

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20125

PHARMACOLOGY REVIEW(S)

N. BONGIOVANNI

MAR 10 1992

NDA 20-125

**SUPERVISORY PHARMACOLOGIST'S OVERVIEW:
-PRECLINICAL TOXICOLOGY, PHARMACODYNAMICS, AND
PHARMACOKINETICS OF QUINAPRIL/HYDROCHLOROTHIAZIDE**

Albert DeFelice, Ph.D.
March 4, 1992

SPONSOR: Warner Lambert Co.
Ann Arbor, MI

ORIG. NDA SUBMISSION: 12/13/90
CENTER RECEIPT DATE: 12/19/90

DRUG: Accuretic (Quinapril HCl and Hydrochlorothiazide)

SYNOPSIS: Based on seven animal studies - combination vs. components - and primary pharmacologist's (Dr. W. Van Arsdell) draft review/evaluation, I conclude that, as with other ACE inhibitors, hydrochlorothiazide (HCTZ) did not qualitatively alter toxicology, dynamics, or kinetics of quinapril. Fetotoxicity, in the absence of teratogenicity, persisted at or near maternotoxic combination dosages, and generic pregnancy-related black-box labeling should stand. I see no evidence that quinapril therapeutic safety margin in animals - including the adverse renal/anti-hypertensive dose ratio - is eroded by HCTZ. Seven combination studies - 5 pharmacodynamic and 2 13-week toxicity - did indicate several-fold reductions in both vasodepressor (anti-hypertensive/hypotensive) and toxic dosages when quinapril was combined with HCTZ. Sponsor did not establish whether this was an additive or synergistic vasodepressor interaction, or examine HCTZ-induced hyper-reninemia and exaggerated hypotension as mechanisms for the apparent shift of the dose-toxicity response curve.

I. Pharmacodynamic Interaction:

A. Antihypertensive:

SH Rat: In a 3 day treatment of aortic-cannulated rats, 30 mg/kg of HCTZ evoked no statistically significant MBP reduction, whereas 0.3 mg/kg of neat quinapril reduced MBP by 29 mmHg; however, such dosages in combination reduced MBP by 60 mmHg. Plasma renin activity was raised 3x by HCTZ.

Renal (perinephritic) Hypert. Dog: In a study where 20 mg/kg of quinapril or 30 mg/kg of HCTZ achieved no statistically significant MBP reduction, the combination of 10 mg/kg of each reduced MBP by 32 mmHg.

Evaluation: Results consistent with drug synergy and renin-dependent mechanism of action, but studies insufficiently powered to identify an apparent placebo (training)

effect and the anti-hypertensive tendency of HCTZ per se, and, therefore, to resolve whether effects were additive or synergistic.

B. Hypotensive:

HCTZ given orally to normotensive beagles for 13 weeks at 25 mg/kg/day (approx. 30x combination MRHD) did not significantly lower S/D blood pressure. Quinapril, at 20 mg/kg (approx 30x combination MRHD), depressed S/D BP by ca. 15 mmHg. However, when given in combinations of 20/12.5 and 20/25 mg/kg (clinical ratios, but approx. 30x MRHD), systolic and diastolic BP were both reduced by approx. 35-40 mmHg by week 12, and with a 24 hr-duration at the 20/25 dosage.

C. Diuretic:

Quinapril, at doses up to 1.0 mg/kg (3x HD), did not affect natriuretic activity of HCTZ in saline-loaded normotensive or SH rats, and at higher dosages slightly inhibited natriuresis (or basal Na⁺ excretion) consistent with hypotension.

II. Reproductive Toxicology:

Two Segment II (teratogenicity) studies were performed - rat and rabbit - where quinapril and/or HCTZ was given during early and mid organogenesis only, sparing late gestational/early post-partum periods. Doses were selected based on pilot study maternotoxic dose-ranging.

A. Rat:

Fetotoxicity, i.e., significant decrease in fetal body weight (both sexes) and in incidence of vertebrae/sternbrae ossification (marker of fetal developmental age), but no overt teratogenicity, occurred at maternotoxic dosages which reduced maternal gestational body weight gain by 67% (50 mg/kg quinapril/30 mg/kg HCTZ) or killed dams (2/20 at 150 Q/94 HCTZ). There was no materno/feto-toxicity of the corresponding high dose monotherapies or evidence of skull anomaly in any treated group including the lethal high combination dose. Each treatment involved 20 dams and approx. 175 fetuses.

B. Rabbit:

There was no effect on fetal survival at term (live, dead, resorbed incidences), or mean fetal body weight in dams treated with Quin./HCTZ at up to 0.5/0.3 mg/kg, a dose which reduced maternal gestational weight gain by 40% and which is only 50% less than the matern? Lethal Dose_{40%} in the pilot study. Each treatment involved 20 dams and approx. 120 fetuses, and appears to be adequately powered.

III. 13-Week Toxicity Studies:

A. Rat:

Three 13-wk studies provide data for comparing sub-chronic effects of quinapril alone vs. quinapril/HCTZ in the clinical ratios of 20/12.5 and/or 20/25: (1) a study of up to 20 mg/kg of quinapril and/or 25 mg HCTZ; (2) a study of quinapril per se at 50, 250, and 500 mg/kg; and (3) a study of 50, 100, 250, and 500 mg/kg of quinapril plus HCTZ in approx. 20/12.5 ratio. I conclude from these and related studies that: a) There are no unique dose-related toxicities in the combined vs. high dose quinapril per se group. b) When approx. equi-toxic combination and quinapril per se treatments are compared, quinapril dosages are approx. 0-5 fold lower in the combination than in the monotherapy groups. c) Apparent HCTZ-associated shift in quinapril dose-toxicity response curve is associated with (and may reflect?) documented hyper-reninemic and vasodepressor-enhancing effects of the added thiazide. d) Therapeutic ratio (antihypertensive/adverse-renal dosage ratio) remains, as for quinapril per se, in excess of 100 even including juxta-glomerular changes as adverse.

Parameters affected and relevant mg/kg quinapril dosages - alone (A) or combination (C) - include:

- body wt. decrement: 20 (A) vs. 8 (C)
- JGA hypertrophy: 20 (A) vs. 20 (C)
- Elevated BUN: 50 (A) vs. 20 (C)
- Anemia: 50 (A) vs. 50 (C)
- Increased Kidney/Liver Wts: 500 (A) vs. 100 (C)
- Nephritis/Gastric Ulcers: 500 (A) vs. 500 (C)
- Reduced Heart Wt: 50 (A) vs. 20 (C)

I regard the reduced heart weight, in the absence of histopathology, to likely reflect decreased cardiac afterload (hypotension).

B. Dog:

The approx. 8% control incidence of renal cortical tubular basophilia (a marker of cell turnover) was not increased by either 25 mg/kg/day/13 weeks of HCTZ or up to 20 mg/kg/day/13 weeks of quinapril per se (25x MRHD). I consider the incidence of this finding to be exacerbated when HCTZ was added (12.5 or 25 mg/kg) to the high quinapril dose. Other renal effects - JG cell hypertrophy and renal interstitial edema - occurred at 8 and 20 mg/kg of quinapril, respectively, and these thresholds were not affected by adding HCTZ.

The combination dose-antihypertensive response curve has not been established in hypertensive dogs to estimate therapeutic ratio and safety margins. However, renal

tubular basophilia occurred at approx. 30 x the human maxi. recommended combination dose.

RECOMMENDATION: Approvable. I see no unexpected or undesirable efficacy, pharmacokinetic, or toxicity interaction of quinapril with this diuretic in animals. Fetotoxicity of quinapril/HCTZ or quinapril per se is comparable to other ACE inhibitors, and a generic pregnancy-related warning applies. Gravid animals have not been exposed to the combination or, except for rats, to quinapril alone during the second and third trimesters. Any effects of such exposure, whether direct or pre-renal, on fetal kidney function is unknown.

JSI
Albert E. DeFelice, Ph.D.

cc:

Orig. NDA

HFD-110

HFD-110/CSO

HFD-110/ADeFelice

clb/3/9/92/N20125.SP

PHARMACOLOGIST'S REVIEW COVERSHEET

NDA No.: 20-125
SPONSOR: Parke-Davis/Warner-Lambert
DRUG: Accuretic
CATEGORY: ACE Inhibitor/Diuretic Antihypertensive
EVALUATION: Combination increases toxicity of quinapril

- The submission generally consistent with Agency's
Format Guidelines: Yes (X) No ()

- Appropriate studies submitted: Yes () No (X)

- Primary adverse pharmacological effect: Hyperplasia/hypertrophy of afferent artery/JGA.
Body weight decrement at high doses.

- Target organs in toxicity studies:

Kidney: Increased kidney and liver organ weight measurements. Increased BUN, CPK, LDJ, creatinine. Decreased RBC, Hct, Hgb. Tubular dilation. Hypertrophy/Hyperplasia JGA.

Stomach: Focal gastric erosions.

- Reproductive or developmental toxicity: Yes (X) No ()

If yes, explain briefly: Maternotoxic, fetotoxic, abortion, stunted embryos, focal hematomas.

Kidney: Dilated pelvis, focal chronic nephritis.

- Carcinogenicity studies:

Number of studies: Rat: Mouse: Other:
Results: +, -, ± (0) (0) (0)

Site of tumors: Accupril is a combination of two approved drugs.

- Subchronic/Chronic blood level studies:

Rat: X Dog: X Other:

Rats: F/M - plasma level ratio is 5/1.

Dogs: F/M - ratio is 1/2.

- GLP Problems: Yes () No (X)

- OTHER COMMENTS: Male intubation deaths. Moribund sacrifices.

A measure of possible erosion of safety margin is to be recommended. A mouse study is to be recommended to define the kidney damage.

REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA.

Wm. C. Van Arsdel III
March 20, 1992

ORIGINAL NDA SUBMISSION DATED: December 13, 1990.
DIVISION RECEIPT DATE: December 19, 1990.
REVIEWER RECEIPT DATE: January 2, 1991.

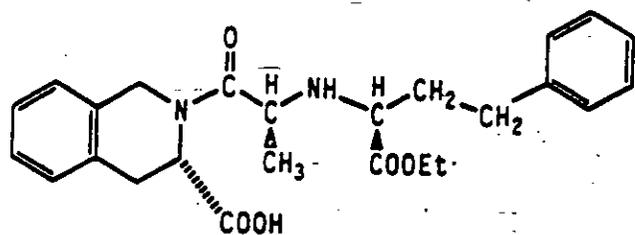
SUMMARY BASIS FOR APPROVAL: Submission Date, December 18, 1991.

SPONSOR: Parke-Davis Research Division of Warner Lambert Co., Ann Arbor, MI.

NAME OF DRUG: Trade: ACCURETIC (quinapril hydrochloride and hydrochlorothiazide fixed combination) TABLETS)
Generic: Quinapril/hydrochlorothiazide tablets.
Codes: CI-955; CI-906 (Quinapril); CI-570 (hydrochlorothiazide); and CI-928 (Quinaprilat, the active metabolite of Quinapril).

REFERENCE NDA: 19,885, Quinapril.

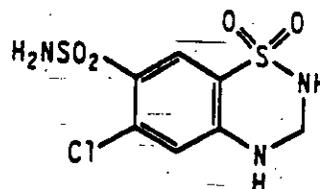
CHEMICAL NAMES and CHEMICAL STRUCTURES:



quinapril hydrochloride

HCl

and



hydrochlorothiazide

FORMULATION:

Quinapril/hydrochlorothiazide tablets will be in three sizes; 20/25, 10/12.5, and 20/12.5 mg tablets (Q/HCTZ), respectively. Other ingredients include Lactose NF Hydrous, Magnesium Carbonate USP, Povidone USP, Magnesium Stearate NF, Crospovidone NF, Candellilla wax FCC, and water.

PHARMACOLOGICAL CLASS: Angiotensin Converting Enzyme (ACE) inhibitor in fixed combination with a diuretic.

INDICATION: Hypertension.

DOSAGE: This fixed-dose combination is not indicated for initial therapy. Usual maintenance doses are 20/12.5 and 20/25 mg/kg/day. Maximum dose recommendation is 40/50 mg/kg (0.8 mg/kg, Quinapril, based on a 50 kg patient).

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 (1365; RR250-01510.

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 # 1424; RR-250-01570.

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SPONSOR'S TABULAR SUMMARIES OF NONCLINICAL PHARMACOLOGY, TOXICOLOGY, PHARMACOKINETICS, AND DRUG METABOLISM STUDIES ARE AS FOLLOWS:

TABLE 1. Tabular Summary of Nonclinical Pharmacology and Toxicology Studies

Species (Reference)	Doses mg/kg PO Quinapril/HCTZ	Test Model	Results	Study Report	
				RR Number	LOCATION VOLUME PAGE
Antihypertensive Efficacy					
Rat (13)	0.3/30	Spontaneously hypertensive	Modest reductions in blood pressure with quinapril alone (0.3 mg/kg) (-29 mm Hg); HCTZ (30 mg/kg) did not significantly lower blood pressure; PRA increased. Coadministration over 3 days produced significant antihypertensive effects (-58 mm Hg).	740-02484	NDA 1.12
Dog (6,14-16)	10/10	Renal hypertensive HCTZ pretreated	Quinapril/HCTZ combination decreased mean arterial blood pressure (-32 mm Hg).	740-02378	NDA 1.12
				740-00936	NDA 1.12
				740-00938	NDA 1.12
				740-02377	NDA 1.12
Renal Actions					
Rat (17)	0.1-3.0/1.0	Normotensive	Quinapril had no effect on the diuretic or natriuretic activity of HCTZ.	740-02586	This NDA 1.7
Rat (18)	0.1-3.0/0.3	Spontaneously hypertensive	Quinapril had no effect on the diuretic or natriuretic activity of HCTZ.	740-02694	This NDA 1.7

TABLE 2. Tabular Summary of All Toxicology Studies
(Page 1 of 5)

Species Strain Sex/group Total	Doses ^a (mg/kg) [route]	Vehicle	MLD (mg/kg)	Results	Study Report		
					RR Number	VOLUME	LOCATION PAGE
Acute Toxicity Studies							
Mouse B ₆ C ₃ F ₁ 10M + 10F N = 100	595/372 750/468 945/590 1190/743 1500/936 [oral]	0.5% methyl- cellulose	M=1063/664 F=1073/669	Depression, prostration, dehydration, hypothermia, dyspnea. No gross pathologic changes. Most deaths occurred within 24 hours.	250-01471	NDA 1.28	
Rat Wistar 10M + 10F N = 160	992/620 1250/781 1575/984 1984/1240 2500/1562 3150/1969 3969/2481 5001/3126 [oral]	0.5% methyl- cellulose	M=4640/2896 ^b F=4640/2896 ^b	Depression, ptosis, dyspnea, generalized congestion. Most deaths occurred within 48 hours.	250-01484	NDA 1.28	
Dog Beagle 1M + 1F N = 2	25/15 to 400/250 ^c [oral]	Gelatin Capsule	>400/250 ^d	Emesis, reduced food consumption, body weight loss, +BUN and creatinine, gastric ulcers/erosions, renal tubular degeneration. No deaths.	250-01485	NDA 1.28	

^a Doses reflect ratio of quinapril/hydrochlorothiazide

^b Estimated from MLDs calculated from combined bulk quinapril/HCTZ doses.

^c Escalating dose regimen

^d No deaths; MLD assumed to be greater than 400/250 mg/kg.

TABLE 2. Tabular Summary of All Toxicology Studies
(Page 2 of 5)

Species Strain Sex/group Total	Doses ^a (mg/kg) [route]	Vehicle	MLD (mg/kg)	Results	Study Report		
					RR Number	VOLUME	LOCATION PAGE
Multidose Toxicity Studies							
Rat Wistar 20M+20F N = 200	VC <u>Quinapril/HCTZ:</u> 50/31, 100/62, 250/156, 500/312 [oral]	0.5% methyl- cellulose	13	+Body weight gain, +food consumption, +kidney weights; necrosis, gastritis, or erosion in the stomach; tubular and interstitial mononuclear cell infiltrates, hypertrophy of arterioles of the JGA.	250-01507	NDA 1.29	
Rat Wistar 10M+10F N = 200	VC <u>HCTZ:</u> 25 <u>Quinapril:</u> 1.6, 8, 20, <u>Quinapril/HCTZ:</u> 1.6/1, 8/5, 8/10, 20/12.5, 20/25 [oral]	0.5% methyl- cellulose	13	+Body weight gain, +BUN, +heart weights, and +kidney weights in quinapril/HCTZ- treated males and/or females; +kidney weights in HCTZ-treated females; and hypertrophy of JGA and the renal afferent arteriole at all high doses.	250-01571	This NDA 1.7	

Abbreviations: VC = vehicle control; JGA = juxtaglomerular apparatus; HCTZ = hydrochlorothiazide; BUN = blood urea nitrogen,
↓ = decreased; ↑ = increased

TABLE 2. Tabular Summary of All Toxicology Studies
(Page 3 of 5)

Species Strain Sex/group Total	Doses ^a (mg/kg) [route]	Vehicle	MLD (mg/kg)	Results	Study Report		
					RR Number	VOLUME	LOCATION PAGE
Multidose Toxicity Studies (continued)							
Dog Beagle 1M+1F N = 6	Quinapril/HCTZ: 25/16, 75/47, 150/94 [oral]	Gelatin Capsule	2	+BUN; renal tubular dilatation at low and high dose, interstitial mononuclear cell infiltrate and hemorrhage in the kidney at high dose.	250-01497	NDA 1.29	05005132
Dog Beagle 3M+3F N = 24	VC Quinapril/HCTZ: 25/16, 75/47, 150/94 [oral]	Gelatin Capsule	13	Dehydration, depression, anorexia, emesis, emaciation, diarrhea; +BUN and creatinine; JGA hypertrophy, tubular dilatation, necrosis, and interstitial edema of the kidney at all doses; oral and gastric erosions and ulcerations, and gastric mineralization at all doses.	250-01510	NDA 1.29	05005204
Dog Beagle 3M+3F N = 60	VC HCTZ: 25 Quinapril: 1.6, 8, 20 Quinapril/HCTZ: 1.6/1, 8/5, 8/10, 20/12.5, 20/25 [oral]	Gelatin Capsule	13	+Serum potassium or chloride at all high doses; multifocal renal cortical tubular basophilia and tubular dilation at high dose quinapril/HCTZ, JGA hypertrophy at all doses of quinapril and quinapril/HCTZ.	250-01570	This NDA 1.8	001

Abbreviations: VC = vehicle controls; JGA = juxtaglomerular apparatus; HCTZ = hydrochlorothiazide; + = increased; - = decreased; BUN = blood urea nitrogen

TABLE 2. Tabular Summary of All Toxicology Studies
(Page 4 of 5)

Species Strain Sex/group Total	Doses ^a (mg/kg) [route]	Vehicle	MLD (mg/kg)	Results	Study Report			
					RR Number	VOLUME	LOCATION PAGE	
Reproductive Toxicity Studies								
Dose Range-Finding								
Rat	VC	0.5% methyl-cellulose	Gestation Days 6 through 15	Dose-related reduction of body weight gain of 22% to 98% versus controls. Fetal weights 14% less than controls in high dose group with corresponding increase in incidence of stunted fetuses.	745-01578	This NDA	1.9	021
SD	3.61/4.51							
SF	7.22/9.05							
N = 30	36.11/45.14							
	108.33/135.42 216.67/270.83 [oral]							
Teratology								
Rat	UC, VC	0.5% methyl-cellulose	Gestation Days 6 through 15	Maternal deaths at 150/93.8 mg/kg (2/20). Decrease in body weight gain at 50/31.3 and 150/93.8 mg/kg. Decrease in mean female fetal body weight at 5/3.1 mg/kg and in both sexes at 50/31.3 and 150/93.8 mg/kg. Incomplete skeletal ossification at 50/31.3 and 150/93.8 mg/kg. No teratogenicity.	745-01647	This NDA	1.9	152
SD	5/3.1							
20 F	50/31.3							
N = 14	150/93.8							
	0/93.8 150/0 [oral]							

^a Doses reflect ratio of quinapril/hydrochlorothiazide
Abbreviations: UC = untreated control; VC = vehicle control; SD = Sprague-Dawley

TABLE 2. Tabular Summary of All Toxicology Studies
(Page 5 of 5)

Species Strain Sex/group Total	Doses ^a (mg/kg) [route]	Vehicle	MLD (mg/kg)	Results	Study Report			
					RR Number	VOLUME	LOCATION PAGE	
Reproductive Toxicity Studies (continued)								
Dose Range-Finding								
Rabbit	VC	0.5% methyl- cellulose	Gestation Days 6 through 18	Marked maternal toxicity: death, abortion, body weight loss at 1.0/0.63 and 1.5/0.94 mg/kg.	745-01424	This NDA	1.10	001
NZW	0.1/0.06							
SF	1.0/0.63							
N = 20	1.5/0.94 [oral]							
Teratology								
Rabbit	UV	0.5% methyl- cellulose	Gestation Days 6 through 18	Maternal toxicity: reduced body weight gain at 0.5/0.31 mg/kg (41%) and 0.5/0 mg/kg (28%); abortion at 0.5/0.31 mg/kg (5.5%) and at 0.5/0 mg/kg (5.3%). No developmental toxicity or teratogenicity.	745-01671	This NDA	1.10	131
NZW	VC							
20F	0.05/0.03							
N = 140	0.1/0.06							
	0.5/0.31							
	0/0.31 0.5/0 [oral]							

^a Doses reflect ratio of quinapril/hydrochlorothiazide
Abbreviations: UC = untreated control; VC = vehicle control; NZW = New Zealand White

TABLE 3. Tabular Summary of Pharmacokinetic and Drug Metabolism Studies

Study (Reference)	Species (N)	Dose (mg/kg)	Design	Results	Study Report	
					RR Number	LOCATION VOLUME PAGE
Pharmacokinetics Quinapril/HCTZ Drug Interaction	Dog (3)	50 Q 25 HCTZ 50 Q + 25 HCTZ	3 X 3 Latin Square	Absorption of quinapril, subsequent conversion to quinaprilat, and elimination were not altered by HCTZ. The extent of HCTZ absorption was increased and renal clearance decreased following concomitant administration with quinapril.	764-00606	NDA 1.36 05007309

Q = quinapril

APPEARS THIS WAY
ON ORIGINAL

INTRODUCTION: Accuretic™ is a fixed dose combination of quinapril and hydrochlorothiazide for use in hypertension.

Quinapril hydrochloride (quinapril), an orally active nonsulfhydryl, nonpeptide ACE inhibitor developed by Parke-Davis for use in hypertension, is an ester prodrug which undergoes rapid metabolic hydrolysis to yield its active diacid (deesterified), quinaprilat. Other metabolites related to quinapril have been identified, but quinaprilat, the principal active metabolite, is the most important. Quinapril produces dose-related decreases in mean blood pressure in two-kidney one-clip renal hypertensive and spontaneously hypertensive rats. The onset of antihypertensive activity in both rats and dogs is rapid, of long duration, and with less than a 25% increase in heart rate.

Quinapril and its active metabolite, quinaprilat, interferes with the normal biochemistry of the renin-angiotensin-aldosterone (RAA) system by inhibiting ACE activity. The concentration which produces 50% inhibition (IC₅₀) for quinaprilat in human plasma ranges between 5.9×10^{-10} and 6.4×10^{-10} M. The diuretic may increase the dependence of blood pressure regulation on the RAA system.

Hydrochlorothiazide is a well known diuretic and antihypertensive agent with multiple mechanisms such as reduction of interstitial fluid volume, alteration of sodium balance, and decreased vascular wall stiffness. The mechanism of the antihypertensive effect of thiazides is unknown. Hydrochlorothiazide does not usually affect normal blood pressure. Hydrochlorothiazide affects the distal renal tubular mechanism of electrolyte reabsorption. Plasma renin activity and plasma aldosterone levels rise in response to thiazide therapy.

PHARMACOLOGY OF QUINAPRIL

In normal conscious dogs with normal plasma renin activity levels, oral quinapril (10 mg/kg) did not produce consistently significant changes in any of the cardiovascular parameters measured. Mongrel dogs tested with 0.003 to 0.03 mg/kg, i.v., quinaprilat (metabolically active moiety of quinapril) for renal function (i.v. saline bolus, 10 ml/kg, inulin, 200 mg/kg, i.v., and PAH, 8 mg/kg), did not reveal significant differences in mean arterial pressure (MAP), HR, or plasma renin activity (PRA); glomerular filtration rate (GFR), effective renal plasma flow (ERPF), or filtration fraction (FF). Treatment increases compared to controls included urine volume (70% over controls) and sodium excretion (60% increase over controls).

Quinapril produced dose dependent decreases in mean arterial blood pressure in renal hypertensive rats. Heart rate was not elevated reflexly despite the marked reduction in arterial blood pressure. The effectiveness of Quinapril was best demonstrated in hypertensive models which exhibited high plasma renin activity (about 35 ng AI/ml/hour), such as two-kidney, one-clip renal hypertensive rat and the diuretic-treated perinephritic hypertensive dog. While doses of 0.03 and 0.1 mg/kg reduced BP in that rat model (which was enhanced by selection only of rats with a mean arterial blood pressure of more than 170 mm Hg), 0.3 was statistically significantly different from control values and considered to be the effectiveness threshold.

Low plasma renin activity is found to be present in deoxycorticosterone acetate (DOCA) treated hypertensive rats, a model in which oral quinapril at 30 mg/kg for eight days failed to prevent the development of hypertension. In a one-kidney DOCA salt hypertensive rat model (plasma renin activity about 2 ng AI/ml/hour), quinapril at 30 mg/kg/day, orally, for 12 days, had no influence on the rapidly increasing blood pressure, thus supporting a renin dependent mechanism of action for quinapril.

TOXICOLOGY OF QUINAPRIL:

The toxicity profile of quinapril, discussed in NDA _____, is similar to that of other ACE inhibitors. Orally, quinapril is about twice as toxic to mice as to rats acutely, but the acute toxicity in rodents is relatively low (rat plasma rapidly metabolizes quinapril). Repeated dosing elicits gastric irritation, juxtaglomerular apparatus (JGA) hypertrophy/hyperplasia, tubular degeneration in the kidney, and reduced red cell parameters and reduced heart weights in rodents and dogs. Other manifestations of toxicity include dog hepatic lesions; rat fetal weight loss, hydronephrosis, and hydroureter; rabbit maternal/fetal loss and abortion at 0.5 mg/kg (1/3 the maximum human therapeutic dose); and, in the in-vitro V79 Chromosome aberration assay, clastogenic effects at cytotoxic doses.

