10mg/kg/day. rhIGF-1 induced pheochromocytoma in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels. Increased incidence of keratoacanthoma in the skin in males at doses of 4 and 10mg/kg/day, and in females at dose of 10mg/kg/day; and mammary gland carcinoma in both males and females at 10mg/kg/day were also observed. Compare with the historical control data, the only positive findings in this carcinogenicity study were the pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg. Therefore, the NOEL for the carcinogenicity of rhIGF-1 was 1mg/kg/day in males and 4mg/kg for females. Doses of 4 and 10mg/kg/day exceeded the MTD based on mortality. Therefore, no drug-related tumors were observed at tolerated doses.

In the reproductive toxicity studies, rhIGF-1 as MKC-031 was administered intravenously to rats at doses of 0.25, 1 and 4mg/kg/day to conduct the fertility study (segment I). Embryo-fetal toxicity studies were conducted in rats at doses of 1, 4, and 16mg/kg/day, and in rabbits at doses of 0.125, 0.5 and 2.0mg/kg/day. In the fertility study, a dose-dependent increase of body weight, body weight gain, and food consumption in both male and female rats at doses of 1 and 4mg/kg/day were noted. There were no significant difference between control and treatment groups in estrus count and estrous cycle during pre-mating administration period, no difference in copulation index, indices of insemination and fertility. For the embryos and fetuses, a dose-dependent increase of the index of dead/resorbed fetuses was observed. There were no significant differences between control and treatment groups in the number of corpora lutea, the number of implantations, and implantation index and the number of live fetuses.

In the embryo fetal development study in rat, a transient decrease in spontaneous movement at the high dose of 16mg/kg was observed during the administration period in dams. No drug-related changes of body weights, food consumption, or gross pathology were noted. In F1 fetuses, there were no drug-related changes of sex ratio, body weight, rate of incidence of pups exhibiting external, visceral or skeletal abnormalities or skeletal variations. No drug-related effects on growth and development of pups were noted. In liveborn F1 pups, the differentiation index for opening of the eyelid on day 14 after birth was significantly low at high dose of 16mg/kg (47.2% vs 71.3% in control). However, this differentiation index on day 17 after birth was 100%. There were no other drug-related changes of body weight after birth, viability, external differentiation, reflexes, behavioral tests and reproductive performance tests. In the embryo fetal development study in rabbits, no drug-related animal deaths or drug-related findings of clinical signs, body weight change or food consumption were noted. However, the food consumption of animals in which a decrease in the amount of feces was noted in the general condition observations was remarkably lower than the other animals. There were many early resorbed embryos and the index of dead embryos/fetuses showed significantly higher values in dams at dose of 2.0mg/kg. There were no significant differences between the control group and each test article treatment group in the number of corpora lutea, number of implantations, number of live fetuses and placenta weights. There were no drug-

related findings of sex ratio, body weight, external, visceral or skeletal examinations of live fetuses.

The NOAEL for fertility was 4mg/kg/day. The systemic exposure in animals receiving this dose was 5 times the MRHD based on BSA. Increases in early fetal resorptions were observed with all dose levels. No embryo fetal developmental abnormalities were observed in rats with doses up to 16 mg/kg/day (20 times the MRHD based on BSA). In the rabbit study, the NOAEL for dam reproductive ability was 2.0mg/kg (8 times the MRHD based on BSA) and the NOAEL for fetal toxicity was 0.5mg/kg (2 times the MRHD based on BSA). There was no teratogenicity of MKC-031 at doses up to 2.0mg/kg.

In the local tolerance studies, Intravenous and subcutaneous injection with different formulation were performed in rabbits. Subcutaneous injection of acetate-containing buffers resulted in mild to moderately severe inflammatory changes in the muscular and hypodermal layers in the rabbit. The acetate plus mannitol preparation induced the mildest reaction. Subcutaneous injections of citrate buffers did not induce similar inflammatory changes. In the acute subcutaneous tolerance study, subcutaneous administration of 0.5ml of IGF-1 solution at concentration of 10mg/ml produced no local irritation in rabbits. In the intravenous injection study, administration of IGF-1 solution intravenously via the marginal ear vein for 1 or 5 days in a volume of 0.1 or 1ml at the concentration of 5mg/ml did not result in irritation that could be directly associated with the test material when compared with results of the effects of saline injection.

In the hemolytic and compatibility in vitro studies, mecasermin in a vehicle solution at a concentration of 5mg/ml does not cause hemolysis of human or canine erythrocytes and is compatible with human and canine serum and plasma.

2.6.6.10 Tables and Figures

Tables and figures have been incorporated into the respective study reviews.

2.6.7 TOXICOLOGY TABULATED SUMMARY

[pivotal studies pertinent to the primary indication and core pharmacology studies relevant to the primary pharmacodynamic effect, as available and as provided by the sponsor]

*Appears This Way
On Original*

2.6.7.1 Toxicology Overview

Test Article: Mecasermin Page 1 of 3

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg) ^a	GLP Compliance	Testing Facility	Study Number
Single Dose Toxicity	Mouse CD1 (ICR)BR/VAF Plus®	Intravenous	NA	0, 0.01, 0.1, 1.0, 10.0	Yes	—	89-008-0320 (Table 2.6.7.3)
	Rat/Crl:CD® BR/VAF Plus®	Subcutaneous	NA	0, 0.1, 0.3, 1.0, 10.0	Yes	—	90-219-0320 (Table 2.6.7.3)
		Subcutaneous	NA	0, 0.1, 0.3, 1.0, 10.0	Yes	—	93-119-0320 (Table 2.6.7.5)
		Intravenous	NA	0, 0.03, 0.1, 0.3, 1.0	No	Genentech	88-149-0320-563 (Table 2.6.7.3)
		Intravenous	NA	0, 0.01, 0.1, 1.0, 10.0	Yes	—	89-009-0320 (Table 2.6.7.3)
		Intravenous	NA	0, 0.001, 0.1	Yes	—	89-339-0320 (Table 2.6.7.3)
	Rat/Sim (SD)FHR (Albino)	Intravenous	NA	0, 0.01, 0.1, 1.0, 10.0	No	Genentech	89-663-0320-563 (Table 2.6.7.3)
	Rabbit/NZW	Intravenous	NA	1.0	No	Genentech	89-166-0320-563 (Table 2.6.7.3)
	Dog/Beagle	Subcutaneous	NA	0, 0.1, 0.3, 1.0	Yes	—	90-221-0320 (Table 2.6.7.3)
		Intravenous	NA	5.0	No	—	89-099-0320 (Table 2.6.7.3)
		Intravenous	NA	0, 0.005, 0.05, 0.5, 2.5	Yes	—	89-010-0320 (Table 2.6.7.3)
	Monkey/Rhesus	Subcutaneous	NA	0.5	Yes	—	93-335-0320 (Table 2.6.7.5)

^a Unless otherwise specified. For Single Dose Toxicity and Repeat Dose Toxicity, the highest No-Observed-Adverse-Effect-Level (NOAEL) is underlined. NA = Not applicable

2.6.7.1 Toxicology Overview (Continued)

Test Article: Mecasermin Page 2 of 3

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day) ^a	GLP Compliance	Testing Facility	Study Number
Repeat Dose Toxicity	Rat CD® BR/VAF Plus®	Subcutaneous	1, 5 or 7 Days	0, 1.0, 2.0	Yes	Genentech	91-086-0320 (Table 2.6.7.6)
		Subcutaneous	4 Weeks	0, 0.2, 0.6, 2.0	Yes	—	90-230-0320 (Table 2.6.7.7.1)
		Subcutaneous	4 Weeks	0, 0.1, 0.3, 1.0	Yes	—	93-120-0320 (Table 2.6.7.7.2)
		Subcutaneous	26 Weeks	0, 0.25, 1.0, 4.0	Yes	—	96-267-0320 (Table 2.6.7.7.3)
		Intravenous	10 Days	0, 1.0	Yes	—	89-011-0320 (Table 2.6.7.6)
		Intravenous	4 Weeks	0, 0.05, 0.25, 1.0	Yes	—	89-013-0320 (Table 2.6.7.7.4)
	Rat CD® (SDR)SPF®	Intravenous	13 Weeks	0, 0.25, 1.0, 4.0	Yes	—	96-266-0320 (Table 2.6.7.7.5)
	Dog/Beagle	Subcutaneous	4 Weeks	0, 0.1, 0.3, 1.0	Yes	—	90-233-0320 (Table 2.6.7.7.6)
		Subcutaneous	26 Weeks	0, 0.15, 0.3, 0.6	Yes	—	96-268-0320 (Table 2.6.7.7.7)
		Intravenous	10 Days	0, 0.5	Yes	—	89-012-0320 (Table 2.6.7.6)
		Intravenous	4 Weeks	0, 0.025, 0.125, 0.5	Yes	—	89-014-0320 (Table 2.6.7.7.8)
		Intravenous	13 Weeks	0, 0.025, 0.125, 0.5	Yes	—	93-334-0320 (Table 2.6.7.7.9)
Genotoxicity (in vitro)	Chinese Hamster Lung Fibroblasts	In vitro	NA	3.8 – 487 µg/mL	Yes	—	96-334-0320 (Table 2.6.7.8)

^a Unless otherwise specified. For Single-Dose Toxicity and Repeat-Dose Toxicity, the highest No-Observed-Adverse-Effect-Level (NOAEL) is underlined.

2.6.7.1 Toxicology Overview (Continued)

Test Article: Mecasermin Page 3 of 3

Type of Study	Species and Strain	Method of Administration	Duration of Dosing	Doses (mg/kg/day)	GLP Compliance	Testing Facility	Number
Genotoxicity (in vivo)	Mouse (ICR)	Intravenous	Single Dose	0, 24.4, 48.7, 97.4	Yes	—	96-355-0320 (Table 2.6.7.9)
Carcinogenicity	Rat (CD# BR/VAF Plus#)	Subcutaneous	84-104 Weeks	0, 0.25, 1.0, 4.0, 10.0	Yes	—	96-177-0320 (Table 2.6.7.10)
Initiation-Promotion	Rat (CD# BR/VAF Plus#)	Subcutaneous	5 Months	0, 0.25, 1.0, 4.0, 10.0	No	Genantech	96-453-0320 ^a (Table 2.6.7.10.1)
Reproductive Toxicity	Rat (CD# SD#) SFF#	Intravenous	*	0, 0.25, 1.0, 4.0	Yes	—	96-269-0320 (Table 2.6.7.12.1)
		Intravenous	F: G7-G17 ^b	0, 1.0, 4.0, 16.0	Yes	—	96-270-0320 (Table 2.6.7.12.2)
	NZW Rabbits	Intravenous	F: G6-G18 ^b	0, 0.125, 0.5, 2.0	Yes	—	95-353-0320 (Table 2.6.7.12.3)
Local Tolerance	NZW Rabbits	Subcutaneous	NA	NA	No	Genantech	88-477-0320-565 (Table 2.6.7.16)
		Subcutaneous	Single Dose	~ 2.0	Yes	—	93-118-0320 (Table 2.6.7.16)
		Intravenous	1 or 3 Days	~ 0.5, 5 mg	Yes	—	89-016-0320 (Table 2.6.7.16)
Other: Hemolytic Potential	Dog and Human	In Vitro	NA	5 mg/mL	Yes	—	89-015-0320 (Table 2.6.7.17)

^a Males: 9 weeks prior to mating through copulation. Females: 2 weeks prior to mating through Gestation Day 7

^b G = Gestation Day

^c Study stopped early and only preliminary results available.

NA = Not applicable

OVERALL CONCLUSIONS AND RECOMMENDATIONS

Non-clinical studies in this application included pharmacology, ADME and toxicokinetics, and toxicology.

In the pharmacology studies, the primary pharmacodynamic endpoint, bone growth, and secondary pharmacodynamic endpoints including hypoglycemic effects, body weight gain, and effects on immune function were evaluated. In addition, drug interaction studies with mecasermin and rhGH, AZT, and dexamethasone were conducted during early development programs for various metabolic indications. The pharmacological activities of mecasermin observed in several laboratory species are consistent with the fact that the amino acid sequence of IGF-1 and IGF receptors are highly conserved among species. Mecasermin's effects on bone growth were demonstrated in growth-deficient animal models (hypophysectomized and dwarf dw/dw rats) with results similar to those seen in humans. Investigation of secondary pharmacological endpoints indicated that mecasermin treatment results in acute decreases in blood glucose, increased weight gain, and enhanced immune function. The relevance of the nonclinical pharmacology studies to the human therapeutic use of mecasermin is supported by the demonstration of dose-related pharmacological activity in all species tested.

The ADME/toxicokinetic studies were conducted in adult normal Sprague Dawley rats, New Zealand White rabbits (males), Beagle dogs (males and females), and rhesus monkeys (males). The SC and IV routes of administration were investigated. IGF-1 is highly (95% to 99%) bound to a family of IGF-1 binding proteins (IGFBPs) in both animals and humans. The alterations in IGFBP profiles may have significant effects on IGF-1's pharmacokinetics. Free IGF-1 is cleared more rapidly than IGF-1 bound to the endogenous binding proteins. In rat and dogs, the total IGF-1 generally increased proportionately with increasing dose, but the

percent that was free increased greater than proportionately from a low approximately 3% at 0.1mg/kg/day to a high of approximately 73% at 10.0mg/kg/day.

After SC doses as high as 10.0mg/kg/day, mecasermin was relatively quickly absorbed. The total and free IGF-1 T_{max} ranged from 15 to 238 minutes in rat, rabbit rhesus monkey, and dog. Mean C_{max} and AUC for total and free IGF-1 increased with increasing doses in rats and dogs. The SC bioavailability for total and free IGF-1 was 38 to 57% in rats and rabbits. Mean total and free IGF-1 C_{max} and AUC values were unchanged or higher at steady state on multiple dosing than after single doses in rats and dogs. Similar predose and 24-hour total and free IGF-1 concentrations suggested that no drug accumulation was noted. Gender-related differences in mean total and free IGF-1 C_{max} and AUC values were observed in rats after SC administration with males have higher exposure, but not in dogs. The half life of free IGF-1 was shorter than the total IGF-1 T_{1/2}. The mean SC T_{1/2} for total IGF-1 was 41 to 604 minutes in rats and 198 to 558 minutes in dogs, and decreased with increasing dose, consistent with a higher percentage of free mecasermin with increasing dose. The mean SC T_{1/2} for free IGF-1 was 23 to 140 minutes in the rat and 48 to 78 minutes in the dog, and did not change with increasing dose. The IV T_{1/2} values in rats were 43 to 96 minutes and 20 minutes for total IGF-1, and free IGF-1, respectively. The volume of distribution of IGF-1 was relatively small in rats after IV administration, likely due to its high protein bindings. The total IGF-1 V_d was 19 to 165ml/kg and the V_{ss} was 50 to 825ml/kg. The free IGF-1 V_d was 149ml/kg and the V_{ss} was 203ml/kg. Studies of the induction or inhibition of liver enzymes involved in the metabolism of conventional drugs were not conducted for mecasermin. The excretion of IGF-1 in urine, bile, feces, or milk after mecasermin administration was not performed. Based on the current data, the ADME/toxicokinetics program conducted for mecasermin provides adequate support for the approval of this application.

In toxicology studies with single doses, there were no mice deaths at doses up to 10mg/kg i.v. No rats died at doses up to 1mg/kg by either iv or sc administration. At dose of 10mg/kg, iv or sc, rats died due to hypoglycemia. There were no rabbit deaths with doses up to 1mg/kg, iv. In the dog study, animal died at 5mg/kg, sc due to hypoglycemia. In monkey study after sc administration, 0.5mg/kg was well tolerated.

