

CENTER FOR DRUG EVALUATION AND RESEARCH

APPLICATION NUMBER: NDA 20753

PHARMACOLOGY REVIEW(S)

Division of Oncology Drug Products, HFD-150
REVIEW AND EVALUATION OF PHARMACOLOGY AND TOXICOLOGY DATA
Review #2

Keywords: steroidal aromatase inhibitor, treatment of advanced breast cancer; label comments

NDA: 20,753

Serial No. 000 Type: NDA Letter Dated 11/30/98 Received by CDR: 12/3/98

Information to be conveyed to the sponsor: YES

Reviewer: John K. Leighton, Ph.D.

Date Review Completed: 10/4/99

Sponsor: Pharmacia and Upjohn

Manufacturer: Pharmacia and Upjohn, Kalamazoo, MI

Drug: Code name: FCE 24304; PNU-155971

Generic name: exemestane

Trade name: Aromasin

Chemical name: 6-methylenandrosta-1,4-diene-3,17-dione

CAS Registry no. 107868-30-4

Molecular formula/weight: C₂₀H₂₄O₂; 296.41

Structure:

Related INDs, NDAs:

IND

Drug Class:

aromatase inhibitor

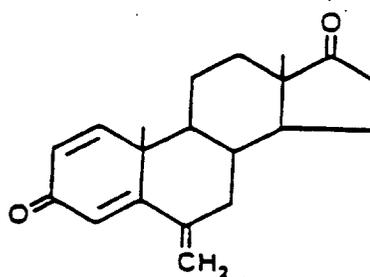
Indication:

"For the treatment of advanced breast cancer in postmenopausal women whose disease has progressed following antiestrogen therapy."

Clinical Formulation:

Final formulation tentative;
 formulation of core shown below:

Exemestane	25 mg
↓ mannitol	mg
↓ hydroxypropylmethylcellulose	mg
↓ polysorbate 80	mg
↓ crospovidone	mg
↓ colloidal silicon dioxide	mg
↓ microcrystalline cellulose	mg
↓ sodium starch glycolate	mg
↓ magnesium stearate	mg



Route of administration and dosage form: oral, sugar coated tablet

Proposed clinical protocol: 25 mg/day (0.4 mg/kg/day, 60 kg person)

Previous review: IND Dr. Lee-Ham review of 4/9/91; Dr. Leighton reviews of 12/15/98 and 3/22/99 and NDA review 9/30/99

Labeling Comments:

This review contains Pharmacology/Toxicology comments on the sponsor's proposed label for exemestane. The NDA has been previously reviewed (see Review #1, 9/30/99). Referenced line numbers refer to the sponsor's numbering system in their original proposed label.

Redacted

1

pages of trade

secret and/or

confidential

commercial

information

The following sentence should be inserted at the end of the OVERDOSAGE section.

Convulsions were observed after single doses of exemestane of 400 mg/kg and 3000 mg/kg in mice and dogs (approximately 80 and 4000 times the recommended human dose on a mg/m² basis), respectively.

RECOMMENDATION: The pharmacology/toxicology data supports approval of exemestane for the treatment of advanced breast cancer.

/S/

10/4/99

John K. Leighton, Ph.D., DABT Date
Biologist

/S/

9/4/99

Paul A. Andrews, Ph.D. Date
Pharmacology/Toxicology Team Leader

cc:
IND ORIG. and Div. File
HFD-150

- /AMartin
- /AStaten
- /JKLeighton
- /PAAndrews

Studies reviewed in this submission:

PHARMACOLOGY

	Title	Vol.	Page	Study no.
1	Endocrine characterization of FCE 24304: effects on aromatase, 5 α -reductase and desmolase and steroid receptor-binding affinity.	1.1	5 1 137	204i
2	Effects of exemestane on the activity of aromatase and other steroidogenic enzymes <i>in vitro</i> .	1.1	5 1 303	9850123
3	Comparative effects of exemestane on aromatase activity in fibroblasts of human breast adipose tissues, breast cancers and placental microsomes.	1.1	5 1 333	9850124
4	Inhibitory effect of exemestane on the aromatase activity of human mammary tumor cells.	1.1	5 1 350	147098009
5	Aromatase inhibition and androgen receptor binding of 17-hydroxexemestane (FCE 25071) and other potential metabolites of exemestane (FCE 24304)	1.1	5 1 359	217i
6	Hormonal and antibormal activity of FCE 24304 in the experimental animal.	1.2	5 2 251	205i
7	Binding of ¹⁴ C-PNU 155971 to human serum albumine and α_1 -acid glycoprotein.	1.30	5 30 028	9750070
8	A study of the inhibition of specific forms of cytochrome P450 by exemestane.	1.31	5 31 200	9550346

SAFETY PHARMACOLOGY

1	Effects of FCE 24304 on the central nervous system.	1.1	5 1 388	202i
2	Convulsant and pro-convulsant effects of FCE 24304 in mice.	1.2	5 2 169	210i

PHARMACOKINETICS

1	Pharmacokinetic studies on FCE 24304. Absorption, distribution, metabolism and excretion in rats and dogs.	1.28	5 28 075	823i
2	Study of absolute bioavailability of PNU 155971 (FCE 24304) in the female rat.	1.28	5 28 356	9650297
3	Ancillary toxicokinetic study after single and repeated (4-week) oral administration of FCE 24304 in the female rat.	1.26 1.28	5 26 301 5 28 296	9650132
4	Absorption of ¹⁴ C-FCE 24304 after single oral administration in comparison with i.v. dose in the dog.	1.29	5 29 001	807i
5	Plasma levels of FCE 24304 in female Beagle dogs during a 52-week oral toxicity study. ¹	1.29	5 29 073	809i
6	Biliary excretion and metabolic pattern of ¹⁴ C-FCE 24304 in the rat after oral dosing.	1.30	5 30 122	806i
7	Urinary metabolism of [¹⁴ C]-FCE 24304 in the dog after intravenous and oral administration.	1.30	5 30 185	808i
8	Biliary metabolites of ¹⁴ C-Exemestane after oral administration to female beagle dogs.	1.30	5 30 291	9750055

¹ Data submitted as part of Toxicology report 416i.

TOXICOLOGY

1	Palatability study of FCE 24304 for 4 weeks in mice.	1.3	5 3 218	9650020
2	Palatability study of FCE 24304 for 4 weeks in rats.	1.6	5 6 280	9650021
3	13-week toxicity study by diet in rats (DRF).	1.7 1.8	5 7 001 5 8 001	9650370
4	52-week oral toxicity study in female beagle dogs followed by a 6-week recovery period.	1.17	5 17 001	416i

REPRODUCTIVE TOXICOLOGY

1	Dosage-range finding study of FCE 24304 administered orally via gavage to CDBR VAF/Plus rats (pilot study for segment I and segment III)	1.18	5 18 001	418i
2	Fertility and general reproduction (including a "behavioral" postnatal evaluation) of FCE24304 administered orally by gavage to CDBR VAF/Plus female rats (segment I evaluation)	1.19 1.20	5 19 001 5 20 001	417i
3	Preliminary teratogenesis study by oral route in rats.	1.21	5 21 001	406i
4	Teratogenesis study of FCE 24304 by oral route in rats	1.21 1.22	5 21 089 5 22 001	414i
5	Preliminary teratogenesis study of compound FCE 24304 given orally to the rabbit.	1.23	5 23 001	407i
6	Assessment of possible embryotoxic or teratogenic effects in rabbits by the oral route.	1.23	5 23 107	411i

GENETIC TOXICOLOGY (Note: these studies have been previously reviewed (#000); additional data is captured within this review)

