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Blood, liver, kidney, femur, and ovaries were assayed from female Wistar rats following oral administration of uranyl nitrate. Three uranium concentrations were studied for six time periods ranging from 4 hours to 240 hours following gavage. Uranium burdens of tissues were determined by neutron activation and delayed fission neutron counting of dried samples. Blood, liver, and ovaries all fell below the minimum level of detection. Femur burdens were converted to skeletal burdens using an empirically determined factor of 19.6. Uptake of uranium by the skeleton and kidneys increased to a peak value followed by a gradual elimination. The maximum skeletal burdens at 30, 3, and 0.3 mg U/kg body weight were 6.6, 3.3, and 0.7 μg U, respectively. The maximum kidney burdens at 30, 3, and 0.3 mg U/kg body weight were 8.5, 6.3, and 0.6 μg U, respectively. The

gastrointestinal absorption of uranium was estimated using the sum of the kidney and skeletal burdens. The maximum GI absorption was between 0.3 and 2.1 percent of the dose administered. The biokinetics of female rats were congruous with data from male rats. Evaluating the absorption and kinetics of orally administered uranium is important because substantial concentrations of natural uranium are sometime found in human drinking water.

Uranium Biokinetics in Gavaged Young Adult Female Rats

by

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INTRODUCTION

Uranium compounds are ubiquitous in nature. Uranium is present in some quantity in all rocks, no matter what type (Ja55). The average uranium concentration in low-silica igneous rock is 1 µg/g, in intermediate igneous rock is 2 µg/g, in high-silica igneous rock is 4 µg/g, in sedimentary rock is 2 µg/g, and in petroleum is 0.1 µg/g (Gi63). Phosphate rock may have concentrations as high as 120 µg/g (Ro79). Extensive use of fertilizers from such deposits has increased uranium concentrations in North American rivers (Spa72). Uranium can leach out of mill tailings into streams or soak into ground water, although this problem is small in comparison to the presence of naturally occurring uranium in water (Mo77; Mo78). The uranium-to-salinity ratio of the oceans is constant within 4% at a 9.21×10^{-8} g U/salinity corresponding to 3.2 µg U/l for sea water of 35% salinity (Tu71).

Water, used for drinking purposes, could come into contact with uranium from any of these sources. Uranium has been measured in drinking water and food (We67; Ham72; Co83b). However, any adverse effects of uranium in drinking water have not been extensively studied

(La86). Because people are at potential risk to uranium exposure, the metabolism of uranium should be investigated.

Welford and Baird (We67) measured the uranium content in tap water from New York City and typical adult diets from New York City, Chicago, and San Francisco. They found the 0.032 µg U/l (2.1×10^{-2} pCi U/l) in New York City tap water to be insignificant compared to the food intake of 1.3 µg U/day (463.8 µg U/yr). Assuming an average adult drinks 2 l/day, this would result in 23.4 µg U/yr (16 pCi U/yr) from drinking water. The amount from food was 463.8 µg U/yr (311 pCi U/yr), about 20 times greater than the annual dose from drinking water. The dietary intakes from Chicago and San Francisco were 1.4 and 1.3 µg U/day (522.9 and 462.5 µg U/yr), respectively. Hamilton (Ham72) found the annual dietary intake from the United Kingdom to be 0.99 µg U/day (360 µg U/yr).

Cothern and Lappenbusch (Co83b) reviewed direct measurements of uranium in drinking water made by the U.S. EPA and the Canadian Radiation Protection Bureau, and inferred drinking water concentrations of uranium from nearby ground water and surface water measured by Oak Ridge National Laboratory as part of the National Uranium Resource Evaluation program. Directly measured uranium concentrations in drinking water from 19 U.S.

cites were usually less than 1 pCi/l and the highest was 3.2 pCi/l. The range of uranium in surface water from 68 stations in Canada was 0.67 to 2.9 pCi/l. The Oak Ridge study found uranium concentrations in domestic water to range from 0.07 to 652 pCi/l. The average concentration was 2 pCi/l and the median was 0.1 - 0.2 pCi/l. Clearly, most sites contained smaller concentrations of uranium although a few sites were very high. Cothern and Lappenbusch concluded that uranium is found in ground and surface water due to natural occurrence, and in some cases due to human activities such as mining and milling uranium. They estimated that, of the 59,812 community water supplies in the U.S., between 25 and 650 exceed 20 pCi/l, 100 - 2,000 exceed 10 pCi/l, and 2500 - 5000 exceed 5 pCi/l.

