CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20-726

PHARMACOLOGY REVIEW(S)

DIVISION OF ONCOLOGY DRUG PRODUCTS
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Labeling Review

NDA No. 20-726

Information to be conveyed to sponsor: Yes

Reviewer: Margaret E. Brower, Ph.D.

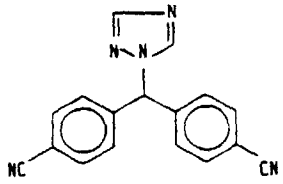
Sponsor: Ciba-Geigy Corporation, Summit NJ

Drug Name: Primary: Letrozole Other Names: Femara, CGS 20267

Chemical Name: 4,4'-[(1H-1,2,4-triazolyl-1-yl)methylene]bis-benzonitrile

CAS Number: 112809-51-5

Structure:



CGS 20 267

Molecular Weight and Formula: 285.3 $C_{17}H_{11}N_5$

Related INDs/NDAs: IND , IND

Pharmacologic Class: Aromatase inhibitor

Indication: Metastatic breast cancer in postmenopausal women

Route of Administration: oral tablet

LABELLING REVISIONS--1ST DRAFT

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JUL 10 1997

DIVISION OF ONCOLOGY DRUG PRODUCTS, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Labeling Review

NDA No. 20-726 /July 1, 1997 (MC)

Information to be conveyed to sponsor: Yes

Reviewer: Margaret E. Brower, Ph.D.

Sponsor: Ciba-Geigy Corporation, Summit NJ

Drug Name: Primary: Letrozole Other Names: Femara, CGS 20267

Chemical Name: 4,4'-[(1H-1,2,4-triazolyl-1-yl)methylene]bis-benzonitrile

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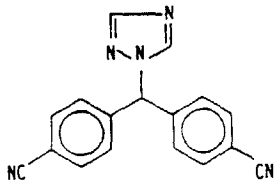

CGS 20 267

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LABELLING REVISIONS--1ST DRAFT + CHANGES

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Margaret E. Brower, Ph.D.
Pharmacology
June 18, 1997

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HFD-150
/PAndrews
/MBrower
/DSpillman

Paul A. Andrews 9/15/97

JUN 18 1997

DIVISION OF ONCOLOGY DRUG PRODUCTS, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Labeling Review

NDA No. 20-726 / 6.4.97 Labeling

Information to be conveyed to sponsor: Yes

Reviewer: Margaret E. Brower, Ph.D.

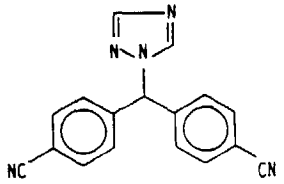
Sponsor: Ciba-Geigy Corporation, Summit NJ

Drug Name: Primary: Letrozole Other Names: Femara, CGS 20267

Chemical Name: 4,4'-[(1H-1,2,4-triazolyl-1-yl)methylene]bis-benzonitrile

CAS Number: 112809-51-5

Structure:



CGS 20 267

Molecular Weight and Formula: 285.3 $C_{17}H_{11}N_5$

Related INDs/NDAs: IND IND (Ciba-Geigy)

Pharmacologic Class: Aromatase inhibitor

Indication: Metastatic breast cancer in postmenopausal women

Route of Administration: oral tablet

LABELLING REVISIONS--1ST DRAFT + CHANGES

( )

Margaret E. Brower, Ph.D.
Pharmacology
June 18, 1997

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/MBrower
/DSpillman

JUN 10 1997

DIVISION OF ONCOLOGY DRUG PRODUCTS, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA

Original NDA Review

NDA No. 20-726

Date(s) of Submission: NDA dated: 7/24/96 (N) , 7/31/96 (BP)
Received by CDER: 7/25/96 ; 8/2/96
Received by reviewer: 7/29/96

Information to be conveyed to sponsor: Yes

Reviewer: Margaret E. Brower, Ph.D.

Date Review Completed: May 21, 1997

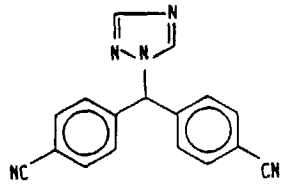
Sponsor: Ciba-Geigy Corporation, Summit NJ

Drug Name: Primary: Letrozole Other Names: Femara, CGS 20267

Chemical Name: 4,4'-[(1H-1,2,4-triazolyl-1-yl)methylene]bis-benzonitrile

CAS Number: 112809-51-5

Structure:



CGS 20 367

Molecular Weight and Formula: 285.3 $C_{17}H_{11}N_5$

Related INDs/NDAs: IND IND (Ciba-Geigy)

Pharmacologic Class: Aromatase inhibitor

Indication: Metastatic breast cancer in postmenopausal women

Route of Administration: oral tablet

Clinical Formulation:

Component (tablet core)	Amount
Letrozole	2.5
colloidal anhydrous silica	
cellulose	
lactose monohydrate	

magnesium stearate
maize starch
sodium starch glycolate

Proposed Dosage: 2.5mg (1.54mg/m^2 based on 60kg human) administered daily until tumor progression

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----------Review----------

Previous Reviews, Dates and Reviewers:
IND
IND
IND

Studies reviewed in this NDA:

I. Pharmacology

ERS-3/88	CGS 20267 (Aromatase inhibitor): Selectivity of aromatase inhibition in hamster ovaries *in vitro* (Vol 12, p. 88)
ERS-4/88	CGS 20267 (Aromatase inhibitor): Effects on steroid production in rat adrenal fragments *in vitro* (Vol 12, p. 94)
ERS-5/88	CGS 20267 (Aromatase Inhibitor): *In vitro* and *in vivo* inhibition of aromatase (Vol 12, p. 79)
-	Highly selective inhibition of estrogen biosynthesis by CGS 20267, a new non-steroidal aromatase inhibitor (Vol 12, p. 135)
ERS-3/89	CGS 20267 (Aromatase inhibitor): Effects on plasma corticosterone and aldosterone in ACTH-stimulated male rats (Vol 12, p. 148)
45/91	CGS 20267(Aromatase inhibitor): effect of 14-day treatment on organ weights and serum luteinizing hormone in female rats (Vol 12, p. 118)

II. Safety Pharmacology

CVS 56/88	General pharmacological effects of CGS 20267 (Anti-tumor agent) (Vol 12, p. 208)
69/92	CNS-evaluation of CGS 20267 (aromatase inhibitor) (Vol 12, p. 243)

III. Pharmacokinetics/Toxicokinetics

A. Pharmacokinetics

DM(EM) 3/1994	Absorption and disposition of [^{14}C]CGS 20267 in male mice (Vol 36, p. 72)
DM(EU) 7/1995	Absorption and disposition of ^{14}C-labelled CGS 20267 in female rats (Vol 36, p. 164)
36/1990	Absorption and disposition of 14C-labelled CGS 20267 substance in rats and dogs (Vol 36, p. 98)
CRB R 42/1989	Plasma kinetics of unchanged CGS 20267 in rats following intravenous and oral administration of a single 1mg/kg dose (Vol 36, p. 201)
CRB R 8/1992	Disposition of CGS 20267 and its metabolite, CGP 44645, in rats and dogs after iv or oral administration of 14C-CGS 20267 (Vol 36, p. 215)
CRB R 12/1990	Plasma concentrations of unchanged CGS 20267 in rats following oral administration of single 0.3, 3 and 30mg/kg doses. Investigation on the presence of the potential hydroxy metabolite, CG 44645 in plasma (Vol 37, p. 1)
BPK(F) 1994/029	In vitro binding of letrozole to human serum proteins, human erythrocytes and serum proteins from rat, dog, baboon, mouse and rabbit (Vol 37, p. 155)
DMET(EU) 3/1996	In vitro characterization of the human cytochrome P450 isozyme(s) responsible for the biotransformation of CGS 20267 to CGP 44645 (Vol 37, p. 245)
DMET(US) 96003	Evaluation of a new chemical entity as an inhibitor of human P450 enzymes (Vol 38, p. 18)
DM(EU) 16/1994	Long-term elimination of ^{14}C-labelled CGS 20267 in female rats (Vol 38, p. 127)

B. Toxicokinetics

BPK(F)1995/009	Letrozole plasma concentrations in male and female dogs on days 1 and 14 following letrozole intravenous administration of daily doses of 0.02, 0.2 and 2mg/kg body weight in a 14-day toxicity study (Vol 37, p.71)

CRB R 31/1990	Trough plasma concentrations of unchanged CGS 20267 in dogs given repeated doses of 0, 0.03, 0.3 or 3mg/kg once daily for 3 months. Investigation on the presence of the hydroxy metabolite, CGP 44645 in plasma (Vol 38, p. 253)
CRB R 46/1993	Plasma concentrations in male and female mice on weeks 2 and 10 following CGS 20267 oral administration via gavage of daily doses of 0.6, 6 and 60mg/kg body weight in a 13-week toxicity study (Vol 36, p. 252)
CRB R 15/1993	CGS 20267 plasma concentrations in male and female rats on days 1, 207 and 365 following CGS 20267 administrations of single and repeated once daily doses of 0.3, 3 and 30mg/kg body weight in a 6/12-month oral toxicity study (Vol 38, p. 197)
CRB R 22/1993	CGS 20267 plasma concentrations in male and female dogs on days 1, 182 and 364 following CGS 20267 administrations of single and repeated once daily doses of 0.03, 0.3 and 3mg/kg body weight in a 6/12-month oral toxicity study (Vol 37, p. 109)
BPK(F) 1994/028	Letrozole plasma concentrations in male and female rats on weeks 14, 53 and 78 following letrozole oral administration via gavage of daily doses of 0.1, 1 and 10mg/kg body weight in a 104-week carcinogenicity study (Vol 38, p. 150)
BPK(F) 1995/006	Letrozole plasma concentrations in male and female mice on weeks 26, 54 and 78 following letrozole oral administration via gavage of daily doses of 0.6, 6 and 60mg/kg body weight in a 104-week oral carcinogenicity study (Vol 36, p. 306)

IV. Toxicology

A. Single dose Toxicity

896022	Acute oral toxicity study in mice (Vol 13, p. 1)
896023	Acute oral toxicity study in rats (Vol 13, p. 13)
896024	Acute intraperitoneal toxicity study in rats (Vol 13, p. 25)
896025	Acute oral toxicity study in dogs (Vol 13, p. 38)

B. Multiple dose Toxicity

93010	13-week oral toxicity study in mice (Vol 13, p. 48)
946092	14-day intravenous toxicity study in rats (Vol 19, p. 1)
886120	28-day oral dose rangefinding study in rats (Vol 19, p. 225)

916010 6/12-month oral toxicity study in rats (Vol 22, p. 1)
946093 14-day intravenous toxicity study in dogs (Vol 29, p. 1)
886121 28-day oral dose rangefinding study in dogs (Vol 29, p. 212)
916015 6/12-month oral toxicity study in beagle dogs (Vol 31, p. 1)

V. Carcinogenicity

96001 104-week oral carcinogenicity study in mice (MIN 934020) (Vol 14, p. 1)
95059 104-week oral carcinogenicity study in rats (MIN 924172) (Vol 25, p. 1)

VI. Reproductive Toxicity

95042 An oral dose-rangefinding study for the effects on embryo and fetal development in rats (MIN 954026) (Vol 33, p. 1)
96010 An oral study for effects on embryo and fetal development in rats (MIN 954027) (Vol 33, p. 122)
956003 An oral dose-rangefinding study for effects on embryo and fetal development in rabbits (MIN 954024) (Vol 34, p. 113)
96025 An oral study for the effects on embryo and fetal development in rabbits (Separate submission) يوليو ٣١، ١٩٩٥ (BP)

VII. Genetic Toxicity

AFP 12 CGS 20267: Bacterial mutagenicity test (Vol 35, p. 1)
896031 Salmonella/mammalian-microsome mutagenicity test (OECD-conform) (Vol 35, p. 14)
956144 Salmonella and Escherichia/mammalian-microsome mutagenicity test (OECD-conform) (Vol 35, p. 44)
896032 Chromosome studies on Chinese hamster ovary cell line CCL 61 in vitro (OECD-conform) (Vol 35, p. 101)
926313 Cytogenetic studies on Chinese hamster ovary cell line in vitro (EC-conform) (Vol 35, p. 136)
896033 Gene mutation test with Chinese hamster cells V79 (OECD-conform) (Vol 35, p. 177)
896030 Micronucleus test, rat (OECD-conform) (Vol 35, p. 229)

VIII. Special toxicity studies

896324 Test for abnormal toxicity in mice (EC-conform) (Vol 35, p. 278)
946105 5-day intravenous irritation study in rabbits (Vol 35, p. 285)
946922 Acute eye irritation/corrosion study in the rabbit (Vol 35, p. 307)
946923 Acute dermal irritation/corrosion in the rabbit (Vol 35, p. 321)

Studies not reviewed in this NDA:

I. Pharmacology

BS 24	Effects of CGS 20267 (letrozole) and CGP 63606 (anastrozole) on DMBA-induced mammary carcinomas in Sprague-Dawley rats (Vol 12, p. 194)
-	Receptors reconsidered, A 20-year perspective (Vol 12, p. 1)
-	Steroid hormone receptors in breast cancer treatment strategy (Vol 12, p. 35)
-	Source of estrogen production in postmenopausal women (Vol 12, p. 47)
-	Aromatase inhibitors in cancer treatment (Vol 12, p. 55)
-	Arimidex: a potent and selective fourth-generation aromatase inhibitor (Vol 12, p. 70)
-	Initiation of precocious sexual maturation in the immature rat treated with dehydroepiandrosterone (Vol 12, 100)
-	Steroid induction of gonadotropin surges in the immature rat. I. Priming effects of androgens (Vol 12, p. 111)
-	The role of estrogen in the feedback regulation of follicle-stimulating hormone secretion in the female rat (Vol 12, p. 142)
-	Evidence that corticosterone is not an obligatory intermediate in aldosterone biosynthesis in the rat adrenal (Vol 12, p. 153)
BS23	Comparison of the effects of a 14-day treatment with CGS 20267 (letrozole) and CGP 63606 (anastrozole) on uterine weight in adult female rats (Vol 12, p. 157)
-	CGS 16949A, a new non-steroidal aromatase inhibitor: effects on hormone-depeendent and -independent tumors in vivo (Vol 12, p. 165)
BR 56/91	CGS 20267(aromatase inhibitor): Effects on mamary tumor-tearing rats (Vol 12, p. 170)
BR 6/89	Interactions of CGS 20267 (aromatse inhibitor) with neurotransmitter receptors in vitro (Vol 12, p. 230)

II. Pharmacokinetics/Toxicokinetics

CRBR 11/1990	CGS 20267: determination of CGS 20267 and two potential metabolites, CGP 44645 and CGP 44646, in human plasma by high-performance liquid chromatography (Vol 36, p. 1)
CRBR 15/1991	CGS 20267: Determination of CGS 20267 and one potential metabolite, CGP 44645, in human and dog urines by high-performance liquid chromatography (Vol 36, p. 23)
DM 32/1991	CGS 20267: Preparative separation and isolation of the O-glucuronide of CGP 44645, the main metabolite of CGS 20267, from the urine of a rabbit treated with CGP 44645 (Vol 36, p.44)
B79/1989	CGS 20267: In vitro glucuronidation of CGP 44645 by incubation with rat hepatocytes (Vol 36, p. 53)

B101/1989	CGS 20267: Synthesis of the O-sulfate of CGP 44645 (Vol 36, p. 56)
B86/1988	CGS 20267: Synthesis of the carbon-14 labelled compound (Vol 36, p.59)
DMET(US) 96015	A pilot 3-week oral hepatic microsomal enzyme induction study in female rats (MIN 961024) (Vol 37, p. 414)
BPK(F) 1995/013	Letrozole plasma concentrations in male and female rats on days 1 and 14 following letrozole intravenous administration of daily doses of 0.03, 0.3 and 3mg/kg body weight in a 14-day toxicity study (Vol 36, p. 322)
CRBR 14/1990	Plasma concentrations of unchanged CGS202676 in rats following oral administration of single and repeated doses of 0.3, 3 and 30mg/kg once daily for 30 days (Vol 37, p. 26)
BPK(F) 1995/023	Letrozole plasma concentrations in female rabbits on gestation day 17 following letrozole oral daily doses of 0.06, 0.6 and 6mg/kg body weight on days 7-19 of gestation to evaluate effects of embryo and fetal development in a dose-range finding study (Vol 37, p. 49)
BPK(F) 1995/009	Letrozole plasma concentrations in male and female dogs on days 1 and 14 following letrozole intravenous administration of daily doses of 0,02, 0.2 and 2mg/kg body weight in a 14-day toxicity study (Vol 37, p. 71)
DMET(EU) 1/1996	In vitro biotransformation of CGS 20267 using mouse, rat, dog and human liver fractions (Vol 37, p. 192)
DMET US) 95020	In vitro metabolism by human liver slices (Vol 37, p. 222)
CB 90/07	The effects of CGS 20267 on selected biochemical and morphological liver parameters following administration to male rats at two dose levels for various periods of time (Vol 37, p. 287)
DMET (US) 964014	A pilot 2-week oral hepatic microsomal enzyme induction study in dogs (Vol 37, p.414)
CB 94/50	Spectral interaction of CGS 20267 with microsomal cytochrome P450 from human liver (Vol 38, p. 1)
BS20/1996	Inhibition of aromatase in vitro (Vol 38, p. 117)
BPK(F) 1994/003	Pharmacokinetics of letrozole in breast cancer patients receiving daily oral doses of 0.1, 0.5 or 2.5mg for 6 weeks and then increased to 0.25, 1 or 5mg for the duration of patient response to treatment (Vol 38, p.276)