In the multiple dose chronic toxicological studies, the majority of effects noted were consistent with the expected physiological or exaggerated pharmacological effect which is hypoglycemia. Other significant adverse side effects included increases in body weight/body weight gain and/or food consumption, mild changes of clinical chemistry and hematological parameters, and increases in some organ weights including liver, kidney, heart, thymus, and/or spleen that correlated with the body weight changes secondary to the anabolic activities of IGF-1. Hematology changes seen in one or both species consisted of higher hemoglobin and hematocrit, lower white blood cell and absolute lymphocyte counts, and/or lower eosinophil counts. Clinical chemistry changes consisted of transient alterations in serum electrolytes (sodium, chloride, potassium, phosphorus, and/or calcium), protein, albumin, and/or triglycerides. In most instances, the clinical pathology changes noted in the repeat dose studies were reversible. There are few histopathological changes associated with increases in organ weight and the organ weight increases were reversible. Histological changes identified in the 26-week study with SC administration of mecasermin in the rat were confined to an increase

in thymic cortical area at the end of the treatment period following sc doses of 4.0mg/kg. This increase is correlated with the increase in thymic weight and cellularity. In dogs in the 26-week study, histological changes consisted of adrenal hyperplasia with fibrosis which was noted at sc doses at 0.3mg/kg/day and above. Adrenal medullary fibrosis but not hyperplasia was still present in dogs treated with 0.6mg/kg/day, 5 weeks following the cessation of treatment. In the 13-week iv repeated dose toxicology studies, no drug-related organ weights and histopathological findings were noted in rats. In dog, there was a significant shortening of the APTT in males at dose of 0.5mg/kg/day and a significant lengthening of the prothrombin time in females at dose of 0.5mg/kg. Histopathological findings at the end of treatment period included cerebrum, cerebellum, medulla oblongata, heart, lungs, stomach, cecum, and liver in animals at dose of 0.5mg/kg/day. Most of these findings are related to slight to moderate hemorrhage and/or slight lymphocyte infiltration. The NOAELs in the 26-week sc repeated dose toxicology studies was 4mg/kg in rats and 0.15mg/kg in dogs. The systemic exposure was 15X and 3X for the MRHD in rats and dogs, respectively. In the 13-week iv repeated dose toxicology studies, the NOAEL was 4mg/kg in rats and 0.125mg/kg in dogs.

Mecasermin was tested negative in both in the in vitro chromosome aberration assay in either direct method or metabolic method with or without S9 mixture and in vivo micronucleus assay in mouse bone marrow cells. In two year carcinogenicity study of rhIGF-1 with rats, rhIGF-1 induced pheochromocytoma in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg, and keratoacanthoma in males at doses of 4 and 10mg/kg. The NOEL for the carcinogenicity of rhIGF-1 was 1mg/kg/day in males and 4mg/kg for females. Doses of 4 and 10mg/kg/day exceeded the MTD based on mortality. Therefore, high doses were evaluated and no drug-related tumors were observed at tolerated doses.

In the fertility study, mecasermin caused a dose-dependent increase of body weight, body weight gain, and food consumption in both male and female rats at doses of 1 and 4mg/kg/day. There were no significant difference between control and treatment groups in estrus count and estrous cycle during pre-mating administration period, no difference in copulation index, indices of insemination and fertility. For the embryos and fetuses, a dose-dependent increase of the index of dead/resorbed fetuses was observed. There were no significant differences between control and treatment groups in the number of corpora lutea, the number of implantations, and implantation index and the number of live fetuses. In the embryo fetal development study in rat, a transient decrease in spontaneous movement at dose of 16mg/kg during the administration period was observed in dams. No drug-related changes of body weights, food consumption, or gross pathology were noted. There were no drug-related changes of body weights, food consumption, or gross pathology. In F1 fetuses, there were no drug-related changes of sex ratio, body weight, rate of incidence of pups exhibiting external, visceral or skeletal abnormalities or skeletal variations. No drug-related effects on growth and development of pups were noted. In liveborn F1 pups, the differentiation index for opening of the eyelid on day 14 after birth was significantly low at dose of 16mg/kg (47.2% vs 71.3% in control). However, this differentiation index on day 17 after birth was 100%. There were no other drug-related changes of body weight after birth, viability, external differentiation, reflexes, behavioral tests and reproductive performance tests. In the study in rabbits, no drug-related animal death or drug-related findings of clinical signs, body weight change or food consumption were noted. However, the food consumption of animals in which

a decrease in the amount of feces was noted in the general condition was remarkably lower than the other animals. There were many early resorbed embryos and the index of dead embryos/fetuses showed significantly high values in dams at dose of 2.0mg/kg. There were no significant differences between the control group and each test article treatment group in the number of corpora lutea, number of implantations, number of live fetuses and placenta weights. There were no drug-related findings of sex ratio, body weight, external, visceral or skeletal examinations of live fetuses. The NOAEL for fertility was 4mg/kg/day. The systemic exposure was 5 times the MRHD based on BSA (TK study was not performed in these reproductive toxicology studies). Increases in early fetal resorptions were observed with all dose levels. No embryo fetal developmental abnormalities were observed in rats with doses up to 16 mg/kg/day which is 20 times the MRHD based on BSA. In rabbit study, the NOAEL for dam reproductive ability was 2.0mg/kg and the NOAEL for fetuses was 0.5mg/kg, and there was no teratogenicity of MKC-031 at doses up to 2.0mg/kg.

Subcutaneous injection of acetate-containing buffers resulted in mild to moderately severe inflammatory changes in the muscular and hypodermal layers in the rabbit. The acetate plus mannitol preparation induced the mildest reaction. Subcutaneous injections of citrate buffers did not induce similar inflammatory changes. In the acute subcutaneous tolerance study, subcutaneous administration of 0.5ml of IGF-1 solution at concentration of 10mg/ml produced no local irritation in rabbits. In the intravenous injection study, administration of IGF-1 solution intravenously via the marginal ear vein for 1 or 5 days in a volume of 0.1 or 1ml at the concentration of 5mg/ml did not result in irritation that could be directly associated with the test material when compared with results of the effects of saline injection. In addition, mecasermin in a vehicle solution at a concentration of 5mg/ml does not cause hemolysis of human or canine erythrocytes and is compatible with human and canine serum and plasma.

Unresolved toxicology issues (if any): No outstanding or unresolved pharmacology or toxicology issues.

Recommendations: Approval

Suggested labeling:

Mutagenesis: INCRELEX™ was not clastogenic in the in vitro chromosome aberration assay and the in vivo mouse micronucleus assay.

Impairment of fertility: INCRELEX™ was administered intravenously to rats at doses of 0.25, 1 and 4mg/day to conduct the fertility study. ☐

J

Pregnancy Category C: Embryo-fetal toxicity studies were conducted in SD rats with doses of 1, 4, and 16mg/kg/day, and in New Zealand White rabbits with doses of 0.125, 0.5 and 2.0mg/kg/day administered intravenously. No embryo-fetal developmental abnormalities were observed in rats with doses up to 16 mg/kg/day (20 times the MRHD based on body surface area [BSA] comparison). In the rabbit study, the NOAEL for maternal toxicity was 2.0mg/kg (8 times the MRHD based on BSA) and the NOAEL for fetal toxicity was 0.5mg/kg (2 times the MRHD based on BSA). INCRELEX™ displayed no teratogenicity at doses up to 2.0mg/kg (8 times the MRHD based on BSA).

The effects of INCRELEX™ on an unborn child have not been ☐ studied. Therefore, there is insufficient medical information to determine whether there are significant risks to a fetus. ☐

Nursing Mothers: There have been no studies conducted with INCRELEX™ in nursing mothers. INCRELEX™ should not be administered to a nursing woman.

Signatures (optional):

Reviewer Signature _____

Supervisor Signature _____, Concurrence Yes ____ No ____

Appendix:

I. Dose-ranging study report: Dose selection was based on the hypoglycemia response of IGF-1. In the previous studies, a single dose of 10mg/kg administered subcutaneously to fasting rats resulted in 50% mortality. In non-fasted rats, doses of up to 64mg/kg are not lethal but do result in severe prolonged hypoglycemia. Since rats age, there will be periodic episodes in which the rats will not eat sufficiently overnight, 10mg/kg was chosen as the high dose to avoid an unacceptable deaths during the latter half of this 2-year study. Based on the 26 weeks general toxicity study, 4mg/kg was chosen as the middle dose in which it induced limited toxicity including increase in body weight, food consumption and organ weights, and the thymic pathology. The low dose of 0.25mg/kg was chosen in an attempt to obtain a no-effect level.

II. ECAC report:

Date of Meeting: July 5, 2005

Committee: David Jacobson-Kram, Ph.D., HFD-024, Chair
Joseph Contrera, Ph.D., HFD-901, Member
Abby Jacobs, Ph.D., HFD-024, Member
Terry Peters, D.V.M., HFD-520, Alternate Member
Jeri El-Hage, Ph.D., HFD-510, Pharmacology Supervisor
Shen Xiao, Ph.D., Presenting Reviewer

Author of Draft: Shen Xiao, Ph.D.

The following information reflects a brief summary of the Committee discussion and its recommendations. Detailed study information can be found in the individual review.

NDA#: 21-839

Drug Name: INCRELEX (mecasermin)

Sponsor: Tercica Medica Inc

Background:

Human insulin like growth factor-1 (IGF-1) is a 7.649 kDa non-glycosylated protein of 70 amino acids with three intra-molecular disulfide bridges and is structurally homologous to proinsulin. Recombinant human (rh) IGF-1 or mecasermin is produced by recombinant DNA technology in *E. coli* and is identical in structure to endogenous human IGF-1. The actions of IGF-1 as a "somatomedin" are obligate for growth hormone (GH) to be able to stimulate bone and body growth. rhIGF-1 is indicated for the long term treatment of growth failure in children with primary IGF-1 deficiency (Primary IGFD). Mecasermin tested negative for genotoxic potential in both the in vitro chromosome aberration assay in the CHL/IU fibroblast cell line and in the in vivo mouse micronucleus assay (Ames test was not performed).

In the two year carcinogenicity study of rhIGF-1 in SD rats, the drug was administered subcutaneously at doses of 0.25, 1, 4, and 10mg/kg/day. These doses are 0.3, 1.3, 3.6, and 7.4 times the human maximum recommended dose (0.12mg/kg twice daily), based on the total IGF-1 AUC (not the free form of IGF-1). Because of low survival, the high dose groups of males and females were terminated during Week 84 and the remaining male groups during Week 99. The remaining

female groups were terminated during Week 105. In addition, because of low survival, dose administration was discontinued for approximately 4 weeks prior to termination for males at 1.0mg/kg/day and for approximately 7 and 13 weeks for males and females at 4mg/kg/day, respectively.

Rat Carcinogenicity Study:

In the 104-week subcutaneous carcinogenicity study with rhIGF-1 in rats, rhIGF-1 induced adrenal pheochromocytomas in males at doses of 1.0mg/kg/day and higher, and in females at all dose levels. Statistically significant increased incidences of keratoacanthomas in the skin of males at doses of 4 and 10mg/kg/day, and of mammary gland carcinoma in males at 10mg/kg/day were also observed. Only the incidences of pheochromocytomas in males at doses of 4 and 10mg/kg and in females at dose of 10mg/kg and keratoacanthomas in males at doses of 4 and 10mg/kg exceeded the highest incidences seen in the Charles River historical database. The doses of 4 and 10mg/kg/day exceeded the MTD, based on dose-related increased in mortality (secondary to hypoglycemia)

Executive CAC Recommendations and Conclusions:

The Committee concluded that the rat carcinogenicity study was adequate.

The Committee concurred that the pheochromocytomas in both males and females, keratoacanthomas in the skin of males, and mammary gland carcinoma in males were drug related. The Committee noted that these tumor findings were only observed at doses that exceeded the MTD, based on excess mortality secondary to IGF-1 induced hypoglycemia.

David Jacobson-Kram, Ph.D.
Chair, Executive CAC

cc:\n
NDA# 21-839/Division File, HFD 510
Jeri El-Hage/Team leader, HFD-510
Shen Xiao/Reviewer, HFD-510
Enid Galliers/CSO/PM, HFD-510
ASeifried, HFD-024

III. Alternative study protocols and CAC report: NA**IV. Tables for microscopic findings in carcinogenicity study:**

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

[] 6281-377
Genentech 96-177-0329

PAGE: 1

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
H1GF-1 IN RATS

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED				
SEX: ALL; GROUP: ALL; WEEKS: ALL DEATH: ALL; FIND: ALL; SUBSET: ALL		SEX: MALE				
GROUP: -1- -2- -3- -4- -5-						
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75
** TOP OF LIST **						
BONE, FEMUR (FB)		NUMBER EXAMINED:	75	73	75	74
		NOT REMARKABLE:	73	71	69	74
--OSTEODYSTROPHY, FIBROUS			1	2	5	0
--HYPEROSTOSIS			1	1	1	0
BONE, HUMER (FH)		NUMBER EXAMINED:	75	72	75	74
		NOT REMARKABLE:	55	48	61	57
--HYPERCELLULARITY, NARROW			19	22	11	14
--INFARCT			0	0	2	0
--INFLAMMATION, LYMPHOHISTIOCYTIC			0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	4	2	0
BONE, STERNUM (SB)		NUMBER EXAMINED:	75	74	75	75
		NOT REMARKABLE:	73	72	70	75
--OSTEODYSTROPHY, FIBROUS			1	1	4	0
--HYPEROSTOSIS			1	1	1	0
BONE, STERNUM (SE)		NUMBER EXAMINED:	75	74	75	75
		NOT REMARKABLE:	62	41	59	52
--HYPERCELLULARITY, NARROW			22	27	14	20
--INFARCT			0	0	1	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	4	2	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

[] 6281-377
Genentech 96-177-0329

PAGE: 2

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
H1GF-1 IN RATS

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED				
SEX: ALL; GROUP: ALL; WEEKS: ALL DEATH: ALL; FIND: ALL; SUBSET: ALL		SEX: MALE				
GROUP: -1- -2- -3- -4- -5-						
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75
EYE (EY)		NUMBER EXAMINED:	75	75	75	74
		NOT REMARKABLE:	58	60	57	53
--INFLAMMATION, CHRONIC-ACTIVE, CORNEAL			19	9	9	10
--INFLAMMATION, SUPPURATIVE, UVEAL			2	1	1	0
--INFLAMMATION, CHRONIC, RETINAL			0	1	0	0
--MINERALIZATION, CORNEAL			0	1	2	2
--OSSIFICATION, SCLERAL			0	0	1	1
--ATROPHY, RETINAL, UNILATERAL			1	0	0	0
--CATARACT			4	2	4	4
--CONGESTION, IRIS			2	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	2	0
BRAIN (BR)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	43	47	41	49
--COMPRESSION, VENTRAL			27	24	25	18
--DILATATION, VENTRICULAR			17	13	26	4
--NECROSIS, WHITE MATTER			1	0	2	0
--GLIOSIS, WHITE MATTER			0	0	2	0
--NECROSIS, HIPPOCAMPUS			0	0	0	0
--NECROSIS, GRAY MATTER			0	0	2	1
--GLIOSIS, GRAY MATTER			0	0	1	1
--HEMORRHAGE			0	1	0	0
--MINERALIZATION			2	0	0	0
--E-GRANULAR CELL TUMOR			2	1	2	1
--M-MALIGNANT GRANULAR CELL TUMOR			2	0	0	0
--M-OLIGODENDROGLIOMA			1	0	0	0
--CONTINUED ON NEXT PAGE --			0	1	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-377-0320