Emesis was the dose-limiting clinical manifestation in dogs. Gastric erosions and ulcers were noted in dogs and rats at 30 and 160 times the maximum daily human dose of 1.6 mg/kg. (For the purposes of this summary, multiples of the human dose refer to the maximum, recommended daily dose in a 50 kilo patient.) Gastrointestinal irritation was attributed to local effects since it was not observed in intravenous studies.

Increased plasma renin and increased size and granularity of juxtaglomerular (JG) cells in the kidney were noted in all species tested. Sponsor contends that this change reflects pharmacologic stimulation of renin-containing cells due to loss of feedback inhibition by angiotensin II, thus, the presence of JGA changes does not alter the assessment of a nontoxic dose. JGA changes did not reverse after a four-week drug withdrawal period in the 52-week rat study. According to sponsor, this observation was not unexpected, since JGA changes occurring after chronic administration of high doses of captopril regressed slowly and did not show complete reversal after drug withdrawal periods of up to six months. However, sponsor then contends that quinapril effects on JGA and renin production tended to reverse by 104 weeks. Renal degenerative changes were observed with quinapril at high doses in acute and subacute studies in rodents and dogs, and in chronic rodent studies at approximately 3 to 5 times the human dose (JG granularity, hyperplasias, and nephritis). In repeated-dose dog studies, slight to mild increases in blood urea nitrogen (BUN) at doses in excess of 60 times the human dose were not associated with increases in creatinine or histologic evidence of renal toxicity and were attributed to prerenal causes. Quinapril also may reduce renal clearance of HCTZ as well as BUN.

Sponsor considers that the quinapril no-effect dose in dogs for kidney toxicity over three months was greater than 15 times (25/1.6 mg/kg) the human dose, and over one year was 6 times (10/1.6 mg/kg) the human dose. In rats, an increase in severity (over that of controls) of chronic progressive nephropathy was apparent in both sexes at 100 mg/kg after 104 weeks, but the 10 mg/kg dose, the lowest dose used in rats, exhibited JG pathologies, thus, there were no no-effect levels. In the 2 year mouse study an increase in severity of chronic progressive nephropathy was apparent in females at 35 and 75 mg/kg, and in males at 75 mg/kg after 104 weeks, JG pathology appeared at the lowest dose tested in females (5 mg/kg), but not males. One of 5 H-D (5 mg/kg) rabbits had an acute renal failure and all dose levels (0.5, 1.0, and 5.0 mg/kg) exhibited JG pathology. Apparently there is no no-effect dose level on record for any species.

Sponsor noted that severe effects of anemia and thrombocytopenia or leukopenia with bone marrow hypocellularity observed in dogs given captopril weren't seen with quinapril; however, quinapril observations were obtained at much lower doses, and dose relationships were not discussed.

Hepatic changes were noted in three dogs treated for 13 to 52 weeks with quinapril at doses 60 to 80 times the human dose. Liver weight increases in subacute rodent studies occurred without histopathologic changes.

Maternal and fetal toxicity in the rabbit has been noted with quinapril and the toxic dose is less than the clinical dose.

In the preliminary 13-week mouse study there were no drug-related effects on the general health or behavior of the treated animals, and no drug-related gross pathologic changes were observed. Treatment-related decreases in both relative and absolute heart weight were observed in all treated male groups and in female groups at 125, 250, and 500 mg/kg. Hyperplasia of the JGA was observed in all drug-treated groups (no no-dose effect), an effect considered by sponsor to be a pharmacological effect. In addition, there was an increased incidence of focal chronic nephritis in all treatment groups. Based on weight-gain suppressions in the 13-week study, doses of 5, 35, and 75 mg/kg/day were selected for the long-term carcinogenicity bioassay. In the mouse carcinogenicity study, the body weight decrement was less than 4% for all groups and clinical signs and pathologies were unremarkable. Mean heart weights were decreased in the treated male groups (-4%, -10%, & -12%, respectively, in L-, M-, & H-D groups), and -8% in both the M- and H- dose female groups (L-D increased 10%).

Treated mice had a dose related increase in incidence and severity with respect to nephritis, in addition to justaglomerular hypertrophy/hyperplasia and tubular basophilia. Malignant lymphomas were of equivalent incidence between control and treatment groups but the treated mice died earlier in a dose related manner (parallels nephritis effect).

In the rat carcinogenicity study, the only differences between the doses with respect to intercurrent mortality were caused by dose related increases in male intubation deaths. Terminal body weights were 91 to 107% of control weights and reduction of feed consumption did not exceed 3% in the treated groups. Biochemical alterations at 100 mg/kg included increased BUN and plasma renin in males, decreased serum-glucose levels in females, and minor increases in CPK and LDH levels in males given 50 mg/kg or more.

Increased kidney weights in high dosed males, and anticipated renal histopathologies seen in previous studies (JG hypertrophy/hyperplasia and increased granularity of the JGA and interlobular arteries) were found at all dose levels, but heart-weight decreases were not, and no unanticipated toxicity was evident at 10 or 50 mg/kg. Pulmonary edema and hemorrhage due to gavage dosing errors were 15% more numerous in the pathologist's report sheets than reported in the summary, and more numerous in treated groups in a dose related manner than in controls. Two rare tumors (normal incidence less than 1%), mesenteric lymph node/hemangioma and uterine leiomyosarcoma, were found to be significant by the FDA statistician at the 0.05 level. The skin/subcutaneous lipoma (benign) at the 0.01 significance level was the only tumor that exceeded controls using Fisher's Exact Pair-Wise Comparison test.

Quinapril in an in vitro nonmammalian cell system observing point mutation in Salmonella typhimurium TA-1535, TA-100, TA-1537, TA-1538, and TA-98 with a system limit concentration of 10,000 mcg/plate (which proved not cytotoxic to most strains) exhibited no significant increases in revertant counts with any of the quinapril concentrations.

Quinapril in an in vitro mammalian cell system assay using Cricetulus griseus lung cells exposed for three hours to doses up to 1400 ug/ml in the absence or presence of rat liver S9 fraction, with cytotoxicity at 1400 ug/ml and higher, did not show significant increases in the mutant frequencies under metabolic activation. Although there was a significant positive dose-response trend; a twofold background frequency wasn't attained in the presence or absence of S-9.

In male Cricetulus griseus lung cells used to evaluate induction of structural chromosome aberrations (SCA), quinapril concentrations above 1200 ug/ml were cytotoxic, but the incidence of SCA was not elevated for either the non activation phase or for the activation phase.

In clastogenicity studies in an in vitro Mammalian Cell System with Arochlor 1254-induced rat liver S9, prepared according to the method of Ames with a bacterial mutagenesis titration, quinapril did not increase the SCA incidence.

In an In Vitro mammalian cell system using Cricetulus griseus CHO (ovary) cells in a nonactivation and activation assay, cytotoxicity was observed at doses of CI-906 above 700 mcg/ml but quinapril in the test did not manifest direct or indirect genotoxic (altered gene recombination) activity under conditions of this assay.

Clastogenicity observed in an in vitro chromosome aberration assay occurred only at cytotoxic doses and was not considered biologically significant.

Quinaprilat (major metabolite of quinapril) does not exhibit greater toxicity upon repeated intravenous administration at doses equivalent to those generated in oral quinapril studies. The degradation products, impurities, and diketopiperazine metabolites of quinapril were also tested and found to have low acute toxicity.

REPRODUCTION SEGMENT I:

Fertility/reproduction (Segment I) studies in CD rats with Quinapril doses of 0 (UTC), 0 (VC), 10, 50 and 100 mg/kg/group/day, led to the following drug associated findings: Five treated F₀ generation rats died; one male and 2 females in the H-D group, one female in the M- and one in the L-D group (zero control deaths). Cause of death was not determined. Swollen ventral necks were seen in 2 H-D males (attributed to viral infection by examining pathologist) and hair loss in one H-D female, otherwise, there were no treatment related clinical signs or gross necropsy findings that sponsor would consider dose related. In the 12/group C-sectioned dams, mean numbers of corpora lutea, implantations, viable fetuses, their fetal body weights and sex distributions were not significantly different between treated and control groups. The incidence of non-gravid dams among viable females allowed to deliver was 6/11, 2/11, 4/11 and 0/10, respectively, in the control, L-, M-, and H-D groups. Treated F₀ females allowed to deliver had a slight increase in postimplantation loss. There were no drug-related effects on length of gestation, parturition, litter size, fetal body weights, sex distribution, nesting or nursing behavior, or fertility indices of treated F₀ males or females when compared to the control groups. There were no significant differences in F₁ fetal malformations, developmental variations, or behavioral indices between treated and control groups. No deaths or significant clinical reactions occurred among treated F₁ offspring during maturation; growth was normal in both sexes. Body weight gains during pregnancy, fertility rate, and litter parameters of F₁ offspring, as well as sex ratios, fetal wastage, and body weights of the F₂ offspring from treated groups were comparable to controls. There were no statistically significant differences in the incidence of external abnormalities in F₂ fetuses between the treated and control groups.

It was concluded that under the conditions of this study, quinapril did not induce adverse effects on maternal clinical signs or gross necropsy findings or on fertility or reproductive parameters at the dose levels selected.

In an exploratory dose range finding teratology study, 5/dose level, in pregnant rats with quinapril administered on days 6 to 15 of pregnancy at doses of 100, 200, 400, 600 and 800 mg/kg/day, two dams given 800 and one given 600 mg/kg/day died during the dosing period (cause of death was not determined). Dose-related, transient postdose salivation was observed in 19 of the 25 treated animals. During predosing gestation days 0-6 all groups exhibited similar weight gains (29-33 grams) but during the first week of dosing, gains, not dose related, varied from 9 to 39 grams. A compensatory post-dosing (days 15-21) week provided overall gestation period gains of 97 to 121 grams, gains which were similar across groups in the postdosing period and not dose related. Food intake was similar in the test groups. Hydroureter was more frequent at 600 and 800 mg/kg/day and hydronephrosis at 800 mg/kg/day than at lower doses, but sponsor did not consider this condition to be drug related. While data is not provided in a manner to allow separations of animals with these pathologies, it is to be noted that most litters were unaffected in the three groups receiving the lower doses of 100, 200, and 400 mg/kg, there being only 5 cases (1.7/group) of hydronephrosis or hydroureter listed (occurring in only 2 to 4 litters out of the 15). In contrast, most of the surviving dams in the 2 highest doses had affected litters. At 600

mg/kg, 9 cases of hydroureter were found in 3 of 4 litters and hydronephrosis in 1 of 4 litters; and at 800 mg/kg, 2 of 3 litters exhibited 4 cases of hydronephrosis and 1 of 3 litters had hydroureter (2 pups) -- with any contribution in the dead remaining unaccounted:

Incidence of hydronephrosis and hydroureter:

Dose, mg/kg/day (No Control):	100	200	400	600	800
Number of Litters:	5	5	5	4	3
Number of Live Fetuses:	63	66	68	43	43
Hydronephrosis/Hydroureter Fetuses	2 (3%)	0	3 (5%)	10 (23%)	6 (14%)

In general, no other gross, visceral, or skeletal malformations were apparent, but a hydroureter/hydronephrosis pathology dose relationship can be postulated.

In a teratology study using CD inseminated rats dosed with 0 (UTC), 0 (VC), 50, 150, and 300 mg/kg/gestation days 6 to 15, one H- and one M-D dam died due to dosing trauma (a dose-related response previously noted for quinapril). Findings included transient post-dose salivation observed on a nondose-related basis in the drug-treated groups. Other sporadic clinical signs observed in treated animals include urine scald, reduced fecal output, soft feces, rhinorrhea, and rales. Maternal toxicity was evidenced as reduced body weight gain during the dosing period (days 6-15), with the greatest reduction in dams given 150 or 300 mg/kg; however, post dosing compensation recovery reduced the difference. Gestational period daily food intake was reduced less than 10% in treated groups. Litter and fetal parameters were unaffected, fetal examination revealed no drug-related external abnormalities, and no gross visceral or skeletal abnormalities were observed.

In an exploratory dose range-finding study with artificially inseminated rabbits given 10, 15, 25, 50, 100, 200 and 400 mg/kg/day Quinapril on days 6 to 18 of pregnancy (sacrificed on Day 21 of gestation), twenty-eight of 37 does died during the experimental period and one was sacrificed in poor condition. In addition, 2 does were lost to dosing error and one because of fractured vertebrae and paralysis of the hind limbs (cumulative T = 31/37). Hyperpnea and anorexia were frequently associated with treatment at all dose levels. Additional clinical signs included abortion, rales, dyspnea, diarrhea or soft stool, urine scald, bloody or mucoid vaginal discharge, weakness, lethargy, emaciation, dehydration, prostration, hypokinesia, nasal discharge, convulsion, and hair loss. During the treatment period, body weight gain was reduced among the surviving pregnant does. Gross pathologic findings in the does were multifocal mucosal ulceration or erosion of the stomach at all dose levels. Abnormal findings among preterm fetuses included ecchymotic subcutaneous hemorrhages, generalized hemorrhage, omphalocele, and open eyes. Only two of the four pregnant does that survived to term sacrifice had viable normal fetuses.

Because of the marked maternal toxicity, a second range-finding study was conducted with quinapril, gestation days 6 through 18 at doses of 0, 1, 2, 4, 6, or 8 mg/kg/day, resulting in seven deaths (cause not determined).

Findings: Dose, Mg/kg/day	0	1	2	4	6	8
Number/group	5	5	5	5	5	5
Number/gravid/group	5	4	4	5	4	5
Number died (and as % of gravid)	0	0	0	2 (40%)	2 (50%)	3 (60%)
Aborted prior to (death) or sacrifice	0	0	0	(1)	(1)	1
Does with viable fetuses	5	4	2	2	2	1
Viable fetuses/doe	6.6	6.8	3.8	6.3	3.0	8.0
Implantations/doe	6.6	8.0	8.5	9.3	3.0	8.0

One doe in each of the 4, 6, and 8 mg/kg groups aborted prior to death or scheduled sacrifice, otherwise, there were no clinical signs or gross pathologic observations associated with treatment. Mean maternal body weight and body weight change in the does given 1 mg/kg were comparable to the control group. Mean weight loss occurred at doses of 2, 4, 6 and 8 mg/kg during the treatment period. During the overall gestational period, only the 2 and 4 mg/kg groups had weight losses. No differences in the number of viable and nonviable fetuses, resorptions, implantations, or corpora lutea were found between the 1 mg/kg and control groups. Embryotoxicity occurred at 2 and 4 mg/kg, as shown by increased number of does with resorptions, decreased viable fetuses, and increased postimplantation losses. Due to maternal toxicity and reduced fetal sample size at 6 and 8 mg/kg, meaningful comparisons of reproductive parameters could not be made. This study confirmed quinapril intolerance to pregnant rabbits. Based on these findings, 1 mg/kg was selected as a tolerable dose level for the definitive study.

Groups of 14 artificially inseminated rabbits were given 0, 0.5, 1.0, and 1.5 mg/kg/day quinapril on gestation Days 6 through 18. Seven does died between gestation days 17 and 27; two H-, four M-, and one L-D, but no control group deaths. No significant clinical signs of toxicity were apparent in animals that died; causes of death were not determined. The L-D doe that died (day 27) aborted one to two days before death. The only other abortion occurred on Day 21 in a M-D doe. Nasal discharge and hair loss were noted in all groups. Pathologic findings included pulmonary congestion in all groups and pitted kidneys in control, 0.5, and 1.0 mg/kg groups. Pitted kidneys were attributed to endemic encephalitozoan (*Nosema*) infection and considered to be incidental. During dosing and throughout the overall gestation period, all treated groups displayed maternal body weight loss relative to controls. In the posttreatment period, maternal body weight loss was noted only in animals given 0.5 or 1.5 mg/kg. Mean postimplantation loss was increased in the 1.0 and 1.5 mg/kg groups (Sponsor: due to an increase in total number of early resorptions) with a corresponding decrease in the mean number of viable fetuses when compared to the control group. No apparent differences were found in the incidences of external, visceral, or skeletal malformations, or incidence of developmental variations between the treated and control groups. Quinapril did not elicit a corporal teratogenic response in this study, but maternal toxicity and embryotoxicity were observed at all dose levels, (with no no-effect dose level in evidence), and significantly at doses of 1.0 and 1.5 mg/kg. Quinapril is about 500 times as toxic to the rabbit as Captopril, and is toxic at a dose level less than 1/3 that of the human clinical dose.

Dose, Mg/kg/day	0	0.5	1.0	1.5
Number/group	14	14	14	14
Number/gravid/group	14	14	13	12
Number died	0	1	4	2
Nongravid	0	0	1	0
Gravid	0	1	3	2
Number aborted	0	1	1	0
Does at C-section (No. nongravid)	14	13	10	12 (2)
Does with resorptions only	0	0	2	1
Does with viable fetuses	14	13	7	9
Viable fetuses/doe	7.3	7.0	5.4	6.2
Implantations/doe	8.1	7.5	7.9	8.4
Corpora lutea/doe	11.6	12.9	12.8	13.6
Mean fetal body weight (gms)	31.4	31.4	32.3	29.6

A Perinatal and postnatal study on pregnant CD, rats with 20/group given zero, 25, 75, and 150 mg/kg quinapril/day on gestation days 15 through day 20 of lactation had no mortality, significant clinical signs of toxicity, or drug-related clinical signs in any group. Three animals each in control, 25, and 75 mg/kg groups were nongravid. No drug-related abnormalities were noted at necropsy. Mean maternal weights in treated groups were comparable to controls, and no significant effect was observed on nesting or nursing behavior, pregnancy rate, length of gestation, or litter size.

Mean pup body weight was significantly decreased in all treated groups at each interval of the nursing period, except at birth in the 25 mg/kg group. Neonatal survival at birth and throughout lactation was comparable among all groups. Twenty-nine pups were found dead or missing. One mid-dose and one high-dose pup had multiple malformations. Sponsor did not consider these findings treatment-related because of the low incidences seen in the study and compared with the historical rates of anomalies in this laboratory. No dead pups with abnormalities were found in the control or low-dose groups. A slight but significant decrease in offspring body weights was observed in newborn rats following treatment of the dams from gestation Day 15 through lactation Day 20 with dose levels of 25 to 150 mg/kg.

No drug-related clinical signs were observed in a modified peri- postnatal study in four groups of Sprague-Dawley CD rats given 0 or 150 mg/kg/day, quinapril. Maternal effects such as body weight gains, food consumptions, organ weights, gross- and histo-pathological variations (except JG cell hypertrophy/hyperplasia) were not considered to be drug related. There were no significant differences between control and treated groups with respect to numbers of corpora lutea, number of implant sites, litter size, percent preimplantation loss, and percent survival to term.

According to the sponsor, apparent greater values seen in post implantation loss and number of resorptions in the treated dams were not statistically significant. All gravid animals delivered on gestation days 21-23, and mean day of gestation was comparable across groups. However, signs of toxicity were present as can be seen by the following selected data for gestation (G) and lactation (L):

Treatment days (Group No.):	VC (1)	G15-G21 (3)	G15-L21 (2)	L0-L21 (4)
No. observed/group:	30	15	30	25
No. C-sectioned, day 21:	5	5	5	5
Live born, mean	14.0	14.2	13.5	11.9
Post implantation loss	6.6	8.2	11.4	17.0
Dam, Died (after death of pups)	1	0	0	0
Dams, Euthanized, cause:				
all pups dead or missing	2	0	5	4
Did not deliver	0	0	3	1
F ₀ Dams, histopath: JG H/H:	0	8	1	3

Term fetuses from all groups appeared normal upon external examination. No abnormalities were observed during microscopic examination of kidney specimens obtained from control and treated fetuses. In contrast, treated neonates which received the drug during gestation and lactation (group 2) and during lactation only (group 4) and sacrificed during lactation, exhibited a treatment duration related increase in kidney histopathologies, as follows:

Sacrifice; lactation day number:	6	13	21
Tubular dilation:			
control	0	0	0
group 2	0	6/10	3/10
group 4	0	1/10	2/10
Glomerulosclerosis:			
control	0	0	0
group 2	0	2/10	0
group 4	0	0	0
Dilated Pelvis:			
control	0	1/10	1/10
group 2	2/10	2/10	1/10
group 4	0	1/10	2/10
JG Cell Hypertrophy/ Hyperplasia:			
control	0	0	0
group 2	0	0	10/10
group 4	0	0	5/10

The administrations of 150 mg/kg/day under the conditions of this experiment resulted in mean plasma quinaprilat concentrations of 30600 to 64100 ng/ml in the dams one hour after dosing. Mean foetal plasma quinaprilat concentrations were 225 and 306 nanograms/ml in groups 2 & 3, respectively, less than 1% of the dam's concentrations of quinaprilat. The Dam's milk ranged from 3 to 5% (1520-1960 nanograms/ml) of the plasma drug concentrations from the same dams.

Toxicity increases when quinapril is administered concomitantly with diuretics, the increased toxicity is likely to reflect additive effects of ACE inhibition, blood volume reduction, electrolyte changes, and observed or expected alterations of blood pressure, GFR, and urine output.

PHARMACOLOGY OF COMBINATION, QUINAPRIL/HYDROCHLOROTHIAZIDE:

In pilot studies in two-kidney perinephritic hypertensive dogs, oral doses up to 20 mg/kg of either quinapril or enalapril or 30 mg/kg HCTZ did not lower blood pressure. Previous experience with this model had shown inconsistent renin elevation, while treatment with a diuretic such as HCTZ caused a sustained elevation in plasma renin activity. A day after administration of 5 mg/kg HCTZ, b.i.d., 10 mg/kg of either quinapril or enalapril when used together with 10 mg/kg of HCTZ produced marked antihypertensive activity.

In conscious dogs with high preexisting plasma renin activity (8.9 ng AI/ml/hr) induced by sodium restriction (diet, less than 10 mEq Na/day) and diuretic treatment (furosemide, 5 mg/kg, oral, every other day, one week; then one day prior to once a week testing), oral quinapril at doses of 0.3 to 3.0 mg/kg dose-dependently reduced total peripheral resistance by 17% to 28% and mean arterial blood pressure by 8% to 19% while modestly raising heart rate (3 to 13%) and cardiac output (10% to 13%).

In spontaneously hypertensive rats, quinapril produced dose-dependent antihypertensive actions over an oral dose range of 0.3 to 10 mg/kg, but the magnitude of the fall in blood pressure was less than that observed in renal hypertensive rats. Coadministration with HCTZ significantly enhanced the antihypertensive activity. Antihypertensive actions were maintained over an eight day period without tolerance development. Quinapril did not affect the urinary excretion of either vasodilatory prostanoids or indices of kinin formation. A marked reduction in serum ACE activity was accompanied by an increase in plasma renin activity and a decrease in urinary aldosterone.

COMPARATIVE COMBINATION STUDIES:

Spontaneously hypertensive rats were given threshold antihypertensive doses (0.2 or 0.3 mg/kg/day) of Quinapril, ineffective antihypertensive doses of HCTZ (30 mg/kg/day), or both together; a combination with a significantly greater response than either Quinapril or HCTZ alone. These apparently synergistic effects appeared on the second day and were fully manifest on the third day. HCTZ alone caused a 3-fold increase in plasma renin activity.

In renal hypertensive dogs (bilateral renal encapsulation) four weeks after operation, quinapril alone at oral doses of up to 20 mg/kg did not lower blood pressure; HCTZ at 10 mg/kg had no significant effects on blood pressure; but Quinapril and HCTZ, both 10 mg/kg, produced marked antihypertensive activity.

A Multiple-Dose Study to Assess the Functional Interaction of Quinapril and Hydrochlorothiazide in Saline-Loaded Normotensive Rats; RR 740-02586; 11/04 to 11/30/88; Issue Date: 8/29/89. (male S-D; 12/dose group).

Saline loaded normotensive rats were administered 1.0 mg/kg HCTZ and/or 0.1, 0.3, 1.0, and 3.0 mg/kg quinapril. HCTZ alone significantly increased urine volume and sodium excretion with respect to vehicle treated rats.

Quinapril alone had no effect on urine volume with doses of 0.1 to 1.0, but 3.0 mg/kg, p.o., uniformly reduced urine volume (44%), sodium excretion (41%), potassium excretion (24%), percent volume load excreted (43%), and percent sodium load excreted (38%, Table 1, RR 740 02586; or 48%, Table 3, RR-REG-X 740 02909).

Except for the potassium, these values are probably related to the reduced urine volume at this dose. The combination provided a dose effect difference in response to the HCTZ increase and quinapril decreases. There was a dose related decrease in the HCTZ-increased urine volume similar to the dose related decrease seen in quinapril only treated rats; while the HCTZ-increased excretions of sodium and potassium were potentiated at lower dosages of quinapril, but not at 3.0 mg/kg. Sponsor has submitted differing tables with different submissions. The 1.29 mEq/kg/5hr for 0.1 mg/kg quinapril with 1.0 mg/kg HCTZ (Table 3) is probably incorrect; 1.54 mEq/kg/5hr for 0.1 mg/kg quinapril with 1.0 mg/kg HCTZ for Table 1 is probably correct.

