

**CENTER FOR DRUG EVALUATION AND
RESEARCH**

APPLICATION NUMBER:

21-667

PHARMACOLOGY REVIEW



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: 21,667
SERIAL NUMBER: 000
DATE RECEIVED BY CENTER: August 11, 2003
DRUG NAME: Glutamine / L-glutamine
INDICATION: Oral glutamine is indicated for short bowel syndrome

SPONSOR: Nutritional Restart Pharmaceutical, L.P.
Durham, NC

DOCUMENTS REVIEWED: Vol. 1.1-1.6

REVIEW DIVISION: Division of Gastrointestinal and
Coagulation Drug Products (HFD-180)

PHARM/TOX REVIEWER: Ke Zhang, Ph.D.

PHARM/TOX SUPERVISOR: Jasti Choudary, B.V.Sc., Ph.D.

DIVISION DIRECTOR: Robert Justice, M.D., M.S.

PROJECT MANAGER: Tanya Clayton

Date of review submission to Division File System (DFS):
May 6, 2004

TABLE OF CONTENTS

EXECUTIVE SUMMARY 1

3.1 INTRODUCTION AND DRUG HISTORY 4

3.2 PHARMACOLOGY 5

3.3 PHARMACOKINETICS/TOXICOKINETICS 13

3.4 TOXICOLOGY 16

 3.4.1 Overall toxicology summary..... 16

 3.4.2 Single-dose toxicity..... 17

 3.4.3 Repeat-dose toxicity..... 17

 3.4.4. Genetic toxicology..... 44

 3.4.6. Reproductive and developmental toxicology..... 45

3.6 ALL CONCLUSIONS AND RECOMMENDATIONS 47

**APPEARS THIS WAY
ON ORIGINAL**

EXECUTIVE SUMMARY

1. Recommendations

1.1 Recommendation on approvability

From a preclinical standpoint, approval of oral glutamine is recommended for short bowel svndrome _____

1.2 Recommendation for nonclinical studies: None

1.3 Recommendations on labeling

Sponsor should be asked to revise the labeling as recommended.

2. Summary of nonclinical findings

The following summary is based on literature reports submitted by the sponsor.

2.1 Pharmacologic Activity:

Glutamine, a non-essential amino acid, is widely distributed throughout the body and it is important in normal gastrointestinal cell structure, function, and regeneration. It is considered as a "conditionally essential" amino acid since the nutritional requirement of glutamine is markedly increased in catabolic conditions. The pharmacology studies demonstrated that treatment with dietary glutamine promotes electrolyte and nutrient absorption, and stimulates mucosal cell proliferation and regeneration of the small intestinal mucosa in rats with the resected small intestine. The trophic effects were synergistically elevated when glutamine is given in combination of growth hormone in rats with resected small intestine. These results suggest that the trophic effects of glutamine are certainly beneficial for patients with short bowel syndrome.

The pharmacokinetic studies indicated that oral absorption of glutamine was demonstrated with peak plasma glutamine level at ~1-1.5 hours after oral dose in rats. Glutamine was distributed in the liver, lung, kidney, heart, spleen, muscle,

and brain following dietary administration in rats. Glutamine can cross the placenta in rats. Glutamine is formed in the body through the condensation of a glutamate and an ammonia molecule by glutamine synthetase with hydrolysis of ATP. In the reverse process, glutaminase deaminated glutamine to glutamate and ammonia. Approximately 66% radioactivity (^{15}N) of glutamine was recovered in the urine following intravenous administration of radio-labeled glutamine in rats. Majority of the radioactivity (94%) was associated with urinary urea and only ~4% was as ammonia.

2.2 Toxicological Findings

The acute oral toxicity studies were conducted with glutamine in mice, rats, and rabbits. LD_{50} values were provided in the report. The oral LD_{50} values were 20.3 (females) and 21.7 (males) g/kg in mice, 7.5 (males) and 10.5 g/kg in rats, and 18.8 g/kg in male rabbits. Signs of acute toxicity and minimal lethal dose were not identified in this report.

In the 30-day oral toxicity study in rats, glutamine was given to rats by oral gavage at 4, 6, and 10 g/kg/day for 30 days. Glutamine was lethal at 10 g/kg/day dose due to "administration of the test substance suspension in large volumes" ("physical problems"). Catarrh in the stomach was noted in the L-GM groups but not in the control group.

In the 180-day oral toxicity study in rats, glutamine was given to rats by oral gavage at 2 and 4 g/kg/day for 180 days. Catarrh in the stomach and infiltration of inflammatory cell and edema in gastric submucosa were identified in both glutamine groups but not in control group. Catarrh in the stomach was noted on both days 90 and 180.

**APPEARS THIS WAY
ON ORIGINAL**

PHARMACOLOGY/TOXICOLOGY REVIEW**3.1 INTRODUCTION AND DRUG HISTORY**

NDA number: 21,667

Review number: 01

Sequence number/date/type of submission: August 8, 2003

Information to sponsor: Yes () No (x)

Sponsor and/or agent: Nutritional Restart Pharmaceutical, L.P.
Durham, NC

Manufacturer for drug substance:



Reviewer name: Ke Zhang

Division name: Division of Gastrointestinal and Coagulation
Drug Products

HFD #: 180

Review completion date: May 6, 2004

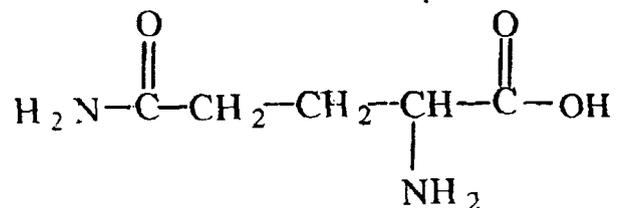
Drug:

Trade name: L-glutamine powder

Chemical name: (S)-2-aminoglutaramic acid

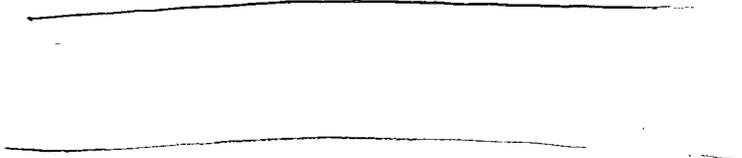
Molecular formula/molecular weight: C₅H₁₀N₂O₃ and 146.15

Structure:

Relevant INDs/NDAs/DMFs: IND 54,284 (oral glutamine), IND 48,750
(recombinant human growth hormone).

Drug class: Amino acid.

Indication: Oral glutamine is indicated for short bowel syndrome



Clinical formulation: Glutamine for oral administration is formulated as a white crystalline powder in a paper-foil-plastic laminate packet. Each packet of oral glutamine contains 5 g of L-glutamine.

Route of administration: Oral powder dissolved in water (5 g glutamine powder in 8 oz water).

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

Studies reviewed within this submission:

PRECLINICAL STUDIES AND TESTING LABORATORIES:

Type of Study	Study #	Lot #	lab	Page #
Pharmacology	Published reports			5
Pharmacokinetics	Published reports			13
Acute Toxicity:				
Acute oral toxicity studies in mice, rats, and rabbits	Published report		1	17
Subacute/subchronic/chronic Toxicity:				
30-day oral toxicity study in rats	Published report		1	17
180-day oral toxicity study in rats	Published report		1	27

1 =

3.2 PHARMACOLOGY

3.2.1 Brief summary

Glutamine, a non-essential amino acid, is widely distributed throughout the body and it is important in normal gastrointestinal cell structure, function, and regeneration. It is considered as a "conditionally essential" amino acid since the nutritional requirement of glutamine is markedly increased

in catabolic conditions. The pharmacology studies demonstrated that treatment with dietary glutamine promotes electrolyte and nutrient absorption, and stimulates mucosal cell proliferation and regeneration of the small intestinal mucosa in rats with the resected small intestine. The trophic effects were synergistically elevated when glutamine is given in combination of growth hormone in rats with resected small intestine. These results suggest that the trophic effects of glutamine are certainly beneficial for patients with short bowel syndrome.

3.2.2 Primary pharmacodynamics

Glutamine is a non-essential amino acid widely distributed throughout the body and makes up about 50% of the human body's free amino acid pool. The typical daily dietary intake of glutamine is ~5-10 g. Glutamine is important in normal gastrointestinal cell structure, function, and regeneration. Under normal condition, glutamine level in the body is maintained by dietary intake and synthesis from endogenous glutamate. However, the nutritional requirement of glutamine is dramatically increased in catabolic condition such as short bowel syndrome. Thus, it is referred as a "conditionally essential" amino acid. The pharmacological activities of glutamine alone and in combination of growth hormone were evaluated in the animal models of intestinal resection.

Effects of glutamine alone

To investigate the effects of glutamine on the growth-related factors after intestinal resection (Dig Surg 1999;16:197-203), rats were fed diets with and without glutamine (4%) for 2 days after the intestinal resection (60% of middle part of small bowel removed). Epidermal growth factor, transforming growth factor- α , insulin-like growth factors I and II, peptide YY, and enteroglucagon were analyzed in mucosa from the proximal jejunum, distal ileum, and in portal plasma 72 hours after resection. Animals in the control groups (transected animals and non-operated animals) were also fed diets with and without 4% glutamine. The results indicated that treatment with glutamine significantly increased peptide YY, epidermal growth factor, and enteroglucagon in the portal plasma and ileal mucosa in the resected animals as compared to the non-glutamine treated animals. The results were presented in Tables 2 and 4 in this publication and these tables are attached below.

Table 2. The mean (SEM) portal plasma concentrations of peptides in resected, transected and control rats receiving a postoperative diet free of glutamine or supplemented with glutamine (4%)

	PYY pmol/l	EG pmol/l	EGF pmol/l	IGF-I pmol/l	IGF-II nmol/l	TGF- α pmol/l
Resected						
No glutamine (n = 11)	37.7 (6.3)	22.5 (2.0)	9.8 (0.88)	432 (24)	24.9 (0.8) ^{b-d}	0.034 (0.004)
4% glutamine (n = 12)	63.9 (7.0) ^{b-d}	31.9 (1.8) ^{a,c}	10.5 (1.1) ^a	521 (46)	17.3 (1.8)	0.032 (0.007)
Transected						
No glutamine (n = 10)	19.5 (1.7) ^a	18.5 (1.4)	8.8 (0.89)	617 (24)	14.2 (1.0)	0.039 (0.002)
4% glutamine (n = 10)	18.1 (1.4)	17.1 (1.6)	8.2 (1.1)	615 (27)	17.7 (1.4)	0.036 (0.002)
Controls						
No glutamine (n = 10)	39.2 (7.8)	27.5 (2.6)	8.08 (0.59)	597 (57)	15.3 (2.0)	0.039 (0.005)
4% glutamine (n = 10)	18.7 (4.0)	20.8 (2.6)	5.8 (0.75)	541 (86)	18.6 (2.1)	0.039 (0.004)

PYY = Peptide YY; EG = enteroglucagon; EGF = epidermal growth factor; IGF = insulin-like growth factor; TGF- α = transforming growth factor- α .

Significant differences vs. the control group receiving the same diet: ^a p < 0.05; ^b p < 0.01.

Significant differences vs. the transected group receiving the same diet: ^c p < 0.01.

Significant difference vs. the resected group not receiving the same diet: ^d p < 0.05.

Table 4. Mean (SEM) ileal mucosa levels of peptides in resected, transected and control rats on a postoperative diet free of glutamine or supplemented with glutamine (4%)

	PYY pmol/g	EG pmol/g	EGF pmol/g	IGF-I pmol/g	IGF-II pmol/g	TGF- α pmol/g
Resected						
No glutamine (n = 11)	42.3 (3.0)	19.5 (2.57)	0.17 (0.01)	2.45 (0.24)	3.84 (0.56)	0.18 (0.03)
4% glutamine (n = 12)	61.4 (5.4)	30.8 (3.37)	0.25 (0.04) ^a	2.83 (0.32)	5.29 (0.55) ^b	0.24 (0.05)
Transected						
No glutamine (n = 10)	75 (20)	21.9 (6.2)	0.16 (0.04)	2.41 (0.49)	2.17 (0.18)	0.22 (0.03)
4% glutamine (n = 10)	145 (38)	44.2 (9.2) ^c	0.15 (0.02)	3.1 (0.5)	2.45 (0.27)	0.37 (0.04)
Controls						
No glutamine (n = 10)	128 (19)	29.4 (4.24)	0.12 (0.02)	2.44 (0.34)	2.04 (0.31)	0.22 (0.03)
4% glutamine (n = 10)	124 (22)	24.3 (3.00)	0.13 (0.02)	2.49 (0.37)	3.01 (0.42)	0.28 (0.04)

PYY = Peptide YY; EG = enteroglucagon; EGF = epidermal growth factor; IGF = insulin-like growth factor; TGF- α = transforming growth factor- α .

Significant difference vs. the control group receiving glutamine: ^a p < 0.05.

Significant difference vs. the transected group receiving glutamine: ^b p < 0.05.

Significant difference vs. the resected group not receiving glutamine: ^c p < 0.05.

Epidermal growth factor stimulates mucosal cell proliferation and promotes electrolyte and nutrient absorption. Enteroglucagon also stimulates small intestinal growth.

Increased Peptide YY slow GI transient and gastric emptying which would be beneficial after resection. Therefore, treatment with dietary glutamine would promote the nutrient absorption and small intestine growth by increasing peptide YY, epidermal growth factor, and enteroglucagon in the portal plasma and ileal mucosa.

The effects of dietary glutamine on mucosal regeneration were evaluated in rats with 80% mid-jejunoileal resection (J Clin Biochem Nutr; 1993; 15:219-225). The mucosal regeneration was determined by measuring the levels of bromodeoxyuridine labelling indices and alkaline phosphatase activity in the residual jejunal mucosa. Treatment with dietary glutamine (7.5 g/100 g) for 7 days significantly increased bromodeoxyuridine labelling indices and mucosal alkaline phosphatase activity as compared to the glutamine free diet. The results were presented in Table 4 in this publication and this table is attached below.

Table 4. Bromodeoxyuridine labelling indices of the residual jejunal epithelium and alkaline phosphatase activity in the homogenate of the residual jejunal mucosa from experimental rats fed a glutamine-free or a glutamine-enriched elemental diet for 7 consecutive days following 80% resection of the jejunioileum.

Indicator of mucosal regeneration	Glutamine-free elemental diet group	Glutamine-enriched elemental diet group	P
Bromodeoxyuridine labelling index	0.19 ± 0.02	0.32 ± 0.07	< 0.01
Alkaline phosphatase activity (International units/mg protein)	0.16 ± 0.03	0.24 ± 0.04	< 0.05

Values are expressed as mean ± SD from five rats in each experiment.

