Investigator's Brochure

for

[^{18}F]Fluoroestradiol

AN INVESTIGATIONAL POSITRON EMISSION TOMOGRAPHY (PET) RADIOPHARMACEUTICAL FOR INJECTION, INTENDED FOR USE AS AN *IN VIVO* DIAGNOSTIC FOR IMAGING ESTROGEN RECEPTORS IN TUMORS

IND [Your IND Number provided by FDA]

IND Sponsor: [Your Name and Address Here]

IB Edition Number: [Number]
IB Edition Date/Release Date: [Date]

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1. Summary

16α-[^{18}F]-fluoro-17β-estradiol (FES) is a radiolabeled imaging agent that has been used with positron emission tomography (PET) to investigate tumor estrogen receptor (ER) activity. FES binding to sex steroid binding protein (SBP or SHBG) is nearly identical to that of estradiol[1]. Studies have shown that the strength of FES binding to the ER is also nearly identical to estradiol[2]. In breast cancer, the uptake of FES, measured by PET, has been shown to correlate with ER expression in biopsy material assayed by *in vitro* radioligand binding[3] or by immunohistochemistry[4]. [^{18}F]FES has not been marketed in the United States and, to the best of our knowledge there has been no marketing experience with this drug in other countries. Over 500 patients are known to have received this drug, either as reported through the published literature, under the RDRC (Radioactive Drug Research Committee) program or under Investigational New Drug (IND) application, without adverse effects. Fluorine-18 labeled FES is synthesized with high specific activity so the quantity of estrogenic material injected with the radiopharmaceutical is < 5 μg[2,5]. Between 3 and 6 mCi of [^{18}F]FES is administered in a nominal volume of 20 mL of phosphate buffered saline containing less than 15% ethanol for a single PET scan.

Radiation from typical ^{18}F PET exposure is minimal compared to the Food and Drug Administration (FDA) suggested limits (below) and are thought to present a very small risk to the recipient. The radiation absorbed effective dose equivalent to the whole body from intravenously injected [^{18}F]fluoroestradiol is estimated to be 0.022 mSv/MBq (488 mrem for a 6 mCi injection). The critical organ is the liver, with an average absorbed dose of 0.13 mGy/MBq. The organ and total body doses associated with FES PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures[6,7,8,9] and are well below the maximum suggested individual study and annual total body dose of 30 and 50 mGy, respectively, suggested for investigational radiopharmaceuticals by the FDA[10].

[^{18}F]FES uptake is advancing our understanding of the role of functional ER expression in cancer. This knowledge will help in the design of therapeutic trials to improve treatment outcomes, and has the long term potential to help clinicians plan, target, and evaluate therapies. Future investigations will evaluate the use of [^{18}F]FES PET imaging to direct patient treatment and to evaluate the efficacy of specific therapies *in vivo*.

A standard 14-day repeat dose toxicology study of [^{19}F]fluroestradiol in rodents at daily doses that were 25 and 100 times the maximum human dose on a surface area basis, representing a cumulative maximum dose of 1400 times the human dose, demonstrated that FES is non-toxic in a preclinical setting.

2. Introduction

The investigational radiopharmaceutical is [^{18}F]fluoroestradiol; 16-alpha-[^{18}F]-fluoro-17-beta-estradiol; ([^{18}F]FES). [^{18}F]FES is a lipophilic molecule that acts similarly *in vivo* to estradiol and binds to estrogen receptors. This radiopharmaceutical is under investigation as a noninvasive diagnostic agent for assessment of the estrogen receptor content of tumors using positron emission tomography (PET). As a noninvasive agent, the entire body can be scanned for estrogen receptors without requiring biopsy and can sample any imageable tumors in the body. Biopsy methods can only sample a limited number and volume of tumors. The advantages of *in vivo* assessment of estrogen receptors include avoiding sampling error, and assessing the entire tumor volume receptor status rather than part of the tumor (addressing the heterogeneity of ER expression), and assessing the biological activity of the receptor at diagnosis and in response to treatment[11]. This imaging information may prove useful to determine the value of hormonal therapy for cancer that targets estrogen receptors in individual patients.

In the last 25 years, more than 20 fluorinated estrogen derivatives have been proposed for imaging studies. The most promising radiolabeled estrogen analog identified to date is 16-α-[^{18}F] fluoro-17-β-fluoroestradiol (FES), which has good ER binding affinity and can be prepared in high effective specific activity[12]. The [^{18}F]fluoroestradiol is a sterile, intravenously (IV) injectable solution in a volume of ≤ 20 mL containing of 0.15 M phosphate buffered saline: < 15% ethanol (v:v). The injectable dose of [^{18}F]FES is generally 6 mCi (220 MBq), with an allowable range of 3 to 6 mCi (110 – 220 MBq) of [^{18}F]fluoroestradiol and with a specific activity greater than 170 Ci/mmol at the time of injection. FES is the only active ingredient. There is no evidence that nonradioactive and radioactive FES molecules display different biochemical behavior.

3. Physical, Chemical, and Pharmaceutical Properties and Formulation

3.1. Agent Description

[^{18}F]FES is a lipophilic molecule that acts similarly to estradiol *in vivo* and binds to estrogen receptors. This radiopharmaceutical is under investigation as a noninvasive diagnostic agent for noninvasive assessment of the estrogen receptor content of tumors using positron emission tomography (PET) imaging.

The [^{18}F]fluoroestradiol is a sterile, IV injectable solution with a volume of ≤ 20 mL containing 0.15 M phosphate buffered saline: < 15% ethanol (v:v). The injected dose of [^{18}F]FES is generally 6 mCi (185 MBq) with an allowable range of 3 to 6 mCi of [^{18}F]fluoroestradiol. The drug product solution is stored at room temperature in a gray

butyl septum sealed, sterile, pyrogen-free glass vial with an expiration time of 8 hours. The mass of injected drug is ≤ 5 μg (≤ 17 nmol) of FES.

3.2. Chemical Structure

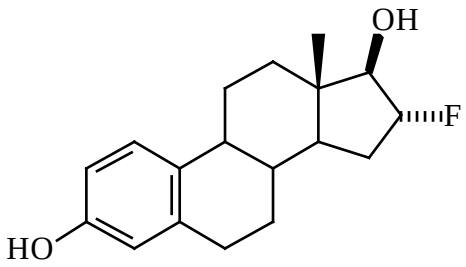

Figure 3.1. 16-α-[^{18}F]fluoro-17-β-fluoroestradiol

3.3. Final Product Specifications

Table 3.1. Specifications for [^{18}F]fluoroestradiol solution

Property	Specification
Chemical Purity (particulates)	Clear and Colorless
pH	6 – 8
Residual Kryptofix® [2.2.2]	< 50 μg/mL Kryptofix®
Radiochemical Purity (HPLC)	> 95%
Chemical Purity (HPLC)	FES < 5 μg per injected dose, other UV absorbing impurities beyond HPLC void volume (280 nm) ≤ 5 μg per injected dose
Radiochemical Purity (TLC)	R_f > 0.5 Purity ≥ 95%
Radionuclidic Purity	Measured half-life 100 – 120 minutes
Residual Solvent Levels	Acetone < 5,000 ppm Acetonitrile < 400 ppm
Bacterial Endotoxin	< 175 EU per dose
Sterility	No growth observed in 14 days, also must pass filter integrity test

Table 3.2. Components of the final [^{18}F]FES drug solution

Components	Grade	Amount in Final Product
[^{18}F]FES, 16-alpha-[^{18}F]-fluoro-17-beta-estradiol	Same as for [^{19}F]FES	nominally 6 mCi (3.0 to 6.0 mCi allowed)
[^{19}F]FES, 16-alpha-[^{19}F]-fluoro-17-beta-estradiol	NSC# 743445	≤ 5 µg
Ethanol, absolute	USP*	<15% by volume
Saline for injection	USP	0.075 M
Sodium phosphate	USP	0.075 M
Water for Injection	USP	Remainder

*United States Pharmacopeia (USP)

Table 3.3. Limit of impurities in the final [^{18}F]FES drug product

Impurities	Acceptance Criteria
Kryptofix® [2.2.2]	< 50 µg/mL
Acetonitrile	< 400 ppm
Acetone	< 5000 ppm
Other Impurities by HPLC / UV Absorbance at 280 nm	< 5 µg total per patient dose

[^{18}F]-fluoroestradiol (FES) is a radiopharmaceutical designed for imaging estradiol binding to estrogen receptors (ERs) *in vivo*. Its molecular weight is 290.4 Daltons. FES has chemical properties very similar to estradiol. The relative binding affinity (RBA, FES/estradiol) for the estrogen receptor is 0.8[13]. The metabolism of FES and estradiol are similar[14,15] with elimination primarily by conjugation in the liver, followed by renal clearance of the glucuronide. Measurements of the relative binding affinity for the blood transport protein, sex hormone binding globulin (SHBG), was 10% relative to estradiol[16]. An average of 45% of circulating FES is bound to SHBG[17], similar to estradiol[18]. In the sections that follow, the pharmacology and toxicity of estradiol in addition to FES is reviewed.

FES is produced with a specific activity greater than 170 Ci/mmol and the injected mass dose is less than or equal to 5 µg (17 nmole). The requirement for high specific activity, low mass dose, assures that only a small fraction of the estrogen receptors (ER) are occupied during a PET imaging study. If the receptor approaches saturation, then FES uptake would no longer reflects receptor concentration. A 5-µg dose is far below any known toxicity for fluoroestradiol or other ER ligands.

4. Nonclinical Studies

4.1. Nonclinical Pharmacology of FES and Estradiol

The extensive body of literature on the pharmacology and toxicity of fluoroestradiol and of estradiol is summarized below. Estradiol is a naturally occurring substance with biochemical and pharmacologic properties nearly identical to FES. It is important to interpret toxicity data for FES relative to reported toxicity for estradiol in the context of the intended use of FES as a single-dose-administration agent for diagnostic imaging. In this setting, FES reaches physiologic levels (i.e., greater than post-menopausal levels) only on a transient basis. This must be viewed in the context of many years of exposure to physiologic levels of estradiol in most women.

4.2. Pharmacokinetics and Metabolism of FES in Animals

The pharmacology of FES has been studied in rats. In rats, FES is rapidly metabolized to more polar species[19]. By 60 minutes after injection, less than 15% of circulating radioactivity is due to [^{18}F]FES; the remainder is metabolites. Injection of blood from rats obtained 2 hours after injection into different rats showed that the metabolites did not accumulate in ER-rich tissues, such as the uterus, that could be blocked by estradiol[19]. This suggests that the metabolites are conjugates or other species that do not bind to ER, as compared to unconjugated oxidation products such as the estrone, which would be expected to bind to ER.

Immature Sprague-Dawley female rats received a single injection of [^{18}F]FES and were sacrificed at 30, 60, and 120 min after injection. The results of tissue distribution assays are presented in Table 4.1 as percent injected dose per gram[13]. [^{18}F]FES was found to have high binding selectivity to target estrogen receptor rich tissues.

Table 4.1. Biodistribution of 16α-[^{18}F]fluoro-17β-estradiol in immature female rats, % I.D. per gram (mean ± s.d.)[*]

Tissue	½ hr[†]	1 hr[†]	1 hr[‡] (low dose)	1 hr[§] (+E2)	2 hr[†]
Blood	0.16 ± 0.03	0.14 ± 0.07	0.14 ± 0.07	0.50 ± 0.24	0.10 ± 0.05
Uterus	4.87 ± 1.17	4.67 ± 1.50	7.09 ± 1.04	0.61 ± 0.12	8.58 ± 7.01
Ovaries	1.62 ± 0.410	1.59 ± 0.60	2.48 ± 0.52	0.45 ± 0.13	2.25 ± 1.52
Muscle	0.21 ± 0.09	0.17 ± 0.08	0.42 ± 0.26	0.12 ± 0.02	0.18 ± 0.15
Liver	2.10 ± 0.67	1.29 ± 0.25	1.24 ± 0.36	2.02 ± 0.56	1.26 ± 0.88
Spleen	0.14 ± 0.04	0.11 ± 0.04	0.15 ± 0.05	2.18 ± 0.03	0.17 ± 0.15
Kidney	1.22 ± 0.33	0.81 ± 0.14	1.07 ± 0.13	0.74 ± 0.12	0.57 ± 0.22
Esophagus	0.18 ± 0.04	0.23 ± 0.09	0.81 ± 0.79	0.40 ± 0.07	0.22 ± 0.17
Lung	0.24 ± 0.06	0.17 ± 0.04	0.29 ± 0.07	0.41 ± 0.16	0.17 ± 0.07
Bone	0.18 ± 0.06	0.17 ± 0.07	0.27 ± 0.09	0.23 ± 0.07	0.26 ± 0.15

[*] Five animals were used at each time point.