Cothern and Lappenbusch (Co83b) compare the annual intake of uranium from food with that from water. Assuming the average concentration of uranium in drinking water to be 2 pCi/l, and a daily consumption of 2 l/day, the average person would ingest 1460 pCi/yr from drinking water. The annual intake from food is approximately 360 µg/yr or 240 pCi/yr. The annual intake from drinking water would then be 6 times greater than from food. This average concentration of uranium in drinking water is 95 times greater than reported by Welford and Baird (We67) for New York City. Cothern and

Lappenbusch's median concentration (0.1 - 0.2 pCi/l) would be 5 to 10 times greater than Welford and Baird.

Uranium is potentially toxic in two ways, radioactively and chemically. Radiation primarily affects the bone because of the longer residence time of uranium in bone (Du75). The overall radiological risk from ingesting water with a uranium concentration of 10 pCi/l is 34 x 10^{-6} premature deaths per lifetime (Co83a). Mays et al. (Ma85) calculated 0.1 to 5 excess bone sarcomas per 1 million persons exposed to a lifetime daily ingestion of 5 pCi of uranium. Their best estimate is 1.5 excess bone sarcomas per 1 million persons. In contrast, 750 naturally occurring bone sarcomas are expected per 1 million persons (Ma85). Although the potential for radiotoxicity exists, chemical toxicity would occur before radiation effects could develop. Chemical damage to the kidney is more severe and occurs at lower doses than the radiation damage to the skeleton for natural uranium (Du75; Wr85). Nephritis, catalasuria, albuminuria, polyuria and glucosuria all result from chemical damage to the kidney from uranium poisoning (Lu58; Hu73; Du75).

Uranium in nature occurs in several oxidation states. Both U^{+4} and U^{+6} are stable in solution. The hexavalent form as the uranyl ion (UO_2^{++}) is the principal ion in aqueous solutions (Du75) and in animal

tissues (Yu73). Hence, uranyl nitrate ($UO_2(NO_3)_2 \cdot 6H_2O$) has been used most often in previous investigations.

Uranium can enter the body by inhalation, injection, or ingestion. In the industrial setting, inhalation of uranium dust or aerosols is the primary route for uranium exposure (Yu73). Hence, most of the research of uranium metabolism has been from inhaled or injected uranium (Yu73; Wr85).

Hursh and Spoor (Hu73), Yuile (Yu73), Durbin and Wrenn (Du75), and Wrenn et al. (Wr85) all have written review articles which summarize much of the uranium metabolism literature. Hursh and Spoor (Hu73) made a thorough review of the literature covering data on human metabolism of uranium. Yuile (Yu73), cited many animal studies discussing topics such as tolerance to uranium following repeated doses, differing sensitivities related to age, sex, and animal strain, and the distribution and excretion of uranium. He also discussed uranium complexes with proteins and bicarbonate in the plasma, and the mechanism of uranium action in the body. Durbin and Wrenn (Du75) summarized the results of animal experiments on the biological behavior of uranium. Metabolism, acute and chronic chemical toxicity, and radiation effects were discussed. They also provided an introductory summary on the chemical states of uranium. Wrenn et al. (Wr85),

summarized the literature covering the metabolism of uranium and radium. They presented models of uptake for both acute and chronic exposures as well as listed models by others.

A few studies have focused upon ingested uranium. Hamilton (Ha48) found an acute dose of uranium to be eliminated from the kidneys in 5 days and from the skeleton in 60 days, in rats following parenteral or oral administration. He also made an extensive analysis of bone deposition.

In addition to examining uptake, elimination, and distribution, a few investigators have made estimates of the GI absorption. Fish et al. (Fi60) gave uranyl fluoride in water to mongrel dogs. They found a 1.55% GI absorption of uranium into the bloodstream from doses of 0.7 mg uranium per kg body weight. They concluded that the pattern of urinary excretion of absorbed uranium is statistically the same whether the uranium reaches the bloodstream from absorption in the GI tract or from direct injection.