The bromodeoxyuridine labelling indices is an indicator of the regenerative ability of epithelium. The results suggest that dietary glutamine accelerates the regeneration of the small intestinal mucosa as indicated by increase in bromodeoxyuridine labelling indices. The mucosal alkaline phosphatase is a brush-border enzyme and its activity is elevated by fat feeding. Histopathological examination of the liver also revealed that the fat infiltration in the liver was more severe in the control group than that in the glutamine treatment group. The later findings imply that glutamine treatment prevents the liver from fat infiltration and this preventive effect may be related to the increased activity of alkaline phosphatase.

In another study (Can J Gastroenterol; 1994; 8(2):108-114), rats underwent anastomosis by removing 1 cm ileum and performing

end-to-end anastomosis. These rats were fed with either glutamine (4 g/kg/day) or glycine (4 g/kg/day) diets for 5 days before surgery and 2 days after surgery. The fluid absorption, morphometrics (villous height, villous number), glutaminase activity, DNA and protein content in ileum and jejunum were measured. The results indicated that treatment with glutamine significantly increased villus height, DNA:protein ratio, and glutaminase activity in jejunum but not in ileum as compared to the glycine treatment in the non-surgical animals (not in rats with anastomosis). The authors believed that the resection and anastomosis may cause transient impairment of the functions of the small intestine. The results indicated that oral supplement of glutamine produces beneficial effects in jejunum (proximal intestine) in the normal non-surgical rats.

Effects of glutamine and growth hormone in combination

To investigate the potential synergistic effects of glutamine and insuline-like growth factor-I (IGF-I), rats with 80% small bowel resected were fed diet with glutamine (28 g/100 g total amino acids) and treated subcutaneously with recombinant human IGF-I for 7 days (Ziegler et al, 1996; Am. J. Physiol 271:G866-875). DNA and protein contents in ilea and plasma and ileal weights were determined. The results indicated that glutamine and IGF-I each significantly and synergistically increased the DNA content in ilea and ileal weight. The results were presented in Table 2 and Figures 1 in this publication, which are attached below.

Table 2. Whole body weight change and ileum wet weight after small bowel resection

Study Group	Body Weight, g		Ileal Wet Weight/cm ²
	Initial	Change	
Control	258 ± 6	-5 ± 4	44 ± 2
Resected	261 ± 5	-18 ± 2*	99 ± 3*
Resected/IGF-I	252 ± 3	+2 ± 2*†	103 ± 6*
Resected/high Gln	253 ± 5	-12 ± 2*†‡	94 ± 5*
Resected/IGF-I + Gln	251 ± 6	-3 ± 1†‡	120 ± 6‡

See text for details of study group dietary and hormonal regimes. IGF-I, insulin-like growth factor-I. *P < 0.01 vs. control; †P < 0.01 vs. resected; ‡P < 0.01 vs. resected/IGF-I. §P < 0.01 vs. all other groups by Fisher's protected least squares difference test.

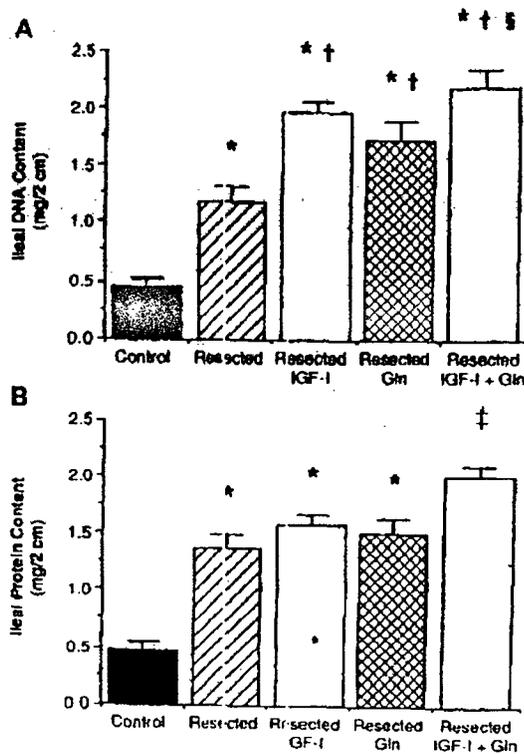


Fig. 1. Full-thickness DNA and protein content were determined in an ileal segment between 1 and 3 cm distal to the surgical anastomosis after 7 days of diet-hormone infusion. A: ileal DNA content per centimeter ($n = 8$ control group; $n = 7$ other groups) in the 5 experimental groups. See Dietary/hormonal regimens for explanation of groups. IGF-I, insulin-like growth factor-I; Gln, glutamine. * $P < 0.01$ vs. control; † $P < 0.01$ vs. resected; § $P < 0.05$ vs. resected/high Gln. B: ileal protein content per centimeter ($n = 8$ control group; $n = 7$ other groups) in the 5 experimental groups. * $P < 0.01$ vs. control; † $P < 0.01$ vs. all other groups.

The results suggest that combination of glutamine and IGF-I would synergistically enhance intestinal growth in resected small bowels.

In a study published in Journal of Surgical Research (Xin Zhou et al, 2001; 99:47-52), rats with 85% small bowel resected received liquid glutamine via a gastrotomy tube (20 g/l, 5 ml/rat) and growth hormone (0.3 IU, bid) subcutaneously 3 days after resection. Glutamine and growth hormone were given to rats for a total of 9 days. Growth hormone significantly increased body weight, jejunal and ileal villous height, mucosal thickness, and plasma level of insulin-like growth factor I. These parameters were not significantly altered by glutamine supplement. However, treatment with both hormone and glutamine

in combination produced further increases in these parameters. These results were presented in Figures 1, 2, and 3 in this publication. These figures are attached below.

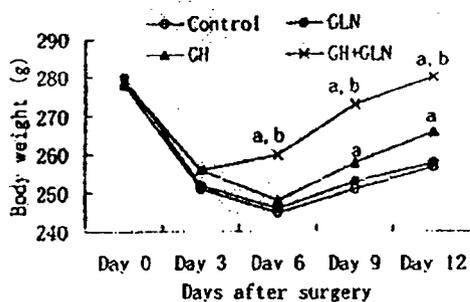


FIG. 1. Changes in body weight of rats with massive intestinal resection, receiving control luminal liquid diet, glutamine-enriched diet, growth hormone treatment, or combined glutamine supplementation and growth hormone treatment ($n = 8$ in each group). * $P < 0.05$ vs Control. * $P < 0.05$ vs GH.

APPEARS THIS WAY
ON ORIGINAL

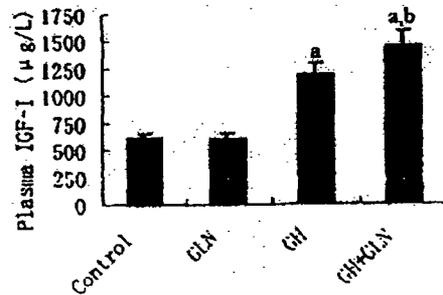


FIG. 2. Plasma insulin-like growth factor level on Day 12 in rats with massive intestinal resection, receiving control luminal liquid diet, glutamine-enriched diet, growth hormone treatment, or combined glutamine supplementation and growth hormone treatment ($n = 8$ in each group). * $P < 0.05$ vs Control. ^a $P < 0.05$ vs GH.

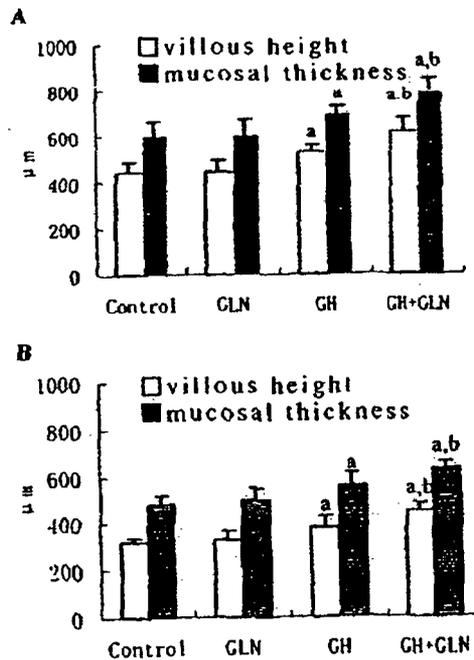


FIG. 3. Villous height and mucosal thickness of remnant jejunum (A) and ileum (B) in rats with massive intestinal resection, receiving control luminal liquid diet, glutamine-enriched diet, growth hormone treatment, or combined glutamine supplementation and growth hormone treatment ($n = 8$ in each group). * $P < 0.05$ vs Control. ^a $P < 0.05$ vs GH.

The results indicated that glutamine in combination with growth hormone produces synergistic trophic effects of the resected small intestine. The trophic actions of glutamine on resected

small intestine are certainly beneficial for patients with short bowel syndrome.

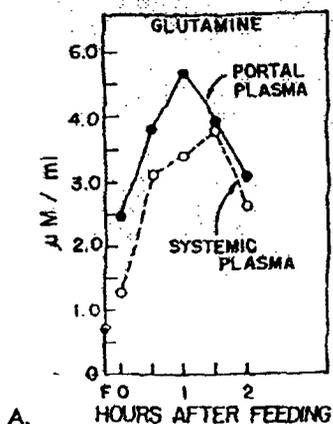
3.3 PHARMACOKINETICS

3.3.1 Brief summary

Glutamine level in the body is maintained by dietary intake and synthesis from endogenous glutamate. The typical daily dietary intake of glutamine is ~5-10 g. Oral absorption of glutamine was demonstrated with peak plasma level of glutamine reached at ~1-1.5 hours after dosing in rats. Glutamine was distributed in the liver, lung, kidney, heart, spleen, muscle, and brain following dietary or intravenous administration in rats. Glutamine is formed in the body through the condensation of a glutamate and an ammonia molecule by glutamine synthetase with hydrolysis of ATP. In the reverse process, glutaminase deaminated glutamine to glutamate and ammonia. Approximately 66% radioactivity (^{15}N) of glutamine was recovered in the urine following intravenous administration of radio-labeled glutamine in rats. Majority of the radioactivity (94%) was associated with urinary urea and only ~4% was as ammonia.

3.3.3 Absorption

The oral absorption of glutamine was demonstrated in rats following oral gavage of 20% aqueous suspension of glutamine (Archives of Biochemistry and Biophysics, 1962, 97:442-448). The results indicated that portal and systemic plasma levels of glutamine peaked at 1-1.5 hours after dosing and the portal plasma level of glutamine was higher than that in the systemic plasma. The results were presented in Figure 2A in this publication. This figure is attached below.



The percentage of oral absorption and the oral bioavailability were not determined in this study.

3.3.4 Distribution

The results from a study with dietary glutamine in rats indicated that increase in the glutamine level was observed in the liver, lung, kidney, heart, spleen, and muscle following dietary glutamine at 28.5 and 57 mg/day (Tigerman H., et al., 1950, the copy of the article was provided by the sponsor but the journal name, volume, and pages cannot be identified). The results were summarized in Table I in this publication. This table is attached below.

TABLE I
Effect of Ingested Glutamine upon Rat Tissue Glutamine from Eight Rats

Tissue	Glutamine, mg. per cent per group		
	Liver.....	98	102
Lung.....	82	83	107
Kidney.....	106	109	121
Heart.....	205	210	253
Spleen.....	167	188	199
Muscle.....	187	190	203
Glutamine ingested, gm. per kg. ration.....	0	1.9	3.8

The distribution of glutamine in the brain, liver, kidney, and muscle was also demonstrated following intravenous administration of glutamine at 7% in rats (Schwerin P., et al, 1950, the copy of the article was provided by the sponsor but the journal name, volume, and pages cannot be identified). The

results of this study showed that the highest level of glutamine was detected in the liver and kidney at 10 minutes after dosing. The glutamine level was also increased in the brain, muscle, and blood. The results were summarized in Table II in this study. This table is attached below.

TABLE II
Glutamate Acid (Acid) and Glutamine (Amide) Concentration in Rat Organs after Intravenous Injection of Glutamic Acid or Glutamine

Values expressed as mg. per 100 gm.

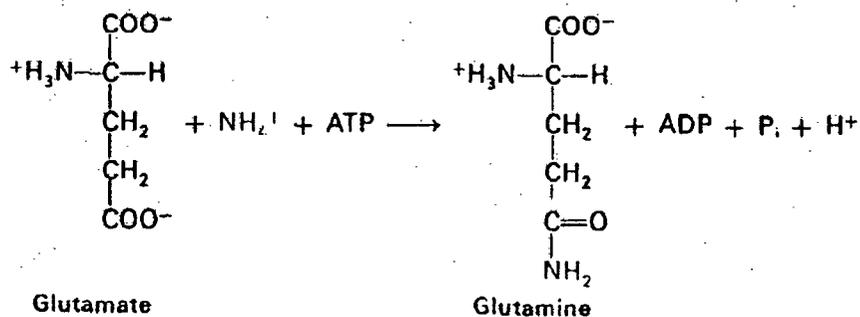
Min.	Brain		Liver		Muscle		Kidney		Blood	
	Acid	Amide	Acid	Amide	Acid	Amide	Acid	Amide	Acid	Amide
0*	152 ±16†	57 ±8.1	49 ±14	55 ±3.7	18 ±2.6	40 ±12	96 ±4.5	22 ±11	3 ±0.7	6.1 ±1.4
Glutamic acid administered										
10	120	65	94	38	40	35				
10	144	83	99	31	56	53	400	23	109	5.4
15	122	58	63	80	18	42	490	38	63	7.5
15	111	34	111	65	64	55	520	43	90	7.1
20	140	56	72	45						
20	158	62	114	44			424	39	85	6.6
30	116	37					510	47	68	6.2
60	131	67					260	40	4.8	4.3
Glutamine administered										
10	120	68	210	210	18	68				
15	161	78	370	92			219	99	6.1	32
15	165	85					146	239	6.7	86
20	140	84	300	73	13	53				
30	136	86	157	128			160	185	4.4	34
30	155	88					274	174	6.1	36
60	147	73					94	21	3.6	7.5

* Each average in this row represents six groups of three animals each, except for liver and muscle for which five and three groups were used, respectively.
 † Standard deviation. The bold-faced values differ from the control values significantly ($P < 0.05$) (10).

Glutamine can cross the placenta in rats.

3.3.5 Metabolism

Glutamine level in the body is maintained by dietary intake and synthesis from endogenous glutamate. Glutamine is formed in the body through the condensation of a glutamate and an ammonia molecule by glutamine synthetase with hydrolysis of ATP. In the reverse process, glutaminase deaminated glutamine to glutamate and ammonia. This process is depicted in the following figure.



