Summary of Preclinical Data for 21-[^{18}F]fluoro-16α,17α-[(*R*)-1′-α-furylmethylidene)dioxy]-19-norpregn-4-ene-3,20-dione (FFNP)

Prior to human studies, preclinical toxicology and biodistribution/radiation dosimetry studies were performed in small animals. These experiments were performed in accordance with a protocol approved by the Washington University Animal Studies Committee.

Toxicology Studies

The preclinical toxicology evaluation of FFNP consisted of acute toxicity studies in female B6C3F1 mice and female Golden Syrian hamsters. Animal toxicity studies employed ~40,000 times the maximum mg/kg mass to be administered to humans (1×10^{-4} mg/kg) in both hamsters and mice. Three production batches of [^{18}F]FFNP with an average specific activity of 6.9 Ci/mmol, were prepared and stored until the radioactivity had decayed. These were pooled to give 3.6 mL (2.293 mg). There were 5 animals in each mouse and hamster group. A volume of 0.12 mL (0.077mg) was injected per mouse and 0.6 mL (0.382 mg) per hamster. Animals were injected intravenously with either decayed [^{18}F]FFNP or saline as a control. Mice and hamsters were observed for one hour post injection. Both mice and hamsters were observed and weighed several times each week during the two weeks post injection, and then euthanized. Statistical analyses including F-test and t-test were performed.

Mouse and hamster blood samples were taken post mortem by cardiac puncture. Whole blood samples were collected in Microtainer brand tubes with EDTA and gently mixed to disperse the anticoagulant. Blood for serum analysis was collected in Microtainer brand serum separator tubes and centrifuged to separate the serum from the packed cells. Complete blood counts (CBCs) included white blood cell count and differential;, red blood cell count, hemoglobin, hematocrit, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hematocrit, and platelet count. CBCs were performed at Washington University

using the Division of Comparative Medicine (DCM) BioChem ImmunoSystems 9000 Hematology Analyzer.

Serum samples were transferred into cuvettes, sent to the St. Louis University (SLU) Veterinary Laboratory, and analyzed using their Roche Mira Classic Chemistry Analyzer. If necessary, samples were refrigerated prior to analysis. The following tests were chosen to monitor the clearance organs. aspartate transaminase and alanine aminotransaminase as indicators of possible liver damage; total bilirubin and alkaline phosphatase as indicators of gallbladder function; creatinine and blood urea nitrogen as indicators of kidney function; and total protein as an indicator of both liver and kidney function.

Pre-necropsy examination included skeletal palpation, observation of the hair coat, skin, body fat, ears, and hydration, as well as checking for diarrhea and nasal or ocular discharge. Gross necropsy examinations included respiratory system, digestive system, musculoskeletal system, urinary system, genital system, heart, brain, thymus, spleen, lymph nodes, adrenal gland, pituitary and eye. These were performed by a Division of Comparative Medicine veterinary pathologist at Washington University. Samples of brain, heart, kidney, liver, ovaries, adrenals and uterus from each animal were immersion fixed in 10% neutral buffered formalin. Fixed samples were then embedded in paraffin, sectioned into 5 μ slices, and mounted on glass slides for staining with hematoxalin and eosin blue. Each slide contained multiple (2-5) slices of the organ examined. Histopathological examination was performed by a veterinary pathologist.

Mouse Results

Following injection with either saline (control group) or decayed [^{18}F]FFNP (treated group), there were no apparent differences between the control mice and the treated mice. All mice showed no acute response and appeared normal for one hour after injection. Mice were asymptomatic for any disease and were active, alert and well groomed throughout the study.

For the CBCs, the results for all mice in both groups fell within the normal ranges for all tests performed. Using student's t-test at a 95% confidence limit, there were no statistically significant differences between the two groups. The veterinary pathologist observed no unusual results. The specific chemistry tests analyzed from the serum samples (defined above) also showed no statistically significant differences between the two groups using student's t-test at a 95% confidence limit.

Pre-necropsy examinations showed no abnormalities. Gross necropsy results were normal. Histopathological examination of brain, heart, kidney, liver, ovaries, adrenals and uterus samples of all mice showed no abnormalities. The veterinary pathologist observed no unusual results and no significant differences between the two groups.

Hamster Results

There were signs of edema and bruising at the site of injection for several hamsters in both treatment groups 24 hours post injection, but it did not appear to interfere with either their mobility or ability to eat or drink. Weight loss was also seen in both groups, probably due to anesthesia used for injection. Weight loss did not correlate with bruising or edema. All edema had disappeared 48 hours post injection, but some bruising was still visible. Three days post injection all hamsters were fine.