Harrison and Stather (Har81) found the GI absorption of uranyl nitrate to be 0.77% and uranium dioxide to be 0.11% in golden Syrian hamsters. They also have determined the uranium distribution in hamsters injected with uranyl nitrate, looking

specifically at the liver, femora, gut, and carcass. The kidneys were not separated from the carcass.

Sullivan (Su80a) found a GI absorption of 0.061% in Wistar rats which were fed ad libitum. The sum of liver, skeleton, and urine was taken as indication of the amount absorbed from the GI tract. He reported no significant fecal contamination of urine and the rats excreted more uranium in the urine than was retained in the skeleton and liver at 7 days postadministration. The kidneys, which were found to contain a significant amount of uranium by LaTouche et al. (La86), however, were not sampled. The percent absorption was 100 times greater (6.7%) in neonatal rats (Su80b).

LaTouche et al. (La86) noted that the presence of food in the GI tract greatly affected the absorption of uranium from drinking water. Rats that had been fed prior to gavage showed great variation in GI absorption. They concluded that absorption of uranium into the body is most significant when the upper GI tract is empty such as following an overnight fast. They found the GI absorption to be between 0.6 and 2.8% over the range of doses studied in male Wistar rats starved 12 hours before gavage and 4 hours afterward. They determined absorption as the sum of the skeletal and kidney burdens because of contamination of urine by feces. This

resulted in a conservative estimate of GI absorption because uranium excreted in the urine was not measured.

A few experiments have been made of human GI absorption of uranium. Some of the human investigations include Butterworth's (Bu58) study of human GI absorption of uranium in which a volunteer drank 1 gm of uranyl nitrate in 200 ml of water. The subject became ill, and vomited, thereby introducing considerable error into the experiment. Nevertheless, Butterworth calculated a 1% GI absorption from the results. In a more controlled experiment, Hursh et al. (Hu69) postulated that human GI absorption of uranium is between 0.5 and 5%. Their data also suggested that absorption decreases with increasing age, based upon 4 subjects.

In a human study by Luessenhop et al. (Lu58), 99% of the uranyl nitrate injected intravenously was cleared from the blood stream in twenty hours. Having left the blood, 6 to 14% of the uranium was deposited in the skeleton, 16% in the kidneys, and 49 to 84% excreted in urine. They also gave a detailed description of the chemical damage to the kidney by uranium. Wrenn et al. (Wr85) concluded from a consensus model that the average human GI absorption of uranium is most likely between 1 and 2% of the amount ingested.

Uranium may be assayed by several different methods. Radiochemical analysis (Yu73), autoradiography (Yu73), fluorometry (Ne48; We67; Hu69), alpha scintillation counting (Har81; Su80a,b), and delayed neutron counting (DNC) (Ham72; Nel84; La86) have all been used for uranium assay. Fluorometry has been the most widely used method for biological and environmental samples because of its excellent sensitivity. However, it suffers from interference from other metals and requires tedious sample preparation.

DNC has equally good sensitivity as fluorometry and fewer analytical steps for easier and quicker sample preparation, as well as higher precision when compared to all the other methods (Id79). The DNC method has been used primarily for geological samples, but is appropriate for biological samples, as well (Nel84). Background counts in the DNC method result from gamma sensitivity, nitrogen-17 decay, and electronic noise (Bi78). With proper discriminator settings on the single channel analyzer and a delay time of 20 seconds for ^{17}N decay, the minimum levels of detection are in the nanogram range (Bi78). The sensitivity of the DNC system can be enhanced by either increasing the reactor power level or increasing the enrichment of uranium-235 (Nel84).

The purpose of this study was to investigate oral administration of uranyl nitrate in female rats using delayed neutron counting. Young-adult female Wistar rats were used because another study in this lab (La86) had accumulated a large amount of data on the biokinetics of uranium in young-adult male Wistar rats. This presented the opportunity for comparison of male and female rats under the same conditions.

Specifically, the objectives of this study were to:

1) Determine the pattern of uptake and elimination of uranium administered orally by gavage;
2) Determine the extent of GI absorption of uranium;
3) Compare the results from female rats to data from male rats.

MATERIALS AND METHODS

Young, unmated female rats of the Wistar strain, weighing an average of 191 grams were used in this study. The actual weight range was 171-231 grams. The rats were secured from Simonsen Laboratory, Inc. (Gilroy, California).