The high level of glutamine synthetase activity is seen in the skeletal muscle where glutamine is stored and released to other tissues. Glutamine is mainly disposed through conversion to glutamate and then urea. It was demonstrated (Berenbom M, et. Al.; 1949, the copy of the article was provided by the sponsor but the journal name, volume, and pages cannot be identified) that approximately 66% radioactivity (^{15}N) of glutamine was recovered in the urine following intravenous administration of radio-labeled glutamine in rats. Majority of the radioactivity (94%) was associated with urinary urea and only ~4% was as ammonia.

3.3.6 Excretion

A radio-labeled study (Berenbom M, et. Al.; 1949, the copy of the article was provided by the sponsor but the journal name, volume, and pages cannot be identified) indicated that approximately 66% radioactivity (^{15}N) of glutamine was recovered in the urine following intravenous administration of radio-labeled glutamine in rats. Majority of the radioactivity (94%) was associated with urinary urea and only ~4% was as ammonia.

3.4 TOXICOLOGY

3.4.1 Overall toxicology summary

Sponsor did not conduct any toxicity studies with glutamine but provided the following study reports.

General toxicology:

3.4.2 Single-dose toxicity

Study title: Acute oral toxicity studies in mice, rats, and rabbits:

Key study findings: These studies were conducted by _____ LD₅₀ values were provided in a table in Volume 1.6 and this table is attached below. Signs of acute toxicity and minimal lethal dose were not identified in this report.

I. Acute toxicity:

Animal	Administration	Sex	LD ₅₀ value (g/kg)
Mouse	p.o.	male	21.7
		female	20.3
	i.v.	male	4.5
		female	4.5
Rat	p.o.	male	7.5
		female	10.5
Rabbit	p.o.	male	18.8

3.4.3 Repeat-dose toxicity

RAT:

Study title: 30-day oral toxicity study in rats



Key study findings: In this study, glutamine was given to rats by oral gavage at 4, 6, and 10 g/kg/day for 30 days. Glutamine was lethal in the high dose due to "administration of the test substance suspension in large volumes" ("physical problem"). Catarrh in the stomach was noted in the L-GM groups but not in the control group.

Methods: To evaluate the repeated dose toxicity of --110 (N-acetyl-L-glutamine aluminum complex) in rats, --110 was given to Sprague Dawley rats (10/sex/group) by oral gavage at 0, 0.4, 1, 2, 4, 6, and 10 g/kg/day for 4 weeks. --110 was developed as a drug for treatment of peptic ulcers. L-glutamine (L-GM) was used as a control in this study. L-GM was given to rats (10/sex/group) by oral gavage at 4, 6, and 10 g/kg/day for 30 days. Animals were not treated on Sunday. L-GM was suspended in 5% aqueous gum arabic solution at 25-50% and volume of 0.16-2 ml/100 g body weight was given by oral gavage. In this study, animals were observed daily. Body weight, food and water consumption were determined every 3 days. Hematology, clinical chemistry, and urinalysis were conducted at the termination. All animals were necropsied at termination and organ weights were determined. Histopathology was conducted in all animals. It is noticed that the data from glutamine dose group of 6 g/kg/day and --110 dose group of 1 g/kg/day were not presented in the tables in the result section.

Results:

1. Clinical Signs/Mortality: Two females and one male treated with L-GM at 10 g/kg/day died due to administration of the test substance suspension in large volumes. "Physical problems" were noted in these animals.

2. Body Weights: Sponsor provided mean body weight values during the 30-day period. The mean body weight was 250 g, 251 g, and 240 g (males) and 188 g, 191 g, and 192 g (females) in the 4, 6 and 10 g/kg L-GM groups, respectively. The mean body weight was 259 g for males and 190 g for females in the control group. The mean body weight was about 7% lower in the high dose males as compared to the control.

3. Food and Water Consumption: The food consumption was slightly lower in the high dose L-GM males (19.6 g/rat/day) as compared to the control (22.1 g/rat/day). There were no treatment related changes in water consumption.

4. Hematology: There were no treatment related changes in L-GM groups. The results were presented in Tables 6 and 7 in this publication and these tables are attached below.

Table 6 Subacute toxicity of 110 in male rats administered orally
Hematological test

Day of exam.		30 days								
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.	10.
		Control D.W.	110 0.4 g/kg	110 2.0 g/kg	110 4.0 g/kg	110 8.0 g/kg	110 16.0 g/kg	110 32.0 g/kg	L-GM 4.0 g/kg	L-GM 10.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5	5
R.B.C. ($10^6/mm^3$)	Mean S.D.	670. 74.	702. 46.	696. 25.	709. 69.	669. 32.	662. 61.	695. 26.	725. 23.	
Hematocrit (percent)	Mean S.D.	44.8 1.7	44.6 2.1	43.8 1.2	43.9 3.2	43.6 1.5	43.0 2.4	44.4 1.0	45.6 1.4	
Hemoglobin (g/dl)	Mean S.D.	16.1 0.3	16.2 0.4	15.6 0.6	15.0 1.2	15.2* 0.6	15.3* 0.7	15.9 0.2	16.0 0.5	
W.B.C. ($10^3/mm^3$)	Mean S.D.	115.2 43.4	103.4 24.1	111.0 9.9	124.8 26.7	120.0 31.6	100.8 12.4	94.8 10.4	91.2 6.8	
Hemogram Lymphocyte (percent)	Mean S.D.	86.5 3.2	88.1 1.0	87.0 2.6	73.2 11.7	74.2 13.2	84.7 7.0	83.7 4.4	83.3 6.8	
Monocyte (percent)	Mean S.D.	3.4 1.2	2.2 1.0	2.8 1.0	3.9 0.8	2.3 1.0	2.1 1.0	2.4 1.2	2.6 1.4	
Eosinophile (percent)	Mean S.D.	0.7 0.7	0.3 0.4	1.0 0.8	0.5 0.4	0.7 0.4	0.2 0.2	0.4 0.6	0.3 0.2	
Basophile (percent)	Mean S.D.	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.0	0.0 0.9	0.0 0.0	0.0 0.0	
Neutrophile Total (percent)	Mean S.D.	9.5 2.8	9.4 0.7	9.3 1.7	22.4* 11.5	23.2 12.2	12.9 6.5	13.5 3.7	14.0 7.8	
Band (percent)	Mean S.D.	1.9 1.9	2.1 1.2	1.4 1.4	2.0 0.9	1.8 1.0	1.1 1.0	4.3 3.9	3.8 3.0	
Segment (percent)	Mean S.D.	7.6 1.4	7.3 1.2	7.8 2.7	20.4* 12.5	21.4 11.9	11.9 6.7	9.2 1.9	10.0 6.7	
Blood plate ($10^3/mm^3$)	Mean S.D.	72. 17.	72. 10.	71. 8.	88. 18.	83. 18.	80. 11.	80. 8.	78. 8.	
Prothrombin time (second)	Mean S.D.	20.3 1.6	20.3 2.0	20.7 2.9	21.2 1.6	21.2 1.4	23.3 2.6	22.1 1.7	23.0 4.9	

** significantly different from control data ($p < 0.01$)
 * significantly different from control data ($p < 0.05$)
 S.D. Standard deviation
 P.B.C. (Red blood cell)
 W.B.C. (White blood cell)

Table 7 Subacute toxicity of —110 in female rats administered orally
Hematological test

Day of Exam.		30 days								
Group dose		1.	2.	3.	4.	5.	6.	7.	8.	10.
Item		Control D.W.	—110 0.4 g/kg	—110 2.0 g/kg	—110 4.0 g/kg	—110 8.0 g/kg	—110 16.0 g/kg	L-GM 4.0 g/kg	L-GM 10.0 g/kg	
No of animal exam.		5	5	5	5	5	5	5	5	5
R. B. C. (10 ⁶ /mm ³)	Mean	643.	596.	599.	664.	605.	622.	694.	664.	
	S. D.	48.	21.	17.	42.	70.	18.	51.	40.	
Hematocrit (percent)	Mean	42.8	38.2	40.0	42.0	40.6	40.6	43.4	42.2	
	S. D.	3.7	2.0	2.6	1.5	3.4	1.6	3.1	2.6	
Hemoglobin (g/dl)	Mean	15.2	13.3*	14.0	14.5	13.7	14.1	15.2	14.6	
	S. D.	0.9	0.9	1.2	0.8	0.9	0.9	1.3	0.9	
W. B. C. (10 ⁶ /mm ³)	Mean	79.6	71.8	76.0	81.0	79.4	72.0	82.6	74.0	
	S. D.	17.7	14.6	14.4	13.4	14.9	14.9	17.4	13.9	
Hemogram Lymphocyte (percent)	Mean	85.8	87.5	89.5	87.5	87.0	82.4	89.6	84.9	
	S. D.	5.5	3.4	3.7	6.7	5.2	4.3	0.7	6.3	
Monocyte (percent)	Mean	2.2	2.6	2.2	2.4	3.0	2.6	1.3	1.4	
	S. D.	1.4	1.6	0.7	2.4	1.6	1.2	1.0	1.0	
Eosinophile (percent)	Mean	1.0	0.6	1.3	0.3*	0.3*	0.5	0.9	0.5	
	S. D.	0.5	0.6	0.9	0.2	0.2	0.5	0.7	0.5	
Basophile (percent)	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	S. D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
Neutrophile (percent)	Mean	11.1	9.3	7.5	9.8	9.7	14.5	8.2	13.2	
	S. D.	3.8	4.0	3.4	4.3	3.7	4.5	0.9	5.7	
Band (percent)	Mean	3.1	1.9	2.4	2.4	2.1	3.3	2.5	3.7	
	S. D.	1.2	1.5	1.5	1.8	2.0	1.5	1.2	3.1	
Segment (percent)	Mean	8.0	7.4	5.1	7.4	7.6	11.2	5.7	9.5	
	S. D.	3.8	2.6	2.7	4.4	3.4	3.6	1.2	4.6	
Blood plate (10 ⁶ /mm ³)	Mean	78.	80.	75.	79.	67.	67.	96.	88.	
	S. D.	8.	14.	18.	16.	8.	26.	22.	13.	
Prothrombin time (second)	Mean	20.9	21.4	20.9	20.7	22.5	23.4	21.5	21.9	
	S. D.	1.5	1.4	1.5	0.5	3.3	3.1	1.9	1.9	

** significantly different from control data ($p < 0.01$)* significantly different from control data ($p < 0.05$)

S. D. Standard deviation

R. B. C. (Red blood cell)

W. B. C. (White blood cell)

5. Clinical Chemistry: Decrease in bromfulein retention time in the L-GM groups in the bromsulfalein retention test (liver function test) was not clinically meaningful. All other liver function tests were normal in the L-GM groups. There were no clear treatment related changes in other parameters in the L-GM groups. The results were summarized in Tables 8 and 9. These tables are attached below.

Table 8 Subacute toxicity of ---110 in male rats administered orally
Blood chemical test

Day of exam.		30 days								
Group dose		1.	110		110		110		8.	10.
Item		Control D. W.	0.4 g/kg	2.0 g/kg	4.0 g/kg	6.0 g/kg	10.0 g/kg	4.0 g/kg	10.0 g/kg	L-GM
No of animal exam.		5	5	5	5	5	5	5	5	5
Total protein (g/dl)	Mean S. D.	6.3 0.2	6.1 0.1	5.5** 0.1	5.5** 0.2	5.4** 0.1	5.3** 0.4	6.0 0.3	6.1 0.1	
A/G	Mean S. D.	0.92 0.05	1.05** 0.06	1.04** 0.04	1.07* 0.10	1.05* 0.05	1.21* 0.08	0.99 0.07	0.03 0.10	
AL.P. (K.A. unit)	Mean S. D.	15.2 3.0	16.3 1.7	13.7 1.6	12.8 1.7	15.8 1.4	20.7 3.8	17.4 2.7	18.2 4.8	
S-G.P.T. (I.U.)	Mean S. D.	32.4 4.3	31.6 2.9	28.4 3.7	34.0 10.5	42.4 27.1	35.2 13.5	30.4 4.5	35.2 18.7	
S-G.O.T. (I.U.)	Mean S. D.	130.4 19.4	138.4 19.3	133.6 8.7	148.0 23.8	158.0 51.8	146.4 21.8	134.4 20.2	136.8 17.9	
B.S.P. (percent)	Mean S. D.	4.2 1.3	4.2 0.8	5.0 1.8	3.5 1.0	6.2 3.0	4.4 1.9	6.1 1.7	4.0 2.0	
Blood sugar (mg/dl)	Mean S. D.	142 10.	151. 8.	115. 13.	89. 13.	96. 21.	103. 21.	141. 5.	159. 31.	
B.U.N. (mg/dl)	Mean S. D.	13.3 1.5	15.5 1.6	15.5 1.4	21.1** 3.1	21.0* 5.1	24.6** 4.0	15.1 1.8	14.3 1.6	
N.P.N. (mg/dl)	Mean S. D.	34.0 1.9	36.2 3.1	38.0 3.0	41.0** 0.9	42.2** 1.6	42.2** 3.0	36.2 1.7	34.4 2.2	
Serum electrolyte Sodium (mEq/l)	Mean S. D.	139.4 1.0	139.0 0.6	139.4 0.5	138.6 0.5	138.2 0.4	139.0 1.1	138.6 0.5	139.2 1.6	
Potassium (mEq/l)	Mean S. D.	5.28 0.13	5.8 0.37	5.22 0.34	5.50 0.35	5.28 0.48	5.00 0.23	5.20 0.14	5.36 0.22	

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S. D. standard deviation

A/G (Albumin/Globulin)

AL.P. (Alkaline phosphatase)

S-G.P.T. (Serum glutamic pyruvic transaminase)

S-G.O.T. (Serum glutamic oxalacetic transaminase)

B.S.P. (Bromsulfalein retention test)

B.U.N. (Blood urea nitrogen)

N.P.N. (Nonprotein nitrogen)

Shown in Tables 8 and 9.