	Title	Vol.	Page	Study no.	Rev. #
1	Gene mutation in <i>Salmonella typhimurium</i> on FCE 24304 (Ames test).	1.23	5 23 249	303i	1
2	DNA repair test with FCE 24304 in rat hepatocyte primary cultures.	1.24	5 24 082	302i	1
3	Micronucleus test in mouse bone marrow cells after oral administration of FCE 24304.	1.24	5 24 124	301i	1
4	Metaphase chromosome analysis in mouse bone marrow cells after oral administration of FCE 24304	1.24	5 24 144	306i	1

Studies not reviewed within this submission:

PHARMACOLOGY

	Title	Vol.	Page	Study no.
1	Comparison of the effects of the irreversible aromatase inhibitor exemestane with stamestane and MDL 18962 in rats with DMBA-induced mammary tumors.	1.1	5 1 176	—
2	Effect of the irreversible aromatase inhibitor FCE 24304 on DMBA-induced mammary tumors in ovariectomized rats treated with testosterone.	1.1	5 1 182	206i
3	Effect of oral treatment with the aromatase inhibitor exemestane on DMBA-induced mammary tumors in ovariectomized rats treated with testosterone.	1.1	5 1 199	218i
4	Effects of the irreversible aromatase inhibitor FCE 24304 in rats with DMBA-induced mammary tumors.	1.1	5 1 214	201i
5	Comparison of the effects on the irreversible aromatase inhibitor FCE 24304 with SH 489 and MDL 18962 in rats with DMBA-induced mammary tumors.	1.1	5 1 234	207i
6	Antitumor activity of combined treatment with exemestane and tamoxifen on DMBA-induced mammary tumors in rats.	1.1	5 1 255	215i
7	Effect of combined treatment with the irreversible aromatase inhibitor FCE 24304 and the antiprolactin drug cabergoline in rats with DMBA-induced mammary tumors.	1.1	5 1 275	208i

8	Novel aromatase and 5 alpha-reductase inhibitors. J Steroid Biochem Molec Biol 1994; 49:289-294.	1.1	5 1 297	—
9	The <i>in vitro</i> effect of the aromatase inhibitor exemestane on P388 and LoVo tumor cells.	1.1	5 1 376	216i
10	Effects of single or repeat oral dosing with exemestane on ACTH-stimulated corticosterone levels in rat	1.2	5 2 278	219i (9650173)
11	Antigenicity study of FCE 24304.	1.2	5 2 294	213i
12	6-Methylenandrosta-1,4-diene-3,17-dione (FCE 24304): a new irreversible aromatase inhibitor. J Steroid Biochem 1988: 30, 391-394	1.2	5 2 357	—
12	Novel irreversible aromatase inhibitors. Ann NY Acad Sci 1990;595:357-367.	1.2	5 2 361	—
14	Various published manuscripts	1.32	5 32 001	—

SAFETY PHARMACOLOGY

1	General pharmacological studies of FCE 24304.	1.2	5 2 001	214i
2	Cardiovascular safety of the aromatase inhibitor FCE 24304.	1.2	5 2 182	209i
3	Gastrointestinal and choleric effects of FCE 24304.	1.2	5 2 219	203i
4	Effects of oral FCE 24304 on diuresis in rats	1.2	5 2 238	211i
5	FCE 24304 (Vehicle): study of the potential hemolytic effects of vehicle benzyl alcohol-ethylene glycol monoethyl ether-ethanol, 1:7:2, diluted 1:1 with saline, in male rats after a single intravenous administration.	1.26	5 26 001	9650231
6	FCE 24304 (Vehicle): study of the potential hemolytic effects of vehicle benzyl alcohol-ethylene glycol monoethyl ether-ethanol, 1:7:2, diluted 1:1 with saline, in Beagle dogs after a single intravenous administration.	1.26	5 26 074	9650230
7	PNU 155971 (FCE 24304-exemestane): study of the potential hemolytic effects of several vehicles after a single intravenous administration in the male rat.	1.26	5 26 144	9650241
8	Preliminary effects on <i>in vitro</i> blood osmotic fragility.	1.26	5 26 200	9550248
9	Effects on <i>in vitro</i> blood osmotic fragility.	1.26	5 26 216	6550262

PHARMACOKINETICS

Pharmacokinetic parameters

1	Plasma levels of PNU-155971 (FCE 24304) in female beagle dogs during a 4-week oral ancillary toxicokinetic study.	1.26 1.29	5 26 361 5 29 304	9750069
2	Methods for the determination of exemestane in blood and urine – several studies	1.27	5 27-001	114i, 122i, 124i, 9550282, 9559131, V94.666
3	Stability of FCE 24304 in plasma and urine – several studies	1.27	5 27 342	820i, 821i, 9550135, 9550136
4	Preliminary data on plasma levels in the rat.	1.27	5 27 417	801i
5	Preliminary pharmacokinetic study in the rat.	1.27	5 27 429	803i
6	Preliminary pharmacokinetic study in the dog.	1.28	5 28 435	802i
7	Plasma levels of FCE 24304 in female beagle dogs during a 4-week oral ancillary toxicokinetic study.	1.29	5 29 115	9550147
8	The pharmacokinetics of radioactivity in female dogs following oral administration of ¹⁴ C-FCE 24304.	1.29	5 29 221-	9550289

Absorption

1	Absorption, elimination and urinary metabolic pattern of ¹⁴ C-FCE 24304 after oral and I.V. administration in rats.	1.28	5 28 048	805i
2	Absorption and excretory balance of [¹⁴ C]-FCE 24304 in female rabbit.	1.28	5 28 399	9550341
3	Study of absolute bioavailability of PNU 155971 (FCE 24304) in the female dog.	1.29	5 29 253	9650302
4	The absorption, pharmacokinetics and excretion of ¹⁴ C-FCE 24304 in the female cynomolgus monkey.	1.29	5 29 368	816i

Distribution

1	The disposition of ¹⁴ C-FCE 24304 in the female rat.	1.30	5 30 054	815i
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Metabolism

1	Urine metabolism of ¹⁴ C-FCE 24304 after oral administration to female rats.	1.30	5 30 145	9650137
2	Plasma metabolism of ¹⁴ C-FCE 24304 after oral administration to rats.	1.30	5 30 168	9650156
3	Urine metabolism of FCE 24304 after oral administration of the ¹⁴ C-labelled compound in dogs.	1.30	5 30 210	811i
4	Further identification of FCE 24304 metabolites in dog urine. (Part II)	1.30	5 30 241	9550330
5	Plasma metabolism of ¹⁴ C-Exemestane after oral administration to female beagle dogs.	1.30	5 30 274	9650258
6	Biliary metabolism of ¹⁴ C-Exemestane after oral administration to female beagle dogs. Identification of polar metabolites by	1.30	5 30 309	9750067
7	Urine metabolism of ¹⁴ C-FCE 24304 after intravenous and oral administration to cynomolgus monkeys.	1.30	5 30 338	9650133
8	Effects of FCE 24304 on rat hepatic microsomal cytochromes P-450.	1.30	5 30 380	804i
9	"In vitro" metabolism of FCE 24304 in 9000 x G fraction of rat liver homogenates.	1.31	5 31 001	822i
10	Identification of the form(s) of cytochrome(s) P450 involved in the oxidative metabolism of FCE 24304 in human liver.	1.31	5 31 021	9750052
11	Investigation of the inducing properties of FCE 24304 for selected drug metabolizing enzymes in male and female rat liver.	1.31	5 31 053	812i
12	<i>In vitro</i> studies to determine the potential inhibition of human drug metabolising cytochromes P450 by exemestane (PNU 155971), letrozole (PNU 191826) and anastrozole (PNU 192331).	1.31	5 31 227	9850104
13	Cis-flupentixol metabolites in rat plasma and bile. The first proof of glutathione conjugation at the exocyclic double bond. Drug Metabolism and Disposition 1991; 19: 154-162	1.31	5 31 277	---