Following injection with either saline (control group) or decayed [^{18}F]FFNP (treated group), there were no apparent differences between the control hamsters and the treated hamsters. All hamsters showed no acute response and appeared normal for one hour after injection. Swelling/bruising at the injection site was noted in both treatment groups, but was not unexpected given the large volume (0.6 mL) injected. The hamsters were asymptomatic for any diseases during this study. Hamsters were alert and well groomed throughout the study.

All hamsters gained weight over the course of the study. The final average weight for the control hamsters was 128.9 grams (119.3-136.7 g); final average weight for treated hamsters was 133.9 grams (125.7-148 g). Using the student's t-test at a 95% confidence limit, there were no statistically significant differences between the two groups at any point during the study. The CBC results for all hamsters in both groups fell within the normal ranges for all tests performed. Using student's t-test at a 95% confidence limit, there were no statistically significant differences between the two groups. The veterinary pathologist observed no unusual results.

For blood chemistry tests, there were no statistically significant differences between the two groups, using student's t-test at a 95% confidence limit.

Pre-necropsy exams showed no abnormalities. Gross necropsy results were normal. Histopathological examination of brain, heart, kidney, liver, ovaries, adrenals and uterus samples of all hamsters showed no lesions. Mild to moderate endometrial proliferation was seen in the uterus of three treated hamsters and three control hamsters. According to the veterinary

pathologist, this may have been due to stage of the estrus cycle. There were no significant differences between the two groups.

Animal Biodistribution Studies and Dosimetry

Six groups of mature female Sprague-Dawley rats (n=4 or 5 per group with average weight of 200g) were injected intravenously with approximately 50 μCi in 160 μL via tail vein. Animals groups were dissected at the following time points: 15 min, 30 min, 1 hr, 2 hr, 4hr and 6 hr post injection. Animals were euthanized by cervical dislocation and all major organs were harvested, weighted and counted for radioactivity. The decay-corrected measured radioactivity was then expressed as percent injected dose for each organ and plotted as a function of time to produce the time activity curves. The time activity curves were then fitted by a combination of exponentials to best represent the data. These fitted functions were then integrated analytically to yield the organ residence times. Animals were followed in metabolism cages where feces and urine were collected. The animal dissection data organ residence times were then used with OLINDA/EXM to yield human radiation dose estimates for the standard adult female model.

Animal Dosimetry Estimation

Organ residence times as measured from the rat dissection experiment are presented in Supplemental Table 1. The largest accumulation is observed in the liver, muscle (unspecific) and gastrointestinal tract. A total of 102 min of residence times were collected in the different organs. The cumulative residence time in the urine and feces (excreted) were 5.62 and 0.158 min, respectively. A remaining 56 min of residence time was associated to the remainder of the body and includes the measured activity in the blood and carcass. The organ radiation doses for the standard MIRD human female model are presented in Supplemental Table 2.

Supplemental Table 1. Organ residence times from rat biodistribution.