[00977] Table 9 Subacute toxicity of -110 in female rats administered orally
Blood chemical test

Day of exam.		30 days							
Group dose		1. Control	2. -110	3. -110	4. -110	5. -110	6. -110	8. L-GM	10. L-GM
Item		D.W.	0.4 g/kg	2.0 g/kg	4.0 g/kg	6.0 g/kg	10.0 g/kg	4.0 g/kg	10.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Total protein (g/dl)	Mean	5.9	6.2	5.5*	5.3*	5.1*	5.4	6.0	6.0
	S. D.	0.2	0.2	0.3	0.4	0.4	0.4	0.1	0.2
A/G	Mean	1.10	0.98	1.01	1.04	1.11	1.23	1.06	1.01
	S. D.	0.06	0.10	0.08	0.11	0.12	0.10	0.07	0.08
AL. P. (K. A. unit)	Mean	9.1	8.0	9.0	10.2	10.8	15.8	9.4	11.8
	S. D.	3.0	2.1	1.4	4.0	1.1	5.5	1.9	2.6
S-G. P. T. (I. U.)	Mean	28.0	24.0	26.0	29.2	31.2	32.0	19.6	17.6
	S. D.	3.2	5.7	4.2	5.2	8.8	8.3	9.2	8.1
S-G. O. T. (I. U.)	Mean	133.5	125.6	136.0	137.2	145.2	146.8	139.2	116.8
	S. D.	12.4	15.0	20.5	37.3	20.1	13.8	17.1	32.2
B. S. P. (Percent)	Mean	3.8	2.8	1.8**	1.2**	2.0*	3.6	2.3*	1.3**
	S. D.	0.6	0.7	0.8	0.2	1.0	1.9	0.9	0.1
Blood sugar (mg/dl)	Mean	117.	129.	107.	103.	102.	91.*	127.	142.*
	S. D.	17.	15.	12.	10.	13.	4.	22.	12.
B. U. N. (mg/dl)	Mean	13.9	14.8	14.3	18.5	19.8*	21.4**	16.6	13.8
	S. D.	0.8	1.2	1.8	4.6	3.9	3.4	2.8	2.8
N. P. N. (mg/dl)	Mean	33.8	32.8	35.2	36.4	39.0*	40.0**	36.2	33.8
	S. D.	2.9	2.7	7.9	7.7	1.4	2.0	3.5	2.0
Serum electrolyte sodium (mEq/l)	Mean	138.8	139.0	138.8	138.8	139.0	137.2	138.8	138.2
	S. D.	0.8	0.6	0.8	1.2	0.6	1.3	0.8	0.8
Potassium (mEq/l)	Mean	5.00	5.22	5.28	5.22	5.44	5.32	5.08	5.26
	S. D.	0.23	0.07	0.28	0.33	0.35	0.84	0.70	0.43

** Significantly different from control data (p < 0.01)

* Significantly different from control data (p < 0.05)

S. D. Standard deviation

A/G (Albumin/Globulin)

AL. P. (Alkaline phosphatase)

S-G. P. T. (Serum glutamic pyruvic transaminase)

S-G. O. T. (Serum glutamic oxalacetic transaminase)

B. S. P. (Bromsulfalein retention test)

B. U. N. (Blood urea nitrogen)

N. P. N. (Nonprotein nitrogen)

6. Urinalysis: There were no treatment related changes in L-GM groups. The results were summarized in Tables 10 and 11. These tables are attached below.

Table 10 Subacute toxicity of -110 in male rats administered orally
Urine test

Day of exam.		30 days								
Group dose		1. Control	2. -110	3. -110	4. -110	5. -110	6. -110	8. L-GM	10. L-GM	
Item		D.W.	0.4 g/kg	2.0 g/kg	4.0 g/kg	6.0 g/kg	10.0 g/kg	4.0 g/kg	10.0 g/kg	
No. of animal exam.		5	5	5	5	5	5	5	5	
Occult blood	Mean	+	-	-	-	-	-	+	-	
Ketone	Mean	-	-	-	-	-	-	-	-	
Glucose	Mean	-	-	-	-	-	-	-	+	
Protein (mg/dl)	Mean	19.	47.	38.	39.	47.	33.	6.	66.	
	S.D.	14.	44.	33.	32.	44.	36.	12.	118.	
pH	Mean	7.9	7.6	8.2	8.5	8.6	8.7*	7.7	7.9	
	S.D.	0.5	0.5	0.7	0.4	0.4	0.4	0.4	0.8	
SP GR.	Mean	1.02	1.04	1.03	1.04*	1.05*	1.03*	1.02	1.05	
	S.D.	0.01	0.03	0.01	0.01	0.02	0.01	0.01	0.04	

** Significantly different from control data ($p < 0.01$)
 * Significantly different from control data ($p < 0.05$)
 S.D. standard deviation
 Scale for occult blood and ketone by labstix.
 --negative, +=small, #=moderate, ##=large.
 Scale for glucose by Labstix
 --negative, +=light, #=medium, ##=dark.

Table 11 Subacute toxicity of -110 in female rats administered orally
Urine test

Day of exam.		30 days								
Group dose		1. Control	2. -110	3. -110	4. -110	5. -110	6. -110	8. L-GM	10. L-GM	
Item		D.W.	0.4 g/kg	2.0 g/kg	4.0 g/kg	6.0 g/kg	10.0 g/kg	4.0 g/kg	10.0 g/kg	
No. of animal exam.		5	5	5	5	5	5	5	5	
Occult blood	Mean	+	-	+	+	-	-	-	-	
Ketone	Mean	-	-	-	-	-	-	-	-	
Glucose	Mean	-	-	-	-	-	-	-	-	
Protein (mg/dl)	Mean	27.	6.	0.	0.	0.	0.	0.	0.	
	S.D.	38.	12.	0.	0.	0.	0.	0.	0.	
pH	Mean	8.0	7.7	8.7	8.6	8.8*	8.5	8.4	7.9	
	S.D.	0.5	0.6	0.4	0.4	0.2	0.4	0.4	1.0	
SP GR.	Mean	1.03	1.04	1.02	1.03	1.02	1.03	1.05	1.05	
	S.D.	0.01	0.01	0.00	0.01	0.01	0.02	0.02	0.02	

** Significantly different from control data ($p < 0.01$)
 * Significantly different from control data ($p < 0.05$)
 S.D. Standard deviation
 Scale for occult blood and ketone by labstix.
 --negative, +=small, #=moderate, ##=large.
 Scale for glucose by labstix
 --negative, +=light, #=medium, ##=dark.

7. Organ Weights: There were no obvious treatment related changes in L-GM groups. The results were presented in Tables 12 and 13. These tables are attached below.

Table 12 Subacute toxicity of — 110 in male rats administered orally
Organ weight (g)

Day of exam.		30 days							
Group dose		1. Control	2. -110	3. -110	4. -110	5. -110	6. -110	8. L-GM	10. L-GM
Item		D.W.	0.4 g/kg	2.0 g/kg	4.0 g/kg	6.0 g/kg	10.0 g/kg	4.0 g/kg	10.0 g/kg
No. of Animal exam.		5	5	5	5	5	5	5	5
Heart	Mean	1.20	1.17	1.10	1.00*	0.96*	0.82**	1.23	1.27
	S. D.	0.12	0.09	0.10	0.04	0.08	0.09	0.16	0.09
Lung	Mean	1.30	1.08*	1.15	1.35	1.19	0.98**	1.17	1.28
	S. D.	0.15	0.05	0.11	0.38	0.15	0.10	0.09	0.18
Spleen	Mean	0.93	0.72*	0.77	0.68*	0.61**	0.52**	0.78	0.70*
	S. D.	0.12	0.10	0.08	0.09	0.06	0.12	0.11	0.07
Liver	Mean	11.34	11.45	11.17	10.08	8.84**	8.21**	11.23	11.96
	S. D.	1.01	0.99	0.78	1.07	0.70	0.90	1.07	0.74
Kidney (right)	Mean	1.26	1.25	1.22	1.07*	0.95**	0.89**	1.31	1.30
	S. D.	0.07	0.09	0.06	0.11	0.08	0.13	0.15	0.02
Kidney (left)	Mean	1.29	1.25	1.23	1.05**	1.00**	0.92**	1.28	1.29
	S. D.	0.05	0.10	0.08	0.10	0.04	0.16	0.12	0.05
Thymus	Mean	0.56	0.54	0.52	0.46*	0.34*	0.33**	0.53	0.56
	S. D.	0.03	0.05	0.10	0.07	0.13	0.09	0.12	0.09
Brain	Mean	1.54	1.52	1.49	1.46	1.45	1.41*	1.57	1.63
	S. D.	0.06	0.04	0.10	0.05	0.10	0.09	0.10	0.08
Adrenal (right)	Mean	0.023	0.024	0.028	0.031*	0.028	0.033*	0.027	0.029*
	S. D.	0.004	0.004	0.005	0.004	0.005	0.006	0.004	0.002
Adrenal (left)	Mean	0.023	0.024	0.028	0.031*	0.028	0.033*	0.027	0.029*
	S. D.	0.004	0.004	0.005	0.004	0.005	0.006	0.004	0.002
Sex organ (right)	Mean	1.25	1.32	1.21	1.24	1.20	1.10	1.28	1.29
	S. D.	0.07	0.02	0.04	0.05	0.02	0.15	0.06	0.02
Sex organ (left)	Mean	1.26	1.33	1.23	1.25	1.23	1.11*	1.31	1.31
	S. D.	0.07	0.05	0.04	0.05	0.03	0.10	0.02	0.02

** Significantly different from control data ($p < 0.01$)
* Significantly different from control data ($p < 0.01$)
S. D. Standard deviation

Table 13. Subacute toxicity of -110 in female rats administered orally
Organ weight (g)

Day of exam.		30 days								
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.	9.
		Control D.W.	-110 0.4 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	-110 6.0 g/kg	-110 10.0 g/kg	-110 4.0 g/kg	-110 10.0 g/kg	L-GM 4.0 g/kg
No. of animal exam.		5	5	5	5	5	5	5	5	5
Heart	Mean S. D.	0.92 0.14	0.89 0.10	0.88 0.09	0.92 0.11	0.82 0.11	0.81 0.09	0.98 0.17	1.06 0.12	
Lung	Mean S. D.	1.04 0.09	1.09 0.10	1.03 0.13	1.14 0.10	1.11 0.13	1.02 0.18	1.10 0.14	1.42* 0.22	
Spleen	Mean S. D.	0.69 0.10	0.59 0.07	0.67 0.21	0.76 0.09	0.59 0.11	0.62 0.17	0.71 0.10	0.76 0.19	
Liver	Mean S. D.	7.38 0.47	7.74 0.66	8.43* 0.46	8.92* 0.83	8.11 0.83	7.80 1.08	7.95 0.67	9.43* 1.38	
Kidney (right)	Mean S. D.	0.76 0.03	0.76 0.03	0.78 0.04	0.82 0.04	0.77 0.09	0.77 0.07	0.83 0.07	0.95 0.19	
Kidney (left)	Mean S. D.	0.78 0.05	0.76 0.09	0.79 0.09	0.79 0.04	0.77 0.07	0.78 0.08	0.84 0.05	0.92 0.18	
Thymus	Mean S. D.	0.51 0.06	0.36 0.11	0.37* 0.05	0.37* 0.06	0.36* 0.06	0.34** 0.06	0.53 0.08	0.53 0.09	
Brain	Mean S. D.	1.47 0.11	1.53 0.05	1.46 0.10	1.45 0.05	1.52 0.12	1.39 0.05	1.52 0.02	1.50 0.09	
Adrenal (right)	Mean S. D.	0.028 0.002	0.029 0.002	0.030 0.000	0.033 0.003	0.032* 0.002	0.045 0.016	0.035* 0.005	0.038 0.010	
Adrenal (left)	Mean S. D.	0.028 0.002	0.028 0.002	0.030 0.000	0.033 0.008	0.032* 0.002	0.045 0.016	0.035* 0.005	0.038 0.010	
Sex organ (right)	Mean S. D.	0.057 0.017	0.057 0.020	0.062 0.009	0.062 0.010	0.049 0.011	0.040 0.008	0.060 0.016	0.067 0.010	
Sex organ (left)	Mean S. D.	0.057 0.017	0.057 0.020	0.062 0.009	0.062 0.010	0.049 0.011	0.040 0.008	0.060 0.016	0.068 0.009	

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S. D. Standard deviation

8. Gross Pathology: There were no treatment related changes in L-GM groups.

9. Histopathology: Catarrh in the stomach was noted in the L-GM and -110 groups but not in the control group. In addition, infiltration of inflammatory cell and edema in gastric submucosa and intestinal metaplasia were found in the -110 group but not in the control and L-GM groups. The results were summarized in Table 16-1. This table is attached below.

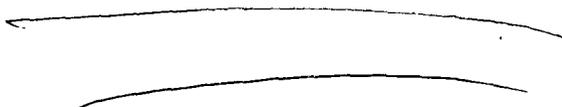
Table 16-1 Subacute toxicity of α -110 in rats administered orally
 Histopathological changes and the number of animals observed lesions

Days of exam.	30 days							
	Control	α -110 (g/kg)					L-GM (g/kg)	
		0.4	2.0	4.0	6.0	10.0	4.0	10.0
Number of animals exam.	5 5	5 5	5 5	5 5	5 5	5 5	5 5	5 5
Spleen								
Slight congestion	2 4	1 1	3 3	2 3	2 3	5 5	2 4	5 3
Slight proliferation of reticulum cell	4 5	3 4	3 5	5 3	3 5	5 5	5 5	5 5
Liver								
Slight fatty infiltration in liver cell	4 3	5 4	4 5	4	2	2 2	3 5	4 4
Slight infiltration of round cell in Glisson's capsule	1 1				1	1		1
Kidney								
Slight vacuolation of tubular epithelium	4 4	1 3	2 5	3 5	5 4	4 5	1 4	3 5
Slight hyalin drop degeneration	3	3	1					
Slight protein cast	2			2		2		
Stomach								
Catarrh								
trace		1		1	1		2	1
slight				1 1		2 1	1	1
moderate			1		1	1	1	
remarkable			2	1	2	1 1		1
Infiltration of inflammatory cell in submucosa								
trace			2	1	1 1	1		
slight			1		1	1		
moderate			1	1	1	3 1		
remarkable			4 1	1 4	1 4	1 2		

Days of exam.	30 days							
	Control	-110 (g/kg)					L-GM (g/kg)	
		0.4	2.0	4.0	6.0	10.0	4.0	10.0
Edema in submucosa								
trace		1				1		
slight		2 1	3		2 3	1		
moderate			1			1		
Intestinal metaplasia								
trace			2		1 1	1 2		
slight								
moderate						1 1		
remarkable						2		
Thymus Slight proliferation of reticulum cell		1			1 3	1		

Blank=0

Study title: 180-day oral toxicity study in rats



Key study findings: In this study, L-GM was given to rats by oral gavage at 2 and 4 g/kg/day for 180 days. Catarrh in the stomach and infiltration of inflammatory cell and edema in gastric submucosa were identified in the both glutamine groups but not in control group. The catarrh in the stomach was noted on both days 90 and 180.