Excretion

1	The excretion of radioactivity in the urine of female dogs following oral administration of ¹⁴ C-FCE 24304.	1.31	5 31 122	813i
2	¹⁴ C-FCE 24304: Plasma radioactivity concentrations and biliary excretion in dogs following oral administration.	1.31	5 31 165	9650399

TOXICOLOGY**Single dose**

1	Local tolerance study after single intravenous and perivenous injection in the dog	1.24	5 24 178	9650033
2	Acute eye irritation study in the rabbit.	1.26	5 26 238	9750257
3	Acute dermal irritation study in the rabbit.	1.26	5 26 244	9750258
4	Delayed dermal sensitization test in guinea pig (Magnusson and Kligman).	1.26	5 26 250	9750310

Repeat dose

1	Four week toxicity study of FCE 24304 given daily to rats by oral route (additional doses).	1.6	5 6 001	408i
2	Two-week intravenous toxicity study in the rat.	1.25	5 25 001	9650016

Studies previously reviewed

Note: all volume and page nos. in tables below refer to NDA location; Review nos. refer to IND

PHARMACOKINETICS

	Title	Vol.	Page	Study no.	Rev. #
1.	Ancillary toxicokinetic study after repeated (4-week) dietary administration of PNU 155971 (ex FCE 24304) in the female rat	NS ²	—	METPK 172/96	2
2.	Plasma levels of FCE 24304 in rats during a 52-week oral toxicity study.	1.27	5 27 445	814i	2
3.	Plasma levels of FCE 24304 in rats during a 4-week oral ancillary study.	1.27	5 27 483	817i	2
4.	Plasma levels of FCE 24304 (exemestane) in a 13-week dietary dose range finding study in rats. ¹	1.28	5 28 001	818i	2
5.	Plasma protein binding of ¹⁴ C-FCE 24304 in man, mouse, rat, rabbit and dog.	1.30	5 30 001	810i	2
6	Ancillary toxicokinetic study after repeated (4-week) dietary administration of PNU 155971 in the female mouse.	NS	—	9750078	3

¹Data also submitted as part of toxicology report no. 9650370, NDA vol. 1.7-1.8; ² not submitted to NDA

TOXICOLOGY**Single dose**

1	Acute oral and intraperitoneal toxicity study in mice.	1.3	5 3 133	402i	1
2	Acute oral and intraperitoneal toxicity of FCE 24304 study in rats.	3.1	5 3 165	403i	1
3	Study of the acute toxicity of FCE 24304 given orally to the beagle.	3.1	5 3 198	401i	1

Repeat dose

1	Four-week toxicity study of FCE 24304 given daily to rats by oral route.	1.5	5 5 001	405i	1
2	Four-week oral toxicity study of FCE 24304 given daily to beagle dogs by the oral route.	1.9	5 9 001	404i	1
3	26-week oral toxicity study in rats followed by a 4-week recovery period.	1.9 1.10	5 9 084 5 10 001	409i	1
4	26-week oral toxicity study in dogs followed by a 4-week recovery period.	1.15 1.16	5 15 144 5 16 001	410i	1
5	52-week chronic oral toxicity study of FCE 24304 in rats with a 6-week recovery.	1.11- 1.15	5 11 001- 5 15 001	415i	2
6	13-week oral toxicity study in the mouse (dose range finding).	NS	—	9850371	3
7	13-week toxicity study by diet in mice (DRF).	1.4	5 4 001	419i	3

Genetic Toxicology

1	Gene mutation in <i>Salmonella typhimurium</i> on FCE 24304 (Ames test).	1.23	5 23-249	303i	1
2	DNA repair test with FCE 24304 in rat hepatocyte primary cultures.	1.24	5 24 082	302i	1
3	Micronucleus test in mouse bone marrow cells after oral administration of FCE 24304.	1.24	5 24 124	301i	1
4	Metaphase chromosome analysis in mouse bone marrow cells after oral administration of FCE 24304	1.24	5 24 144	306i	1
5	Gene mutation test in V79 Chinese Hamster cells with FCE 24304.	1.24	5 24 001	304i	2
6	FCE 24304: Gene mutation test in <i>Escherichia coli</i> .	1.23	5 23 278	307i	2
7	Metaphase chromosome analysis on FCE 24304 in human lymphocytes cultured in vitro	1.24	5 24 049	305i	2

Note: Portions of this review were excerpted directly from the sponsor's submission.

INTRODUCTION AND DRUG HISTORY

Exemestane is an irreversible aromatase inhibitor structurally related to the natural substrate, androstenedione, differing by the presence of a C6 methylene group in the inhibitor. This cytochrome P450 enzyme catalyzes the conversion of C19 androgens (androstenedione and testosterone) to C18 estrogens (estrone and estradiol). In premenopausal women, aromatase inhibition is initially followed by increased estrogen synthesis in the ovary because of increased pituitary gonadotropin stimulation. In postmenopausal women, most estrogen synthesis occurs in peripheral tissue not under gonadotropin regulation and thus not subject to feedback stimulation. The specific inhibition of exemestane may overcome some problems encountered with less specific aromatase inhibitors such as aminoglutethimide.

The sponsor is planning to conduct carcinogenicity studies with exemestane in rats and mice. The Exec CAC reviewed the protocols for these studies on December 22, 1998, and March 16, 1999, respectively.

PHARMACOLOGY

The studies reviewed below were chosen in part due to their citation by the sponsor in their version of the label.

Binding of ¹⁴C-PNU 155971 to human serum albumine and α_1 -acid glycoprotein. Study no. 9750070. Study initiated May 20, 1996. Study completed May 22, 1996. Conducted by the sponsor according to GLPs (OECD/Italian).

Conclusion: Exemestane binds to HSA and AAG in a concentration-independent manner. The sponsor states that both proteins contribute equally to the binding. However, this statement should be considered in the context of the experimental design; no kinetic binding analysis or mixing experiments were performed. The different plasma concentrations of the two proteins *in vivo* will also affect the relative % binding (AAG, plasma concentration 0.05 g/dL (Duche et al., *J Chromatogr B Biomed Sci Appl* 1998, 715:103-9); albumin, plasma concentration 3.4-5.0 g/dL (Berne and Levy, *Textbook of Physiology*, 1993: Mosby Year Book, p 473)).

% binding of ^{14}C -FCE 24304 to human plasma proteins as determined by equilibrium dialysis.

Concentration ng/mL	HSA (40 g/L)	AAG (2 g/L)
10	93.4	94.5
50	93.9	94.8
200	93.6	94.2
500	93.5	94.1
1000	93.6	93.8

A study of the inhibition of specific forms of cytochrome P450 by exemestane. Study no. 9550346. Report dated December, 1995. Conducted by ^f (affiliation unstated) for the sponsor (non GLP). ^f with extensive experience in cytochrome P450 assays.

Conclusion: None of the drug-metabolizing P450 enzymes tested were significantly inhibited by exemestane.

Maximum percent inhibition of exemestane¹ on human liver P450 activities.

Activity	P450 isoform	Liver sample number					
		1	2	3	4	5	6
Phenacetin O-deethylase	1A2	24.3	5.3	14.3	18.3	19.1	27.4
Tolbutamide 4-hydroxylase	2C9	5.8	0.0	0.7	11.8	12.9	12.8
Debrisoquine 4-hydroxylase	2D6	6.4	0.6	0.0	3.2	6.7	3.3
Chlorzoxazone 6-hydroxylase	2E1	1.9	10.4	0.0	10.5	8.6	5.0
Terfenadine hydroxylase	3A4	8.3	6.9	1.8	0.0	1.0	2.4

¹ Exemestane was used at concentrations of up to 50 ng/mL.