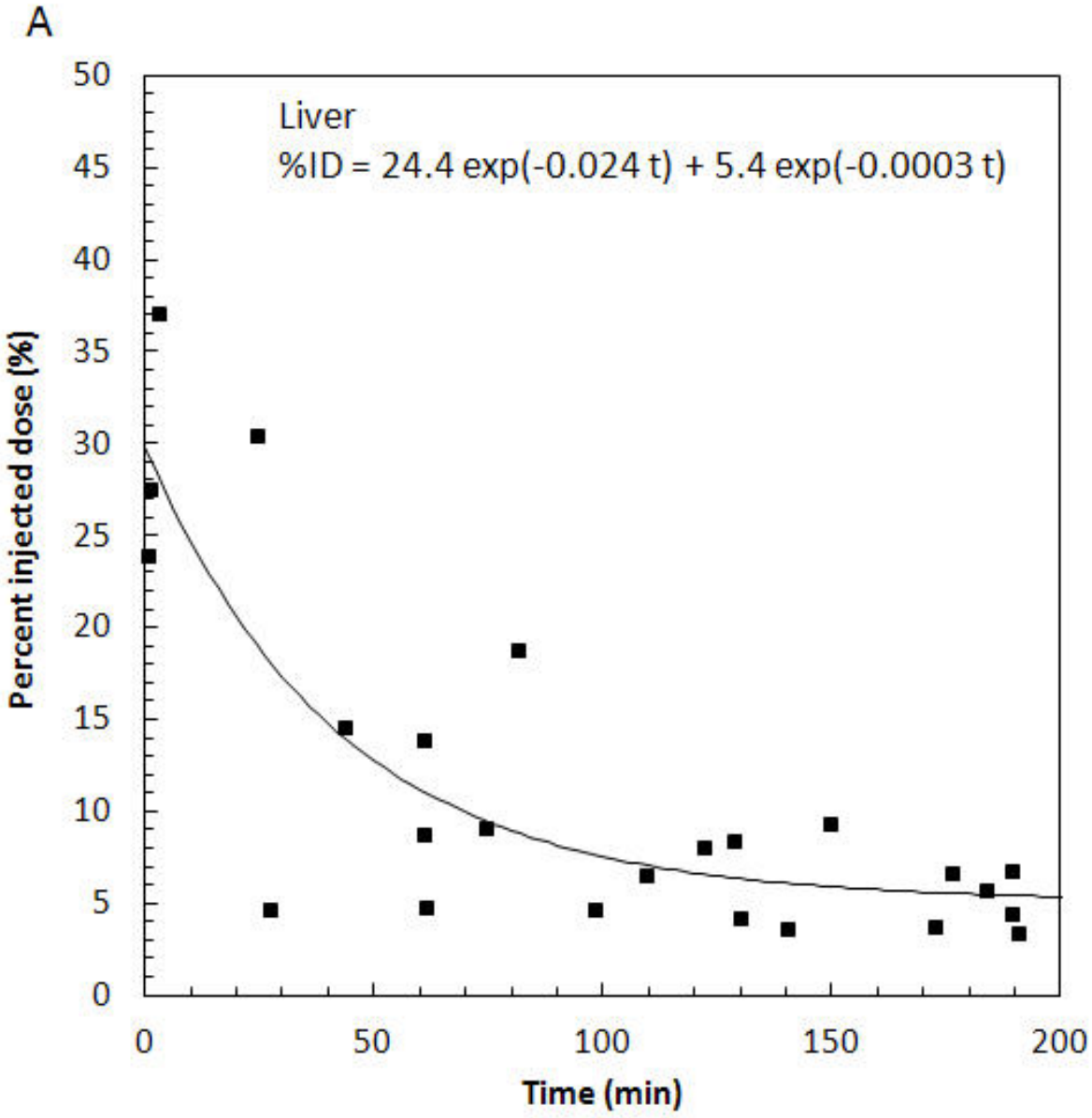
Organ	**Residence Times (min)**
Blood	0.51 +- 0.18
Lungs	0.35 +- 0.07
Liver	8.1 +- 1.2
Spleen	0.12 +- 0.03
Kidneys	0.27 +- 0.09
Bladder wall	0.12 +- 0.10
Muscle	11.4 +- 3.2
Heart wall	0.13 +- 0.04
Brain	0.26 +- 0.07
Tibia	0.07 +- 0.03
Fibula	0.006 +- 0.003
Red Marrow	0.004 +- 0.003
Uterus	1.08 +- 0.40
Ovaries	0.19 +- 0.08
Adrenals	0.062 +- 0.05
Thyroid	0.03 +- 0.02
Pancreas	0.33 +- 0.14
Thymus	0.16 +- 0.04
Stomach	0.87 +- 0.90
Small Intestines	10.6 +- 5.3
Upper large intestines	47.3 +- 14.1
Lower Large Intestines	20.4 +- 6.6
Remainder	56.4 +- 34.2