[†] Injected with 50 μCi 16α-[^{18}F]Fluoro-17β-estradiol

[‡] Injected with 5 μCi 16α-[^{18}F]Fluoro-17β-estradiol

[§] Injected with 50 μCi 16α-[^{18}F]Fluoro-17β-estradiol + 15 μg estradiol, E2.

Nineteen mature female rats with 7,12-dimethylbenz[a]anthracene (DMBA) induced mammary tumors received a single injection of [^{18}F]FES, and tissue and tumor uptake was measured at 3 hours post injection as % injected dose per gram of tissue and tumor to blood and tumor to non-target tissue ratios. The correlation between [^{18}F]FES and ER content at 3 hours was poor[19]. However, VanBrocklin et al[20] studied 69 female Sprague-Dawley rats age 22 – 30 days that were injected with 50 μCi of [^{18}F]FES and sacrificed at one-hour post injection to measure alphafetoprotein (AFP) serum concentration and determine the effects of AFP binding on blood activity levels of [^{18}F]FES. A strong positive correlation was seen between serum AFP concentration and [^{18}F]FES blood activity levels, suggesting that the anticipated correlation between uptake of the tracer and ER content may be compromised by the presence of unbound metabolites in the blood and by endogenous molecules that possess high affinity binding sites for phenolic steroids including estrone and estradiol, such as AFP in rats or SHBG in humans[20].

Time activity curves for animal models are not directly applicable to human uptake for [^{18}F]FES due to the lack of sex hormone binding globulin (SHBG) in rats. Human time activity curves for blood in humans are shown in Section 5.2.

4.3. Toxicology

4.3.1 FES Animal and *In Vitro* Testing

To facilitate advancing the agent beyond phase 1 studies and to validate that FES is non-toxic in a preclinical setting, a 14-day repeat-dose toxicology study of ^{19}F-fluroestradiol was performed in rodents at daily doses that were 25 and 100 times the maximum

human dose on a surface area basis, a cumulative maximum dose of 1400 times the human dose.

Table 4.2. Summary of absolute dose, weight dose, and surface area dose

	Absolute dose	Weight dose µg/kg	Surface area dose µg/m^2
60 kg human	5 µg	0.083	3.08
250 gram rat	12.8 µg	51	308
Factor	2.56 x	614 x	100 x
Cumulative dose	36.4 x	8,596 x	1400 x

No toxicity was apparent in this repeat-dose rodent study. We have also performed the *in vitro* hERG, Ames, and mouse TK studies utilizing the maximum stable concentration of the drug in ethanol, 8 ng/ml. (For reference, the maximum instantaneous blood concentration of a 5-µg dose of FES in a 70-kg human would be approximately 1 ng/ml.)

14-day Repeat-Dose Toxicology Study In Rats With Micronucleus Assessment

Fluoroestradiol in the vehicle (15% ethanol:85% saline) was administered by intravenous injection once daily for 14 consecutive days to two groups (Groups 2 and 3) of Sprague-Dawley CD®(SD)IGS BR rats at 13 and 51 µg/kg, respectively. An additional group (Group 4) was administered cyclophosphamide at 30 mg/kg (positive control for micronucleus test) by an intraperitoneal injection on the last day of dosing. A concurrent control group (Group 1) received the vehicle on a comparable regimen as the test article groups. The dosage volume was 2.0 mL/kg for Groups 1 – 3 and 5.0 mL/kg for Group 4. Groups 1 – 3 each consisted of 5 animals/sex, and Group 4 consisted of 2 males. Animals were observed twice daily for mortality and moribundity. Clinical examinations were performed daily at the time of dosing and individual body weights and feed consumption were recorded at selected intervals. At the end of the dosing period, all animals were humanely euthanized. Clinical pathology evaluations were performed on all animals in Groups 1 – 3 at necropsy. Complete necropsies were conducted on all animals in Groups 1 – 3, and selected organs were weighed. Selected tissues were examined microscopically from all animals in Groups 1 and 3. Bone marrow smear slides were prepared from all animals for micronuclei determination.

There were no signs of toxicity at the doses tested on this study. No adverse clinical observations were noted during the study. There were no test article-related changes in body weights or feed consumption. Clinical pathology parameters were unaffected by test article administration, and there were no toxicologically relevant organ weight changes. All macroscopic and microscopic findings observed were considered spontaneous and/or incidental in nature and unrelated to test article administration, as they were consistent with normal background lesion in clinically normal rats of the age

and strain used on this study. Therefore, based on the results of this study, the no-observed-effect level (NOEL) for intravenous administration of fluoroestradiol to rats for 14 consecutive days was greater than 51 µg/kg/day.

Effects of 16alpha-Fluorestradiol on Cloned hERG Potassium Channels Expressed in Human Embryonic Kidney Cells

The objective of this study was to examine the *in vitro* effects of 16alpha-fluoroestradiol on the hERG (human ether-á-go-go-related gene) channel current (a surrogate for IKr, the rapidly activating, delayed rectifier cardiac potassium current). 16alpha-fluoroestradiol inhibited hERG current by (Mean + SEM, n = 3) 1.4 + 0.2% at 8 ng/mL versus 0.3 + 0.1% in control. hERG inhibition at 8 ng/mL was statistically significant ($P < 0.05$) when compared to vehicle control values but much less than the positive control at 80.4 ± 0.1%. The IC_{50} for the inhibitory effect of 16alpha-fluoroestradiol on hERG potassium current could not be determined due to solubility limitations of 16alpha-fluoroestradiol in HB-PS + 0.3% ethanol, but it is estimated to be greater than 8 ng/mL. (For reference, the maximum instantaneous blood concentration of a 5-µg dose of FES in a 70-kg human would be approximately 1 ng/mL.)

The positive control (60 nM terfenadine) inhibited hERG potassium current by (Mean + SD; n = 2) 80.4 + 0.1%. This result confirms the sensitivity of the test system to hERG inhibition.

4.3.2. Animal Toxicity Studies: Estradiol

Estrogens, including the natural hormones estradiol and estrone, are carcinogenic in laboratory animals. 17β-estradiol (E2) and its esters have been tested in mice, rats, hamsters, guinea pigs, and monkeys by oral and subcutaneous administration[3]. Several studies have focused on estrogen carcinogenesis in the liver and kidney in which multiple effects of estrogens and their metabolites appear to contribute in varying degrees to the development of tumors[4]. In contrast, synthetic estrogens such as 2-fluoroestradiol and 4-fluoroestradiol are poor carcinogens in the same animal model systems compared to native estradiol and synthetic steroidal estrogens such as ethinyl estradiol and diethylstilbestrol (DES) because the fluorine blocks their metabolism to more toxic intermediates when substituted in these positions[21,22].

A large number of animal toxicity studies have been performed for estradiol, largely to investigate tumorigenicity with chronic administration. These toxicity studies are outlined in Table 4.3.

Table 4.3. Summary of 17β-estradiol animal toxicity studies

Species	Gender	Age	N	Dose	Route	Duration	Outcomes	Threshold exposure	Study
Syrian golden hamster	male	3 to 4 weeks	68 (10 control) 18 - Estradiol 15 - 2-Fluoroestradiol 15 - 4-Fluoroestradiol	25 mg pellet	sc implant	224-345 days	renal cancers	Estradiol 25 mg at > 224 days 2-fluoroestradiol > 25 mg 4-fluoroestradiol 25 mg at > 345 days	Liehr[21] 1983
BALB/c mice	male	6 weeks	(1) 32 castrate (2) 60 castrate (3) 46 intact (4) 40 castrate (5) 43 intact	5 mg pellet (20% E2 80% cholesterol)	Implant (1) intrasplenic (2) none (3) L axilla (4) L axilla (5) L axilla	119-384 days	Leydig cell tumors	5 mg at > 130 days > 379 days > 130 days > 130 days > 237 days > 237 days	Huseby[23] 1980
C3H/HeJ mice	virgin female	6 weeks	1152	per day in food 0 100 µg/kg 1000 µg/kg 5000 µg/kg	P.O. QD	3-130 weeks	Pathologic changes and cancers cervix, uterus breast, bone	5000 µg/kg at > 52 weeks	Highman[2] 1978
C3H/HeJ mice	virgin female	6 weeks	1080	per day in food 0 100 µg/kg 1000 µg/kg 5000 µg/kg	P.O. QD	26-130 weeks	Pathologic changes and cancers cervix, uterus ovary, breast, other	5000 µg/kg > 40 weeks	Highman[24] 1980
C3H/HeJ mice	virgin female	6 weeks	1080	0 100 µg/kg 1000 µg/kg 5000 µg/kg	P.O. QD	18-24 months	trabecular proliferation Osteofibrosis cancer in bone	1000, 5000 µg/kg at 18 months	Highman[5] 1981
Alderley Park Rats	female	21 days	10	20 µg/kg	3 x sc injection	24-30 hours	bone marrow changes	> 20 µg/kg	Ashby[25] 1997
CBA mice	male	8 to 12 weeks	78	MMS 100 mg/kg DMSO 2.5 ml/kg 10 mg/kg 100 mg/kg 150 mg/kg	Single ip injection	24-30 hours	bone marrow changes	> 150 mg/kg	
B6C3F1 mice	male	NS	5 per dose	corn oil 312.5 mg/kg 625 mg/kg 1250 mg/kg	3 x ip injection	24 hours	bone marrow changes	> 1250 mg/kg	Shelby[26] 1997
F344 rats	male	NS	5 per dose	corn oil 312.5 mg/kg	3 x ip injection	24 hours	bone marrow changes	> 1250 mg/kg	

Species	Gender	Age	N	Dose	Route	Duration	Outcomes	Threshold exposure	Study
				625 mg/kg 1250 mg/kg					
B6C3F1 mice	female	NS	5 per dose	DMSO 0.10 mg/kg 1.0 mg/kg 10 mg/kg	Single ip injection	30 hours	bone marrow changes	> 10 mg/kg	
B6C3F1 mice	male	NS	5 per dose	DMSO 0.10 mg/kg 1.0 mg/kg 10 mg/kg	Single ip injection	30 hours	bone marrow changes	> 10 mg/kg	
Syrian golden hamster	castrate male	Adult	6	20 mg pellet	sc implant	8.7-9 months	renal cancers	minimum oncogenic level 1.8 µg/ml at > 8 months;	Li[27] 1984
							renal tubular damage	at > 1.5 months	
Macaca mulatta monkey	female	Adult	8	575-825 mg	sc implant	24-28 months	mammary cancers	> 825 mg	IARC[3] 1979

We have been unable to find any reported LD_{50} for the native substance 17β-estradiol. Ethinyl estradiol is a synthetic derivative of the natural estradiol. Quantitatively, the major metabolic pathway for ethinyl estradiol, both in rats and in humans, is aromatic hydroxylation, as it is for the estradiol**.** The acute LD_{50} of ethinyl estradiol is 2,952 mg/kg in rats and 1,737 mg/kg in mice[28]. Deaths of rodents given these large acute doses of ethinyl estradiol have been attributed to liver and kidney failure[29]. Premonitory clinical signs include apathy, abnormal breathing and gait, emaciation, and eventually, convulsions. These levels are five orders of magnitude above our proposed human doses.

In summary, review of the extensive toxicity data on estradiol in animals suggests that the toxicity of the compound comes largely in the form of induced tumors after chronic daily dose of 1,000 µg/kg or more, as summarized in Table 4.3. By comparison, [^{18}F]FES will be used for single-dose imaging studies at a maximum dose of 5 µg, corresponding to less than 0.1 µg/kg in an average 56-kg female.

Direct toxicity from estradiol is less common and was seen in the form of increased trabecular bone growth or renal tubular damage. The latter was seen for a 20-mg estradiol pellet implanted in 85 – 90 g hamsters. Minimum toxic plasma levels for long-term exposure were approximately 2 µg/mL. FES plasma levels in imaging studies are expected to reach a peak of 0.3 ng/mL (limit of injection 0.833 ng/mL) and be less than 15 pg/mL by one hour after injection. The dosing and levels of FES for imaging studies will be single-administration, transient, and range from 2,000 to (more typically), over 5,000 times less than levels where toxicity has been observed in long-term exposure studies in a number of animal models.