Effects of exemestane on the activity of aromatase and other steroidogenic enzymes *in vitro*. Study no. 9850123. Report dated August 31, 1998. Conducted by the sponsor (non GLP). In addition to exemestane, other aromatase inhibitors were examined for comparative purposes for enzyme inhibitory activity.

Conclusion: Exemestane inhibits rat and human aromatase with no apparent effect on other steroidogenic enzymes. Of the aromatase inhibitors tested, letrozole was the most potent inhibitor of human aromatase and aminoglutethimide the least potent.

Mean IC₅₀s (nM) for inhibition of steroidogenic enzymes.

Compound	Aromatase	Aromatase	3 β -HSD-I	21-hydroxylase	11 β -hydroxylase	18-hydroxylase	17 β -HSD
Species	human	Rat	human	rat	rat	Rat	human
Exemestane	25.4	42.9	16,820	>100,000	72,500	84,500	>30,000
Formestane	21.9	72.8	10,568	—	—	—	>30,000
Anastrozole	13.7	6.0	>30,000	>100,000	>100,000	>100,000	>30,000
Letrozole	2.1	2.3	>30,000	>30,000	3,800	3,430	>30,000
Amino-glutethimide	2022	162.0	>30,000	>30,000	12,050	18,330	>30,000
Miconazole	20.4	—	—	2,920	180	160	—

Methods: Estrogenic and antiestrogenic activity were determined by the uterine weight assay in immature rats given FCE 24304 alone or in combination with estradiol benzoate. Androgenic and antiandrogenic activity were determined by prostate, seminal vesicle and levator ani muscle weights in immature castrated male rats given FCE 24304 alone or in combination with testosterone propionate. Progestational and antiprogestational activity were determined by endometrial proliferation in estradiol valerate-primed immature rabbits given FCE 24304 alone or in combination with progesterone. Glucocorticoid and antiglucocorticoid activity were determined by liver glycogen content in adult male rats given FCE 24304 alone or in combination with dexamethasone.

Effect of FCE 24304 on prostate and seminal vesicle weight (androgenic activity) and levator ani muscle weight (anabolic activity) in immature castrated male rats treated for 7 days. Data are mean organ or organ:body weight ratio (n = 7-14 animals/group)

Compound	Route	Dose mg/kg/d	Absolute and relative organ weight					
			Prostate		Seminal Vesicles		Levator ani	
			mg	mg%	mg	mg%	mg	mg%
vehicle	--	--	12.1	12.2	9.0	9.1	37.3	37.4
FCE 24304	sc	1	23.9	24.2	9.9	10.0	34.8	34.8
		3	36.4*	36.0*	12.4	12.4	48.7	48.8
		10	50.2*	50.0*	17.4*	17.4*	75.2*	75.2*
		30	58.1*	58.5*	26.8*	26.9*	85.0*	85.5*
	p.o.	30	23.6	23.7	11.4	11.4	44.0	43.4
		100	37.6*	36.8*	10.4	10.2	47.1	46.2
Testost. propionate	p.o.	0.03	20.2	20.4	11.0	11.1	36.8	37.1
		0.1	32.3*	31.9*	15.0*	14.7*	57.7*	54.8*
		0.3	56.0*	53.6*	34.3*	32.7*	80.7*	76.8*

* p < 0.01 vs. vehicle treated group

Endocrine characterization of FCE 24304: effects on aromatase, 5 α -reductase and desmolase and steroid receptor-binding affinity. Study no 204i. Report dated February 29, 1988. Conducted by (non-GLP).

Conclusion: The studies demonstrate that exemestane is an effective inhibitor of aromatase, with few effects on other enzymes in the steroidogenic pathway. In addition, the binding affinity studies demonstrate that parent exemestane should mediate few, if any, effects through this mechanism.

ED₅₀ (mg/kg) for ovarian aromatase inactivation in PMSG-stimulated rats.

Compound	s.c.	p.o.
FCE 24304	1.8	3.7

In vitro inhibition of rat prostatic 5 α -reductase and rat adrenal desmolase (P450SCC).

Enzyme	FCE 24304 concentration (μ M)	% inhibition	IC ₅₀ (μ M)
5 α -reductase	30	6	--
P450SCC	10	0	> 100
	30	1	
	100	8	

Relative steroid receptor binding affinity (RBA) of FCE 24304.

Receptor	Standard	RBA ₂ (%) ¹
Androgen	Dihydrotestosterone	0.22
Estrogen	Estradiol	0.005
Progestin	Progesterone	0.1
Glucocorticoid	Dexamethasone	0.075
Mineralcorticoid	Aldosterone	0.12

¹ calculated as % potency ($[\text{IC}_{50} \text{ standard} / \text{IC}_{50} \text{ FCE 24304}] \times 100$)

Comparative effects of exemestane on aromatase activity in fibroblasts of human breast adipose tissues, breast cancers and placental microsomes. Study no. 9850124. Report dated September 4, 1998. The study authors are William Miller (U Edinburgh) and Enrico di Salle (Pharmacia and Upjohn). The study was performed at the (non-GLP).

Conclusion: Exemestane inhibited aromatase in placental microsomes, breast cancer biopsies, and cultured adipose tissue fibroblasts in a dose-dependent manner.

IC₅₀s (nM) for aromatase inhibitors.

Compound	Placental microsomes	Breast Cancer Tissues	Cultured Breast Adipose Tissue Fibroblasts
Exemestane	45.0	12.2	5.3
Anastrozole	15.5	7.7	14.0
Letrozole	3.8	2.3	0.72
Fadrozole	5.3	--	--

Inhibitory effect of exemestane on the aromatase activity of human mammary tumor cells. Study no. 1470-98-009. Report dated March 9, 1998. Conducted by the sponsor (non GLP).

Conclusion: Exemestane inhibited MCF-7 aromatase activity in a dose-dependent manner, with an IC₅₀ of 32.9 nM.

PHARMACOLOGY SUMMARY

Exemestane is capable of inhibiting aromatase activity in both stromal and epithelial breast cells *in vitro*, consistent with literature reports of the presence of aromatase in both cell populations. Exemestane did not inhibit P450s involved in drug metabolism or other steroidogenic enzymes. The major metabolites of exemestane identified to date were less potent aromatase inhibitors than the parent compound. One metabolite, FCE 25071, was found to bind to the androgen receptor and to have androgenic activity *in vitro*. *In vitro*, exemestane was found to bind to serum albumin and α_1 -acid glycoprotein to the same extent, but the relative affinities of exemestane for each protein was not assessed.

SAFETY PHARMACOLOGY

Effects of FCE 24304 on the central nervous system. Study no. 202i. Study dated March 18, 1988. Conducted by (non GLP).

Conclusion: FCE 24304 induced mixed behavioral symptoms. Doses up to 200 mg/kg in mice and rats caused slight excitation whereas higher doses caused a slight decrease in spontaneous activity and clonic convulsions in mice. Mice appeared more sensitive than rats to CNS effects of FCE 24304.

Species: CD (SD)BR male rats; CD.1 (CR)BR male mice
 Age/weight: not specified: 140-160 g rats; 20-25 g mice
 Drug: FCE 24304
 Dosage: see table
 Schedule: single, repeat dose
 Route: oral gavage, i.p.