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TABLE 1

Observed Mean (± SE) (N = 6) Renal Excretory Response to Saline Load in Male Rats Treated with Quinapril and Hydrochlorothiazide

Quinapril dose (mg/kg)	HCTZ dose (mg/kg)	Urine Output (ml/5 hr)	Sodium Excretion (mEq/5 hr)	Potassium Excretion (mEq/5 hr)	% Volume Load Excreted	% Sodium Load Excreted
0 (vehicle)	0	5.9±0.8†	0.76±0.18†	1.16±0.15	122.4±14.2†	99.6±19.7†
0.1	0	5.2±0.8†	0.62±0.11†	0.97±0.11†	108.7±15.8†	83.4±14.5†
0.3	0	5.6±1.1†	0.70±0.79†	1.11±0.14	130.2±19.0†	103.8±11.6†
1.0	0	5.2±0.7†	0.65±0.10†	1.10±0.07	108.7±14.4†	86.4±12.0†
3.0	0	3.3±0.3*†	0.45±0.06†	0.88±0.10†	69.4±6.5*†	61.8±7.3*†
0 (HCTZ alone)	1.0	9.3±0.8*	1.21±0.09*	1.29±0.07	194.6±15.4*	163.6±10.1*
0.1	1.0	9.3±1.1*	1.47±0.09*	1.56±0.09*	191.8±23.9*	196.6±9.1*
0.3	1.0	8.1±0.9*	1.44±0.11*	1.41±0.05	191.4±19.3*	195.5±14.7*
1.0	1.0	8.8±1.0*	1.40±0.19*	1.46±0.15	182.9±20.3*	188.4±22.3*
3.0	1.0	6.9±0.4**	1.22±0.20*	1.35±0.12	140.9±6.4†	160.7±22.1*

Data are expressed as mean (± SE) (N = 6).
 * p < 0.05 relative to vehicle
 † p < 0.05 relative to hydrochlorothiazide alone
 RR 29861:1-3

RR-REG-X 740-02909
 Quinapril/HCTZ
 Tablets

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TABLE 3. Renal Excretory Response to Saline Load in Male Rats Treated with Quinapril and Hydrochlorothiazide (HCTZ)

Quinapril Dose (mg/kg)	HCTZ Dose (mg/kg)	Urine Volume (ml/kg/5 hr)	Sodium Excretion (mEq/kg/5 hr)	Potassium Excretion (mEq/kg/5 hr)	% Volume Load Excreted	% Sodium Load Excreted
0	0	5.9±0.8†	0.76±0.18†	1.16±0.15	122±14†	100±20†
0.1	0	5.2±0.8†	0.62±0.11†	0.97±0.11†	108±16†	83±15†
0.3	0	5.6±1.1†	0.70±0.79†	1.11±0.14	130±19†	104±12†
1.0	0	5.2±0.7†	0.65±0.10†	1.10±0.07	109±14†	86±12†
3.0	0	3.3±0.3*†	0.45±0.06†	0.88±0.10†	69±7*†	62±9*†
0	1.0	9.3±0.8*	1.21±0.09*	1.29±0.07	196±15*	164±10*
0.1	1.0	9.3±1.1*	1.47±0.09*	1.56±0.09*	192±24*	197±9*
0.3	1.0	8.1±0.9*	1.44±0.11*	1.41±0.05	191±19*	196±145*
1.0	1.0	8.8±1.0*	1.40±0.19*	1.46±0.15	183±20*	186±22*
3.0	1.0	6.9±0.4**	1.22±0.20*	1.35±0.12	141±6†	161±22*

Data are expressed as mean ± SE (N = 6).
 * p < 0.05 relative to saline (0.9% NaCl)
 † p < 0.05 relative to hydrochlorothiazide alone

A Multiple-Dose Study to Assess the Functional Interaction of Quinapril and Hydrochlorothiazide in Saline-Loaded Spontaneously Hypertensive Rats:
RR-740-02694; 3/20 to 4/06/89; Report Date 9/1/89.

Male, S-D, 12 to 20/group, Vehicle and four separate doses of quinapril (0.1, 0.3, 1.0, and 3.0 mg/kg, PO) were administered orally to rats with or without a single dose of HCTZ (0.3 mg/kg) and challenged with a 10 mL/kg saline load.

Results: Urine output was significantly elevated in animals treated with HCTZ alone relative to saline controls. Quinapril alone increased urine volume (60% above control) only at the 0.3 mg/kg dose level (lower and higher doses tended to decrease urine volume). The elevated HCTZ (0.3 mg/kg) induced urine volume was unaffected by the addition of 0.1 and 0.3 mg/kg of quinapril, but tended to be decreased (25%) in HCTZ animals treated with quinapril at 1.0 and 3.0 mg/kg. HCTZ alone increased sodium excretion four fold above vehicle control. Quinapril alone doubled sodium excretion at 0.3 mg/kg, only, and was mildly natriuretic at all doses in the spontaneously hypertensive rat. Quinapril did not significantly increase the natriuretic response to HCTZ in this rat model. The patterns of potassium excretion paralleled those of urine volume. Sponsor has submitted differing tables with different submissions. The 241 % sodium load excreted for 0.3 mg/kg HCTZ alone (Table 4, RR-REG-X 740-02909) is probably incorrect; the 187 % sodium load excreted for 0.3 mg/kg for 0.1 mg/kg HCTZ alone, as shown in Table 1 (RR 740-02694) is probably correct. (See table 1) The combination of quinapril plus HCTZ had significantly greater antihypertensive activity than either agent alone.

TABLE 1. Observed Mean (\pm SE) (N = 6) Renal Excretory Response to Saline Load in Male Hypertensive Rats Treated with Quinapril and Hydrochlorothiazide

Quinapril Dose (mg/kg)	HCTZ Dose (mg/kg)	Urine Output (ml/kg/5 hr)	Sodium Excretion (mEq/kg/5 hr)	Potassium Excretion (mEq/kg/5 hr)	% Volume Load Excreted	% Sodium Load Excreted
0 (vehicle, N = 10)	0	5.0 \pm 1.0	0.48 \pm 0.11	0.23 \pm 0.06	56 \pm 13	45 \pm 11
0.1 (N = 6)	0	3.8 \pm 0.6	0.65 \pm 0.10	0.31 \pm 0.06	55 \pm 8	62 \pm 5
0.3 (N = 7)	0	6.4 \pm 0.6*	0.80 \pm 0.10*	0.50 \pm 0.07	99 \pm 5*	90 \pm 10*
1.0 (N = 7)	0	3.7 \pm 0.8	0.68 \pm 0.12	0.36 \pm 0.10	53 \pm 11	65 \pm 12
3.0 (N = 6)	0	3.4 \pm 0.7	0.61 \pm 0.11	0.27 \pm 0.05	49 \pm 9	58 \pm 10
0 (HCT alone, N = 9)	0.3	12.7 \pm 0.7*	2.00 \pm 0.07*	0.60 \pm 0.08*	181 \pm 7*	187 \pm 6*
0.1 (N = 6)	0.3	12.6 \pm 0.7*	2.51 \pm 0.08*†	0.73 \pm 0.06*	187 \pm 11*	241 \pm 6*
0.3 (N = 6)	0.3	12.0 \pm 1.4*	2.67 \pm 0.10*	0.67 \pm 0.08*	185 \pm 22*	253 \pm 12*†
1.0 (N = 6)	0.3	9.3 \pm 0.8*†	2.44 \pm 0.08*†	0.66 \pm 0.03*	130 \pm 9*†	236 \pm 9*
3.0 (N = 6)	0.3	9.1 \pm 0.9*	2.09 \pm 0.16*	0.64 \pm 0.06*	136 \pm 14*†	203 \pm 11*

Data are expressed as mean (\pm SE)
* p < 0.05 relative to vehicle alone
† p < 0.05 relative to hydrochlorothiazide alone
MS 25861:15

RR 740-02694

COMBINATION (QUINAPRIL HCl, HYDROCHLOROTHIAZIDE) ACUTE STUDIES.

The purpose of combination studies is to determine whether the safety or efficacy of one drug in the combination is changed by the other; the focus of this review will be on the effect of dose and differences.

Acute Oral Toxicity Study of CI-955 in Mice (CI-955, a 40/25 ratio of a combination of Quinapril and Hydrochlorothiazide, respectively).

Testing Facility: Warner Lambert/Parke-Davis Pharmaceutical Research Division, Sheridan Park, Ontario.

Study Number: Sheridan Park Study No. 1351. Research Report No. RR-250-01471. Date of report: 7/08/87.

Test Article: CI-955, Lot CL 045026 (A 40/25 ratio of a combination of Quinapril and Hydrochlorothiazide, respectively).

Study Dates: Initiated 5/27/86). Completed 6/11/86.

GLP Compliance: GLP compliance attested.

Animals: Random bred, barrier raised, B₆C₃F₁ mice, approximate age 42 days, weighing 15 to 21 g, randomly assigned to 5 groups, 10/sex/group.

Mode of Administration of Test Agent: Via Gavage as a suspension in a 0.5% methylcellulose solution, at a volume of 1 ml/20 g.

Dose Levels: CI-939, single dose, at 2586, 2052, 1629, 1293, and 1026 mg/kg (Quinapril levels of 1500, 1190, 945, 750, and 595 mg/kg, respectively), 14 day observation. Dosages were selected on the basis of the Mouse MLD (1500 mg/kg) Quinapril content (mixture equivalent of 2586 mg/kg; Ref. 250-01332).

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects during study period.

Interim sacrifice: No.

Drug Associated Findings: Results: Clinical signs include depression, prostration, weakness, dehydration, hypothermia, and dyspnea. Neither an asymptomatic nor a non-lethal dose was obtained for either sex. MLD, mg/kg, oral, mouse, 14 day observation, approximately 1850, female 1849 (1486-2700), male 1833 (95% confidence intervals not obtained). This is equivalent to 1110 mg/kg quinapril content, which, compared to the MLD of Quinapril (1450 to 2150 mg/kg), indicates that the combination may increase toxicity in the mouse.

Acute Oral Toxicity Study of CI-955 (Quinapril-Hydrochlorothiazide Combination) in Rats

Testing Facility: Warner Lambert/Parke-Davis Pharmaceutical Research Division, Sheridan Park, Ontario.

Study Number: Sheridan Park No. 1352, Research Report RR-250-01484, 7/08/87.

Test Article: CI-955, Lot CL 045026 (A 40/25 ratio, Quinapril/HCTZ).

Study Dates: Initiated 6/18/86). Completed 7/16/86.

GLP Compliance: GLP compliance attested.

Animals: Random bred, BR, SPF, Wistar albino rats, approximate age, 42 days, weight 96 to 153 g, randomly assigned, 8 groups, 10/sex/group.

Mode of Administration of Test Agent: Via Gavage as a suspension in a 0.5% methylcellulose solution, each animal received a single dose of 30 ml/kg.

Dose Levels: CI-955 administered as a single dose to 8 groups of 10 rats/dose level, at 8622, 6843, 5431, 4310, 3420, 2715, 2155, and 1710 mg/kg (active Quinapril levels of 5001, 3969, 3150, 2500, 1984, 1575, 1250, and 992 mg/kg, respectively); observed for 14-days.

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects during study period.

Interim sacrifice: No.

Drug Associated Findings: Results: Clinical signs include depression, ptosis of the eyelids, lacrimation, epistaxis, noisy breathing or dyspnea, abdominal distention, and paleness. Dose levels of 1710 and 2155 were asymptomatic for both sexes. Doses up to 2715 mg/kg were non-lethal. The MLD, mg/kg, and confidence limits () of CI-955, oral, rat, 14 day observation, was 8511.2 (6653.1 - 16471.2) for females, and 7493.2 (6071.1 - 11816.9) for males or approximately 8000 for both sexes. This is equivalent to 4800 mg/kg quinapril content, which, compared to the MLD of Quinapril (M, 4280; F, 3541 mg/kg), indicates that the combination may decrease toxicity in the rat.

Exploratory Oral-Rising Dose Toxicity Study of CI-955 in Beagle Dogs.

Testing Facility: Warner-Lambert/Parke-Davis Pharmaceutical Research Division, Sheridan Park, Ontario.

Study Number: Sheridan Park Study No. 1353.

Part I. Research Report No. RR-250-01485. Date of report: 3/16/87.

Part II. Research Report No. RR-MEMO 764-00943. Date of report: 1/29/88.

Test Article: CI-955, Lot CL 045026 (A 40/25 ratio of a combination of Quinapril and Hydrochlorothiazide, respectively).

Study Dates: Initiated 7/04/86; Completed 7/21/86.

GLP Compliance: GLP compliance attested.

Animals: Beagle Dogs, 14 months of age, weighing 8.4 (F), 10.3 (M) kg.

Mode of Administration of Test Agent: Administered orally in gelatin capsules on a mg/kg body weight/day basis, as follows:

Dose Levels/day; Part I.

Day:	1	2	3	4	5	6	7	8	9
Mg/Kg:	43.1	43.1	43.1	86.2	86.2	172.4	172.4	258.6	258.6
Q. Eq.	25			50		100		150	
Day:	10	11	12	13	14	15	16	17	
Mg/Kg:	258.6	344.8	344.8	517.2	517.2	689.6	689.6	689.6	
Q. Eq.		200		300		400			

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects. Ophthalmic examinations were done pretest and at termination. BP and ECGs were taken pretest and on Days 1, 8, 15, and at termination. Body weights were recorded daily. Food consumption was assessed daily, visually. Hematological, clinical biochemical, and urinalyses were performed prior to dosing, at the end of 1 and 2 weeks, and at termination.

Interim sacrifice: No.

Mortality: Neither dog died during study; no behavioral changes were observed.

Drug Associated Findings: No evidence of toxicity was seen for the first nine days of the study (43.1 to 258.6 mg/kg). Emesis was seen in the male from day 10 and in the female from day 14. Depression was seen in the male on the 17th day. Body weight losses and reduced food consumption were observed. On the ECG, the QT interval was prolonged in the male on day 17. Progressive increases were seen in the BUN and creatinine levels in the male from day 14 and less severe in the female on day 17. RBC count, Hgb, and Hct levels progressively decreased in the female starting on day 7, but remained within the normal range. In the male urine, occult blood was seen on collection days 14 and 17, and glucose on day 17. Organ weights were within normal range. On gross examination, the stomach revealed multifocal pyloric erosion and congestion in both dogs, and in addition, multifocal gastric ulceration and hemorrhage were evident in the male. Renal changes in the male exhibited marked diffuse degeneration of epithelial cells lining the proximal convoluted tubules. The affected cells were markedly swollen with focal necrosis and sloughing and loss of the brush border. Interstitial edema and dilatation of tubules was also seen in the male. (Comparisons are difficult because the small number of dogs to compare reduces the power of statistical evaluation, and these studies were not provided with the same dosing schedule as the 14-day Rising Dose Quinapril study (NDA 19 885) where a 3-day prefix of 25 mg/kg (Quinapril moiety) has been added, and the 150 mg/kg (Q Moiety) dose is given 3 instead of 2 days. Quinapril alone seems to be less toxic than the combination, because in that study, emesis had occurred after doses of 150 mg/kg, with elevated serum phosphorous, creatinine, and BUN levels after 400 mg/kg in the Female and pathologic findings consisted of minor gastric erosions and/or ulcers, pancreatic acinar degranulation, renal tubular dilatation, and, in the female, interstitial lymphocytic infiltration in the renal pelvis).

Part II. Plasma CI-928 and Hydrochlorothiazide (CI-570) Concentrations in Dogs During an Exploratory Oral Rising Dose Toxicology Study With the Combination Product CI-955 - Sheridan Park Toxicology Study No. 1353.

Testing Facility: Parke-Davis Pharmaceutical Research Division/Warner-Lambert Co., Ann Arbor, MI.

Study Number: Research Report RR-MEMO 764-00943; Date of report: 1/29/88.
Period Covered: 3/87 - 5/87.

Mode of Administration of Test Agent: Administered orally in gelatin capsules on a mg/kg body weight/day basis, part II, as follows:

METHOD One beagle dog of each gender received peroral doses of CI-955, starting at 43.1 mg/kg/day and increasing to 689.6 mg/kg/day over 17 days. The amounts of quinacril and hydrochlorothiazide administered on each day are listed in the following table.

CI-955 Dose (mg/kg)	Days Dosed	Amount (mg) of Active Drug	
		Quinacril	Hydrochlorothiazide
43.1	1-3	25	16
86.2	4-5	50	31
172.4	6-7	100	62
258.6	8-10	150	94
344.8	11-12	200	125
517.2	13-14	300	185
689.6	15-17	400	250

Doses were given in hard gelatin capsules and adjusted daily for changes in body weight.

Heparinized blood samples were drawn prior to each CI-955 dose and at 1, 2, 4, 8, and 24 hours postdose on Days 1, 6, 11, and 15. Plasma was collected, frozen, and transferred to the PCM department for storage at -20°C until assayed for CI-928 and hydrochlorothiazide concentrations using validated RIA and HPLC methods, respectively. The limits of quantitation for CI-928 and hydrochlorothiazide were 6.25×10^{-3} µg/ml and 0.25 µg/ml, respectively. Complete assay validation results are given in Appendices 1 and 2.

Drug Associated Findings: Plasma CI-928 and HCTZ concentrations are listed in Tables 1 and 2 and individual CI-928 and HCTZ pharmacokinetic parameters are summarized in Tables 3 and 4, respectively. CI-955 concentrations were detectable throughout the 24-hour sampling period at each dose level. Concentrations did not increase linearly with increasing dose. Since neither component was administered alone, it is not clear whether toxicity and drug levels reflect only interactive effect (e.g. altered renal clearance of either agent).

TABLE 1

Plasma CI-928 Concentrations in Beagle Dogs Maintained
On Single Rising Oral Doses for 17 Days
(Toxicology Study 1353)

Day	Dose (mg/kg)	Dog	Gender	CI-928 Concentration (µg/ml)					
				Pre-dose	1-hr	2-hr	4-hr	8-hr	24-hr
1	43.1	2457	F						
		2458	M						
6	172.4	2457	F						
		2458	M						
11	344.8	2457	F						
		2458	M						
15	689.6	2457	F						
		2458	M						

* Below the limit of quantitation ($<6.25 \times 10^{-3}$ µg/ml)

TABLE 2

Plasma Hydrochlorothiazide Concentrations in Beagle
Dogs Maintained On Single Rising Oral Doses for 17 Days
(Toxicology Study 1353)

Day	Dose (mg/kg)	Dog	Gender	Hydrochlorothiazide Concentration (µg/ml)					
				Pre-dose	1-hr	2-hr	4-hr	8-hr	24-hr
1	43.1	2457	F						
		2458	M						
6	172.4	2457	F						
		2458	M						
11	344.8	2457	F						
		2458	M						
15	689.6	2457	F						
		2458	M						

* Below the limit of quantitation (<0.25 µg/ml)

TABLE 3

Pharmacokinetic CI-928 Parameters Following Single Rising
Oral Doses of CI-955 for 17 Days
(Toxicology Study 1353)

Day	Dose (mg/kg)	Dog	Gender	C _{max}	t _{max}	λ _z	AUC(0-∞)
1	43.1	2457	F	47.5	1	0.422	159
		2458	M	37.0	1	0.390	132
6	172.4	2457	F	115	1	0.305	551
		2458	M	121	1	0.430	486
11	344.8	2457	F	176	2	0.255	1068
		2458	M	201	2	0.317	1079
15	689.6	2457	F	188	2	0.497	771
		2458	M	65.7	2	0.128	591

C_{max} = maximum observed plasma concentration (μg/ml)
t_{max} = time to reach C_{max} (hr)
λ_z = apparent elimination rate constant (1/hr)
AUC(0-∞) = area under the plasma concentration-time curve
from time zero to infinity (μg·hr/ml)

TABLE 4

CI-570 Pharmacokinetic Parameters Following Single Rising
Oral Doses of CI-955 for 17 Days
(Toxicology Study 1353)

Day	Dose (mg/kg)	Dog	Gender	C _{max}	t _{max}	λ _z	AUC(0-∞)
1	43.1	2457	F	2.78	1	0.349	10.9
		2458	M	3.29	1	0.425	10.7
6	172.4	2457	F	10.6	2	0.334	44.4
		2458	M	9.18	2	0.341	37.5
11	344.8	2457	F	11.2	4	0.155	135
		2458	M	19.6	2	0.215	208
15	689.6	2457	F	16.9	2	0.142	146
		2458	M	16.6	2	0.321	104

C_{max} = maximum observed plasma concentration (μg/ml)
t_{max} = time to reach C_{max} (hr)
λ_z = apparent elimination rate constant (1/hr)
AUC(0-∞) = area under the plasma concentration-time curve
from time zero to infinity

COMBINATION (Quinapril HCl, 40/25, Hydrochlorothiazide) CHRONIC STUDIES.

Thirteen-Week Daily Repeated Dose Oral Toxicity Study With CI-955 in Rats.

Testing Facility: Warner-Lambert/Parke-Davis Pharmaceutical Research, Sheridan Park, Ontario.

Study Number: Sheridan Park # 1363; Research Report No. RR-250-01507. Date of report: 8/18/87. Research Report No. RR MEMO-764-00946, 1/29/88.

Test Articles: CI-955, Lot No. CL-045026 (A 40/25 ratio of a combination of Quinapril, CI-906, and Hydrochlorothiazide, CI-570, respectively).

Study Dates: Initiated 10/29/86; completed 2/25/87.

GLP Compliance: GLP compliance attested.

Animals: Wistar Albino Rats, RB, BR, SPF, 5 groups, 20/sex/group, about 42 days of age and weighing 123-186 gms. Sacrifices, 10/sex/group at 13 weeks (main study); 5 (withdrawal study) at 17 weeks after 4 weeks of no treatment, and 5/sex/group for drug blood level determinations.

Mode of Administration of Test Agent: Via Gavage in 0.5% methyl cellulose administered at a volume of 10 ml/kg.

Dose Levels: 5 dose levels, 0, 86, 172, 431, and 862 mg/kg/day CI-955 (Active Quinapril, 0, 50, 100, 250, and 500 mg/kg, respectively), 7 days/week/13 weeks.

Observations/Measurements: Animals were observed twice daily for signs of drug toxicity and systemic effects during the 13-week study period. Body weights were recorded pretest, weekly, and at sacrifice. Individual food consumptions were recorded weekly. Ophthalmic examinations were carried out for each animal at pretest and during weeks 13 and 17. Hematological, clinical biochemical, and urinalyses were evaluated for each animal at weeks 13 and 17. Animals were sacrificed at 13 and 17 weeks of treatment. Gross and histopathology and organ weight measurements were performed on all animals.

Interim sacrifice: No.

Drug Associated Findings: Mortality: Eighteen animals, one hydrocephalic, before week 1, and 17 from dose related intubation accidents:

Dose quinapril content, mg/kg:	500	250	100	50	0
Dosing Accidents, Deaths:					
F	3	2	1	0	1
M	5	3	1	0	1
Week of Death:					
F	1,1,1	2,3	2	-	2
M	1,2,8,9,12	2,2,3	5	-	7

The intubation deaths occur in a dose related manner. No significant clinical signs or ophthalmic findings were noted during the 13 week period or the 4-week withdrawal period. According to sponsor's summary, based on body weight-gain suppression in comparison to controls, females for the doses of

862, 431, 172, and 86 mg/kg/day, respectively, exhibited weight-gain suppressions of 6, 13, 19, and 21%, (in an inverse dose related manner), and males, 21, 21, 24, and 20% (not in a dose related manner). These weight gain suppressions are all consistently about 20% for all treatment groups except for the two highest dose female groups (which were less). Body weights:

Dose, quinapril content, mg/kg:	500	250	100	50	0
Week 0, -F	131	132	131	131	131
Week 13, F (% wt. decrement)	261 (4%)	255	244	241 (11%)	271
Weight Change (from table)	131	122	113	110	140
Week 0, M	173	172	172	173	172
Week 13, M (% wt. decrement)	447 (14%)	447	434 (17%)	452	519
Weight Change (from table)	274	276	262	279	347
Withdrawal Body Weights:					
Week 13, F	266	256	245	252	278
Week 17, F	279	279	265	280	303
Change, Grams:	13	23	20	27	24
Week 13, M	476	455	451	443	528
Week 17, M	512	497	523	516	559
Change, Grams:	36	42	72	74	30

Water balance or edema is not mentioned; however, in the four week treatment withdrawal segments, the females given the highest dose (which had shown the least weight-gain suppression) now exhibited a 46% suppression of weight gain with respect to controls, the lower three female dosage groups were statistically equivalent to the controls while the male treated groups all showed greater gain than the controls during this period. (Such a rapid turn-around weight change for the H-D F group is difficult to explain without further information; however, edema and water balance might be the first considerations). All treated groups in both sexes had increased BUN-values at 13 weeks (in contrast to 250 and 500 mg/kg only in males only with quinapril only), but were near normal at 17 weeks (after 4-week withdrawal). Absolute and relative kidney weights were increased in both sexes in a non-dose related manner. The absolute and relative liver weights were increased in the three highest dose level groups of females, while only the relative liver weights were increased over control values in all 4 treated male groups. Significant non-dose related renal lesions which were found in the treated groups of both sexes consisted of basophilic cortical tubules (especially in the males), interstitial fibrosis and mononuclear cell infiltrates. Hypertrophy of the afferent arteriole of the juxtaglomerular apparatus was observed in all treated groups of both sexes; hypertrophy of interlobular arterioles occurred only in the two highest dosed groups.

Part II. Plasma CI-928 and Hydrochlorothiazide (CI-570) Concentrations in Rats During Thirteen Weeks of Oral Dosing With the Combination Product CI-955: Sheridan Park Toxicity Study 1363.

Testing Facility: Parke-Davis Pharmaceutical Research Division/Warner-Lambert Co., Ann Arbor, MI.

Study Number: Research Report No. RR-MEMO 764-00946. Date of report: 1/29/88. Period Covered: 11/87 - 12/87.

Two hour post dose plasma CI-928 (quinapril diacid, quinaprilat) concentrations increased with increasing dose and ranged from micrograms/ml. Male plasma CI-928 concentrations were consistently higher than those of females in the same dosing group. This was especially true for the low dose group in which males had plasma levels about 5 X as high as those in the female group, and may represent the area of a threshold. Predose CI-928 concentrations ranged from (with only 2 values above 0.80) mcgms/ml, indicating slight CI-928 accumulation, but with great individual variation.

CI-955 and (Quinapril Moiety) in Dose/Day; Plasma Quinaprilat in mcg/ml.

mg/kg	Quinaprilat: Predose (mcg/ml)		2-Hour Postdose	
	Male	Female	Male	Female
86 (50)	0.10--0.46	0.01--0.05	16.34 (10.9 - 21.7)	3.42 (0.3 - 7.1)
172 (100)	0.08--0.16	0.05--0.12	19.13 (8.8 - 28.0)	13.66 (8.5 - 19.1)
431 (250)	0.53--1.39	0.03--0.50	41.26 (23.0 - 62.8)	23.11 (5.5 - 48.3)
862 (500)	0.55--1.14	0.13--0.61	72.10 (68.6 - 75.6)	68.30 (66.8 - 74.0)

Two hour post dose plasma HCTZ concentrations increased with increasing dose and ranged from micrograms/ml. Male plasma HCTZ concentrations were slightly higher than those of females in the same dosing group. Predose HCTZ concentrations ranged from below the limit of quantitation to 2.39 microgms/ml and indicated negligible HCTZ accumulation.