106-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
XHOFF-I IN RATS

PAGE: 3

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	75	75	75	75
** FROM PREVIOUS PAGE **			75	75	75	75
BRAIN (BR)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	43	47	41	52
--M-ASTROCYTOMA			0	0	2	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	0	1	0
SPINAL CORD (SC)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	74	72	75	74
--GLIOSIS, WHITE MATTER			1	0	0	0
--HEMORRHAGE			0	1	0	0
--M-ASTROCYTOMA			0	2	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	0	1
KIDNEY (KN)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	1	2	1	2
--NEPHROPATHY, CHRONIC PROGRESSIVE			71	72	72	69
--DILATATION, PELVIC			8	11	10	8
--MINERALIZATION, TUBULAR			5	1	10	0
--MINERALIZATION, PELVIC			7	2	3	1
--PYELONEPHRITIS			4	2	8	5
--NECROSIS, PAPILLARY			1	0	1	0
--NECROSIS, TUBULAR			2	0	0	0
--CONGESTION			0	0	1	1
--CYST			14	10	20	13
--ECTOPIC ADRENAL CORTEX			0	0	1	0
--ECTASIA, TUBULAR			0	1	0	0
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-377-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
XHOFF-I IN RATS

PAGE: 4

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-
		NUMBER:	75	75	75	75
** FROM PREVIOUS PAGE **			75	75	75	75
KIDNEY (KN)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	1	2	1	2
--INFARCT			1	2	3	1
--HYALINE DEPLET ACCUMULATION			1	1	0	0
--HYPERPLASIA, TUBULAR EPITHELIUM			0	0	1	0
--HYPERPLASIA, TRANSITIONAL CELL			7	5	6	6
--B-PAPILLOMA, TRANSITIONAL CELL			1	0	0	0
--B-ADENOMA, TUBULAR CELL			0	0	1	0
--B-CARCINOMA, TUBULAR CELL			0	0	1	0
--M-LIPUSARCOMA			2	0	0	0
--M-OSTEOSARCOMA			0	2	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	2	4
--THROMBUS			1	1	4	0
LIVER (LI)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	8	6	6	10
--INFLAMMATION, CHRONIC			21	19	19	17
--INFLAMMATION, SUPPURATIVE			0	0	1	0
--HEMATOPOIETIC, EXTRAMEDULLARY			1	1	6	2
--NECROSIS, COAGULATIVE, RANDOM			7	5	9	4
--NECROSIS, HEPATOCELLULAR, CENTRILOBULAR			2	1	1	1
--INFARCT			1	0	0	0
--THROMBUS			1	0	0	0
--FIBROSIS, CAPSULAR			1	0	0	0
--PIGMENT, SUPPUR CELL			1	1	4	1
--VACUOLATION, HEPATOCELLULAR			0	1	1	0
** CONTINUED ON NEXT PAGE **			20	26	29	18

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0120

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KH18F-1 IN RATS

PAGE: 5

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -2- -3- -4- -5-		NUMBER: 75 75 75 75		75 75		75 75		75 75	
** FROM PREVIOUS PAGE **		NUMBER EXAMINED:		75 75		75 75		75 75		75 75	
LIVER (LI)		NOT REMARKABLE:		5 6		8 10		12			
--CYST		1		0		0		0		0	
--DEGENERATION, CYSTIC		13		11		11		1		2	
--DILATATION, SINUSOIDAL		17		13		13		7		1	
--CONGESTION		12		8		10		4		7	
--HYPERPLASIA, BILE DUCT		40		35		37		41		45	
--FOCUS, CLEAR CELL		0		0		1		1		1	
--FOCUS, BASOPHILIC CELL		12		8		4		7		3	
--FOCUS, EOSINOPHILIC CELL		1		2		1		0		0	
--FOCUS, MIXED CELL		1		2		1		0		0	
--ABNORMAL, LOOSE DEVELOPMENT		1		0		0		0		0	
--B-ADENOMA, HEPATOCELLULAR		1		0		0		1		1	
--M-CARCINOMA, HEPATOCELLULAR		2		0		1		0		0	
--I-MESOTHELIOMA		0		1		0		0		0	
--N-SCHWANNOMA		0		1		0		0		0	
--N-CARCINOMA		0		0		1		0		0	
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1		4		2		4		0	
TRACHEA (TR)		NUMBER EXAMINED:		75 75		75 75		75 75		75 75	
		NOT REMARKABLE:		75 74		73 74		75 75			
--INFLAMMATION, SUPPURATIVE		0		0		1		0		0	
--I-CARCINOMA, C-CELL		0		1		0		0		0	
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0		0		1		1		0	

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0120

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KH18F-1 IN RATS

PAGE: 6

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-		NUMBER: 75 75 75 75 75		75 75		75 75		75 75	
		NUMBER EXAMINED:		75 75		75 75		75 75		75 75	
		NOT REMARKABLE:		74 75		75 74		74 74			
ESOPHAGUS (ES)		NUMBER EXAMINED:		75 75		75 75		75 74		75 74	
		NOT REMARKABLE:		74 75		75 74		74 74			
--DILATATION		1		0		0		0		0	
--LUMEN, SUPPURATIVE EXUDATE		0		0		0		1		0	
THYROID (TY)		NUMBER EXAMINED:		75 75		75 75		75 75		75 75	
		NOT REMARKABLE:		28 17		23 29		17			
--INFLAMMATION, VASCULAR		1		0		1		0		0	
--CYST, ULTIMOBRANCHIAL		31		42		14		33		10	
--CYST, FOLLICULAR		4		5		6		8		5	
--CONGESTION		0		0		1		1		0	
--HYPERPLASIA, C-CELL		9		10		5		4		1	
--HYPERPLASIA, FOLLICULAR CELL		1		2		1		7		2	
--B-ADENOMA, C-CELL		18		11		15		6		0	
--N-CARCINOMA, C-CELL		0		3		0		1		0	
--N-ADENOMA, FOLLICULAR CELL		1		3		0		1		0	
--N-CARCINOMA, FOLLICULAR CELL		0		0		1		0		2	
--X-HEMATOPOIETIC NEOPLASIA (SEE 'HEMATO NEOPLASIA' FOR TYPE)		0		0		2		1		0	
PARATHYROID (PT)		NUMBER EXAMINED:		65 72		73 71		74		74	
		NOT REMARKABLE:		31 41		29 33		25		25	
--INFARCT		0		0		1		0		0	
--HYPERPLASIA		0		5		0		1		1	
--I-CARCINOMA		0		1		0		0		0	
--X-HEMATOPOIETIC NEOPLASIA (SEE 'HEMATO NEOPLASIA' FOR TYPE)		0		1		0		0		0	
** CONTINUED ON NEXT PAGE **											

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-I IN RATS

PAGE: 7

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX:	MALE				
		GROUP:	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75	75
** FROM PREVIOUS PAGE **							
PARATHYROID (PT)		NUMBER EXAMINED:	69	74	73	71	74
		NOT REMARKABLE:	31	43	43	33	36
--ONE EXAMINED			35	24	42	35	36
LACRIMAL GL. EX (EO)		NUMBER EXAMINED:	74	75	75	75	75
		NOT REMARKABLE:	11	6	7	7	11
--INFILTRATE, LYMPHOCYTIC			43	50	49	49	42
--ABSCESSES			0	0	0	1	0
--CONGESTION			0	0	0	2	1
--HARDERIAN GLAND ALTERATION			56	66	63	57	62
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	2	2	0
LUNG (LP)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	44	29	42	35	38
--INFILTRATE, MACROPHAGE, ALVEOLAR			22	29	20	17	18
--INFLAMMATION, INTERSTITIAL			2	1	0	0	2
--INFLAMMATION, SUPPURATIVE			0	1	0	1	1
--PERIVASCULAR INFILTRATE, LYMPHOCYTIC			0	6	10	9	9
--PERIVASCULAR INFILTRATE, EOSINOPHIL			0	0	0	1	0
--INFLAMMATION, VASCULAR			0	0	0	0	1
--FIBROSIS, PLEURAL/SUBPLEURAL			0	0	0	0	1
--FIBROSIS, BRONCHIOAL			0	2	1	0	0
--CONGESTION			1	0	1	1	0
--HEMORRHAGE			2	5	3	5	10
--THROMBUS			0	1	0	0	3
** CONTINUED ON NEXT PAGE **			1	1	0	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-I IN RATS

PAGE: 8

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX:	MALE				
		GROUP:	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75	75
** FROM PREVIOUS PAGE **							
LUNG (LU)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	44	29	42	39	38
--PIGMENT			0	1	0	0	2
--METAPLASIA, OSSIOUS			0	2	0	1	1
--MINERALIZATION, SEPTAL			1	1	0	0	0
--HYPERTROPHY, MUSCLE, ARTERIOLE			3	1	3	4	7
--HYPERPLASIA, TYPE II PNEUMOCYTE			1	0	1	0	0
--B-ADENOMA, BRONCHIOAL-ALVEOLAR			0	1	0	0	0
--M-CARCINOMA, BRONCHIOAL-ALVEOLAR			0	0	1	0	0
--M-FIBROSARCOMA			0	0	1	0	0
--M-PNEUROMYOCYTOMA			0	1	0	0	0
--M-CARCINOMA, C-CELL			0	0	0	2	0
--M-CARCINOMA			0	1	0	0	0
--M-MESOTHELIOMA			0	1	0	0	0
--M-MALIGNANT PARAGANGLIOMA			0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	1	2	4	4
THYROID (TH)		NUMBER EXAMINED:	69	68	70	74	71
		NOT REMARKABLE:	59	58	55	60	59
--INFLAMMATION, VASCULAR			0	1	0	1	0
--CONGESTION			1	1	1	4	8
--ECTOPIC PARATHYROID			0	0	1	0	0
--HYPERPLASIA, EPITHELIAL			0	5	10	5	4
--HYPERPLASIA, LYMPHOCYTIC, MEDULLA			2	0	0	0	0
--M-CARCINOMA			0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			2	2	2	4	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 95-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ENIG-1 IN RATS

PAGE: 2

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ---MALE---					
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-
		NUMBER:	75	75	75	75	75
SPLEEN (SP)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	35	34	44	42	48
--PIGMENT, INCREASED			18	13	16	23	23
--HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED			17	24	15	7	4
--HEMATOPOIESIS, EXTRAMEDULLARY, DECREASED			0	1	0	1	3
--CONGESTION			0	0	0	0	1
--CYST, SEROUSAL			3	4	1	1	0
--FIBROSIS, CAPSULAR			1	0	0	1	1
--HYPERPLASIA, LYMPHOCTIC			1	0	1	0	1
--HYPERPLASIA, STROMAL CELL			0	0	0	1	0
--H-HEMANGIOSARCOMA			1	0	0	0	1
--M-SARCOMA, UNDIFFERENTIATED			0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	2	4	0
--INFARCT			0	2	0	0	0
LN, MESENTERIC (MS)		NUMBER EXAMINED:	74	74	74	75	75
		NOT REMARKABLE:	67	68	51	70	70
--FIBROSIS			0	0	0	1	0
--CONGESTION			1	2	2	1	2
--THROMBUS			0	0	1	0	0
--DILATATION, SINUSOIDAL			1	0	1	1	4
--PIGMENT, INCREASED			1	0	2	1	2
--HEMORRHAGE			3	1	0	1	1
--HYPERPLASIA, LYMPHOCTIC			1	0	3	0	2
--H-HEMANGIOSARCOMA			0	1	0	0	0
--H-CARCINOMA			0	0	1	0	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 95-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ENIG-1 IN RATS

PAGE: 10

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ---MALE---					
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-
		NUMBER:	75	75	75	75	75
** FROM PREVIOUS PAGE **							
LN, MESENTERIC (MS)		NUMBER EXAMINED:	74	74	74	75	75
		NOT REMARKABLE:	67	68	61	70	70
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	0	3	2	0
ADRENAL, CORTEX (AC)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	41	49	50	55	61
--DEGENERATION, CYSTIC, CORTICAL			16	10	11	4	5
--NECROSIS, CORTICAL			0	0	1	1	0
--ATROPHY, CORTICAL, UNILATERAL			2	0	0	0	1
--HEMATOPOIESIS, EXTRAMEDULLARY			1	2	0	3	1
--CONGESTION			0	0	1	1	0
--HEMORRHAGE			0	0	1	0	0
--HYPERPLASIA, CORTICAL			13	9	10	8	4
--HYPERPLASIA, CORTICAL			7	4	1	4	3
--H-ADENOMA			1	1	0	1	2
--H-CARCINOMA			1	0	0	0	0
--H-CARCINOMA			0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	1	2	0
--ONE EXAMINED			0	0	1	0	0
ADRENAL, MEDULLA (MA)		NUMBER EXAMINED:	75	75	75	74	75
		NOT REMARKABLE:	52	56	42	51	43
--HYPERPLASIA, MEDULLARY			5	0	10	13	15
--D-PRECHROMOCYTOMA			10	12	10	25	18
--H-MALIGNANT PRECHROMOCYTOMA			2	0	0	1	1

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-177
Genentech 96-177-0120

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ZINC-1 IN RATS

PAGE: 11

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75				
** FROM PREVIOUS PAGE **		75 75 75 75 75				
ADRENAL, MEDULLA (MA)		NUMBER EXAMINED: 75 75 75 74 75				
		NOT REMARKABLE: 52 56 52 31 43				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 0 1 0 0				
--ONE EXAMINED		5 0 3 2 2				
PITUITARY (PI)		NUMBER EXAMINED: 74 75 75 74 74				
		NOT REMARKABLE: 14 13 22 21 33				
--CYST		7 4 4 6 3				
--MINERALIZATION		5 0 1 0 0				
--CONGESTION		2 0 0 3 0				
--HEMORRHAGE		0 0 1 0 0				
--HYPERPLASIA		17 12 8 7 28				
--B-ADENOMA		41 48 41 40 33				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 1 2 2 0				
AORTA (AO)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 72 73 69 75 73				
--MINERALIZATION		3 2 6 0 2				
HEART (HT)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 8 7 4 6 6				
--CARDIOMYOPATHY, DEGENERATIVE		57 67 70 57 63				
--DILATATION		1 0 1 0 0				
--MINERALIZATION		0 1 3 2 0				
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-177
Genentech 96-177-0120

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ZINC-1 IN RATS

PAGE: 12

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75				
** FROM PREVIOUS PAGE **		75 75 75 75 75				
HEART (HT)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 8 7 4 6 6				
--METAPLASIA, OSSEOUS		0 0 1 0 0				
--HYPERPLASIA, MESOTHELIAL		0 0 1 0 1				
--B-ATRIOCAVAL MESOTHELIOMA		1 0 3 1 1				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 1 1 0				
--THROMBUS, ATRIAL		0 1 1 2 0				
TONGUE (TO)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 66 68 54 64 71				
--INFILTRATE, LYMPHOCYTIC		2 2 0 1 2				
--INFLAMMATION, VASCULAR		5 2 3 2 0				
--MINERALIZATION		3 3 4 0 0				
--DEGENERATION, MUSCLE		0 0 0 0 1				
--CYST, EPITHELIAL INCLUSION		0 0 1 0 0				
--B-LIPOMA		0 0 0 1 0				
--B-PAPILLOMA, SQUAMOUS CELL		0 0 0 1 0				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 1 2 0				
MUSCLE, SKELETAL (SM)		NUMBER EXAMINED: 75 73 75 74 73				
		NOT REMARKABLE: 54 67 66 70 73				
--INFILTRATE, LYMPHOPLASMATIC		2 1 3 0 2				
--DEGENERATION/NECROSIS		3 2 2 0 3				
--ATROPHY		0 0 0 1 0				
--M-RHABDOMYOSARCOMA		1 0 0 0 0				
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
TRIOF 1 IN RATS