Methods: To evaluate the repeated dose toxicity of -110 (N-acetyl-L-glutamine aluminum complex) in rats, -110 was given to Sprague Dawley rats (11/sex/group) by oral gavage at 0, 0.2, 0.4, 1, 2, and 4 g/kg/day for 180 days. L-glutamine (L-GM) was used as a control in this study. L-GM was given to rats (11/sex/group) by oral gavage at 2 and 4 g/kg/day for 180 days.

L-GM was suspended in 5% aqueous gum arabic solution at 50% and volume of 0.2-0.8 ml/100 g body weight was given by oral gavage. Animals were not treated on Sunday. In this study, animals were observed daily. Body weight, food and water consumption were determined every 3 days. Three animals per sex per group were sacrificed on days 90, and 210 and 5 animals per sex per group were sacrificed on day 180. Hematology, clinical chemistry, and urinalysis were conducted on days 90, 180, and 210 after treatment. All animals were necropsied at scheduled termination. Organ weights were determined on day 180. Histopathology was conducted in all animals.

Results:

1. Clinical Signs: There were no treatment related changes in L-GM group and there were no death in this study.
2. Body Weights: Sponsor provided mean body weight values during the 180-day period. The mean body weight was 453 g and 455 g (males) and 288 g and 284 g (females) in the 2 and 4 g/kg L-GM groups, respectively. The mean body weight was 463 g for males and 299 g for females in the control group. The mean body weight was about 5% lower in the high dose females as compared to the control.
3. Food and Water Consumption: The food consumption was slightly lower in the high dose L-GM females (14.7 g/rat/day) as compared to the control (16.1 g/rat/day).
4. Hematology: Slight decrease in hematocrit (10-11%) was noted in the high dose males as compared to the control. There were no clear treatment related changes in other parameters in the L-GM groups. The results were summarized in Tables 6, 7, 8, and 9. These tables are attached below.

Table 8 Chronic toxicity of -110 in male rats administered orally (90 days)

Hematological test

Day of exam.		90 days								
		Group dose		1.	2.	3.	4.	5.	6.	7.
Item		Control	-110	-110	-110	-110	-110	-110	L-GM	L-GM
		D.W.	0.2 g/kg	0.4 g/kg	1.0 g/kg	2.0 g/kg	4.0 g/kg	2.0 g/kg	4.0 g/kg	
No of animal exam.		3	3	3	3	3	3	3	3	3
R.B.C.	Mean	779	803	780	811	790	801	802	758	
(10 ⁶ /mm ³)	S.D.	25	51	4	43	11	35	10	15	
Hematocrit	Mean	45.3	46.0	44.7	44.0	45.0	45.0	45.0	41.3*	
(percent)	S.D.	0.5	0.8	0.9	1.4	0.0	0.5	2.2	1.9	
Hemoglobin	Mean	15.7	16.5	15.1	15.5	15.4	15.7	15.8	15.1	
(g/dl)	S.D.	0.3	0.5	0.4	0.5	0.6	0.8	0.1	0.4	
W.B.C.	Mean	154.0	120.7	184.0	147.3	129.0	145.3	142.3	124.3	
(10 ⁶ /mm ³)	S.D.	43.5	6.1	56.7	35.1	14.9	12.8	10.4	24.6	
Hemogram										
Lymphocyte	Mean	88.5	80.8	58.8	77.3	76.2*	80.5	72.5**	84.3	
(percent)	S.D.	1.6	7.0	18.7	5.7	4.7	5.6	2.9	3.8	
Monocyte	Mean	1.7	1.8	3.0	3.0	3.7	3.7	3.2	3.5	
(percent)	S.D.	0.9	0.8	1.4	2.0	2.7	2.5	3.1	1.1	
Eosinophile	Mean	0.5	0.0	0.3	0.7	0.5	1.3	0.7	0.2	
(percent)	S.D.	0.4	0.0	0.2	0.6	0.4	0.5	0.6	0.2	
Basophile	Mean	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	
(percent)	S.D.	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	
Neutrophile										
Total	Mean	9.3	17.3	37.8	3.5*	19.5**	14.5	23.8**	12.0	
(percent)	S.D.	0.6	7.6	19.2	1.9	2.3	5.2	1.2	3.5	
Band	Mean	2.5	3.2	5.3	3.5	2.3	1.8	4.0	0.8**	
(percent)	S.D.	0.4	1.0	2.8	1.9	1.9	1.3	1.5	0.2	
Segment	Mean	6.8	14.2	32.5	15.5**	17.2**	12.7	19.8**	11.2	
(percent)	S.D.	1.0	6.7	17.4	1.8	1.0	4.2	2.5	3.3	
Blood plate	Mean	93	106	95	94	93	104	110	80	
(10 ⁶ /mm ³)	S.D.	1	9	17	8	5	17	22	14	
Prothrombin time	Mean	17.1	17.1	18.3	16.5	16.4	13.8**	17.1	17.3	
(second)	S.D.	0.5	0.9	1.7	0.6	0.5	0.8	1.6	1.4	

** Significantly different from control data (p<0.01)

* Significantly different from control data (p<0.05)

S.D. - Standard deviation

R.B.C. (red blood cell)

W.B.C. (white blood cell)

Table 7 Chronic toxicity of —-110 in female rats administered orally (90 days)

Hematological test

Day of exam.		90 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	—110 0.2 g/kg	—110 0.4 g/kg	—110 1.0 g/kg	—110 2.0 g/kg	—110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		3	3	3	3	3	3	3	3
R.B.C.	Mean	595	722	717	673	697	691	706	685
(10 ⁶ /mm ³)	S.D.	15	10	17	25	5	15	12	5
Hematocrit	Mean	40.7	41.3	41.0	40.7	40.7	40.7	41.0	39.7
(percent)	S.D.	0.5	0.5	0.8	0.9	0.5	0.5	0.8	0.9
Hemoglobin	Mean	14.8	16.3*	14.9	14.4	14.3	14.1	14.9	14.7
(g/dl)	S.D.	0.6	0.1	0.5	0.7	0.4	0.6	0.2	0.1
W.B.C.	Mean	109.0	81.0	126.0	95.3	112.7	101.7	102.7	100.0
(10 ³ /mm ³)	S.D.	23.0	6.2	16.6	5.4	10.5	7.3	13.2	19.0
Hemogram									
Lymphocyte	Mean	77.5	78.8	78.5	80.5	75.7	83.2	78.8	77.7
(percent)	S.D.	1.9	3.0	2.1	1.9	8.0	5.2	1.4	4.3
Monocyte	Mean	1.8	2.3	1.8	1.2	1.7	0.7	1.8	2.3
(percent)	S.D.	0.8	1.0	0.2	0.5	0.6	0.6	0.6	1.4
Eosinophile	Mean	1.7	0.7	1.5	0.8	1.5	0.7	0.2*	0.8
(percent)	S.D.	0.6	0.2	1.1	0.8	0.7	0.9	0.2	0.8
Basophile	Mean	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
(percent)	S.D.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neutrophile									
Total	Mean	19.0	16.5	17.8	17.5	21.2	15.5	19.2	19.2
(percent)	S.D.	2.9	2.9	2.9	2.5	7.6	4.9	1.2	3.8
Band	Mean	4.0	3.7	2.0*	2.2	2.8	1.3*	2.7	2.8
(percent)	S.D.	0.8	0.8	0.4	2.1	1.2	0.2	1.0	2.2
Segment	Mean	15.0	12.8	15.8	15.3	18.3	14.2	16.5	16.3
(percent)	S.D.	2.1	3.5	2.5	2.5	6.3	4.8	1.5	1.5
Blood plate	Mean	101	110	96	88	89	96	91	76
(10 ³ /mm ³)	S.D.	10	5	16	19	10	12	10	12
Prothrombin time	Mean	18.7	22.0	18.9	20.7	18.8	21.0	20.9	19.9
(second)	S.D.	1.7	7.1	2.1	0.8	2.0	2.4	2.7	4.9

** Significantly different from control data (p<0.01)

* Significantly different from control data (p<0.05)

S.D. = Standard deviation

R.B.C. (red blood cell)

W.B.C. (white blood cell)

Table 8 Chronic toxicity of ___-110 in male rats administered orally (180 days)

Hematological test

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	-110 0.2 g/kg	110 0.4 g/kg	110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
R.B.C. (10 ⁶ /mm ³)	Mean S.D.	765 85	876 100	752 39	718 40	636* 29	626 89	654* 38	668 36
Hematocrit (percent)	Mean S.D.	46.4 1.0	45.8 1.5	45.8 1.2	43.4* 1.9	39.4** 1.7	37.8** 3.9	41.6** 1.6	41.2** 2.0
Hemoglobin (g/dl)	Mean S.D.	15.7 0.5	16.0 0.5	15.7 0.5	15.3 0.7	14.3** 0.6	13.6* 1.3	15.3 1.5	15.8 1.9
W.B.C. (10 ³ /mm ³)	Mean S.D.	119.0 14.0	88.8* 16.7	110.6 22.0	118.0 11.2	82.4** 5.4	98.4 23.0	73.0** 10.8	70.4** 23.8
Hemogram									
Lymphocyte (percent)	Mean S.D.	82.5 5.8	79.8 4.9	77.1 3.8	76.6 7.5	78.5 5.0	81.8 8.4	78.1 5.2	83.3 6.1
Monocyte (percent)	Mean S.D.	2.0 1.1	1.2 1.1	2.0 1.0	2.1 0.9	2.0 1.4	1.6 0.9	1.8 1.3	1.4 0.5
Eosinophile (percent)	Mean S.D.	0.4 0.4	0.7 0.5	0.5 0.4	0.9 0.9	0.6 0.5	1.1 0.8	0.6 1.0	0.6 0.8
Basophile (percent)	Mean S.D.	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	0.0 0.0
Neutrophile									
Total (percent)	Mean S.D.	15.1 6.5	17.9 5.7	20.4 3.7	20.4 7.5	18.9 4.5	15.5 8.7	19.5 5.8	14.7 5.6
Band (percent)	Mean S.D.	3.1 2.2	1.2 0.9	3.2 0.7	2.6 1.2	4.4 1.2	2.5 2.3	2.9 1.5	2.7 1.5
Segment (percent)	Mean S.D.	12.0 4.8	16.7 5.1	17.2 4.2	17.8 7.0	14.5 4.4	13.0 6.4	16.6 5.1	12.0 5.7
Blood plate (10 ³ /mm ³)	Mean S.D.	50 12	84* 21	72 21	54 10	57 13	54 13	58 18	93** 18
Prothrombin time (second)	Mean S.D.	17.7 2.6	18.9 0.8	17.1 1.7	17.4 1.2	17.8 2.2	16.5 2.0	17.2 2.2	17.2 1.1

** Significantly different from control data (P<0.01)

* Significantly different from control data (P<0.05)

S.D.~standard deviation

R.B.C. (red blood cell)

W.B.C. (white blood cell)

Table 9 Chronic toxicity of α -110 in female rats administered orally (180 days)

Hematological test

Day of exam.		180 days							
Items	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	α -110 0.2 g/kg	α -110 0.4 g/kg	α -110 1.0 g/kg	α -110 2.0 g/kg	α -110 4.0 g/kg	L-GN 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
R.B.C. ($10^6/mm^3$)	Mean S.D.	677 33	703 90	668 59	655 38	739* 37	716 45	722 37	750** 22
Hematocrit (percent)	Mean S.D.	42.2 2.8	41.4 1.5	41.4 1.5	41.0 1.1	44.6 1.5	45.4 1.4	45.0 1.5	44.6 1.4
Hemoglobin (g/dl)	Mean S.D.	14.9 0.8	14.5 0.5	14.9 0.5	14.7 0.2	15.8 0.3	16.0* 0.4	15.8 0.8	16.0 0.5
W.B.C. ($10^3/mm^3$)	Mean S.D.	106.0 23.6	75.0 19.5	81.8 16.7	76.4 11.5	121.0 11.2	127.8 22.4	157.4* 32.4	103.8 17.8
Hemogram									
Lymphocyte (percent)	Mean S.D.	69.0 16.6	79.3 2.7	80.5 6.2	83.0 7.4	83.9 4.9	80.7 6.5	67.4 15.4	83.7 4.3
Monocyte (percent)	Mean S.D.	2.6 1.1	1.4 1.4	2.2 1.0	2.2 1.3	1.6 1.0	2.7 1.2	2.0 1.2	2.2 0.7
Eosinophile (percent)	Mean S.D.	0.7 0.5	1.2 0.4	0.4 0.6	0.8 0.7	0.7 0.6	0.3 0.4	0.2 0.2	0.2 0.2
Basophile (percent)	Mean S.D.	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	0.0 0.0
Neutrophile									
Total (percent)	Mean S.D.	27.7 16.2	18.5 3.5	16.9 5.9	14.0 7.2	13.8 4.6	16.3 6.7	30.4 15.8	13.9 4.0
Band (percent)	Mean S.D.	2.9 2.0	2.8 2.3	2.2 1.7	1.2 1.9	1.6 1.0	1.2 0.9	3.6 1.9	1.0 0.6
Segment (percent)	Mean S.D.	24.8 14.8	15.7 4.2	14.7 5.9	12.8 6.7	12.2 3.8	15.1 5.9	26.8 14.0	12.9 3.9
Blood plate ($10^6/mm^3$)	Mean S.D.	67 12	76 25	66 27	71 26	77 16	72 22	70 15	45* 14
Prothrombin time (second)	Mean S.D.	18.9 2.5	20.0 1.5	16.7 1.8	15.9 1.3	16.1 1.3	16.3 2.1	16.5 1.5	16.6 0.8

** Significantly different from control data ($p < 0.01$)* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

R.B.C. (red blood cell)

W.B.C. (white blood cell)

5. Clinical Chemistry: Serum glutamic pyruvic transaminase was significantly increased in the high dose males (26.8 I.U.) on day 180 (but not on day 90) as compared to the control (12 I.U.). There were no clear treatment related changes in other

parameters in the L-GM groups. The results were presented in Tables 11, 12, 13, and 14. These tables are attached below.