Test	Species	Dose mg/kg	Schedule	Findings
Irwin's	Mice and rats	12.5, 25, 50, 100, 200, 400, 800, 1600 (p.o.) 100 (i.p.)	Single	<u>Mice</u> Mortality: HD 6/6 within 2 hr from respiratory depression ≤ 200 po: ↑ irritability, tail pinch response ≥ 400 po 100 ip: slight depression; ↓ spontaneous activity, righting reflex; meiosis; preconvulsive symptoms (Straub tail, tremors, ↑ muscle tone, hind-limb paresis); clonic convulsions (> 60 min); dose dependent <u>Rats:</u> ≤ 200: slight irritability, hyperactivity, ↑ reflex response 1600 po, 100 ip: ↓ spontaneous activity ≥ 400 po, 100 ip: ↑ hind paw flushing
Body and skin temperature	Mice and rats	As above	Single	<u>Mice</u> Hypothermia from 400 mg/kg; dose dependent
Rotarod	Rats	10, 30, 100	Single D x 5	Unremarkable
Locomotor	Mice	10, 30, 100	Single	Unremarkable
Convulsions ^a	Mice	10, 30, 100	Single	Unremarkable
Phenobarbital interaction (45 mg/kg ip)	Rats	10, 30, 100	Single D x 5	Unremarkable

^a pentylenetetrazole induced tonic extensor convulsions and mortality

Convulsant and pro-convulsant effects of FCE 24304 in mice. Study no 2T01. Study dated June 28, 1990. Conducted by (non GLP). This study was designed to confirm previous observations.

Conclusions: FCE 24304 had convulsant effects (clonic convulsions). FCE-24304 showed marked, dose-related proconvulsant activity through interaction with pentylenetetrazole.

Species: CD.1 (CR)BR male mice
 N: 20/dose
 Age/weight: not specified: 20-25 g
 Drug: FCE 24304
 Dosage: 100, 200, 400, 800 or 1200 mg/kg
 Schedule: single
 Route: oral gavage

Dose mg/kg	0	100	200	400	800	1200	0	100	200	400	800	1200
PTZ ^a	-	-	-	-	-	-	+	+	+	+	+	+
# mice with clonic convulsions	0	0	0	0	2	14	19	17	19	19	20	20
# mice with tonic convulsions	0	0	0	0	0	0	2	1	3	5	10	16
Mortality	0	0	0	0	0	0	1	1	3	5	10	15

^a pentylenetetrazole 70 mg/kg i.p.

PHARMACOKINETICS

Pharmacokinetic studies on FCE 24304. Absorption, distribution, metabolism and excretion in rats and dogs. Study no. 823i. Report dated September 29, 1993. Conducted by (non-GLP).

In addition to blood levels, the concentration of radioactivity in tissues, fetuses, milk, feces, urine and bile; the plasma protein binding ratio (rat, dog and human); distribution into blood cells with time; and presence of metabolites were also determined. Structures of potential metabolites (FCE 25071, 27247, 27278, 27353, 27472, 27474, 27560, 27561 and 27562) are provided in the submission.

Conclusion: After a single dose, AUC/dose of total radioactivity does not increase with increasing dose in female rats. AUC and C_{max} in male rats were 30% and 50%, respectively, of that found in female rats. AUC increased with repeat dosing, reaching a steady state after the 12th dosing. Steady state plasma concentrations were 4-6.6 times higher than found after the first dose. Absorption of exemestane appeared more efficient in dogs than in rats, based on the higher C_{max} and AUC in this species.

Radioactivity concentration in the blood showed its first peak at 1 hour and declined thereafter. Absorption may be affected by feeding condition. Exemestane undergoes rapid and extensive metabolism and is primarily eliminated through the fecal route. Exemestane and/or its metabolites are subject to enterohepatic circulation. With the exception of the increased levels of radioactivity found in the rat maternal ovary (693% 6 hrs post dosing), maternal tissue concentrations of radioactivity in non-pregnant fasted female rats were similar to pregnant fasted rats. Exemestane and its metabolites are rapidly transferred to the fetal compartment. Radioactivity was also detected in the milk of lactating rats and, at some timepoints, at concentrations higher than found in maternal plasma. In all samples collected, unknown metabolites were the major component, and species differences between rats and dogs were observed in the metabolic fate of FCE 24304.

Species: Sprague-Dawley rats
 n: 3/dose/timepoint
 Age/weight: ♂ 8 weeks old, 240g
 ♀ 7-8 weeks, 190g; pregnant - mated at 9 weeks, 260 g (13th day of pregnancy) or 310 g (18th day of pregnancy); lactating - 10th-11th day post delivery, 300 g
 Drug: ¹⁴C-FCE 24304
 Labeled - Purity > 95%, lot CP-1451; unlabeled - lot 9001 G226

Route	oral gavage	oral gavage	i.v. (via metatarsal or caudal vein)
Dosage	0.2, 1 or 5 mg/kg	1 mg/kg	1 mg/kg
Schedule	Single	daily for 21 days	Single

Pharmacokinetic parameters calculated from total radioactivity in blood of fasted rats.

	Sex	AUC (0-∞) ng eq hr/mL	AUC/dose	t _{max} ¹ hr	t _{1/2} (6-24 hr) hr	C _{max} ¹ ng eq/mL
0.2 mg/kg (p.o.)	♀	223	1115	1	6.4	14
1 mg/kg (p.o.)	♀	748	748	1	7.1	42
5 mg/kg (p.o.)	♀	4300	860	1	5.3	164
1 mg/kg (p.o.)	♂	222	222	1	12	20
1 mg/kg (p.o.) (non-fasted)	♀	435	435	2	9.7	18
1 mg/kg (p.o.; D x 21) (non-fasted)	♀	6350	6350	1	22	48
1 mg/kg (i.v.)	♀	3180	3180	—	8.4	177 ²

¹ first peak; a second peak, sometimes larger than the first, was observed at 4-8 hours post dosing;

² C_{15min}

Excretion of radioactivity (0-120 hrs) as % of dose in urine and feces in rats and dogs.

Rats	Sex	Urine	Feces	Total	Carcass
0.2 mg/kg (p.o.)	♀	19.6	76.9	96.4	0.9
1 mg/kg (p.o.)	♀	18.1	81.8	99.9	0.8
1 mg/kg (p.o.)	♂	10.5	89.8	100.3	0.2
5 mg/kg (p.o.)	♀	16.3	82.2	98.5	0.3
1 mg/kg (p.o.) (non-fasted)	♀	15.0	81.0	96.0	0.4
1 mg/kg (p.o.; D x 21) (non-fasted)	♀	10.3	89.8	100.1	0.2
1 mg/kg (i.v.)	♀	26.4	75.3	101.6	0.9
Beagle dog					
1 mg/kg (p.o.)	♀	26.6	73.7	100.3	—

Radioactivity concentration (ng eq/g or mL) in tissues of fasted female rat administered a single oral dose (1 mg/kg) of ¹⁴C-FCE 24304 on the 18th day of gestation.

Tissue	1 hr post dosing	6 hr post dosing
Maternal plasma	41	58
Maternal blood	34	53
Maternal liver	1633	956
Maternal kidney	274	290
Maternal ovary	286	944
Placenta	78	76
Amnionic fluid	11	22
Fetus	27 ¹	70 ²
Fetal blood	24	51
Fetal liver	68	72
Fetal kidney	25	53

¹ 0.01% of total dose/fetus; ² 0.03% of total dose/fetus

Radioactivity concentration (ng eq/mL) in plasma and milk of non-fasted lactating rats administered a single oral dose (1 mg/kg) of ¹⁴C-FCE 24304 on the 10th-11th day of gestation.

Time	Milk	Plasma
15 min	10	12
1 hr	17	20
2	14	16
6	38	26
24	9	6
48	ND ¹	ND

¹ < 3 ng eq/mL

Species: female beagle dogs
 n: 3
 Age/weight: 10 months/8 kg
 Drug: ¹⁴C-FCE 24304
 Dosage: 1 mg/kg
 Schedule: single
 Route: oral gavage

Pharmacokinetic parameters calculated from total radioactivity in blood and plasma of dogs.