PAGE: 13

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
NUMBER		75	75	75	75	75
** FROM PREVIOUS PAGE **		-	-	-	-	-
MUSCLE, SKELETAL (SM)	NUMBER EXAMINED:	75	73	75	74	73
	NOT REMARKABLE:	69	67	69	70	70
--M-FIBROSARCOMA		0	1	0	0	0
--N-FIBROSARCOMA		0	0	1	0	0
--I-MESOTHELIOMA		0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	1	3	0
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	75	73	75	74	73
	NOT REMARKABLE:	75	72	74	74	73
--DEGENERATION		0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	1	0	0
PANCREAS (PA)	NUMBER EXAMINED:	75	75	75	74	75
	NOT REMARKABLE:	40	47	54	51	61
--INFLAMMATION, VASCULAR		5	2	1	3	0
--FIBROSIS		0	0	1	1	1
--ATROPHY, ACINAR		20	16	12	19	7
--ECTASIA, DUCTAL		1	1	1	1	3
--FOCUS, BASOPHILIC		5	5	6	3	5
--HYPERPLASIA, ACINAR CELL		0	0	2	1	0
--HYPERPLASIA, ISLET CELL		0	2	0	0	0
--B-ADENOMA, ISLET CELL		5	2	0	1	0
--M-CARCINOMA, ISLET CELL		1	5	0	1	0
--B-ADENOMA, ACINAR CELL		1	0	0	0	0
--N-CARCINOMA		0	0	1	0	0
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
TRIOF 1 IN RATS

PAGE: 14

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
NUMBER		75	75	75	75	75
** FROM PREVIOUS PAGE **		-	-	-	-	-
PANCREAS (PA)	NUMBER EXAMINED:	75	75	75	74	75
	NOT REMARKABLE:	40	47	54	51	61
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	1	2	0	0
STOMACH, GL (ST)	NUMBER EXAMINED:	75	75	75	75	75
	NOT REMARKABLE:	62	59	50	44	47
--EROSION/ULCERATION		2	7	6	11	20
--INFLAMMATION, VASCULAR		1	0	2	2	0
--INFLAMMATION, ACUTE		0	4	2	0	0
--CONGESTION		2	2	5	19	6
--HEMORRHAGE		0	1	1	0	0
--EDEMA		3	2	0	0	0
--FIBROSIS		0	0	0	1	0
--DEGENERATION, MUSCULARIS		0	0	2	0	0
--MINERALIZATION		4	3	0	0	0
--HYPERPLASIA, EPITHELIAL, GLANDULAR		1	0	0	1	0
--N-CARCINOMA		0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	2	0	0
STOMACH, NONGL (SN)	NUMBER EXAMINED:	75	75	75	75	75
	NOT REMARKABLE:	66	66	69	70	73
--EROSION/ULCERATION		5	2	5	3	5
--INFLAMMATION, ACUTE		1	0	1	0	0
--INFLAMMATION, VASCULAR		1	0	0	0	1
--HEMORRHAGE		1	0	0	0	0
** CONTINUED ON NEXT PAGE **						

TABLE 21
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rhIGF-I IN RATS

PAGE: 15

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----					
		GROUP: -1- -2- -3- -4- -5-					
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75	75
** FROM PREVIOUS PAGE **			75	75	75	75	75
STOMACH, NOEGL (SU)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	65	68	65	70	73
--EDEMA			9	4	1	2	0
--MINERALIZATION			1	1	0	0	0
--CYST			1	0	0	0	0
--HYPERKERATOSIS, SQUAMOUS MUCOSA			0	0	0	0	1
--HYPERPLASIA, SQUAMOUS MUCOSA			1	2	1	2	3
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	1	0	0
DUODENUM (DU)		NUMBER EXAMINED:	74	73	73	73	72
		NOT REMARKABLE:	74	73	72	72	72
--ATROPHY, MUCOSAL			0	0	1	0	0
--ECTOPIC PANCREAS			0	0	0	1	0
JEJUNUM (JE)		NUMBER EXAMINED:	65	65	67	48	53
		NOT REMARKABLE:	58	60	66	48	52
--INFLAMMATION, CHRONIC-ACTIVE			1	0	0	0	0
--M-CARCINOMA			0	0	1	0	1
ILEUM (IL)		NUMBER EXAMINED:	71	72	67	53	61
		NOT REMARKABLE:	71	71	66	52	60
--PARASITISM			0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	1	1	1	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rhIGF-I IN RATS

PAGE: 16

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----					
		GROUP: -1- -2- -3- -4- -5-					
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75	75
			75	75	75	75	75
CECUM (CB)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	72	74	72	73	74
--INFLAMMATION, CHRONIC-ACTIVE			0	1	2	0	0
--INFLAMMATION, VASCULAR			2	0	1	2	0
--PARASITISM			0	0	0	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	0	0	1	0
COLON (CO)		NUMBER EXAMINED:	75	75	74	75	75
		NOT REMARKABLE:	69	73	72	69	74
--INFLAMMATION, CHRONIC-ACTIVE			1	0	0	0	0
--INFLAMMATION, VASCULAR			0	0	1	2	0
--PARASITISM			2	1	1	3	1
--M-CARCINOMA			0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	0	1	0
RECTUM (RE)		NUMBER EXAMINED:	74	75	75	74	74
		NOT REMARKABLE:	65	66	69	66	69
--INFLAMMATION, VASCULAR			3	3	1	1	0
--ATROPHY, MUCOSAL			0	0	0	1	0
--PARASITISM			6	6	6	6	5
SALIV GL. SUBMAX (SG)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	75	73	71	72	75
--INFLAMMATION, CHRONIC-ACTIVE			0	0	1	0	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ZNIQF-1 IN RATS

PAGE: 17

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION	GROUP:	-1- -2- -3- -4- -5-				
		NUMBER:	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75
SALIV GL; SUBMAX (SG)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	75	73	72	72
--ATROPHY, ACINAR			0	0	0	0
--INFARCT			0	0	1	0
--I-FIBROSARCOMA			0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	1	2	2
LN, SUBMAXILLARY (MN)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	13	11	13	10
--HYPERPLASIA, PLASMOCYTIC			54	59	52	54
--HYPERPLASIA, LYMPHOCTIC			7	3	7	4
--CONGESTION			9	10	8	6
--HEMORRHAGE			4	0	0	1
--DILATATION, SINUSOIDAL			0	3	1	3
--N-FIBROSARCOMA			1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	3	2	3
--ONE EXAMINED			2	4	3	4
INJECTION SITE (IS)		NUMBER EXAMINED:	74	75	75	75
		NOT REMARKABLE:	2	1	1	2
--FIBROSIS			72	70	72	66
--INFLAMMATION, CHRONIC			66	60	66	43
--INFLAMMATION, GRANULOMATOUS			16	10	5	10
--INFLAMMATION, ULCERATIVE			2	2	0	0
--INFLAMMATION, EXUDATIVE, EPIDERMAL			0	1	0	0
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ZNIQF-1 IN RATS

PAGE: 18

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION	GROUP:	-1-	-2-	-3-	-4-	-5-
		NUMBER:	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75
INJECTION SITE (IS)		NUMBER EXAMINED:	74	75	75	75
		NOT REMARKABLE:	2	1	1	0
--ABSCESSES			0	0	0	1
--CONGESTION			10	3	6	7
--HEMORRHAGE			43	38	31	29
--NECROSIS, SUBCUTANEOUS MUSCLE			8	1	5	4
--ATROPHY, SUBCUTANEOUS MUSCLE			25	21	15	13
--FOREIGN BODY			17	7	2	6
--ATROPHY, ADRENAL			0	0	0	0
--CYST, EPIDERMAL INCLUSION			0	0	1	0
--HYPERPLASIA, EPIDERMAL			3	1	2	0
--B-KERATOSACRITHOMA			0	0	0	2
--B-ADENOMA, BASAL CELL			0	0	0	0
--B-FIBROMA			1	0	0	0
--M-FIBROSARCOMA			1	0	1	2
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	2	0
SKIN (SK)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	61	51	50	45
--INFLAMMATION, ULCERATIVE			0	1	2	0
--INFLAMMATION, CHRONIC			0	2	1	0
--ABSCESSES			0	1	1	0
--EDEMA			1	0	0	0
--FIBROSIS			0	0	0	0
--ATROPHY, ADRENAL			0	1	2	1
--FOREIGN BODY			1	0	0	1
** CONTINUED ON NEXT PAGE **			0	0	1	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

5281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ENIGP-1 IN RATS

PAGE: 19

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ---MALE---									
		GROUP: -1- -2- -3- -4- -5-									
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75									
** FROM PREVIOUS PAGE **		75 75 75 75 75									
SKIN (SK)		NUMBER EXAMINED: 75 75 75 75 75									
		NOT REMARKABLE: 61 51 59 45 44									
--CYST, EPIDERMAL INCLUSION		1 1 0 4 6									
--HYPERKERATOSIS		0 0 0 0 2									
--HYPERPLASIA, EPIDERMAL		0 1 2 3 2									
--B-ADENOMA, BASAL CELL		0 0 1 2 0									
--B-PAPILLOMA, SQUAMOUS CELL		1 1 3 1 4									
--B-KERATOCARCINOMA		1 1 3 9 18									
--B-ADENOMA, SEBACEOUS GLAND		1 2 0 2 1									
--B-TRICHOEPITHELIOMA		0 0 1 0 0									
--M-CARCINOMA, BASAL CELL		0 0 0 0 1									
--M-CARCINOMA, SQUAMOUS CELL		0 0 0 1 0									
--B-LIPOMA		1 0 0 0 0									
--S-FIBROMA		0 4 3 4 2									
--M-FIBROSARCOMA		4 4 3 4 4									
--M-SCHWANNOMA		0 1 0 1 2									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 2 2 0									
URINARY BLADDER (UB)		NUMBER EXAMINED: 75 74 75 75 75									
		NOT REMARKABLE: 72 66 64 67 70									
--INFLAMMATION, SUPPURATIVE		0 3 0 3 1									
--INFLAMMATION, CHRONIC-ACTIVE		0 1 3 1 0									
--INFILTRATE, LYMPHOPLASMACYTIC		1 2 3 2 1									
--DILATATION		0 1 0 0 0									
--CONGESTION		0 2 1 3 4									
--HEMORRHAGE		0 1 2 0 1									
** CONTINUED ON NEXT PAGE **											

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

5281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ENIGP-1 IN RATS

PAGE: 20

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ---MALE---									
		GROUP: -1- -2- -3- -4- -5-									
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75									
** FROM PREVIOUS PAGE **		75 75 75 75 75									
URINARY BLADDER (UB)		NUMBER EXAMINED: 75 74 75 75 75									
		NOT REMARKABLE: 72 66 64 67 70									
--CALCULUS		1 1 0 1 0									
--EMBRYONAL DUCT REMNANT		1 0 0 0 0									
--METAPLASIA, SQUAMOUS		0 1 0 0 0									
--HYPERPLASIA, TRANSITIONAL CELL		1 3 8 8 2									
--B-PAPILLOMA, TRANSITIONAL CELL		0 0 1 0 0									
--M-LEIOMYOSARCOMA		1 0 0 0 0									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 0 0 0									
PROSTATE (PE)		NUMBER EXAMINED: 75 75 74 75 75									
		NOT REMARKABLE: 13 12 19 18 44									
--INFLAMMATION, SUPPURATIVE		53 42 41 24 79									
--INFILTRATE, LYMPHOPLASMACYTIC		34 26 29 23 11									
--INFLAMMATION, CHRONIC-ACTIVE		0 0 1 2 0									
--EDEMA		0 1 0 0 0									
--CONGESTION		0 0 0 0 1									
--HEMORRHAGE		0 1 1 0 1									
--SECRETION, INCREASED		0 0 1 0 0									
--SECRETION, DECREASED		1 2 0 1 0									
--METAPLASIA, MUCINOUS		1 0 1 0 0									
--B-ADENOMA		1 0 0 0 0									
--M-CARCINOMA		0 0 1 0 0									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 1 1 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0329

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 21

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75				
SEMINAL VESICLES (SV)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 56 58 48 57 58				
--INFLAMMATION, SUPPURATIVE		3	4	5	4	3
--INFILTRATE, LYMPHOPLASMATIC		0	1	1	1	2
--INFLAMMATION, CHRONIC-ACTIVE		0	0	1	0	1
--SECRETION, INCREASED		5	6	10	8	9
--SECRETION, DECREASED		12	7	11	4	1
--CONGESTION		0	0	0	0	1
--HEMORRHAGE		0	0	1	0	0
--M-CARCINOMA		0	0	0	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	1	0	0
--N-CARCINOMA		0	0	1	0	0
TESTIS (TE)		NUMBER EXAMINED: 75 75 75 74 75				
		NOT REMARKABLE: 43 50 39 41 54				
--ATROPHY/DEGENERATION, BILATERAL		10	6	11	5	6
--ATROPHY/DEGENERATION, UNILATERAL		2	4	5	7	1
--INFLAMMATION, VASCULAR		15	10	22	11	4
--INFLAMMATION, SUPPURATIVE		1	0	0	0	0
--MINERALIZATION		25	10	18	11	6
--HYPERPLASIA, SERTOLI CELL		0	0	0	1	0
--HYPERPLASIA, INTERSTITIAL CELL (LEYDIG CELL)		1	3	2	1	5
--B-HEMANGIOMA		0	0	0	1	0
--B-INTERSTITIAL CELL TUMOR		3	2	4	5	2

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 22

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75				
EPIDIDYMIDES (EP)		NUMBER EXAMINED: 75 75 75 74 75				
		NOT REMARKABLE: 49 48 45 43 59				
--HYPOSPERMIA, BILATERAL		8	4	9	5	4
--HYPOSPERMIA, UNILATERAL		1	4	3	5	0
--INFILTRATE, LYMPHOPLASMATIC		16	20	18	13	10
--INFLAMMATION, VASCULAR		2	0	2	2	0
--INFLAMMATION, SUPPURATIVE		1	1	0	0	0
--INFLAMMATION, GRANULOMATOUS		0	1	0	0	0
--MINERALIZATION		1	0	0	0	0
--M-CARCINOMA		0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	2	2	0
--ONE EXAMINED		1	0	0	0	0
CAUSE OF DEATH (CD)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 0 0 0 0 0				
--SCHEDULED SACRIFICE		34	21	15	13	11
--UNDETERMINED		8	15	18	21	39
--PITUITARY ADENOMA		16	18	29	20	1
--MAMMARY GLAND-CARCINOMA		0	0	0	0	1
--KIDNEY NEPHROPATHY, CHRONIC, PROGRESSIVE		4	2	11	7	11
--KIDNEY-PYELONEPHRITIC		0	0	1	1	2
--BRAIN-OLIGODENDROGLIOMA		0	0	1	0	0
--BRAIN-ASTROCYTOMA		0	0	1	0	0
--BRAIN-GLIOMATOUS CELL TUMOR		1	0	0	0	0
--BRAIN-NECROSIS, HIPPOCAMPEUS		0	0	1	0	1
--BRAIN-NECROSIS, WHITE MATTER		1	0	2	9	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KH10F-1 IN RATS