Table 11. Chronic toxicity of —110 in male rats administered orally (90 days)

		Blood chemical test							
Day of exam.		90 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	—110 0.2 g/kg	—110 0.4 g/kg	—110 1.0 g/kg	—110 2.0 g/kg	—110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		3	3	3	3	3	3	3	3
Total protein (g/dl)	Mean	6.2	6.7	6.5	6.2	5.9	6.0	6.5	6.4
	S.D.	0.2	0.1	0.2	0.0	0.2	0.2	0.2	0.3
A/G	Mean	0.80	0.69	0.64	0.86	0.89	0.98**	0.75	0.79
	S.D.	0.05	0.08	0.23	0.06	0.04	0.02	0.03	0.02
ALP. (K.A. unit)	Mean	11.3	11.1	14.6	11.5	11.1	8.2*	15.9	13.3
	S.D.	1.3	2.5	5.3	1.2	1.2	0.5	3.9	2.5
S-G.P.T. (I.U.)	Mean	33.3	14.0	36.7	48.7	66.7	19.3	42.7	45.3
	S.D.	23.1	4.3	9.8	30.7	42.1	5.2	10.4	23.1
S-G.O.T. (I.U.)	Mean	144.0	93.3	140.0	162.7	173.3	150.7	171.3	150.7
	S.D.	22.4	13.3	28.3	30.3	44.2	10.4	9.0	22.6
B.S.P. (percent)	Mean	6.0	9.1	12.3	11.3	12.7*	6.1	14.8	10.6
	S.D.	3.1	3.8	2.1	0.1	0.4	0.7	4.1	3.2
Blood sugar (mg/dl)	Mean	147	131	145	157	141	144	151	155
	S.D.	6	8	8	8	13	6	6	4
B.U.N. (mg/dl)	Mean	12.1	12.9	13.2	13.8	13.1	15.4*	12.6	13.3
	S.D.	0.5	0.9	0.8	1.9	1.4	1.4	1.1	1.1
N.P.N. (mg/dl)	Mean	32.3	34.4	35.3*	35.3	33.3	36.3*	33.7	34.7
	S.D.	0.5	1.0	0.9	2.1	0.9	1.2	1.7	2.1
Serum electrolyte									
Sodium (mEq/l)	Mean	140.3	139.3	140.3	140.0	141.0	139.7	139.7	139.3
	S.D.	0.5	0.5	0.5	0.8	1.4	0.5	0.9	0.5
Potassium (mEq/l)	Mean	5.07	4.77	5.23	5.13	4.93	5.17	4.87	5.20
	S.D.	0.21	0.05	0.37	0.12	0.05	0.12	0.12	0.45

** Significantly different from control data ($P < 0.01$)

* Significantly different from control data ($P < 0.05$)

S.D. = Standard deviation

A/G (Albumin/Globulin)

ALP. (Alkaline phosphatase)

S-G.P.T. (Serum glutamic pyruvic transaminase)

S-G.O.T. (Serum glutamic oxalacetic transaminase)

B.S.P. (Bromsulfalein retention test)

B.U.N. (Blood urea nitrogen)

N.P.N. (Nonprotein nitrogen)

Table 12 - Chronic toxicity of -110 in female rats administered orally (90 days)

Blood chemical test

Day of exam.		90 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	-110 0.2 g/kg	-110 0.4 g/kg	-110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		3	3	3	3	3	3	3	3
Total protein (g/dl)	Mean	6.4	6.7*	6.4	6.2	6.0	5.7**	6.3	6.5
	S.D.	0.1	0.0	0.3	0.1	0.3	0.1	0.0	0.1
A/G	Mean	0.91	0.90	0.87	0.92	0.86	0.84	0.90	0.86
	S.D.	0.00	0.03	0.06	0.04	0.09	0.05	0.08	0.04
ALP. (K.A. unit)	Mean	6.7	6.4	5.8	5.0	6.1	5.1	5.9	5.5
	S.D.	1.6	1.7	1.2	0.4	1.5	0.6	0.9	1.3
S-G.P.T. (I.U.)	Mean	26.7	8.0*	34.0	36.0	38.0	18.7	38.0	34.7
	S.D.	8.2	2.8	11.8	18.8	12.8	6.8	5.9	6.2
S-G.O.T. (I.U.)	Mean	136.0	88.7*	162.0	144.0	161.3	128.7	140.0	139.3
	S.D.	17.0	10.9	7.1	24.7	18.6	6.6	12.8	20.7
B.S.P. (percent)	Mean	7.5	2.1**	3.6*	3.5*	4.2*	5.5	5.2	6.4
	S.D.	1.1	1.0	1.5	1.3	1.1	0.4	2.2	3.7
Blood sugar (mg/dl)	Mean	149	133	147	143	136	123*	153	151
	S.D.	6	8	11	8	11	10	2	19
B.U.N. (mg/dl)	Mean	14.4	11.8	12.5	13.7	13.3	13.4	12.5	12.4
	S.D.	2.1	1.1	0.9	2.2	1.2	1.6	0.5	0.6
N.P.N. (mg/dl)	Mean	34.3	33.2	33.3	33.3	32.7	32.0	30.7	30.7
	S.D.	2.9	1.9	1.7	2.4	2.1	2.9	0.5	0.9
Serum electrolyte									
	Sodium (mEq/l)	Mean S.D.	138.3 0.9	138.3 1.2	139.7 0.5	138.0 0.8	139.0 1.4	140.0 0.0	138.7 0.5
Potassium (mEq/l)	Mean S.D.	4.93 0.05	4.77 0.41	4.97 0.17	4.90 0.14	4.97 0.09	4.70* 0.08	4.80 0.41	5.07 0.26

** Significantly different from control data ($p < 0.01$)* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

A/G (Albumin/Globulin)

ALP. (Alkaline phosphatase)

S-G.P.T. (Serum glutamic pyruvic transaminase)

S-G.O.T. (Serum glutamic oxalacetic transaminase)

B.S.P. (Bromsulfalein retention test)

B.U.N. (Blood urea nitrogen)

N.P.N. (Nonprotein nitrogen)

Table 13 Chronic toxicity of — 110 in male rats administered orally (180 days)

Blood chemical test

Day of exam.		180 days							
		1. Control D.W.	2. — 110 0.2 g/kg	3. — 110 0.4 g/kg	4. — 110 1.0 g/kg	5. — 110 2.0 g/kg	6. — 110 4.0 g/kg	7. L-GM 2.0 g/kg	8. L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Total protein (g/dl)	Mean	6.6	6.6	6.5	6.3	5.9**	5.2**	6.5	6.4
	S.D.	0.2	0.2	0.3	0.2	0.2	0.2	0.2	0.2
A/G	Mean	0.89	0.79	0.94	0.92	1.01	1.04	0.81	1.06
	S.D.	0.18	0.09	0.32	0.27	0.14	0.20	0.34	0.14
ALP. (K.A. unit)	Mean	17.3	16.0	18.3	14.7	15.2	9.1*	20.9	18.3
	S.D.	4.6	2.2	2.9	4.6	1.7	3.0	6.8	3.6
S-G.P.T. (I.U.)	Mean	12.0	22.0	23.6	32.4	48.8**	36.0**	42.4	26.8*
	S.D.	8.7	7.5	10.8	15.5	12.5	4.9	30.2	5.7
S-G.O.T. (I.U.)	Mean	106.8	96.4	126.0*	120.0	141.2**	143.6**	133.2	115.6
	S.D.	6.5	18.9	10.0	22.8	10.7	19.7	22.4	10.8
B.S.P. (percent)	Mean	6.4	8.5	10.1	9.7	11.2	14.1*	8.4	9.7
	S.D.	3.7	4.6	4.7	7.1	3.3	3.8	5.4	3.9
Blood sugar (mg/dl)	Mean	125	140	122	128	122	118	141	134
	S.D.	9	16	7	9	7	5	27	15
B.U.N. (mg/dl)	Mean	12.2	14.9	12.8	12.0	13.0	14.0	14.8	13.7
	S.D.	2.1	2.4	1.3	0.8	1.3	1.7	2.2	1.7
N.P.N. (mg/dl)	Mean	34.4	33.4	35.7	36.7	34.9	35.7	37.7	37.2
	S.D.	2.1	2.4	2.4	2.3	3.1	1.0	3.9	1.7
Serum electrolyte									
Sodium (mEq/l)	Mean	140.2	139.4	139.0*	138.8	139.0	138.6	137.6*	139.0
	S.D.	0.8	1.4	0.6	1.0	1.1	1.6	1.7	1.4
Potassium (mEq/l)	Mean	5.14	5.28	5.28	5.22	5.16	5.08	5.10	5.12
	S.D.	0.52	0.23	0.17	0.29	0.17	0.23	0.22	0.35

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D.—Standard deviation

A/G (Albumin/Globulin)

ALP. (Alkaline phosphatase)

S-G.P.T. (Serum glutamic pyruvic transaminase)

S-G.O.T. (Serum glutamic oxalacetic transaminase)

B.S.P. (Bromsulfalein retention test)

B.U.N. (Blood urea nitrogen)

N.P.N. (Nonprotein nitrogen)

Table 14. Chronic toxicity of —110 in female rats administered orally (180 days)
Blood chemical test

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	—110 0.2 g/kg	—110 0.4 g/kg	—110 1.0 g/kg	—110 2.0 g/kg	—110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Total protein	Mean	6.8	7.2	6.6*	6.1**	5.9**	5.5**	6.6	6.7
(g/dl)	S.D.	0.1	0.6	0.2	0.3	0.3	0.3	0.3	0.1
A/G	Mean	1.02	1.10	1.24*	1.12	1.11	1.16	1.07	1.10
	S.D.	0.14	0.07	0.11	0.25	0.09	0.09	0.21	0.19
AL.P.	Mean	12.0	4.6**	8.3	6.8*	5.7**	5.2**	10.1	10.9
(K.A. unit)	S.D.	3.6	1.0	2.9	1.1	0.8	0.8	2.3	2.7
S-G.P.T.	Mean	29.2	22.4	48.8	45.6	50.0*	53.2	53.2	40.0
(I.U.)	S.D.	3.5	10.9	26.7	18.7	12.1	37.2	32.0	13.7
S-G.O.T.	Mean	126.0	111.2*	146.0	140.0	160.0**	143.2	136.0	133.2
(I.U.)	S.D.	6.1	8.4	31.1	20.7	13.9	26.8	24.7	16.2
B.S.P.	Mean	4.1	6.2	12.1*	10.8	6.5	5.4	3.4	4.4
(percent)	S.D.	1.7	3.9	5.0	6.2	4.3	4.3	0.5	2.4
Blood sugar	Mean	133	136	132	120	116*	121	137	130
(mg/dl)	S.D.	13	11	12	8	5	13	22	13
B.U.N.	Mean	12.6	15.4	13.8	15.1	10.9	12.5	13.7	13.8
(mg/dl)	S.D.	2.7	1.6	2.8	3.6	1.3	1.9	3.8	2.9
N.P.N.	Mean	34.4	32.2	34.1	34.7	31.6	31.3	34.4	33.2
(mg/dl)	S.D.	3.8	1.7	1.7	2.4	1.8	2.0	3.7	2.0
Serum electrolyte									
Sodium	Mean	139.2	138.0	138.0	138.2	139.2	138.6	138.2	138.6
(mEq/l)	S.D.	1.3	1.4	2.8	1.0	1.6	1.6	1.0	1.4
Potassium	Mean	4.84	4.92	4.94	4.86	4.86	4.90	5.02	5.06
(mEq/l)	S.D.	0.27	0.22	0.30	0.17	0.28	0.17	0.15	0.28

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p > 0.05$)

S.D.—Standard deviation

A/G (Albumin/Globulin)

AL.P. (Alkaline phosphatase)

S-G.P.T. (Serum glutamic pyruvic transaminase)

S-G.O.T. (Serum glutamic oxalacetic transaminase)

B.S.P. (Bromsulfalein retention test)

B.U.N. (Blood urea nitrogen)

N.P.N. (Nonprotein nitrogen)

6. Urinalysis: There were no treatment related changes in L-GM group. The results were presented in Tables 16, 17, 18, and 19. These tables are attached below.

Table 16 Chronic toxicity of $\bar{110}$ in male rats administered orally (90 days)
Urine test

Day of exam.		90 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	$\bar{110}$ 0.2 g/kg	$\bar{110}$ 0.4 g/kg	$\bar{110}$ 1.0 g/kg	$\bar{110}$ 2.0 g/kg	$\bar{110}$ 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		3	3	3	3	3	2	3	3
Urine volume (ml/16 hr)	Mean S.D.	4.1 1.0	3.1 1.4	6.7 2.4	2.3 1.0	3.4 1.4	3.2 0.8	7.0 4.2	3.0 1.6
Occult blood	Mean	-	-	+	+	-	-	-	-
Ketones	Mean	-	-	-	-	-	-	-	-
Glucose	Mean	-	-	-	-	-	-	-	-
Protein (mg/dl)	Mean S.D.	30 0	13 12	22 12	77 33	53 33	50 50	53 33	53 33
pH	Mean S.D.	7.2 0.6	7.0 0.0	7.5 0.7	7.3 0.5	7.8 0.8	8.5 0.5	8.5 0.4	7.8 0.6
SPGR.	Mean S.D.	1.06 0.01	1.02* 0.01	1.03 0.00	1.10 0.03	1.08 0.02	1.10 0.10	1.07 0.05	1.09 0.03

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. Standard deviation

Scale for occult blood and ketons by labstix: -- Negative, + Small, # Moderate, ## Large.

Scale for glucose by labstix: -- Negative, + Light, # Medium, ## Dark.