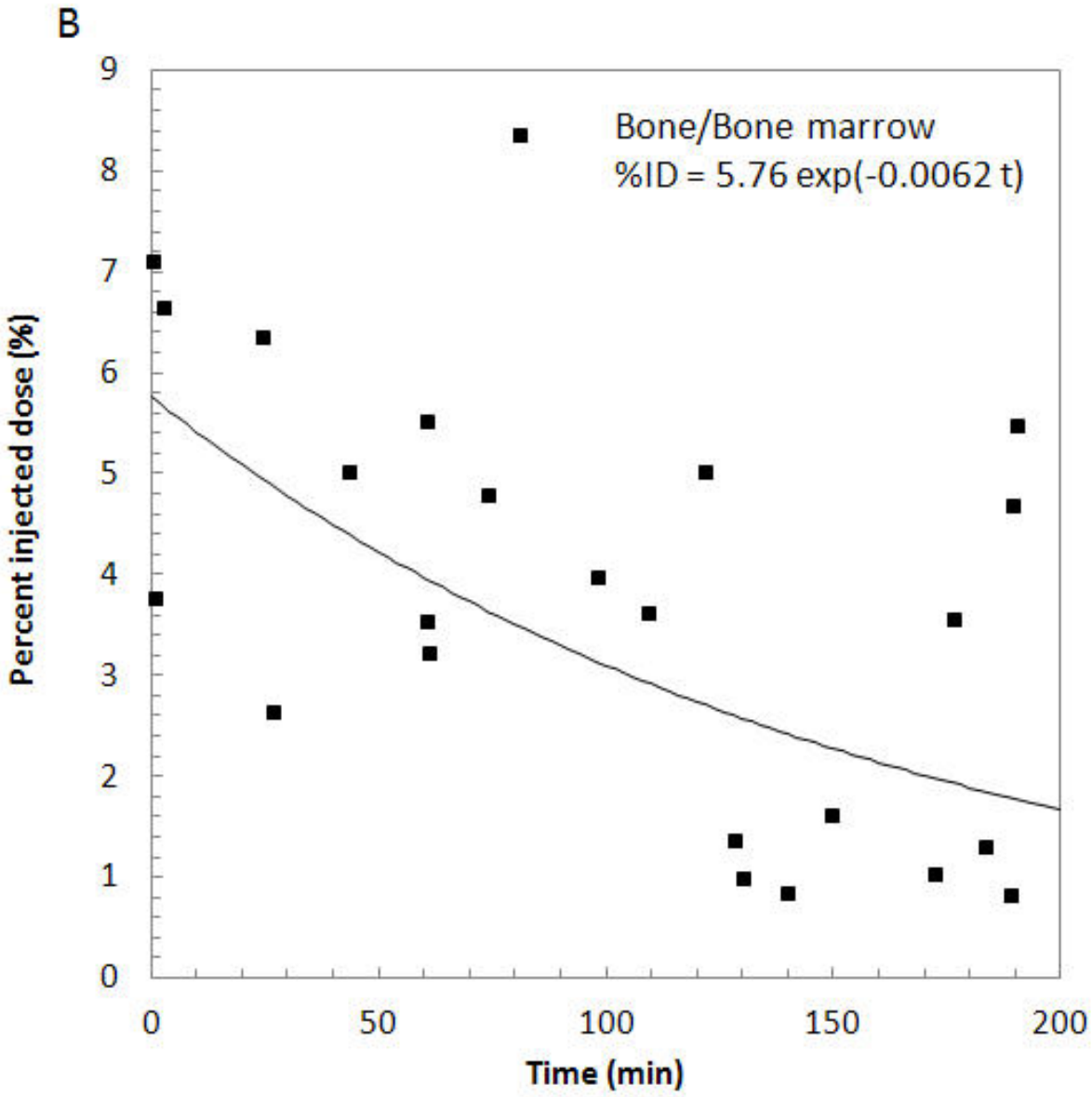
Supplemental Table 2.. Human organ radiation doses estimated from rat biodistribution data in mGy/MBq for the adult female human model. Numbers can be multiplied by 3.7 to give estimates in rad/mCi or rem/mCi injected.

Organ	**Organ Dose (mGy/MBq)**
Adrenals	0.021
Brain	0.003
Breasts	0.007
Gall bladder	0.032
Lower Large Intestines	0.235
Small Intestines	0.087
Stomach	0.024
Upper Large Intestines	0.362
Heart wall	0.009
Kidneys	0.015
Liver	0.031
Lungs	0.007
Muscle	0.011
Ovaries	0.089
Pancreas	0.023
Red marrow	0.015
Osteogenic cells	0.019
Skin	0.007
Spleen	0.011
Thymus	0.027
Thyroid	0.009
Urinary bladder wall	0.017
Uterus	0.063
Whole Body	0.015
Effective Dose (mSv/MBq)	0.065