PAGE: 23

TABLE INCLUDES:		--- NUMBER - OF - ANIMALS - AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
NUMBER:		75	75	75	75	75	75	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75	75	75	75	75	75
CAUSE OF DEATH (CD)		75	75	75	75	75	75	75	75	75	75
NUMBER EXAMINED:		75	75	75	75	75	75	75	75	75	75
NOT REMARKABLE:		0	0	0	0	0	0	0	0	0	0
--LEUKEMIA, LGL		0	0	0	1	0					
--LEUKEMIA, GRANULOCYTIC		0	0	1	1	0					
--LYMPHOMA		1	2	1	3	0					
--HISTIOCYTIC SARCOMA		0	1	0	0	0					
--MALIGNANT FIBROUS HISTIOCYTOMA		2	0	0	0	0					
--AUDITORY SSB GL-CARCINOMA		1	0	1	1	0					
--SKIN-SQUAMOUS CELL CARCINOMA		1	0	0	1	0					
--SKIN-FIBROSARCOMA		1	2	0	1	3					
--SKIN-SCHWANNOMA		8	1	0	3	1					
--SKIN-FIBROMA		0	1	0	0	0					
--ADRENAL-PHEOCHROMOCYTOMA		0	0	0	2	0					
--ADRENAL-CARCINOMA, CORTICAL		1	0	0	0	0					
--THYROID-CARCINOMA, C-CELL		0	1	0	0	0					
--SKELETAL MUSCLE-FIBROSARCOMA		0	1	0	0	0					
--SKELETAL MUSCLE-RHABDOMYOSARCOMA		1	0	0	0	0					
--LIVER-COAGULATIVE NECROSIS		1	0	0	0	0					
--ABDOMINAL CAVITY-SCHWANNOMA		0	0	0	0	1					
--MESOTHELIOMA		0	0	0	0	0					
--SPLEEN - SARCOMA		0	0	0	0	0					
--CARDIOMYOPATHY, DEGENERATIVE		1	0	0	0	1					
--PROSTATE-SUPPURATIVE INFLAMMATION		0	1	0	0	0					
--URINARY BLADDER-LEIOMYOSARCOMA		1	0	0	0	0					

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KH10F-1 IN RATS

PAGE: 24

TABLE INCLUDES:		--- NUMBER - OF - ANIMALS - AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									
		75 75 75 75 75									
HEMATIC NEOPLASIA (HM)		NUMBER EXAMINED: 75 75 75 75 75									
		NOT REMARKABLE: 71 66 72 69 75									
--M-LYMPHOMA		2 3 1 3 0									
--M-LEUKEMIA, LARGE GRANULAR LYMPHOCYTIC		0 0 0 1 0									
--M-LEUKEMIA, GRANULOCYTIC		0 0 1 1 0									
--M-HISTIOCYTIC SARCOMA		0 3 0 1 0									
--M-MALIGNANT FIBROUS HISTIOCYTOMA		2 1 1 0 0									
FOOT/FOOTPAD (FP)		NUMBER EXAMINED: 25 23 21 19 12									
		NOT REMARKABLE: 0 0 0 0 0									
--INFLAMMATION, ULCERATIVE		24 21 17 15 10									
--INFLAMMATION, CHRONIC ACTIVE		1 2 3 3 2									
--JOINT INFLAMMATION, CHRONIC ACTIVE		0 0 0 2 0									
--HYPERKERATOSIS		1 1 3 4 3									
--EXOSTOSIS		0 1 0 1 0									
TAIL (TI)		NUMBER EXAMINED: 12 2 0 3 7									
		NOT REMARKABLE: 1 0 0 0 0									
--INFLAMMATION, ULCERATIVE		5 0 1 1 2									
--CYST, EPIDERMAL INCLUSION		4 0 5 2 3									
--ABSCESS		3 2 3 0 0									
--HYPERKERATOSIS		4 1 3 2 2									
--INFLAMMATION, CHRONIC ACTIVE		1 0 0 1 1									
--HYPEROSTOSIS		3 0 0 1 0									
--HYPERPLASIA, EPIDERMAL		0 0 1 0 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 94-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGF-1 IN RATS

PAGE: 25

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX:	-----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-
		NUMBER:	75	75	75	75	75
MAMMARY, MALE (MP)		NUMBER EXAMINED:	19	24	16	16	15
		NOT REMARKABLE:	5	8	4	4	7
--GALACTOCELE			12	13	8	11	2
--ABSCESS			0	0	0	1	0
--METAPLASIA, SQUAMOUS			0	0	0	1	0
--HYPERPLASIA, GLANDULAR			3	1	0	1	2
--FIBROADENOMA			1	2	3	2	1
--M-CARCINOMA			0	1	0	0	4
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	1	1	1	0
JOINT, OTHER (JT)		NUMBER EXAMINED:	3	1	2	5	2
		NOT REMARKABLE:	0	0	0	0	0
--INFLAMMATION, CHRONIC-ACTIVE, SYNOVIAL			1	1	1	2	2
--EXOSTOSIS			2	1	2	2	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	0	1	0
BONE, OTHER (BO)		NUMBER EXAMINED:	0	0	1	0	0
		NOT REMARKABLE:	0	0	1	0	0
CALVARIUM/SKULL (CA)		NUMBER EXAMINED:	2	0	2	2	0
		NOT REMARKABLE:	1	0	0	0	0
--HYPEROSTOSIS			0	0	1	1	0
--B-OSTEOMA			1	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	0	0	1	0
** CONTINUED ON NEXT PAGE **							

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 94-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGF-1 IN RATS

PAGE: 26

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX:	-----MALE-----				
		GROUP:	-1-	-2-	-3-	-4-	-5-
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75	75
** FROM PREVIOUS PAGE **							
CALVARIUM/SKULL (CA)	NUMBER EXAMINED:	2	0	2	2	0	
	NOT REMARKABLE:	1	0	0	0	0	
--HEMORRHAGE, DURA		0	0	1	0	0	
CAVITY, ORAL (OC)	NUMBER EXAMINED:	1	1	0	0	0	
	NOT REMARKABLE:	0	0	0	0	0	
--CYST, EPITHELIAL, GINGIVA		0	1	0	0	0	
--X-FIBROSARCOMA		1	0	0	0	0	
DENTAL, INCISOR (DI)	NUMBER EXAMINED:	0	0	0	1	0	
	NOT REMARKABLE:	0	0	0	0	0	
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0	
ARTERY, OTHER (AO)	NUMBER EXAMINED:	4	1	4	2	0	
	NOT REMARKABLE:	0	0	0	0	0	
--INFLAMMATION, CHRONIC		2	1	2	2	0	
--CONGESTION		0	1	0	0	0	
--MINERALIZATION		3	0	2	0	0	
--THROMBUS		1	1	2	1	0	
AORTIC ARCH (AA)	NUMBER EXAMINED:	1	1	2	0	0	
	NOT REMARKABLE:	0	0	0	0	0	
--MINERALIZATION		1	1	2	0	0	

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 36-177-0120

PAGE: 27

164-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH PHGF-1 IN RATS					
--- NUMBER OF ANIMALS AFFECTED ---					
TABLE INCLUDES:					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL					
SEX: -----MALE-----					
GROUP: -1- -2- -3- -4- -5-					
NUMBER: 75 75 75 75 75					
ORGAN AND FINDING DESCRIPTION					
CAVITY, THORACIC (TA)					
NUMBER EXAMINED: 1 1 2 1 0					
NOT REMARKABLE: 0 0 0 0 0					
--HEMORRHAGE, MEDIASTINUM					
--M-MESOTHELIOMA					
--M-MALIGNANT PARANGLIOMA					
--B-CARCINOMA					
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)					
CAVITY, ABDOM (PC)					
NUMBER EXAMINED: 0 0 5 2 1					
NOT REMARKABLE: 0 1 0 0 0					
--NECROSIS, FAT					
--INFLAMMATION, CHRONIC ACTIVE					
--HEMORRHAGE, OMENTUM					
--EDEMA					
--EMBRYONAL DUCT REMNANT					
--M-SCHWANNOMA					
--M-HEMANGIOSARCOMA					
--M-MALIGNANT PARANGLIOMA					
--N-CARCINOMA					
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)					
ADIPOSE TISSUE (AT)					
NUMBER EXAMINED: 0 5 3 1 0					
NOT REMARKABLE: 0 5 3 1 0					

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 36-177-0320

PAGE: 28

164-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH PHGF-1 IN RATS					
--- NUMBER OF ANIMALS AFFECTED ---					
TABLE INCLUDES:					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL					
SEX: -----MALE-----					
GROUP: -1- -2- -3- -4- -5-					
NUMBER: 75 75 75 75 75					
ORGAN AND FINDING DESCRIPTION					
MESENTERY (MY)					
NUMBER EXAMINED: 1 0 1 0 0					
NOT REMARKABLE: 1 0 1 0 0					
AUDITORY SEE GL (AS)					
NUMBER EXAMINED: 1 0 1 1 0					
NOT REMARKABLE: 0 0 0 0 0					
--M-CARCINOMA					
COAGULATING GL (CG)					
NUMBER EXAMINED: 0 0 3 4 0					
NOT REMARKABLE: 0 0 0 0 0					
--INFLAMMATION, SUPPURATIVE					
--N-CARCINOMA					
PREPUTIAL GLAND (PG)					
NUMBER EXAMINED: 1 0 0 0 0					
NOT REMARKABLE: 0 0 0 0 0					
--INFLAMMATION, CHRONIC ACTIVE					
VAS DEFERENS (VD)					
NUMBER EXAMINED: 0 0 1 0 0					
NOT REMARKABLE: 0 0 0 0 0					
--X-HEMATOPOIETIC NEOPLASIA					
0 0 1 0 0					

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 29

TABLE INCLUDES:		-- NUMBER OF ANIMALS AFFECTED --									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----									
		GROUP: -1- -2- -3- -4- -5-									
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75									
-----		-----									
URETHRA (UR)		NUMBER EXAMINED:		0	1	0	1	1			
		NOT REMARKABLE:		0	0	0	0	0			
--PROTEINACEOUS MATERIAL				0	0	0	1	0			
--INFLAMMATION, SUPPURATIVE				0	1	0	0	1			
--HYPERPLASIA, TRANSITIONAL CELL				0	1	0	0	0			
--METAPLASIA, SQUAMOUS				0	1	0	0	0			
URETER (UE)		NUMBER EXAMINED:		1	0	2	1	2			
		NOT REMARKABLE:		0	0	0	0	0			
--INFLAMMATION, SUPPURATIVE				1	0	0	0	1			
--INFILTRATE, LYMPHOPLASMATIC				0	0	2	0	0			
--DILATATION				1	0	2	1	2			
--HYPERPLASIA, TRANSITIONAL CELL				1	0	2	0	0			
LN, INGUINAL (IN)		NUMBER EXAMINED:		3	2	1	2	2			
		NOT REMARKABLE:		1	1	0	0	2			
--HYPERPLASIA, PLASMATIC				2	1	0	1	1			
--CONGESTION				2	1	0	2	1			
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)				0	0	1	0	0			
LN, MEDIASTINAL (ML)		NUMBER EXAMINED:		6	7	8	8	7			
		NOT REMARKABLE:		1	0	0	0	0			
--HYPERPLASIA, PLASMATIC				1	1	0	2	1			
--HYPERPLASIA, LYMPHOCTIC				1	1	1	2	0			
** CONTINUED ON NEXT PAGE											

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 30

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----									
		GROUP: -1- -2- -3- -4- -5-									
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75									
-----		-----									
** FROM PREVIOUS PAGE **											
LN, MEDIASTINAL (ML)		NUMBER EXAMINED: 6 7 8 8 7									
		NOT REMARKABLE: 1 0 0 0 0									
--HEMORRHAGE		3 3 0 2 4									
--CONGESTION		0 1 2 1 2									
--DILATATION, SINUSOIDAL		3 1 2 1 1									
--PIGMENT, INCREASED		0 1 0 1 0									
--X-CARCINOMA		0 0 1 0 0									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 3 2 3 0									
LN, ANT MES/PANC (AP)		NUMBER EXAMINED: 3 4 4 1 3									
		NOT REMARKABLE: 1 0 1 0 0									
--CONGESTION		0 0 1 0 0									
--HEMORRHAGE		1 1 0 0 3									
--DILATATION, SINUSOIDAL		1 0 0 0 0									
--X-CARCINOMA, ISLET CELL		0 1 0 0 0									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 2 2 1 0									
LN, RENAL (RL)		NUMBER EXAMINED: 10 3 7 9 3									
		NOT REMARKABLE: 0 0 0 0 0									
--HYPERPLASIA, PLASMACYTIC		3 0 2 3 0									
--HEMORRHAGE		6 1 3 5 2									
--CONGESTION		0 0 1 0 1									
--DILATATION, SINUSOIDAL		1 1 4 4 1									
--PIGMENT, INCREASED		1 0 0 1 0									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 1 2 2 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

Genentech 6281-277
Genentech 96-177-0220

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGP-1 IN RATS

PAGE: 31

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
		NUMBER: 75 75 75 75 75				
LN, LUMBAR/ILTAC (LM)		NUMBER EXAMINED: 10 11 12 14 5				
		NOT REMARKABLE: 1 0 0 1 0				
--HYPERPLASIA, PLASMACYTIC		5	6	4	4	2
--HYPERPLASIA, LYMPHOCYTIC		3	0	0	0	1
--INFLAMMATION, SUPPURATIVE		0	0	0	2	0
--CONGESTION		0	1	1	2	1
--HEMORRHAGE		0	0	1	1	0
--DILATATION, SINUSOIDAL		6	6	6	9	3
--PIGMENT, INCREASED		0	1	2	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	1	2	2	0
LN, TRACHEOBRON (TB)		NUMBER EXAMINED: 0 2 0 0 0				
		NOT REMARKABLE: 0 0 0 0 0				
--HEMORRHAGE		0	1	0	0	0
--HYPERPLASIA, LYMPHOCYTIC		0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	0	0	0
LN, AXILLARY (AX)		NUMBER EXAMINED: 6 9 5 13 10				
		NOT REMARKABLE: 1 2 0 4 5				
--HYPERPLASIA, PLASMACYTIC		1	3	2	6	4
--HYPERPLASIA, LYMPHOCYTIC		1	0	0	0	0
--CONGESTION		1	4	1	3	2
--HEMORRHAGE		1	1	1	1	1
--DILATATION, SINUSOIDAL		1	1	0	0	1
--PIGMENT INCREASED		1	0	1	1	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

Genentech 6281-277
Genentech 96-177-0220

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGP-1 IN RATS

PAGE: 32

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----MALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
		NUMBER: 75 75 75 75 75				
LN, AXILLARY (AX)		NUMBER EXAMINED: 8 9 5 10 10				
		NOT REMARKABLE: 1 2 0 4 5				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	1	1	0
LN, ILSOCEAL (IC)		NUMBER EXAMINED: 0 0 0 1 0				
		NOT REMARKABLE: 0 0 0 0 0				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0
LN, OTHER (LN)		NUMBER EXAMINED: 0 1 0 2 1				
		NOT REMARKABLE: 0 0 0 0 0				
--HYPERPLASIA, LYMPHOCYTIC		0	0	0	1	1
--N-MESOTHELIOMA		0	1	0	0	0
--N-CARCINOMA, C-CELL		0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	2	0	1	0
LN, POPLITEAL (PP)		NUMBER EXAMINED: 2 5 1 3 2				
		NOT REMARKABLE: 0 0 0 0 0				
--HYPERPLASIA, PLASMACYTIC		2	5	1	3	2
--DILATATION, SINUSOIDAL		0	2	0	1	1
MENINGES (MG)		NUMBER EXAMINED: 0 0 0 1 0				
		NOT REMARKABLE: 0 0 0 0 0				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	0	0	1	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
R102-1 IN RATS