4.3.3. Animal Toxicity Studies: Other Fluorinated Estradiols

Fluorine has been tested as a substituent for modifying estrogens to inhibit metabolism, since the element can be substituted for a hydrogen with little or no change in the tertiary conformation of the molecule. In many positions, this ensures recognition of the modified estrogens by hormone receptors so test compounds maintain their estrogenic activity. Liehr et al synthesized 2-fluoroestradiol and 4-fluoroestradiol to test the possible prevention of estrogen-induced carcinogenesis[21]. Estrogenicity of the molecules was measured using two bioassays; increase in uterine wet weight in ovariectomized immature rats, and weight of testes of the male Syrian hamster. Immature female rats 20 – 21 days of age and 35 – 40 g were ovariectomized and then injected with doses ranging from 0.003 to 3.0 μg/100 g body weight of estradiol or 2-fluoroestradiol. Animals were sacrificed 24 hours after injection of the test material and uterine wet weights were measured as an index of estrogenic activity. Both compounds produce the same maximum response using this assay for estrogenic activity. 2-Fluoroestradiol had an ED_{50} of approximately 0.1 μg/kg body weight compared to a value of 0.02 – 0.03 μg/kg for E2[30].

4.3.4. Genotoxicity and Mutagenicity: FES and Estradiol

Bacterial Reverse Mutation Assay

Fluoroestradiol, was tested in the Bacterial Reverse Mutation Assay using *Salmonella typhimurium* tester strains TA98, TA100, TA1535, and TA1537 and *Escherichia Coli* tester strain WP2 uvrA in the presence and absence of Aroclor-induced rat liver S9. The assay was performed in two phases, using the plate incorporation method. The first phase, the initial toxicity-mutation assay, was used to establish the dose-range for the confirmatory mutagenicity assay and to provide a preliminary mutagenicity evaluation. The second phase, the confirmatory mutagenicity assay, was used to evaluate and confirm the mutagenic potential of the test article.

Ethanol was selected as the solvent of choice based on solubility and compatibility with the target cells.

In the initial toxicity-mutation assay, the maximum dose tested was 1.25 μg per plate; this dose was achieved using a concentration of 0.025 mg/mL and 50 μL plating aliquot. The dose levels tested were 0.00050, 0.0015, 0.0050, 0.015, 0.050, 0.15, 0.50, and 1.25 μg per plate. The test article formed soluble and clear solutions in ethanol from 0.000010 to 0.025 mg/mL. In the initial toxicity-mutation assay, no positive mutagenic response was observed. Neither precipitate nor appreciable toxicity was observed. Based on the findings of the initial toxicity mutation assay, the maximum dose plated in the confirmatory mutagenicity assay was 1.25 μg per plate.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed. The dose levels tested were 0.015, 0.050, 0.15, 0.50, and 1.25 µg per plate. Neither precipitate nor appreciable toxicity was observed.

Under the conditions of this study, test article fluoroestradiol was concluded to be negative in the Bacterial Reverse Mutation Assay.

***In Vitro* Mammalian Cell Gene Mutation Test**

Fluoroestradiol, was tested in the L5178Y/TK+/- Mouse Lymphoma Mutagenesis Assay in the absence and presence of Aroclor-induced rat liver S9. The preliminary toxicity assay established the concentration range for the mutagenesis assays. The mutagenesis assays were used to evaluate the mutagenic potential of the test article.

Ethanol was selected by the sponsor as the solvent for the test article was soluble in ethanol at approximately 1.0 mg/mL, the maximum concentration prepared for the preliminary toxicity assay.

In the preliminary toxicity assay, the maximum concentration of fluoroestradiol in treatment medium was 8.0 ng/mL. No visible precipitate was present at any concentration in treatment medium. Selection of concentrations for the mutation assay was based on reduction of suspension growth relative to the solvent control and maximum concentration requested by the sponsor. No substantial toxicity, i.e., suspension growth of < 50% of the solvent control, was observed at any concentrations with or without S9 activation.

Based on the results of the preliminary toxicity assay, the concentrations treated in the initial mutagenesis assay ranged from 0.15 to 8.0 ng/mL for both the non-activated and S9-activated cultures with a 4-hour exposure. No visible precipitate was present at any concentration in treatment medium. The concentrations chosen for cloning were 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL with and without S9 activation. No cloned cultures exhibited mutant frequencies > 90 mutants per 106 clonable cells over that of the solvent control. There was no concentration-related increase in mutant frequency.

Based on the results of the preliminary toxicity assay, the concentrations treated in the extended treatment assay ranged from 0.15 to 8.0 ng/mL for non-activated cultures with a 24- hour exposure. No visible precipitate was present at any concentrations in treatment medium. The concentrations chosen for cloning were 1.0, 2.0, 4.0, 6.0, and 8.0 ng/mL. No cloned cultures exhibited mutant frequencies > 90 mutants per 106 clonable cells over that of the solvent control. There was no concentration-related increase in mutant frequency.

The trifluorothymidine-resistant colonies for the positive and solvent control cultures from both assays were sized according to diameter over a range from approximately 0.2

to 1.1 mm. The colony sizing for the MMS and DMBA positive controls yielded the expected increase in small colonies (verifying the adequacy of the methods used to detect small colony mutants) and large colonies.

Under the conditions of this study, test article fluoroestradiol was concluded to be negative in the L5178Y/TK+/- Mouse Lymphoma Mutagenesis Assay.

A literature review using the Registry of Toxic Effects of Chemical Substances [RTECS] and the National Library of Medicine's Medline yielded no specific analysis for genotoxicity or mutagenicity of 16-alpha-fluoroestradiol. Toxicity data have been reported regarding the use of 2-fluoroestradiol and 4-fluoroestradiol[21,22].

In summary, multiple studies support the role of estradiol and its catechol estrogen metabolites as weak mutagens and carcinogens in *in vivo* and *in vitro* experiments. However, these data, combined with the recent preclinical toxicology and mutagenicity work reported above, also suggest a low frequency of mutagenic events related to estradiol, even with high concentrations and repeated exposures. [^{18}F]FES is therefore extremely unlikely to pose a mutagenic or carcinogenic threat in single-dose exposures such as those required in FES PET.

Studies have detected low level alterations to DNA; however, these changes occurred with chronic exposure to estradiol concentrations of 25 µg/mL or greater. This level is 2 – 3 orders of magnitude greater than FES levels encountered in PET, and exposure for PET imaging is transient. The mass of drug used in the FES imaging studies is well below any levels where genotoxicity was observed.

5. Effects in Humans

5.1. Pharmacology of FES and Estradiol

The pharmacology of FES is best understood by analogy to estradiol. Estradiol is a naturally occurring steroid that comes from two sources: (1) synthesis in the ovary in pre-menopausal women and (2) conversion from adrenal steroids, largely through aromatization (and aromatase enzymes)[31,32] in a variety of tissues, most notably fat, breast tissue, and breast cancers. Pre-menopausal levels of estradiol vary widely depending upon the phase of the menstrual cycle, reaching levels as high as 500 pg/mL (1.7 nM) mid-cycle. In post-menopausal women, and in men, levels are generally less than 30 pg/mL (0.1 nM).

Estradiol is very lipophilic and is generally present in slightly higher concentration in tissues with higher fat content. Circulating estradiol is largely protein bound with high affinity but low capacity to SHBG and with low affinity but high capacity to albumin[18,33].

Much of circulating estradiol is bound to SHBG and the remainder is bound to albumin[18]. FES is an estrogen analog used as a diagnostic agent to image regional estradiol binding to ER and is closely related to estradiol[13]. Estradiol exerts its physiologic effect by binding to ER, a nuclear receptor. ER is selectively expressed in a variety of tissues, most notably the breast, uterus, ovaries, bone, and pituitary.

The molecular mechanism of estradiol action through the ER is becoming clearer[34]. ER has two receptor subtypes — alpha and beta. ER alpha serves largely as an activator of downstream events related to breast and female sex organ function. The function of ER beta is less well understood and may, in some cases, inhibit ER alpha by forming a heterodimer with ER alpha. Estradiol binding to ER alpha in the nucleus results in dimerization of the receptor and this allows interaction with specific DNA sequences, termed the estrogen-response elements (ERE)[34], leading to selective regulation of target gene expression. A variety of co-regulators interact with the ER homodimer and the ERE and can affect the level of target gene expression. Different co-factor levels are believed to be responsible for the varied action of estrogens in different target tissues, in addition to structural changes of the ER with ligand binding, leading to the sometimes differing responses to different ER ligands[34]. In the uterus, estrogens bound to ER stimulate endometrial growth and are critical in maintaining a functioning uterine-placental unit during pregnancy.

Ovarian synthesis of estradiol is a key component of female endocrine function in a complex feedback loop with the pituitary. Estradiol also promotes new bone formation and is important in maintaining bone mineral density, especially in women. Estrogens affect the cardiovascular system, largely through their beneficial effect on serum lipids. In the breast, estradiol promotes ductal epithelial cell proliferation and is a key component stimulating lactation. Estrogens are established growth factors for endometrial and many breast cancers. Approximately 60% of breast cancers express ER, and estradiol and other estrogens provide a key stimulus for tumor growth and an opportunity for endocrine-based therapy. This last effect is the impetus for developing a diagnostic agent for imaging ER expression in breast cancer patients that led to our investigation of FES for use in PET diagnostic imaging[34].

Because FES is not intended for therapeutic effect and is not used in sufficient concentration to elucidate a physiologic effect, mechanisms of action beyond metabolism and binding to ER have not been studied. However, the mechanism of action for FES can be inferred from animal and human studies, and the similarity of FES to estradiol in its metabolism and in its binding to ER and to SHBG. The binding of FES to the ER, both *in vitro* and *in vivo*, has been extensively studied and validated by comparison to estradiol and to standard *in vitro* assays of ER expression[13, 16, 19,35]. Studies evaluating the biological behavior of FES using *in vitro* competitive binding assays showed that FES has a high affinity for the estrogen receptor. Antagonism with estradiol helped to define the specific and non-specific binding of FES to target tissues[36]. FES relative binding affinity (fluoroestradiol equilibrium binding constant/estradiol

equilibrium binding constant) is in the range of 0.8 to 0.9[13, 19,37]. Competitive binding assays of [^{18}F]FES with estradiol have been performed and have related this to the molar concentration of FES as measured by LC-MS.

The binding affinity of FES to the ER is 0.8 of the binding affinity of estradiol to the ER, and FES non-specific binding is similar to estradiol[13]. In immature rats, specific ER binding is shown by high uterine uptake (uterus to blood 39 +/- 16 at one hour) that is reduced by co-injection of estradiol, showing that the two compounds compete for the same receptor[13]. Non-target tissue uptake is low (uterus to non-target ratio 28 +/- 4.8 at one hour). Quantitative estimates of FES uptake in human tumors show that FES uptake measured by PET is proportional to tumor ER expression and correlates with *in vitro* assays of ER expression[35].

5.2. Pharmacokinetics of FES and Estradiol

Typical blood FES concentration after a 6 mCi injection is 1 µCi/mL (< 3 pmoles/mL) peak, and by 60 minutes after injection it is < 150 fmoles/mL[38]. For the typical specific activity, 1000 Ci/mmole, this corresponds to peak blood FES levels of 290 pg/mL (1 pmole/mL) and 15 pg/mL (50 fmole/mL) at 60 minutes, and the limiting-case (highest level) values of 833 pg/mL and 42 pg/mL at one hour. This compares to pre-menopausal mid-cycle estradiol levels of 62 – 534 pg/mL and post-menopausal levels of 20 – 88 pg/mL (UW Laboratory Medicine: http://byblos.labmed.washington.edu/bcard/search.asp). Males are expected to have levels similar to post-menopausal women[39]. Thus the short-term exposure to estrogen from an FES injection as part of an FES PET study transiently yields physiologic levels of fluoroestradiol, decreasing to sub-physiologic levels after 60 minutes.

The metabolism of estradiol has been well characterized[15]. Like other steroids, estradiol has high uptake in the liver. Metabolism occurs largely in the liver, with two key components: (1) oxidation at the 17 position to form the estrone and (2) hydroxylation at the 2 and 16 positions to form hydroxy estradiols or estrones. There is rapid interconversion, both in the liver and periphery, to form estriol and 16-epiestriol. Estradiol, estrone and estriol are the predominant estrogenic species and are often referred to collectively as the "classical estrogens". The formation of sulfate conjugates at hydroxyl sites is an important route of estrogen metabolism and leads to excretion into the bile. Estrogen conjugates formed in the liver are secreted into bile and have highly efficient enterohepatic circulation, with only 7 percent of administered estradiol excreted in the feces in tracer studies[15]. This enterohepatic circulation serves as a reservoir for estrogens and is important in regulating estrogen levels[15]. Glucuronides are also formed in the liver, to a lesser extent than sulfates. Excretion of estrogen glucuronides in the urine is the primary route of elimination in humans and is a source of estrogens for hormone replacement therapy (conjugates from equine urine, Premarin®).