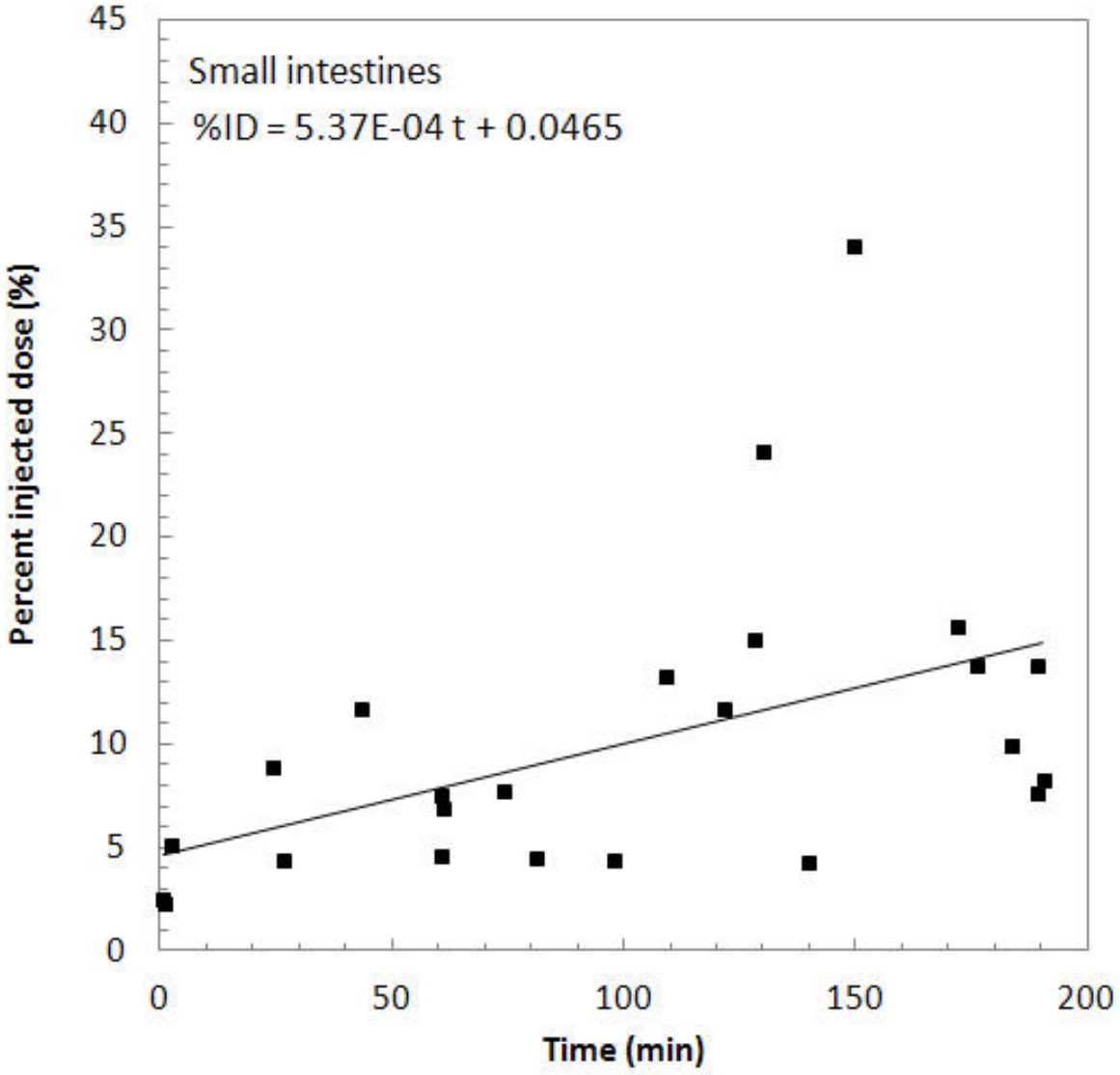
Time activity Curves for FFNP from PET images in Breast Cancer Patients

The following figure contains the FFNP time-activity curve data from the human PET imaging studies performed in adult women with breast cancer who participated in the dosimetry arm of the study.