Studies previously reviewed:

36/1990	Absorption and disposition of 14C-labelled CGS 20267 substance in rats and dogs (Vol 36, p. 98) (Re-reviewed)
CRBR 42/1989	Plasma kinetics of unchanged CGS 20267 in rats following intravenous and oral administration of a single 1mg/kg dose (Vol 36, p. 201)
CRBR 31/1990	Trough plasma concentrations of unchanged CGS 20267 in dogs given repeated doses of 0, 0.03, 0.3,or 3mg/kg once daily for 3 months. Investigation on the presence of the hydroxy metabolite, CGP 44645 in plasma (Vol 38, p. 253)
896024	Acute intraperitoneal toxicity study in rats (Vol 13, p. 25)
896056	3-month oral toxicity study in rats (Vol 20, p. 1)

896026 3-month oral toxicity study in dogs (Vol 30, p. 1)

I. PHARMACOLOGY

ERS-3/88 **CGS 20267 (Aromatase inhibitor): Selectivity of aromatase inhibition in hamster ovaries *in vitro*** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1988)

ERS-4/88 **CGS 20267 (Aromatase inhibitor): Effects on steroid production in rat adrenal fragments *in vitro*** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1988)

ERS-5/88 **CGS 20267 (aromatase Inhibitor): *In vitro* and *in vivo* inhibition of aromatase** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1988)

- **Highly selective inhibition of estrogen biosynthesis by CGS 20267, a new non-steroidal aromatase inhibitor** (Bhatnagar AS, Hausler A, Schieweck K, Lang M, Bowman R, Steroid Biochem Molec Biol, 1990; 37:1021-7)

ERS-3/89 **CGS 20267 (Aromatase inhibitor): Effects on plasma corticosterone and aldosterone in ACTH-stimulated male rats** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1989)

45/91 **CGS 20267(Aromatase inhibitor): effect of 14-day treatment on organ weights and serum luteinizing hormone in female rats** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1991)

Letrozole is a non-steroidal competitive inhibitor of aromatase. When compared to aminoglutethimide *in vitro*, CGS20267 was found to competitively inhibit human placental microsomal aromatase from 150 to 250 times more effectively. Estrogen production was inhibited up to 600 times more effectively with CGS20267.

In LH-stimulated hamster ovarian tissue, CGS20267 inhibited estradiol production *in vitro* at 0.1μM with an IC_{50} of of 0.02μM; progesterone production was not significantly affected up to 350μM.

In vivo, CGS 20267 did not affect plasma levels of corticosterone or aldosterone at a dose of 4mg/kg administered orally to ACTH-treated rats (1000 times higher than the ED_{50} for aromatase inhibition *in vivo*). A 14-day treatment with 1mg/kg administered po to untreated rats, interrupted ovarian cyclicity and depressed uterine weight to that observed 14 days following ovariectomy. In female rats with estrogen-dependent DMBA- induced mammary tumors, 0.1mg/kg CGS 20267, administered po for 42 days caused almost complete regression of tumors.

Treatment of Tif: rats with up to 1mg/kg CGS 20267 for 14 days resulted in a reduction of uterine and pituitary weights and elevation of serum LH equivalent to those levels observed in untreated ovariectomized rats. These changes were dose related and were a result of the reduction of circulating estrogens.

In another study, CGS 20267 was not found to inhibit adrenal steroidogenesis (corticosterone and aldosterone) in rat adrenal fragments *in vitro* at concentrations 3 orders of magnitude higher than those required for inhibition of estrogen production.

II. SAFETY PHARMACOLOGY

CVS 56/88 **General pharmacological effects of CGS 20267** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1988)

69/92 **CNS-evaluation of CGS 20267 (aromatase inhibitor)** (Conducted by Ciba-Geigy Limited, Basle, Switzerland, 1988)

Lethality was observed in cats following single oral doses of 10mg/kg or 30mg/kg CGS 20267; death was preceeded by depressed heart rate and blood pressure, and severe arrhythmias. There were no effects on heart rate or blood pressure at doses of 1 or 3mg/kg. CGS 20267 increased the rate and force of contraction of guinea pig atria; these effects were markedly attenuated with pretreatment of the atria with propranolol. This cardiostimulatory effect appeared to have been the result of catecholamine release. In concentrations ranging from μmol/L, CGS 20267 did not alter the tone of isolated guinea pig ileum. Urine volume and electrolyte excretion in conscious rats were not affected following oral dosing of 3mg/kg CGS 20267.

CGS 20267 did not exert any significant effects on behavior, sedation or memory of male mice treated with 10mg/kg po. Male rats treated with the same dose did not exhibit effects in the rotarod or the motility tests. CGS 20267 caused a transient increase in body temperature of these animals 2h following dosing.

III. PHARMACOKINETICS/TOXICOKINETICS

DM(EM) 3/1994 **Absorption and disposition of [^{14}C]CGS 20267 in male mice.** Conducted by Pharma Research, Preclinical safety/drug metabolism, Ciba-Geigy Limited, Basle, Switzerland in 1994.

Methods

species: ♂ albino Tif: mice

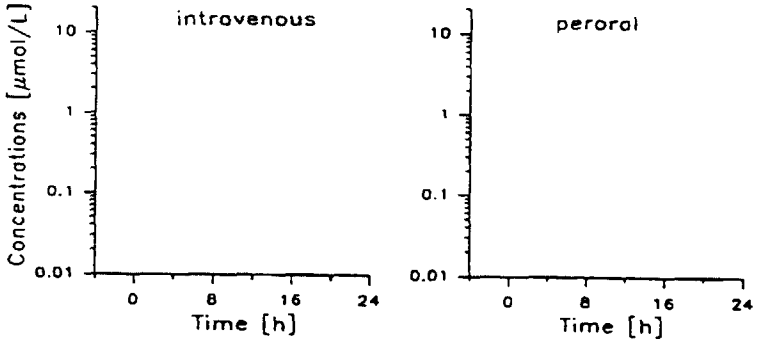
drug: ^{14}C-labelled CGS 20267, batch Z-855.10A, purity 99%

dosage: 1mg/kg
vehicle: polyethylene glycol, saline (iv dose); cornstarch (oral dose)
wt: 25-42g
route: iv, gavage
assay:

Results

CGS 20267 was absorbed approximately 70-100% from the GI tract of mice following peroral administration as determined from plasma concentration (C_{max}) and urinary excretion following comparative iv and gavage dosing.

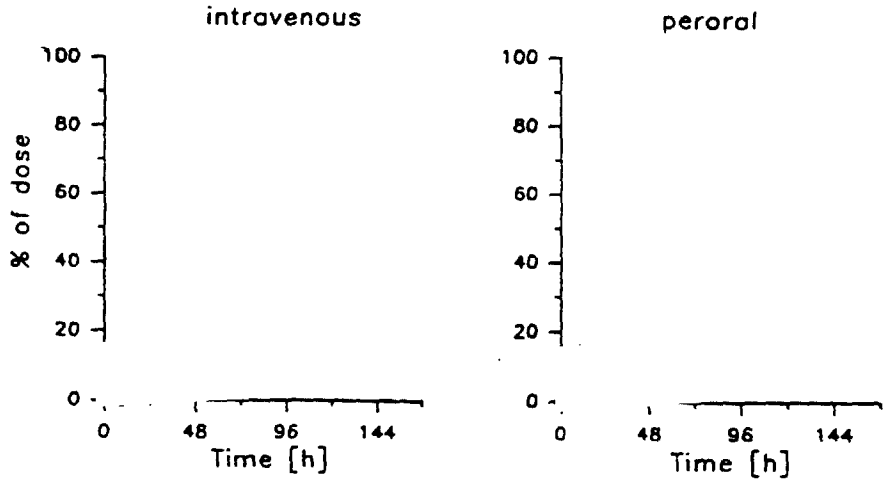
Five minutes following iv administration, the plasma [^{14}C] CGS 20267 ranged between μmol/l; between hr, mean concentrations declined from 1.7 to 0.9μmol/l, were ~ 0.04μmol/l at 24hr and were below the LOQ at 48hr. In comparison, from 30mins to 4hr following oral administration, mean plasma concentrations ranged from 0.46 to 0.59μmol/l, increased to 0.95μmol/l at 6hr, declined to 0.06 at 24hr and were below the LOQ at 48hr. Plasma concentrations were lower and more constant over a longer duration with oral dosing until 24hr when levels declined in a manner similar to iv administration.



The distribution patterns of radioactivity in organs and tissues were qualitatively similar 5 minutes and 6 hours following iv and po administration of [14C]CGS 20267, respectively (see table below). Letrozole concentrations were consistently highest in liver and adrenal at these times following dosing.

Comparative distribution of letrozole 5 mins following iv administration and 6 hrs following po administration		
Tissue	Mean [^{14}C] concentration (nmol/g) of letrozole	
	5 mins following iv administration	6 hrs following po administration
Blood	1.76	0.90
Plasma	2.15	1.08
Liver	18.08	15.61
Kidney	4.90	2.16
Adrenal	8.37	4.17
Aorta	6.39	1.77
Small intestine	6.45	2.40
Brown fat	5.98	1.60

Within 7 days of dosing, 93.4 (iv) and 96.7% (po) of the total letrozole dose was excreted via urine and feces; 82 (iv) and 85.4% (po) of the total was excreted within 24hrs. The bulk of the drug was recovered from the urine (73.7% iv and 71% po) within 24hrs following administration. The patterns of metabolites in urine fractions were similar following iv and po administration of letrozole. The unchanged parent drug accounted for 33 to 63% of the dose eliminated within 24hrs. In addition, the carbinol metabolite (CGP 44645) was 1.2-3.7%, the O-glucuronide was 8-16% and an unidentified metabolite was 1-3% of the dose. The ratio of unchanged drug to total (free and conjugated) carbinol metabolite was ~4:1.



DM(EU) 7/1995 Absorption and disposition of ^{14}C-labelled CGS 20267 in female rats. Conducted by Ciba-Geigy Limited, Basle, Switzerland in 1995.

Methods

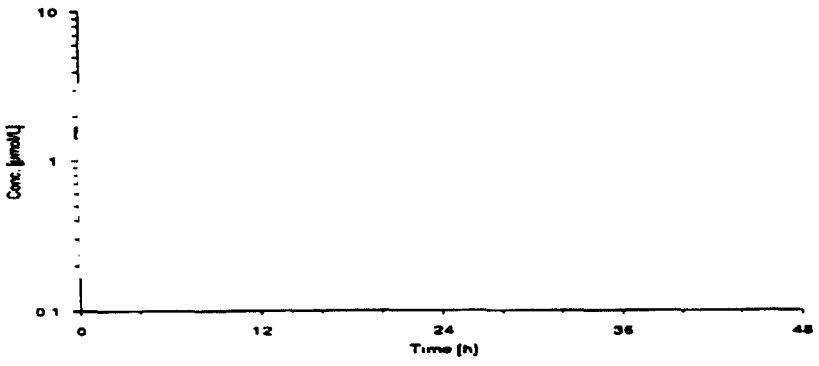
species: ♀ albino Tif: rats
drug: ^{14}C-labelled CGS 20267, batch Z-855.10A, purity 98%
dosage: 1mg/kg (single iv dose: 2.2mg ^{14}C-CGS 20267 dissolved in 4g polyethylene glycol followed by 2g 0.9% saline, concentration of 0.37mg/g letrozole, dose= 0.52g solution /200g BW); single oral dose: 6.1mg ^{14}C-CGS 20267 suspended in 17g polyethylene glycol followed by 8.5g water, concentration of 0.24mg/g letrozole, dose= 0.84g solution/200g BW
wt, age: 178-193g, 6 weeks
route: iv, gavage
assay: bile duct cannulated for bile collection (method not described)

Results:

Absorption of labeled letrozole was approximately 55% in ♀rats administered the drug via gavage, as indicated by the sum of the proportions of label excreted with bile and urine within 96hrs of administration. In comparison, as observed in the study reviewed above, CGS 20267 was absorbed approximately 70-100% from the GI tract of mice following peroral administration as determined from plasma concentration and urinary excretion following comparative iv and gavage dosing.

Five minutes following iv administration in rats, the plasma [^{14}C] CGS 20267 ranged between µmol/l; between hr, mean concentrations declined from 1.7 to 0.9µmol/l, were ~ 0.04µmol/l at 24hr and were below the LOQ at 48hr. In comparison, from 30mins to 4hr following oral administration, mean plasma concentrations ranged from 0.46 to 0.59µmol/l, increased to 0.95µmol/l at 6hr, declined to 0.06 at 24hr and were below the LOQ at 48hr. Plasma concentrations were lower and more constant over a longer duration with oral dosing until 24hr when they declined in a manner similar to iv administration.

Letrozole was evenly distributed throughout the body irrespective of the route of administration with the exception of adrenals and liver which exhibited 13- and 4-fold the ^{14}C concentration in blood (1.9µmol/L), respectively. The comparative kinetics of letrozole in the blood following iv and oral administration is shown below. Male rat data derived from Study 36/1990 (NDA page 13) and was included for comparative purposes.



Comparative distribution of letrozole in ♀rats 5 mins following iv administration and 4 and 24hrs following po administration			
Tissue	Mean [^{14}C] concentration (nmol/g) of letrozole		
	5 mins following iv administration	4 hrs following po administration	24hrs following po administration
Blood	1.90	1.56	0.98
Plasma	2.12	na	1.12
Liver	7.71	5.73	4.57
Adrenal	24.90	29.28	17.59
Aorta	6.26	na	3.59
Salivary	5.27	6.04	2.71

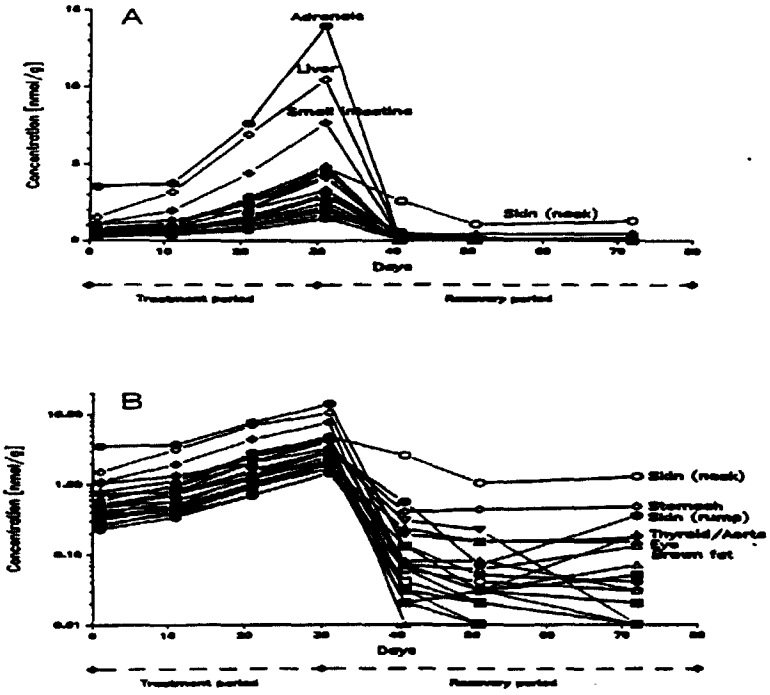
Elimination of labelled letrozole in urine and feces was 90% of the dose 7 days following either iv or oral administration in ♀ rats. The clearance of labelled letrozole from blood, plasma and tissues and excretion in urine and feces was markedly slower in female compared to male rats. The terminal T½ of labelled letrozole in blood and plasma was reported to be 40-50hrs in ♀ as compared to 7-8hrs in ♂. Accordingly, systemic exposure in females was [approximately 4-fold] higher than in males.