Table 17 Chronic toxicity of $\bar{110}$ in female rats administered orally (90 days)
Urine test

Day of exam.		90 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	$\bar{110}$ 0.2 g/kg	$\bar{110}$ 0.4 g/kg	$\bar{110}$ 1.0 g/kg	$\bar{110}$ 2.0 g/kg	$\bar{110}$ 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		2	3	2	2	3	3	2	3
Urine volume (ml/16 hr)	Mean S.D.	3.4 2.3	7.2 1.8	1.1 0.2	1.8 0.3	2.8 1.7	2.6 0.6	3.0 -0.1	2.7 1.6
Occult blood	Mean	-	-	-	+	-	-	-	-
Ketones	Mean	-	-	-	-	-	-	-	-
Glucose	Mean	-	-	-	-	-	-	-	-
Protein (mg/dl)	Mean S.D.	65 35	5 0	30 0	18 12	13 12	5 0	18 12	45 40
pH	Mean S.D.	5.3 1.2	6.7 0.5	8.3 0.3	6.5 0.0	5.7 1.2	8.7* 0.5	6.5 2.5	6.8 0.8
SPGR. (mg/dl)	Mean S.D.	1.12 0.01	1.02** 0.01	1.07 0.01	1.08 0.01	1.09 0.06	1.04* 0.02	1.06 0.01	1.07 0.02

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. Standard deviation

Scale for occult blood and ketons by Labstix: -- Negative, + Small, # Moderate, ## Large

Scale for glucose by labstix: -- Negative, + Light, # Medium, ## Dark

Table 18 Chronic toxicity of -110 in female rats administered orally (180 days)
Urine test

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	-110 0.2 g/kg	-110 0.4 g/kg	-110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Urine volume (ml/16 hr)	Mean	1.2	5.3	2.6	2.5	7.2*	4.3	3.3	4.4*
	S.D.	1.7	4.4	1.8	2.8	3.8	2.7	4.1	1.3
Occult blood	Mean	--	--	--	--	--	--	--	--
Ketones	Mean	--	--	--	--	--	--	--	--
Glucose	Mean	--	--	--	--	--	--	--	--
Protein (mg/dl)	Mean	100	21**	20**	24**	9**	14**	67	34**
	S.D.	0	40	12	12	11	13	41	35
pH	Mean	6.0	6.9**	6.5	6.2	7.6**	8.4**	6.3	6.4
	S.D.	0.0	0.5	0.4	0.4	0.7	0.8	0.4	0.4
SP.GR.	Mean	1.11	1.06	1.08	1.10	1.04*	1.06	1.11	1.09
	S.D.	0.04	0.05	0.03	0.05	0.02	0.03	0.04	0.04

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

Scale for occult blood and ketons by labstix: -- Negative, + Small, # Moderate, ## Large

Scale for glucose by labstix: -- Negative, + Light, # Medium, ## Dark

Table 19 Chronic toxicity of -110 in male rats administered orally (180 days)
Urine test

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
		Control D.W.	-110 0.2 g/kg	-110 0.4 g/kg	-110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Urine volume (ml/16 hr)	Mean	6.0	6.5	6.2	4.8	5.5	8.6	10.8	11.5
	S.D.	3.7	1.3	4.6	2.6	4.1	4.8	9.5	5.8
Occult blood	Mean	--	--	--	+	--	--	+	--
Ketones	Mean	--	--	--	--	--	--	--	--
Glucose	Mean	--	--	--	--	--	--	--	--
Protein (mg/dl)	Mean	79	58	30	58	29	10	39	20
	S.D.	111	34	0	34	37	10	32	12
pH	Mean	6.3	7.0	6.0	6.7	8.0**	8.7**	6.4	6.7
	S.D.	0.4	1.3	0.0	0.4	0.6	0.4	0.5	0.4
SP.GR.	Mean	1.06	1.06	1.07	1.08	1.09	1.06	1.08	1.06
	S.D.	0.03	0.01	0.03	0.03	0.06	0.03	0.04	0.01

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

Scale for occult blood and ketons by Labstix: -- Negative, + Small, # Moderate, ## Large

Scale for glucose by labstix: -- Negative, + Light, # Medium, ## Dark

7. Organ Weights: There were no obvious treatment related changes in L-GM groups. The results of the terminal organ

weights were summarized in Tables 25, 26, 27, and 28. These tables are attached below.

Table 25 Chronic toxicity of -110 in male rats administered orally (180 days)

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
	D.W.	Control	-110 0.2 g/kg	-110 0.4 g/kg	-110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Heart	Mean	1.86	1.88	1.73	1.75	1.69	1.62	1.78	1.71
	S.D.	0.15	0.13	0.15	0.16	0.16	0.32	0.16	0.27
Lung	Mean	2.03	2.27	1.72	1.80	1.79	1.90	2.60	1.80
	S.D.	0.32	0.53	0.13	0.36	0.26	0.58	0.97	0.22
Spleen	Mean	1.06	1.05	1.01	1.00	1.01	0.84	1.12	0.99
	S.D.	0.17	0.14	0.06	0.10	0.06	0.14	0.19	0.11
Liver	Mean	16.97	18.06	16.06	15.42	15.11	13.92*	16.49	15.58
	S.D.	1.29	2.53	1.96	0.91	1.07	1.77	0.71	1.98
Kidney (right)	Mean	1.88	1.85	1.74	1.66	1.64	1.52*	1.87	1.75
	S.D.	0.19	0.14	0.06	0.11	0.11	0.24	0.15	0.13
Kidney (left)	Mean	1.85	1.83	1.85	1.71	1.71	1.53	1.87	1.67
	S.D.	0.21	0.13	0.09	0.11	0.14	0.24	0.27	0.29
Thymus	Mean	0.19	0.28	0.23	0.21	0.22	0.23	0.22	0.27
	S.D.	0.08	0.11	0.17	0.10	0.10	0.12	0.13	0.14
Brain	Mean	1.76	1.89	1.81	1.70	1.81	1.69	1.81	1.83
	S.D.	0.15	0.10	0.09	0.30	0.03	0.16	0.02	0.03
Adrenal (right)	Mean	0.037	0.034	0.029	0.027	0.024	0.032	0.031	0.026
	S.D.	0.017	0.004	0.007	0.008	0.004	0.004	0.004	0.005
Adrenal (left)	Mean	0.039	0.034	0.028	0.027	0.028	0.031	0.031	0.026
	S.D.	0.021	0.004	0.007	0.008	0.002	0.005	0.004	0.005
Sex organ (right)	Mean	1.58	1.68	1.57	1.53	1.51	1.47	1.56	1.57
	S.D.	0.08	0.05	0.15	0.07	0.02	0.10	0.07	0.07
Sex organ (left)	Mean	1.51	1.65	1.58	1.55	1.40	1.51	1.56	1.59
	S.D.	0.10	0.11	0.14	0.08	0.20	0.10	0.04	0.07

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

Table 25 Chronic toxicity of —110 in male rats administered orally (180 days)
Relative organ weight (mg/100 g)*

Day of exam.		180 days							
Item	Group dose	1. Control D.W.	2 -110 0.2 g/kg	3 -110 0.4 g/kg	4 -110 1.0 g/kg	5 -110 2.0 g/kg	6 -110 4.0 g/kg	7. L-GM 2.0 g/kg	8. L-GM 4.0 g/kg
	No of animal exam.	5	5	5	5	5	5	5	5
Heart	Mean S.D.	303 17	276 31	286 23	307 38	289 33	319 28	301 30	287 48
Lung	Mean S.D.	335 78	337 101	286 26	317 70	303 27	370 64	447 193	303 40
Spleen	Mean S.D.	176 42	155 25	168 14	174 18	173 16	167 15	190 41	168 23
Liver	Mean S.D.	2762 203	2634 262	2644 62	2692 201	2580 200	2781 181	2787 189	2621 358
Kidney (right)	Mean S.D.	307 48	272 20	289 26	290 29	283 49	303 26	318 40	296 35
Kidney (left)	Mean S.D.	303 51	267 18	308 25	299 32	294 50	306 28	317 58	281 53
Thymus	Mean S.D.	30 12	40 14	36 21	38 20	36 14	44 17	39 24	44 21
Brain	Mean S.D.	287 31	277 24	301 28	299 65	311 33	340 35	306 18	309 23
Adrenal (right)	Mean S.D.	6.2 3.5	5.0 0.8	4.8 1.1	4.8 1.6	4.1 0.7	6.5 1.2	5.2 0.7	4.4 0.9
Adrenal (left)	Mean S.D.	6.6 4.2	5.0 0.8	4.7 1.2	4.8 1.6	4.8 0.6	6.3 1.3	5.2 0.7	4.4 0.9
Sex organ (right)	Mean S.D.	258 31	248 30	262 38	268 21	259 31	298 39	263 17	264 20
Sex organ (left)	Mean S.D.	247 32	243 32	263 33	270 20	240 47	307 44	263 17	267 21

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

* B.W. (Body weight)

Table 27 Chronic toxicity of —110 in female rats administered orally (180 days)

Organ weight (g)

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
	D.W.	Control	-110 0.2 g/kg	-110 0.4 g/kg	-110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Heart	Mean S.D.	1.22 0.19	1.06 0.10	1.13 0.20	1.22 0.22	1.23 0.07	1.14 0.09	1.20 0.17	1.19 0.09
Lung	Mean S.D.	1.82 0.48	1.51 0.15	1.56 0.41	1.41 0.23	1.42 0.17	1.40 0.13	1.51 0.15	1.87 0.60
Spleen	Mean S.D.	0.78 0.13	0.60 0.13	0.80 0.13	0.81 0.11	0.80 0.09	0.74 0.12	0.81 0.07	0.86 0.11
Liver	Mean S.D.	9.88 2.46	8.69 1.23	9.23 2.13	10.16 2.67	9.43 0.61	9.47 0.48	9.45 0.79	9.58 0.58
Kidney (right)	Mean S.D.	0.95 0.08	0.96 0.11	0.93 0.08	1.11 0.31	0.98 0.05	1.05 0.10	0.97 0.08	1.02 0.06
Kidney (left)	Mean S.D.	1.01 0.08	0.91 0.09	0.97 0.09	1.15 0.39	0.96 0.06	1.04 0.10	0.99 0.09	1.00 0.05
Thymus	Mean S.D.	0.20 0.08	0.20 0.03	0.15 0.02	0.17 0.03	0.23 0.06	0.18 0.05	0.26 0.10	0.20 0.11
Brain	Mean S.D.	1.68 0.05	1.65 0.13	1.69 0.05	1.77 0.17	1.66 0.10	1.70 0.03	1.70 0.07	1.71 0.08
Adrenal (right)	Mean S.D.	0.032 0.007	0.032 0.004	0.034 0.004	0.031 0.004	0.031 0.005	0.034 0.005	0.027 0.004	0.032 0.004
Adrenal (left)	Mean S.D.	0.034 0.004	0.032 0.004	0.034 0.004	0.031 0.004	0.031 0.005	0.036 0.005	0.027 0.004	0.026 0.008
Sex organ (right)	Mean S.D.	0.078 0.038	0.044 0.005	0.064 0.013	0.063 0.010	0.059 0.009	0.056 0.009	0.054 0.011	0.060 0.006
Sex organ (left)	Mean S.D.	0.062 0.013	0.040 0.009	0.063 0.014	0.061 0.007	0.057 0.007	0.054 0.009	0.052 0.010	0.070 0.015

** Significantly different from control data ($p < 0.01$)

* Significantly different from control data ($p < 0.05$)

S.D. = Standard deviation

Table 28 Chronic toxicity of —110 in female rats administered orally (180 days)
Relative organ weight (mg/100 g)*

Day of exam.		180 days							
Item	Group dose	1.	2.	3.	4.	5.	6.	7.	8.
	D.W.	Control	-110 0.2 g/kg	-110 0.4 g/kg	-110 1.0 g/kg	-110 2.0 g/kg	-110 4.0 g/kg	L-GM 2.0 g/kg	L-GM 4.0 g/kg
No of animal exam.		5	5	5	5	5	5	5	5
Heart	Mean S.D.	327 33	280 30	312 15	343 36	347 25	338 24	326 34	335 61
Lung	Mean S.D.	483 106	402 74	431 82	397 33	403 64	420 63	410 30	475 123
Spleen	Mean S.D.	208 20	163 54	222 12	228 12	225 26	217 23	221 16	243 59
Liver	Mean S.D.	2618 390	2309 446	2533 193	2838 434	2661 223	2815 189	2573 110	2662 189
Kidney (right)	Mean S.D.	254 14	254 42	261 37	311 51	276 24	312** 27	265 28	284 34
Kidney (left)	Mean S.D.	271 17	241 33	273 35	320 70	271 33	310 30	270 25	281 44
Thymus	Mean S.D.	55 19	54 16	41 7	47 6	64 15	54 16	71 27	55 25
Brain	Mean S.D.	453 43	441 77	482 78	502 26	469 60	506 45	465 38	479 59
Adrenal (right)	Mean S.D.	8.5 1.2	8.5 1.7	9.6 1.3	8.8 1.3	8.7 1.1	10.2 2.2	7.4 1.3	6.9 1.0
Adrenal (left)	Mean S.D.	9.1 0.2	8.5 1.7	9.6 1.3	8.8 1.3	8.7 1.1	10.8 2.1	7.4* 1.3	7.4 2.6
Sex organ (right)	Mean S.D.	19.59 7.20	11.91 2.85	18.21 4.65	17.75 1.23	16.84 3.63	16.63 2.61	14.72 2.80	16.85 2.93
Sex organ (left)	Mean S.D.	16.46 2.03	10.78* 3.34	18.00 4.97	17.27 1.09	16.26 3.46	16.06 2.78	14.15 2.52	19.30 3.18

** Significantly different from control data ($P < 0.01$)

* Significantly different from control data ($P < 0.05$)

S.D. = Standard deviation

* B.W. (Body weight)

8. Gross Pathology: There were no treatment related changes in L-GM groups.

9. Histopathology: Catarrh in the stomach and infiltration of inflammatory cell and edema in gastric submucosa were identified in both glutamine groups but not in control group. Catarrh in

the stomach was noted on both days 90 and 180. In addition, slight fatty infiltration in the liver cells (one low dose rat and one high dose rat), slight vacuolation of tubular epithelium (two high dose rats), and slight protein cast in the kidney (one high dose rat) were also found in the glutamine groups on day 180. The results were presented in Table 31. This table is attached below.

Table 31 Chronic toxicity of -110 in rats administered orally
Histopathological changes and the number of animals observed lesions

Item	Group dose	90 days								180 days								210 days						
		Control	-110 (g/kg)				L-GM (g/kg)				Control	-110 (g/kg)				L-GM (g/kg)				Control	-110(g/kg)			
			0.20	0.41	0.72	0.41	0.20	0.41	0.72	0.41		0.20	0.41	0.72	0.41	0.20	0.41	0.72	0.41					
Number of animal exam.		3	3	3	3	3	3	3	3	5	5	5	5	5	5	5	5	5	3	3	3	3	3	
Spleen																								
Slight congestion		1	1	1	1	2	2	2	2	5	5	4	4	4	4	5	2							
Slight proliferation of reticulum cell		3	1	2	2	2	3	2	2	1	5	5	4	5	3	1								
Liver																								
Slight fatty infiltration in liver cell			2	1	1	1	1	1							1	1	1							
Kidney																								
Slight vacuolation of tubular epithelium				1	1		1	1	1	1	1	1	1	1	4	1								
Slight protein cast									2	1		1	1	4	1									
Stomach																								
Catarrh trace			1	1		1	1	1		2	2	1	3		2	2		2		1	3			
slight		1			2		2	1		1	2		1		1	1			2	1	1			
moderate		1		1							2					1			1		1			
remarkable					1	2						1							1					
Infiltration of inflammatory cell and edema in submucosa																								
trace			1	1							1				2	1			2	1	1			
slight											3				1	2				1	3			
moderate		1	1	1	2							1								1	1			
remarkable				2	3	3				1	3	2	5	4	5									
Intestinal metaplasia																								
trace												1							1	2	3			
slight					1		1					1		1						1				
moderate																								
remarkable				1	2	3						2	3	5						1	1			

Blank=0, --=Non exam.