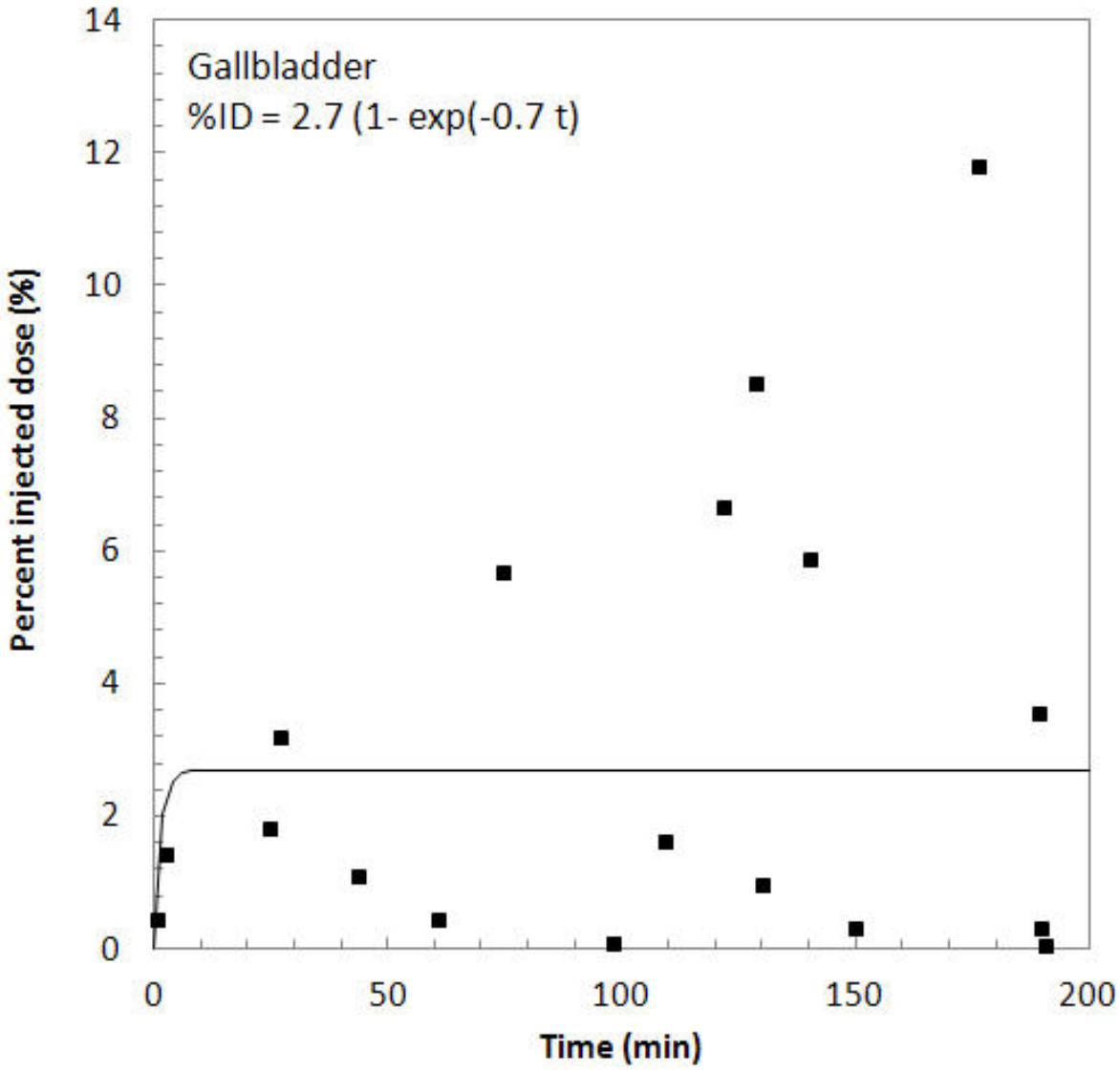


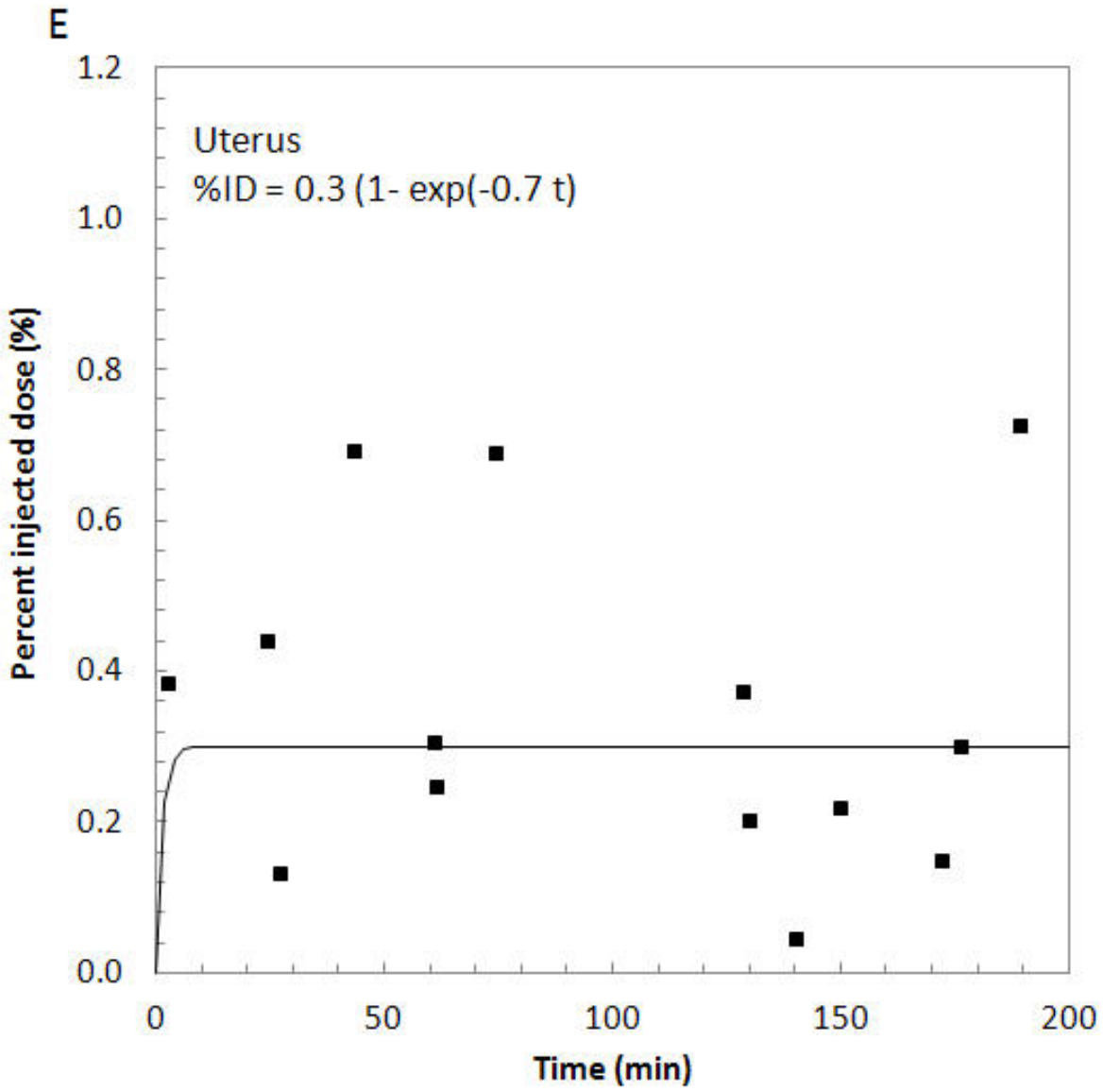

B
Bone/Bone marrow
%ID = 5.76 exp(-0.0062 t)
Percent injected dose (%)
Time (min)
0
1
2
3
4
5
6
7
8
9