36/1990 **Absorption and disposition of 14C-labelled CGS 20267 substance in rats and dogs**. Conducted by Pharma research and development, Ciba-Geigy, Basel, Switzerland in 1990. Previously reviewed by H.R.Prasanna.

Studies in dogs and rats indicated that letrozole was rapidly absorbed from the GI tract. In rats, kinetics of letrozole in blood and plasma was characterized by a broad plateau, suggesting enterohepatic cirulation of the compound and a $t_{1/2}$ of 7-9 hrs. High letrozole levels were observed in the adrenals and liver. Following dosing in rats for up to 30 days, concentrations of letrozole did not indicate steady state, but were 3-8 fold higher than after a single dose. Following termination of dosing, clearance occurred within 7 days with similar porportions with urine and feces. [^{14}C] activity was primarily due to the O-glucuronic acid conjugate of the carbinol metabolite, CGP 44645. Unconjugated CGP 44645 and unchanged CGS 20267 (< 5% of the dose) were observed in lower concentrations.

In dogs, only 25-60% of the dose was excreted within 7 days following dosing. The major letrozole activity retained in the body was primarily due to unchanged drug. Urinary radioactivity in dogs was primarily the 0-glucuronic acid conjugate of CGP 44645 and unchanged CGS 20267 in a ratio of 3:2.

Concentrations of total radioactive substances in blood plasma, organs and tissues of male rats 24 hours after the 1st, 10th, 20th and 30th and 10, 20 and 41 days after the 30th once daily peroral administration of 1 mg/kg of ^{14}C-labeled CGS 20 267. (Means of 2-3 animals per time point)
A : linear, B : semi-logarithmic representation



CRB R 8/1992 **Disposition of CGS 20267 and its metabolite, CGP 44645, in rats and dogs after iv or oral administration of 14C-CGS 20267.** Conducted by Biopharmaceutical Research Center, Ciba Geigy, Malmaison, France in 1992.
species: Tif: rats (8 iv, 5 oral dosing) and beagle dogs (1 iv, 3 oral dosing)
dosage: 0.1, 1mg/kg iv and oral in rats; 0.1mg/kg iv, 0.1 and 3mg/kg oral in dogs
assay:

Pharmacokinetic parameters of letrozole in dogs following iv and oral administration							
Dose/# anim	C_{max} (µmol/l)	T_{max} (h)	$AUC_{0\text{-}168h}$ (µmol·h/l)	$AUC\infty$ (µmol·h/l)	$AUC\infty$/dose (µmol·h/l)	$T_{1/2}$ (h)	Cl (ml/min/kg)
0.1(iv)/ N=1	-	-	18.6	24	3.33	79.1	0.26
0.1(oral)/N=2	0.05-0.056	8-24	3.25-6.15	4.64-8.83	0.95-1.50	59.8-93.4	-
3.0(oral)/N=1	3.97	24	388	493	2.62	71.	

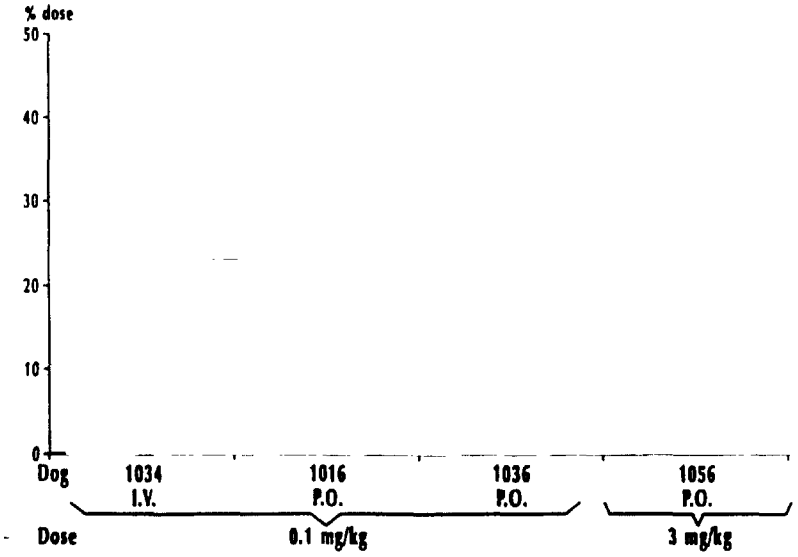
Pharmacokinetic parameters were not analyzed for rodents.

The predoninant 14C-component of rat and dog urine was identified as the o-glucuronic acid conjugate of the carbinol metabolite , CGP 44645. The study author considered conjugated CGP 44645 to be unique to the urine. The differences in the urinary excretion of rats and dogs were a result of species difference in the rate of biotransformation of letrozole.

DISPOSITION OF CGS 20 267 AND ITS METABOLITE, CGP 44 645, IN RATS AND DOGS, AFTER I.V. OR ORAL ADMINISTRATION OF ^{14}C-CGS 20 267 (C.R.B. PROJECT X393).

FIGURE 6

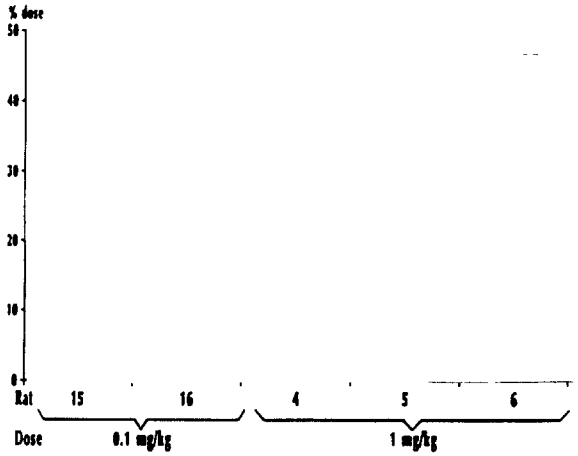
Urinary excretion of CGS 20 267 and CGP 44 645 in dog.



DISPOSITION OF CGS 20 267 AND ITS METABOLITE, CGP 44 645, IN RATS AND DOGS, AFTER I.V. OR ORAL ADMINISTRATION OF ^{14}C-CGS 20 267 (C.R.B. PROJECT X393).

FIGURE 7

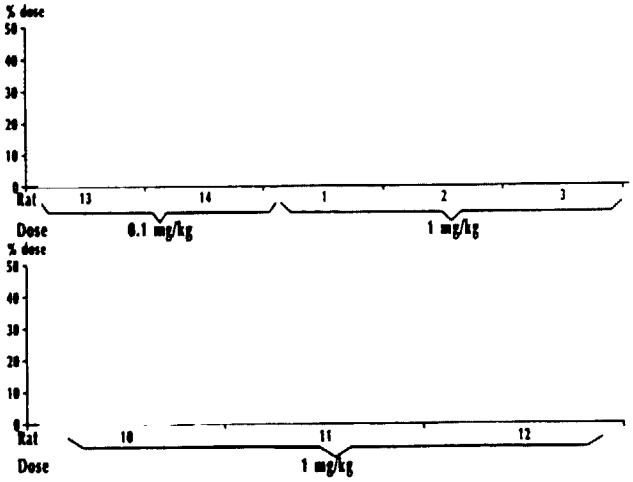
Urinary excretion of CGS 20 267 and CGP 44 645 in rat after oral administration.



DISPOSITION OF CGS 20 267 AND ITS METABOLITE, CGP 44 645, IN RATS AND DOGS, AFTER I.V. OR ORAL ADMINISTRATION OF ^{14}C-CGS 20 267 (C.R.B. PROJECT X393).

FIGURE 8

Urinary excretion of CGS 20 267 and CGP 44 645 in rat after i.v. administration.



CRB R 12/1990 **Plasma concentrations of unchanged CGS 20267 in rats following oral administration of single 0.3, 3 and 30mg/kg doses. Investigation on the presence of the potential hydroxy metabolite, CG 44645 in plasma.** Conducted at Bioanalytics and pharmacokinetics, Ciba-Geigy, Malmaison, France in 1990.

species: Tif: ♂ rats

dosage: 0.3, 3, 30mg/kg CGS 20267 (batch BE 1844/7H)

samples collected: 0.5, 2, 4, 8, 24, 32 and 48h postdose

Difficulty with separation between the hydroxy metabolite, CGP 44645 and unchanged drug.

Low concentrations of the hydroxy metabolite were detected in plasma following administration of the HD (30mg/kg) only.

Pharmacokinetic parameters of rats administered letrozole orally			
parameter	0.3mg/kg	3mg/kg	30mg/kg
C_{max} (µmol/l)	0.60	4.9	39.2
T_{max} (h)	2	2	8
AUC_{0-48} (µmol·h/l)	9.38	81.6	724
$T_{1/2}$ (h)	6.8	8.5*	7.5
Cl (ml/min/kg)	1.64	1.99*	2.38

*N=2; all other indices, N=3

BPK(F) 1994/029 In vitro binding of letrozole to human serum proteins, human erythrocytes and serum proteins from rat, dog, baboon, mouse and rabbit. Conducted at Bioanalytics and pharmacokinetics, Ciba-Geigy, Malmaison, France in 1995.

Protein binding in serum from rat, dog, baboon, mouse and rabbit was 56.2, 54.0, 49.4, 53.7, and 53.3%, respectively. In comparison, protein binding in human serum was 58.3% for concentrations ranging from ng/ml. Binding in human serum albumin was 55.1%. Protein binding was primarily a result of albumin; binding was negligible with alpha-1-acid glycoprotein and gamma globulin. No further data were provided.

DMET(EU) 3/1996 In vitro characterization of the human cytochrome P450 isozyme(s) responsible for the biotransformation of CGS 20267 to CGP 44645. Conducted by Drug metabolism and exploratory toxicology, Ciba-Geigy, Basle, Switzerland in 1996.

The *in vitro* biotransformation of CGS 20267 to the carbinol metabolite CGP 44645 was investigated in rat and human liver microsomes. CGS 20267 was a poor substrate for the biotransformation to CGP 44645 both in rat and human microsomes. The formation of CGP 44645 was not saturable; a K_m of ~200µmol/l was estimated by the study author for the biotransformation of CGS 20267 to the carbinol metabolite.

Ketoconazole was identified as an inhibitor of the biotransformation in rat and human microsomes. The human cytochrome P450 isozymes, CYP3A4 and CYP2A6 were indicated to catalyze the biotransformation of CGS 20267 to CGP 44645. Saturation was observed with CYP2A6 at concentrations above 12.5µmol/L.

DMET(US) 96003 Evaluation of a new chemical entity as an inhibitor of human P450 enzymes. Conducted by

CGS 20267 acts as a competitive inhibitor for CYP2A6 and CYP2C19 in human liver microsomes, using coumarin and S-mephenytoin, respectively, as the test substrates. The K_i for CYP2A6 was 0.12µM, indicating that letrozole may act as a potent inhibitor of this isoenzyme at potentially clinically relevant concentrations (considering peak plasma concentration of 0.11µM following a 2.5mg dose in humans). The K_i for CYP2C19 was 9µM, suggesting that letrozole is a significantly less potent inhibitor of this isoenzyme when compared to CYP2A6. The disposition of agents metabolized by these isozymes may be influenced by concurrent administration of letrozole in humans.

DM(EU) 16/1994 Long-term elimination of ^{14}C-labelled CGS 20267 in female rats.

Conducted by Drug metabolism and exploratory toxicology, Ciba-Geigy, Basle, Switzerland in 1994.

species: Tif: rats (3females)

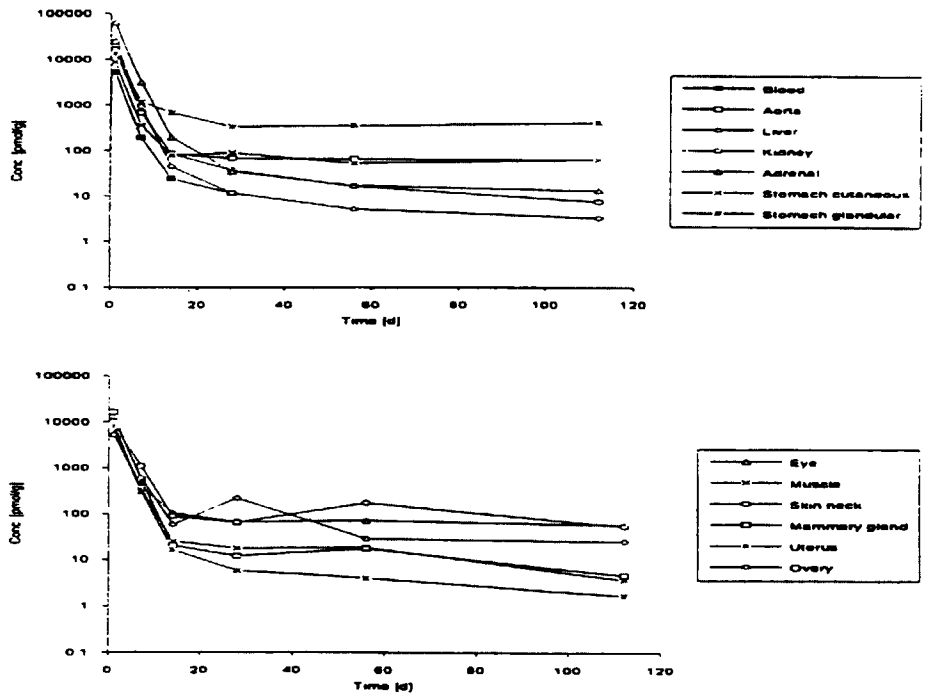
dosing: 5mg/kg

sample collection: 1, 7, 14, 28, 56 and 112 days following dosing

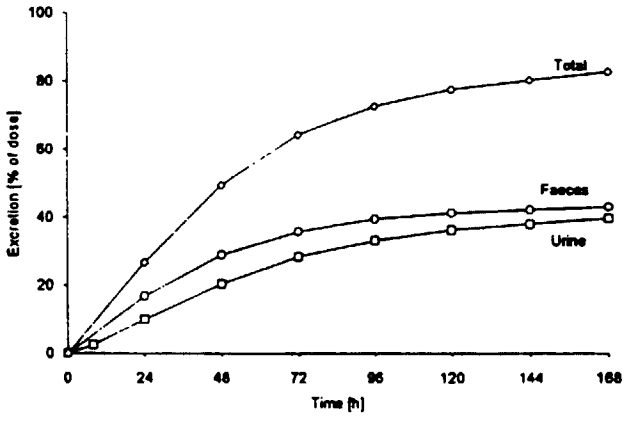
The mean ^{14}C concentration of letrozole in blood was 5.1nmol/g within 24 hrs of dosing. Concentration of letrozole in tissues and organs examined was 2-6 fold higher than in blood. The

elimination of letrozole from organs and tissues was multiphasic with the major decline in concentration up to day 14 following dosing followed by a very slow terminal decline between days 28 and 112 post dose. The slowest decline was observed in skin, stomach and eye. The elimination of letrozole in female rats was slower than that previously observed in male rats.

Long-term elimination of radioactive substance(s) from organs and tissues of female rats after peroral administration of 5 mg/kg [^{14}C]CGS 20 267 (means of 3 animals).



Cumulative excretion of radioactive substance(s) in urine and faeces of female rats after peroral administration of 5 mg/kg [^{14}C]CGS 20 267 (means of 3 animals).