PAGE: 33

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: MALE									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-		NUMBER: 75 75 75 75 75							
CRANIAL NERVE(S) (CN)				NUMBER EXAMINED:		0 0 0 1 0					
				NOT REMARKABLE:		0 0 0 1 0					
BILE DUCT (BD)				NUMBER EXAMINED:		0 0 0 2 0					
				NOT REMARKABLE:		0 0 0 1 0					
--DILATATION						0 0 0 1 0					
NARROW VERTTEBRA (VN)				NUMBER EXAMINED:		0 0 0 1 0					
				NOT REMARKABLE:		0 0 0 0 0					
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)						0 0 0 1 0					
SUBCUTANEOUS TIS (SQ)				NUMBER EXAMINED:		1 1 0 1 0					
				NOT REMARKABLE:		1 1 0 1 0					
DIAPHRAGM (DP)				NUMBER EXAMINED:		0 0 1 0 0					
				NOT REMARKABLE:		0 0 0 0 0					
--N-CARCINOMA						0 0 1 0 0					
PERICARDIAL SAC (PS)				NUMBER EXAMINED:		0 0 1 0 0					
				NOT REMARKABLE:		0 0 1 0 0					

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0120

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
R102-1 IN RATS

PAGE: 34

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: MALE									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-		NUMBER: 75 75 75 75 75							
BONE, RIB (RI)				NUMBER EXAMINED:		0 0 0 1 0					
				NOT REMARKABLE:		0 0 0 0 0					
--DYSPLASIA, CHONDROCYTE						0 0 0 1 0					
PINNA (PN)				NUMBER EXAMINED:		0 0 0 2 0					
				NOT REMARKABLE:		0 0 0 0 0					
--OSSEOUS METAPLASIA, CARTILAGE						0 0 0 1 0					
--B-PAPILLOMA, SQUAMOUS CELL						0 0 0 1 0					

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-177
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGF-1 IN RATS

PAGE: 35

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75
** TOP OF LIST **						
BONE, FEMUR (FB)	NUMBER EXAMINED:	75	70	74	74	75
	NOT REMARKABLE:	75	69	73	73	74
--INFLAMMATION, CHRONIC, ARTICULAR CARTILAGE		0	0	0	1	0
--EXOSTOSIS		0	0	0	0	1
--HYPEROSTOSIS		0	1	0	0	0
--OSTEODYSTROPHY, FIBROUS		0	0	1	0	0
MARROW, FEMUR (FM)	NUMBER EXAMINED:	75	70	74	74	75
	NOT REMARKABLE:	46	41	38	47	50
--HYPERCELLULARITY, MARROW		28	27	35	27	25
--INFARCT		0	1	1	0	0
--FIBROSIS		1	0	0	0	0
--INFLAMMATION, LYMPHOCYTIC		1	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	0	0	0
BONE, STERNUM (SB)	NUMBER EXAMINED:	75	75	75	74	75
	NOT REMARKABLE:	75	75	74	74	75
--OSTEODYSTROPHY, FIBROUS		0	0	1	0	0
MARROW, STERNUM (SM)	NUMBER EXAMINED:	75	75	75	74	75
	NOT REMARKABLE:	48	43	42	53	57
--HYPERCELLULARITY, MARROW		27	31	31	21	18
--INFLAMMATION, HISTIOCYTIC		1	0	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	0	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-177
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGF-1 IN RATS

PAGE: 36

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER:	75	75	75	75
EYE (EX)		NUMBER EXAMINED:	75	75	75	75
	NOT REMARKABLE:	67	66	69	70	67
--INFLAMMATION, CHRONIC-ACTIVE, CORNEAL		0	1	0	0	2
--MINERALIZATION, CORNEAL		4	6	2	2	1
--INFLAMMATION, SUPPURATIVE, UVEAL		0	0	1	0	1
--HEMORRHAGE		0	0	0	0	1
--CATARACT		3	1	2	2	4
--OSSIFICATION, SCLERAL		1	1	0	0	0
--PHTHISIS BULBI		0	0	1	0	0
--ATROPHY, RETINAL, UNILATERAL		0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	0	0	0
--ONE EXAMINED		0	0	0	1	0
BRAIN (BX)	NUMBER EXAMINED:	75	75	75	75	75
	NOT REMARKABLE:	14	25	19	25	17
--COMPRESSION, VENTRAL		57	54	53	45	34
--DILATATION, VENTRICULAR		40	32	29	23	6
--GLIOSIS, GRAY MATTER		0	1	0	1	0
--NECROSIS, GRAY MATTER		0	0	0	4	6
--NECROSIS, HIPPOCAMPUS		0	0	0	2	6
--INFILTRATE, LYMPHOCYTIC		1	0	0	0	1
--HEMORRHAGE		1	2	0	1	1
--MINERALIZATION		0	1	1	0	0
--EMBOLUS, SUPPURATIVE		0	0	0	0	2
--X-OLIGOENDROGLIOMA		0	0	0	1	0
--X-ASTROCYTOMA		1	0	0	0	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 55-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
B10GF-1 IN RATS

PAGE: 37

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-
NUMBER:		25	75	75	75	75
** FROM PREVIOUS PAGE **		25	75	75	75	75
BRAIN (BR)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--M-NEUROBLASTOMA		0	0	1	0	0
--M-MALIGNANT RETICULOSIS		1	0	0	0	0
--M-MENINGEAL SARCOMA		1	0	0	0	0
--I-PITUITARY CARCINOMA		3	3	2	2	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	0	0
SPINAL CORD (SC)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--FIBROSIS, PERIVASCULAR		0	1	0	0	0
--GLIOSIS, WHITE MATTER		1	5	1	0	0
--NECROSIS, WHITE MATTER		0	0	1	0	0
--HEMORRHAGE		1	1	1	1	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	1	0	0
KIDNEY (KD)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--NEPHROPATHY, CHRONIC PROGRESSIVE		61	63	66	69	67
--MINERALIZATION, TUBULAR		12	20	15	23	15
--MINERALIZATION, PELVIC		32	45	40	43	35
--PYELONEPHRITIS		4	2	0	1	0
--DILATATION, PELVIC		2	4	5	2	2
--CYST		2	3	3	5	5
--FIBROSIS, CAPSULAR		1	0	2	0	0
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 55-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
B10GF-1 IN RATS

PAGE: 38

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-
NUMBER:		25	75	75	75	75
** FROM PREVIOUS PAGE **		25	75	75	75	75
KIDNEY (KD)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--NECROSIS, TUBULAR		2	2	3	2	1
--EMULOUS, SUPPURATIVE		0	0	0	0	0
--INFLAMMATION, SUPPURATIVE		0	0	0	0	0
--INFARCT		1	0	0	0	0
--ECTASIA, TUBULAR		2	0	0	0	0
--CONGESTION		1	0	0	0	0
--THROMBUS		0	0	0	1	0
--PIGMENT		1	0	0	0	0
--HYPERPLASIA, TUBULAR EPITHELIUM		0	0	0	1	0
--HYALINE DROPLET ACCUMULATION		0	1	0	0	0
--HYPERPLASIA, TRANSITIONAL CELL		7	7	8	10	1
--M-LIPOSAAROMA		0	1	0	1	0
--M-CARCINOMA, TUBULAR CELL		0	0	1	3	0
--M-CARCINOMA, TRANSITIONAL CELL		0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	1	1	0	0
LIVER (LI)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--INFLAMMATION, CHRONIC		12	27	24	26	13
--HYPERPLASIA, BILE DUCT		25	22	20	20	24
--VASCULITIS, HEPATOCYLLULAR		30	20	21	12	5
--HEMATOPOIESIS, EXTRAMEDULLARY		12	19	24	21	13
--CONGESTION		9	7	6	7	8
--DILATATION, SINUSOIDAL		13	22	20	12	7
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
MIGF-1 IN RATS

PAGE: 39

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: --- FEMALE ---				
ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-
NUMBER EXAMINED:		75	75	75	75	75
NOT REMARKABLE:		75	75	75	75	75
LIVER (LI)		75	75	75	75	75
NOT REMARKABLE:		5	9	12	12	23
--ECTASIA, BILE DUCT		0	0	0	2	1
--CYST		1	0	0	0	0
--POLYARTHYROCYTOSIS, HEPATOCELLULAR		5	3	4	1	0
--PIGMENT, KUPFFER CELL		1	0	0	0	1
--FIBROSIS, CAPSULAR		2	2	1	1	0
--DEGENERATION, CYSTIC		0	0	1	1	0
--NECROSIS, RANDOM		5	1	1	2	3
--NECROSIS, COAGULATIVE		0	0	1	1	0
--NECROSIS, HEPATOCELLULAR, CENTRILOBULAR		0	2	2	3	0
--INFARCT		0	0	2	1	0
--FOCUS, BASOPHILIC CELL		12	18	10	12	14
--FOCUS, EOSINOPHILIC CELL		4	3	2	1	1
--FOCUS, MIXED CELL		1	0	0	1	1
--FOCUS, CLEAR CELL		2	1	1	1	2
--ANOMALY, LOBE DEVELOPMENT		0	0	0	0	1
--B-ADENOMA, HEPATOCELLULAR		0	0	0	2	1
--N-LEIOMYOSARCOMA		0	0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	2	2	0	1
TRACHEA (TR)		75	75	75	75	75
NUMBER EXAMINED:		75	74	75	75	75
NOT REMARKABLE:		75	74	75	75	75
--DEGENERATION, EPITHELIAL		0	1	0	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
MIGF-1 IN RATS

PAGE: 40

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: --- FEMALE ---				
ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-
NUMBER EXAMINED:		75	75	75	75	75
NOT REMARKABLE:		75	75	75	75	75
ESOPHAGUS (ES)		75	75	75	75	75
NUMBER EXAMINED:		75	75	75	75	75
NOT REMARKABLE:		75	75	75	75	75
THYROID (TY)		75	75	75	75	75
NUMBER EXAMINED:		75	75	75	75	75
NOT REMARKABLE:		11	31	23	33	32
--CYST, ULTIMOBRANCHIAL		50	27	32	27	24
--CYST, FOLLICULAR		0	1	3	3	1
--ECTOPIC THYMUS		0	1	0	0	0
--INFLAMMATION, VASCULAR		0	0	0	2	0
--INFLAMMATION, SUPPURATIVE		0	0	0	0	1
--HYPERPLASIA, C-CELL		15	8	11	7	8
--HYPERPLASIA, FOLLICULAR CELL		0	2	2	1	1
--B-ADENOMA, FOLLICULAR CELL		0	1	2	0	1
--M-CARCINOMA, FOLLICULAR CELL		0	1	1	2	1
--B-ADENOMA, C-CELL		16	13	14	11	7
--M-CARCINOMA, C-CELL		0	2	0	2	0
--ONE EXAMINED		1	0	0	0	0
PARATHYROID (PT)		75	70	70	74	29
NUMBER EXAMINED:		75	70	70	74	29
NOT REMARKABLE:		43	18	41	19	15
--HYPERPLASIA		0	1	4	4	0
--B-ADENOMA		1	0	0	0	0
--ONE EXAMINED		27	31	26	16	14

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-277-0320

PAGE: 41

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----					
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-
		NUMBER:	75	75	75	75	75
LACRIMAL GL. EX (EO)		NUMBER EXAMINED:	75	75	74	75	75
		NOT REMARKABLE:	55	61	53	52	58
--HARDERIAN GLAND ALTERATION			10	7	13	11	11
--INFILTRATE, LYMPHOCYTIC			13	9	16	14	7
--INFLAMMATION, SUPPURATIVE			0	0	0	0	1
--ATROPHY, ACINAR			1	0	1	0	0
--ONE EXAMINED			0	0	0	1	1
LUNG (LU)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	35	40	41	39	43
--INFILTRATE, MACROPHAGE, ALVEOLAR			24	27	21	20	9
--INFLAMMATION, INTERSTITIAL			2	1	1	0	0
--INFLAMMATION, SUPPURATIVE			0	1	2	0	0
--PERIVASCULAR INFILTRATE, EOSINOPHIL			1	0	0	2	0
--PERIVASCULAR INFILTRATE, LYMPHOCYTIC			0	11	5	13	9
--FIBROSIS, BRONCHIOCLAR			0	1	0	0	0
--MINERALIZATION, SEPTAL			0	0	2	0	0
--METAPLASIA, OSSSEOUS			2	0	0	1	2
--CONGESTION			0	2	4	3	4
--HEMORRHAGE			0	0	0	1	2
--THROMBUS			0	0	2	0	2
--HYPERTROPHY, MUSCULAR, ARTERIOLE			10	4	3	8	11
--HYPERPLASIA, TYPE II PNEUMOCYTE			0	0	0	1	2
--N-CARCINOMA			0	0	2	2	1
--N-YOLK SAC CARCINOMA			0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			2	1	1	1	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-277-0320

PAGE: 42

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---					
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----					
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-
		NUMBER:	75	75	75	75	75
THYMUS (TH)		NUMBER EXAMINED:	74	70	74	74	73
		NOT REMARKABLE:	27	14	23	29	26
--CYST, ULTIMOBRANCHIAL			0	1	0	0	0
--HYPERPLASIA, EPITHELIAL			45	50	51	08	36
--HYPERPLASIA, LYMPHOCYTIC, CORTX			0	0	1	0	0
--HYPERPLASIA, LYMPHOCYTIC, MEDULLA			1	2	0	8	1
--CONGESTION			0	0	1	1	5
--HEMORRHAGE			0	0	0	0	1
--D-THYMOMA			0	2	0	2	1
--S-FIBROMA			0	2	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	0	3	0
SPLEEN (SP)		NUMBER EXAMINED:	75	75	75	75	75
		NOT REMARKABLE:	8	5	3	19	15
--PIGMENT, INCREASED			50	46	45	38	41
--HEMATOPOIESIS, EXTRAMEDULLARY, INCREASED			23	33	38	24	21
--HEMATOPOIESIS, EXTRAMEDULLARY, DECREASED			2	0	0	3	1
--CYST, SEROSAL			0	0	0	1	0
--FIBROSIS, CAPSULAR			0	0	4	0	0
--INFARCT			0	0	1	0	0
--M-SCHNAPPOMKA			0	0	1	0	0
--M-HEMANGIOSARCOMA			0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			1	2	2	5	1

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-177-0120

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rhIGF-1 IN RATS

PAGE: 43

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									
LN, MESENTERIC (MS)		NUMBER EXAMINED: 75 74 75 75 75									
		NOT REMARKABLE: 62 59 56 64 53									
-- CONGESTION		3 0 1 0 1									
-- HEMORRHAGE		0 0 1 0 5									
-- PIGMENT, INCREASED		12 13 18 9 14									
-- INFLAMMATION, CHRONIC-ACTIVE		0 0 0 1 0									
-- DILATATION, SINUSOIDAL		1 1 0 1 1									
-- HYPERPLASIA, RETICULOENDOTHELIAL		0 0 0 0 1									
-- INFILTRATE, EOSINOPHIL, SINUSOID		0 0 0 0 1									
-- X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 2 0 0 2									
ADRENAL, CORTX (AC)		NUMBER EXAMINED: 75 75 75 75 75									
		NOT REMARKABLE: 9 7 6 22 23									
-- DEGENERATION, CYSTIC, CORTICAL		64 64 56 49 42									
-- HEMATOPOIESIS, EXTRAMEDULLARY		0 0 3 3 3									
-- INFILTRATE, LYMPHOCYTIC, CAPSULE		1 0 0 0 0									
-- HYPERTROPHY, CORTICAL		4 1 4 6 6									
-- HYPERPLASIA, CORTICAL		6 6 5 2 4									
-- NECROSIS, CORTICAL		0 1 1 1 2									
-- CONGESTION		0 0 1 2 2									
-- MINERALIZATION		0 3 1 0 0									
-- ATROPHY, CORTICAL, UNILATERAL		0 1 1 0 0									
-- ABSCESS		0 0 0 1 2									
-- E-ADENOMA		1 2 1 0 1									
-- X-CARCINOMA		0 0 3 0 0									
-- X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 1 4 0 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 56-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rhIGF-1 IN RATS