FES metabolism has also been studied in humans. Metabolite analysis of blood and urine was performed in patients undergoing [^{18}F]FES PET studies[14]. Results were similar to rat data, showing that FES is rapidly metabolized to polar species, with less than 20% of blood radioactivity in the form of [^{18}F]FES by 60 minutes after injection. There is also net clearance of both FES and labeled metabolites from the blood via hepatic uptake, biliary excretion, and urinary excretion of polar conjugates (Figure 5.2). By 120 minutes after injection, circulating FES is less than 5% of peak values, and the total of FES and labeled metabolites is less than 40% of the peak. Clearance rates of intravenous FES and intravenous estradiol are similar (Figures 5.1 and 5.2); for both compounds circulating levels have decreased to less than 5% of peak levels by 60 minutes after injection. Analysis of metabolites excreted in the urine sampled 90 – 120 minutes after injection has been done using glucuronidases to dissociate the glucuronide conjugates and acid hydrolysis to break the sulfate conjugation bond. These experiments recovered mostly [^{18}F]FES, with a small percentage of a more polar substance not identified in the studies[14]. These results suggest that, on the time scale of PET imaging, FES is metabolized primarily to non-oxidized conjugated FES.

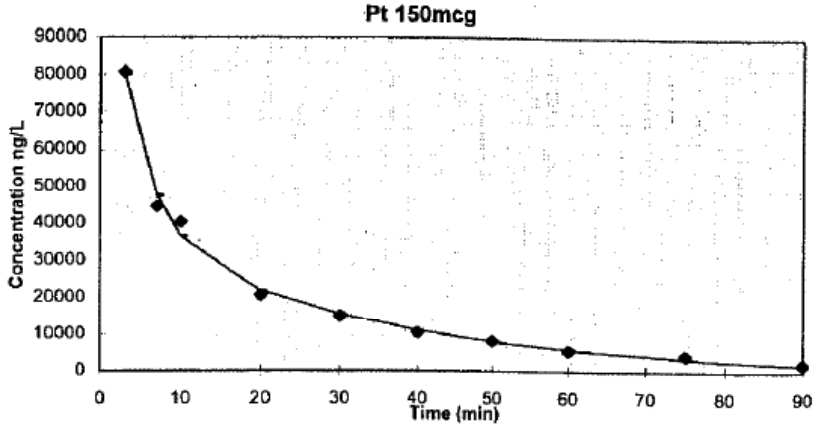

Figure 5.1. Serum concentrations versus time profile for a patient receiving a 150 μg dose of estradiol. The solid line denotes theoretical fit using a two-compartment model. Y-axis units are ng/L (pg/mL). The plasma $T_{1/2\beta}$ (longer clearance component) for estradiol is reported as 27.45 minutes by White[40].

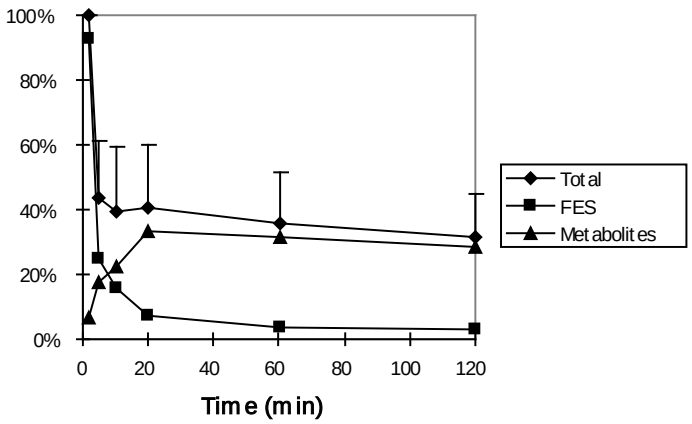

Figure 5.2. Total metabolized and unmetabolized circulating activity versus time, decay corrected to injection (FES, solid square; metabolites, solid triangle; total, solid diamond). Activity was normalized to peak injected FES for each patient. The error bars are the SE for 15 patients[14].

In summary, FES biochemistry, ER binding affinity, and metabolism are very similar to estradiol, suggesting that data on estradiol biochemistry and pharmacology are applicable to FES. Any differences between results for FES and estradiol appear to arise from the fact that only short-term (1 – 2 hours), transient kinetics and metabolism of the radiolabeled [^{18}F]FES are relevant to its use in PET. Studies of estradiol physiology suggest that exposures of several hours to days are needed to elucidate physiologic effects and thus longer-term, equilibrium kinetics and metabolism are most relevant. Because of the ^{18}F half-life limitation, oxidation plays only a minor role in [^{18}F]FES metabolism and liver conjugation is responsible for enterohepatic circulation and prompt excretion in urine over the life of ^{18}F.

Biodistribution studies in humans further support the concept that FES metabolism is similar to that of estradiol. The liver rapidly takes up FES with subsequent excretion into bile[14,38] (Figure 5.3). From sequential images of the biodistribution of FES using PET, it was shown that FES passed into the bile and moved through the small intestine[38]. Very little, if any, radioactivity was seen in the large intestine, suggesting highly efficient enterohepatic circulation, similar to that of estradiol. Similar results were found using 16-α-radioiodo-17-β-estradiol in a swine model[14,41].

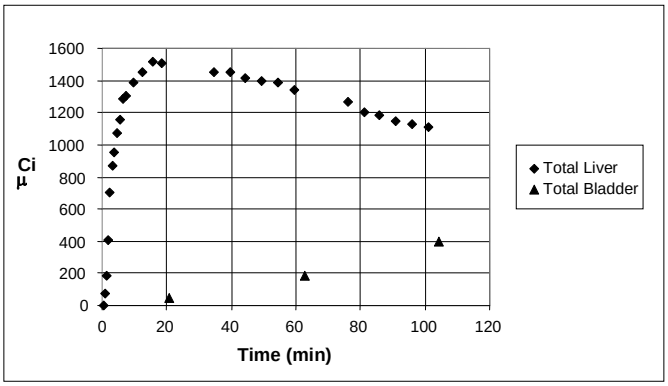

Figure 5.3. Total liver and bladder activity as a function of time after FES injection for a patient injected with an approximately 6,000 µCi (6 mCi) total dose.[14]

Radiopharmaceuticals labeled with ^{18}F must be evaluated for direct dehalogenation reactions leading to loss of [^{18}F]-fluoride from the estradiol. Since F^- has very high uptake in bone, even small amounts of circulating $^{18}F^-$ would be visible as bone uptake in PET images. No uptake above background was seen in bone with FES PET imaging. Thus defluorination is not a factor in FES metabolism.

Although FES protein binding studies have been performed in rodent species[20], rodents lack SHBG, and labeled estrogen binding differs for rodents versus humans[33,42]. Studies of FES binding to SHBG in humans support behavior similar to estradiol[17]. An average of 45% ± 15% of circulating FES is bound to SHBG. This fraction is dependent upon the plasma concentration of SHBG and follows a predictable relationship based on a model of free FES in equilibrium with FES bound to albumin and SHBG[17]. A fit of the data on percent FES bound to SHBG versus SHBG plasma concentration yielded an estimated dissociation constant for FES-SHBG similar to the published value for estradiol-SHBG[17,18].

5.3. Safety and Efficacy of FES

[^{18}F]FES could potentially exert toxic effects through one of three mechanisms: (1) radiation exposure to tissues from the radioactive label[38], (2) physiologic actions mediated through the ER, and (3) directly toxic or mutagenic effects of FES metabolites. Radiation exposure from [^{18}F]FES at activity doses used in PET (6 mCi, typical) is low, and is comparable to other nuclear medicine procedures[38]. Radiation exposure is discussed in detail in Section 5.4. With respect to the other two mechanisms of toxicity, FES injected as a bolus for PET imaging transiently reaches physiologic concentrations, but returns to sub-physiologic levels within an hour after injection. As such, toxic effects due to actions mediated through the ER and directly toxic effects of metabolites will be far less than those of natural ER ligands.

Although the toxicity of FES has not been studied, the toxicity of estradiol has been widely studied and published. Given the biochemical and pharmacologic similarity

between FES and estradiol, the low mass administered and short-term exposure to FES resulting from PET studies, the estradiol toxicity literature serves as an appropriate gauge for any potential toxicity of FES. In this section, we review human safety studies, and prior experience with FES in humans from several centers.

5.3.1. Estradiol Human Toxicity

5.3.1.1. Oral Administration of Estrogen

Exogenous estrogens are most commonly prescribed in an oral form and are well absorbed from the gastrointestinal tract. After administration of 2 mg of oral estradiol, serum concentrations of 50 to 100 pg/mL are achieved for 24 hours. This is similar to the serum concentrations for a normal menstrual cycle although menstrual cycle peak values reach 500 pg/mL)[43]. The most common side effects of orally administered estrogens are gynecomastia, edema, anorexia, weight changes, nausea and vomiting, abdominal cramps, and feeling of bloating. Serious side effects may include thromboembolic events, cardiovascular disease, hypercalcemia, cerebrovascular disease, breast cancer, and endometrial cancer. A meta-analysis of multiple observational studies of post-menopausal hormone replacement therapy, most often including conjugated estrogen, reported an increase in relative risk of stroke, thromboembolic events, breast cancer, and endometrial cancer that increased with duration of treatment. Many of these studies did not specify whether hormone replacement therapy was prescribed as unopposed estrogen therapy or as combined estrogen and progesterone therapy[44]. These results were consistent with recently published data from the Women's Health Initiative (WHI) study of unopposed conjugated estrogen therapy where relative risks for stroke and thromboembolic events were increased; however, in the WHI study the risk of breast cancer was not elevated for this population[45].

Common side effects of treatment with oral estradiol are summarized in Table 5.1 for a subset of observational studies containing only oral estradiol. Studies that included concomitant treatment with progesterone, treatment with oral conjugated equine estrogen (CEE) or transdermal estradiol are not relevant to FES administered for imaging studies and are not reviewed here. The four studies listed in Table 5.1 were randomized, double-blind, placebo-controlled studies evaluating the use of oral estradiol for treatment of hot flashes in postmenopausal women. Chung et al[46] studied 100 surgically menopausal Chinese women. Participants were given 6 months of oral estradiol 2 mg/QD and 6 months of placebo in alternating order. Serum estradiol levels were measured pre- and post-therapy and were increased significantly post-therapy although exact values were not reported. Headache and mild dizziness were reported as side effects[46].Freedman and Blacker[47] studied 24 postmenopausal women who were randomly assigned in a double-blind fashion to receive either 1.0 mg/QD oral estradiol (n = 12) or placebo (n = 12) for 90 days to evaluate effects of estradiol on menopausal hot flashes. No specific side effects to the treatment were reported[47].

Notelovitz et al[48,49] studied 333 healthy menopausal women, 40 – 60 years of age, to evaluate effects of estradiol on menopausal hot flashes. The study was a double-masked, randomized, placebo-controlled trial completed over 12 weeks of treatment. Patients were assigned to one of the following doses: placebo (n = 66), or oral micronized estradiol of 0.25 mg QD (n = 68), 0.5 mg QD (n = 64), 1 mg/QD (n = 67) or 2 mg QD (n = 68). Serum estradiol levels were reported pre therapy and after 4, 8, and 12 weeks of treatment and reported as the steady state mean of the available data for each subject. Vaginal bleeding and breast pain were the most frequently reported adverse events. Bleeding was reported in 14% in the placebo group, 10% of the 0.25 mg group, 6% of the 0.5 mg group, 21% of the 1 mg group, and 37% in the 2 mg group. Breast pain was reported in 12% of the 2 mg group but in only 3 – 6% of other groups[48,49]. Notelovitz and Mattox et al studied 145 post-menopausal women in a double-blind, randomized, multicenter study of estradiol therapy and the effect on vasomotor and vulvovaginal symptoms of menopause[49]. Subjects were randomized to placebo, 0.5 mg QD or 1 mg QD of oral estradiol for a period of 12 weeks. All groups reported atypical bleeding with a higher percentage in the 1 mg QD arm of the study. One patient in this arm also developed endometrial cancer. There was no difference in the reported rate of headaches or abdominal pain between the groups[48,49].