CRB R 15/1993 CGS 20267 plasma concentrations in male and female rats on days 1, 207 and 365 following CGS 20267 administrations of single and repeated once daily doses of 0.3, 3, and 30mg/kg body weight in a 6/12-month oral toxicity study. Conducted by the Biopharmaceutical Research Center (CRB), Ciba Geigy, Rueil-Malmaison, France in 1993 according to GLP.

species: Tif: rats (satellite group of 5 animals/sex/dose)
drug: CGS 20267, batch 800289
dosage: 0.3, 3 and 30mg/kg (0.01, 0.1, 1mg/ml suspensions in 3% corn starch)
samples collected: 2, 4, 8 and 24 hrs postdose on day 1; predosing, 2, 4, 8, 12 and 24 hrs postdosing on days 207 (7 months) and 365 (study termination) + 32 and 48h postdose on day 365
assay:
LOQ: µmol/L

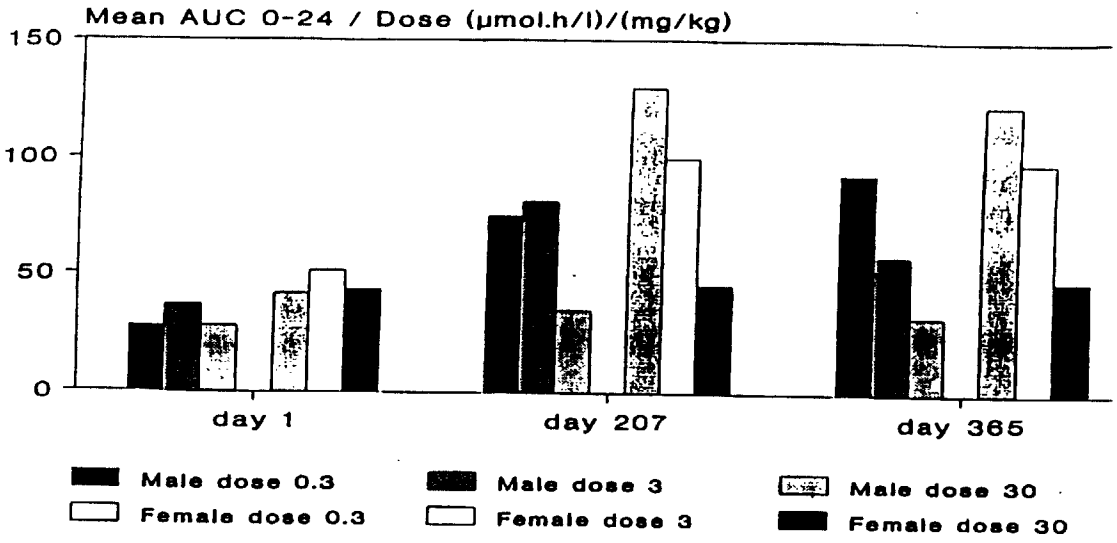
When letrozole was administered orally once daily for 1 year, the plasma kinetics of the parent drug changed in a dose-and sex-dependent manner as shown in the following tables and graph. Plasma concentrations (C_{max}) were higher in females compared to male rats; a high degree of inter-animal variability was observed. Observed AUC were dose-proportional in males and females on day 1 only. The exposure, as indicated by the dose-normalized AUC, was 40 to 55% higher in females. This may be related to the higher $T_{½}$ observed in females. AUC were higher on days 207 and 365 compared to day 1; the dose-response was similar on day 1 for all doses in males and females, but decreased with increasing dose on days 207 and 365. For each dose, the AUC observed on day 207 was not significantly modified by further dosing up to day 365. In general, the median T_{max} decreased during dosing in both sexes at all doses. In males, the $T_{½}$ on days 207 and 365 were higher than day 1 with the LD and MD, but comparable with the HD. The $T_{½}$ was shorter on days 207 and 365 with the HD in both males and females; no accumulation was apparent with the HD. The data suggest induction of metabolism at the HD which counteracts the accumulation seen at the lower doses.

Previous studies in rats have indicated that systemic exposure to metabolites of letrozole following oral administration has been low, with the unchanged drug accounting for most of the circulating radioactivity. As was previously suggested, the plasma clearance of letrozole appears to be dependent on the dose and duration of treatment.

Plasma concentration (μmol/L) in rats administered letrozole for 52 weeks							
Day	Hour	Males			Females		
		LD	MD	HD	LD	MD	HD
1		0.157	2.73	23.8	0.447	5.73	59.7
207		0.610	6.77	25.8	1.28	9.40	36.7
		0.801	7.96	28.6	1.46	9.89	40.8
365		0.827	5.12	29.7	1.32	9.99	47.4
		0.955	5.59	24.7	1.38	10.1	46.7

AUC (0-24), Dose-normalized AUC (0-24) and T½ of rats administered letrozole for 52 weeks						
Day of Dosing	Males			Females		
	LD	MD	HD	LD	MD	HD
AUC (0-24) (μmol.h/l)						
1	8.37	110	837	12.5	154	1300
207	22.5	243	1040	38.9	301	1370
365	28.0	174	973	37.0	295	1430
Dose-normalized AUC (0-24) [(μmol.h/l)/(mg/kg)]						
1	27.9	36.7	27.9	41.7	51.3	43.3
207	75.0	81.0	34.7	130	100	45.7
365	93.3	58.0	32.4	123	98.3	47.7
T½ (h)						
1	10	14	18	42	57	ND
207	45	42	19	93	36	22
365	46	36	18	68	38	28
365*	45	28	14	64	41	22

*calculated with 48h plasma concentration on day 365



CRB R 22/1993 **CGS 20267 plasma concentrations in male and female dogs on days 1, 182 and 364 following CGS 20267 administrations of single and repeated once daily doses of 0.03, 0.3 and 3mg/kg body weight in a 6/12-month oral toxicity study.** Conducted by the Biopharmaceutical Research Center, Ciba-Geigy, Malmaison, France in 1993 according to Swiss GLP.

species: beagle dogs (2/sex), 6-8 months of age
drug: CGS 20,267, batch 800289
dosage: 0.03, 0.3, 3mg/kg administered perorally (letrozole mixed with lactose) in gelatine capsules once daily, 7 days/week for 12 months (administered prior to feeding)
assay: measurement of letrozole in plasma collected prior to dosing and 2, 8 and 24 hrs following dosing on study days 1, 182, and 364
LOQ: µmol/l

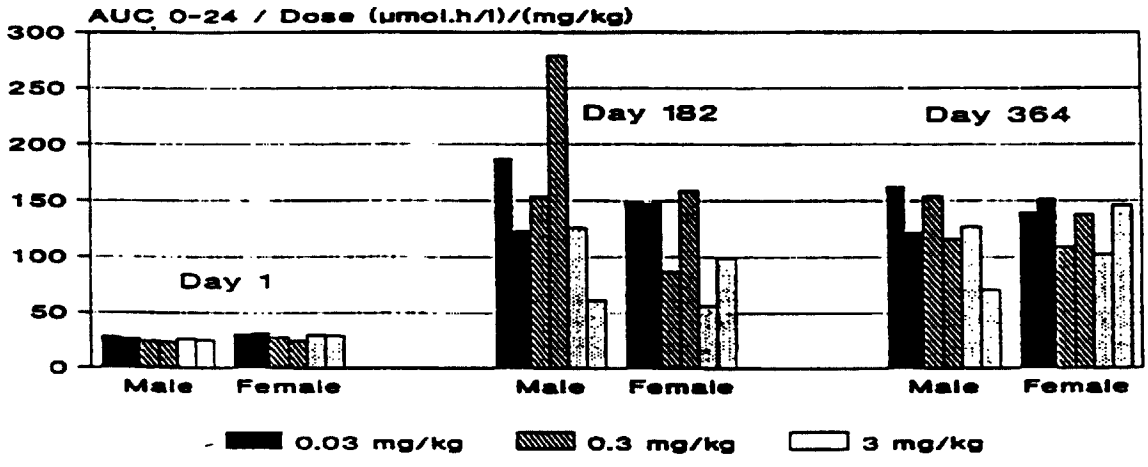
Plasma concentrations were incorrectly labeled as nmol/L in study (µmol/L in appendix).

Plasma concentrations (C_{max}) of male and female dogs were comparable at 0 and 24 hours on days 182 and 364; C_0 , C_{24} at days 182 and 364 were higher than C_{24} on day 1 for all dose groups. Plasma concentrations and observed AUC were dose-proportional in males and females on day 1 only; C_{max} on day 182 were comparable to those on day 364. Plasma profiles were similar for males and females for all doses at all treatment days. AUC for males and females were comparable on days 182 and 364 and higher than AUC on day 1; the steady-state observed on day 182 was not modified by further daily treatment to day 364. The dose-response, as indicated by the dose-normalized AUC, was similar between sexes. The dose-normalized AUC were similar on day 1 for males and females at all doses, but generally decreased with increasing dose on days 182 and 364 in both sexes. The dose-response suggested an induction of metabolism at the highest doses on days 182 and 364. Estimated T½ (comparison of previous single dose data to multiple dose data of this study) indicated drug elimination of 82, 70 and 92h

(2.9 to 3.8 days) for doses of 0.03, 0.3 and 3mg/kg, respectively. T½ was not dose or time dependent in dogs.

Plasma concentrations (µmol/L) in dogs administered letrozole for 12 months							
Day	Hour	Males/Dose (mg/kg)			Females/Dose (mg/kg)		
		0.03	0.3	3	0.03	0.3	3
1		0.036	0.335	3.33	0.043	0.356	3.89
182		0.184	3.08	11.4	0.196	1.31	8.65
		0.173	2.43	11.5	0.186	1.53	9.07
364		0.166	1.73	12.1	0.173	1.46	15.5
		0.169	1.58	10.9	0.173	1.48	14.9

AUC (0-24) and Dose-normalized AUC (0-24) of dogs administered letrozole for 12 months						
Day of Dosing	Males			Females		
	0.03	0.3	3	0.03	0.3	3
AUC (0-24) (µmol.h/l)						
1	0.832	7.22	76.6	0.948	7.81	88.6
182	4.69	65.1	281	4.47	36.8	233
364	4.28	40.6	297	4.38	37.1	372
AUC (0-24) [(µmol.h/l)/(mg/kg)]						
1	27.8	24.1	25.5	31.6	26	30
182	156	216.5	93.5	149	122.6	77.5
364	142.5	135	98.7	146	123.5	124



BPK(F) 1994/028 **Letrozole plasma concentrations in male and female rats on weeks 14, 53 and 78 following letrozole oral administration via gavage of daily doses of 0.1, 1 and 10mg/kg body weight in a 104-week carcinogenicity study.** Conducted by Bioanalytics and Pharmacokenetics, Ciba Geigy, Malmaison, France in 1995 according to Swiss GLP.

species: rats (10 animals/sex/dose)

drug: CGS 20267, batch 800192

dosage: 0.1, 1, 10mg/kg (0.01, 0.1, 1mg/ml suspensions in 3% corn starch)

samples collected: predose, 2, 4, 8 and 24 hrs postdose during weeks 14, 53, and 78

assay:

LOQ: µmol/L

Plasma concentrations (C_{max}) at each time point indicated substantial inter-animal variability; variability was lower at week 14. T_{max} was variable: from 2-8hrs in males and 2-24hrs in females. At all doses, plasma concentrations in females were higher than those in males, which were also observed during the 12 month toxicity study in rats. Similarly, exposure, as indicated by dose-normalized AUC, was higher in females. Dose normalized AUC decreased with increasing dose; however, for a given dose, the systemic exposure to letrozole increased with the duration of dosing. This may be explained by the induction of letrozole metabolism following repeated administration. (Exception to ↓ in dose response with ↑ in dose was observed at 1mg/kg in ♀ at week 14, ♂ and ♀ at week 53 and ♂ at week 78) Predose concentrations of letrozole were higher at week 78 compared to concentrations at week 53 which were higher compared to concentrations at week 14. There was slight drug accumulation over the duration of sampling.

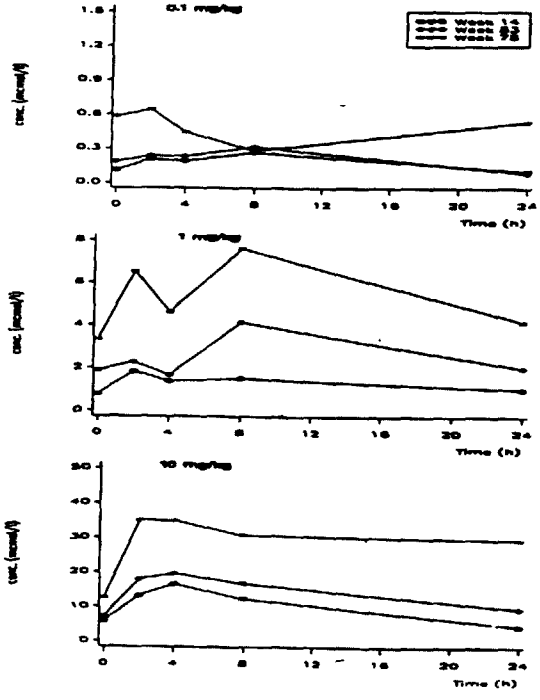
Range of plasma concentrations and corresponding CV (%) in male and female rats administered letrozole for 103 weeks

Dose	Week	Males		Females	
		Plasma concen[a]	CV (%)[a]	Plasma concen[a]	CV (%)[a]
0.1	14		9.22-48.2		14.1-26.0
	53		14.8-65.8		24.8-76.1
	78		29.0-95.5		10.3-52.0
1	14		19.2-45.9		31.6-98.2
	53		28.4-84.6		15.5-51.3
	78		28.1-85.3		29.1-68.7
10	14		9.76-58.0		12.3-40.8
	53		17.6-64.9		33.2-61.0
	78		34.4-76.6		12.4-52.9

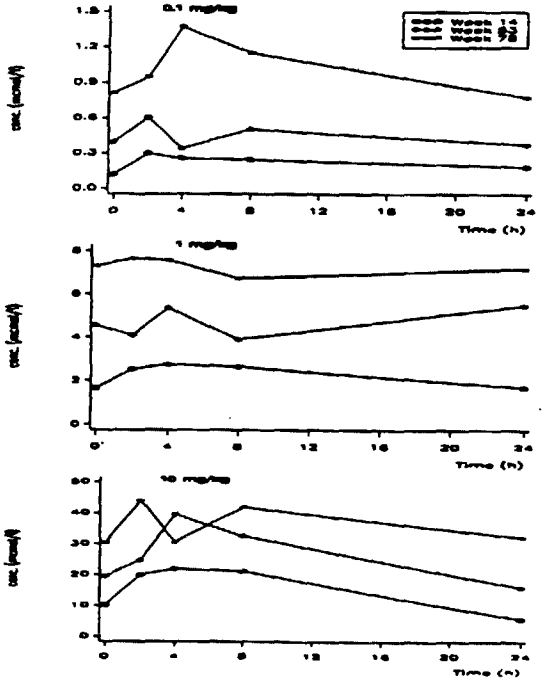
[a] Range of 4 samples measured at 2, 4, 8 and 24 hrs following dosing

AUC and normalized AUC				
Dose	Males		Females	
	AUC (0-24h) (µmol.h/L)	Normalized AUC (µmol.h/L) /(mg/kg)	AUC (0-24h) (µmol.h/L)	Normalized AUC
Week 14				
0.1	4.53	45.3	5.38	53.8
1.0	32	32	55.1	55.1
10	242	24.2	374	37.4
Week 53				
0.1	5.16	51.6	10.6	106
1.0	69	69	112	112
10	347	34.7	644	64.4
Week 78				
0.1	10.3	103	24.5	245
1.0	140	140	170	170
10	732	73.2	889	88.9

Letrozole mean plasma concentrations in male rats on weeks 14, 53 and 78 after daily administration of 0.1, 1 and 10 mg/kg



Letrozole mean plasma concentrations in female rats on weeks 14, 53 and 78 after daily administration of 0.1, 1 and 10 mg/kg



BPK(F) 1995/006 **Letrozole plasma concentrations in male and female mice on weeks 26, 54 and 78 following letrozole oral administration via gavage of daily doses of 0.6, 6 and 60mg/kg body weight in a 104-week oral carcinogenicity study** . Conducted by Bioanalytics and Pharmacokenetics, Ciba Geigy, Malmaison, France in 1995 according to Swiss GLP.

species: mice (control: 5animals/sex, LD, MD, HD: 15animals/sex)

drug: CGS 20267, batch 800192

dosage: 0.6, 6, 60mg/kg/day

samples collected: 2, 6, 24 hrs postdose during weeks 26, 54, 78 and 79; control samples collected at 2 hrs during the indicated weeks

assay:

LOQ: µmol/L

The plasma concentrations (C_{max}) increased with increasing dose and were comparable to the C_{max} values observed in a previous study measured on weeks 2 and 10 in mice administered the same doses. Unlike the plasma concentrations of rats, there were no apparent gender differences in C_{max}. There was no letrozole accumulation following repeated dosing.

Table 5: **Mean (n = 1 to 5) letrozole plasma concentrations (µmol/L) in mice**

104-week oral carcinogenicity study in mice following letrozole administration via gavage of daily doses of 0.6, 6 and 60 mg/kg b.w.