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RR-MEMO 764-00946

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TABLE 1

Plasma CI-928 (quinapril diacid) Concentrations (µg/ml) in Wistar Rats Following Daily Administration of CI-955 (Sheridan Park Toxicology Study 1363)

Daily Dose (mg/kg)	Gender	Animal Number	Week 13	
			Pre-dose	2-Hr
86	F	36184	0.01	
		36185	0.05	
		36187	0.03	
		36188	0.03	
	M	36284	0.14	
		36285	0.46	
		36286	0.37	
		36287	0.10	
172	F	36164	0.12	
		36165	0.11	
		36166	0.06	
		36167	0.05	
		36168	0.07	
	M	36264	0.16	
		36265	0.11	
		36266	0.12	
		36267	0.14	
		36268	0.08	
431	F	36144	0.07	
		36145	0.12	
		36146	0.50	
		36147	0.03	
		36148	0.16	
	M	36244	0.80	
		36245	0.53	
		36246	0.71	
		36247	1.39	
		36248	0.56	
862	F	36125	0.34	
		36126	0.61	
		36127	0.13	
	M	36128	0.45	
		36224	1.14	
		36227	0.55	

Limit of quantitation (6.25×10^{-3} µg/ml)

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TABLE 2

Plasma Hydrochlorothiazide Concentrations (µg/ml) in Wistar Rats Following Daily Administration of CI-955 (Sheridan Park Toxicology Study 1363)

Daily Dose (mg/kg)	Gender	Animal Number	Week 13	
			Pre-dose	2-Hr
86	F	36184	BLQ	
		36185	BLQ	
		36187	BLQ	
		36188	BLQ	
	M	36284	0.38	
		36285	0.45	
		36286	BLQ	
		36287	BLQ	
172	F	36164	BLQ	
		36165	0.26	
		36166	BLQ	
		36167	BLQ	
		36168	BLQ	
	M	36264	0.43	
		36265	BLQ	
		36266	0.31	
		36267	0.43	
		36268	BLQ	
431	F	36144	0.32	
		36145	0.58	
		36146	0.74	
		36147	0.59	
		36148	0.46	
	M	36244	1.04	
		36245	1.27	
		36246	2.39	
		36247	0.98	
		36248	0.70	
862	F	36125	1.13	
		36126	0.78	
		36127	1.90	
	M	36128	2.20	
		36224	1.59	
		36227	1.26	

BLQ = Below limit of quantitation (0.25 µg/ml)

Thirteen-Week Daily Repeated Dose Oral Toxicity Study With CI-955 in Rats.

Testing Facility: Parke-Davis Research Institute, Division of Warner-Lambert Canada Inc. Mississauga, Ontario.

Study Number: Sheridan Park # 1425; RR-250-01571; 12/15/89.

Test Articles: CI-955, Lot NRs CL 045026 & CM 006019 (A 40/25 ratio of a combination of Quinapril, CI-906, Lot No. X 44219 & X43891 and Hydrochlorothiazide, CI-570, Lot No. 85081022, respectively).

Study Dates: Initiated 01/31/89; completed 05/09/89.

GLP Compliance: GLP compliance (Canadian) attested.

Animals: Wistar Albino Rats, RB, BR, SPF, 10 groups, 10/sex/group, about 42 days of age and weighing 137-250 gms.

Mode of Administration of Test Agent: Via Gavage in 0.5% methyl cellulose administered at a volume of 10 ml/kg.

Dose Levels: 10 dose groups, with Quinapril/and/or CI-570 (HCTZ), 0, -0-/25, 1.6/-0-, 1.6/1, 8/-0-, 8/5, 8/10, 20/-0-, 20/12.5, and 20/25 mg/kg/day, respectively, 7 days/week/13 weeks.

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects during the 13-week study period. Body weights were recorded pretest, weekly, and at sacrifice. Individual food consumptions were recorded weekly. Ophthalmic examinations were carried out for each animal at pretest and during week 13. Hematological, clinical biochemical, and urinalyses were evaluated for each animal at week 13. Animals were sacrificed at 13 weeks of treatment. Gross and histopathology and organ weight measurements were performed on all animals.

Interim sacrifice: No.

Drug Associated Findings: No drug related deaths. No significant clinical signs or ophthalmic findings were noted during the 13 week period. Males given doses containing 22 mg/kg/day of quinapril, whether alone or with 12.5 or 25 mg/kg of HCTZ, exhibited 12 to 15% lower mean body weights than comparable controls, thus the effect of quinapril on weight changes was not influenced by the addition of HCTZ. Females did not exhibit significant differences between treated and control weight values. Sponsor's Weight gain suppressions are consistently 20% for all treatment groups except for the two highest dose female groups (which were less). No significant differences in overall food consumption was apparent. No drug related hematological changes were apparent. Mild increases (26 to 40%) in BUN were seen in females receiving 34.5 mg/kg of the mixture, and for both males and females receiving 47 mg/kg of the mixture (both 20 mg/kg; Q Eq.). This was not seen with the ingredients separately, and in males only at higher dosages (250 and 500 mg/kg) with quinapril alone, and can be considered a combination effect. Male average heart weights were slightly, but significantly lighter than controls in only two groups; those receiving 20 Q/12 H and 8 Q/10 H mg/kg combinations.

These doses are lower than those seen in previous quinapril studies (such as the 13 week study where dosages of 50, 250, and 500 mg/kg all exhibited heart weights 18 to 27% less than controls, and the carcinogenicity studies which did not exhibit a comparative decrease in heart weights after 10, 50, and 100 mg/kg). Female groups (which in previous quinapril studies such as the 13 week study with dosages of 50, 250, and 500 mg/kg all exhibited heart weights 18 to 22% less than controls) did not exhibit any significant differences in the heart weights between control and treated. Kidney weights were slightly, but significantly higher in only 2 male and 2 female groups, with the largest change in both sexes in the highest dose (47 mg/kg) combination treated rats. Combination dosages of 34.5 and 47 mg/kg (20 mg/kg Q) exhibited hypertrophy of the afferent arteriole and hypertrophy of the juxtaglomerular apparatus; with dosages containing (8 mg/kg quinapril) not exhibiting this characteristic.

The maximum human therapeutic dose is 0.8 mg/kg quinapril content representing a 10 fold dose multiple of no-effect animal toxicity dose over the clinical dose.

Exploratory 2 Week Oral Toxicity Study of CI-955 in Beagle Dogs.

Testing Facility: I. Warner-Lambert/Parke-Davis Pharmaceutical Research, Sheridan Park, Ontario. II. Ann Arbor, MI.

Study Number: I. Sheridan Park study # 1362; Research Report No. RR-250-01497. Date of report: 6/09/87. II. Research Report No. RR-MEMO-764-00936. Date of report: 1/29/88.

Test Articles: CI-955 Lot No. X 43981 (A 40/25 ratio Quinapril/HCTZ)

Study Dates: I. Initiated 9/26/86; completed 10/10/86.

GLP Compliance: -GLP compliance attested.

Animals: Beagle Dogs, 3 groups, 1/sex/group, approximately 13 months of age, weighing 6.8 to 11.6 kg at start of study.

Mode of Administration of Test Agent: CI-955, orally, in gelatin capsules.

Dose Levels: Orally, gelatin capsules, 43.1, 129.3, and 258.6 mg/kg/day of CI-955 (equivalent to 25, 75, & 150 mg/kg/day of CI-906), 7 days/week/14 days.

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects during the 14 day study period. Body weights were recorded pretest, weekly, and at sacrifice. Individual food consumption was measured daily. Heart rate determinations and ophthalmic and ECG examinations were done pretest, postdose and before termination. Hematological, clinical biochemical, and urinalyses were evaluated for each animal before initiation of dosing, and before termination.

Interim sacrifice: No.

Drug Associated Findings: No significant clinical signs or ophthalmic changes were observed at terminal examination. All animals consumed their assigned rations each day. Body weight-gain losses were from 2 to 4% of pretest values. Heart rates, individual cardiac intervals and amplitudes, except for the P-wave amplitude, were not significantly different from pretest values. During the second week, the P-wave amplitude in 2/3 females (H- and L-D) reportedly was increased from predose values for 2 hours after dosing. In the M- & H-D groups, treatment related findings include increased BUN in 2/4, and slightly increased LDH values in 4/4. Other biochemical, hematology, urinalyses, organ weights, and gross pathology were unremarkable. Significant histopathology findings were limited to the kidney with tubular dilatation and interstitial mononuclear cell infiltrate and hemorrhage in the H-D male, and tubular dilatation in the H and L-D female.

Part II. CI-928 and Hydrochlorothiazide (CI-570) Plasma Concentrations in Male and Female Beagle Dogs Following Oral Dosing With the Combination Product CI-955 - Sheridan Park Toxicity Study 1362.

Testing Facility: Parke-Davis Pharmaceutical Research Division/Warner-Lambert Co., Ann Arbor, MI.

Study Number: Research Report No. RR-MEMO 764-00936. Date of report: 1/29/88. Period Covered: 3/87 - 4/87.

Conclusions: Two hour post dose plasma CI-928 (quinaprilat) concentrations increased with increasing quinapril dose, but with great individual variation.

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TABLE 1

CI-928 Plasma Concentrations in Beagle Dogs Maintained
on Single Daily Oral CI-955 Doses for 14 Days
(Toxicology Study 1362)

Daily CI-955 Dose (mg/kg)	Dog	Gender	CI-928 Concentrations (µg/ml)						
			Day 1			Day 14			
			Pre Dose	2-hr	24-hr	Pre Dose	2-hr	24-hr	
43.1	2481	F	bq1						
	2484	M	bq1						
129.3	2480	F	bq1						
	2483	M	bq1						
258.6	2479	F	bq1						
	2482	M	bq1						

bq1 - Below quantitation limit ($<6.25 \times 10^{-3}$ µg/ml)

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RR-MEMO 764-00936

TABLE 2

Hydrochlorothiazide (CI-570) Plasma Concentrations in
Beagle Dogs Maintained on Single Daily Oral CI-955 Doses
for 14 Days (Toxicology Study 1362)

Daily CI-955 Dose (mg/kg)	Dog	Gender	Hydrochlorothiazide (CI-570) Concentrations (µg/ml)						
			Day 1			Day 14			
			Pre Dose	2-hr	24-hr	Pre Dose	2-hr	24-hr	
43.1	2481	F	bq1						
	2484	M	bq1						
129.3	2480	F	bq1						
	2483	M	bq1						
258.6	2479	F	bq1						
	2482	M	bq1						

bq1 - Below quantitation limit (<0.25 µg/ml)

Thirteen-Week Oral Toxicity Study of CI-955 in Beagle Dogs.

Testing Facility: Warner-Lambert/Parke-Davis Pharmaceutical Research, Sheridan Park, Ontario.

Study Number: Sheridan Park No. 1365; Research Report RR-250-01510, 9/10/87.

Test Article: CI-955, Lot CL 045026 (A 40/25 ratio of Quinapril/HCTZ).

Study Dates: Initiated 11/27/86; Completed 3/05/87.

GLP Compliance: GLP compliance attested.

Animals: Beagle Dogs, 4 groups, 3/sex/group, approximately 11 months of age and weighing between 8.3 and 11.0 kg at start of study.

Mode of Administration of Test Agent: CI-955, in gelatin capsules.

Dose Levels: 0 (VC), 43.1, 129.3, and 258.6 (surviving animals dosed for only 6 weeks) mg/kg/day of CI-955 (active CI-906 content: 0, 25, 75, and 150 mg/kg, respectively), 7 days/week, administered in gelatin capsules for 13 weeks.

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects. Ophthalmic examinations were done pretest and at termination. Blood pressure and ECGs were taken pretest, Day 1, week 6, and at termination. Body weights were determined at pretest, weekly and at sacrifice. Food consumption was determined daily. Clinical biochemical, Hematological, and urinalyses were performed pretest, during week 6, and at termination. Extra determinations were performed on moribund animals as indicated.

Interim sacrifice: No.

Mortality: No deaths, but there were 7 moribund sacrifices, 2 F (weeks 2 & 3) and 1 M (week 3), H-D; 2 F (weeks 6 & 8) and 1 M (week 3), M-D; and one F (week 6), L-D.

Drug Associated Findings: Moribund signs prior to sacrifice included dehydration, depression, inappetence, emesis, weakness, emaciation, diarrhea, a 12 - 28% body weight loss, and ulceration of the oral mucous membranes. Animals surviving to termination exhibited sporadic emesis, oral ulcerations, and in the H-D males, body weight losses of 11-19%. Except for H-D males, treatment group body weight losses were not consistently different from the controls. Electrocardiograms and heart rates were unremarkable. Moribund animals had marked increases in BUN levels, increased creatinine levels, and decreased sodium and chloride levels. Animals surviving to termination exhibited sporadic elevations of BUN and creatinine and decreased sodium, but other clinical biochemical, hematological, urinalyses and organ weight findings were unremarkable. In all moribund sacrifice animals, significant gross and microscopic oral, gastric, and kidney lesions were observed. The oral lesions, seen in all dose groups, were characterized by multifocal areas of mucosal erosion or ulcerations with moderate or marked inflammation. The gastric lesions, noted in all dose groups, were characterized by multifocal areas of mucosal erosion or ulcerations with hemorrhage and necrosis of the

glandular epithelium. Five of the dogs also had gastric mineralization involving one or more of the following regions: middle and deep mucosa, muscularis, muscularis mucosa, sub mucosa, submucosal blood vessels and gastric smooth muscles. Renal tubular degenerative changes characterized by mild to marked dilatation of the cortical tubules with individual cell necrosis, cytoplasmic vacuolation, and diffuse interstitial edema. Terminal sacrifice animals were generally unremarkable except for lighter heart weights, gastric erosions in one H-D male, and renal changes (tubular dilatation, edema, fibroplasia, and interstitial mononuclear cell infiltrates) in all H- and M-D animals and hypertrophy of the JG cells in all treatment groups. Persistence of H-D toxicity after 6-weeks off drug is to be noted. The actual doses of quinapril (0, 25, 75, and 150 mg/kg) in this combination were less than what was administered in the previous 13 week quinapril study (25, 125, and 250 [div] mg/kg) which did not have moribund animals, severe weight losses, and had gastric ulcers in only 1/6 H-D, and 2/6 M-D dogs; these toxic effects at the lower doses were more marked in the combination treated dogs, indicating increased toxicity by the combination of quinapril with HCTZ.

Part II. Plasma CI-928 and Hydrochlorothiazide (CI-570) Concentrations in Dogs During 13 Week Oral Dosing With the Combination Product CI-955 - Sheridan Park Toxicity Study 1365.

Testing Facility: Parke-Davis Pharmaceutical Research Division/Warner-Lambert Co., Ann Arbor, MI.

Study Number: RR-MEMO 764-00944; 2/03/88 (Period Covered: 3/87 - 11/87).

At the 13 week testing period, post dose (2, 4, 8, and 24 hours) plasma CI-928 (quinaprilat) concentrations increased with increasing quinapril dose, but with great individual variation (see Table 1). Higher predose concentration values were correlated with higher post dose concentrations.

TABLE 1

Plasma CI-928 Concentrations ($\mu\text{g}/\text{ml}$) in Beagle Dogs Following Daily Administration of CI-955 for 13 Weeks

(Toxicology Study 1365)

Daily Dose (mg/kg)	Gender	Animal Number	Pretest	Week 6	Week 13				
					Predose	2 hr	4 hr	8 hr	24 hr
43.1	F	2491	bql	0.95	0.44	0.67	0.45	0.11	0.27
		2492	0.008	0.31					
	M	2503	0.010	0.22	0.45	0.11	0.27		
		2504	bql	0.12					
		2505	bql	0.12					
129.3	F	2489	bql	1.10	1.60	0.49	0.45		
	M	2501	bql	2.44					
		2502	bql	0.81					
258.6	F	2486	bql	1.17	1.29	3.58	0.59		
		2498	bql	1.74					
	M	2499	bql	0.60					

bql = below quantifiable limit ($<6.25 \times 10^{-3} \mu\text{g}/\text{ml}$)

TABLE 3

Plasma CI-928 Concentrations ($\mu\text{g/ml}$) in Beagle Dogs
Sacrificed Prior to Study Termination

Daily Dose (mg/kg)	Gender	Animal Number	Week of Sacrifice	Pretest	Week 6	At Termination
43.1	F	2493	6	0.019	-	3.36
129.3	F	2488	8	bql	2.59	17.6
	F	2490	6	bql	-	7.58
	M	2500	3	bql	-	6.04
258.6	F	2485	2	bql	-	52.0
		2487	3	bql	-	7.60
	M	2497	3	bql	-	7.12

bql = below-quantitation limit ($<6.25 \times 10^{-3} \mu\text{g/ml}$)

Thirteen-Week Repeated Dose Oral Toxicity Study of CI-955 in Beagle Dogs.

Testing Facility: Parke-Davis Research Institute, Division of Warner-Lambert
Canada Inc. Mississauga, Ontario.

Sheridan Park Study # 1424; RR-250-01570; Report Date December 1, 1989.

Test Article: CI-955 (A 40/25 ratio of Quinapril/HCTZ).

Study Dates: Initiated 2/23/89; Completed 6/01/89.

GLP Compliance: GLP compliance attested.

Animals: Beagle Dogs, 10 groups, 3/sex/group, approximately 8 to 15 months of
age and weighing between 5.4 and 10.8 kg at start of study.

Mode of Administration of Test Agent: Oral, in gelatin capsules.

Dose Levels:

Table 1 - Compound Administration

Group	Treatment	Bulk Dose (mg/kg/day)	CI-906 Human Dose Multiple	Active CI-906 (mg/kg/day)	Active CI-570 (mg/kg/day)	Number of Animals/Sex
I	Control	0		0	0	3
II	CI-570	25		0	25	3
III	CI-906	1.8	1 x	1.6	0	3
IV	CI-906	8.8	5 x	8	0	3
V	CI-906	22.0	12.5 x	20	0	3
VI	CI-955-1	2.8	1 x	1.6	1	3
VII	CI-955-1	13.8	5 x	8	5	3
VIII	CI-955-1	34.5	12.5 x	20	12.5	3
IX	CI-955-2	18.8	5 x	8	10	3
X	CI-955-2	47.0	12.5 x	20	25	3

Observations/Measurements: Animals were observed daily for signs of drug toxicity and systemic effects. Ophthalmic examinations were done pretest, week 6, and at termination. Detailed clinical examinations were performed at weeks 4, 8, and 13. Blood pressures and ECGs were taken pretest/predose, and 2 hours post-dose at weeks 1, 6, and 12. Body weights were determined at pretest, weekly and at sacrifice. Consumption of food and water was determined daily. Clinical biochemical, Hematological, and urinalyses were performed pretest, during weeks 1, 6, and 12. Extra determinations were performed on moribund animals as indicated.

Interim sacrifice: No.

Mortality: No deaths.

Drug Associated Findings:— There were no significant differences in clinical signs, food and water consumption, body weight changes, ophthalmic findings, hematology, electrocardiograms and heart rates; organ weights and gross pathologies were unremarkable. Significant decreases in group mean serum potassium values were seen only in animals receiving 25 mg/kg HCTZ, and in group mean serum chloride values during the first week in animals receiving 25 mg/kg HCTZ. Increased hypertrophy of the juxtaglomerular Apparatus was not seen with 25 mg/kg HCTZ alone, and in only one of 6 dogs in the group receiving 8.8 mg/kg of quinapril. Dog groups receiving 20 mg/kg of quinapril, or any combination of 8 and 20 mg/kg of quinapril with HCTZ, exhibited at least 5/6 dogs with increased hypertrophy of the juxtaglomerular apparatus, thus indicating that the addition of HCTZ potentiates an increase of hypertrophy of the juxtaglomerular apparatus. Other histopathologies were unremarkable.

Plasma Quinapril (CI-906), Quinaprilat (CI-928), and Hydrochlorothiazide (CI-570) Concentrations in Male and Female Beagle Dogs Following Oral Administration of Quinapril Alone, Hydrochlorothiazide Alone, CI-955-1 (Quinapril 1.6/Hydrochlorothiazide 1.0 Ratios), and CI-955-2 (Quinapril 0.8/Hydrochlorothiazide 1.0 Ratios), for Thirteen-Weeks.

Testing Facility: Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI.

Study Number: Sheridan Park No. 1424; RR-MEMO 764-01426, 2/16/90.

Test Articles: CI-906, CI-570, CI-928, CI-955-1, and CI-955-2.

Study Dates: Initiated 02/16/89; Completed 1/08/90.

GLP Compliance: GLP compliance attested.

Animals: Beagle Dogs, 10 groups, 3/sex/group.

Mode of Administration of Test Agents or Placebos: In gelatin capsules.

Dose Levels:

TABLE 1. Doses of Quinapril, HCTZ, CI-955-1, and CI-955-2 Administered to Beagle Dogs for 13 Weeks: Sheridan Park Toxicology Study 1424

Group	Treatment	Bulk Dose ^a (mg/kg/day)	Active Dose Q/HCTZ (mg/kg/day)	Active Dose Quinapril (mg/kg/day)	Active Dose HCTZ (mg/kg/day)	Number Animals Male/Female
I	Control	0	0	0	0	3/3
II	CI-570	25	25	0	25	3/3
III	CI-906	1.8	1.6	1.6	0	3/3
IV	CI-906	8.8	8	8	0	3/3
V	CI-906	22	20	20	0	3/3
VI	CI-955-1	2.8	2.6	1.6	1	3/3
VII	CI-955-1	13.8	13	8	5	3/3
VIII	CI-955-1	34.5	32.5	20	12.5	3/3
IX	CI-955-2	18.8	18	8	10	3/3
X	CI-955-2	47	45	20	25	3/3

^a Bulk dose is based on the factor of 1.1 for the conversion of quinapril HCl to the active moiety quinapril base
Q Quinapril

Observations/Measurements: Quinapril and quinaprilat were assayed using a validated gas chromatographic method with electron capture detection and quantified using peak-height ratio method with CI-907 as internal standard. The lower quantitation limit of quinapril and quinaprilat was 5 ng/ml. HCTZ was assayed using a validated high-performance liquid chromatographic method with UV detection, and quantified using the peak-height-ratio method with hydroflumethiazide serving as internal standard. The lower quantitation limit of HCTZ was 25 ng/ml.

Interim sacrifice: No.

Mortality: No deaths.

Drug Associated Average Findings: Plasma concentrations of HCTZ, quinapril and quinaprilat in ng/mL.

Active Dose Quinapril/HCTZ	Sex	Component (hour)	Week 1		Week 6		Week 12	
			0	2	0	2	0	2
0/25 mg/kg	M	HCTZ	ND	3373	BLQ	3433	BLQ	3200
	F	HCTZ	ND	768	BLQ	2936	BLQ	2826
1.6/1.0	M	HCTZ	BLQ	121	BLQ	77	BLQ	79
	F	HCTZ	BLQ	305	BLQ	79	BLQ	132
8.0/5.0	M	HCTZ	BLQ	825	BLQ	509	ND	620
	F	HCTZ	BLQ	844	BLQ	516	ND	613
20/12.5	M	HCTZ	ND	2117	BLQ	1464	BLQ	1604
	F	HCTZ	ND	1714	BLQ	1807	BLQ	1653
8/10	M	HCTZ	BLQ	1240	BLQ	931	BLQ	1237
	F	HCTZ	BLQ	1300	BLQ	1330	BLQ	940
20/25	M	HCTZ	BLQ	4683	BLQ	2260	BLQ	3855
	F	HCTZ	BLQ	3823	BLQ	3510	BLQ	4520

1.6/-0-	M	Quinapril	BLQ	# 5.6	BLQ	# 6.1	BLQ	# 7.7
	F		BLQ	7.7	BLQ	# 8.0	BLQ	# 7.5
8/-0-	M	Quinapril	BLQ	31	BLQ	38	BLQ	30
	F		BLQ	43	BLQ	32	BLQ	26
20/-0-	M	Quinapril	BLQ	100	BLQ	100	BLQ	109
	F		BLQ	68	BLQ	101	BLQ	188
1.6/1.0	M	Quinapril	BLQ	10	BLQ	BLQ	BLQ	4
	F		BLQ	16	BLQ	12	BLQ	5
8.0/5.0	M	Quinapril	BLQ	23	BLQ	44	ND	23
	F		BLQ	27	BLQ	25	ND	27
20/12.5	M	Quinapril	BLQ	72	BLQ	42	BLQ	59
	F		BLQ	131	BLQ	129	BLQ	116
8/10	M	Quinapril	BLQ	15	BLQ	23	BLQ	29
	F		BLQ	24	BLQ	26	BLQ	29
20/25	M	Quinapril	BLQ	89	BLQ	78	BLQ	62
	F		BLQ	118	BLQ	109	BLQ	63

= Greater than.

1.6/-0-	M	Quinaprilat	BLQ	1281	19.6	669	6.3	841
	F		BLQ	1183	18.1	718	34.6	1149
8/-0-	M	Quinaprilat	BLQ	3460	91.1	4773	81.7	3997
	F		BLQ	4804	24.2	4735	31.3	6633
20/-0-	M	Quinaprilat	BLQ	21950	44.3	13533	36.9	21500
	F		BLQ	20433	128.6	20667	103.3	19413
1.6/1.0	M	Quinaprilat	BLQ	842	6.7	559	10.1	606
	F		BLQ	1149	9.7	1360	6.2	1108
8.0/5.0	M	Quinaprilat	BLQ	6197	12.8	4910	13.0	5230
	F		BLQ	5267	36.4	4337	88.4	8640
20/12.5	M	Quinaprilat	BLQ	23067	49.7	<u>11891</u>	52.0	<u>12160</u>
	F		BLQ	19667	79.2	21800	59.2	20567
8/10	M	Quinaprilat	BLQ	4550	18.5	3724	12.3	6897
	F		BLQ	5927	20.6	6287	42.2	4930
20/25	M	Quinaprilat	BLQ	22767	61.7	<u>12430</u>	62.2	<u>13543</u>
	F		BLQ	22100	125.	27800	81.6	26233

In general, plasma concentrations increased with increasing dose for all tested constituents. Quinapril and HCTZ concentrations were below the limits of quantification in all predose samples collected. Quinaprilat, on the other hand, was detectable in these samples and increased with increasing dose levels of quinapril. Aside from a slightly lower male plasma level of HCTZ after 6 weeks on a combination of 20/12.5 mg/kg and after 6 and 12 weeks on a combination of 20/25 mg/kg of quinapril/HCTZ, there is not much difference between the plasma levels of HCTZ when combined with quinapril; however, both doses in the combination were increased together and no conclusions can be drawn regarding set doses of HCTZ with respect to increasing doses of quinapril. In males given 20 mg/kg quinapril in combination with 0, 12.5, or 25 mg/kg of HCTZ, there is a slight HCTZ treatment related decrease in the plasma levels of quinaprilat after 6 and 12 weeks on the program (underlined in above table). This decrease is not seen in the females, resulting, in effect, that after 6 and 12 weeks on the drug, the females have about 2 X the blood level that the males have; an observation that may be of importance for pregnancy considerations. These observations differ slightly from the conclusions of the sponsor who did not perceive in the combinations any sex differences or any evidence of a pharmacokinetic drug-drug interaction.

CARCINOGENICITY STUDIES:

Sponsor is not submitting carcinogenicity studies because this product is a combination of approved drugs deemed non-carcinogenic, although statistical findings of increased incidence of mesenteric lymph node hemangiomas and skin/subcutaneous lipomas in rats at the highest dose of only 100 mg/kg of Quinapril is mentioned in the Quinapril labeling. Sponsor is presuming that an increase in effectiveness by this combination will not potentiate or create possible carcinogenic effects, but no data is available for evaluation.