Table 5.1. Adverse events from selected trials of oral estradiol[50]

Number of Patients	Drug	Dose	Duration	Adverse Events	Estradiol Blood Levels (pg/mL)	Source
100	Oral estradiol	2 mg/QD	6 month	Headaches Dizziness	Not reported	Chung 1996[46]
24	Oral estradiol	1 mg/QD	90 days	None reported	Pre = 8.3 ± 2.2 Post = 131.7 ± 22.3	Freedman 2002[47]
333	Oral estradiol	0.25 mg/QD (1) 0.5 mg/QD (2) 1 mg/QD (3) 2 mg/QD (4)	12 weeks	Atypical bleeding (1) 10% (0.25 mg) (2) 6% (0.5 mg) (3) 21% (1 mg) (4) 37% (2 mg) Breast tenderness 3-6% (<2 mg) (4) 12% (2 mg)	Median steady state Pre Rx = 6 (1) = 20 (2) = 33 (3) = 49 (4) = 97	Notelovitz 2000a[48]
145	Oral estradiol	0.5 mg/QD 1 mg/QD	12 weeks	Atypical bleeding, 1 case of endometrial ca, headaches, abdominal pain	Not reported	Notelovitz 2000b[49]

It is important to focus on the typical blood FES concentration achieved in PET, which after a 6 mCi injection is 1 µCi/mL (< 3 pmoles/mL) peak, and which, by 60 minutes after injection, is less than 150 fmoles/mL. For the limiting-case specific activity, this corresponds to a peak blood FES level of 833 pg/mL and 42 pg/mL at one hour,

compared to pre-menopausal mid-cycle estradiol levels of 62 to 534 pg/mL and post-menopausal levels of 20 to 88 pg/mL. Males have levels similar to post-menopausal women. Thus the short-term exposure to estrogen from an FES injection as part of an FES PET study transiently yields physiologic levels of the estrogenic steroid, decreasing to sub-physiologic levels after 60 minutes.

In summary, FES used in diagnostic imaging studies is generally limited to no more than four administrations per subject, with blood levels that reach the levels seen in the oral dosing studies for less than one hour. There is a body of data on oral administration of estradiol with blood levels ranging from 25 – 100 pg/mL recorded adverse events with administration over 12 weeks or more. With the exception of one case of endometrial cancer, a known side effect of chronic estrogen administration, toxic effects were largely minor.

5.3.1.2. Intravenous Administration of Estrogen

Reports of intravenous administration of estrogens are rare; it is used in this form largely in the setting of acute dysfunctional uterine bleeding. Two studies have documented acute toxicities resulting from intravenous bolus doses. White and colleagues studied pharmacokinetics and tolerability of 17β-estradiol in eight postmenopausal women[40]. Estradiol was administered in doses of 25, 50, 100, or 200 μg peripherally over a five second period. Peak serum levels were not reported, however, the authors did document approximate dosage proportionality with respect to serum area under the curve (AUC). Estradiol at 25 μg resulted in an AUC of 361.69 μg•min/L and the level was 512.48 μg•min/L in a patient who received a 50 μg dose. An adverse event was reported in only one patient who experienced mild discomfort at the injection site immediately following her dose. This reaction lasted 3 – 4 seconds and did not recur.

Intravenous administration of Premarin® 25 mg versus placebo in 34 patients with dysfunctional uterine bleeding resulted in mild adverse reactions reported for seven of 18 treated patients (39%) versus two of 16 (13%) in the placebo group[51]. Adverse effects in the Premarin® treated patients included flushing, euphoria, dizziness, drowsiness, and taste disturbances. The mean changes from baseline following injection of Premarin® versus placebo for blood pressure, pulse and respiratory rate were not statistically significant. Intravenous estradiol combined with oral estradiol was given in post-menopausal women with recurrent ischemia and history of unstable angina[52]. This study reported a low incidence of headache, edema, vaginal bleeding, and a 23% incidence of breast tenderness and mood changes. Results of these three studies are shown in Table 5.2.

Table 5.2. Adverse events from selected trials of intravenous estradiol

Number of Patients	Drug	Dose	Duration	Adverse Events	Estradiol Blood Levels	Source
8	IV estradiol	25 μg 50 μg 150 μg 300 μg	Single injection	No reported AE	Baseline = 42.2 ng/L 60 mins post inj = 689.6 ng/L	White 1998[40]
18	IV Premarin® (Conjugated Equine Estradiol)	25 mg	Dual injection	Flushing, drowsiness, euphoria, dizziness, nausea	Not reported	Devore[51] 1982
100	IV estradiol + Oral estradiol	1.25 mg + 1.25 mg/QD	Single injection + 21 days of oral E	flushing 7% headache 11% edema 11% vaginal bleeding 10% breast tenderness 23% mood changes 5%	Not reported	Schulman 2002[52]

The White et al study is most relevant to the use of FES for PET[40]. For this study up to 300 μg of estradiol was administered intravenously. Blood levels of estradiol were up to 690 ng/L (pg/mL) at 60 minutes post injection, and there were no reported adverse events. For FES used for PET, the typical dose is 1.5 μg or less, with a maximum dose of 5 μg, and for the dose limit injection the blood level at 60 minutes should be 42 pg/mL. PET studies also indicate that the tissue distribution and blood clearance of [^{18}F]FES is similar to IV estradiol.

5.3.2. [^{19}F]FES Human Safety Studies

We are unaware of, nor did a literature search show, any human studies of [^{19}F]FES safety in humans beyond that for carrier [^{19}F]-FES associated with the [^{18}F]FES human studies described below.

5.3.3. [^{18}F]Fluoroestradiol Human Imaging Studies

A summary of the reported human imaging studies using [^{18}F]FES is presented in Table 5.3 and then described more fully in the following paragraphs.

Table 5.3. Published manuscripts reporting [^{18}F]FES human imaging

Clinical Condition	No. of Patients	MBq Injected	Specific Activity	μmoles Injected	Reference
Endometrial Carcinoma	19	Approx 185 MBq	100 – 200 GBq/μmol	Not Reported	Tsujikawa 2010[54] (Japan)
Endometrial Cancer	22	185 MBq	100 – 200 GBq/μmol	.002 – .004 μmol	Tsujikawa 2009[67] (Japan)
Breast Cancer	59	222 MBq	Not Reported	Not Reported	Dehdashti 2009[64] (St Louis, MO)
Breast Cancer	17	125.8 – 233.1 MBq (avg. 196.1)	Not Reported	Not Reported	Peterson 2008[63] (Seattle, WA)
Endometrial Cancer	2	185 MBq	Not Reported	Not Reported	Tsujikawa 2008[66] (Japan)
Endometrial Cancer	38	185 MBq	100 – 200 GBq/μmol	002-.004 μmol	Tsujikawa 2008[62] (Japan)
Normal Endometrium	16	185 MBq	> 111 GBq/μmol	Not Reported	Tsuchida 2007[53] (Japan)
Breast Cancer	20	300 – 400 MBq	Not Reported	Not Reported	Kumar 2007[65] (Canada)
Metastatic Breast Cancer	47	222 MBq	≥ 1000 mCi/μmol	0.006 μmol	Linden 2006[55] (Seattle, WA)
Breast Cancer	49	56 – 296 MBq	1000 – 2000 mCi/μmol	0.0015 – 0.004 μmol	Mankoff 2001[38] (Seattle, WA)
Breast Cancer	40	222 MBq	Not Reported	0.006 μmol	Mortimer 2001[56] (St. Louis, MO)
Breast Cancer	18	≤ 222 MBq	Not Reported	≤ 0.006 μmol	Tewson 1999[17] (Seattle, WA)
Metastatic Breast Cancer	11	222 MBq	Not Reported	0.006 μmol	Dehdashti 1999[57] (St. Louis, MO)
Primary or Metastatic Breast Cancer	15	≤ 222 MBq	Not Reported	≤ 0.006 μmol	Mankoff 1997[14] (Seattle, WA)
Meningioma	6	148 – 296 MBq	4.3 – 11.1 Ci μmol^{-1}	Not Reported	Moresco 1997[58] (Milan, Italy)
Primary or Metastatic Breast Cancer	43	222 MBq	Not Reported	0.006 μmol	Mortimer 1996[59] (St. Louis, MO)
Primary or Metastatic Breast Cancer	53	222 MBq	Not Reported	0.006 μmol	Dehdashti 1995[60] (St. Louis, MO)
Metastatic Breast Cancer	16	222 MBq	Not Reported	≤ 0.006 μmol	McGuire 1991[61] (St. Louis, MO)
Primary Breast Cancer	13	92.5 – 222 MBq	Not Reported	≤ 0.006 μmol	Mintun 1988[35] (St. Louis, MO)
Total*	504				

*Although every attempt to eliminate duplication was made, it is possible that some patients are counted twice due to representation in multiple publications.

Early studies established a correlation between FES uptake and *in vitro* assay of ER expression[35] and documented the biodistribution and radiation dosimetry of FES[38]. Several studies documented the metabolism, clearance, and serum protein binding of FES in humans[17,38]. Other studies demonstrated heterogeneous uptake of FES in advanced breast cancer as a reflection of heterogeneous ER expression[57, 60]. One paper measured FES uptake in meningiomas[58]. Finally, other studies have measured FES uptake in patients treated with hormonal therapy[61, 56, 55]. The general conclusion from the studies summarized above is that [^{18}F]FES PET images identified estrogen receptor positive tissue that was heterogeneously distributed within human tumors. These data may be helpful in identifying patients who will benefit from endocrine therapy for their cancer and predict the likelihood of response to specific treatment hormonal regimens.

In a paper published in 2010, Tsujikawa et al[54] reported on the correlation between the uptake of 16α-[^{18}F]fluoro-17β-oestradiol (FES) and expression of estrogen receptors, as well as other related immunohistochemistry markers. Nineteen patients with endometrioid adenocarcinoma underwent preoperative PET studies with FES and FDG. Standardized uptake values (SUVs) for each tracer and the regional FDG to FES SUV ratio were calculated using images after coregistration. FES uptake showed a significantly positive correlation with expression of estrogen receptor α (ERα). The FDG to FES ratio showed a significantly negative correlation with expression of ERα and progesterone receptor B (PR-B). The FES uptake and FDG to FES ratio did not correlate with expression of ERβ, Ki-67 or glucose transporter 1 (GLUT1). FDG uptake was not correlated with any of the immunohistochemical scores. The PR-B score was strongly correlated with the ERα score. Well-differentiated carcinoma (grade 1) showed a significantly higher FES uptake and significantly lower FDG to FES ratio than moderately or poorly differentiated carcinoma (grade 2 – 3). None of the PET parameters were significantly different between advanced-stage carcinoma (≥ stage IB) and early-stage carcinoma (IA) based on the Féderation International de Gynécologie et d'Obstétrique (FIGO) staging classification. Differentiation grade was the most closely correlated parameter to FES uptake and FDG to FES ratio by multivariate analyses. The authors concluded FES PET combined with FDG would be useful for non-invasive evaluation of ERα distribution, as well as ERα function, which reflects differentiation grade in endometrial carcinoma[54].

In a study of 16 female healthy volunteers published in 2007, Tsuchida et al[53] administered a single dose of FES to investigate the relationship between endometrial and myometrial FES uptake and menstrual phase or endogenous estrogen level. Endometrial SUV was significantly higher in the proliferative phase than in the secretory phase (6.03 ± 1.05 vs. 3.9 ± 1.29, P = .022). In contrast, there was no significant difference in myometrial SUV when the proliferative and secretory phases were compared (P = .23). Further, there was no correlation between SUV and endogenous estrogen level in the proliferative phase. The authors concluded that the change of ER concentration relative to menstrual cycle as characterized by FES PET was consistent with those from previous reports that used an immunohistochemical technique. These

data suggest that FES PET is a feasible, noninvasive method for characterizing changes in ER concentration[53].