Time (h)	0.6 mg/kg b.w.		6 mg/kg b.w.		60 mg/kg b.w.	
	M	F	M	F	M	F
Week 26 (days 177-178)						
2	1.04	1.13	12.5	11.2	108	96.1
6	0.602	0.567	7.88	6.62	83.4	121
24	0.078	ND	1.12	1.05	8.13	12.5
Week 54 (days 372-373)						
2	0.898	0.918	11.4	10.7	107	116
6	0.643	0.640	8.36	9.14	82.7	100
24	0.078	0.093	1.19	1.77	5.89	17.2
Week 78 (day 545) and Week 79 (day 546)						
2	1.19	1.19	12.9	11.5	81.8	101
6	0.633	0.744	8.22	9.47		116
24	0.065	0.100	0.839	2.38		5.75

M : male mice ; F : female mice ; ND : not detected.

CRBR 46/1993 **Plasma concentrations in male and female mice on weeks 2 and 10 following CGS 20267 oral administration via gavage of daily doses of 0.6, 6 and 60mg/kg body weight in a 13-week toxicity study**. Conducted by Ciba-Geigy, Malmaison, France in 1994)

Similar pharmacokinetic profiles were observed for male and female mice.

C_{max}, AUC and normalized AUC in male and female mice following oral administration of letrozole for 13 weeks						
Dose (mg/kg)	C_{max} (µmol/L)		AUC_{0-12} (µmol.h/L)		AUC_{0-12}/Dose (µmol.h/L)/(mg/kg)	
	week 2	week 6	week 2	week 6	week 2	week 6
0.6	0.559	0.786	4.06	5.33	6.77	8.88
6	7.09	8.52	49.2	64.1	8.20	10.7
60	69.7	70.7	622	614	10.4	10.2

Pharmacokinetics summary

Peroral absorption of CGS 20267 was more complete in mice when compared to rats and dogs; however, the elimination of labeled letrozole from blood and plasma of mice was more rapid than these other species. In addition, letrozole appeared to be metabolized to a lesser extent in mice compared to rats or dogs. Following oral administration of 0.6-60mg/kg letrozole to mice for up to 18 months, the systemic exposure was essentially dose-proportional and the kinetics were independent of dose, time or gender. Plasma concentrations of letrozole in mice were lower and more constant over a longer duration following oral administration until 24hr when levels declined in a manner similar to iv administration. The distribution patterns of letrozole were greatest in liver and adrenal (10 to 17 and 5-fold the concentration in blood, respectively) at 5min and 6 hrs following dosing.

Following single doses of ^{14}C-labeled letrozole to male or female rats, the concentrations of label decreased very slowly during the first 4-8hrs; thereafter, the t½ was 7-8hrs for males and 40-50hrs for females. This may have been the result of slower absorption or the enterohepatic circulation of letrozole. Upon repeated oral administration to rats of either gender, dose- and time-dependent kinetics were observed; these were probably a result of both metabolism induction and drug accumulation. As with mice, letrozole was evenly distributed throughout the body irrespective of the route of administration with the exception of the adrenals and liver which exhibited 13-and 4-fold the letrozole concentration in blood, respectively. Accumulation of letrozole in the skin of the neck was observed following 10-30 days of treatment; this site in rodents is considered to be predisposed to external contamination by orally administered letrozole. The clearance of labeled letrozole from blood, plasma and tissues, and excretion in urine and feces was markedly slower in female compared to male rats. Accordingly, systemic exposure in females was approximately 4-fold higher than in males.

In dogs, the kinetics of letrozole was generally dose-proportional; there appeared to be no gender difference. Letrozole was eliminated very slowly (T_{max} up to 2 hrs; t½ not provided) from the plasma following single iv doses of 0.02-2mg/kg; the plasma elimination half-life of letrozole appeared to be independent of dose or treatment duration. Following administration of an oral dose of 0.1mg/kg letrozole, only 25-60% of the dose was excreted within 7 days following dosing. As with mice and rats, letrozole was evenly distributed throughout the body of dogs

irrespective of the route of administration.

Systemic exposure to metabolites of letrozole is low. In mice, rats and dogs, the concentration of total radioactivity in plasma approached the concentration of unchanged letrozole following administration of the ^{14}C-label. The major metabolite, which is inactive in inhibiting aromatase, is 4, 4'-methanolbisbenzonitrile (CGP 44645). It is eliminated primarily as its glucuronide into urine. Carbinol CGP 44645 and the O-glucuronide of the carbinol metabolite were similarly excreted into the urine of mice, rats and dogs. The ratio of unchanged letrozole to total carbinol metabolite (CGP 44645 and O-glucuronide) was ~4:1 in the urine of ♂ mice, ~1:6 in the urine of ♂ rats and ~2:3 in the urine of ♂ dogs.

In human and rat liver microsomes, formation of CGP 44645 was inhibited by ketoconazole and TAO, known inhibitors of CYP3A4. In human liver microsomes, letrozole appeared to competitively inhibit CYP2A6 and CYP3A4.

The ratio of renal to fecal excretion of letrozole varied between species; the ratio was ~1:1 in male or female rats, ~2:1 in the dog and about 8:1 in mice. The rates of excretion of labelled letrozole following dosing differed between species. Clearance of parent drug from plasma decreased in the order: mouse > male rat > female rat > dog. Following single doses, the t½ of letrozole was approximately 4-5hr in mice, 7-10h in male rats, 20-50h in female rats and 60-90h in dogs.

Letrozole binds to plasma proteins in all species, primarily as a result of albumin. Binding in human serum was 60% compared to 56, 54, 50, 54 and 53% in rat, dog, baboon, mouse and rabbit, respectively. Protein binding was negligible with α-1-acid glycoprotein and gamma globulin. The concentration in the erythrocyte fraction of humans is approximately 80% of that in plasma.

Comparative pharmacokinetic parameters in mice, rats and dogs following multiple oral dosing for 52-54 weeks

Species	Dose (mg/kg)	C_{max} (µmol/L)	AUC_{0-24} (µmol.h/l)	AUC_{0-24} normalized	T½ (h)
Rat	0.3	0.8-1.3	28.0-37.0	93.3-123	46-68
	3	5.1-10.0	174-295	58.0-93.3	36-38
Dog	0.3	1.5-1.7	37.1-40.6	123.5-135	-
	3	12.1-15.5	297-372	98.7-124	-
Mice	0.6	0.89-0.92	-	-	-
	6	10.7-11.4	-	-	-

- = not determined

IV. TOXICOLOGY

A. Single Dose Toxicity

The following 4 studies were conducted by Ciba-Geigy Limited, Basle, Switzerland according to Swiss GLP. They were previously reviewed by HR Prasanna in 1991.

896022 **Acute oral toxicity study in mice.**
896023 **Acute oral toxicity study in rats.**
896024 **Acute intraperitoneal toxicity study in rats.**
896025 **Acute oral toxicity study in dogs.**

Single dose toxicity in 3 species dosed orally with letrozole			
Species/strain	LD_{10} (mg/kg)		Observations
	♂	♀	
Mouse/ Tif:MAGf(SPF)	>2000	<2000 (LD_{20}=2000; 1/5 within 24hr)	↓motor activity, irregular respiration, muscular hyptonia, piloerection, inhibition of pain response, hyperemia of mucosa and skin, arched back, cool body; recovery 3 days
Rat/ Tif:RAlf(SPF)[a]	>2000	>2000	↓motor activity, ataxia, dyspnea, muscular hypotonia, inhibition of pain response, ruffled coat, salivation, hyperemia of mucosa and skin, ↑defecation cool body; recovery 5 days
Dog/beagle[b]	-	-	200mg/kg- death due to respiratory failure within 48hr/dosing; preceeded by convulsions, tachycardia,hyperthermia; 100mg/kg- hyperactivity, ataxia, tonic-clonic convulsions, tremors, labored respiration, muscular hypertonia, ↓motor activity, ↑urinary output; recovery day 12

[a] Acute ip rat study LD_{50}~500mg/kg; LD_{10} between 50-500mg/kg; mice and rats dosed orally via gavage
[b] Dosed via capsule; not possible to determine LD_{10} since only 1 ♂dog/dose treated, death at 200mg/kg

B. Multiple Dose Toxicity

946092 **14-day intravenous toxicity study in rats.** Conducted by Ciba-Geigy Limited, Basle, Switzerland in 1995 according to GLP.

886120 **28-day oral dose rangefinding study in rats.** Conducted by Ciba-Geigy Limited, Basle, Switzerland in 1989 according to GLP.

946093 **14-day intravenous toxicity study in dogs.** Conducted by Ciba-Geigy

Limited, Basle, Switzerland in 1995 according to GLP.

886121 **28-day oral dose rangefinding study in dogs.** Conducted by Ciba-Geigy Limited, Basle, Switzerland in 1989 according to GLP.

Comparison of the toxicity of oral and intravenous letrozole administration in rodent and non-rodent animal models						
Animal/ Route/ Duration	Dosing	Mortality/ Clinical Observa	Body Wt./ Food Consump	Hematol/ Clin Chem	Organ wts	Histopath
rat/iv/14d[a]	.03,.3,3mg/kg1X/d (.18,1.8,18mg/m^2) N=5/sex/group-main 6/sex/group-satellite	1deathHD♀; HD:↓activity, dyspnea,pallor, recumbency	MD+HD gain ↓♂, ↑♀; FC↓	HD♂ and ♀ slight↓RBC; all doses♀-↓↓CHE HD↓ protein,album ♀>sensitiv	↓MD,HD uterine wt ↑ovary wt all dose	HD:small uteri, ↓estrus, hyperpl mammary(MD+HD),↑mitosis/liver
rat/oral/28d[a]	.5,5,50mg/kg1X/d (3,30,300mg/m^2) N=5/sex/group	HD♀:stiff gait, arched back, muscular hypotonus, constant diestrus	gain↓♂+ HD♀,↑LD MD♀; FC ↓♂, HD♀	♀-leucocytes↑, ↑ASAT,ALAT,AP, free testosterone, ↓CHE	HD-sl↑ liver, sl↓ epididym sem ves, prostate; dose-rela↓ uterine wt	hypertrophy,fatty change/liver HD;↑height of epithelial cells of thyroid M/HD♀; adrenal vacuoliza./HD♂, hypertrophy/pituit/ HD♀;atrophy/ vagina M/HD ♀
dog/iv/14d	.02,.2,2mg/kg1X/d (.4,4,40mg/m^2) N=3/sex/group	None relevant	None rel.	None relevant	dose rela↑ ovary	M/HD enlarged ovaries,cysts, atrophy vagina; all dosed ♂: hyperplasia/ Leydig c/testes
dog/oral/ 28d	5mg/kg1X/d(100mg/ m^2)N=2/sex/group	Minor observa	None rel.	Sl variation WBC; ↑ALAT,ASAT, triglycer,α2-glob, tot prot, album, calcium; free testosterone↑	↑kidney, liver, ovary, ↓thymus, adrenal, uterine	moderate-marked tubular dilatation, prolifera/tubular epithe, nephritis/ kidney;inflamma, necrosis/liver; ↓degenera/thymus; adrenal atrophy; hypertrophy, hyperpl/Leydig c; atrophy/uterus, vagina;ovarian cyst, undevel mammary gl

[a] Tif: rats

It is difficult to compare the route of letrozole administration using the studies listed above since oral dosing in rats is >15-fold iv administration, oral dosing in dogs is 2.5-fold iv administration and the period of oral dosing is 2-fold the duration of iv administration. However, comparing the above data in general, it appears that administration of letrozole via the oral route results in increased toxicity involving the liver, kidney and adrenal in rats and dogs. Adrenal atrophy and/or vacuolization may indicate interference with steroid synthesis. Hypertrophy and fatty changes of the liver observed in rodents may be a result of enzyme induction. Necrosis of the liver observed in dogs at 5mg/kg was not exhibited in longer term studies at lower dose levels. Kidney changes, which were observed in rodent and non-rodent species, may have been the result of lowered estrogen levels.

91-6010 6/12-Month Oral Toxicity Study in Rats. Conducted by Ciba-Geigy Limited, Basel, Switzerland in 1995 according to OECD GLP (with exception of toxicokinetic analyses [performed by ; x-ray examinations and bone analyses [performed by I. Wiesenburg and K. Muller, Bone Metabolism, Pharma Research, Ciba-Geigy Limited, Basel] and investigation of vaginal smears [performed by A. Bhatnagar, Endocrinology, Pharma Research]).

Methods

species: Tif: rats (40/sex/dose including 10/sex/dose for interim sacrifice, 20/sex/dose for main study, 5/sex/dose for recovery, 5/sex/dose for determination of plasma levels, hormone parameters (♂ and ♀), and vaginal smears (♀)).
drug: Letrozole lot # 800289; control (Klucel HF)
dosage: 0.3, 3.0, and 30mg/kg for 364 days
age, wt: 6-8 weeks, 158-270g
route: gavage 1X daily
recovery: minimum of 28 days
basis of dose selection: 3-Month Oral Toxicity Study in Rats, # 89-6056

Observations

mortality	daily
clinical signs	twice daily
body weights	daily
food consumption	twice weekly
hematology	pretest, weeks 6, 13, 27, 38, 53 and 57
clinical chemistry	pretest, weeks 6, 13, 27, 38, 44 (ALAT, ASAT, AP only), 53 and 57
urinalysis	♂ - weeks 2, 7, 11, 23, 35 and 52 in control and HD, week 56 in all groups; ♀ - week 2 control and HD, weeks 7, 11, 23, 35 and 52 LD and MD, week 56 all dose
ophthalmoscopy	pretest, weeks 24, 51, and 56 + weeks 12 and 37 in control and HD

organ weights	interim sacrifice at 6months, final sac at 12months and following 4 weeks of recovery
gross pathology	interim sacrifice at 6months, final sac at 12months and following 4 weeks of recovery
histopathology	see histopathology table of organs
toxicokinetics	blood samples collected from satellite group of 5rats/sex/group on day 1 and 6 months at 2, 4, 8, and 24 hrs following dosing and at study termination at 2, 4, 8, 12, 24, 32, and 48 hrs following dosing for drug plasma levels (reviewed in Pharmacokinetics/Toxicokinetics section of NDA)

Results

Mortality and clinical observations

Five HD ♀ were sacrificed moribund at 8-9 months as a result of bone fractures; macroscopically bones appeared soft and friable and bony callus formation was observed microscopically. Additional deaths during the study included 2 HD ♂ with malignant lesions (1 mammary adenocarcinoma on study day 235 and 1 metastisized malignant lymphoma of the hemolymphoreticular system on study day 188), 1 LD and HD ♂ and 1MD ♀ with myocarditis, 1 MD ♀ with purulent inflammation and 1 LD ♀ with cystitis. In addition, 4 animals (1 control and 3 HD animals) died from general poor health, and 8 animals (4 HD) from technician error.

Clinical Observations of rats administered letrozole for 12 months								
Findings	Control		3mg/kg		10mg/kg		30mg/kg	
	♂	♀	♂	♀	♂	♀	♂	♀
Decreased activity	-	1/40	2/40	-	1/40	3/40	40/40	40/40
Muscular hypotonia	-	1/40	1/40	-	-	2/40	2/40	40/40
Skin lesions/alopecia	4/40	9/40	5/40	4/40	10/40	4/40	22/40	25/40
Retracted leg(s)	-	-	-	-	-	1/40	1/40	7/40*
Chromodacryorrhea	1/40	-	1/40	-	1/40	2/40	6/40	6/40
Chromorhinorrhea	-	-	-	-	-	-	1/40	2/40
Salivation	-	2/40	-	-	2/40	2/40	5/40	5/40
Piloerection	-	2/40	1/40	-	2/40	3/40	1/40	-
Eye opacity	3/40	1/40	3/40	6/40	3/40	2/40	3/40	7/40

* 5 animals sacrificed moribund as a result of bone fractures

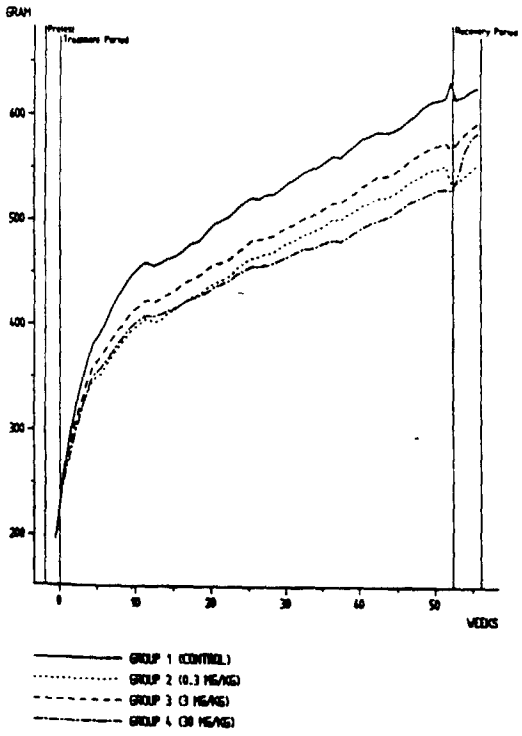
Many of the clinical observations observed during letrozole treatment were dose related with significantly increased incidence at the HD (decreased activity, muscular hypotonia, skin lesions, retracted legs). In addition, many of the clinical observations were exhibited throughout the study or during the last 6 months of dosing.