COMPARATIVE TOXICITY STUDY IN FEMALE RABBITS. Sponsor has not provided a study to compare the toxicity of the Quinapril/HCTZ compound with a 2 week Quinapril rabbit study; therefore, no comparative evaluation can be made.

REPRODUCTION AND TERATOLOGY:

Segment I: No Segment I drug-in-combination studies have been provided by the sponsor; therefore, no comparative evaluation between Quinapril and Q/HCTZ can be made. In the Quinapril alone rat study, with only 0, 10, 50 and 100 mg/kg dosing, there were 5 treatment related (cause unknown) deaths. These deaths occurred at 100 mg/kg (1/12 male and 2/24 female deaths), at 50 (1/24 F), and at 10 mg/kg (1/24 F), indicating a materno toxicity of Quinapril. A segment I study for the combination is to be recommended.

Exploratory Oral Dose Range Finding Study in Pregnant Rats With CI-955.

Testing Facility: Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI.

Study # 1447; RR-745-01578; Report Date March 02, 1990.

Test Articles: CI-955 (A 40/25 ratio of Quinapril/HCTZ).

Study Dates: Initiated, 10/09/89, Completed 11/02/89.

GLP Compliance: GLP compliance attested.

Animals: S-D CD female rats, 6 groups, 5/group, approximately 12 weeks old, average weight 249 grams (range 346 to 433 gm), were inseminated (1/1) by untreated male rats at least 14 weeks of age and weighing 394 grams.

Mode of Administration of Test Agent: Oral, via gavage.

Dose Levels: Quinapril was administered via gavage, once daily, days 6 thru 15 of pregnancy to pregnant females, as follows:

Groups of five sperm-positive females were administered daily doses of 0 (vehicle) or CI-955 as designated below.

TABLE 1. Group and Animal Identification

Group	No. of Females	Treatment	CI-955 (mg/kg)	CI-906 (mg/kg)	CI-370 (mg/kg)	Animal Numbers
1	5	Vehicle	0	0	0	42749-42753
2	5	CI-955	8.1	3.61	4.51	42754-42758
3	5	CI-955	16.3	7.22	9.05	42759-42763
4	5	CI-955	81.3	36.11	45.14	42764-42768
5	5	CI-955	243.8	108.33	135.42	42769-42773
6	5	CI-955	487.5	216.67	270.83	42774-42778

OBSERVATIONS/MEASUREMENTS:

Maternal toxicity was assessed by effects on appearance (clinical observations), body weight, food consumption, gross necropsy findings, and reproductive outcome (e.g., abortion/total resorption). Developmental toxicity was assessed by effects on embryo/fetal survival (pre/postimplantation loss and survival at term), body weight, sex distribution, and external malformations or variations. Malformations are defined as developmental deviations which (1) are gross structural changes, (2) may be incompatible with life, and (3) are generally rare in occurrence. Variations are structural alterations which occur infrequently but more often than malformations, and have no significant biological effect on body conformity, function, or general well-being.

Mortality: No deaths.

Drug Associated Findings: Five animals had hair loss during the study; one given 487.5 mg/kg, two, 243.8, one, 16.3 mg/kg, and one vehicle control. No other clinical signs were observed. Maximum weight decrement of maternal body weight (9%) with respect to controls was seen in the group receiving 243.8 mg/kg. (Table T - 1)

Exploratory Oral Dose Range Finding Study in Pregnant Rats with CI-955

8.1. Table T-1. Maternal Body Weight

Treatment Dose (mg/kg)	Vehicle		CI-955				
	0	8.1	16.3	81.3	243.8	487.5	
Gestation Days							
0	246.0 ^a ±3.4	243.2 ±3.2	290.0 ±7.5	290.8 ±7.1	290.2 ±3.1	293.0 ±2.9	
6	271.2 ±4.7	267.8 ±4.5	280.2 ±6.1	275.2 ±7.4	279.2 ±3.8	280.8 ±1.9	
11	292.6 ±6.7	276.2 ±4.3	282.6 ±6.8	280.2 ±9.9	289.0 ±3.5	274.6 ±5.8	
15	312.2 ±6.6	297.0 ±5.7	312.2 ±6.1	295.4 ±13.1	290.2 ±5.2	299.4 ±4.6	
21	387.6 ±13.5	380.6 ±10.7	400.0 ±15.2	381.2 ±12.0	374.4 ±13.7	380.2 ±6.9	

^a Values represent mean ± SE (grams)
Five pregnant animals per group

151.6 137.4 150.0 130.4 124.2 127.2

Induced suppression of total food intake (less than 1%) was most severe at 8.1 mg/kg. Small thyroids were reported in the treated animal groups at an incidence of 0, 40%, 0, 20%, 60%, and 60%, respectively, for control through highest dose, respectively. All animals were pregnant. None of the treated or control animals delivered early or had total litter resorption. Mean numbers of corpora lutea, implantation sites, and live, dead, or resorbed fetuses were comparable across groups. Pre- and Postimplantation losses were variable across groups and no treatment or dose relationships were apparent. Developmental toxicity (reduced mean fetal weights) also occurred at these doses. According to the sponsor, the maximum tolerated dose in this study was 243.8 mg/kg.

Exploratory Oral Dose Range Finding Study in Pregnant Rats with CI-955

8.5. TABLE T-5. Reproductive and Litter Data (Page 1 of 2)

Treatment Daily Dose (mg/kg)	Vehicle		CI-955			
	0	8.1	16.3	81.3	243.8	487.5
Maternal Parameters						
Number gravid	5	5	5	5	5	5
Number nongravid	0	0	0	0	0	0
Number died	0	0	0	0	0	0
Number sacrificed before term	0	0	0	0	0	0
Number delivered early	0	0	0	0	0	0
Number examined at term sacrifice (Day 21)	5	5	5	5	5	5
Number with resorptions only	0	0	0	0	0	0
Number with viable litters	5	5	5	5	5	5
Corpora lutea	15.2±0.9 ^a	15.4±0.9	17.2±1.0	17.4±1.2	18.0±1.6	17.4±1.1
Implantations	14.4±0.7	14.0±0.8	16.4±0.8	16.2±1.2	14.0±2.9	15.8±0.9
Live fetuses ^b	13.0±0.8	14.0±0.5	15.8±0.9	14.4±0.9	13.4±2.9	14.4±1.0
Dead fetuses	0	0	0	0	0	0
Resorptions	1.4±0.2	0.8±0.6	0.6±0.4	1.8±0.8	0.6±0.2	1.4±0.2
Litter size ^{b,c}	13.0±0.8	14.0±0.5	15.8±0.9	14.4±0.9	13.4±2.9	14.4±1.0
Preimplantation loss (%) ^d	5.0±2.2	3.7±1.5	4.3±3.0	6.9±2.4	25.7±12.6	8.5±4.3
Postimplantation loss (%) ^e	10.0±2.0	4.9±3.4	3.7±2.5	10.4±4.3	8.9±6.7	9.2±1.9

^a Mean ± Standard Error (where applicable)

^b Excludes litters with total resorptions

^c Litter size = live plus dead fetuses

^d Preimplantation loss = [(number of corpora lutea - implant sites)/corpora lutea] x 100

^e Postimplantation loss = [(number of implant sites - viable fetuses)/implant sites] x 100

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Exploratory Oral Dose Range Finding Study in Pregnant Rats with CI-955

8.5.

TABLE T-5. Reproductive and Litter Data
(Page 2 of 2)

Treatment Daily Dose (mg/kg)	Vehicle		CI-955			
	0	8.1	16.3	81.3	243.8	487.5
Fetal Parameters						
Mean fetal weight (g)						
Males (live)	5.4±0.2	5.1±0.1	5.1±0.2	5.0±0.2	4.9±0.2	4.8±0.1
Females (live)	5.1±0.2	4.9±0.1	4.8±0.2	4.9±0.1	4.4±0.1	4.4±0.1
Sex ratio (S)						
Males (live)	42.4±6.3	37.4±5.4	49.3±1.5	52.3±8.3	58.6±4.3	40.5±5.4
Females (live)	57.6±6.3	62.6±5.4	50.7±1.5	47.7±5.3	41.7±4.3	59.5±5.4
Survival of fetuses at term (S) ^f	100	100	100	100	100	100
Malformed fetuses/litters	0	1/1 ^g	0	0	0	0
Variations						
Stunted (weight <4 g)	2/1 ^h	1/1	1/1	1/1	2/1	7/3
Focal hematomas	4/2	5/3	24/3	5/3	16/3	11/4

^f Survival at term = (live at term sacrifice/(live + dead)) x 100^g Fetus 42756-B: one facial papilla absent, agnathia, microstomia, aglossia, one nare present^h Number of fetuses/number of litter-affectedTeratology Study in Rats with CI-955.Testing Facility: Parke-Davis Pharmaceutical Research Division of Warner Lambert Co., Ann Arbor, MI.Study Number: 1468; Research Report RR-745-01647; 10/18/90. Test Article: CI-955, Lot CLO45026, a powder blend ratio of 1.6 mg CI-906/1.0 mg CI-570.Study Dates: Initiated 01/01/90; completed 02/01/90.GLP Compliance: GLP compliance attested.Animals: S-D, :CD-BR, sexually mature female rats; 7 groups, 20/group, weighing an average of 254 grams (range 208 to 314 gm), were inseminated (1/1 ratio) by untreated 3 month old male rats weighing 372 grams (range 208 to 314 gm) at start of study.Mode of Administration of Test Agent: Via Gavage in 0.5% methyl cellulose.Dose Levels: CI-906/CI-570 (= CI-955) at doses of 0/0 (UTC), 0/0 (VC), 5/3.1 (= 8.1), 50/31.3 (= 81.3), 150/93.8 (= 243.8), 0/93.8, and 150/0 mg/kg/day, gestation days 6 thru 15. Selected on the basis of an exploratory oral dose range-finding study in pregnant rats in which there were no deaths or significant clinical signs occurring during the study. The high dose was expected to cause some degree of maternal toxicity (reduced body weight-gain and food intake). The low dose was estimated to be a no adverse effect dose and the mid-dose an intermediate between them.

Maternal clinical observations, body weight, and food consumption were monitored throughout the study. On gestation Day 21, cesarean sections were performed and the number of corpora lutea, implantation sites, early and late resorptions, and live and dead fetuses were recorded. Live term fetuses were sexed, weighed, and examined for external, visceral, and skeletal variations and malformations. Reproductive and developmental parameters, including the incidence of malformations and variations, were compared between treated and the vehicle control groups.

Maternal toxicity was assessed by effects on clinical observations, body weight, food consumption, gross necropsy findings, and reproductive outcome (e.g., total resorption). Developmental toxicity was assessed by effects on embryo/fetal survival (pre/postimplantation loss and survival at term), body weight, sex distribution, and structural malformations and variations. External, visceral, and skeletal findings were judged to be either malformations or variations. Malformations are defined as developmental deviations which 1) are gross structural changes, 2) may be incompatible with life, and 3) are generally rare in occurrence. Variations are structural alterations which occur infrequently but more often than malformations, and have no significant biological effect on body conformation, function, or general well-being. An increase in the incidence of malformed offspring, indicative of teratogenicity, is characterized by an increase in all of the following: 1) percent malformed offspring per litter, 2) number and percent of litters with malformed offspring, and 3) number of offspring or litters with a particular malformation that appears to increase with dose. A dose-related increase in the incidence of variations was considered an indication of developmental toxicity.

Interim sacrifice: No.

Mortality: Four dams died. One, day 11, given 150 mg/kg Quinapril, probably a gavaging error. Three (CI-955), on day 18, one dam given 8.1 (Q, 5) mg/kg (no clinical signs, but with malocclusion), and 2 given 243.8 (Q, 150) mg/kg (which exhibited weight loss and reduced food intake).

Drug Associated Findings: Reduced fecal output was seen in a few CI-955 treated animals, but no drug related clinical signs were observed. Maternal toxicity was evidenced as reduced body-weight-gain during the dosing period (days 6-15), with the greatest reduction in dams given 81.3 and 243.8 mg/kg; however, post dosing compensation recovery removed significant differences. Gestational period daily food intake was reduced significantly in treated groups, except for the H-D quinapril (not dose related). Litter and fetal parameters were unaffected, and fetal examination revealed no drug-related external abnormalities. No gross visceral or skeletal abnormalities were observed (Tables T-7 to 10); however, there was an increased incidence of kidney pelvis dilation notations in all groups of HCTZ treated pups.

9.7. TABLE T-7. Reproductive and Litter Data^a

Treatment Dose (mg/kg)	Untreated		Vehicle			CI-955		CI-570	CI-906
	-	0	8.1	81.3	243.8	93.8	150		
Maternal Parameters									
Mated	20	20	20	20	20	20	20	20	20
Nonpregnant	0	0	1	0	3	0	0	0	1
Pregnancy rate (%)	100	100	95	100	85	100	95	100	95
Pregnant animals at term	20	20	18	20	15	20	20	20	18
With resorption only	0	0	0	0	0	0	0	0	0
With viable litters	20	20	18	20	15	20	20	20	18
Corpora lutea	15.8±0.5	15.3±0.5	16.2±0.5	16.3±0.5	15.7±0.4	15.8±0.5	16.1±0.6	16.1±0.6	16.1±0.6
Implant sites	14.8±0.6	14.2±0.7	14.9±0.5	15.3±0.5	14.8±0.5	13.8±0.8	14.9±0.6	14.9±0.6	14.9±0.6
Live fetuses	14.3±0.6	13.3±0.7	14.1±0.5	14.3±0.6	13.9±0.6	13.2±0.6	14.1±0.7	14.1±0.7	14.1±0.7
Dead fetuses	0	0	0	0	0	0	0	0	0
Resorptions	0.5±0.2	0.9±0.2	0.8±0.2	1.0±0.2	0.9±0.1	0.6±0.1	0.8±0.3	0.8±0.3	0.8±0.3
Litter size ^b	14.3±0.6	13.3±0.7	14.1±0.5	14.3±0.6	13.9±0.6	13.2±0.6	14.1±0.7	14.1±0.7	14.1±0.7
Preimplantation loss ^c (%)	6.7±2.3	8.4±3.2	7.5±1.4	6.4±1.9	5.5±2.0	12.2±4.3	7.7±1.6	7.7±1.6	7.7±1.6
Postimplantation loss ^d (%)	3.7±1.4	7.0±1.6	5.8±1.5	7.0±1.5	6.4±1.9	6.5±2.5	6.0±2.0	6.0±2.0	6.0±2.0
Fetal Parameters									
Survival at term ^e (%)	100	100	100	100	100	100	100	100	100
♂ male fetuses	47.9±3.1	51.8±4.1	56.1±3.3	51.7±2.9	51.7±3.1	43.1±3.7	43.3±2.5	43.3±2.5	43.3±2.5
♀ female fetuses	82.1±3.1	48.4±4.1	43.9±3.1	48.3±2.9	48.3±3.1	56.9±3.7	56.7±2.5	56.7±2.5	56.7±2.5
Fetal body weight (g)									
males	5.0±0.1	5.2±0.1	4.9±0.1	4.7±0.2*	4.6±0.1*	5.1±0.1	5.0±0.1	5.0±0.1	5.0±0.1
females	4.8±0.1	5.0±0.1	4.7±0.1*	4.5±0.2*	4.4±0.1*	4.7±0.1	4.8±0.1	4.8±0.1	4.8±0.1

^a Mean ± S.E. (when applicable)
^b Litter size = live plus dead fetuses
^c Preimplantation loss = ((number of corpora lutea - implant sites)/corpora lutea) X 100
^d Postimplantation loss = ((number of implant sites - viable fetuses)/implant sites) X 100
^e Survival at term = (live at term sacrifice/(live + dead)) X 100
* Significantly different from vehicle control at p < 0.0224 by trend test, two-tailed

Study 1468: Oral Teratology Study in Rats With CI-955
9.8. TABLE T-8. External and Visceral Findings in Offspring

Treatment Dose (mg/kg)	Untreated		Vehicle			CI-955		CI-570	CI-906
	-	0	8.1	81.3	243.8	93.8	150		
Fetuses examined	285	286	294	285	208	284	293		
Litters examined	20	20	18	20	15	20	18		
Malformed fetuses/litters	2/2	0	1/1	0	1/1	1/1	1/1		
Number of fetuses/Number of litters affected									
Malformations									
Eye - microphthalmia	1/1 ^a	--	--	--	--	--	--	--	--
Ear - ectopic	1/1 ^a	--	--	--	--	--	--	--	--
Nose - atresia of nares	1/1 ^a	--	--	--	--	--	--	--	--
Jaw - micrognathia	--	--	--	--	--	1/1 ^b	1/1 ^c	--	--
- agnathia	1/1 ^a	--	--	--	--	--	--	--	--
Mouth - stomia	1/1 ^a	--	--	--	--	--	--	--	--
Vibrissal Pad - malformed	1/1 ^a	--	--	--	--	--	--	--	--
Heart - interventricular septal defect	--	--	--	--	--	--	--	--	--
Spleen - agenesis	--	--	--	--	--	--	1/1 ^c	--	--
Testis - decreased size	1/1	--	--	--	--	--	1/1 ^c	--	--
Vessel - interrupted aortic arc	--	--	1/1	--	--	--	--	1/1 ^c	1/1
Variations									
Stunted (<4.0g)	9/6 ^a	0	5/3	22/7	25/8	0/6 ^c	12/8		
Hematoma - external	18/8	24/9	7/6	24/12	12/7	16/6	19/10		
Eye - appears hemorrhagic	--	--	--	--	--	1/1	--		
Edema - general	--	--	--	1/1	--	--	--		
Head - dome shaped	--	--	--	--	--	1/1	--		
Facial papilla - absent	--	--	--	--	--	--	1/1		
Forelimb - fluid-filled cyst between digits	--	--	--	--	--	1/1	--		
Heart - misshapen	--	--	--	--	1/1	--	--		
- discolored	1/1 ^a	--	--	--	--	1/1 ^c	--		
- slight amt. blood/pericardium	--	1/1	--	--	--	--	--		
Vessel(s) - malposition	--	1/1	--	--	3/2	--	2/2		
Small intestines - misshapen	1/1	--	--	--	--	--	--		
Spleen/pancreas - blood clot between	--	--	--	--	--	--	--		
Liver - discolored	--	2/2	1/1	--	--	--	1/1		
- lobulated lobe	--	--	--	--	--	--	1/1		
Spleen - discolored	--	--	--	1/3	--	--	--		
Kidney - dilated pelvis	1/1	1/1	6/4	5/3	3/2	5/4	1/1		
- with reduced papilla	1/1	--	--	1/1	1/1	1/1	--		
Ureter - dilated	30/11	21/11	26/12	33/13	35/9	22/12	14/6		
- convoluted	--	--	--	--	--	--	--		
Adrenal - discolored	1/1	--	--	--	1/1	--	1/1		

^a Fetus 43553-7
^b Fetus 43621-9
^c Fetus 43641-6

9.9. TABLE T-9. Skeletal Findings in Offspring

Treatment Dose (mg/kg)	Untreated	Vehicle	CI-955			CI-570	CI-906
			8.1	81.3	243.8	93.8	150
Fetuses examined	196	184	175	195	145	182	173
Litters examined	20	20	18	20	15	20	18
Malformed fetuses/litters	2/2	0	0	0	4/2	4/3	2/2
Number of fetuses/Number of litters affected							
Malformations							
Skull bone(s) - malformed	1/1 ^a	--	--	--	2/2 ^d	1/1 ^b	--
- fused	1/1 ^a	--	--	--	1/1 ^d	3/2 ^{b,c}	--
- agenesis	1/1 ^a	--	--	--	--	--	--
Vertebrae - malformed	2/2 ^a	--	--	--	1/1 ^d	--	--
- fused	1/1	--	--	--	1/1 ^d	--	1/1
- agenesis	--	--	--	--	1/1 ^d	--	--
- one less presacral	--	--	--	--	2/1 ^e	1/1	1/1
Rib(s) - branched	--	--	--	--	--	1/1 ^c	--
- fused	--	--	--	--	1/1 ^d	--	--
- agenesis	--	--	--	--	1/1 ^d	1/1 ^c	1/1
Sternebrae - fused	--	--	--	--	--	1/1 ^b	--
Variations							
Skull bone(s) - malpositioned	1/1	--	--	--	--	--	--
- extra ossification site	1/1	--	1/1	--	--	1/1	3/2
- immature form	8/4	1/1	6/1	7/2	1/1	2/2	2/2
- unossified	22/8	11/8	14/7	6/3	--	17/5	--
Vertebrae - misshapen centra	--	3/2	2/2	6/5	1/1	3/2	5/4
- dumbbell shaped centra	2/1	5/4	2/2	2/2	1/1	1/1	4/3
- extra presacral	1/1	--	--	--	--	1/1	2/2
- immature form	2/1	2/1	--	6/1	3/2	1/1	1/1
- centra bifid	1/1	2/2	1/1	3/3	1/1	2/2	2/2
- unossified	57/17	22/11	21/14	64/17	49/14	34/13	28/12
Pelvic girdle - immature form	--	--	--	7/2	--	--	--
Ribs - short 13th	6/4	3/3	7/4	10/4	10/4	5/4	2/1
- bent	2/2	--	--	--	--	--	--
- extra well-formed lumbar	1/1	--	--	--	--	--	1/1
- extra rudimentary lumbar	2/2	1/1	3/2	3/3	1/1	2/2	5/4
- extra cervical	1/1	1/1	--	--	--	--	--
- extra rudimentary cervical	3/3	7/4	2/2	5/3	8/3	3/1	1/1
Sternum - focal fusion	--	--	--	--	--	--	1/1
- asymmetric form	2/2	--	--	--	--	2/2	--
- misaligned	--	--	--	--	1/1	--	--
Digit - misaligned	--	1/1	--	--	--	--	--

- ^a Fetus 43593-7
- ^b Fetus 43641-6
- ^c Fetus 43651-2
- ^d Fetus 43621-9
- ^e Fetus 43624-8,11

9.10. TABLE T-10. Skeletal Ossification Parameters in Offspring

Treatment Dose (mg/kg)	Untreated	Vehicle	CI-955			CI-570	CI-906
			8.1	81.3	243.8	93.8	150
Fetuses examined	196	184	175	195	145	182	173
Litters examined	20	20	18	20	15	20	18
Number ossified/litter ^a							
Cervical centra	3.3 ±0.4	3.4 ±0.3	3.5 ±0.2	2.6 ±0.3	2.9 [*] ±0.3	3.3 ±0.4	3.1 ±0.3
Sternebrae	5.9 ±0.03	6.0 ±0.01	5.9 ±0.03	5.8 [*] ±0.09	5.8 [*] ±0.08	5.9 ±0.10	6.0 ±0.02
Proximal phalanges							
- forelimbs	4.6 ±0.4	5.1 ±0.4	4.1 ±0.4	5.0 ±0.4	4.8 ±0.4	4.4 ±0.4	5.1 ±0.4
- hindlimbs	0.9 ±0.3	1.2 ±0.4	0.6 ±0.2	0.8 ±0.2	0.5 ±0.3	1.0 ±0.2	0.6 ±0.2
Metacarpals							
- forelimbs	8.0 ±0.01	8.0 ±0.02	8.0 ±0.0	8.0 ±0.03	8.0 ±0.0	7.9 ±0.10	8.0 ±0.02
- hindlimbs	9.1 ±0.2	9.3 ±0.1	9.8 ±0.1	9.0 ±0.1	8.8 ±0.2	9.1 ±0.2	8.9 ±0.2

* Significantly different from vehicle control at p < 0.0204 for trend test, two-tailed
^a Mean ± SE

Exploratory Oral Dose Range Finding Study in Pregnant Rabbits With CI-955.

Testing Facility: Parke-Davis Pharmaceutical Research Division of Warner Lambert Co., Ann Arbor, MI.

Study Number: 1465; Report No. RR-745-01424, 07/31/90. Test Article: CI-955 Lot CL 045026, a powder blend ratio of 1.6 mg CI-906/1.0 mg CI-570 (CI-906/PD 109,452-2; CI-570/PD 035599).

Study Dates: Initiated 11/21/89); completed 02/02/90.

GLP Compliance: GLP compliance attested.

Animals: Artificially inseminated New Zealand white female 29 week old rabbits weighing an average of 3.8 kg (3.4 - 4.4 kg).

Mode of Administration of Test Agent: Via Gavage in 0.5% methyl cellulose administered at a volume of 1 mL/kg.

Dose Levels: CI-955 was administered on days 6 to 18 of pregnancy to pregnant females, 5/dose level, at doses of 0.16, 1.63, and 2.44 mg/kg/day (0.1, 1.0, and 1.5 mg/kg quinapril content). The dams were sacrificed on Day 21 of gestation and maternal and fetal parameters were evaluated.

Observations/Measurements: All females were observed daily during treatment for signs of drug induced toxicity and systemic effects. Clinical observations were recorded. Body weights were recorded pretest and on gestation days 0, 6, 9, 12, 15, 18, 24, and 30. Individual food consumptions were recorded at 3-day intervals. Animals with food spillage were excluded from calculations. Gross necropsies were performed on all animals that died or were euthanatized during the study. No tissues were processed for histological examination. Animals were euthanatized on day 30 of gestation and the number of live and dead or resorbing fetuses and number of corpora lutea and implant sites were recorded. Fetuses were sexed, weighed, externally examined, and dissected for visceral abnormalities.

Interim sacrifice: No.

Mortality: Three animals died and four aborted; deaths occurring on gestation days 22 or 26. Two died and one aborted when given 1.63 (1.0 Q) mg/kg (M-D) and one died and three aborted when given 2.44 (1.5 Q) mg/kg (H-D). The M-D doe that died exhibited total resorption.

Drug Associated Findings: Feces were absent or reduced in 1, 1, 3, and 6 animals with control, L-, M-, or H-D groups, respectively. During the 12 day treatment period, treated does lost weight in a dose related manner, with the H-D group losing 10%, but compensatory post-treatment adjustment occurred in all treatment groups. For the entire gestation period, body weight gains were comparable for most groups, but the H-D group lost 6% of it's starting weight. Total food intake was reduced by treatment in a non-dose related manner. A maximum tolerated dose in this study was estimated to be between 0.16 and 1.63 mg/kg.