3.4.4. Genetic toxicology

Study title: Amino Acid excess increase Sister-Chromatid Exchagnes (SCEs) in human lymphocytes (Mutation Resaerch; 1996; 372:75-78).

The purpose of this study was to determine the effects of excessive amounts of amino acids including glutamine on SCEs in the cultures of human lymphocytes. The results indicated that addition of 10, 50, and 100 µg/ml of glutamine slightly increased the SCEs (11.05-11.50 SCEs/metaphase) as compared to the control (8.15 SCEs/metaphase) but this increase was not concentration dependent. There was no positive control in this study. The results were presented in Table 1 in this report. This table is attached below.

Table 1
Influence of amino acids on SCE frequency in human lymphocytes

Amino acids (t.)	Original content (µg/ml) ^a	SCE/metaphase(mean ± SE)				SCE ratios (mean)	
		Control	Treatment (µg/ml excess) ^b				
			10	50	100	Average	
Essential							
Arginine	146	8.15 ± 2.18	11.89 ± 2.21	12.00 ± 2.59	12.25 ± 3.23	12.07 ± 2.68	1.48
Cystine	37	8.15 ± 2.18	14.00 ± 2.35	13.85 ± 2.82	14.25 ± 2.95	14.03 ± 2.71	1.73
Isoleucine	37	6.50 ± 1.43	12.15 ± 2.62	12.90 ± 3.75	12.40 ± 3.75	12.48 ± 3.37	1.93
Leucine	37	6.50 ± 1.43	9.61 ± 1.85	10.05 ± 2.50	10.40 ± 3.62	10.02 ± 2.66	1.52
Lysine	29	6.50 ± 1.43	11.25 ± 2.26	11.56 ± 2.94	12.01 ± 2.32	11.61 ± 2.51	1.79
Methionine	11	6.50 ± 1.43	9.10 ± 2.65	9.65 ± 2.43	9.75 ± 2.69	9.50 ± 2.59	1.45
Phenylalanine	11	6.62 ± 2.20	7.85 ± 1.95	8.00 ± 2.10	8.21 ± 2.22	8.02 ± 2.09	1.21
Threonine	15	8.15 ± 2.18	10.65 ± 2.10	10.95 ± 2.84	11.25 ± 3.23	10.95 ± 2.72	1.34
Tryptophan	4	6.62 ± 2.20	8.81 ± 2.24	9.27 ± 2.88	9.55 ± 2.23	9.21 ± 2.45	1.38
Histidine	11	6.50 ± 1.43	9.45 ± 1.93	9.60 ± 2.58	9.72 ± 1.90	9.59 ± 2.14	1.48
Tyrosine	15	8.15 ± 2.18	13.44 ± 3.22	13.14 ± 2.38	13.25 ± 2.69	13.28 ± 2.76	1.63
Valine	15	6.50 ± 1.43	10.25 ± 1.94	10.37 ± 2.72	10.65 ± 2.45	10.42 ± 2.37	1.61
Glutamine	219	8.15 ± 2.18	11.24 ± 1.89	11.50 ± 2.20	11.05 ± 2.28	11.26 ± 2.12	1.40
Nonessential							
Glutamic acid	15	6.62 ± 2.20	8.95 ± 1.32	9.30 ± 2.29	9.85 ± 2.35	9.37 ± 1.97	1.43
Omithine	-	6.62 ± 2.20	10.25 ± 2.49	10.14 ± 3.38	10.15 ± 2.72	10.18 ± 2.86	1.57
Hydroxyproline	15	6.50 ± 1.43	9.15 ± 1.95	9.95 ± 2.02	10.00 ± 1.85	9.70 ± 1.94	1.47
Proline	15	6.50 ± 1.43	10.85 ± 1.95	11.40 ± 2.50	11.61 ± 2.62	11.29 ± 2.36	1.73
Alanine	-	6.50 ± 1.43	10.15 ± 2.66	10.15 ± 2.92	10.20 ± 2.24	10.11 ± 2.61	1.56
Aspartic acid	15	6.50 ± 1.43	9.15 ± 1.45	9.45 ± 2.32	9.71 ± 2.76	9.26 ± 2.18	1.45
Serine	22	6.50 ± 1.43	9.65 ± 2.45	9.30 ± 2.75	10.05 ± 2.84	9.67 ± 2.68	1.52
Glycine	7	6.62 ± 2.20	8.21 ± 1.87	8.09 ± 2.04	8.33 ± 1.35	8.21 ± 1.75	1.21

For all treatments, p < 0.01.

^a Representing the original final concentrations of amino acids in culture medium calculated based on the original content of each amino acid in RPMI 1640.

^b The final concentrations of the additional dose of each amino acid applied

The small and concentration-independent increase may not be of any clinical significance.

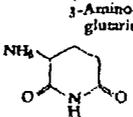
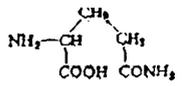
3.4.6. Reproductive and developmental toxicology

Study title: Studies on the relationship between the chemical structure and embryotoxic activity of thalidomine and related compounds (R.L. Smith, et.a., In: Robson JM, et. Al., eds. Embryopathic Activity of Drugs. Ed. Boston: Little, Brown and Company; 1965:194-209).

Key study findings: Treatment with L-glutamine orally at 150 mg/kg/day during gestation days 7-12 in pregnant rabbits was not teratogenic. However, this study is not considered as a valid study since the treatment with glutamine during gestation days 7-12 did not cover the entire organogenesis of rabbits (gestation days 6-20 in rabbits).

The aims of the studies were to evaluate embryotoxicity of thalidomine and structure related compounds including L-glutamine. In these studies, pregnant rabbits were given L-glutamine orally at 150 mg/kg/day during gestation days 7-12. The results indicated that treatment with L-glutamine was not teratogenic at this dose. However, this study is not considered as a valid study since the treatment with glutamine during gestation days 7-12 did not cover the entire organogenesis (gestation days 6-20). And the dose of glutamine tested (150 mg/kg/day) was not sufficient high. The results were presented in Table 5 in this report. This table is attached below.

Table 5
Embryotoxic activity of 3-aminoglutarimide and L- and DL-glutamine

Compound	No. of rabbits	Im-plantations	Re-ceptions	Mal-formed	Normal
 3-Amino-glutarimide	4	37	4	0	33
DL-Glutamine	5	37	6	0	31
L-Glutamine	5	41	2	0	39
					

Treatment: 150 mg./kg. orally on days 7-12 of pregnancy.

LABELING:

The labeling is according to 21 CFR, Subpart B. The following revisions in the labeling are recommended.

1. Sponsor's Version:

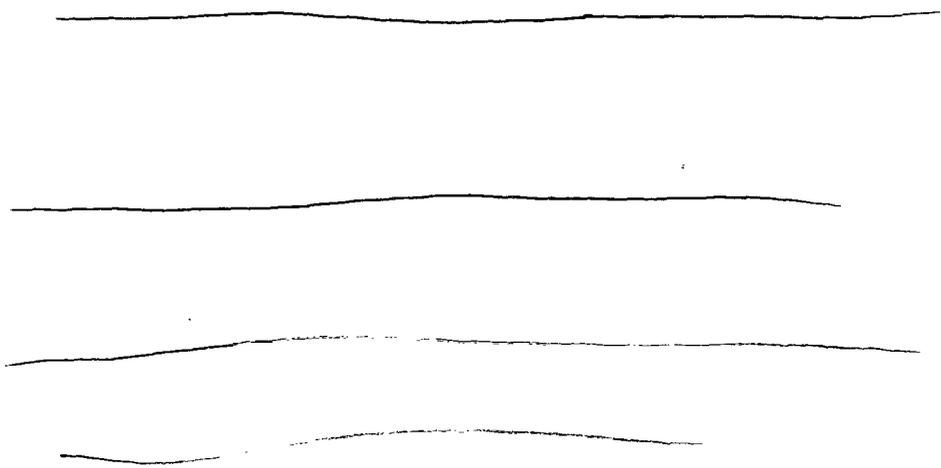
CARCINOGENESIS, MUTAGENESIS, IMPAIRMENT OF FERTILITY

[Redacted text]

2. Sponsor's Version:

Pregnancy Category B

[Redacted text]



3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Glutamine is a non-essential amino acid widely distributed throughout the body and makes up about 50% of the human body's free amino acid pool. Glutamine is important in normal gastrointestinal cell structure, function, and regeneration. Under normal condition, glutamine level in the body is maintained by dietary intake and synthesis from endogenous glutamate. The typical daily dietary intake of glutamine is ~5-10 g. However, the nutritional requirement of glutamine is dramatically increased in catabolic condition such as short bowel syndrome. Thus, it is considered as a "conditionally essential" amino acid. The review of this NDA is based on the literature reports submitted by the sponsor. The pharmacology studies demonstrated that treatment with dietary glutamine promotes electrolyte and nutrient absorption, and stimulates mucosal cell proliferation and regeneration of the small intestinal mucosa in rats with the resected small intestine. The trophic effects were synergistically elevated when glutamine is given in combination of growth hormone in rats with resected small intestine. These results suggest that the trophic effects of glutamine are certainly beneficial for patients with short bowel syndrome.

In the present NDA, sponsor is seeking for approval to market oral glutamine for short bowel syndrome as a cotherapy

with recombinant human growth hormone (rhGH) to reduce or eliminate the requirement for parental nutrition and to increase gut absorption of nutrients. In support of this NDA, sponsor did not conduct any preclinical studies but provided published reports from literature including pharmacology, pharmacokinetics, single acute oral dose toxicity studies in mice, rats, and rabbits, 28-day and 180-day oral toxicity studies in rats, and a sister chromatid exchange study in human lymphocytes.

The pharmacokinetic studies indicated that oral absorption of glutamine was demonstrated with peak plasma level of glutamine at ~1-1.5 hours after oral dose in rats. Glutamine was distributed in the liver, lung, kidney, heart, spleen, muscle, and brain following dietary or intravenous administration in rats. Glutamine can cross the placenta in rats. Glutamine is formed in the body through the condensation of a glutamate and an ammonia molecule by glutamine synthetase with hydrolysis of ATP. In the reverse process, glutaminase deaminated glutamine to glutamate and ammonia. Approximately 66% radioactivity (¹⁵N) of glutamine was recovered in the urine following intravenous administration of radio-labeled glutamine in rats. Majority of the radioactivity (94%) was associated with urinary urea and only ~4% was as ammonia.

The acute oral toxicity studies were conducted with glutamine in mice, rats, and rabbits. LD₅₀ values were provided in this report. The oral LD₅₀ values were 20.3 (females) and 21.7 (males) g/kg in mice, 7.5 (males) and 10.5 g/kg in rats, and 18.8 g/kg in male rabbits. The clinical signs of acute toxicity and minimal lethal dose were not given.

In the 30-day oral toxicity study in rats, glutamine was given to rats by oral gavage at 4, 6, and 10 g/kg/day for 30 days. Glutamine was lethal in the high dose due to administration of the test substance suspension in large volumes ("physical problem"). Catarrh in the stomach was noted in the glutamine groups but not in the control group.

In the 180-day oral toxicity study in rats, glutamine was given to rats by oral gavage at 2 and 4 g/kg/day for 180 days. Catarrh in the stomach and infiltration of inflammatory cell and edema in gastric submucosa were identified in both glutamine groups but not in control group. Catarrh in the stomach was noted on both days 90 and 180.

A genetic toxicity study to determine the effects of excessive amounts of amino acids including glutamine on sister-chromatid exchanges (SCEs) in human lymphocytes indicated that addition of 10, 50, and 100 µg/ml of glutamine slightly increased the SCEs (11.05-11.50 SCEs/metaphase) as compared to the control (8.15 SCEs/metaphase) but this increase was not concentration dependent. The clinical significance of the small and concentration-independent increase of SCEs is not clear.

In the present NDA, sponsor is seeking approval to market oral glutamine for short bowel syndrome as a cotherapy with recombinant human growth hormone (rhGH) to reduce or eliminate the requirement for parental nutrition and to increase gut absorption of nutrients. Treatment with oral glutamine alone should be continued to sustain the achieved increase in gut absorption and reduction of parenteral nutrition requirements for additional 12 weeks to 3 years. In support of this NDA, sponsor did not conduct any preclinical studies but provided published reports from literature including 30-day and 180-day oral toxicity studies in rats. The results of the 30-day and 180-day oral toxicity studies in rats indicated that treatment with oral glutamine produced catarrh in the stomach at 4 and 10 g/kg/day in the 28-day oral toxicity study and 2 and 4 g/kg/day in the 180-day oral toxicity study. The required chronic toxicity study in nonrodent species is not available. However, glutamine, a "conditionally essential" amino acid, is widely distributed throughout the human body and makes up about 50% of the human body's free amino acid pool. The typical daily dietary intake of glutamine is ~5-10 g. Oral glutamine has been marketed in the U.S.A. as an over-the-counter dietary supplement. Therefore, from a preclinical standpoint, approval of oral glutamine is recommended for short bowel syndrome as a cotherapy with rhGH for 4 weeks followed by additional 12 weeks with glutamine alone to reduce or eliminate the requirement for parental nutrition and to increase gut absorption of nutrients. Long term effects of large dose of glutamine are not known. Sponsor should be asked to revise the labeling as recommended.

Recommendations:

1. From a preclinical standpoint, approval of oral glutamine is recommended for short bowel syndrome as a cotherapy with recombinant human growth hormone for 4 weeks followed by additional 12 weeks with glutamine alone to reduce or eliminate the requirement for parental nutrition and to increase gut absorption of nutrients.

2. Labeling should be revised as recommended.

Ke Zhang, Ph.D. Date
Pharmacologist, HFD-180

Comments:

Jasti B. Choudary, B.V.Sc., Ph.D. Date
Supervisory Pharmacologist, HFD-180

CC:
NDA
HFD-180
HFD-181/CSO
HFD-180/Dr. Choudary
HFD-180/Dr. Zhang
HFD-048/Dr. Viswanathan

R/D INIT. J. CHOUDARY 5/4/04
C:\DATA\WORD\21667.DOC

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

/s/

Ke Zhang
5/6/04 10:21:05 AM
PHARMACOLOGIST

Jasti Choudary
5/7/04 12:52:36 PM
PHARMACOLOGIST

NDA 21-667

Nonclinical Inspections

This section is not applicable.

0
ISI
5.18.04
Tanya Clayton
Regulatory Project Manager 0

NDA 21-667

CAC/ECAC Report

This section is not applicable.

0
/S/ 1 5.18.04

Tanya Clayton
Regulatory Project Manager