In a study published in 2008 by Tsujikawa et al[62] FES and FDG PET studies were performed in 38 patients with benign and malignant uterine tumors to compare differences in tracer accumulation. Regional values of tracer uptake were evaluated by using standardized uptake value (SUV). Patients with endometrial carcinoma showed significantly greater mean SUV for FDG (9.6 ± 3.3) than for FES (3.8 ± 1.8) ($P < .005$). Patients with endometrial hyperplasia showed significantly higher mean SUV for FES (7.0 ± 2.9) than for FDG (1.7 ± 0.3) ($P < .05$). Patients with leiomyoma showed significantly higher mean SUV for FES (4.2 ± 2.4) than for FDG (2.2 ± 1.1) ($P < .005$), and patients with sarcoma showed opposite tendencies for tracer accumulation. Tracer uptake in patients with endometrial carcinoma was significantly higher for FDG ($P < .001$) and significantly lower for FES ($P < .05$) when compared with values in patients with endometrial hyperplasia. On the other hand, patients with sarcoma showed a significantly higher uptake for FDG ($P < .005$) and a significantly lower uptake for FES ($P < .05$) compared with patients with leiomyoma. The authors concluded that ER expression and glucose metabolism of uterine tumors measured by using PET showed opposite tendencies, and that PET studies utilizing both FES and FDG could provide pathophysiologic information for the differential diagnosis of uterine tumors[62]. These results demonstrate the potential predictive capability of FES PET.

In another study by Peterson et al published in 2008[63], [^{18}F]fluoroestradiol uptake was compared with ER expression assayed *in vitro* by immunohistochemistry (IHC) with both qualitative and semiquantitative measures. Seventeen patients with primary or metastatic breast cancer were studied with dynamic [^{18}F]FES PET; cancer tissue samples, collected close to the time of imaging, were assayed for ER expression by IHC. For each tumor, partial-volume-corrected measures of [^{18}F]FES uptake were compared with ER expression measured by three different ER scoring methods: qualitative scoring (0 – 31), the Allred score (0 – 10), and a computerized IHC index. The authors noted that there was excellent agreement ($r^2 = 0.99$) between observers using IHC as well as the different methods of measuring ER content ($P < 0.001$), and they concluded that there is good agreement between [^{18}F]FES PET and ER expression measured by IHC, and that [^{18}F]FES imaging may be a useful tool for aiding in the assessment of ER status, especially in patients with multiple tumors or for tumors that are difficult to biopsy[63].

In a study by Dehdashti et al published in 2009[64], 51 post-menopausal women with advanced estrogen receptor positive breast cancer were studied. Patients underwent FES PET and FDG PET at baseline and repeat FDG PET after 30 mg estradiol. Tracer uptake was measured as the standardized uptake value (SUV). Patients were subsequently treated with either an aromatase inhibitor or fulvestrant. PET results were correlated with responsiveness to endocrine therapy. Per study criteria, 17 patients responded and 34 patients did not respond to endocrine therapy. Four responders and one non-responder had a clinical flare reaction, while only the responders

demonstrated metabolic flare. After estradiol challenge, a significantly higher mean (± SD) percent change in SUV for FDG was noted in responders (20.9 ± 24.2) compared with non-responders (-4.3 ± 11.0, $P < 0.0001$). On FES PET, a higher tumor SUV was noted in responders (3.5 ± 2.5) compared with non-responders (2.1 ± 1.8, $P = 0.0049$). There was significantly longer overall survival in patients with metabolic flare than in those without flare regardless of type of endocrine therapy ($P = 0.0062$). The authors concluded that baseline tumor FES uptake and metabolic flare after an estradiol challenge are both predictive of responsiveness to endocrine therapy in ER+ breast[64] cancer.

In a study by Kumar et al published in 2007[65] an improved automated radiosynthesis methodology for [^{18}F]FES was developed. Stability studies of the resulting injectable form were performed up to 24 hours after dose formulation under normal storage conditions. A comparison of FES versus FDG PET imaging was then conducted in ER+ breast cancer patients. The results of the improved synthesis methodology were favorable and the subsequent PET imaging suggested specificity of FES for ER+ tumors versus FDG[65].

In a 2008 paper Tsujikawa et al[66] reported two postmenopausal patients under suspicion of endometrial carcinoma on the basis of cytology and/or magnetic resonance imaging (MRI), who were on tamoxifen treatment since undergoing surgery for breast cancer. Pelvic MRI suggested endometrial carcinomas, whereas FDG and FES-PET showed no abnormal tracer accumulation. A postoperative histopathologic examination revealed that the lesions were endometrial hyperplasias with no malignant findings. They concluded that FES PET enabled them to evaluate endometrial ER expression noninvasively. The evaluation of ER expression using FES PET requires careful attention regarding the influence of hormonal therapy because tamoxifen greatly affects FES accumulation of even endometrial hyperplasia, which should be an FES-avid lesion[66].

Another study published by Tsujikawa et al in 2009 investigated whether [^{18}F]FES and [^{18}F]FDG PET reflect clinic-pathologic features in patients with endometrial tumors[67]. A total of 22 patients with endometrial adenocarcinoma and nine with endometrial hyperplasia underwent [^{18}F]FES PET for estrogen receptor imaging and [^{18}F]FDG PET. The diagnostic accuracy of MRI findings for clinical staging was also compared. They found that although the SUV for [^{18}F]FDG was significantly lower in endometrial hyperplasia than in carcinoma, a statistically significant difference between high-risk and low-risk carcinoma was observed only in SUV for [^{18}F]FES. High-risk carcinoma showed a significantly greater [^{18}F]FDG to [^{18}F]FES ratio (3.6 ± 2.1) than did low-risk carcinoma (1.3 ± 0.5, $P < 0.01$) and hyperplasia (0.360.1, $P < 0.005$). Low-risk carcinoma showed a significantly higher [^{18}F]FDG to [^{18}F]FES ratio than hyperplasia ($P < 0.0001$). In receiver-operating-characteristic (ROC) analysis, the most accurate diagnostic PET parameter for predicting high-risk and low-risk carcinoma was the [^{18}F]FDG to [^{18}F]FES ratio. The optimal [^{18}F]FDG/[^{18}F]FES cutoff value of 2.0, determined by ROC analysis, revealed 73% sensitivity, 100% specificity, and 86% accuracy, which was better than the 77% accuracy

for MRI. The [^{18}F]FDG to [^{18}F]FES ratio of 0.5 yielded a correct diagnosis for carcinoma from hyperplasia with 100% accuracy. They concluded that endometrial carcinoma reduces estrogen dependency with accelerated glucose metabolism as it progresses to a higher stage or grade, that the [^{18}F]FDG to [^{18}F]FES ratio reflects tumor aggressiveness, and that this index will be useful for making noninvasive diagnoses and deciding the appropriate therapeutic strategy for patients with endometrial carcinoma[67].

5.3.4. [^{18}F]FES Human Safety Studies

Approximately 500 subjects are represented in the published studies. These studies include some of the local experience at the University of Washington. To date 267 patients have been studied with [^{18}F]FES at the University of Washington Medical Center. Other than infrequent transient intravenous site discomfort and an "alcohol taste", there have been no adverse events related to [^{18}F]FES administration.

Although lab values have not been routinely measured pre- and post-FES PET scans as part of the PET procedure, many patients at the institution have undergone serial measurements of renal and liver function, differential blood counts and assay of electrolytes as part of their clinical management. To estimate toxicity risk, 109 consecutive patients who underwent FES PET scans between 2002 and 2005 were examined. Of these 109, 30 patients had hematology and serum chemistry values measured both before FES PET scanning (median 16 days prior) and within 21 days after the infusion of [^{18}F]FES (median 10.5 days post scan).

Measurements of renal and liver function (serum creatinine, SGOT, SGPT, and alkaline phosphatase) showed no clinically significant changes pre- versus post-FES infusion in this group of patients. Three patients had elevated alkaline phosphatase prior to FES infusion, due to extensive bony metastatic disease, and these patients continued to have elevated levels post FES infusion with no clinically significant change. Differential blood counts (platelet counts, WBC, neutrophils, hemoglobin, and hematocrit) were examined. These showed a number of patients with abnormal blood values prior to FES PET scanning; however, this was expected in a heavily pre-treated population undergoing salvation therapy for metastatic breast cancer. There were no clinically significant changes in blood counts seen post-[^{18}F]FES infusion compared with the pre-imaging values. Thirty patients were chosen at random for more detailed analysis. The mean FES injected dose for these 30 patients was 4.8 mCi with a mean specific activity of 1,130 Ci/mmole. Data for the 30 patients are tabulated in Table 5.4. Pre-FES lab values were collected an average of 16 days prior to the FES PET scan. Post-FES lab values were collected an average of 10 days after FES injection.

Table 5.4. Representative lab values from patients undergoing [^{18}F]FES infusion (from University of Washington data)

Test	UW Lab Reference Range	Pre-FES Median	Pre-FES Range	Post-FES Median	Post-FES Range	Paired t-test
Calcium*	8.9 – 10.2 mg/dL	9.1	7.4 – 10.0	9.1	8.3 – 9.9	0.67
Urea Nitrogen*	8 – 21 mg/dL	14.0	8.0 – 155.0	14	8.0 – 31.0	0.40
Creatinine	0.3 – 1.2 mg/dL	0.8	0.6 – 1.2	0.8	0.6 – 1.2	0.65
Hematocrit*	36 – 45 %	36.0	29.0 – 47.0	35.0	26.0 – 44.0	0.23
Hemoglobin*	11.5 – 15.5 g/dL	12.0	9.1 – 16.3	11.7	9.3 – 15.1	0.28
Platelet Count*	150 – 400 THOU/µL	225.5	101 – 378	220.5	95.0 – 344	0.69
WBC*	4.3 – 10.0 THOU/µL	4.25	2.26 – 11.01	4.29	2.52 – 8.44	0.40
Neutrophils*	1.8 – 7.0 THOU/µL	2.52	0.97 – 10.46	2.36	1.46 – 6.62	0.94
GPT*	6 – 40 U/L	22.0	7.0 – 77.0	21.0	12.0 – 72.0	0.71
GOT*	15 – 40 U/L	18.5	11.0 – 74.0	19.0	11.0 – 67.0	0.97
Alkaline Phosphatase*	34 – 121 U/L	59.0	29.0 – 198	68.0	32.0 – 210	0.11
Total Protein*	6.0 – 8.2 g/dL	7.2	5.1 – 8.0	7.1	5.9 – 8.1	0.51

* Range of values not within normal limits pre-FES due to previous chemotherapy toxicities

In summary, there has been no evidence or reports of toxicity other than transient intravenous site discomfort and transient taste or smell sensation in University of Washington experience or in the published literature for [^{18}F] FES used for PET.

5.3.5. Adverse Events and Monitoring for Toxicity

The only related adverse events reported for diagnostic [^{18}F]FES administration as described in this Investigator's Brochure in over 500 patients studied are IV site discomfort and transient taste disturbance. Thus no significant adverse effects are expected as a result of the administration of [^{18}F]FES.

Based upon the chemistry, dosing and administration schema, clinical data, and pharmacology of [^{18}F]FES, the following events should be considered 'highly unlikely' for purposes of subject/patient informed consent with regard to risks:

- Local tissue injury and possible infection due to accidental extravasation of the dose;
- Allergic reaction which may be life threatening;
- Transient changes in sense of taste or smell;
- Injury or increased long term risk of a new cancer due to radiation exposure.

5.3.6. [^{19}F]FES Human Toxicity

The published literature does not have any reports of administration of [^{19}F]FES to humans. The safety of FES in this IND is predicated on animal studies and similarities among related chemical entities. In addition, since [^{19}F]FES occurs as part of the production of [^{18}F] FES and accounts for almost all of the measured mass of FES in FES PET studies, human experience with [^{18}F]FES therefore includes experience with [^{19}F] FES.

5.3.7. [^{18}F]FES Human Toxicity

Because the half-life of fluorine-18 is only 110 minutes, toxicity studies are not possible with the radiolabeled agent. Pharmacologic details of FES in patients are provided in Section 5.2 and Figures 5.1 – 5.3. The radiation dose associated with [^{18}F]FES is discussed separately in Section 5.4.

5.4. Biodistribution and Radiation Dosimetry of FES

^{18}F is a positron emitting radionuclide with a half-life of 110 minutes. The tracer [^{18}F] FES is used to measure estrogen receptor expression in tumors and normal tissues.

5.4.1. Study Population and Biodistribution of [^{18}F]FES

The uptake of [^{18}F]FES in normal human tissues has been measured and used to estimate the radiation absorbed dose associated with the imaging procedure. Dosimetry studies were performed at the University of Washington and have been peer-reviewed and published in the *Journal of Nuclear Medicine*[38].