PAGE: 44

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									
ADRENAL, MEDULLA (MA)		NUMBER EXAMINED: 73 74 74 73 74									
		NOT REMARKABLE: 65 60 48 48 49									
-- HEMATOCTYST		1 0 0 0 1									
-- INFILT, LYMPHOCYTIC		0 0 0 0 1									
-- HYPERPLASIA, MEDULLARY		1 3 11 15 15									
-- B-PHEOCHROMOCYTOMA		1 0 6 0 13									
-- X-MALIGNANT PHEOCHROMOCYTOMA		0 0 0 1 0									
-- ONE EXAMINED, MEDULLA		4 6 12 0 3									
-- X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 0 0 0 0									
PITUITARY (PI)		NUMBER EXAMINED: 75 75 75 74 75									
		NOT REMARKABLE: 3 3 3 3 15									
-- HYPERPLASIA		6 5 4 9 13									
-- ANGIOECTASIS		5 6 4 2 5									
-- CONGESTION		0 0 0 4 2									
-- CYST		1 4 3 4 6									
-- E-ADENOMA		62 62 61 58 43									
-- X-CARCINOMA		3 2 3 2 1									
-- I-MENINGEAL SARCOMA		1 0 0 0 0									
-- X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 0 0 0									
AORTA (AO)		NUMBER EXAMINED: 75 75 75 75 75									
		NOT REMARKABLE: 75 76 72 74 75									
-- MINERALIZATION		0 1 3 1 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 45

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
		NUMBER: 75 75 75 75 75				
		NOT REMARKABLE: 16 14 13 17 13				
HEART (HT)	NUMBER EXAMINED:	75	75	75	75	75
	NOT REMARKABLE:	16	14	13	17	13
--CARDIOMYOPATHY, DEGENERATIVE		56	61	56	56	41
--THROMBUS, ATRIAL		0	2	1	0	0
--EMBOLUS, SUPPURATIVE		0	0	0	0	1
--THROMBUS, AORTIC VALVE		0	0	0	0	1
--MINERALIZATION		0	0	2	1	0
--M-CARCINOMA		0	0	0	1	0
--M-YOLK SAC CARCINOMA		0	0	1	0	0
--M-ENDOCARDIAL SCHWANNOMA		0	0	1	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	1	1	1	0
TONGUE (TO)	NUMBER EXAMINED:	75	75	75	75	75
	NOT REMARKABLE:	72	72	71	69	73
--MINERALIZATION		0	0	2	0	0
--INFILTRATION, VASCULAR		0	2	1	4	0
--INFILTRATE, LYMPHOCTYTIC		2	0	0	1	1
--INFILTRATION, ULCERATIVE		1	1	0	1	0
--INFILTRATION, SUPPURATIVE		0	0	0	0	1
--B-PAPILLOMA, SQUAMOUS CELL		0	0	1	0	0
MUSCLE, SKELETAL (SM)	NUMBER EXAMINED:	75	74	74	75	75
	NOT REMARKABLE:	73	70	72	73	71
--INFILTRATE, LYMPHOPLASMATIC		4	2	0	0	1
--INFLAMMATION, ACUTE, PERIMYSIUM		1	0	0	0	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 46

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
		NUMBER: 75 75 75 75 75				
		NOT REMARKABLE: 70 72 72 73 71				
** FROM PREVIOUS PAGE **	NUMBER EXAMINED:	75	74	74	75	75
MUSCLE, SKELETAL (SM)	NOT REMARKABLE:	70	72	72	73	71
--DEGENERATION/NECROSIS		1	1	1	2	1
--HEMORRHAGE		0	0	0	0	1
--M-FIBROSARCOMA		0	0	1	0	0
--M-RHABDYSARCOMA		0	1	0	0	0
--M-UNDIFFERENTIATED SARCOMA		0	0	0	0	1
--I-CARCINOMA		1	0	0	0	0
NERVE, SCIATIC (SN)	NUMBER EXAMINED:	75	74	74	75	75
	NOT REMARKABLE:	75	74	73	75	75
--DEGENERATION		0	0	1	0	0
PANCREAS (PA)	NUMBER EXAMINED:	75	75	75	75	74
	NOT REMARKABLE:	56	57	63	62	60
--ATROPHY, ACINAE		9	12	6	11	4
--FOCUS, BASOPHILIC		4	2	1	2	5
--HYPERPLASIA, ACINAE CELL		0	0	0	0	1
--HEPATOCELLULAR METAPLASIA, ISLET CELLS		0	0	0	0	2
--INFILTRATION, VASCULAR		0	2	1	1	0
--INFILTRATION, SUPPURATIVE		0	0	0	0	1
--DILATATION, DUCTAL		1	1	1	0	1
--HYPERPLASIA, ISLET CELLS		0	1	0	0	0
--B-ADENOMA, ISLET CELL		1	1	0	0	0
--M-CARCINOMA, ISLET CELL		0	1	0	0	0

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGF-1 IN RATS

PAGE: 47

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-
NUMBER:		75	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75
PANCREAS (PA)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	56	57	63	62
--B-ADENOMA, ACINAR CELL			0	0	0	1
STOMACH, GL (ST)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	67	67	64	62
--EROSION/ULCERATION			2	4	4	5
--INFLAMMATION, ACUTE			1	1	0	0
--INFLAMMATION, VASCULAR			0	1	1	0
--CONGESTION			4	2	3	6
--EDEMA			0	0	1	0
--CYST			1	0	0	1
--MINERALIZATION			0	1	3	1
--MALFORMATION, MUCOSA			0	0	0	0
--M-LEIOMYOSARCOMA			0	0	0	1
STOMACH, NONGL (SU)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	73	69	72	70
--EROSION/ULCERATION			2	3	1	2
--INFLAMMATION, ACUTE			1	3	0	1
--HYPERPLASIA, SQUAMOUS			1	2	1	2
--CONGESTION			0	0	0	2
--EDEMA			0	4	1	2
--MINERALIZATION			0	0	1	0
--HYPERPLASIA, ATYPICAL			0	1	0	0
** CONTINUED ON NEXT PAGE **			0	1	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
PHGF-1 IN RATS

PAGE: 48

TABLE INCLUDES:		NUMBER OF ANIMALS AFFECTED				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1-	-2-	-3-	-4-	-5-
NUMBER:		75	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75
STOMACH, NONGL (SU)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	73	69	72	70
--I-LEIOMYOSARCOMA			0	0	0	1
DUODENUM (DU)		NUMBER EXAMINED:	75	75	75	75
		NOT REMARKABLE:	75	75	76	74
--HYPERTROPHY, VILLI			0	0	0	1
JEJUNUM (JE)		NUMBER EXAMINED:	74	73	70	67
		NOT REMARKABLE:	74	72	70	66
--DIVERTICULUM, CONGENITAL			0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	1	0	0
ILEUM (IL)		NUMBER EXAMINED:	75	74	73	71
		NOT REMARKABLE:	75	74	73	71
CECUM (CE)		NUMBER EXAMINED:	75	75	74	75
		NOT REMARKABLE:	74	73	74	73
--INFLAMMATION, ACUTE			1	0	0	0
--INFLAMMATION, VASCULAR			0	1	0	1
--CONGESTION			0	0	0	1
--PARASITISM			0	0	0	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)			0	1	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6261-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 49

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
		NUMBER: 75 75 75 75 75				
COLON (CO)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 75 72 73 73 74				
--PARASITISM		0 0 4 3 2				
--INFLAMMATION, VASCULAR		0 1 0 2 0				
RECTUM (RE)		NUMBER EXAMINED: 74 75 75 75 75				
		NOT REMARKABLE: 70 65 68 70 67				
--INFLAMMATION, VASCULAR		0 1 1 1 0				
--EROSION/ULCERATION		1 0 0 0 0				
--PARASITISM		3 5 6 4 7				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 0 0 0 1				
SALIV GL. SUBMAX (SG)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 74 75 75 75 75				
--INFILTRATE, LYMPHOCTIC		1 0 0 0 0				
LN, SUBMAXILLARY (MN)		NUMBER EXAMINED: 74 75 74 74 75				
		NOT REMARKABLE: 6 0 9 0 0				
--HYPERPLASIA, PLASMOCYTIC		60 63 65 62 67				
--HYPERPLASIA, LYMPHOCTIC		2 4 2 5 2				
--DILATATION, SINUSOIDAL		2 2 4 0 0				
--CONGESTION		14 8 0 14 5				
--HEMORRHAGE		5 3 3 4 1				
--PIGMENT, INCREASED		1 1 0 0 0				
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6261-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rHGF-1 IN RATS

PAGE: 50

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
		NUMBER: 75 75 75 75 75				
LN, SUBMAXILLARY (MN)		NUMBER EXAMINED: 74 75 74 74 75				
		NOT REMARKABLE: 6 0 9 0 0				
--ONE EXAMINED		5 2 2 0 4				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 1 0 0 1				
INJECTION SITE (IS)		NUMBER EXAMINED: 75 75 74 74 75				
		NOT REMARKABLE: 3 8 2 10 6				
--INFLAMMATION, CHRONIC		64 58 63 55 55				
--INFLAMMATION, SUPPURATIVE		0 0 0 1 0				
--INFLAMMATION, ULCERATIVE		2 1 1 1 1				
--INFLAMMATION, GRANULOMATOUS		6 5 4 1 0				
--FIBROSIS		70 64 70 63 64				
--HEMORRHAGE		46 23 48 30 42				
--CONGESTION		4 5 7 6 8				
--EDEMA		1 0 0 0 0				
--ATROPHY, SUBCUTANEOUS MUSCLE		36 28 30 21 30				
--NECROSIS, SUBCUTANEOUS MUSCLE		5 7 5 5 10				
--FOREIGN BODY		4 11 7 4 2				
--HYPERPLASIA, EPIDERMAL		1 2 1 0 2				
--B-ADENOMA, BASAL CELL		0 1 0 0 0				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 0 0 0				
SKIN (SK)		NUMBER EXAMINED: 75 75 75 74 75				
		NOT REMARKABLE: 68 64 63 64 69				
--INFLAMMATION, ULCERATIVE		1 3 2 1 4				
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

4281-377
Genentech 96-177-0320

194-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KHGF-1 IN RATS

PAGE: 51

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
NUMBER:		75	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75
SKIN (SK)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--INFLAMMATION, CHRONIC		1	2	1	1	3
--ABSCESSES		0	1	0	0	0
--ATROPHY, ADNEXAL		3	1	4	2	3
--HEMORRHAGE		0	1	1	0	0
--EDEMA		1	0	0	1	0
--HYPERPLASIA, EPIDERMAL		0	2	1	1	3
--CYST, EPIDERMAL INCLUSION		0	0	0	1	1
--B-FIBROMA		1	2	3	1	1
--M-FIBROSARCOMA		1	0	2	2	2
--M-HEMANGIOSARCOMA		0	0	0	0	1
--M-SCHNANNOMA		0	0	0	1	1
--B-KERATOCARCINOMA		0	1	1	0	1
--B-PAPILLOMA, SQUAMOUS CELL		0	0	0	0	1
--M-CARCINOMA, SQUAMOUS CELL		0	0	0	0	1
--M-AMELANOTIC MELANOMA		0	1	0	0	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	1	0	0
MAMMARY, FEMALE (MF)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--GALACTOCELE		49	55	57	58	52
--HYPERPLASIA, GLANDULAR		43	46	44	40	40
--HYPERPLASIA, ATYPICAL		5	6	5	5	3
--B-MYCEPITHELIOMA		0	0	0	1	0
--B-FIBROADENOMA		22	35	25	23	25
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

194-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KHGF-1 IN RATS

PAGE: 52

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----				
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-				
NUMBER:		75	75	75	75	75
** FROM PREVIOUS PAGE **		75	75	75	75	75
MAMMARY, FEMALE (MF)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--X-CARCINOMA		32	26	29	27	26
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	0	0	0
URINARY BLADDER (UB)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--DILATATION		0	0	0	0	1
--INFLAMMATION, CHRONIC		0	0	1	0	0
--CALCULUS		0	0	1	0	0
--HYPERPLASIA, TRANSITIONAL CELL		1	0	0	0	0
--B-LEIOMYOMA		0	1	0	0	0
UTERUS (UT)		NUMBER EXAMINED:				
		NOT REMARKABLE:				
--INFLAMMATION, ACUTE		0	0	1	3	0
--CYST, ENDOMETRIAL		19	26	27	27	31
--DILATATION, LUMEN		0	1	0	1	4
--VASCULAR DILATATION, MYOMETRIUM		0	1	1	0	0
--METAPLASIA, SQUAMOUS, ENDOMETRIAL		11	10	7	15	16
--FIBROSIS		1	1	2	0	1
--I-HEMANGIOSARCOMA		0	0	0	0	1
--B-POLYT. ENDOMETRIAL, STROMAL		5	2	2	4	2
--X-CARCINOMA		0	0	2	0	0

TABLE 24

Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 36-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
HIGH-1 IN RATS

PAGE: 53

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION	GROUP:	1-	2-	3-	4-	5-
	NUMBER:	75	75	75	75	75
VAGINA (VA)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 55 47 51 54 63				
--INFLAMMATION, CHRONIC-ACTIVE		5	2	2	0	4
--INFLAMMATION, VASCULAR		0	1	0	1	0
--LEUKEN, SUPPURATIVE EXUDATE		16	24	21	20	9
--HYPERPLASIA, STROMAL CELL		0	1	2	0	0
--B-PAPILLOMA, SQUAMOUS CELL		0	0	1	0	0
--B-FOLLY, STROMAL		0	2	0	0	0
--M-STROMAL SARCOMA		1	0	0	0	0
--I-STROMAL SARCOMA		0	1	0	1	1
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1	0	0	0	0
OVARY (OV)		NUMBER EXAMINED: 75 75 75 75 75				
		NOT REMARKABLE: 7 9 11 9 6				
--HYPOACTIVITY		65	62	61	65	65
--CYST, HEMORRHAGIC, CHRONIC		1	0	1	0	0
--CYST, PAROVARIAN		0	0	1	2	0
--CYST, BURSAL		1	1	1	1	1
--CYST, FOLLICULAR		0	1	0	0	0
--INFLAMMATION, VASCULAR		0	0	1	0	0
--HYPERPLASIA, GRANULOSA CELL		0	0	1	0	0
--HYPERPLASIA, STROMAL CELL		0	0	1	0	0
--B-TESTIS CELL TUMOR		0	2	5	6	2
--M-MALIGNANT GRANULOSA/THECA CELL TUMOR		0	1	2	1	0
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0	1	1	0	0
--OWS EXAMINED		3	1	1	0	3

TABLE 24

Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 36-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
HIGH-1 IN RATS