	AUC (0-∞) ng eq hr/mL	t _{max} hr	t _{1/2} (2-72 hr) hr	C _{max} ng eq/mL
Blood	1300	1	—	120
Plasma	2230	1	12	202

Protein binding (%) of ¹⁴C-FCE 24304 (in vitro) in female rat, dog or human plasma.

Concentration ng/mL	Rat	Dog	Human
100	89.5	95.1	95.9
1000	87.9	93.5	96.1

Ratio of protein binding of ¹⁴C-FCE 24304 (in vivo) in female rat and dog plasma (dose 1 mg/kg).

Time	Rat	Dog
1 hr	55.5%	61.6%
6 hr	69.6%	53.8%

Identification and quantification of metabolites in female rats and dogs administered ¹⁴C-FCE 24304 at a single dose of 1 mg/kg.

Species	Tissue	Compound	T _{max} (hr)	C _{max} (ng eq/mL)	% of total
Rat	Plasma	FCE 24304	1	2 ³	2.8
		Anticipated ¹	ND ²		
		RP24	1	16	28.5
		RP29	4	7	11.4
		RP23	4	5	7.8
		RP27	6	7	8.2
		RP22	6	5	5.3
		RP29	6	5	6.2
		Other (25)	—	<4	—
Rat	Urine ⁴ (0-48 hr)	RU71 + 72	—		19.0
		RU64	—		6.0
		RU55	—		4.4
		Other (71)			<3.9
	Feces (0-48 hr)	FCE 24304			0.3
		RF23			4.5
		RF24			3.1
		RF17			2.0
		Other (57)			<1.9
	Dog	Plasma	FCE 24304	0.25	4 ³
FCE 27353			1	8	4.1
FCE 27474			1	6	2.9
DP21			0.25	22	17.5
DP12			0.25	17	13.5

	DP11	0.25	16	12.7
	DP30	2	15	9.4
	DP33	6	11	11.8
	DP32	6	11	11.4
	Other (28)		<9	5.7
Urine (0-48hr)	FCE 27353	--		1.3
	DU37			9.0
	DU33			7.7
	DU12			6.6
	DU42			3.5
	DU58			3.5
	Other (53)			<3.2
Feces (0-48 hr)	FCE 24304			0.2
	DF44			6.0
	DF43			5.0
	DF17			4.8
	DF40			3.6
	DF41			3.3
	DF18			3.2
	Other (38)			<2.4

¹ see above (Page 9) for a list of potential metabolites (e.g., FCE xxxxx); ² none detected; ³ ng/mL; ⁴ enzymatic hydrolysis had no large effects on relative amounts of metabolites

Note: the metabolism of FCE 24304 in rats was affected by multiple dosing (D x 21); these data are not summarized. Also not discussed are the metabolic profile of FCE 24304 in rat liver, kidney and bile.

Study of absolute bioavailability of PNU 155971 (FCE 24304) in the female rat. Study no. 9650297. Report dated January, 1997. Conducted by the sponsor according to GLPs (OECD/Italian).

Conclusion: Exemestane exhibited rapid absorption, low bioavailability (< 5%) and high volume of distribution. Some deviation from dose-proportionality was observed.

Species: Sprague Dawley female rats
n: 4/dose/timepoint
Age/weight: 49-59 days; 190-230 g
Drug: FCE 24304 or ¹⁴C-FCE 24304
Batch 4002L226 (unlabelled) and TRQ6453 (labeled)
Dosage: oral, 3 or 30 mg/kg; i.v., 3 mg/kg
Schedule: single
Route: oral (via gavage) or i.v. (caudal vein)
Pharmacokinetics of unchanged FCE 24304.

	i.v. (3 mg/kg)	p.o. (3 mg/kg)	p.o. (30 mg/kg)
C _{max} (ng/mL)	623.5	3.3	40.1
C _{max} /dose	207.8	1.1	1.3
AUC (ng hr/mL)	298.0	7.6	174.0
AUC/dose	132.7	2.5	5.8
t _{max} (hr)	--	0.5	0.5
t _{1/2} (hr)	12.9	2.8	9.7
CL (mL/min/kg)	126	--	--
V _{ss} (L/kg)	21.0		
F (%)	--	1.9	4.4

Ancillary toxicokinetic study after single and repeated (4-week) oral administration of FCE 24304 in the female rat—Study no. 9650132. Report dated April, 1996. Conducted by the sponsor according to GLP (OECD/Italian).

Conclusion: FCE 24304 was rapidly absorbed after single and repeat dose administration. The AUC of exemestane exhibited dose proportionality at all doses except 315 mg/kg at day 1. A decrease in bioavailability and/or increase in systemic clearance of the drug may occur during repeat dosing.

Species: Sprague Dawley female rats
n: 5/dose/timepoint
Age/weight: 7 week old; 150-180 g
Drug: FCE 24304
 Batch 3002F226; purity > 98.4%
Dosage: 20, 50, 125 or 315 mg/kg/day
Schedule: D x 28
Route: oral (via gavage)

Mean pharmacokinetic parameters

Dose mg/kg	Day 1				Day 28			
	C _{max} ng/mL	t _{max} hr	AUC ng hr/mL	AUC/dose	C _{max} ng/mL	t _{max} hr	AUC ng hr/mL	AUC/dose
20	23.8	0.5	57.3	2.86	26.4	0.5	58.8	2.94
50	122.7	0.5	275.2	5.50	60.6	0.5	136.5	2.73
125	171.1	2	539.6	4.31	52.8	2	288.7	2.31
315	591.0	2	5332.4	16.93	96.8	2	742.3	2.36

Biliary excretion and metabolic pattern of [¹⁴C]-FCE 24304 in the rat after oral dosing. Study no. 806i. Report dated November 1990. Conducted By (non-GLP).

Conclusion: The polar fraction contains most of the biliary radioactivity. With the exception of increasing FCE 25071 (31x), enzymatic hydrolysis (β -glucuronidase/aryl sulfatase) had little effect on the metabolic pattern observed in bile.

51.84% of administered radioactivity appeared in the bile within the first 24 hours; an additional 1% appeared between 24-48 hours post dosing.

Species: Sprague Dawley female rats (bile cannulated)
n: 3
Age/weight: not stated; 200-300 g
Drug: ¹⁴C-FCE 24304
 Batch RT 7955/45; radiochemical purity > 99%
Dosage: 30 mg/kg ¹⁴C-FCE 24304
Schedule: single
Route: oral (via gavage)

Mean relative percent of radioactivity excreted in bile.

Fraction or metabolite	0-1 hr	0-1 hr	7-24 hr	7-24 hr
	Before ¹	After	Before	After
Polar	78.34	68.06	83.32	66.43
F3 ²	2.27	2.84	1.31	4.92
25071	0.00	3.01	0.13	4.03
F4 ²	12.31	16.88	8.08	11.81
24304	6.37	8.27	6.07	11.14
F5	0.70	0.93	1.09	1.67

¹ Before or after enzymatic hydrolysis; ² metabolite also reported in rat urine.

Plasma levels of FCE 24304 in female Beagle dogs during a 52-week oral toxicity study. Study no. 809i. Samples collected in study no. 416i (reviewed in Toxicology below). Report dated May, 1992. No statement of GLP compliance with the analytical phase of the report is included. The aim of the study was apparently to demonstrate absorption of the compound for the toxicology study.

Conclusion: Large variations in plasma samples occurred in animals of the same dose group at all doses tested. In this study FCE 24304 did not substantially accumulate with additional dosing and may induce its own metabolism. FCE 25071 is known to bind to the androgen receptor and to have androgenic activity *in vivo*. Factoring in dosing and body weights, the plasma concentration of this metabolite is within the physiological range of androgens.