Forty-nine women were involved in the study, with ages ranging from 31 to 85. After patients were injected with an average of 204 MBq (5.4 mCi) of FES (range 55.5 – 296 MBq = 1.5 – 8.0 mCi), the estimates of radiation dose to various organs and to the whole body were derived from time-activity curves (TACs) of blood and normal tissue from PET imaging studies of patients with known or suspected breast cancer. The normal tissues in the imaging data that were used for dosimetry were distant from the site of any known tumor.

Table 5.5. Tissue uptake of FES for injection of 37 MBq in standard 56-kg adult female

Organ	Number of Subjects	$\bar{C}$ KBq-hrs/g Mean (SD)	Organ Mass (g)	Ã MBq-hrs Mean (SD)
Breast	47	0.7 (0.3)	361	0.2 (0.1)
Gall Bladder	15	31.7 (16.8)	49	1.6 (0.8)
Intestines*				
1.	4	5.8 (7.0)		
2.	14 (28)	9.8	176 (ULI)	
3.	4 plus pooled	6.6 (6.4)	322 (SI)	3.3 (3.2)
Blood	48	2.0 (0.5)	347	0.7 (0.2)
Heart Wall	48	1.8 (0.6)	241	0.4 (0.1)
Kidneys	3	4.2 (0.7)	248	1.0 (0.2)
Liver	49	18.9 (4.8)	1400	26.4 (6.7)
Lungs	48	1.3 (0.5)	651	0.8 (0.3)
Red Marrow	47	1.2 (0.6)	1050	1.2 (0.6)
Spleen	18	1.1 (0.7)	123	0.1 (0.1)
Bladder*				
1.	2	14.4 (10.1)		
2.	16 (28)	18.3	160	2.5 (1.2)
3.	2 plus pooled	15.7 (7.5)		
Uterus*				
1.	3	4.8 (2.7)		
2.	10 (18)	7.6	79	0.4 (0.2)
3.	3 plus pooled	5.5 (2.6)		
Remainder			50793†	58.9 (7.6)

* These organs also had an additional point from a more sparsely sampled time-activity curve.
1. Fully-sampled curves
2. Pooled additional data (Parentheses indicate the number of points used in the curve).
3. Mean of fully sampled curves plus additional value from pooled data. (These are the numbers used for Table 5.6.)
† Remainder of the body for 56-kg female
SD = Standard Deviation, SI = Small intestine, ULI = Upper Large Intestine, $\bar{C}$ = integrated activity concentrations, Ã = cumulated activities

5.4.2. Estimation of Radiation Absorbed Dose from [^{18}F]FES

All the image biodistribution data were normalized for a 37 MBq injection into a 56-kg woman. The integrated activity concentrations ($\bar{C}$) and cumulated activities (Ã) for the source organs are shown in Table 5.5. Examples of fully sampled curves are shown in Figure 5.4. Combined curves and fits for uterus, intestines, and bladder are shown in Figure 5.5. For the intestines, radioactivity above background was found only in the small intestine. It was difficult, however, given their anatomic locations, to distinguish between upper large intestine and small intestine. Therefore, dosimetry was

conservatively estimated assuming identical TACs for both the upper large intestine (ULI) and the small intestine obtained from region-of-interest (ROI) analysis of presumed small intestine uptake in the PET images. No tracer accumulation was seen in the lower large intestine (LLI), sigmoid colon or rectum, where differentiation from small bowel is easier.

The mean dose, standard deviation, and the 25th and 75th percentiles are presented in Table 5.6. The 25th and 75th percentiles are determined assuming a normal curve with the given mean and standard deviation for each organ. The critical organ is the liver, with an average absorbed dose of 0.13 mGy/MBq. The effective dose equivalent is 0.022 mSv/MBq.

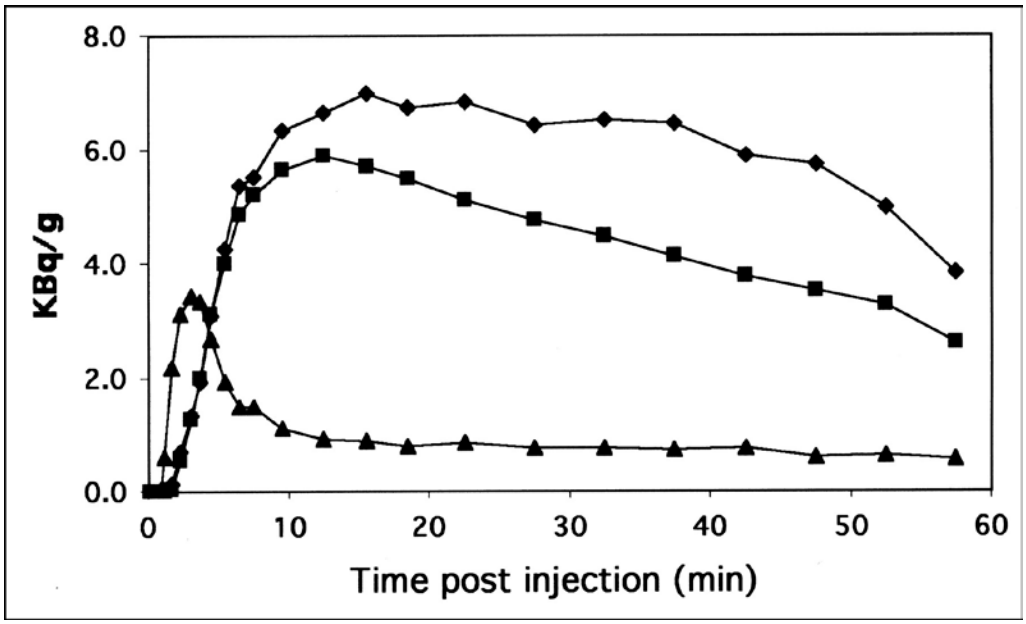

Figure 5.4. Example of tissue-activity curves for FES in the gall bladder (♦), liver (■), and blood (▲). The data are normalized to 37 MBq (1 mCi) of injected activity per 56 kg body weight.

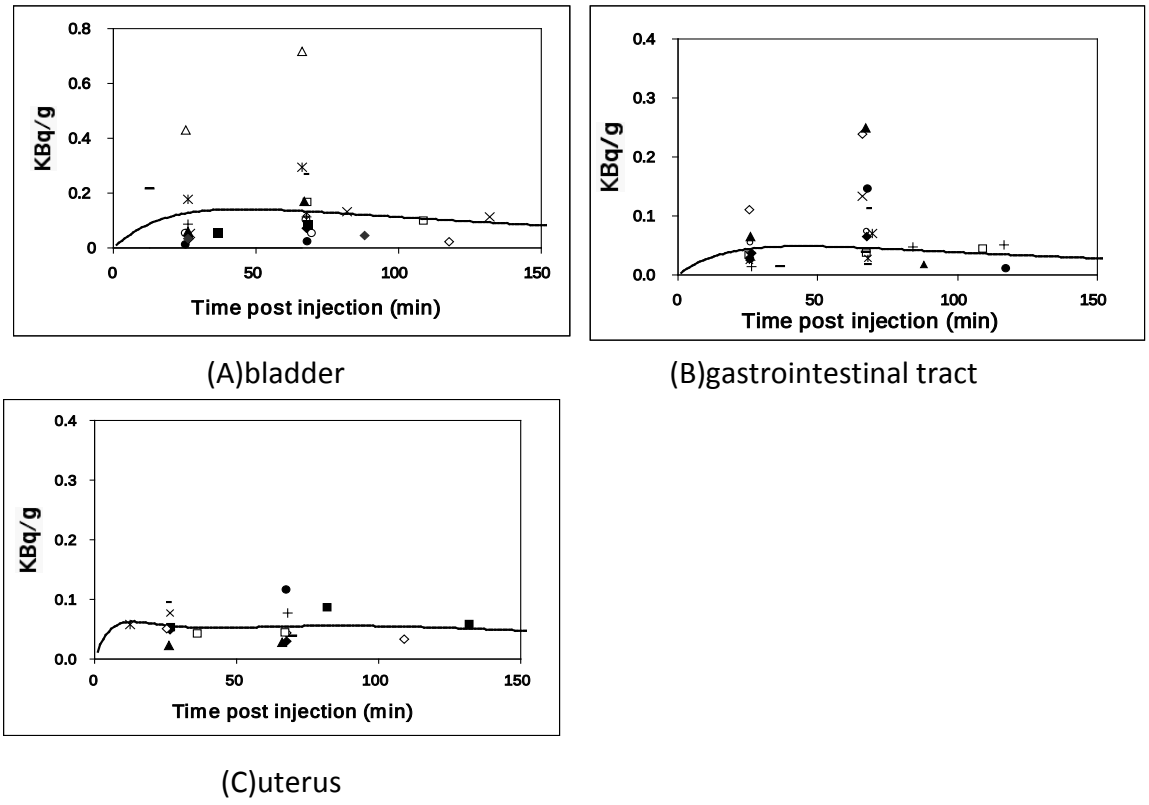

Figure 5.5. FES activity in the bladder (A), gastrointestinal tract (B), and uterus (C). Different symbols are used for different studies. The data have been normalized to 37 MBq injected per 56-kg female body weight. Fits are shown as solid lines. Gastro-intestinal tract and bladder were empirically fit with a rising exponential (Eq. 5.1) and the uterus curve with the function described in Eq. 5.2.

$$C(t) = A\,(1 - e^{-Bt}) \qquad \text{Eq. 5.1}$$

$$C(t) = A[te^{-Bt} + (1 - e^{-Ct})(De^{-Et} + Fe^{-Gt})] \qquad \text{Eq. 5.2}$$

Note that the Y-axis scale for bladder is twice as large as for uterus and intestinal tract. We did not include data from two male patients in our dosimetry calculations. The cumulative activity (areas under the curve), $\bar{c}$, for male subjects, normalized to a standard male weight of 70 kg, fell within the range of values presented for female subjects (data not shown).

Table 5.6. Radiation Absorbed Dose to Organs

Organ	Mean mrad/mCi	Mean mGy/MBq	SD* mGy/MBq	25% mGy/MBq	75% mGy/MBq
Adrenals	85	0.023	0.003	0.021	0.025
Brain	36	0.010	0.001	0.009	0.010
Breasts	32	0.009	0.002	0.008	0.010
Gall Bladder Wall	379	0.102	0.041	0.075	0.134
Lower Large Intestine	45	0.012	0.001	0.011	0.013
Small Intestine	99	0.027	0.015	0.017	0.038
Stomach	50	0.014	0.001	0.013	0.014
Upper Large Intestine	110	0.030	0.016	0.019	0.042
Heart Wall	96	0.026	0.004	0.024	0.029
Kidney	128	0.035	0.004	0.032	0.038
Liver	466	0.126	0.030	0.105	0.149
Lungs	61	0.017	0.002	0.015	0.018
Muscle	79	0.021	0.001	0.021	0.022
Ovaries	66	0.018	0.002	0.016	0.019
Pancreas	84	0.023	0.002	0.021	0.024
Red Marrow	48	0.013	0.002	0.012	0.014
Bone Surface	53	0.014	0.001	0.014	0.015
Skin	18	0.005	0.000	0.005	0.005
Spleen	54	0.015	0.003	0.012	0.017
Testes	44	0.012	0.001	0.011	0.012
Thymus	50	0.014	0.001	0.013	0.014
Thyroid	45	0.012	0.001	0.012	0.013
Urinary Bladder Wall	186	0.050	0.020	0.036	0.066
Uterus	145	0.039	0.013	0.031	0.049
Lens	33	0.009	0.000	0.009	0.009

*SD = Standard Deviation

Effective Dose Equivalent = 0.022 mSv/MBq (0.004 SD)

5.4.3. Sources of Radiation Absorbed Dose in Addition to [^{18}F]FES

In addition to the radiation exposure from the PET scan, there is radiation exposure from the transmission scan that is used to correct the PET scan for density. At the University of Washington either a ^{68}Ge source (PET) or a CT low flux scan (PET/CT) is used. The radiation dose from a single ^{68}Ge scan is approximately 25 mrad and from a CT scan using this low dose technique - 120 kV, 60 mA, 0.8 sec, 20 mm ST, 1.375 pitch, whole body (eyes to thigh) is 490 mrem effective dose (4.9 mSv).

5.4.4. Summary

The organ and total body doses associated with FES PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures[6, 7, 8, 9] and are well below the maximum suggested individual study and

annual total body dose of 30 and 50 mGy, respectively, suggested for investigational radiopharmaceuticals in 21 CFR[10]. Ongoing clinical trials using FES PET imaging will establish its appropriate role in the study of cancer. This analysis indicates that the radiation absorbed dose resulting from the imaging procedure is not a limiting factor and is favorable for further use of this imaging agent. It also suggests that the current recommended maximum dose to provide a good image, approximately 222 MBq (6 mCi), produces an acceptable radiation dose.