Following study week 44, selected animals from test and control groups were tested for viral infection and found to be positive for Kilham Virus; the study author indicated that this finding did not effect the study quality or integrity.

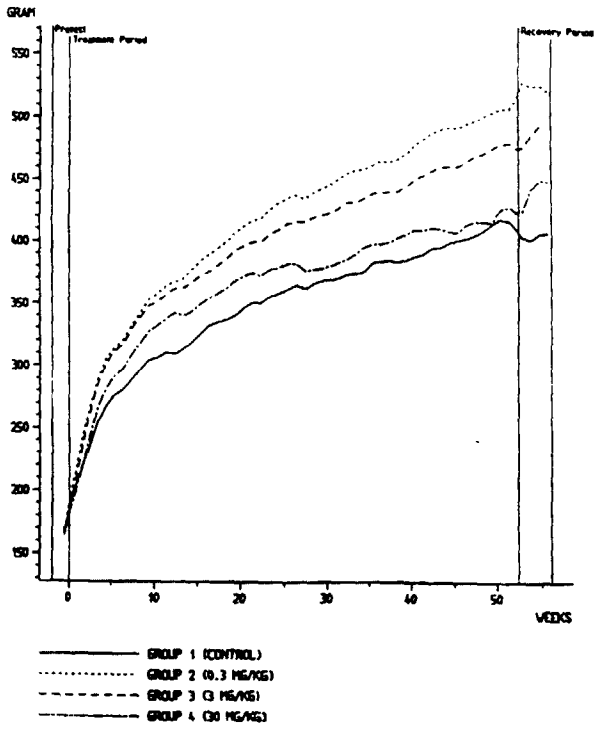
Treated ♀ exhibited diestrus throughout the dosing period; LD and MD, and HD recovery animals resumed cyclicity by recovery weeks 55 and 57, respectively.

Body weight (see graphs):
Body weights of ♂ administered letrozole were significantly depressed from study week 2 to study termination when compared to concurrent controls. At week 26, mean weights of LD and HD animals were ↓ 11 and 12%, respectively; at 53 weeks of dosing, weights continued to be ↓ 15, 10 and 16% in LD, MD, and HD animals, respectively. Following 4 weeks of recovery, weights recovered slightly but were still ↓11 and 7% at the LD and HD, respectively. In contrast, letrozole ↑ body weights in all ♀ groups. Mean ♀ body weights were 20, 15 and 6% higher than concurrent controls in the LD, MD and HD groups, respectively at week 26 and 25, 16 and 3% higher following 53 weeks of dosing. At the end of the recovery period, weights were increased 28, 22, and 10% in LD, MD and HD ♀. Weight change was more pronounced in females at the LD and MD.

BODY WEIGHTS
MALES



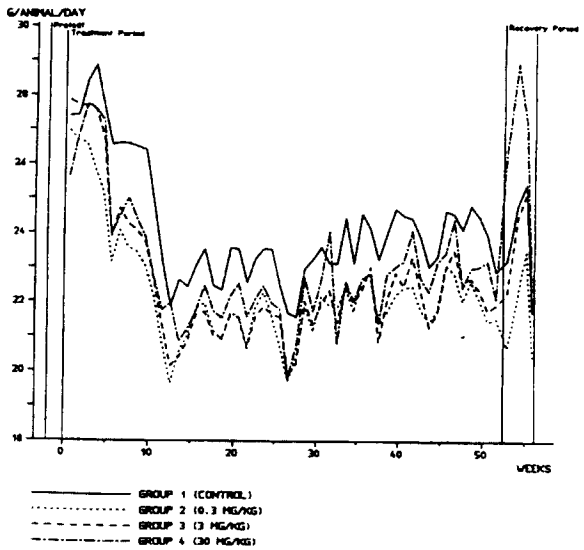
BODY WEIGHTS
FEMALES



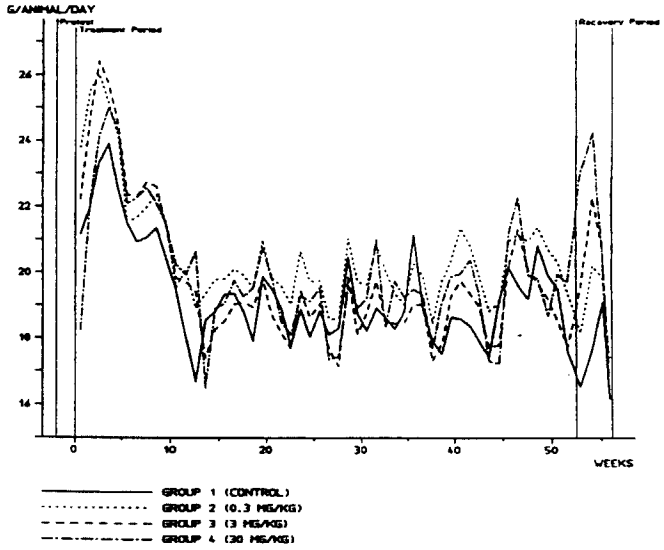
Food Consumption (see graphs):

Food consumption was depressed up to 13% in dosed ♂ consistently from week 4 (LD) and week 6 (MD, HD) to week 28 and sporadically to study termination. Consumption of food was similar to concurrent controls during the recovery period. Food consumption of dosed ♀ was similar to concurrent controls.

FOOD CONSUMPTION
MALES



FOOD CONSUMPTION
FEMALES



Hematology, Clinical Chemistry and Urinalysis:

One HD ♂ sacrificed moribund during study week 27 exhibited depressed red cell and increased white cell parameters; histopathology findings included malignant lymphoma with organ infiltration. Findings in this animal were not related to letrozole administration. Hematological parameters of other dosed ♂ were similar to concurrent controls. Red cell parameters of LD and MD ♀ were slightly increased (hgb, hct ↑ up to 6%, RBC ↑up to 11%) during dosing and recovery when compared to concurrent controls; red cell parameters of HD ♀ were similar to controls. Leukocyte counts of dosed ♀ were increased from 6-54% during the administration of letrozole; following the recovery period, the leukocytes of MD ♀ only continued to be elevated when compared to concurrent controls. Lymphocyte counts of MD and HD ♀ were increased 58% at week 6; lymphocyte counts of all dosed ♀ were increased 12-40% throughout the dosing period and were reversible following recovery. Changes in leukocyte and lymphocyte counts were likely due to a fall in estrogen as a result of aromatase inhibition; ↑ in lymphocytes have been found to occur in ovariectomized rats.

ASAT levels of dosed and control animals were increased throughout the dosing and recovery period; study authors suggested this finding to be a result of a viral infection observed in dosed and control animals. Many of the changes noted in the table below can be attributed to aromatase inhibition and enzyme induction.

% Change in Clinical Chemistry Parameters of Rats Administered Letrozole										
		Week 6			Week 27			Week 53		
parameter	sex	LD	MD	HD	LD	MD	HD	LD	MD	HD
AP	♂	↑6	↑11	↑15		↑10			↑16	
	♀	↑16	↑18	↑40	↑19	↑24	↑34	↑22	↑29	↑56
CHE	♂	↑23	↑29	↑8	↑14	↑23	↑8		↑10	
	♀	↓59	↓61	↓62	↓55	↓55	↓53	↓49	↓50	↓42
Chol	♂			↑23		↑6				↑25
	♀		↑9	↑26		↑19	↑40		↑18	↑68
Trigl	♂	↓8		↓8	↑32	↑37	↑13	↑17	↑39	↑10
	♀	↑7	↑26			↑9	↓15	↑21	↑52	
γ-glob	♂		↓29	↓39						
	♀	↓63	↓70	↓62	↓24	↓18	↓24	↓27	↓28	↓28
T_4[a]	♂	nm	nm	nm		↑22	↑14	↑6	↑38	↑32
	♀	nm	nm	nm		↓5	↓15	↑110	↑140	↑170
LH	♀				↑160	↑130	↑130	↑190	↑200	↑250

nm= not measured
blank= no change from control
[a] T_4=thyroid function

% change in clinical chemistry parameters in rats following recovery (week 57)				
Parameter	Sex	LD (0.3mg/kg)	MD (3mg/kg)	HD (30mg/kg)
AP	♀		↑32	↑25
CHE	♂	↓11	↑14	
	♀	↓14	↓57	↓36
Trigl	♂	↑50	↑56	↑55
	♀	↓18	↑24	↓23
Cholesterol	♂	↓20	↓18	
	♀	↓18	↑50	↑38
γ-globulin	♀		↓22	↓22
T_4	♀	↑36	↑19	↑7
LH	♀		↑78	↑82

blank= no change from control

Leukocytes were observed in the urine of control and dosed ♂ and ♀ from week 35 to study termination.

Organ Weights

Weights of organs relative to brain weights were compared to concurrent controls at interim sacrifice (6 months), final sacrifice (12 months) and following recovery. Weights of organs relative to body weights were not compared since these data reflected the body weight depression of dosed groups.

% change in organ weights relative to brain weights of male rats administered letrozole for 12 months followed by a 1-month recovery									
	Interim sacrifice			Sacrifice following dosing			Recovery sacrifice		
Organ	LD	MD	HD	LD	MD	HD	LD	MD	HD
Kidney	-	-	-	-	-	-	↓21	↓15	↓16
Liver	↓19	↓22	-	-	-	↑17	↓22	↓16	↓9
Spleen	-	-	-	-	-	-	↓23	↓25	↓20
Thyroid	-	-	-	↓17	↓23	↓8	↓25	↓26	↓23
Testes	-	-	-	-	-	-	↓11	↓9	-
Epididymides	-	-	↓25	-	↓11	↓20	↓11	↓9	↓18
Seminal vesicles	-	-	↓60	↓17	↓28	↓51	↓20	↓27	↓44
Thymus	-	-	↓27	-	↓10	↓20	-	-	-
Prostate	-	-	↓40	-	↓27	↓33	↓18	↓13	↓20
Pituitary	-	-	↑27	-	-	↑28	↓14	-	↓18
Axillary lymph node	-	-	↓25	-	-	↓10	-	-	-

% change in organ weights relative to brain weights of females administered letrozole for 12 months followed by a 1-month recovery									
	Interim sacrifice			Sacrifice following dosing			Recovery sacrifice		
Organ	LD	MD	HD	LD	MD	HD	LD	MD	HD
Kidney	↑16	-	↑9	-	-	↑9	↑33	↑27	↑20
Liver	↑18	↑17	↑43	↑14	↑24	↑40	↑15	↑25	↑6
Spleen	-	-	↑9	-	-	↑17	-	-	-
Ovaries	↑8	↑12	↑9	↓16	↓5	-	↑47	↑33	↑21
Uterus	↓50	↓70	↓62	↓52	↓69	↓74	↑11	↓45	↓38
Heart	↑11	-	↑17	↑10	↑8	↑17	-	-	-
Axillary lymph node	-	-	↓36	-	-	-	-	-	-
Pituitary gland	↓21	↓30	↓20	↓19	↓21	↓14	-	-	↓12
Adrenal gland	↓27	↓32	↓29	↓11	↓11	↓19	↓5	↓16	↓23
Thyroid	-	-	-	↑21	-	↑12	-	-	-
Mandibular gland	-	-	-	↑17	↑22	↑32	-	-	-

Gross Pathology

♀- MD at 6 months; all doses at 12 months; MD and HD following recovery- small uterus; HD sacrificed following 8-9 months of dosing- bone fractures

♂- MD, HD at 6 months; HD at 12 months; 1HD animal following recovery- small seminal vesicles

♂ and ♀- all doses at 12 months; recovery ♀- accentuated lobular pattern and yellow discoloration of liver; scabs in dorso-thoracic region of skin (not observed in recovery animals)

Bone Examination

X-ray examination of the foreleg of 5 animals/dose/sex indicated that the bone diameter and weight of HD (30mg/kg) ♂ and ♀ were reduced following dosing. Reductions in bone diameter and weight were observed sporadically and with ↓ severity in MD ♂. There were no changes in bone calcium or hydroxyproline levels in examined animals.

Histopathology

seminal vesicles	slight to moderate atrophy (all doses at 6 and 12 months, MD and HD recovery)
testes	leydig cell hyperplasia (12 months and recovery)
prostate	hyperplasia HD (12 months and recovery)
ovaries	absence of large corpora lutea, sertoli cell hyperplasia, stromal hyperplasia (all doses, all sacrifice)
uterus	atrophy (all doses, all sacrifice)
vagina	epithelial atrophy (anestrus) (all doses, all sacrifice)
mammary gland	proliferation of secretory activity
pituitary	cellular hypertrophy, vacuoles (all doses ♂ and ♀, all sacrifice); changes less pronounced following recovery
thyroid	follicular cell hypertrophy (all doses, 6 and 12 months, ♂ and ♀); reversed following recovery
liver	centrilobular hypertrophy and ↑ incidence and severity of fatty changes (HD ♂ and ♀ at 6 months, all doses ♂ and ♀ 12 months and recovery)
bone marrow	hypercellularity (all doses ♂ and ♀, all sacrifice)
spleen	↑ hematopoiesis (HD 6 month ♂ and ♀; all doses 12 months ♂ and ♀ + control ♀; reversed following recovery)
mesenteric lymph nodes	mast cell hyperplasia (all doses ♂ and ♀, all sacrifice)

Study summary

Alterations were observed in the reproductive organs, liver, thyroid, pituitary, bone marrow, lymph nodes and spleen of males and females. Changes in reproductive system, pituitary and clinical biochemistry were independent of dose and were a result of the inhibition of estrogen synthesis by letrozole, causing estrogen depletion. Increase in body weights and depression of serum cholinesterase in ♀, and leydig cell hyperplasia in ♂ were results of hormonal imbalance. Estrogen is known to have an inhibitory effect on bone marrow;

hypercellularity of bone marrow and stimulation of hematopoiesis in the spleen is possibly related to the inhibition of estrogen syntheis. Bone fractures were observed in HD ♀ 8-9 months following dosing. Bone fragility has been observed in rodents administered antiestrogenic agents but not with other aromatase inhibitors (ie. Arimidex). Hypertrophy of the thyroid and liver correlated with changes in thyroid hormone levels and liver parameters which may be a result of enzyme induction. Fatty liver and correlating liver parameter changes in female rats may also reflect a hormonal effect on lipid metabolism. Relative adrenal weights of MD and HD females remained depressed following recovery; there were no correlating histological changes.

Observations at 26 and 52 weeks of dosing as well as following a recovery period were important in assessing letrozole toxicity in this species.