There were no adverse effects on fetal body weight or sex ratio at term in the survivors of the CI-955 treated groups and no malformations or variations were observed, but there was only one surviving litter in the H-D group, and only 2 surviving litters in the M-D group. The combination appears to be more toxic in study comparisons than Quinapril alone, but missing values and low numbers prevent definition of differences. It is possible that this is only a high dose effect which does not extend into lower doses. Comparisons as follows:

Died (D), Aborted (A)/ No. Study.	Quinapril content of doses, mg/kg:				
	0.05	0.1	0.5	1.0	1.5
1. Quinapril			1A (D)/14	3D, 1A/13	2D/12-
2. Quinapril/HCTZ Equivalent to 1--(X 3)		-0-/4 ? /12	Not done	2D, 1A/5 6D, 3A/15	1D, 3A/4 3D, 9A/12
3. Quinapril/HCTZ Quinapril comparison	-0-/15	-0-/18	1A/18 1A/19	Not done	Not done

Exploratory Oral Dose Range Finding Study In Pregnant Rabbits with CI-955

8.7. Table T-7. Reproductive and Litter Data^a

Treatment Dose (mg/kg)	Vehicle	CI-955		
	0	0.16	1.63	2.44
Maternal Parameters				
Number gravid	4	4	5	4
Number nongravid	1	1	0	1
Number died	0	0	2	1
Number sacrificed before term	0	0	0	0
Number delivered early	0	0	0	0
Number aborted	0	0	1	3
Number examined at term sacrifice (Day 30)	5	5	3	4
Number with resorptions only	0	0	1 ^b	0
Number with viable litters	4	4	2	1
Corpora lutea implantations	9.3±0.7	11.0±0.4	13.0±2.0	12.0
Live fetuses	4.3±1.1	6.5±1.9	9.0±3.0	9.0
Dead fetuses	4.3±1.1	6.0±1.8	8.0±2.0	6.0
Resorptions	0	0	0	0
Litter size ^{c,d}	0	0.5±0.3	1.0±1.0	3.0
Preimplantation loss (%) ^e	4.3±1.1	6.0±1.8	8.0±2.0	6.0
Postimplantation loss (%) ^f	55.4±9.8	40.6±17.6	32.7±12.7	25.0
	0	6.3±3.7	8.3±5.3	33.3
Fetal Parameters				
Mean fetal weight (g)				
Males (live)				
Females (live)	55.9±5.3	48.3±3.3	55.7±5.7	57.1
Sex ratio (%)	55.6±2.8	52.6±6.1	53.6±6.1	51.9
Males (live)				
Females (live)	33.2±11.3	38.5±14.2	48.3±18.3	66.7
Survival of fetuses at term (%) ^g	66.8±11.3	61.5±14.2	51.7±18.3	33.3
Malformed fetuses	100	100	100	100
	0	0	0	0

^a Mean ± Standard Error (where applicable)

^b Animal (4374) died; excluded from calculations

^c Litter size = live plus dead fetuses

^d Excludes litters with total resorptions

^e Preimplantation loss = [(number of corpora lutea - implant sites)/corpora lutea] x 100

^f Postimplantation loss = [(number of implant sites - viable fetuses)/implant sites] x 100

^g Survival at term = [live at term sacrifice/(live + dead)] x 100

Oral Teratology Study in Rabbits With CI-955.

Testing Facility: Parke-Davis Pharmaceutical Research Division of Warner Lambert Co., Ann Arbor, MI.

Study Number: 1495; Report No. RR-745-01671, 11/08/90. Test Article: CI-955 Lot CL 045026, a powder blend ratio of 1.6 mg CI-906/1.0 mg CI-570 (CI-906/PD 109,452-2; CI-570/PD 035599).

Study Dates: Initiated 02/26/89; completed 04/06/90.

GLP Compliance: GLP compliance attested.

Animals: Artificially inseminated New Zealand white female 24 week old rabbits weighing an average of 3.6 kg (3.2 - 4.2 kg).

Mode of Administration of Test Agent: Via Gavage in 0.5% methyl cellulose administered at a volume of 1 mL/kg.

Dose Levels: Criteria of dose selection were based upon the range finding study (above) in which mortality and abortions occurred in both the M- and H-D groups. The H-D was chosen to be 0.81 mg/kg with lower doses of 0.16 and 0.08 mg/kg to evaluate the response.

CI-955 was administered on gestation days 6 to 18 to pregnant females, 20/dose level, at doses of UTC (0), VC (0), 0.08, 0.16, and 0.81 mg/kg/day (0, 0.05, 0.1, and 0.5 mg/kg quinapril content; all 3 test dose levels less than the 0.8 mg/kg recommended maximum human clinical dose). In addition, H-D equivalent groups with quinapril alone (0.5mg/kg) and HCTZ alone (0.31 mg/kg) were added for comparison. Dams were sacrificed on Day 21 of gestation and maternal and fetal parameters were evaluated.

Observations/Measurements: All females were observed daily during treatment for signs of drug induced toxicity and systemic effects. Clinical observations were recorded. Body weights were recorded pretest and on gestation days 0, 6, 9, 12, 15, 18, 24, and 30. Individual food consumptions were recorded at 3-day intervals. Animals with food spillage were excluded from calculations. Gross necropsies were performed on all animals that died or were euthanized during the study. Selected tissues from F₁ fetuses were processed for histological examination. Animals were euthanized on day 30 of gestation and the number of live and dead or resorbing fetuses and number of corpora lutea and implant sites were recorded. Fetuses were sexed, weighed, externally examined (including palat ), and dissected for visceral abnormalities. They were then eviscerated, skinned, cleared, stained with Alizarin Red S, and examined for skeletal defects.

Interim sacrifice: No.

Mortality: None of the Treated or control animals died during the study, but there were two abortions in groups receiving H-D quinapril (0.3 mg/kg), one with quinapril alone (CI-906) and one with the CI-955 combination.

Drug Associated Findings: Body-weight (B-W) changes were minimal during the study. The group mean body-weights were quite similar at the beginning of the experiment, the difference between the lowest and highest group mean B-W value being less than 2%. At no time during the 12 day treatment period did the group mean B-W variation between treated groups and control groups exceed 4%, but the lowest mean B-W values were in the CI-955 H-D (0.5 mg/kg) group. Post treatment compensatory B-W adjustment occurred in all treatment groups, the variation between the group BW means at 30 day sacrifice being less than 1.5 %, but at this time the highest mean B-Ws were in the M- and H-D CI-955 treatment groups. Total food intake was not significantly changed by treatment. The abortions occurred in the groups receiving 0.5 mg/kg Quinapril, one group with and one without HCTZ; otherwise, there were no adverse effects on fetal body weight, sex ratio, or survival at term in these very low dose CI-955 treated groups. Malformations and variations were not dose related. See tables T-7 to T-10.

Study 1495: Oral Teratology Study in Rabbits With CI-955

9.7.

TABLE T-7. Reproduction and Litter Data^a

Treatment Dose (mg/kg)	Untreated	Vehicle	CI-955			CI-570	CI-906
	--	0	0.08	0.16	0.81	0.31	0.5
Maternal Parameters							
Inseminated	20	20	20	20	20	20	20
Nonpregnant	1	0	5	2	2	3	1
Pregnancy Rate (%)	95	100	75	90	90	85	95
Aborted	--	--	--	--	1	--	1
With resorptions only	--	1	--	--	--	--	--
With viable litters	19	19	15	18	17	17	18
Corpora lutea	11.0 ± 0.5	10.4 ± 0.6	9.5 ± 0.6	11.7 ± 0.7	10.8 ± 0.6	10.2 ± 0.7	10.2 ± 0.6
Implant sites	7.3 ± 0.8	7.0 ± 0.5	5.7 ± 0.7	7.3 ± 0.6	8.4 ± 0.7	7.8 ± 0.7	7.0 ± 0.6
Live fetuses ^b	6.6 ± 0.7	6.9 ± 0.5	5.5 ± 0.7	6.8 ± 0.6	8.1 ± 0.6	7.6 ± 0.7	6.7 ± 0.6
Dead fetuses	0.1 ± 0.1	0	0	0	0	0	0
Resorptions	0.6 ± 0.3	0.4 ± 0.2	0.2 ± 0.1	0.6 ± 0.2	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.1
Litter size ^c	6.7 ± 0.7	6.9 ± 0.5	5.5 ± 0.7	6.8 ± 0.6	8.1 ± 0.6	7.6 ± 0.7	6.7 ± 0.6
Preimplantation loss ^d (%)	32.3 ± 6.9	25.8 ± 5.4	39.2 ± 7.1	35.1 ± 5.6	21.2 ± 5.4	23.4 ± 6.2	29.7 ± 5.5
Postimplantation loss ^e (%)	7.2 ± 3.2	11.2 ± 5.4	2.7 ± 1.5	7.9 ± 2.7	3.1 ± 1.5	2.5 ± 1.7	6.5 ± 2.9
Fetal Parameters							
Survival at term ^f (%)	98.7 ± 1.3	100	100	100	100	100	100
Male fetuses (%)	44 ± 6	56 ± 4	59 ± 8	48 ± 5	55 ± 5	51 ± 5	56 ± 6
Female fetuses (%)	56 ± 6	44 ± 4	41 ± 8	52 ± 5	45 ± 5	49 ± 5	44 ± 6
Fetal body weight (g)							
Males	53.5 ± 2.0	53.1 ± 1.2	57.0 ± 1.7	53.7 ± 1.3	49.8 ± 1.8	52.5 ± 1.7	53.9 ± 2.0
Females	52.6 ± 1.4	51.0 ± 0.9	53.9 ± 2.5	51.4 ± 1.6	49.6 ± 1.9	50.3 ± 2.0	51.0 ± 2.1

^a Mean ± S.E. where applicable
^b Total resorptions excluded from calculations
^c Litter size = live plus dead fetuses (total resorption excluded)
^d Preimplantation loss = ((number of corpora lutea - implant sites)/corpora lutea) x 100
^e Postimplantation loss = ((number of implant sites - viable fetuses)/implant sites) x 100
^f Survival at term = (live at term sacrifice/(live + dead)) x 100

Study 1495: Oral Teratology Study in Rabbits With CI-955

9.8. TABLE T-8. External and Visceral Findings in Offspring

Treatment Dose (mg/kg)	CI-955						
	untreated	Vehicle	CI-955			CI-570	CI-906
		0	0.08	0.16	0.81	0.31	0.5
Fetuses examined	127	132	82	122	138	129	120
Litters examined	19	19	15	18	17	17	18
Malformed fetuses/litters	1/1	1/1	0	1/1	0	3/3	1/1

Malformations	Number of fetuses/number of litters affected						
	untreated	Vehicle	CI-955			CI-570	CI-906
Spina bifida	1/1	--	--	--	--	--	1/1
Nose - malformed	--	--	--	1/1 ^a	--	--	--
Jaws - micrognathia	--	--	--	1/1 ^b	--	--	--
Limbs - talipes	--	--	--	--	--	1/1	--
Heart	--	--	--	--	--	1/1	--
Right ventricle enlarged	--	1/1 ^b	--	--	--	--	--
Two cusps on semi-lunar valve	--	1/1 ^b	--	--	--	--	--
Aorta - enlarged	--	1/1 ^b	--	--	--	--	--
Lung - agenesis lobe	--	--	--	--	--	1/1	--
Diaphragm - hernia	--	--	--	--	--	1/1	--
Variations							
Stunted (wt <30 g)	3/2	--	--	--	3/2	2/2	4/2
Head - misshapen	--	--	--	1/1	--	--	--
Eyes - opacity	--	--	--	1/1 ^c	--	--	--
Teeth	--	--	--	1/1	--	--	--
Malpositioned	--	--	--	1/1	--	--	--
Not erupted	--	--	--	1/1	--	1/1	--
Limbs - hematoma	--	--	--	--	1/1	--	--
Heart - slight-fluid in pericardium	--	--	--	--	1/1	1/1	--
Vessel							
Malpositioned	2/2	2/1	2/1	4/2	3/1	3/3	3/2
Accessory	--	--	--	--	1/1	--	--
Lung - azygous lobe absent	3/2	3/2	1/1	--	3/2	10/4	1/1
Gallbladder - accessory	--	--	--	1/1	--	--	--
Kidney							
White material (mineralization)	--	--	--	1/1	--	1/1	--
Dilated pelvis	--	--	--	2/2	--	1/1	1/1
Reduced papilla	--	--	--	1/1	--	--	1/1
Absent papilla	--	--	--	--	--	1/1	--
Ureter							
Dilated	--	--	--	--	--	1/1	--
Malpositioned	--	2/2	--	--	--	--	--
Thymus - enlarged, hemorrhagic (congested)	--	--	1/1	--	--	--	--

^a Fetus 4487-4
^b Fetus 4438-1
^c No histologic correlate

9.9. TABLE T-9. Skeletal Findings in Offspring (Page 1 of 2)

Treatment Dose (mg/kg)	Untreated	Vehicle 0	CI-955			CI-570	CI-906
			0.08	0.16	0.81	0.31	0.5
Fetuses examined	127	132	82	122	138	129	120
Litters examined	19	19	15	18	17	17	18
Malformed fetuses/litters	1/1	2/2	4/2	3/3	8/5	8/5	5/4
<u>Number of fetuses/number of litters affected</u>							
Malformation							
Skull bone(s) - fused	--	--	3/2	1/1	2/1	1/1	--
agenesis	--	--	--	1/1	--	--	--
Scapula - malformed	--	--	--	--	--	2/2	--
Vertebrae - malformed	1/1 ^a	1/1	1/1	1/1	1/1	--	2/2 ^b
- fused	--	1/1	--	2/2	--	2/1	2/2
- duplication/extra bone	--	--	--	--	1/1	--	4/3
- agenesis	--	--	--	2/2	3/2	1/1	--
- one less presacral	--	1/1	--	4/2	--	1/1	--
Ribs - branched	--	1/1	--	1/1	2/2	3/3	--
- malformed	--	--	--	--	--	1/1	--
- fused	--	--	--	--	--	1/1	2/2
- duplication/extra	--	--	--	2/2	--	2/2	--
- agenesis	--	--	--	1/1	1/1	--	--
Sternebrae - fused	--	--	1/1	--	--	1/1	--
Limbs - bent/short	--	--	--	--	--	1/1 ^c	--
Variations							
Skull bone(s) - misshapen	1/1	--	--	1/1	--	--	1/1
- bent hyoid horns	5/3	3/2	1/1	3/3	3/1	10/7	5/4
- extra ossification	--	1/1	2/1	--	1/1	2/1	4/4
- immature form	--	--	--	--	--	1/1	--
- unossified	2/2	--	--	--	--	--	--
Scapula - misshapen	--	--	--	--	--	--	2/2

^a Fetus 4433-6 (spina bifida)
^b Fetus 4553-4 (spina bifida)
^c Fetus 4524-6 (talipes)

9.9. TABLE T-9. Skeletal Findings in Offspring (Page 2 of 2)

Treatment Dose (mg/kg)	Untreated	Vehicle 0	CI-955			CI-570	CI-906
			0.08	0.16	0.81	0.31	0.5
Fetus examined	127	132	82	122	138	129	120
Litters examined	19	19	15	18	17	17	18
<u>Number of Fetuses/Number of Litters Affected</u>							
Vertebrae - misshapen	--	1/1	--	--	1/1	--	2/2
- misaligned	--	--	--	--	1/1	--	--
- extra presacral	3/2	1/1	1/1	--	1/1	3/3	2/2
- extra cervical	--	--	--	--	--	--	1/1
- extra ossification site	--	--	1/1	--	--	--	--
- immature form	--	--	--	--	--	--	1/1
- centra bifid	--	--	--	2/1	--	--	--
Pelvic bone	--	--	--	--	--	1/1	--
- misshapen	--	--	--	--	--	--	--
- immature form	1/1	--	--	--	--	--	--
Ribs	--	--	--	--	--	1/1	--
- thick/wavy	--	--	--	--	--	--	--
- extra cervical	1/1	5/2	--	1/1	2/2	--	2/2
- short 12th	--	1/1	--	--	--	--	--
- extra well-formed lumbar	79/18	54/18	44/12	68/17	71/16	54/14	50/16
- extra rudimentary lumbar	34/14	48/18	19/11	40/15	34/15	44/13	38/16
Sternebrae	--	1/1	--	--	--	--	--
- misshapen	--	2/1	1/1	--	--	--	--
- asymmetric form	--	--	--	--	--	--	--
- extra ossification site	1/1	--	--	--	--	2/1	1/1
- focal fusion	5/3	1/1	--	1/1	2/2	1/1	1/1
- epiphysis	6/3	--	2/2	9/5	8/4	18/7	3/2
Femur - epiphysis	1/1	--	--	--	--	--	--
Limbs (talus) - unossified	2/2	--	--	1/1	2/2	--	--
Digits - unossified	--	--	--	--	--	--	--

Study 1495: Oral Teratology Study in Rabbits With CI-955

9.10. TABLE T-10. Skeletal Ossification Parameters for Offspring

Treatment Dose (mg/kg)	Untreated	Vehicle	CI-955		CI-570	CI-906
	--	0	0.08	0.16	0.81	0.31 0.5
Fetuses examined	127	132	82	122	138	129 120
Litters examined	19	19	15	18	17	17 18
			Number Ossified Litter ^a			
Sternbrae	5.9 ±0.03	5.9 ±0.05	6.0 ±0.02	6.0 ±0.02	6.0 ±0.02	5.9 ±0.03 6.0 ±0.02
Forelimbs - olecranon	0.9 ±0.13	1.0 ±0.13	1.2 ±0.19	0.7 ±0.15	0.7 ±0.15	1.0 ±0.16 0.9 ±0.15
- tuberosities of humerus	3.94 ±0.03	4.00 ±0.00	3.96 ±0.04	3.90 ±0.06	3.88* ±0.06	3.88** ±0.05 3.92 ±0.06
- epiphyses	2.00 ±0.00	2.00 ±0.00	2.00 ±0.00	2.00 ±0.00	1.99 ±0.01	2.00 ±0.00 1.99 ±0.01
Hindlimbs - epiphyses	3.88 ±0.05	3.94 ±0.03	3.94 ±0.04	3.80 ±0.09	3.66* ±0.11	3.81 ±0.09 3.85 ±0.10

^a Mean ± SE

* Significantly different from vehicle control at p < 0.0224 for trend test, two-tailed

** Significantly different from vehicle control at p < 0.0224 for Wilcoxon test, two-tailed

Segment III: No Segment III Quinapril/HCTZ studies have been provided by the sponsor; therefore, no comparative evaluation between Quinapril and Q/HCTZ can be made. In the perinatal and postnatal study on rats with Quinapril alone, with only 0, 25, 75 and 150 mg/kg dosing in groups of 20 pregnant rats, there were no treatment related deaths, but 29 pups were found dead or missing, and one H-D and one M-D pup had multiple malformations. In the modified perinatal - postnatal study in rats that received vehicle or 150 mg/kg Quinapril during gestation days 15 to lactation day 21, or 150 mg/kg during gestation days 15 to 21 or lactation days 0 to 21, there were dams that were euthanized with all pups dead or missing or that did not deliver and some that exhibited histopathology of JG H/H, but term fetuses from all groups appeared normal upon external examination. No abnormalities were observed during microscopic examination of fetal kidney specimens obtained from control and treated fetuses. Some treated neonates which received the drug during gestation and lactation (group 2) and during lactation only (group 4) and sacrificed during lactation, exhibited a treatment-duration related increase in kidney histopathologies, such as tubular dilation, glomerulosclerosis, dilated pelvis, and JG Cell Hypertrophy/Hyperplasia. There appears to be some slight treatment effect. A Segment III reproduction study for the combination is to be highly recommended.

PREGNANCY INFORMATION IN LABELING.

QUINAPRIL/HYDROCHLOROTHIAZIDE: The tenor of this section is misleading because the combined information is compared in differing orders of magnitude of unit measurement. "Teratogenicity studies were conducted in rats and rabbits with up to 150 mg/kg quinapril /.../ in combination with up to 93.8 mg/kg HCTZ /.../." The next sentence discusses "... and in rabbits given 0.5/0.31 mg/kg." "and rabbits" should be removed from the first sentence because the paired dosing information has a 300 fold difference and is not directly comparable. The sentence beginning: "Maternal toxicity, ..." is incomplete, it should be corrected to include the maternal deaths and abortions in both rats and rabbits. The last two sentences in this paragraph should be removed because "fetotoxicity" includes maternal death and abortion and probably physiological teratogenicity as well, and there was an increase in kidney pelvis dilation in all groups of pups receiving the HCTZ. It should be noted that the last rabbit reproduction study used doses (0.05 to 0.5 mg/kg) that were less (1/16 to 5/8) than the recommended human clinical dose (0.8 mg/kg Quinapril content).

QUINAPRIL: It is to be noted that the plasmas of guinea pigs and rats metabolize quinapril while the plasmas of dogs, monkeys, and man do not. Comparable Quinaprilat plasma levels were not obtained from the rats at comparable Quinapril dose levels to that in dogs, monkeys, and man, because Quinaprilat could not be detected in rat plasma at such low quinapril dose loading levels. Clinically comparable Quinaprilat plasma levels in rats require a hundred-fold increase in the Quinapril loading dose, a dose level with a marked sexual difference in that male rats have plasma levels 5 times that attained in the females. For this reason the comparisons of loading dose levels in the proposed labeling (Pregnancy Category C, Quinapril, "(180 times the maximum daily human dose)" in the reproduction labeling information may be misleading (certainly this is true with respect to the rabbit's 1/16 of the the maximum daily human dose). It might be better to state that at doses of quinapril which produce equivalent Quinaprilat plasma levels* in rats there is maternal toxicity, fetotoxicity, and neonatal kidney effects such as tubular dilation and dilated pelvis, and, at all dose levels, JG cell hypertrophy/ hyperplasia. Higher doses (400 to 800 mg/kg) were associated with 5 to 23% hydronephrosis or hydroureter and there was no no-effect dose level in the study. And: "Quinapril was not teratogenic in the rabbit; however, as noted with other ACE inhibitors, maternal toxicity and embryotoxicity were seen in some rabbits at doses as low as 1 mg/kg/day." It is to be recommended that the above statement in the labeling be changed to reflect pathologies; perhaps to the effect that: "Quinapril caused maternal toxicity and embryotoxicity (maternal deaths and abortions) in rabbits at all dose levels from 0.5 (1/3 the MHTD) to 8 mg/kg/day (doses which produce plasma levels of only 0.056 to 0.36 mg/ml*, 2 hours postdose). The study included cleft palates, conjoined twins, an omphalocele, and interventricular septal defects which, because of inadequate survival (numbers), cannot be attributed to either drug or background. In another study at slightly higher doses, abnormal findings among preterm fetuses included ecchymotic subcutaneous hemorrhages, generalized hemorrhage, omphalocele, and open eyes, and only two of the four pregnant does that survived to term sacrifice had viable normal fetuses."

* Note that cross species plasma equivalent levels cannot be compared.

It is possible that neither the rabbit, because of oversensitivity, nor the rat, because of the plasma metabolism of quinaprilat, are suitable species for reproductive safety projection for Accuretic; however, on the basis of the observed maternal and fetal toxicities seen at less than clinical dose recommendations, as well as the fetal abnormalities, this preparation should be contraindicated in pregnancy.

EQUIVALENT PLASMA LEVELS:

SPECIES // Oral Quinapril Dose // MAXIMUM PLASMA LEVELS, mcg/ml:
 // mg/kg, % bioavailable // ¹⁴C, % Quinaprilat, Quinaprilat.

Monkey	3	68%	3.7		
Dog	3	53%	3.5 to 4.5	90%	(3.1 to 4.0)
Dog (i.v.)	0.5				3.3
Dog	3				3.5
Dog	25				27.7 to 31.9
Dog	75				57.2 to 74.6
Dog	100				100.
Rat, Gravid-150					30.6 to 64.1
Foeti					.2 to 0.31
Rabbits, 2 Hours Postdose.					
	0.5				0.056
	1.0				0.098
	5.0				0.356
	5.0 + saline				0.322

APPEARS THIS WAY
ON ORIGINAL

Wistar Rats Were Given Quinapril + HCTZ Orally at a 40/25 Ratio for 13 Weeks and Tested for Quinaprilat (mcg/ml) Predose and 2 Hour Postdose.

Quinapril Content

mg/kg/day	Quinaprilat: Predose (mcg/ml)		2-Hour Postdose (mcg/ml)	
	Male	Female	Male	Female
86	0.10--0.46	0.01--0.05	16.34 (10.9--21.7)	3.42 (0.3--7.1)
172	0.08--0.16	0.05--0.12	19.13 (8.8--28.0)	13.66 (8.5--19.1)
431	0.53--1.39	0.03--0.50	41.26 (23.0--62.8)	23.11 (5.5--48.3)
862	0.55--1.14	0.13--0.61	72.10 (68.6--75.6)	68.30 (66.8--74.0)

Sex differences in Quinaprilat plasma levels of Dogs given quinapril and HCTZ.

Quinapril/HCTZ mg/kg // Weeks:	(Male) Plasma Quinaprilat, mcg/ml			(Female) Plasma Quinaprilat, mcg/ml		
	1	6	12	1	6	12
1.6/-0-	1.3	0.67	0.8	1.2	0.7	1.15
1.6/1.0	0.8	0.56	0.6	1.1	1.4	1.1
8/-0-	3.46	4.77	4.0	4.8	4.7	6.6
8/5.0	6.2	4.9	5.2	5.3	4.3	8.6
8/10	4.5	3.7	6.9	5.9	6.3	4.9
20/-0-	21.9	13.5	21.5	20.4	20.7	19.4
20/12.5	23.1	11.9	12.2	19.7	21.8	20.6
20/25	22.8	12.4	13.5	22.1	27.8	26.2

Man: Composite. (From Biopharmaceutics Review; E.S.: Study 906-191; Volunteers, 12 (7M,5F), mean weight, 78 kg. Sex differences not mentioned; Study 906-254; Volunteers, 18 (9M,9F), ages 20-53. Sex differences not mentioned; Study 906-60; Volunteers, 6, Male, mean body weight, 69.2 Kg, ages 29-45).