PAGE: 54

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----				
ORGAN AND FINDING DESCRIPTION	GROUP:	-1-	-2-	-3-	-4-	-5-
	NUMBER:	75	75	75	75	75
CAUSE OF DEATH (CD)	NUMBER EXAMINED:	75	75	75	75	75
	NOT REMARKABLE:	0	0	0	0	0
--SCHEDULED SACRIFICE		21	25	20	12	10
--UNDETERMINED		1	1	0	4	14
--PITUITARY-ADENOMA		45	32	38	39	33
--PITUITARY-CARCINOMA		1	2	2	2	0
--MAMMARY GLAND-FIBROADENOMA		1	2	1	2	1
--MAMMARY GLAND-CARCINOMA		1	2	2	10	7
--KIDNEY-NEPHROPATHY		0	2	3	4	0
--KIDNEY-PYELONEPHRITIS		1	0	0	0	0
--BRAIN-NEUROBLASTOMA		0	0	1	0	0
--BRAIN-NECROSIS, HIPPOCAMPUS		0	0	0	1	3
--BRAIN-MENINGEAL SARCOMA		1	0	0	0	0
--BRAIN-MALIGNANT RETICULOSIS		1	0	0	0	0
--LYMPHOMA		1	1	0	0	1
--LEUKEMIA, LGL		1	0	1	0	0
--MALIGNANT FIBROUS HISTIOCYTOMA		0	0	1	0	0
--SKIN-INFLAMMATION, CHRONIC		0	0	0	0	1
--SKIN-FIBROMA		0	0	0	0	1
--SKIN-FIBROSARCOMA		0	0	0	0	1
--SKIN-SCHWANNOMA		0	0	1	1	1
--SKELETAL MUSCLE-FIBROSARCOMA		0	0	0	1	0
--SKELETAL MUSCLE-UNDIFFERENTIATED SARCOMA		0	0	1	0	0
--SKELETAL MUSCLE-RHABDOMYOSARCOMA		0	0	0	0	1
--YOLK SAC CARCINOMA		0	1	0	0	0
--CERVIX-STROMAL SARCOMA		0	0	1	0	0
--VAGINA-STROMAL SARCOMA		0	1	1	0	0
** CONTINUED ON NEXT PAGE **		1	0	0	0	0

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

Genentech 6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rhIGF-I IN RATS

PAGE: 55

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX:ALL;GROUP:ALL;WEEKS:ALL		SEX:-----FEMALE-----									
DEATH:ALL;FIND:ALL;SUBSET:ALL											
		GROUP: -1- -2- -3- -4- -5-									
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75									
** FROM PREVIOUS PAGE **		-3- -4- -5- -6- -7-									
CAUSE OF DEATH (CD)		NUMBER EXAMINED: 75 75 75 75 75									
		NOT REMARKABLE: 0 0 0 0 0									
--AUDITORY SERACEOUS GLAND CARCINOMA		0 1 0 0 0									
--HEART-THROMBUS, AORTIC VALVE		0 0 0 0 1									
--STOMACH-LEIOMYOSARCOMA		0 0 0 0 1									
--ADRENAL-CARCINOMA, CORTICAL		0 0 1 0 0									
HEMATO NEOPLASIA (HN)		NUMBER EXAMINED: 75 75 75 75 75									
		NOT REMARKABLE: 73 71 72 72 74									
--M-LYMPHOMA		1 2 0 3 1									
--M-MALIGNANT FIBROUS HISTIOCYTOMA		0 1 1 0 0									
--M-LEUKEMIA, LARGE GRANULAE LYMPHOBLASTIC		1 0 2 0 0									
--M-SARCOMA, HISTIOCYTIC		0 1 0 0 0									
FOOT/FOOTPAD (FP)		NUMBER EXAMINED: 17 13 16 19 13									
		NOT REMARKABLE: 0 0 0 0 0									
--INFLAMMATION, ULCERATIVE		12 13 14 17 17									
--HYPERPLASIA, EPIDERMAL (ACANTHOSIS)		1 0 1 0 0									
--JOINT INFLAMMATION, CHRONIC-ACTIVE		1 1 0 0 1									
--INFLAMMATION, CHRONIC ACTIVE		2 0 2 2 1									
--HYPERKERATOSIS		2 0 1 0 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

Genentech 6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
rhIGF-I IN RATS

PAGE: 56

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: ----- FEMALE -----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									

TAIL (TI)		NUMBER EXAMINED: 1 1 2 3 3									
		NOT REMARKABLE: 0 0 0 0 1									
--INFLAMMATION, ULCERATIVE		0	0	1	1	3					
--CYST, EPIDERMAL INCLUSION		1	0	2	2	1					
--HYPERKERATOSIS		0	1	1	1	1					
--ABSCESS		0	1	1	0	0					
--HYPEROSTOSIS		0	0	0	1	0					
PINNA (PN)		NUMBER EXAMINED: 0 2 1 0 1									
		NOT REMARKABLE: 0 0 0 0 0									
--ULCERATION		0	1	1	0	1					
--INFLAMMATION, CHRONIC		0	2	1	0	1					
--HYPERPLASIA, EPIDERMAL		0	2	0	0	0					
--HEMORRHAGE		0	1	0	0	0					
AUDITORY SEE GL (AS)		NUMBER EXAMINED: 0 1 0 0 1									
		NOT REMARKABLE: 0 0 0 0 1									
--M-CARCINOMA		0	1	0	0	0					
SUBCUTANEOUS TIS (SQ)		NUMBER EXAMINED: 5 0 0 0 1									
		NOT REMARKABLE: 0 0 0 0 0									
--EDEMA		0	0	0	0	1					
--THROMBUS, SUPPURATIVE		0	0	0	0	1					

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ENIGF-1 IN RATS

PAGE: 57

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL;GROUP=ALL;WEEKS=ALL DEATH=ALL;FIND=ALL;SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-				
		NUMBER:	75	75	75	75	75				
JOINT, OTHER (JT)		NUMBER EXAMINED:	1	0	0	1	2				
		NOT REMARKABLE:	0	0	0	0	0				
--EXOSTOSIS			1	0	0	0	1				
--INFLAMMATION, CHRONIC-ACTIVE, SYNOVIAL			0	0	0	1	0				
BONE, OTHER (BO)		NUMBER EXAMINED:	0	0	0	1	0				
		NOT REMARKABLE:	0	0	0	0	0				
--INFLAMMATION, CHRONIC-ACTIVE			0	0	0	1	0				
BONE, RIB (RI)		NUMBER EXAMINED:	0	0	0	0	1				
		NOT REMARKABLE:	0	0	0	0	0				
--DYSPLASIA, CHONDROCYTE			0	0	0	0	1				
CALVARIUM (CA)		NUMBER EXAMINED:	0	0	0	0	2				
		NOT REMARKABLE:	0	0	0	0	0				
--HEMORRHAGE, PERIOSTEAL			0	0	0	0	2				
--INFLAMMATION, ACUTE			0	0	0	0	2				
ADIPOSE TISSUE (AT)		NUMBER EXAMINED:	1	0	0	0	0				
		NOT REMARKABLE:	1	0	0	0	0				

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
ENIGF-1 IN RATS

PAGE: 58

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL;GROUP=ALL;WEEKS=ALL DEATH=ALL;FIND=ALL;SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP:	-1-	-2-	-3-	-4-	-5-				
		NUMBER:	75	75	75	75	75				
CAVITY, ABDOM (PC)		NUMBER EXAMINED:	3	3	4	2	3				
		NOT REMARKABLE:	0	0	0	0	0				
--NECROSIS, FAT			3	3	3	2	2				
--H-HEMANGIOSARCOMA			0	0	0	0	1				
--H-SCHWANNOMA			0	0	1	0	0				
PERITONEUM/CAV (PC)		NUMBER EXAMINED:	0	0	0	0	0				
		NOT REMARKABLE:	0	0	0	0	0				
MESENTERY (MY)		NUMBER EXAMINED:	0	0	1	1	0				
		NOT REMARKABLE:	0	0	1	1	0				
LN, OTHER (LN)		NUMBER EXAMINED:	1	1	0	3	1				
		NOT REMARKABLE:	0	0	0	0	0				
--HYPERPLASIA, PLASMACYTIC			0	0	0	1	0				
--DILATATION, SINUSOIDAL			0	1	0	0	0				
--HYPERPLASIA, LYMPHOCTIC			0	0	0	1	1				
--FIBROSIS			0	0	0	0	1				
--NECROSIS			1	0	0	0	0				
LN, INGUINAL (IN)		NUMBER EXAMINED:	11	0	9	9	4				
		NOT REMARKABLE:	4	1	5	4	3				
--CONGESTION			0	1	2	0	1				

** CONTINUED ON NEXT PAGE **

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KRGF-1 IN RATS

PAGE: 53

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75				
** FROM PREVIOUS PAGE **		-1- -2- -3- -4- -5-				
LN. INGUINAL (IN)		NUMBER EXAMINED: 11 6 9 9 4				
		NOT REMARKABLE: 6 3 5 4 3				
--HYPERPLASIA, PLASMACYTIC		5 2 3 5 0				
--HEMORRHAGE		0 0 0 1 0				
LN. AXILLARY (AX)		NUMBER EXAMINED: 26 31 28 32 26				
		NOT REMARKABLE: 11 12 9 7 9				
--HYPERPLASIA, LYMPHOCTIC		4 3 4 5 5				
--HYPERPLASIA, PLASMACYTIC		6 8 8 14 9				
--INFLAMMATION, VASCULAR		0 1 0 0 0				
--CONGESTION		1 0 4 6 4				
--HEMORRHAGE		3 4 3 4 3				
--DILATATION, SINUSOIDAL		1 3 3 1 2				
--PIGMENT, INCREASED		6 5 6 3 1				
--FIBROSIS		0 0 0 0 1				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 1 0 0				
LN. MEDIASTINAL (ML)		NUMBER EXAMINED: 2 8 12 7 4				
		NOT REMARKABLE: 0 2 9 2 1				
--HYPERPLASIA, PLASMACYTIC		0 1 4 0 1				
--PIGMENT, INCREASED		0 2 4 0 2				
--DILATATION, SINUSOIDAL		1 1 0 1 0				
--INFILTRATE, MAST CELL		0 1 0 1 0				
--CONGESTION		0 2 5 3 1				
--HEMORRHAGE		0 4 7 2 1				
** CONTINUED ON NEXT PAGE **						

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
KRGF-1 IN RATS

PAGE: 59

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---				
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----				
		GROUP: -1- -2- -3- -4- -5-				
ORGAN AND FINDING DESCRIPTION		NUMBER: 75 75 75 75 75				
** FROM PREVIOUS PAGE **		-1- -2- -3- -4- -5-				
LN. MEDIASTINAL (ML)		NUMBER EXAMINED: 2 8 12 7 4				
		NOT REMARKABLE: 0 1 8 2 1				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 1 1 0 0				
LN. RENAL (RL)		NUMBER EXAMINED: 2 2 9 0 0				
		NOT REMARKABLE: 1 0 8 0 0				
--HYPERPLASIA, PLASMACYTIC		0 0 1 0 0				
--CONGESTION		1 2 3 0 0				
--HEMORRHAGE		0 0 2 0 0				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 1 0 0				
--PIGMENT, INCREASED		0 0 1 0 0				
LN. LUMBAR/ILAC (LM)		NUMBER EXAMINED: 8 8 12 11 6				
		NOT REMARKABLE: 1 0 4 3 2				
--HYPERPLASIA, PLASMACYTIC		3 5 5 8 4				
--HYPERPLASIA, LYMPHOCTIC		1 0 2 0 0				
--DILATATION, SINUSOIDAL		0 0 6 0 0				
--CONGESTION		2 0 0 1 1				
--HEMORRHAGE		0 1 1 0 1				
--PIGMENT		0 1 0 0 0				
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		0 1 0 0 2				

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
K100-1 IN RATS

PAGE: 61

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									
LN, TRACHEOBROM (TB)		NUMBER EXAMINED: 0 0 0 0 0									
		NOT REMARKABLE: 0 0 0 0 0									
--DILATATION, SINUSOIDAL		0 0 0 1 0									
--HEMORRHAGE		0 0 0 1 0									
LN, POPLITEAL (PP)		NUMBER EXAMINED: 2 2 1 2 1									
		NOT REMARKABLE: 1 0 0 0 0									
--HYPERPLASIA, PLASMACYTIC		1 2 1 2 1									
--DILATATION, SINUSOIDAL		1 6 6 0 0									
LN, ANT MES/PANC (AP)		NUMBER EXAMINED: 1 4 1 0 0									
		NOT REMARKABLE: 0 1 0 0 0									
--HEMORRHAGE		0 2 0 0 0									
--PIGMENT		0 2 0 0 0									
--X-HEMATOPOIETIC NEOPLASIA (SEE "HEMATO NEOPLASIA" FOR TYPE)		1 1 1 0 0									
LN, ILEOCECAL (IC)		NUMBER EXAMINED: 0 6 0 1 0									
		NOT REMARKABLE: 0 0 0 0 0									
--HYPERPLASIA, LYMPHOCTIC		0 0 6 1 0									
--CONSTRICTION		0 6 0 1 0									
--DILATATION, SINUSOIDAL		0 6 0 1 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
K100-1 IN RATS

PAGE: 62

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									
UTERUS (UB)		NUMBER EXAMINED: 1 1 2 0 0									
		NOT REMARKABLE: 0 0 0 0 0									
--HYPERPLASIA, TRANSITIONAL CELL		1 0 0 0 0									
--INFILTRATE, LYMPHOPLASMACYTIC		1 0 1 0 0									
--DILATATION		0 1 2 0 0									
CERVIX (CV)		NUMBER EXAMINED: 1 3 5 0 0									
		NOT REMARKABLE: 0 0 1 0 1									
--FIBROSIS		1 2 2 6 4									
--HYPERPLASIA, SQUAMOUS MUCOSA		0 0 0 1 0									
--HYPERPLASIA, STROMAL CELL		1 0 1 0 2									
--B-LEIOMYOMA		0 0 0 1 0									
--H-SARCOMA, STROMAL		0 1 1 1 1									
--HYPERKERATOSIS		0 0 0 2 0									
--LUMEN, SUPPURATIVE EXUDATE		0 0 0 1 0									
INNER EAR (IE)		NUMBER EXAMINED: 1 0 1 0 0									
		NOT REMARKABLE: 1 0 1 0 0									
BODY AS A WHOLE (BW)		NUMBER EXAMINED: 0 0 0 0 0									
		NOT REMARKABLE: 0 0 0 0 0									

TABLE 24
Incidence of Microscopic Observations
Carcinogenicity Animals - All Animals

6281-377
Genentech 96-177-0320

104-WEEK SUBCUTANEOUS CARCINOGENICITY STUDY WITH
K100-1 IN RATS

PAGE: 63

TABLE INCLUDES:		--- NUMBER OF ANIMALS AFFECTED ---									
SEX=ALL; GROUP=ALL; WEEKS=ALL DEATH=ALL; FIND=ALL; SUBSET=ALL		SEX: -----FEMALE-----									
ORGAN AND FINDING DESCRIPTION		GROUP: -1- -2- -3- -4- -5-									
		NUMBER: 75 75 75 75 75									
ARTERY, OTHER (AV)		NUMBER EXAMINED: 0 1 2 1 0									
		NOT REMARKABLE: 0 0 0 0 0									
--INFLAMMATION, CHRONIC		0 1 1 1 0									
--MINERALIZATION		0 0 1 1 0									
--THROMBUS		0 0 1 0 0									
BILE DUCT (BD)		NUMBER EXAMINED: 0 1 0 0 0									
		NOT REMARKABLE: 0 1 0 0 0									

** END OF LIST **

**This is a representation of an electronic record that was signed electronically and
this page is the manifestation of the electronic signature.**

/s/

Shen Xiao
7/20/05 05:31:04 PM
PHARMACOLOGIST

Jeri El Hage
7/21/05 09:08:44 AM
PHARMACOLOGIST