916015 6/12-Month Oral Toxicity in Beagle Dogs. Conducted by Ciba-Geigy Limited, Stein, Switzerland in 1994 according to OECD GLP with exception of characterization information of control treatment (lactose) and results of blood level determinations [performed by

Methods

species: beagle dogs, 8 dogs/sex/dose including interim sacrifice at 6 months (2dogs/sex/group), and necropsy following dosing at 12 months (3 dogs/sex/group) and following 4 weeks of recovery (2 dogs/sex/group)
drug: CGS 20267, batch #800289, purity: 99.4% ; analysis of mean concentration of LD and MD mixtures = 80 and 73% of nominal concentration for during study (97.5 and 96.2 prior to study initiation), sponsor indicated analysis incidental - following 12 months, drug concentrations within required limits
dosage: 0, 0.03, 0.30, 3mg/kg
age; weight: ♂ 19-22weeks, 5.8-9.9kg; ♀ 21-30weeks, 5.9-10.9kg
route: gelatin capsule 1X/day, 7 days/week
rationale for dose selection: 3-month oral toxicity study in dogs

Observations

clinical signs	daily
body weights	weekly
food consumption	daily
hematology	pretest, weeks 14, 26, 52 and following recovery (56)
clinical chemistry	pretest, weeks 14, 26, 52 and 56
urinalysis	pretest, weeks 14, 26, 52 and 56
ophthalmoscopy	pretest, weeks 26, 52, and 56
electrocardiography	pretest, weeks 14, 26, 52 and 56
neurological exam	pretest, weeks 26, 52 and 56
organ weights	interim sacrifice at 6months, final sac at 12months and following 4 weeks of recovery
gross pathology	interim sacrifice at 6months, final sac at 12months and following 4 weeks of recovery
histopathology	see histopathology table of organs

toxicokinetics	plasma collected prior to dosing and 2, 8 and 24 hrs following dosing on study days 1, 182, and 364 (reviewed in Pharmacokinetics/Toxicokinetics section of NDA)

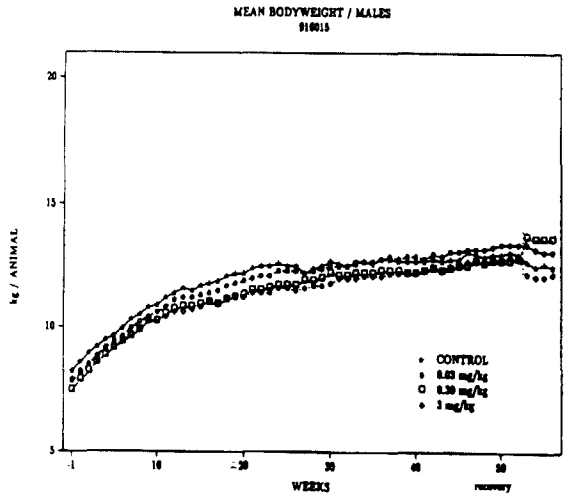
Results

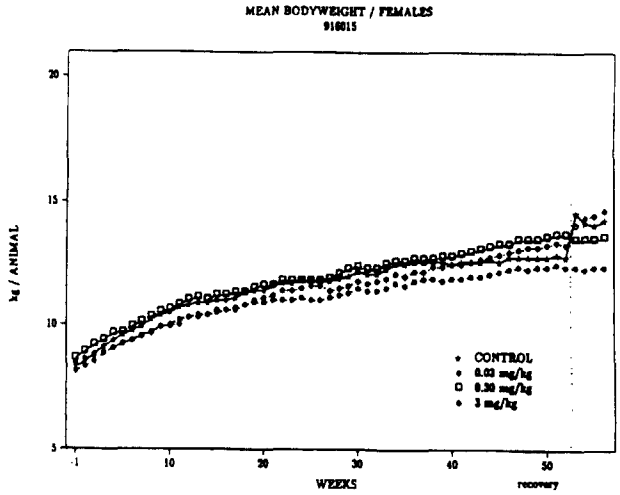
Mortality and clinical observations

There were no unscheduled deaths during the study. Alopecia, inflammation of the skin and crust formation were observed in 1/8 LD ♂ and ♀, 2/8 MD ♀and 1/8HD ♂ and ♀; skin changes were more extensive and severe in HD animals. Punctate hemorrhages were also observed on the footpads of HD animals. Findings occurred from weeks study 20 to 51. Estrus was not observed in HD ♀ and was reduced in other dosed ♀. Vomiting and blood in feces were observed in 1/8 MD and HD ♂ and 3/8 HD ♀ prior to dosing and throughout the study duration.

Body weights

Body weights of MD and HD ♂ were slightly (≤10%) depressed from study week 13 to termination of dosing when compared to concurrent controls; body weight of HD ♂ continued to be slightly (~6%) depressed following recovery. Body weights of LD and HD ♀ were slightly (~6%) depressed from study week 11 to week 41; body weights of LD ♀ remained depressed (≤13%) throughout the study and recovery periods. In general, weights recovered slightly after 6 months.





Food Consumption

Food consumption of dosed animals was similar to that of concurrent controls.

Ophthalmologic, electrocardiographic and neurologic results

Conjunctivitis was observed in all control and treated animals prior to dosing, at weeks 26 and 52 and following recovery. There were no ophthalmologic findings related to dosing with letrozole.

Significant reductions were observed in the QRS-complex of LD and MD ♂ at study week 14 and the QT 500 was significantly ↑ in HD ♂ at week 26. There were no significant changes in heart rate or QT-interval. Study authors indicated that significant changes were not related to letrozole treatment since there was no dose-response relationship of heart rate with these parameters and visual comparison of the EKG tracings with pretest were similar.

Letrozole had no effect on neurological parameters.

Hematology

Red cell parameters were ↓in HD ♂ and ♀ when compared to concurrent controls; parameters were most severely depressed in ♀ at 56 weeks. RBC changes in ♂ did not appear to be.of toxicological significance. WBC and neutophils were ↓ in HD ♀ throughout the study. Platelet counts of HD ♂ and all dosed ♀ were slightly higher than control values at pretest (6-12%), but significantly ↑ from study weeks 14-56 when compared to concurrent controls.

% change in hematology parameters of dogs administered letrozole for 12 months						
Parameter	Males (3mg/kg) at week			Females (3mg/kg) at week		
	26	52	56	26	52	56
RBC	↓8	↓5	↓10	↓9	↓11	↓19
Hgb	↓7	↓5	↓9	↓5	↓9	↓14
Hct				↓8	↓9	↓16
WBC				↓28	↓13	↓16
Neutrophils				↓13	↓10	↓13
Platelets	↑32	↑30	↑7	↑38[a]	↑31[b]	↑19[c]

[a] platelet count of LD♀ ↑22%; MD♀ ↑32% at 26 weeks
[b] platelet count of LD ♀ ↑9%; MD♀ ↑21% at 52 weeks
[c] platelet count of LD ♀ ↑26%; MD♀ ↑42% at 56 weeks

Clinical chemistry

GGT levels of dosed ♀ were significantly ↑ throughout dosing and recovery; increases were most pronounced at 26 weeks. Globulin, α_2 -globulin, ALAT and ALP were ↑at 26 and/or 52 weeks; recovery was not complete at all dose levels by 56 weeks. These changes may be a result of microsomal hepatic enzyme induction. Bilirubin levels were ↓ in MD and HD ♀ at 26 and 52 weeks; recovery was not complete at the HD following the recovery period. Sporadic changes in the clinical chemistry parameters of ♂ dogs were not of toxicological significance.

% Clinical chemistry change in female dogs administered letrozole for 12 months + 1 month recovery[a]									
Parameter	change at 26 weeks			change at 52 weeks			change at 56 weeks[b]		
	0.03	0.3	3.0	0.03	0.3	3.0	0.03	0.3	3.0
GGT (U/l)	↑3.5fold	↑11fold	↑9fold	↑3.5fold	↑6fold	↑3.5fold	↑1.6f	↑2.7f	↑2.4f
Bilirubin (µmol/l)	-	↓28	↓34	-	↓32	↓37	-	-	↓23
Globulin (g/l)	-	↑11	↑7	-	↑37	↑20	-	↑17	↑13
α_2 Glob (g/l)	↑35	↑67	↑71	↑42	↑95	↑89	↑32	↑67	↑98
ALAT (U/l)	-	↑29	↑28	-	-	↑36	-	↑20	↑14
ALP (U/l)	↑18	↑6	↑19	↑30	↑20	↑28	↑63	-	-

[a] compared to concurrent controls
[b]4 weeks recovery following 52 weeks of dosing

Organ Weights

The organ weight changes are listed in the following tables. Liver weights were increased throughout the study in HD♂ and sporadically increased (consistently following recovery) in ♀ at all doses. Ovarian weights were significantly increased, and testicular and epididymal weights were consistently depressed throughout the study. Uterine weights were increased following recovery at all doses. Epididymal weights were inconsistently changed. Most of these changes did not recover following recovery.

Relative organ weights of male dogs administered letrozole for 12 months with 1 month recovery[a]									
Organ	LD			MD			HD		
	6mo	12mo	recov	6mo	12mo	recov	6mo	12mo	recov
Brain				↑13		↓16	↑13		
Liver					↑12		↑23	↑20	↑25
Testes					↓11	↓13	↓38	↓45	↓5
Epididymis	↓19		↓25	↓43	↓30	↓32	↓56	↓39	↓34
Lung							↓17	↓13	↑24
Prostate	↑66		↓22	↑64		↓40	↑43	↓21	

[a] Percent change from concurrent control

Relative organ weights of female dogs administered letrozole for 12 months with 1 month recovery[a]									
Organ	LD			MD			HD		
	6mo	12mo	recov	6mo	12mo	recov	6mo	12mo	recov
Liver			↑44	↑18		↑8	↑6		↑26
Adrenal			↓13		↓33	↓26			↓40
Ovary	↑6-fold		↑3-fold	↑23-fold	↑21-fold	↑5.5-fold	↑20-fold	↑12-fold	↑9-fold
Uterus			↑84	↓72	↑4-fold	↑2.3-fold	↓75	↓71	↑46

[a] Percent change from concurrent control

Gross Pathology

6 months:

♀ Enlarged ovaries 2/2 HD; cystic ovaries 1/2LD, 2/2MD, HD

♂ Small testes 1/2HD

12 months:

♀ Enlarged ovaries 4/4LD, MD, HD; cystic ovaries 1/4LD, 2/4MD, HD
Distended uterus 2/4HD

♂ Small testes 3/4HD

Recovery:

♀ Enlarged ovaries 2/2LD, MD, HD; cystic ovaries 2/2LD, 1/2HD
Uterus enlarged with fluid 1/2HD

Microscopic Pathology

Incidence of histopathological findings of dogs administered letrozole for 12 months with 1 month recovery									
Organ/Finding	LD at dosing interval			MD at dosing interval			HD at dosing interval		
	6 mo. N=2	12 mo. N=4	Recov N=2	6 mo. N=2	12 mo. N=4	Recov N=2	6 mo. N=2	12 mo. N=4	Recov N=2
Testes/tubular atrophy	1	3	1	2	4	2	2	4	2
/Leydig cell hyperplasia	1	4	0	2	4	1	2	4	2
Ovary/hyperpl. corpus luteum	2	3	2	1	4	2	2	4	2
/cystic c.luteum	1	2	1	1	4	0	2	4	1
/follicular cyst	0	2	0	0	2	0	0	2	0
/follicular atrophy	0	1	0	0	2	2	0	4	2
Uterus/atrophy	0	0	0	2	0	0	2	4	0
/pyometra	0	0	0	0	2	0	0	0	0
/glandular cystic hyperplasia of endometrium	0	1	1	0	1	1	0	0	0
Vagina/atrophy	0	0	0	0	0	0	0	4	0
Pituitary/ hypertrophy	2♂ 1♀	1♂ 2♀	1♂ 1♀	1♂ 1♀	3♂ 2♀	1♂ 1♀	2♂ 2♀	4♂ 4♀	4♂ 4♀
Mammary/ hyperplasia	0	2♀	1♀	0	3♀	2♀	0	4♀	1♀
Thymus/atrophy	0	0	0	0	0	0	0	4♀	2♀
Liver/ hypertrophy	0	0	0	1♀	0	0	2♀	2♀	1♀
Kidney/hyaline droplets*	1♀	1♀ 3♂	0 1♂	1♀	2♀ 2♂	0 2♂	2♀ 1♂	2♀ 2♂	1♀ 2♂
Skin/acanthosis	0	0	0	0	0	0	2♂ 1♀	4♂ 4♀	2♂ 1♀

*Not usually observed in ♀ dogs
Other sporadic histological findings were not of toxicological importance.

Administering letrozole for 1 year to dogs resulted in effects not observed at 6 months of dosing. These findings included ↑ incidence of the following: atrophy of testes, ovarian follicles, uterus,

vagina and thymus; hyperplasia of Leydig, ovarian, uterine and mammary cells; ovarian cysts, hypertrophy of pituitary and acanthosis of the skin. Observations at 26 and 52 weeks of dosing as well as following a recovery period were important in assessing letrozole toxicity in this species. Relative adrenal weights of all dosed females were depressed following recovery; there were no correlating histological changes. Significantly increased GGT in females was a result of hepatic enzyme induction and was accompanied by increased relative liver weights and hepatic hypertrophy.

V. CARCINOGENICITY

T/P (US) 95059 CGS 20267: 104-week oral carcinogenicity study in rats (MIN 924172)

Conducted by Preclinical Safety, Pharmaceuticals Division , Ciba-Geigy Corporation, Summit NJ and Rueil-Malmaison, France in 1995 according to GLP.

Methods

species: rats; 60 animals/sex/dose

drug: CGS 20267 (lot No. 800192)

dosage: 0, 0.1, 1.0, 10mg/kg (0.01, 0.1 and 1mg/ml suspensions of aqueous 3% corn starch) at dosing volume of 10ml/kg for 104 weeks

age; weight: 6 weeks; 168-251.2g♂; 139.1-198.4g♀

route: gavage

Observations

clinical signs	daily
body weights	weekly from 2 weeks prior to dosing to week 13, monthly from week 17 to week 104
food consumption	weekly from 1 week prior to dosing to week 13, monthly from week 17 to week 104
water consumption	analysis indicated levels of lead exceeded acceptable limits for 2 month duration during study
physical and auditory examination	weeks -3, 13 and every 13th week to study termination
hematology	determined from 10 rats/sex/dose at weeks 78 and 104
clinical chemistry	determined from 10 rats/sex/dose at weeks 78 and 104
urinalyses	not determined
ophthalmoscopy	conducted on all animals 2 weeks prior to dosing and control and HD animals at weeks 52 and 103
palpable mass examination	every 4 weeks from week 4-40 and biweekly from week42-104
toxicokinetics	predose and 2, 4, 8, and 24 hrs postdose during weeks 14, 53, and 78/79 to determine plasma levels of unchanged letrozole (see Pharmacokinetics section for review)
organ weights	not determined
gross pathology	at sacrifice
histopathology	at sacrifice

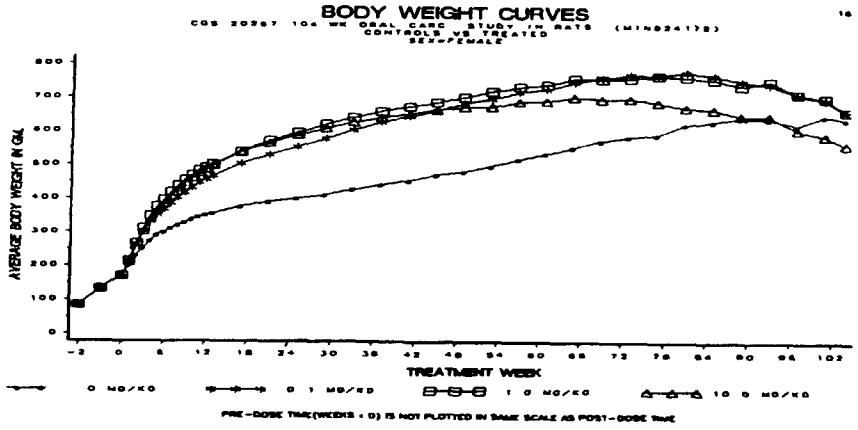
Results

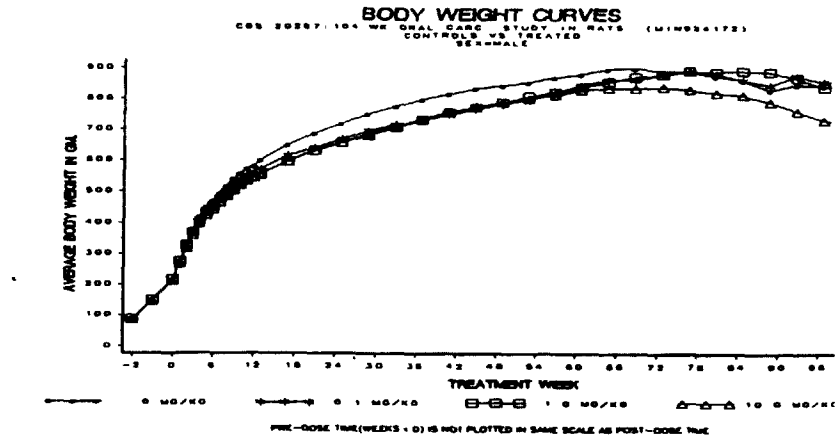
Mortality, clinical observations and physical and auditory examinations

There were no drug-related trends in mortality rates in males or females at any dose level; time to death was similar in control and dosed animals. The incidence of clinical observations in dosed animals were ≤ observations in concurrent controls; noted observations were attributed to animal age and moribund condition prior to death or sacrifice. The incidence of physical changes in dosed animals, including auditory testing, and examination for exudates, abnormal discharge, character of haircoat, posture and behavior was not attributed to administration of letrozole.