Quinapril Treatment:

	2.5 mg	10 mg	20 mg	40 mg	80 mg	160 mg
<u>Quinapril:</u>						
C _{max} , (mcg/ml)	0.025	0.139	0.154	0.239	0.724	0.838
to			0.255	0.645	1.302	1.213
T _{max} , (hr)	.83	0.73	0.67	0.65	0.73	0.67
to			0.75			
AUC, (mcg/hr/ml)	0.047	0.192	0.132	0.318	1.038	0.964
to			0.202	0.803	1.393	1.244
<u>Quinaprilat:</u>						
C _{max} , (mcg/ml)	0.057	0.317	0.590	1.108	2.356	3.945
to			0.620	1.340	2.780	4.372
T _{max} , (hr)	1.7	1.7	1.5	1.55	1.63	1.67
to			1.6			2.00
AUC, (mcg/hr/ml)	0.203	1.062	2.000	3.529	8.819	15.410
to			2.062	4.810	9.617	16.825

PLASMA LEVELS AND SPECIES DIFFERENCES:

The above available spotty data reveals the following: (A) Quinapril dose levels around 3 mg/kg (or man's comparable clinically effective doses of 1 - 1.6 mg/kg) are pharmacologically effective in the dog, rat, monkey, and man. Dog, man, and monkey plasmas do not deesterify quinapril and these species develop quinaprilat plasma levels of 1 - 6 mcg/ml from these effective dosages. Rabbits develop negligible quinaprilat plasma levels after oral doses of 0.5 to 5 mg/kg of Quinapril, but these negligible quinaprilat plasma levels in pregnant rabbits cause death and abortion. Quinaprilat in rat plasma (rapid deesterifier) was not reported for this dose level; apparently doses above 80 mg/kg are required for measurable plasma levels. (B). Extensions of dosages in dogs (25 to 100 mg/kg) produced plasma levels of 31 to 100 mcg/ml. Another dog study (50 mg/kg quinapril with and without HCTZ) had quinaprilat levels about 1/2 that seen above with no apparent HCTZ combination induced differences in the quinaprilat levels. Lower dose levels (0, 1.6, 8, and 20 mg/kg) exhibited no significant differences in clinical signs. Significant decreases in group mean serum potassium values and in group mean serum chloride values during the first week were seen only in animals receiving 25 mg/kg HCTZ. Increased hypertrophy of the juxtaglomerular apparatus was not seen with 25 mg/kg HCTZ alone, and in only one of 6 dogs in the group receiving 8 mg/kg of quinapril. Dog groups receiving 20 mg/kg of quinapril, or any combination of 8 and 20 mg/kg of quinapril with HCTZ, exhibited at least 5/6 dogs in each group with increased hypertrophy of the juxtaglomerular apparatus, thus indicating that the addition of HCTZ potentiates an increase of hypertrophy of the juxtaglomerular apparatus. (C). Pregnant rats at a dose of 150 mg/kg, produce quinaprilat levels of 31 to 63 mcg/ml, while wistar rats, treated with HCTZ, produce only 1/3 those plasma levels of quinaprilat. These HCTZ treated wistar rats, at 88 mg/kg quinapril, reveals a 5 fold male to female sex ratio difference in plasma levels; a sex difference which decreases with increasing dose to an equal response at 888 mg/kg. (D). Contrarily, in dogs, HCTZ addition causes the female to exhibit a higher (2 X) quinaprilat plasma level in comparison to the male. Whether the human sex response might be more like the rat or the dog is not known.

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(E). The complicating factors of differing study design formats, dose level differences, sex, strain, and pregnancy differences, and combination with HCTZ cannot be separated by these studies. (F). Rats and dogs have negligible postdose Quinaprilat plasma levels after 18 to 22 hours.

COMPARISONS BETWEEN GROUPS TREATED WITH QUINAPRIL AND QUINAPRIL/HCTZ:
(Caution is indicated in any comparison of nonconcurrent disparate studies).

HCTZ QUINAPRIL COMBINATION MLD, mouse, oral, 14 day observation.

Condition / Dose, mg/kg:	Q/HCTZ	Q content	Quinapril
MLD	1850	1110	1450 - 2150

This data indicates that the combination may increase toxicity in the mouse.

HCTZ QUINAPRIL COMBINATION MLD, rat, oral, 14 day observation.

Condition	Dose, mg/kg:	Q/HCTZ	Q content	Quinapril
Asymptomatic.		1710 - 2155	992 - 1250	M, 1000 F, Less/1000.
Non lethal.		2715	1575	
MLD		8000	4800	M, 4280 F, 3541

This data indicates that the toxicity of HCTZ combination does not change or may even decrease acute toxicity in the rat.

RAT, 13-WEEK CHRONIC STUDIES: There were 2 combination drug studies, one, Quinapril, 0, 50, 100, 250, & 500 mg/kg/day, equivalent to the Quinapril study but with an extra (100) dose level, and one, a comparative study at much lower dosages of only 1.6, 8, and 20 mg/kg/day Quinapril content, 10/sex/group. The apparent intubation deaths occur in a dose related manner.

Q/HCTZ (Q content)	Dose, Mg/Kg.	Quinapril
2.8 (1.6)		1.6
13.8 (8.0)		8.0
(M) Body Wt. Dec. = 9%		(M) Body Weight Decrement = 2%
(M&F) Inc. Rel. Kdny. Wt.		
18.8 (8.0)		
(M) Body Wt. Dec. = 7%		
(M) Red.: Heart Weight.		
34.5 (20.0)		20.0
(M) Body Wt. Dec. = 12%		(M) Body Weight Decrement = 13%
(M) Red.: Heart Wt.		(M&F) Hypert. Aff.Art./JGA
(F) Inc.: Rel. Kdny Wt.		
(M&F) Hypert. Aff.Art./JGA		

47.0 (20.0)

(M) Body Wt. Dec. = 15%
 (M&F) Inc. BUN.
 (M&F) Inc. Rel. Kdny Wt.
 (M&F) Hypert. Aff.Art./JGA

86 (50)

(M [Dose Rel.], &F) Mod. Inc.: BUN. (M&F) Sl. Inc.: CPK, LDH; (M), BUN.
 (F) Reduction: RBC, Hct, Hgb. Slight Decrease: (M) RBC; (F) Hct.
 Inc. Wts.: (M&F) Kidney; (M) Liver. (M&F) Reduced Heart Weights.

50

172 (100)

Mortality, 1/20 (F), 1/20 (M).
 (All 18 deaths intubation deaths)
 Inc.: (M&F) BUN. Red.: (F) RBC, Hct, Hgb.
 Inc. Wts.: (M&F) Liver & Kidney.

431 (250)

Mortality: 2/20 (F), 3/20 (M).
 [Moribund Sac.: (M) Inc. BUN,
 creatinine, glucose, & K;
 Dec. Na., Neutrophilia.]
 (M&F) Increased: BUN.
 (F) Reduced: RBC, Hct; Hgb.
 (M&F) Inc.: Liver & Kidney Wts.

250

Mortality: 3/12 (F), 2/12 (M).
 (M&F) Transient salivation, rales, and
 rhinorrhea.
 (M&F) Sl. Inc.: CPK, LDH, and BUN.
 (M&F) Red.: RBC, Hct; Inc.: Platelets.
 (M&F) Reduced Heart Weights.
 (F) Increased Liver Weights.

862 (500)

Mortality, 3/20 (F); 5/20 (M).
 (M&F) Increased: BUN.
 (M&F) Reduced: RBC, Hct, Hgb.
 (M) Increased: K.
 (M&F) Inc.: Liver & Kidney Wts.

500

Mortality, 2/12 (F); 6/12 (M).
 (M&F) Transient salivation, rhinorrhea.
 (F) Inc.: CPK, LDH, and (M&F) BUN.
 (M&F) Red.: RBC, Hct; (M): HgGb.
 (M&F) Inc.: Platelets; (F): MCHC.
 (M&F) Red.: Heart Weights.
 (F) Inc.: Liver and Kidney Weights.
 (M) Inc.: Alveol. Macrophage Prolifer.

Pathological findings:

At 500 Mg/Kg, 2 M & 1 F, exhibited mild focal to multifocal gastric parietal/chief cell necrosis. Focal gastric erosion and focal gastric erosion and fibroplasia was seen in one Term. Sac. (M). Non-dose related (M&F) Significant renal lesions consisted of basophilic cortical tubules, interstitial fibrosis and mononuclear cell infiltrates.

(M&F) Hypertrophy of afferent arterioles of th JG apparatus was observed in all treatment groups.

Hypertrophy of interlobular arterioles occurred in the 250 & 500 Mg/Kg groups.

At 500 & 250 Mg/Kg: (M) glandular gastric erosions and/or ulcers, and (MF) Submucosal infiltr. of granulocytes; Autolysis of Sm. & Lg. Intestine; Kidny Interstitial mononuclear infiltration; Nephrocalcinosis; and (M) Pelvic dilation. At 500 Mg/Kg, (M): Interstitial Nephritis.

Mild increases (26 to 40%) in BUN seen in females receiving 34.5 (20, Q) mg/kg of the mixture, and for both males and females receiving 47 (20, Q) mg/kg of the mixture was not seen with the ingredients separately or seen only in males at higher dosages with quinapril alone, and was greater with the increased amount of HCTZ, thus can be considered a combination effect. Male average heart weights were slightly, but significantly lighter than controls in only two groups; those receiving 20Q/12H and 8Q/10H mg/kg of the combinations. These effects are at doses lower than those seen in previous quinapril studies and in the carcinogenicity studies which did not exhibit a decrease in heart weights after 10, 50, and 100 mg/kg. Female groups (which in previous quinapril studies such as the 13 week study with dosages of 50, 250, and 500 mg/kg all exhibited heart weights 18 to 22% less than controls) did not exhibit any significant differences in the heart weights between control and treated. Kidney weights were slightly, but significantly higher in 2 male and 2 female groups, with the largest change in both sexes in the highest dose (47 mg/kg) combination treated rats. Combination dosages of 34.5 and 47 mg/kg (both 20 mg/kg quinapril) exhibited hypertrophy of the afferent arteriol and hypertrophy of the juxtaglomerular apparatus; with dosages containing 8 mg/kg quinapril not exhibiting this characteristic. It can be seen that heart weight changes may be reduced by the combination, but that kidney toxicities are increased by combination with HCTZ in the rat.

RISING-DOSE STUDIES: Beagle dogs, 1/sex, given Quinapril (Escalating dose schedule from 50 to 400 mg/kg/day/for 13 days) or Q/HCTZ (escalating dose schedule with Quinapril moieties from 25 to 400 mg/kg/day/for 17-days):

Q/HCTZ (Q content)	(Dose: Mg/kg)	Quinapril	
43.1 (25)		0	
86.2 (50)		50	
172.4 (100)	Occult Blood, Urinary (M). Dec.: RBC, Hgb, Hct (F).	100	
258.6 (150)	Emesis, (M).	150	Emesis, (M).
344.8 (200)		200	Emesis, (F).
517.2 (300)	Emesis, (F). (M) Increase in Serum BUN and Creatinine; Urinary glucose.	300	Salivation, (F).
689.6 (400)	Depression, (M). Blood Pressure not mentioned. (M): Prolongation of QT interval. (M&F): Increase in BUN and Creatinine. (M): Dec.: Na, K, & Cl.	400	Dec. BP: (F), 157/90 to 75/39. (M), 181/110 to 129/73. Mod. elev. serum phosphatase, Creatine, & BUN; Sl. dec. in Na, K, & Cl. Granular or hyaline casts in Urine. Mild neutrophilia, day 14.
	(M&F): Pathologic findings: multifocal pyloric erosion and congestion.		(M&F): Pathologic findings consisted of minor gastric erosions and/or ulcers.
	(M): Multi-focal gastric ulceration and hemorrhage.		(F): Pancreatic acinar degranulation, renal tubular dilation and interstitial lymphocytic infiltration in the renal pelvis.
	(M): Marked diffuse degeneration of epithelial cells lining proximal convoluted tubules.		

Weight (M): 0.9 Kg
loss: (F): 1.4 Kg

(M): 0.7 Kg
(F): 1.3 Kg

It can be seen that with dogs in a rising dose study, the combination may be slightly more toxic than is Quinapril alone; however, studies are not exactly comparable because of the prefixed 3 days with a lower dose of combination.

DOG, 2-WEEK SUB-CHRONIC STUDIES: Different Quinapril dosages were used in the Quinapril (0, 25, 125, and 250 [div.] mg/kg/day) and Quinapril/HCTZ (Quinapril Content = 0, 25, 75, and 150 mg/kg/day) studies.

Q/HCTZ (Q content) (Dose: Mg/kg) Quinapril

43.1 (25)

(F) Renal tubular dilation and/or hemorrhage and interstitial mononuclear cell infiltration.

(M&F) Liver Parenchymal mononuclear cell infiltration.

25

BP variable, not dose related.
Bone marrow: Sl. Inc. M/E ratio.
(Dec. Erythrocytic, Inc. Band Neut.)

129.3 (75)

(F) Increase: Serum BUN and (M&F), LDH.

(F) Liver Parenchymal mononuclear cell infiltration.

125

Emesis, (1/2 F)

(M&F) BP variable, not dose related.
(M&F) Bone marrow: Inc. M/E ratio.
(1/2 F, 1/2 M), Stomach: Small focal mucosal erosions, hemorrhage and minor foci of inflammation of the lamina propria.

258.6 (150) Emesis, (M).

(M) Increase in Serum BUN and (M&F) LDH.

(M&F) Renal tubular dilation and/or hemorrhage and interstitial mononuclear cell infiltration.

(F) Increased Heart Weight Ratio.

(M&F) Inc. Kidney Wt. Ratio.

(M&F) Kidney Tubular Dilation.

(M&F) Liver Parenchymal and Gall Bladder mononuclear cell infiltration.

250 (divided): Emesis (1/2 F, 1/2 M).

BP variable, not dose related.

Bone marrow: Inc. M/E ratio.

Significant histopathology findings were limited to the kidney with tubular dilation and interstitial mononuclear cell infiltrate and hemorrhage. Apparently kidney toxicities are increased in a 2-week study by HCTZ combination in the dog.

DOG, 13-WEEK CHRONIC STUDIES: Different dosages were used in the Quinapril (0, 25, 125, and 250 [div.] mg/kg/day) and the two Quinapril/HCTZ studies (25/43.1, 75/129.3, and 150/258.6 mg/kg/day in one; and with Quinapril/and/or CI-570 (HCTZ), 0, 0-/25, 1.6/-0-, 1.6/1, 8/-0-, 8/5, 8/10, 20/-0-, 20/12.5, and 20/25 mg/kg/day, 3 sex/dose, in the other, 7 days/week/13 weeks).

Q/HCTZ (Q content)	Dose, Mg/Kg.	Quinapril
0 (0)		0 (2 F) Kidney cortical tubular basophilia.
2.8 (1.6)		1.6 (2 M) Kidney cortical tubular basophilia. (1 M) Kdny cort. tub. basophilia.
13.8 (8)		8.0 (1M, 1F) Kidney cortical tubular basophilia. Hypertrophy of JG cells (2/3 M, 3/3 F). Hypertrophy of JG cells (1/3 M).
18.8 (8)		(2M, 1F) Kidney cortical tubular basophilia. Hypertrophy of JG cells (2/3 M, 3/3 F).
34.5 (20)		20.0 (M) Dec. plasma K, 1st week. (3M, 2F) Kidney changes included cortical tubular basophilia, dilation, fibroplasia, interstitial inflammatory cell infiltrate and edema. Hypertrophy of JG cells (3/3 M, 2/3 F). (1M, 3F) Similar changes except interstitial edema. Hypertrophy of JG cells (2/3 M, 3/3 F).
47.0 (20)		(M&F) Dec. plasma K, 1st week. (3M, 3F) Kidney changes included cortical tubular basophilia, dilation, fibroplasia, interstitial inflammatory cell infiltrate and edema. Hypertrophy of JG cells (3/3 M, 3/3 F).
---	(0) 25 mg/kg, HCTZ:	(M&F) Dec. plasma K and Cl.

Q/HCTZ (Q content) (Dose: Mg/kg) Quinapril

-0- No control moribund sacrifices. :-0- Emesis, (1/3 M, 1/3 F).
 Terminal Sacrifice: Sporadic Emesis (M&F). 1/6, granulation of JG cells.

43.1 (25)

:25

Moribund Sacrifice (1/3 F):

: No treatment moribund sacrifices.

(Mor. Sac. all dose levels: Clinical

:Anorexia, 1/3 F; Emesis, (2/3 M).

signs included dehydration,

:(M&F) BP variable, not dose related.

depression, inappetence, emesis,

:(F) Bone marrow: Inc. M/E ratio.

weakness, emaciation and diarrhea; a

:(F) Dec. Heart Weights; (M) Decreased

12 - 28 % body weight loss; oral

: Heart, Spleen, and Kidney Wts.

mucous membrane ulceration; Markedly

:(M&F) 3/6, granulation of JG cells.

increased BUN, Inc. creatinine; Dec.

Na and Cl; Dec. [4/7] lymphocyte count);

Decrease in Urinary pH; and proteinuria).

T S: Sporadic Emesis (M&F), oral ulcerations (F).

(1 M, 1 F): Slight Increase in Serum BUN;

(F) Dec. Heart Wt.; (1/3 M) Inc. kidney Wt.

(M&F) Mild tubular vacuolation and interstitial edema.

Hypertrophy of JG cells (2/2 F, 1/3 M).

(F) Renal tubular dilation and/or

hemorrhage and interstitial

129.3 (75) (1/3 M, 2/3 F): Moribund Sac.

T-S (2/3 M, 1/3 F): Sporadic Emesis (M&F),

Oral ulcerations (F).

(M&F) Increase in Serum BUN and Creatinine.

(F) Reduced: RBC; Hct, HgGb.

(M&F) Dec. Heart Wt., (2/2 M) Inc. Kidney Wt.

(M&F) Renal tubular dilatation, edema, fibroplasia,

interstitial mononuclear cell infiltration,

tubular atrophy and regeneration.

Hypertrophy of JG cells (1/1 F, 1/2 M).

125

Anorexia, 2/3 F

Emesis, (1/3 F, 2/3 M)

(M&F) BP variable, not dose related.

(1/3 F), Sl. Inc. BUN.

(M), Sl. Inc. Cholesterol.

(1/3 M [Gross liver lesions]),

progressive elevation of Alkaline

PO₄, Aspartate aminotransferase,

and alanine aminotransferase.

(F) Bone marrow: Inc. M/E ratio.

(1/3 F, 1/3 M), small gastric ulcer.

(F) Dec. Heart Weights, (M) Dec.

Heart, Spleen, and Kidney Wts.

:(M&F) 6/6, granulation of JG cells:

(1/3 F, 1/3 M), focal erosions,

gastric mucosa.

258.6 (150) (Survivors dosed only six weeks; 7 weeks recovery).

Moribund Sac. (1/3 M, 2/3 F).

Terminal Sac.: (2/3 M, 1/3 F): (M) Wt. loss, 19% and 11%.

(M&F) Sporadic Emesis; Oral ulcerations (M).

(M&F) Inc. in Serum BUN and Creatinine; (F) Dec. Na.

(M&F) Red.: RBC, Hct, HgGb. (M&F) Dec. Heart Wt.

(1 M): Multifocal gastric mucosal erosions.

(M&F) Renal tubular dilatation, edema, fibroplasia,

interstitial mononuclear cell infiltration,

tubular atrophy and regeneration.

(M&F) Inc. Kidney Wt. Ratio.

(2/2 M) Hypertrophy of JG cells.

(2/2-M) Atrophy of thymic cortex and medulla.

250 (divided)

Anorexia, 1/3 F

Emesis (2/3 F, 1/3 M).

BP variable, not dose related.

(2/3 F, 1/3 M), Sl. Inc. BUN.

(M), Sl. Inc. Cholesterol.

(M&F) Red.: RBC, Hct, HgGb.

(F) Bone marrow: Inc. M/E ratio.

(M) gastric ulcer.

(F) Dec. heart Wts; Inc. Kidney Wts.

(M) Dec. Heart, Spleen, & Kidney Wts.

(M&F) 6/6, granulation of JG cells.

(M&F) hyperplasia/hypertrophy JG.

In the original Quinapril study (5 years earlier), there were no deaths, few clinical signs (sporadic episodes of emesis, anorexia, and changes in fecal consistency), and all animals but one gained weight during the 13 weeks on experiment. Heart weights decreased slightly with increasing dose in both sexes, as did spleen and kidney weights in males; however, spleen and kidney weights in females increased. Clinical laboratory findings included increases in BUN levels seen at Week 8 which returned to normal range by Week 13. Decreased RBC, Hgb, and Hct values were noted in animals of both sexes given 250 mg/kg. One male in the 125 mg/kg dose group had progressive increases in aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase. Bone marrow changes consisted of increased M/E ratios. Gastric ulcers were observed in one male given 250 mg/kg and in one male and one female given 125 mg/kg. Microscopically, one male given 125 mg/kg also had moderate bile duct hyperplasia with minimal to mild periportal fatty change and single hepatocyte necrosis. Other histopathologic changes consisted of a dose related increased incidence of granularity of JG cells (6/6, 6/6, 3/6, and 1/6; H-D to controls, respectively) and, in animals given 250 mg/kg, a mild hyperplasia and hypertrophy of JG cells.

In contrast to the minor effects of Quinapril alone, the Q/HCTZ combination groups treated generally with reduced Quinapril content dosages, had 7 moribund sacrifices. Moribund signs prior to sacrifice included dehydration, depression, inappetence, emesis, weakness, emaciation, diarrhea, a 12 - 28% body weight loss, and ulceration of the oral mucous membranes. Animals surviving to termination exhibited sporadic emesis, oral ulcerations, and in the H-D males, body weight losses of 11-19% (in spite of 7 weeks off the drug treatment), but, except for H-D males, treatment group body weight losses were

not consistently different from the controls. Moribund animals had marked increases in BUN levels, increased creatinine levels, and decreased sodium and chloride levels. Animals surviving to termination exhibited sporadic elevations of BUN and creatinine and decreased sodium, but other clinical biochemical, hematological, urinalyses and organ weight findings were unremarkable. In all moribund sacrifice animals, significant gross and microscopic oral, gastric, and kidney lesions were observed; renal tubular degenerative changes characterized by mild to marked dilatation of the cortical tubules with individual cell necrosis, cytoplasmic vacuolation, and diffuse interstitial edema. Terminal sacrifice animals were generally unremarkable except for lighter heart weights, gastric erosions in one H-D male, and renal changes (tubular dilatation, edema, fibroplasia, and interstitial mononuclear cell infiltrates) in all H- and M-D animals and hypertrophy of the JG cells in all treatment groups (no no-effect dose level).

Results of the low dose comparative study indicate that statistically significant treatment related changes in systolic, mean arterial, or diastolic blood pressures were rarely seen during study, and in general the changes were minor. There were no significant differences in clinical signs, food and water consumption, body weight changes, ophthalmic findings, hematology, electrocardiograms and heart rates; organ weights or gross pathologies. Significant decreases in group mean serum potassium values were seen only in animals receiving 25 mg/kg HCTZ, and in group mean serum chloride values during the first week in animals receiving 25 mg/kg HCTZ. Increased hypertrophy of the juxtaglomerular Apparatus was not seen with 25 mg/kg HCTZ alone, and in only one of 6 dogs in the group receiving 8 mg/kg of quinapril. Dog groups receiving 20 mg/kg of quinapril, or any combination of 8 and 20 mg/kg of quinapril with HCTZ, exhibited at least 5/6 dogs with increased hypertrophy of the juxtaglomerular apparatus, thus indicating that the addition of HCTZ potentiates an increased hypertrophy of the JG apparatus. The lack of moribund animals in the Quinapril groups inspite of consistently higher doses provides indications of increased toxicity of the compound; however, the lack of moribund animals with Quinapril at 250 mg/kg vs moribund animals at all doses from 25 mg/kg and above with the compound doesn't necessarily mean that the combination toxicity is increased ten fold.

PREVIOUS QUINAPRIL TERATOLOGY STUDIES: RATS: 1. Exploratory, Quinapril; RR 745-00505; 5/group; Doses: 0, 100, 200, 400, 600, & 800 mg/kg/day/gestation days 6 - 15. 2. Quinapril; RR 745-00541; 20/group; Doses: 0, 0, 50, 150, 300 mg/kg/day/days 6 thru 15, gestation.

Accuretic 1. Exploratory oral dose range finding study. Dose levels (Quinapril content): 0, 3.6, 7.2, 36.1, 108.3, 216.7 mg/kg, days 6 thru 15, these are lower Quinapril content dosages than used for Quinapril alone.

Accuretic 2. Dose Levels: 0/0 (UTC), 0/0 (VC), 5/3.1 (= 8.1), 50/31.3 (81.3), 150/93.8 (243.8), 0/93.8, and 150/0 mg/kg/day, gestation days 6 thru 15.

Dose, Mg/Kg. Fetuses/Litters = F/L. N = Number of rats. Controls: UNTR, VEHI.
 Q/HCTZ (Q content) (N) [HCTZ content] Quinapril

0 (-0-) 5/G [-0-] Q
 Dam, Hair loss: 1/5.
 Stunted Embryos: F/L, 2/1.
 Focal hematoma: F/L, 4/2.

0 (-0-) 20/G [-0-] (UNTR) Q (UNTR) 20/G
 Stunted embryos: F/L, 9/6.
 1 - malformed, fused and agenesis of skull bones, malformed vertebrae. Q (VEHI) 20/G
 1 - malformed and fused vertebrae.
 Kidney: Dilated pelvis 1/1

0 (-0-) 20/G [-0-] (Vehicle Control).
 Hair loss 2/20.
 Malpositioned blood vessels 1/1.
 Kidney: Dilated pelvis 1/1.

8.1 (3.6) 5/G [4.5]
 Small thymus: 2/5. Wt. Decrement:
 Day 15 = 4%; day 21 = 4%.
 Stunted embryos: F/L, 1/1.
 Focal hematoma: F/L, 5/3.

8.1 (5.0) 20/G [3.1]
 Mortality: 1/20. Non gravid 1/20.
 Stunted embryos: F/L, 5/3.
 1 fetus had absent facial papilla, agnathia, microstomia, aglossia, and absent nare.
 Kidney: Dilated pelvis, 6/4.

16.3 (7.2) 5/G [9.0]
 Hair loss: 1/5.
 Stunted embryos: F/L, 1/1.
 Focal hematoma: F/L, 24/3.

81.3 (36.1) 5/G [45.1]
 Small thymus: 1/5
 Wt. Dec: day 15 = 4%; day 21 = 4%.
 Stunted embryos: F/L, 1/1.
 Focal hematoma: F/L, 5/3.

81.3 (50.0) 20/G [31.3] 50 20/G Stunted: F/L, 1/1.
 Hair loss, 2/20
 Stunted embryos: Fetuses/litters, 22/7.
 Kidney: Dilated pelvis, F/L, 5/3.

243.8 (108.3) 5/G [135.4]
 Hair loss: 2/5. Small thymus: 3/5
 Wt. Dec., day 15 = 10%; day 21 = 6%.
 Foetal Wts. 9% below controls
 Stunted embryos: Fetuses/litters, 2/1.
 Malpositioned blood vessels F/L, 3/2.
 Kidney: Dilated pelvis, F/L, 5/3.
 Focal hematoma: F/L, 16/3.

243.8 (150.0) 20/G [93.8]
 Mortality: 2/20. Hair loss: 8/20.
 Non gravid: 3/20. 4 malformed fetuses:
 (1) micrognathia & multiple malformations
 of skull, vertebrae, & ribs.
 (2) agenesis of presacral vertebrae
 & ribs. (1) malformed skull bones.
 Stunted embryos: Fetuses/litters, 25/8.
 Kidney: Dilated pelvis, F/L, 3/2.