0
50
100
150
200

C

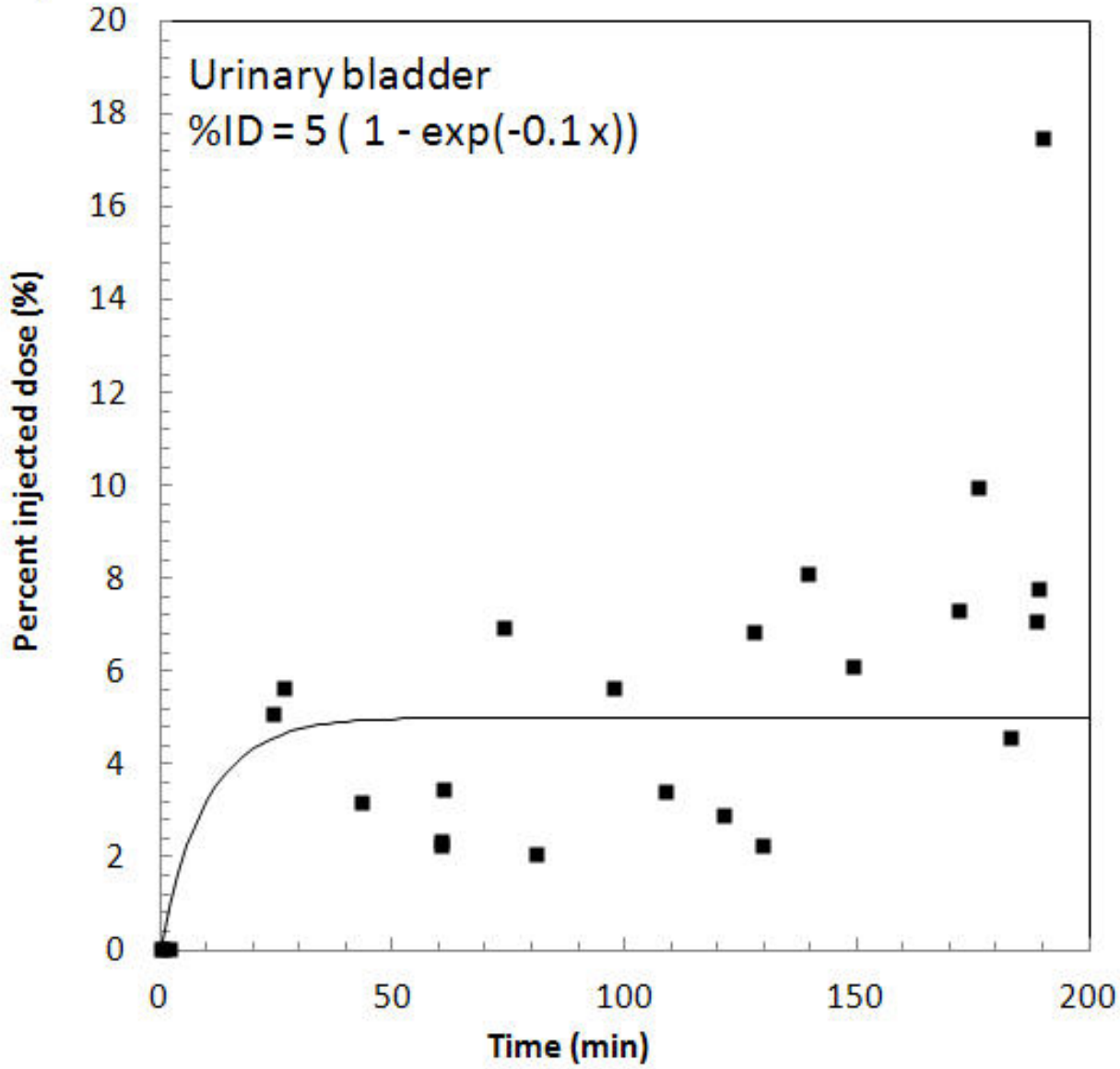

Small intestines
%ID = 5.37E-04 t + 0.0465
Percent injected dose (%)
Time (min)
0
5
10
15
20
25
30
35
40
45
0
50
100
150
200