Species: female beagle dogs
 n: 6/dose
 Age/weight: 7-8 months/7.1-10.2 kg
 Drug: FCE 24304
 Batch #: 9002 G226; purity 97.8%
 Dosage: 30, 120 or 480 mg/kg/day
 Schedule: daily for 52 weeks
 Route: oral gavage (administered to fasted animals)
 Formulation: 5% Methocel/0.4% Tween 80
 Volume: 2 mL/kg

Mean plasma levels (ng/mL) of FCE 24304 during 52 weeks of treatment.

Dose mg/kg/d	Treatment Day 1			Treatment Day 183			Treatment Day 358		
	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
30	73	nd ¹	nd	142	17	4	130	2	nd
120	447	23	4	134	11	6	222	11	nd
480	1942	316	28	238	22	19	873	186	nd

¹ nd = < 10 ng/mL

Mean plasma levels (ng/mL) of an FCE 24304 metabolite, FCE 25071², during 52 weeks of treatment.

Dose mg/kg/d	Treatment Day 1			Treatment Day 183			Treatment Day 358		
	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr	1 hr	4 hr	24 hr
30	18	Nd ¹	nd	24	3	nd	24	nd	nd
120	104	7	4	38	nd	nd	37	-2	nd
480	516	94	6	67	19	nd	143	39	nd

¹ nd = < 10 ng/mL

² FCE 25071 contains a hydroxy group at C17 rather than a keto group at this position as in FCE 24304.

Absorption of ^{14}C -FCE 24304 after single oral administration in comparison with i.v. dose in the dog. Report no 807i. Report dated November 12, 1990. Conducted by _____
(non GLP).

Conclusion: Approximately equal amounts of total radioactivity were recovered in urine and feces within 168 hours after i.v. administration of labeled FCE 24304. Fecal elimination is the primary route of elimination after oral administration. Urinary elimination occurred principally within the first 24 hours after dosing whereas fecal elimination occurred mainly within 48 hours of dosing.

Species: female beagle dogs
n: 3
Age/weight: not stated/9.3-11.1 kg
Drug: ^{14}C -FCE 24304
Dosage: 1 (i.v.) or 30 (oral) mg/kg
Schedule: single
Route: oral gavage and i.v., 4 week washout

	AUC h ng eq/mL	AUC/dose	t _{1/2} hr	C _{max} ng eq/mL	t _{max} hours	F urine ¹	Feces ²	Urine ²
i.v.	5056	5056	95.02				47.37%	34.30%
p.o.	31474	1049 ³		2321	2.25	37%	73.81%	12.94%

¹ index of absorption of radioactivity, as % dose excreted in urine after oral and i.v. dosing; ² total amount recovered from 0-168 hours as % of administered dose; ³ 21% absorption of total radioactivity based on AUC.

Urinary metabolism of [^{14}C]-FCE 24304 in the dog after intravenous and oral administration. Report no 808i. Report dated January, 1992. Conducted by _____
(non GLP). Samples were collected for data used in report 807i.

Conclusion: The parent compound and 7 separate peaks were identified by radio-TLC. The profile of metabolites was similar after oral and intravenous administration. Significantly less % radioactivity was present in each fraction following oral administration vs. intravenous administration when calculated as a % of total radioactivity administered (total of all peaks, 4 vs. 22%, respectively). Intravenous data are not summarized. Enzymatic hydrolysis decreased the amount of radioactivity found in the polar fraction. The sponsor states that no significant differences were observed in metabolite pattern after enzymatic hydrolysis by β -glucuronidase vs. β -glucuronidase/aryl sulfatase (data not presented by the sponsor).

Species: female beagle dogs
n: 3
Age/weight: not stated/9.3-11.1 kg
Drug: ^{14}C -FCE 24304
Dosage: 1 (i.v.) or 30 (oral) mg/kg
Schedule: single
Route: oral gavage and i.v., 4 week washout

Mean % radioactivity peaks in urine as a % of total urinary radioactivity (4-9 hr post-dose collection) following oral administration of FCE 24304. Peaks are shown in decreasing order of polarity.

Enzymatic hydrolysis ¹	Polar	F1	F2	F3	FCE25071	F5	F6	FCE24304
-	64.66	8.53	18.02	5.69	0.51	-	1.98	0.61
+	47.28	13.97	21.44	8.93	3.14	1.57	2.80	0.88

Mean % radioactivity peaks in urine as a % of total radioactivity administered.

-	2.61	0.34	0.72	0.24	0.02	--	0.08	0.02
+	1.97	0.52	0.85	0.35	0.12	0.05	0.10	0.04

¹β-glucuronidase/arylsulfatase

Biliary metabolites of ¹⁴C-exemestane after oral administration to female beagle dogs. Study no. 9750055. Report dated February, 1997. Conducted by the sponsor (non GLP). The biological samples analyzed in this study were collected under study no. 9650399.

The sponsor uses a different nomenclature to refer to the same metabolites in this study, causing some confusion. For cross-reference, the metabolites are: A, 25071; B, 27560; C, 27561; D, 27562; E, 27247; F, 27472; G, 27473; H, 27353; I, 27474; and L, 27278. The structures of these compounds are provided in the submission.

Conclusion: Exemestane is extensively metabolized. The sponsor states that most conjugation was found to be with glucuronic acid as little difference in the metabolite profile was observed between bile extracts incubated with β-glucuronidase/aryl sulfatase vs. β-glucuronidase alone (data not presented by the sponsor). Compounds identified were exemestane and metabolites A-I; based on structural knowledge of these compounds, biotransformation of exemestane occurs through reduction of the 17-keto group and/or epoxidation of the 6-exo double bond followed by hydrolysis and rearrangement.

Species: female beagle dogs (fasted)
 n: 1/timepoint (3 total)
 Drug: ¹⁴C-FCE 24304
 Dosage: 30 mg/kg ¹⁴C-FCE 24304
 Schedule: single
 Route: oral (via gavage)

Bile was collected from 1 female beagle at each post dosing time.

Fraction	% of total radioactivity					
	Enz hydrolysis ¹ →		Time →			
	-	+	-	+	-	+
	0.5 hr	0.5 hr	2 hr	2 hr	12 hr	2 hr
Unknown polar (Unk 1 and 2) ²	56.32	27.19	84.25	36.15	83.17	36.15
Exemestane	3.91	4.27	0.72	2.90	0.30	2.90
A	0.67	1.38	--	1.91	--	1.38
B	3.09	3.28	1.05	1.62	1.8	1.85
C	1.61	1.68	0.69	1.16	1.07	1.30
D	12.98	16.87	1.68	5.5	1.39	5.5
E	0.60	1.68	0.46	3.17	0.57	4.45
F	--	1.08	2.55	2.79	3.21	3.35
G	3.72	4.76	1.19	3.37	1.67	4.10
H	2.05	4.70	0.73	7.09	1.05	4.71
I	3.18	5.16	--	4.22	--	3.27

¹ β-glucuronidase/aryl sulfatase; ² Other unknowns referred to as Unk 3-12.

Pharmacokinetic Summary

Pharmacokinetic parameters

After a single dose of exemestane (oral gavage) in female rats, normalized AUC of parent compound exhibited dose proportionality from 20-125 mg/kg; however, a major deviation was observed at 315 mg/kg/day (the HD). After repeat dosing, normalized AUCs declined 2-fold for 50-125 mg/kg/d and 7-fold for 315 mg/kg/d. This suggests saturation of a metabolic process that is counterbalanced by induction of metabolic processes with repeat administration. In mice, normalized AUC after single and repeat dosing increased with increasing dose (oral gavage) to 50 mg/kg; in the range of 50-450 mg/kg, normalized AUC was dose proportional (see review #3). In mice, normalized AUC decreased with repeat dosing except at the lowest dose tested (15 mg/kg/day), where an increase was observed. In oral feeding studies, normalized AUC decreased with increasing dose, and no major differences between days 7 and 28 were observed.