Body Weight

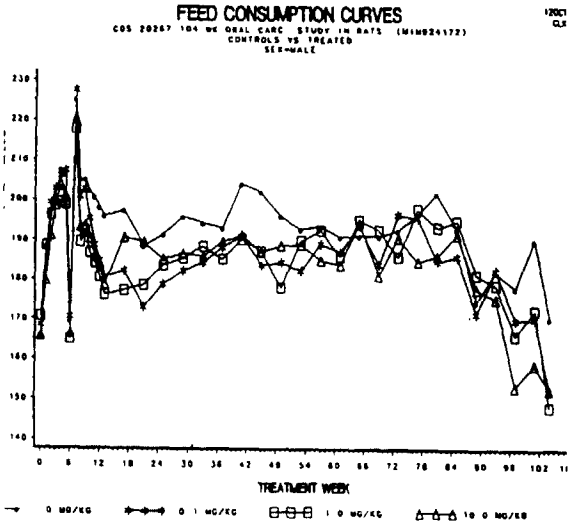
Body weights of HD ♂ were depressed throughout the dosing period when compared to concurrent controls; body weights of LD and MD ♂ were generally depressed to a lesser extent from study day 28 to day 511. The magnitude of depression in body weight between HD and control ♂ was ~7-16%. Body weights of LD and MD ♀ were increased 7-51% throughout the dosing period when compared to concurrent controls. HD weights were similarly increased to week 65 when weights began to decrease; HD weights were similar to concurrent controls at week 89 and 12% below control weights at study termination.

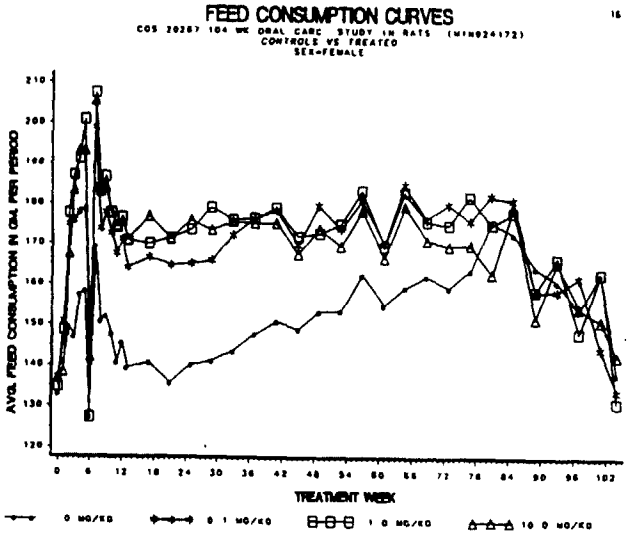




Food Consumption

Drug related depression in male food consumption and increase in female food consumption was associated with concomitant changes in body weight parameters. However, during the period of body weight depression of HD ♀, food consumption was similar to concurrent controls (from weeks 65 to study termination).





Toxicokinetics	Study reviewed in Pharmacokinetics/Toxicokinetics section
Ophthalmology	No treatment related changes

Hematology

Platelet counts of HD ♂ and ♀ were ↑ 21 and 40%, respectively, when compared to concurrent controls at study termination. White cell counts of dosed ♀ were ↑ 40-53% at week 78 and 21-50% at study termination when compared to concurrent controls. Other hematological parameters were similar in dosed and control animals.

Clinical chemistry

% Change in clinical chemistry parameters of rats administered letrozole for 2 years												
Parameter	Males						Females					
	Week 78			Week 103			Week 78			Week 103		
	LD	MD	HD	LD	MD	HD	LD	MD	HD	LD	MD	HD
ALP			↑41		↑26	↑15	↑60	↑42	↑66			↑15
SGPT									↓39			↓13
LDH						↑16	↓16	↑26	↑34	↑25	↑69	
Cholesterol			↑25			↑66	↑50	↑35	↑37	↑14	↑48	↑71
Triglycerides			↑76			↑65	↑24		↓16		↑24	↑38
Creatinine						↑34						
GGT			↑56			↑ 100						
Albumin							↓14	↓16	↓19	↓19	↓15	↓20
Globulin							↑18	↑27	↑32	↑16	↑24	↑36

Organ Weights- There is no indication that organ weights were performed.

Palpable mass examination

A ↓in the incidence of palpable mammary tumors was observed in all treated ♀ which is consistent with the reduction of estrogen levels and the administration of aromatase inhibitors.

Gross Pathology

♀- (all doses) small uteri, enlarged ovaries

♂ and ♀- ≥1.0mg/kg- enlarged liver

Histopathology

The incidence of stromal hyperplasia of the ovary was significantly increased ($p \leq 0.01$, one-sided trend test) in dosed females of all groups; the incidence of benign ovarian stromal tumors was low but .significantly increased (3/60, 5%, $p \leq 0.01$, time adjusted trend test [modified Peto analysis]) in HD females. These changes were probably the result of the inhibition of estrogen synthesis, the lack of negative feedback by estrogen to the pituitary and the resulting

increase in FSH and LH stimulating proliferation of the ovarian stroma. The same mechanism was likely to be responsible for atrophy of the uterus and increased vacuolation of the pituitary in dosed females. Urinary tract changes may also be the result of depressed estrogen levels and genitourinary atrophy. The incidence of papillomas of the urinary bladder was associated with the presence and mechanical irritation of bladder calculi.

Microscopic findings related to inhibition of estrogen synthesis following administration of letrozole to female rats for 104 weeks				
Tissue/finding	control	LD	MD	HD
Ovary (# examined)	60	60	60	60
/benign stromal tumors	0	0	0	3
/stromal hyperplasia	2	51	53	58
Uterus (# examined)	60	60	60	60
/atrophy	1	50	60	59
/polyp	9	1	0	0
Pituitary (# examined)	59	60	60	60
/vacuolation	0	12	20	23
Urinary tract (# examined)	60	60	60	60
/pyelonephritis	2	9	5	8
/urinary bladder inflammation	1	5	7	8
/urinary bladder papilloma	0	4	3	2
/epithelial hyperplasia of urinary bladder	1	11	5	6
/transitional cell carcinoma	0	0	1	0

According to the sponsor, the incidence of hepatocellular adenoma was significantly increased ($p \leq 0.05$, time adjusted trend test) in MD (5/60, 8.3%) and HD (4/60, 6%) males when compared to controls but was not significant when combined with carcinoma. When these data were reanalyzed by the Dept. of Biometrics, FDA, using the method of the exact permutation trend test, the incidence of hepatocellular adenoma in males was not significantly increased ($p=0.11$). Historically, the spontaneous incidence of hepatocellular adenoma and carcinoma in male rats of this strain is 4.2 and 2.6%, respectively (Lang, P. 1992. Spontaneous neoplastic lesions and selected non-neoplastic lesions in the rat.

pub.) The spontaneous incidence of hepatocelluar adenoma and carcinoma in female rats of this strain is 2.2 and 0.4%, respectively. The incidence of hepatocellular carcinoma in HD females (6%, 4/60, p=0.08, time adjusted trend test) was increased when compared to concurrent controls. The incidence of hepatocellular hypertrophy was significantly increased ($p \leq 0.01$, one-sided trend test) in HD males and females. This finding was not analyzed by the Dept. of Biometrics, FDA. The increase of bile duct hyperplasia of treated males was dose related.

The increased incidence of nephropathy observed in HD females was consistent with lowered estrogen levels and elevated androgenic precursors. The increased incidence of hyperplasia of the parathyroid in these animals can be attributed to the nephropathy.

Liver, kidney and parathyroid findings in male and female rats administered letrozole for 104 weeks[a]

Finding	Males				Females			
	contr	LD	MD	HD	contr	LD	MD	HD
Liver/ centrilobular hypertrophy	0	0	5	33	0	4	11	46
/Bile duct hyperplasia	7	9	15	21	14	11	16	21
/Necrosis	11	7	7	10	1	4	10	11
/Vacuolation	13	12	15	27	15	20	18	26
/Hepatocellular adenoma	0	2	5	4	8	4	8	8
/Hepatocellular carcinoma	4	1	3	2	1	2	3	4
/Combined hepatocellular adenoma and carcinoma[b]	4	3	8	5	9	6	11	11
/Centrilobular hypertrophy	0	0	5	33	0	4	11	46
Kidney/ chronic progressive nephropathy	46	51	47	47	39	40	41	52
Parathyroid/ hyperplasia	9	3	4	1	0	5	5	9

[a]N=60

[b] Procedure for collection of neoplastic combinations was not identified

Summary of Neoplastic Findings in Male and Female Rats

(See following pages)

SUMMARY OF FINDING INCIDENCES AND STATISTICAL ANALYSIS
NEOPLASTIC HISTOPATHOLOGY
MALES

BODY SYSTEM / ORGAN / FINDING		DOSE GROUPS 0 mg/kg	0.1 mg/kg	1.0 mg/kg	10 mg/kg	TEST FOR TREND P-VALUE
CARDIO-VASCULAR SYSTEM						
HEART						
MESOTHELIOMA (B)		0/60	1/60	0/60	0/60	
	OBS/EXP:	0.00	4.00	0.00	0.00	0.829
DIGESTIVE SYSTEM						
LIVER						
HEPATOCELLULAR ADENOMA (B)		0/60	2/60	5/60	4/60	
	OBS/EXP:	0.00	0.75	1.62*	1.42*	0.028*
HEPATOCELLULAR CARCINOMA (M)		4/60	1/60	3/60	2/60	
	OBS/EXP:	1.90	0.41	1.02	0.79	0.804
PANCREAS						
ACINAR ADENOMA (B)		3/60	0/60	0/60	0/60	
	OBS/EXP:	4.36	0.00	0.00	0.00	0.994
ISLET CELL ADENOMA (B)		2/60	1/60	2/60	4/60	
	OBS/EXP:	1.00	0.45	0.81	1.73	0.182
ISLET CELL CARCINOMA (M)		1/60	1/60	2/60	3/60	
	OBS/EXP:	0.64	0.59	1.03	1.67	0.144
SALIVARY GLAND						
MYOEPITHELIOMA, MALIGNANT (M)		1/60	0/60	0/60	0/60	
	OBS/EXP:	4.08	0.00	0.00	0.00	1.000
STOMACH						
PAPILLOMA (B)		0/60	0/59	1/60	1/60	
	OBS/EXP:	0.00	0.00	1.65	1.96	0.118

NOTE: THE P-VALUES ON THE REPORT ARE ROUNDED TO THREE DECIMALS. A * OR ** INDICATES A STATISTICAL SIGNIFICANCE AT THE 0.05 OR 0.01 LEVEL, RESPECTIVELY. WHEN RECORDED BESIDE A GROUP OBS/EXP THIS REFLECTS THE COMPARISON WITH CONTROLS.

SUMMARY OF FINDING INCIDENCES AND STATISTICAL ANALYSIS
NEOPLASTIC HISTOPATHOLOGY
MALES

BODY SYSTEM / OPGAN / FINDING		DOSE GROUPS 0 mg/kg	0.1 mg/kg	1.0 mg/kg	10 mg/kg	TEST FOR TREND P-VALUE
ENDOCRINE SYSTEM						
ADRENAL GLAND						
CORTICAL ADENOMA [B]		1/60	1/59	1/60	1/60	
	OBS/EXP:	1.08	1.02	0.94	0.97	0.535
CORTICAL CARCINOMA [M]		1/60	0/59	0/60	0/60	
	OBS/EXP:	5.77	0.00	0.00	0.00	1.000
PHEOCHROMOCYTOMA [B]		8/60	10/59	8/60	8/60	
	OBS/EXP:	1.11	1.21	0.82	0.92	0.753
PHEOCHROMOCYTOMA [M]		1/60	1/59	1/60	1/60	
	OBS/EXP:	1.11	1.03	0.89	0.97	0.553
PARATHYROID						
ADENOMA [B]		5/58	1/54	7/55	4/59	
	OBS/EXP:	1.36	0.25	1.43	0.90	0.508
PITUITARY						
ADENOMA [B], pars distalis		38/58	32/59	32/60	35/59	
	OBS/EXP:	1.36	0.98	0.81	0.95	0.985
CARCINOMA [M]		0/58	0/59	1/60	2/59	
	OBS/EXP:	0.00	0.00	1.14	2.47	0.084
CRANIOPHARYNGIOMA [B]		0/58	1/59	0/60	0/59	
	OBS/EXP:	0.00	4.07	0.00	0.00	0.755
THYROID						
C-CELL ADENOMA [B]		5/60	2/60	1/59	1/60	
	OBS/EXP:	2.28	0.89	0.44	0.44	0.979

NOTE: THE P-VALUES ON THE REPORT ARE ROUNDED TO THREE DECIMALS. A * OR ** INDICATES A STATISTICAL SIGNIFICANCE AT THE 0.05 OR 0.01 LEVEL, RESPECTIVELY. WHEN RECORDED BESIDE A GROUP OBS/EXP THIS REFLECTS THE COMPARISON WITH CONTROLS.

SUMMARY OF FINDING INCIDENCES AND STATISTICAL ANALYSIS
NEOPLASTIC HISTOPATHOLOGY
MALES

BODY SYSTEM / ORGAN / FINDING		DOSE GROUPS 0 mg/kg	0.1 mg/kg	1.0 mg/kg	10 mg/kg	TEST FOR TREND P-VALUE
ENDOCRINE SYSTEM (continued)						
THYROID (continued)						
C-CELL CARCINOMA [M]		0/60	0/60	1/59	0/60	
	OBS/EXP:	0.00	0.00	2.92	0.00	0.577
FOLLICULAR ADENOCARCINOMA [M]		1/60	0/60	0/59	1/60	
	OBS/EXP:	2.47	0.00	0.00	1.98	0.556
FOLLICULAR ADENOMA [B]		0/60	3/60	0/59	1/60	
	OBS/EXP:	0.00	3.09	0.00	0.98	0.565
HEMATOPOIETIC-LYMPHORETICULAR SYSTEM						
SYSTEMIC						
HISTIOCYTIC SARCOMA [M]		3/60	2/60	0/60	3/60	
	OBS/EXP:	1.85	1.03	0.00	1.49	0.727
LYMPHOMA, MALIGNANT [M]		3/60	2/60	9/60	1/60	
	OBS/EXP:	0.88	0.54	2.15	0.27	0.519
THYMUS						
SQUAMOUS CELL CARCINOMA [M]		0/57	1/57	0/58	0/59	
	OBS/EXP:	0.00	4.02	0.00	0.00	0.778
THYMOMA [M]		0/57	1/57	0/58	2/59	
	OBS/EXP:	0.00	1.37	0.00	2.74	0.207
INTEGUMENTARY SYSTEM						
MAMMARY GLAND						
ADENOCARCINOMA [M]		1/49	0/45	0/44	0/41	
	OBS/EXP:	3.97	0.00	0.00	0.00	1.000

NOTE: THE P-VALUES ON THE REPORT ARE ROUNDED TO THREE DECIMALS. A * OR ** INDICATES A STATISTICAL SIGNIFICANCE AT THE 0.05 OR 0.01 LEVEL, RESPECTIVELY. WHEN RECORDED BESIDE A GROUP OBS/EXP THIS REFLECTS THE COMPARISON WITH CONTROLS.

SUMMARY OF FINDING INCIDENCES AND STATISTICAL ANALYSIS
NEOPLASTIC HISTOPATHOLOGY
MALES

BODY SYSTEM / ORGAN / FINDING		DOSE GROUPS 0 mg/kg	0.1 mg/kg	1.0 mg/kg	10 mg/kg	TEST FOR TREND P-VALUE
INTEGUMENTARY SYSTEM (continued)						
MAMMARY GLAND (continued)						
ADENOMA (B)		0/49	1/45	0/44	0/41	
	OBS/EXP:	0.00	3.75	0.00	0.00	0.800
FIBROADENOMA (B)		1/49	2/45	0/44	0/41	
	OBS/EXP:	1.47	2.75	0.00	0.00	0.953
SKIN						
FIBROMA (B)		1/60	4/60	1/60	0/60	
	OBS/EXP:	0.77	2.81	0.60	0.00	0.909
FIBROSARCOMA (M)		1/60	0/60	2/60	2/60	
	OBS/EXP:	0.87	0.00	1.50	1.55	0.246
HEMANGIOSARCOMA (M)		0/60	0/60	0/60	1/60	
	OBS/EXP:	0.00	0.00	0.00	3.89	0.257
KERATOACANTHOMA (B)		3/60	1/60	2/60	0/60	
	OBS/EXP:	2.34	0.69	1.13	0.00	0.956
LIPOMA (B)		0/60	0/60	2/60	2/60	
	OBS/EXP:	0.00	0.00	1.92	1.93	0.067
MYXOMA (B)		1/60	0/60	0/60	0/60	
	OBS/EXP:	4.47	0.00	0.00	0.00	1.000
NERVE SHEATH TUMOR, BENIGN (B)		1/60	0/60	0/60	0/60	
	OBS/EXP:	4.66	0.00	0.00	0.00	1.000

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