5.5. Safety and Toxicity of Other Components of Final [^{18}F]FES Drug Product

The [^{18}F]FES is purified by HPLC using an eluent of 50% ethanol, USP in sterile water for injection. The product [^{18}F]FES is eluted in a volume of 4 to 12 mL into a serum vial containing 15 mL of 0.9% (normal) saline for injection, 0.5 mL of sodium phosphates for dilution and 10 mL of water for injection. The [^{18}F]FES is diluted with 0.9% saline for injection, USP to a volume of nominally 20 mL for injection. The concentration of ethanol in the final injectate is less than 15% by volume, or a maximum of 3.0 mL of ethanol. This is less than one-third of the amount of ethanol in one alcoholic drink and <0.05 mL/kg (< 0.04g/kg) for a standard 56.8-kg woman. In RTECS the LD_{Lo} is given as 1.4 g/kg orally for producing sleep, headache, nausea, and vomiting. Ethanol has also been administered intravenously to women experiencing premature labor (8 g/kg) without producing any lasting side effects[68]. Based upon these reports and experience with over 100 patients over the past decade receiving this amount of ethanol in injectates, we conclude that ethanol will not pose any danger of toxicity in this study.

The other components of the final product solution; sterile water for injection, saline and sodium phosphates are all USP grade. These are all nontoxic for USP grade injectables at the concentrations that will be used. The final product is at pH 7 and the final injection volume is ≤ 20 mL.

The potential contaminants in the final [^{18}F]FES drug product are: acetone, acetonitrile, Kryptofix® [2.2.2], other reaction products. Residual solvents in the final product are limited to 5,000 ppm (µg/mL) of acetone and 400 ppm of acetonitrile. Acetone is used to clean the TRACERLab FX_{F-N} system. Acetonitrile is used to dissolve the Kryptofix® [2.2.2] and is the solvent for the reaction. The permissible level of acetonitrile in the final product is ≤ 400 ppm, the USP permissible level of acetonitrile in 2-[^{18}F]FDG. The allowable level for acetone is < 5,000 ppm. Acetone is a Class 3 solvent. This class of solvents includes no solvent known as a human health hazard at levels normally accepted in pharmaceuticals. Therefore, this limit is based upon the ICH Guideline for Residual Solvents Q3C(R4) (February 2009), page 8[69], where it considers 5,000 ppm in 10 mL, 50 mg or less per day, of Class 3 residual solvents as an acceptable limit without additional justification. All of the residual solvent levels met our acceptance criteria in our initial nine qualification syntheses.

The toxicity for Kryptofix® [2.2.2] has not been reported (RTECS Number Kryptofix® 222 MP4750000). The MSDS for Kryptofix® [2.2.2] lists the LD_{50} for intravenous administration in rats and mice as 35 and 32 mg/kg respectively. In light of these reported toxicities, the FDA has proposed a maximum permissible level of 50 µg/mL of Kryptofix® [2.2.2] in 2-[^{18}F]FDG, as has the USP[70]; therefore, this maximum permissible level will also apply to the [^{18}F]FES final product. All of the Kryptofix levels were < 50 µg/mL in our initial qualification syntheses (n=6).

Although a relatively pure [^{18}F]FES product is obtained, trace amounts of other reaction products may be found in the final product. For this reason an upper limit of 5 µg has been set for the total mass of any other materials in the final injectate that are retained more than 3 minutes on C_{18} HPLC (Betabasic 2X150 mm at 0.3 mL/min) and have UV absorbance at 280 nm. The 5 µg is determined by assuming that the UV absorbing compounds have the same molar extinction coefficient as FES.

5.6. Marketing Experience

To our knowledge, [^{18}F]Fluoroestradiol has not been marketed in the United States.

6. Summary of Data and Guidance for the Investigator

This investigational radiopharmaceutical is [^{18}F]fluoroestradiol, 16-α-[^{18}F]-fluoro-17-β-estradiol, [^{18}F]FES). [^{18}F]FES is a lipophilic molecule that acts similarly *in vivo* to estradiol and binds to estrogen receptors. This radiopharmaceutical is a noninvasive diagnostic agent for assessment of the estrogen receptor (ER) content of tumors using positron emission tomography (PET). The [^{18}F]FES is a sterile, IV injectable solution in phosphate buffered saline. The injected dose of [^{18}F]FES is 6 mCi (185 MBq), with an allowable range of 3 to 6 mCi and a specific activity greater than 170 Ci/mmol at the time of injection. FES is the only active ingredient. There is no evidence that nonradioactive and radioactive FES molecules display different biochemical behavior. The strength of FES binding to the ER and its binding to sex steroid binding protein is nearly identical to that of estradiol. In breast cancer, the uptake of FES, measured by PET has been shown to correlate with ER expression in biopsy material assayed by *in vitro* radioligand binding or by immunohistochemistry. [^{18}F]FES has not been marketed in the United States and, to the best of our knowledge, there has been no marketing experience with this drug in other countries. There are no related imaging products. Approximately 500 patients are known to have received this drug, based upon published reports or under RDRC at the University of Washington, without significant adverse effects.

The ongoing investigational plan is to test the value of [^{18}F]FES PET in evaluating and/or predicting response to therapy for relevant [e.g., E/R +] cancers. The predictive value

and clinical utility of [^{18}F]FES imaging, in concert with estrogen receptor, progesterone receptor (PR), and human epidermal growth factor-2 (HER2) status from tissue assay, and circulating hormone levels will be tested.

Radiation from ^{18}F carries an associated risk to the patient. The radiation absorbed effective dose equivalent to the whole body from [^{18}F]FES injected intravenously is estimated to be 0.022 mSv/MBq (488 mrem for a 6 mCi injection). The critical organ is the liver, with an average absorbed dose of 0.13 mGy/MBq. Dosimetry for other organs is detailed in Section 5.4. The organ and total body doses associated with FES PET imaging are comparable to or lower than those associated with other widely used clinical nuclear medicine procedures and are well below the maximum individual dose suggested for investigational radiopharmaceuticals by the FDA.

Carcinogenesis does not appear to be a risk of administration of [^{18}F]FES as described in this Investigator's Brochure based upon a large number of **animal toxicity studies**, presented above. Although estrogens, including the natural hormones estradiol and estrone, are carcinogenic in laboratory animals, synthetic estrogens such as 2-fluoroestradiol and 4-fluoroestradiol are poor carcinogens in the same animal model systems because the fluorine blocks metabolism when substituted in these positions. [^{18}F]FES is labeled with fluorine at the 16 position of the estradiol. Administration of [^{18}F]FES as described herein, for up to four PET scan procedures, results in intermittent and vastly reduced overall estrogenic exposure compared to regimens known to cause cancer in animals.

The **pharmacology** of FES is best understood by analogy to estradiol, a naturally occurring product synthesized in the ovary in pre-menopausal women and by conversion from adrenal steroids, largely through the action of aromatase enzymes, in a variety of tissues. Pre-menopausal levels of estradiol vary widely during the menstrual cycle, reaching levels as high as 500 pg/mL (1.7 nM) mid-cycle. In post-menopausal women and in men, levels are generally less than 30 pg/mL (0.1 nM). Because FES PET is not intended for therapeutic effect and is not used in sufficient concentration to elucidate a physiologic effect, mechanisms of action beyond metabolism and binding to ER have not been studied.

FES has been evaluated in 14-day repeat-dose animal toxicity, and in *in vitro* genotoxicity and mutogenicity studies. Based upon these studies, as reported in Sections 4.3.1 and 4.3.4 above, FES does not appear to pose a genotoxic or mutagenic risk, nor does it pose a risk for toxicity, when administered in a manner consistent with the PET imaging requirements outlined in this Investigator's Brochure.

Because estradiol is lipophilic, it is generally present in slightly higher concentration in tissues with high fat content. Circulating estradiol is largely protein bound with high affinity but low capacity to SHBG and with low affinity but high capacity to albumin. Estradiol exerts its physiologic effect by binding to the estrogen receptor, ER, which is

selectively expressed in a variety of tissues, most notably the breast, uterus, ovaries, bone, and pituitary. The molecular mechanism of estradiol action through the ER is being increasingly elucidated. It serves largely as an activator of downstream events related to breast and female sex organ function. Estrogens are established growth factors for endometrial and many breast cancers. Approximately 60% of breast cancers express ER, and estradiol and other estrogens provide a key stimulus for tumor growth and an opportunity for endocrine-based therapy. This last effect is the impetus for developing a diagnostic agent for imaging ER expression in breast cancer patients that led to our investigation of FES for use in PET diagnostic imaging.

Typical blood FES concentration after a 6 mCi injection is 1 μCi/mL (< 3 pmoles/mL) peak and by 60 minutes after injection is less than 150 fmoles/mL. For the limiting-case specific activity this corresponds to a peak blood FES level of 833 pg/mL and 42 pg/mL at one hour, comparable to pre-menopausal mid-cycle estradiol levels of 62 to 534 pg/mL and post-menopausal levels of 20 to 88 pg/mL. Males have levels similar to post-menopausal women. Thus the short-term exposure to estrogen from an FES injection as part of an FES PET study transiently yields physiologic levels of the estrogenic steroid, decreasing to sub-physiologic levels after 60 minutes.

[^{18}F]FES metabolism has been studied in humans with results similar to rat data, showing polar metabolites and less than 20% of blood radioactivity as [^{18}F]FES by 60 minutes after injection. There is net clearance of both FES and labeled metabolites from the blood via hepatic uptake, biliary excretion, and urinary excretion of conjugates. By 120 minutes, circulating FES is less than 5% of peak and the total of FES and labeled metabolites is less than 40% of the peak. Clearance rates of intravenous FES and intravenous estradiol are similar. Analysis of metabolites in the urine at 90 – 120 minutes after injection suggests that, on the time scale of PET imaging, FES is metabolized primarily to non-oxidized conjugated FES.

The metabolism of estradiol has been well characterized. It occurs largely in the liver. The formation in the liver of sulfate conjugates at hydroxyl sites is an important route of metabolism. These conjugates are secreted into bile and have efficient enterohepatic circulation that serves as a reservoir for regulating estrogen levels. Glucuronides are also formed in the liver, to a lesser extent than sulfates, and their primary route of elimination is the urine.

Radiopharmaceuticals labeled with ^{18}F must be evaluated for direct dehalogenation reactions leading to loss of [^{18}F]$^{-}$fluoride from the estradiol. Since F^{-} has very high uptake in bone, even small amounts of circulating $^{18}F^{-}$ would be visible as bone uptake in PET images. No uptake above background was seen in bone with FES PET imaging. Thus defluorination is not a factor in FES metabolism.

IV administration of estrogen. Reports of intravenous administration of estrogens are rare; it is used in this form largely in the setting of acute dysfunctional uterine bleeding.

Estradiol administered in doses of 25, 50, 100, or 200 μg peripherally over a five second period documented approximate dosage proportional to serum area under the curve (AUC). Estradiol at 50 μg resulted in an AUC of 512 μg•min/L. An adverse event was reported in only one patient who experienced mild discomfort at the injection site immediately following her dose. This reaction lasted 3 – 4 seconds and did not recur. In another study up to 300 μg of estradiol was administered intravenously and blood levels of estradiol reached 690 pg/mL at 60-minutes post injection. There were no adverse events. For FES used for PET, the typical dose is 1.5 μg or less, with a maximum dose of 5 μg, and for the maximum allowed injection the blood level at 60 minutes should be 42 pg/mL. PET studies indicate that the tissue distribution and blood clearance of [^{18}F]FES is similar to IV estradiol.

In summary, [^{18}F]FES could potentially exert toxic effects through 1 of 3 mechanisms: (1) radiation exposure to tissues from the radioactive label[38], (2) physiologic actions mediated through the ER, and (3) direct toxic or mutagenic effects of FES or metabolites. Radiation exposure from [^{18}F]FES at activity doses used in PET (6 mCi, typical) is low, and is comparable to other nuclear medicine procedures. With respect to the other two mechanisms of toxicity, FES injected as a bolus for PET imaging transiently reaches physiologic concentrations, but returns to sub-physiologic levels within an hour after injection. As such, toxic effects due to actions mediated through the ER and directly toxic effects of metabolites will be far less than those of natural ER ligands. All of the evidence supports the safety of [^{18}F]FES PET imaging.

7. References

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