D


Gallbladder
%ID = 2.7 (1- exp(-0.7 t)
Percent injected dose (%)
Time (min)
0
2
4
6
8
10
12
14
0
50
100
150
200


E
Uterus
%ID = 0.3 (1- exp(-0.7 t)
Percent injected dose (%)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
50
100
150
200
Time (min)

F


Urinary bladder
%ID = 5 (1 - exp(-0.1 x))
Percent injected dose (%)
Time (min)
0
2
4
6
8
10
12
14
16
18
20
0
50
100
150
200

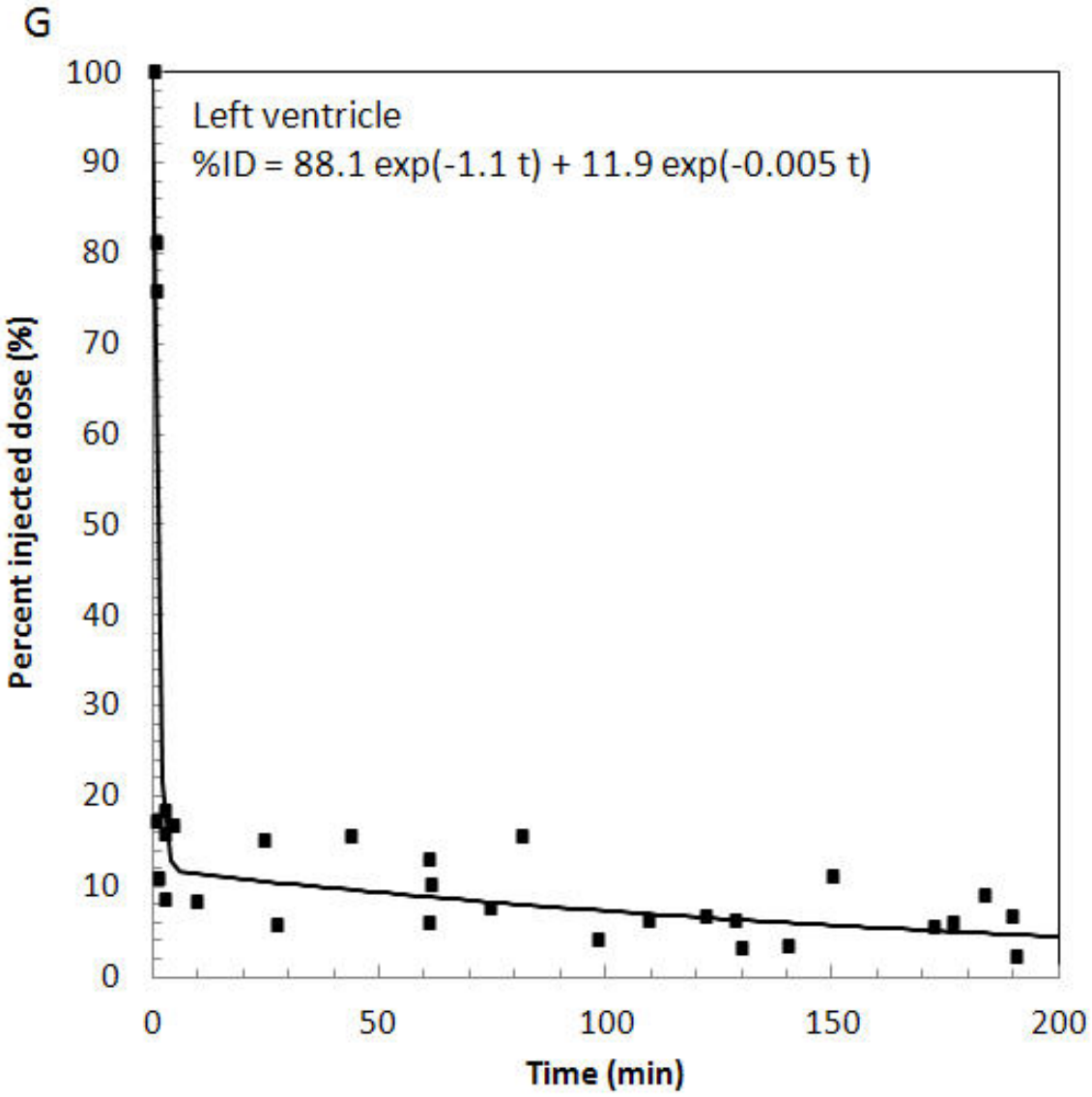


Figure 2 The combined time activity curves along with the clearance or uptake of FFNP are shown for the liver (A), bone/bone marrow (B), small intestines (C), gallbladder (D), uterus (E), whole bladder (F), blood (measured from the left ventricle) (G).