The AUC of total radioactivity increased with repeat dosing, reaching a steady state 12 days after initial dosing. In mice and rats administered exemestane via oral gavage, normalized AUC of the parent compound decreased with repeat dosing.

Comparison of dose (oral gavage) and AUC for exemestane^a in mice, rats and humans.

Mice ^b		Rats		Human ^b	
Dose	AUC day 90	Dose	AUC day 28	Dose	AUC after chronic dosing
mg/kg/day	ng hr/mL	mg/kg/day	ng hr/mL	mg/day	ng hr/mL
15	38	20	58.8	2.5	10.6
50	282	50	136.5	10	35.8
150	1126	125	288.7	25 ^c	104.9
450	2762	315	742.3	50	206.8

^a parent drug only; ^b review #3; ^c proposed human dose

Absorption

The t_{max} of exemestane is reached 0.5-1 hour after oral administration in rats and dogs, indicating that the drug is rapidly absorbed from the GI tract. Species differences include a C_{max} and AUC that are higher in dogs than rats. The AUC, C_{max} and t_{max} of total radioactivity in non-fasted female rats was 58% and 43% of that observed in fasted female rats, respectively, indicating that food delays absorption of exemestane. Bioavailability of exemestane in female rats and female dogs, respectively, was calculated at 1.9% and 3.53% after a single oral dose of 1 mg/kg, and 4.4% and 4.97% after a single oral dose of 30 mg/kg (dog study not summarized).

Distribution

Exemestane is characterized by a large volume of distribution. In female rats, the level of radiolabeled drug (parent and metabolites) was particularly high in liver, adrenals, kidneys and the GI tract. The blood to plasma ratio of drug indicated some uptake of drug material into blood cells. In all species tested, a significant portion of exemestane was bound to serum protein (> 89% in rats, rabbits, dogs, humans and monkeys). Serum albumin and α_1 -acid glycoprotein both bind exemestane *in vitro*.

Radioactivity was also found to substantially distribute to the rat fetuses, with fetal liver concentrations higher than maternal blood concentration. As expected by the lipophilic nature of exemestane, levels of drug in milk of lactating rats was higher than observed in maternal plasma. The maternal ovary of the pregnant rat also had substantially elevated levels of total radioactivity compared to the non-pregnant rat.

Metabolism

Extensive first-pass metabolism of exemestane is suggested by comparison of AUC ratios (oral:i.v.) of total radioactivity (24% rats, 21% dogs) vs. parent compound (< 5% for both species). A large number of metabolites was observed in all biological samples examined. Most metabolites have decreased biological potency compared to the parent compound, with the exception of the androgenic activity of FCE 25071. The rapid metabolism of exemestane probably accounts for its low bioavailability, approximately 5% at a dose of 30 mg/kg in female rats. A decrease in bioavailability and/or increase in systemic clearance was observed with repeat dosing. Sex differences were observed in AUC of male and female rats, but as most studies used only female animals, the nature of sex differences is uncertain.

Most conjugation was found to be with glucuronic acid as arylsulfatase had little effect on the metabolite profile (data not submitted). Based on structural knowledge of identified metabolites, biotransformation of exemestane probable occurs through reduction of the 17-keto group and/or epoxidation of the 6-exo double bond followed by hydrolysis and rearrangement. A number of metabolites have not yet been structurally identified.

Elimination

Dogs appear to have a higher urinary and lower fecal rate of elimination compared to rats. Exemestane is subject to enterohepatic circulation. In rats and dogs, fecal elimination was the most common route of elimination. Most of the administered dose appears in the feces within 48 hours post administration. The plasma half-life of total radioactivity of the terminal phase was 5.3-7.1 hours for rats and 12 hours for dogs. A plasma half-life of parent exemestane in the terminal phase of elimination of 13 hours was observed in rats.

TOXICOLOGY

Palatability study of FCE 24304 for 4 weeks in mice. Report no. 9650020. Study no 911-128. Study dated September 4, 1991. Conducted by (non GLP). An additional aim of this study was to aid in dose selection for longer-term studies.

Conclusion: The sponsor-recommended dose levels for a 13-week study were 25, 100, 400 and 1600 mg/kg/day. The HNSTD for this study was at least 1000 mg/kg/day.

Species: CD-1 (ICR)BR mice
 n: 5/sex/dose
 Age/weight: 6 weeks; ♂ 23.5-31 g, ♀ 18-23 g
 Drug: FCE 24304
 Batch # 9001 G226; purity 98.2%
 Dosage: 100, 300 or 1000 mg/kg/day
 Schedule: daily for 4 weeks
 Route: oral (via feed)

Mortality (2x daily)	None
Clinical Obs. (daily)	Unremarkable
Body weights (daily)	↓ HD ♂ 9.6%; nadir week 3

Food consumption (wkly)	↓ HD ♂ weeks 1-3; nadir week 2, 15% ↓ HD ♀ weeks 1, 3, nadir week 3, 17%
Gross Pathology	Unremarkable

Palatability study of FCE 24304 for 4 weeks in rats. Study no. 9650021. Report dated September 4, 1991. Conducted by _____ (non GLP). An additional aim of this study was to aid in dose selection for longer-term studies.

Conclusion: No palatability problems were noted. The sponsor-recommended dose levels for a 13-week study were 10, 50, 250 and 1250 mg/kg/day. The HNSTD for this study was at least 1000 mg/kg/day.

Species: CD BR rats
 n: 5/sex/dose
 Age/weight: 6 weeks; ♂ 214.7-257.0 g, ♀ 144.1-167.1 g
 Drug: FCE 24304
 Batch # 9001 G226; purity 98.2%
 Dosage: 100, 300 or 1000 mg/kg/day
 Schedule: daily for 4 weeks
 Route: oral (via feed)

Mortality (2x daily)	None
Clinical Obs. (daily)	Unremarkable
Body weights (daily)	↓ vs. C: ♂ HD 30%, MD 8.5%, nadir week 4
Food consumption (wkly)	↓ vs. C: ♂ HD 20%, nadir week 4 ↑ vs. C: LD-HD ♀ week 1, 36%, not dose dependent
Gross Pathology	Unremarkable

PNU-155971 (FCE 34204): 13-week toxicity study by diet in rats (DRF). Report no. 9650370. Study nos. N504 and N504Bis-Q1294. Report dated March 18, 1997. Conducted by the sponsor according to GLPs (OECD/Italian). The purpose of the study was to collect toxicity information in order to establish a dose for a possible carcinogenicity study. However, this oral feeding study was not used for this purpose as exemestane is to be administered in the 2-year carcinogenicity by oral gavage (see Exec CAC minutes, December 22, 1998).

Conclusion: The STD₁₀ for this study was 900 mg/kg/day for both male and female rats. Target organs for exemestane toxicity included the liver, reproductive tract, thymus and possibly the pituitary, spleen and adrenals. The severe body weight depression, particularly in HD animals, and lack of concurrent controls at some doses (MD3 and MD4) complicated interpretation of the findings. A 4-week dietary study indicated that exemestane was palatable at 1000 mg/kg/day, the highest dose tested. This study was briefly summarized in IND review no. 2.

Species: CD BR rats
 n: 10/sex/dose
 satellite: 3 or 6 rats/sex/dose
 Age/weight: 5 weeks; ♂ 132-208 g, ♀ 121-171 g
 Drug: FCE 24304
 Batch # 9004 G226; purity 97.4%