487.5 (216.7) 5/G [270.8]
 Mortality: none. Hair loss, 1/5.
 Small thymus: 3/5.
 Wt. Dec.: day 15 = 7%; day 21 = 3%.
 Foetal Wts. 14% below controls.
 Stunted embryos: Fetuses/Litters, 7/3.
 Focal hematoma: F/L, 11/4.

0 (-0-) 20/G [93.8]
 Hair loss: 1/20.
 4 malformed: 1- malformed and
 fused skull bones and sternbrae,
 1 - fused skull bones and
 agenesis/branched ribs. 1 - fused
 skull bones, and 1 - agenesis of
 presacral vertebrae.
 Stunted embryos: F/L, 8/6.
 Kidney: Dilated pelvis F/L, 5/4.

100 5/G
 Hydronephrosis/hydroureter: 3%.

(150.0) 20/G [-0-]
 Mortality: 1/20. Hair loss: 2/20.
 Non gravid: 1/20.

Stunted embryos: F/L, 12/8
 Malpositioned blood vessels: 2/2.
 Kidney: Dilated pelvis, F/L 1/1.

150 20/G
 Mortality: 1/20. Stunted: F/L, 2/2.

(200) 5/G Stunted: 1/1.

(300) 20/G Mortality: 1/20.
 Stunted: F/L, 14/1.

(400) 5/G
 Hydronephrosis/hydroureter: 5%.
 Stunted: F/L, 6/2.

(600) 5/G Mortality: 1/5.
 One dam with single pup.
 Hydronephrosis/hydroureter: 23%.

(800) 5/G Mortality: 2/5.
 Hydronephrosis/hydroureter: 14%.
 Stunted: F/L, 1/1.

With Quinapril, food consumption was similar for the dosing groups. Hydroureter was seen at 600 mg/kg in 3 of 4 litters which exhibited 9 cases of hydroureter and in 1 of 4 litters, a hydronephrosis; while at 800 mg/kg, 2 of 3 litters exhibited 4 cases of hydronephrosis and 1 of 3 litters had hydroureter in 2 pups. In general, no other gross, visceral, or skeletal malformations were apparent among the offspring. With doses of 0, 0, 50, 150, 300 mg/kg/day/days 6 thru 15, gestation, 20/group, maternal toxicity was evidenced as reduced body-weight gain during the dosing period (days 6-15) with the greatest reduction in dams given 150 or 300 mg/kg. However, post dosing compensation recovery reduced difference. Gestational period daily food intake was reduced less than 10% in treated groups. Litter and fetal parameters were unaffected, and fetal examination revealed no drug-related external abnormalities. No gross visceral or skeletal abnormalities were observed.

Accuretic, exploratory oral dose range finding study, (Q. content): 0, 3.6, 7.2, 36.1, 108.3, 216.7 mg/kg, days 6 thru 15 (these are lower Quinapril dosages than used for Quinapril alone). Five animals had hair loss during the study; one given 216 mg/kg, two given 108, one, 7 mg/kg, and one vehicle control. No other clinical signs were observed. Maximum weight decrement of maternal body weight (6%) with respect to controls was seen in the group receiving 108 mg/kg. Induced suppression of total food intake (less than 1%) was most severe at 3 mg/kg. Small thyroids were reported in the treated animal groups at an incidence of 0, 40%, 0, 20%, 60%, and 60%, respectively, for control thru highest dose, respectively. This had not been reported for tests on Quinapril alone. All animals were pregnant and no animals delivered early or had total litter resorption. Mean numbers of corpora lutea, implantation sites, and live, dead, or resorbed fetuses were comparable across groups. Pre- and Post-implantation losses were variable across groups and no treatment/dose relationships were apparent. Developmental toxicity (reduced mean fetal weights [not seen with Q alone]) also occurred at these doses. CI-955, RR-745-01647; Dosage (Q/HCTZ): 0/0, 0/0, 5/3.1 (8.1), 50/31.3 (81.3), 150/93.8 (243.8), -0-/93.8, and 150/-0- mg/kg/day, gestation days 5 thru 15. Sponsor contends that treated group's mortality might not be drug related. Drug associated findings: Reduced fecal output was seen in a few CI-955 treated animals, but no drug related clinical signs were observed. Maternal toxicity was evidenced as reduced body-weight-gain during the dosing period (days 6-15), with the greatest reduction in dams given 81.3 and 243.8 mg/kg; however, post dosing compensation recovery removed differences of significance. Gestational period daily food intake was reduced significantly in treated groups, except for the H-D quinapril (not dose related). Litter and fetal parameters were unaffected, and fetal examination revealed no drug-related external abnormalities. No gross visceral or skeletal abnormalities were observed; however, there was an increased incidence of kidney pelvis dilation notations in all groups of HCTZ treated pups. Because organ weights were not reported for this group, evaluation of the smaller thyroids seen in the above combination study cannot be made. Q/HCTZ Combination studies were performed at lower Quinapril content dosages than were used for Quinapril alone and direct comparison of results cannot be made; however, the smaller thyroids and the kidney pelvis dilation reported in the lower dosed combination studies but not in the Quinapril studies emphasizes the greater toxicity of the combination product.

TERATOGENICITY STUDIES IN RABBITS

Exploratory dose range-finding studies in pregnant rabbits, gestation days 6 to 18, 5/dose level, with doses of 10, 15, 25, 50, 100, 200 or 400 mg/kg/day, repeated at doses of 0, 1, 2, 4, 6, or 8 mg/kg/day and a 14/dose level teratology study with doses of 0, 0.5, 1.0, and 1.5 mg/kg/day of quinapril.

Dose, Mg/Kg. F/L = Fetuses/Litters. N = No. rabbits. Controls: UNTR, VEHI.

Q/HCTZ (Q content)	(N)	[HCTZ content]	.Quinapril
0 (-0-)	5/G	[-0-]	
Nongravid: 1/5.			
Does with viable fetuses: 4/5.			
Viable Fetuses/Doe: 4.3.			
Resorptions: 0.			
Post implantation loss: 0.			
0 (-0-)	20/G	[-0-] (UNTR)	
Nongravid: 1/20.			
Does with viable fetuses: 19/20.			
Viable Fetuses/Doe: 6.6.			
Resorptions/Doe: 0.6.			
Post implantation loss: 7.2 %.			
0 (-0-)	20/G	[-0-] (VEHI)	Q (VEHI) -5/G
Resorptions, total: 1.			
Nongravid: 0.			
Does with viable fetuses: 19/20.			
Viable Fetuses/Doe: 6.9.			
Resorptions/Doe: 0.4.			
Post implantation loss: 11.2 %.			
0.08 (0.05)	20/G	[0.03]	Q (VEHI) -14/G
Nongravid: 5.			
Does with viable fetuses: 15/20.			
Viable Fetuses/Doe: 5.5.			
Resorptions/Doe: 0.2.			
Post implantation loss: 2.7 %.			
Skull bones fused: F/L, 3/2.			
0.16 (0.1)	5/G	[0.06]	
Nongravid: 1/5.			
Does with viable fetuses: 4/5.			
Viable Fetuses/Doe: 6.0.			
Resorptions/Doe: 0.5.			
Post-implantation loss: 6.3 %.			

0.16 (0.1) 20/G [0.06]
 Nongravid: 2/20.
 Does with viable fetuses: 18/20.
 Viable Fetuses/Doe: 6.8.
 Resorptions/Doe: 0.6.
 Post implantation loss: 7.9 %.
 Kidney, dilated pelvis: F/L, 2/2.
 Skull bones fused: F/L, 1/1.

0 (0.5) 20/G [-0-]
 Nongravid: 1/20. Abortion: 1/20.
 Does with viable fetuses: 18/20.
 Viable Fetuses/Doe: 6.7.
 Resorptions/Doe: 0.3.
 Post implantation loss: 6.5 %.
 Kidney, dilated pelvis: F/L, 1/1.

0 (-0-) 20/G [0.31]
 Nongravid: 3/20.
 Does with viable fetuses: 17/20.
 Viable Fetuses/Doe: 7.6.
 Resorptions/Doe: 0.2.
 Post implantation loss: 2.5 %.
 Kidney, dilated pelvis: F/L, 1/1.
 Ureter, dilated: F/L, 1/1.
 Skull bones fused: F/L, 1/1.

0.81 (0.5) 20/G [0.31]
 Nongravid: 2/20. Abortion: 1/20.
 Does with viable fetuses: 17/20.
 Viable Fetuses/Doe: 8.1.
 Resorptions/Doe: 0.3.
 Post implantation loss: 3.1 %.
 Skull bones fused: F/L, 2/1.

1.63 (1.0) 5/G [0.63]
 Mortality: 2/5. 1 Abortion. NG: 0.
 Does with viable fetuses: 2/5.
 Viable Fetuses/Doe: 8.0.
 Resorptions/Doe: 1.0.
 Post implantation loss: 8.3 %.

2.44 (1.5) 5/G [0.9]
 Mortality: 1/5. 3 Abortions. NG: 1.
 Does with viable fetuses: 1/5.
 Viable Fetuses/Doe: 6.0.
 Resorptions/Doe: 3.0.
 Post implantation loss: 33.3 %.

0.5 14/G
 Mortality: 1 (aborted)/14.
 Does with Resorptions only: 2/5.
 Does with viable fetuses: 13/14.
 Viable Fetuses/Doe: 7.0.
 2 Hydrocephaly, internal (1 Domed head). 1 Conjoined twins.
 1 Cleft palate & coartc. aortic A.

1.0 5/G
 Nongravid (NG): 1/5
 Does with viable fetuses: 4/5.
 Viable Fetuses/Doe: 6.8.

1.0 14/G
 Mortality: 4 (1 NG)/14. 1 Abortion.
 Does with Resorptions only: 2/14.
 Does with viable fetuses: 7/14.
 Viable Fetuses/Doe: 5.4.
 1 Bulbous Aortic arch, IV septal D.
 1 Cleft palate

1.5 14/G
 Mortality: 2/14. Nongravid: 2/14.
 Does with Resorptions only: 1/14.
 Does with viable fetuses: 9/14.
 Viable Fetuses/Doe: 6.2.
 1 Hydrocephaly, Dome shaped head.

- . 2.0 5/G
- . Wt. Loss
- . Nongravid: 1/5
- . Does with Resorptions only: 2/5.
- . Does with viable fetuses: 2/5.
- . Viable Fetuses/Doe: 3.8.

- . 4.0 5/G
- . Mortality: 2 (1 had aborted)/5.
- . Wt. Loss
- . Does with Resorptions only: 1/5.
- . Does with viable fetuses: 2/5.
- . Viable Fetuses/Doe: 6.3.

- . 6.0 5/G
- . Mortality: 2 (1 aborted)/5.
- . Does with viable fetuses: 2/5.
- . Nongravid: 1/5
- . Viable Fetuses/Doe: 3.0.

- . 8.0 5/G
- . Mortality: 3/5; 1 abortion.
- . Does with viable fetuses: 1/5.
- . Viable Fetuses/Doe: 8.0.

- . 10 5/G
- . Mortality: 4/5.
- . Does with viable fetuses: 1.
- . Viable Fetuses/Doe: 3.0.

- . 15 5/G
- . Mortality: 3/5; 2 abortions.

- . 25 5/G
- . Mortality: 5 (1 aborted)/5.

- . 50 5/G
- . Mortality: 4/5; 1 abortion.
- . Does with total resorptions: 2.

- . 100 5/G
- . Mortality: 4 (1 aborted)/5.
- . Does with viable fetuses: 1.
- . Viable Fetuses/Doe: 1.

- . 200 5/G
- . Mortality: 5 (1 aborted)/5.

- . 400 7/G
- . Mortality: 7/7.

In the original Quinapril teratology dose range-finding study in pregnant rabbits at doses of 10, 15, 25, 50, 100, 200 and 400 mg/kg/day/pregnancy days 6 - 18, mortality totaled 31/37 rabbits on the study and hyperpnea and anorexia were frequently associated with treatment at all dose levels. Additional clinical signs included abortion, rales, dyspnea, diarrhea/soft stool, urine scald, bloody/mucoid vaginal discharge, weakness, lethargy, emaciation, dehydration, prostration, hypokinesia, nasal discharge, convulsion, hair loss and reduced body weight gain. Gross pathologies in the does included multifocal mucosal ulceration or erosion of the stomach at all dose levels. Abnormal findings among preterm fetuses included ecchymotic subcutaneous hemorrhages, generalized hemorrhage, omphalocele, and open eyes. Only two of the four pregnant does that survived to term sacrifice had viable normal fetuses. A repeat of that study, with quinapril on gestation Days 6 through 18 at daily doses of 0, 1, 2, 4, 6, or 8 mg/kg/day proved still lethal at the higher levels to both the dam and the fetus without clinical signs or gross pathologic observations associated with treatment. Mean maternal body weight and body weight change in the does given 1 mg/kg were comparable to the control group. Doses of 0, 0.5, 1.0, and 1.5 mg/kg/day on gestation Days 6 through 18 were still lethal at all dose levels, without control group deaths, but no significant clinical signs of toxicity were apparent in animals that died and causes of death were not determined. The doe given 0.5 mg/kg that died on Day 27 aborted one to two days prior to death; another abortion occurred on Day 21 in a doe given 1.0 mg/kg. Mean postimplantation loss was increased in the 1.0 and 1.5 mg/kg groups with a corresponding decrease in the mean number of viable fetuses when compared to the control group. No apparent differences were found in the incidences of external, visceral, or skeletal malformations, or incidence of developmental variations between the treated and control groups. Quinapril did not elicit a corporal teratogenic response in this study, but maternal toxicity and embryotoxicity were observed at all dose levels, (with no no-effect dose level in evidence), and significantly at doses of 1.0 and 1.5 mg/kg. Quinapril is about 500 times as toxic to the rabbit as Captopril, and is toxic at a dose level less than 1/3 that of the human clinical dose. These studies confirmed the intolerance of pregnant rabbits to quinapril.

CI-955 (Quinapril/HCTZ) in pregnant rabbits, 5/group, at dose of 0.16, 1.63, and 2.44 mg/kg/day (0.1, 1.0, and 1.5 mg/kg quinapril content), days 6 to 18 of pregnancy, three animals died and four aborted. There were no adverse effects on fetal body weight or sex ratio at term in the survivors of the CI-955 treated groups and no malformations or variations were observed in the survivors, but there was only one surviving litter in the H-D group, and only 2 in the M-D group. The reasons for the deaths and abortions and physiological differences between such toxicity states remain unknown, but the responses are apparently interrelated. Doses with Quinapril content of 0, 0.05, 0.1, and 0.5 mg/kg were all less than the 0.8 mg/kg recommended combination maximum human clinical dose. Additional H-D equivalent groups with quinapril alone (0.5mg/kg) and HCTZ alone (0.31 mg/kg) were added for comparison. There were two abortions in H-D quinapril (0.5 mg/kg) groups, one with quinapril alone (CI-906) and one with the CI-955 combination. Body weight (B-W) changes were minimal during the study, and total food intake was not significantly changed by treatment. Malformations and variations were not dose related. These comparisons of Quinapril/HCTZ combination with Quinapril indicate that the combination is more toxic than quinapril alone.

In the mouse, comparative MLD studies indicate that the Quinapril/HCTZ combination may significantly increase toxicity of Quinapril. There are no other Q/HCTZ combination mouse studies with which to evaluate chronic comparisons between Quinapril and its combination.

NEPHROPATHY: In the Quinapril 2 year mouse study there was an increased incidence of focal chronic nephritis in all treatment groups. An increase in severity of chronic progressive nephropathy was apparent in Quinapril treated terminal sacrifice females given 35, and in males and females given 75 mg/kg after 104 weeks. Lesion severity was greater in females listed with intercurrent deaths at all dose levels and included observations in the 5 mg/kg dose level (the low dose; thus a no-effect dose level is not available). This finding of increased nephropathy in intercurrent deaths in the treated groups reinforces the observation that treated mice with lethal lymphomas die ("found dead") earlier than controls in a dose related manner.

These chronic progressive nephropathy observations with a titratable dose related damage response indicated that the dose that could be tolerated in mice was limited by the progressive kidney damage. This information was considered adequate rational in not requiring an increase in the doses selected for the 2 year carcinogenicity study even though in that study the body weight decrement was less than 4% for all groups and clinical signs and pathologies were unremarkable. (See Table 2).

TABLE 2. Group Comparisons of Chronic Nephropathy Scores

Treatment Group	Grade of Lesion Severity						Evaluation not possible	Total Number in group
	0	1	2	3	4	5		
INTERCURRENT DEATHS								
Females								
Untreated Controls	3	2	0	0	0	0	4	9
Vehicle Controls	6	1	0	0	0	0	6	13
Quinapril (5 mg/kg)	0	4	0	0	0	0	1	5
Quinapril (35 mg/kg)	1	1	0	0	0	1	3	12
Quinapril (75 mg/kg)	0	0	0	1	3	0	5	9
Males								
Untreated Controls	0	0	1	0	0	0	2	3
Vehicle Controls	0	1	1	0	0	0	2	4
Quinapril (5 mg/kg)	2	0	0	0	0	0	2	4
Quinapril (35 mg/kg)	2	2	1	0	0	0	1	6
Quinapril (75 mg/kg)	0	0	1	1	0	0	3	5
104 WEEK SACRIFICE								
Females								
Untreated Controls	8	33	0	0	0	0		41
Vehicle Controls	5	32	0	0	0	0		37
Quinapril (5 mg/kg)	13	32	0	0	0	0		45
Quinapril (35 mg/kg)	0	1	18	18	0	0		37
Quinapril (75 mg/kg)	0	0	0	9	30	0		39
Males								
Untreated Controls	0	7	30	30	0	0		67
Vehicle Controls	0	9	31	5	0	0		45
Quinapril (5mg/kg)	0	8	35	3	0	0		46
Quinapril (35 mg/kg)	0	3	40	1	0	0		44
Quinapril (75 mg/kg)	0	0	3	41	?	0		45

- Lesion Grade: 0 - Nil
 1 - Minimal
 2 - Mild
 3 - Moderate
 4 - Severe
 5 - End-stage (Maximal)

Comments: E.D. signifies that evaluation of lesion grade was difficult, and E.N.P. that evaluation was not possible because of unsatisfactory staining and/or autolysis due to the intercurrent death of the animal or because of other disease such as amyloidosis or metastatic tumor invasion. An assessment was attempted in cases designated E.D. but not E.N.P.

Mean heart weights were decreased in the treated male groups (-4%, -10%, & -12%, respectively, in L-, M-, & H-D groups), and -8% in both the M- and H-dose female groups (but increased 10% in the L-D, F). The juxtaglomerular hypertrophy/hyperplasia and tubular basophilia pathology appeared at the lowest dose tested in females (5 mg/kg), but not males. HCTZ acts primarily on the kidney and might easily increase the severity of nephropathy in the mouse, but no information is available concerning this question.

Acute renal failure with BUN of 70 mg/dl was seen in 1/3 surviving (but sacrificed days 12 & 13 to save data because 2/5 had died on day 10) H-D (5 mg/kg) rabbits. Although sponsor claimed that just one kidney failure could not be attributed to the drug, the alternative must also be considered, because the cause of death in this group was unknown, the sample size is too small, and kidney damage was seen in other species. In this study, all dose levels (0.5, 1.0, and 5.0 mg/kg) exhibited JG pathology; apparently there is no no-effect dose level on record for any species.

Sponsor noted that severe effects of anemia and thrombocytopenia or leukopenia with bone marrow hypocellularity observed in dogs given captopril weren't seen with quinapril; however, Quinapril observations were made at much lower dose levels than those previously made with captopril, and dose relationships were not discussed by the sponsor. Because of dose differences, effects of anemia and thrombocytopenia or leukopenia with bone marrow hypocellularity that were observed in dogs given captopril and not seen with lower doses of quinapril cannot be ruled out as a general ACE inhibitor effect by the available data.

In the rat carcinogenicity study, the only differences between the doses with respect to intercurrent mortality were caused by dose related increases in male intubation deaths. Pulmonary edema and hemorrhage due to gavage dosing errors were more numerous than reported in the summary, and more numerous in treated groups in a dose related manner than in controls. Clinical signs and symptoms were minor. Terminal body weights were 91 to 107% of control weights and reduction of feed consumption did not exceed 3% in the treated groups. Biochemical alterations at 100 mg/kg included increased BUN and plasma renin in males, decreased serum glucose levels in females, and minor increases in CPK and LDH levels in males given 50 mg/kg or more. Significant biochemical alterations were limited to increased BUN in males and decreased serum glucose levels in females given 100 mg/kg and to minor increases in CPK and LDH levels in males given 50 mg/kg or more. Plasma renin levels were increased significantly only in males given 100 mg/kg. Organ weight changes were limited to increased kidney weights in high dose males. No ophthalmic findings or changes in ECG parameters were detected at 6 months. Terminal ophthalmoscopic evaluation revealed a slightly higher incidence of sacculation of retinal arterioles in treated animals compared to controls. The only drug-related terminal sacrifice gross finding seen in M- and H-D males was a finely pitted cortical surface of the kidney; a dose related increase with a male incidence about 5 to 7 times that of the females. Anticipated renal histopathology seen in previous studies were found at all dose levels, but heart weight decreases were not. Drug-related histopathologic renal changes at 52 weeks in most animals given 10 mg/kg and above were more prominent in males and more pronounced than at 26 weeks and included JG hypertrophy/hyperplasia and increased granularity of the JGA and interlobular arteries.

At 52 and 57 weeks, the occurrence of spontaneous renal degenerative lesions was greater in animals given 50 or 100 mg/kg. The 52 to 104 week sacrifice incidence of medullary mineral deposits (MDM) appeared in M- and H-D males and H-D females.

Sponsor contends that chronic progressive nephropathy (CPN), excluding JGA, is only a modest enhancement of this spontaneous nephropathy in H-D quinapril treated groups of both sexes and is not seen at the lower two doses. The JG changes were still prominent after the reversal period; however, sponsor claims that these JG changes did not progress with continued dosing during the last 52 weeks of the study and that the increased JGA granularity which correlated with elevated plasma renin levels early in the study became less conspicuous. Sponsor contends that this observation was correlated with declining plasma renin activity from week 52 to week 104. It is difficult to draw these conclusions from the data submitted. Glomerulonephrosis, tubular basophilia, and cortical cysts, were more common in males. As in the mice, the kidney was adversely affected by the treatment in a dose related manner. Target organ changes common to both species were JGA hypertrophy and hyperplasia at all treatment levels (no no-effect level) and renal degenerative changes at doses of 35 mg/kg or more in mice and of 50 mg/kg or more in rats.

These 2 year studies were not repeated with the combination and no toxicity comparisons between Quinapril and the Q/HCTZ combination are available. Renal degenerative changes in the kidney appeared in both species, and were more dose sensitive in the mouse than in the rat. With such a clear cut dose dependent difference in the mouse it might be recommended that comparative kidney toxicity studies be undertaken in the mouse to evaluate the effect of the combination on kidney toxicity.

BODY WEIGHTS AND FOOD CONSUMPTION:

Body weight and food consumption decrements in Quinapril treated mice and rats were treatment and dose related, but not marked until 500 mg/kg for female mice and for 250 and 500 mg/kg for male rats. Considerably lower dosages were selected for most chronic studies. The addition of HCTZ in an equivalent study in rats produced non dose-related weight decrements similar for all dose levels in the male groups and in L- and LM-D females; but (inversely) with minimal changes in the HM- and H-D females inspite of a reduced food consumption. Five animals/group were put on on a recovery program after 13 weeks and all groups slowly recovered toward (but did not achieve) normal weight gain potential except the two higher dosed female groups which suddenly lost weight and became equivalent to the other groups. No data was provided, but water balance and kidney function would be likely suspects for such an observation.

SUMMARY AND RECOMMENDATIONS:

Both of the active ingredients of this preparation, Accuretic, are approved drugs and will be given together by countless physicians. Approval of this NDA for a fixed combination would require suitable labeling to indicate the poorly characterized increased toxicity of the combination and/or the generation of more information quantitating that increased toxicity.

For proof of antihypertensive activity in spontaneously hypertensive rats, sponsor compared doses of 0.3 mg/kg Quinapril, 30 mg/kg HCTZ, and in combination for a 1/100 ratio. The dose chosen for the hypertensive dog renal excretory response comparison study was 10 mg/kg for both Quinapril and HCTZ, and in combination for a ratio of 1/1. In rat renal activity studies, doses ranged from 0.1 to 3.0 mg/kg for Quinapril and for only 1.0 mg/kg for HCTZ, then in combination for those levels with ratios varying from 1/10 to 3/1. All toxicology studies used a Q/HCTZ ratio of 40/25. The clinical combination ratios are 40/50 and 60/40.

In the toxicity tests of Quinapril combination, sponsor has reduced Quinapril content (with respect to previous studies with Quinapril) in the doses used and direct Quinapril dose toxicity comparisons cannot always be made. Sponsor has not provided the rationale for reduced Quinapril combination dosing in the toxicity tests with the combination; but, assuming that the reduction represents an estimated potentiating of clinical effect of the HCTZ in the combination, then a comparison of effects of such combination equivalents should be equal. Instead, the lower Quinapril content combination doses appear to be more toxic than the higher doses of Quinapril alone, and a potentiation of toxicity is evident. The combination is more efficacious; however, given this greater toxicity, the question of erosion of the safety margin becomes paramount. Unless the therapeutic gain is greater than the increased toxicity, this combination cannot be recommended. This question cannot be answered from the animal data presented.

It is to be recommended that sponsor provide the rationale for the test dose levels chosen especially with reference to clinical gain. Because comparisons are now made with differing Quinapril levels, further tests are to be recommended to provide direct comparisons of Quinapril with equivalent doses of Quinapril/HCTZ in combination. Such studies would provide a direct estimate of the enhancement of toxicity, allow a toxicity comparison of the combination to any improvement of clinical efficacy, and, to determine the possibility of safety margin erosion.

It is apparent that kidney damage is increased by the combination and the kidney can be considered a target organ, but no good comparison studies are available. Because the mouse appears to be the most sensitive model for kidney damage determinations, it is to be recommended that sponsor make a comparative evaluation of possible kidney damage in the mouse.

The combination increases the maternotoxicity, fetotoxicity and probably is a fetal physiological teratogen at a fraction of the clinical dosages in the rabbit. No comparison studies between Quinapril and the Q/HCTZ combination with respect to Segment I and III reproductive parameters have been submitted. In view of the increased toxicity in other comparisons, the lack of such Segment I and III reproductive safety information would indicate the need for such studies and a recommendation for a contraindication in pregnancy.

/S/

3rd

Wm. C. Van Arsdel III, March 20, 1992.

cc:
Orig. NDA
HFD-502/JWeissinger
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HFD-110
HFD-110/CSO
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3/20/92