Two types of cages were used. Rats kept longer than twenty-four hours were placed individually in metabolic cages, which purportedly separated the urine from the feces. The bottom section was cleaned every other day to keep the feces from sticking to the side and contaminating the urine. Rats being kept for less than twenty-four hours were placed in "shoe box" cages. These containers had a grill in the floor which kept the rats above their urine and feces. This arrangement prevented reingestion of uranium which had been eliminated from the body.

In addition to cleaning cages, animal maintenance included watering and feeding. The rats were allowed free access to tap water at all times. They were given OSU rat chow which had been ground to a fine powder. Solid chunk food was tried first. The rats would gnaw the chunks of food until they were small enough to pull

out of the feeders. Then, they proceeded to finish eating the small chunks of food inside the main part of the cage. Such eating habits created crumbs which mixed with the feces and clogged the urine funnel. To avoid these problems, the food was powdered, forcing the rats to eat in the feeder, keeping all crumbs in the catch bin. The natural uranium content of the food was assayed as 0.81 $\pm$ 0.22 ppm (personal communication from Dr. Y.D. LaTouche).

The rats were starved for twelve hours prior to gavage, in order to avoid problems with uranium binding to undigested food in the GI tract. Previous studies in this laboratory had shown tremendous variation in GI absorption of uranium with male rats given free access to food. Following gavage, rats to be sacrificed by eight hours were given only water, while animals to be maintained longer were given food after four hours.

Uranium was administered as uranyl nitrate by gavage. Three dose groups, 30, 3, and 0.3 mg U/kg body-weight were studied. These doses were achieved by using three different concentrations of uranyl nitrate (3.0, 0.3, and 0.03 mg U/ml). The volume of solution given to each rat (approximately 2 ml) was based upon body weight. For example, a rat weighing 191 grams that was supposed to receive 30 mg U/kg dose would be gavaged

with 1.91 ml of 3.0 mg U/ml uranyl nitrate.

(1.91 ml/0.191 kg) (3.0 mg U/ml) = 30 mg U/kg

The 3.0 mg U/ml solution was natural uranium (0.72% ^{235}U), while both the 0.30 mg U/ml and the 0.03 mg U/ml solutions were of uranium enriched to 3.34% ^{235}U in order to increase the minimum level of detection.

Each dose group was divided into six sampling times: 4, 8, 24, 48, 96, and 240 hours from gavage to dissection. The numbers of animals used in each group varied from seven to nine and are listed in Table 1. The 48- and 96-hour time periods were not done at 30 mg/kg because toxic effects were seen at the 240-hour period for that dose.

The method of sacrifice was suffocation by carbon dioxide followed by cervical dislocation. The tissues studied were the blood, liver, kidney, bone and ovaries. From $^{1}/_{2}$ to 1 ml of blood was sampled by cardiac puncture. Since the liver was too large to fit into a 2 dram sample vial, only the left frontal lobe of the liver was removed. The same lobe was used each time. Both entire kidneys were sampled. The membrane surrounding each kidney was removed. Both ovaries were sampled, following the removal of as much fat as possible. Since the amount of uranium in the ovaries for the 30 mg/kg dose was near the minimum level of

Table 1: Number of animals per treatment group
Shows the number of animals for each dose, at each time period.

DOSE (mgU/kg RAT)	TIME (HOURS POST GAVAGE) 4	8	24	48	96	240
30	7	9	8	0	0	9
3	8	8	8	8	8	8
0.3	8	8	9	7	8	8

detection, ovaries were not sampled for the two lower dose levels.

Skeletal burden was determined by assaying the whole skeletons for one treatment group, and using the right femurs as representative bones for the others. Whole skeletons were assayed bone by bone for the 3 mg/kg dose group at 240 hours. The calculated ratio of uranium content in the right femur to uranium content in the entire skeleton was used for determining whole skeleton burdens in the remaining treatment groups. The femurs were prepared by steaming approximately 40 minutes, and peeling the muscle and cartilage from the bone. Whole skeletons were prepared by removing the skin, viscera, brain, and eyes and feeding the remains to a dermestid beetle colony. After approximately two weeks, the beetles reduced the carcasses to cartilage and bone, leaving no trace of flesh.

After dissection, the samples required only minimal preparation for neutron activation. Initially, the tissues were placed in vials, frozen in liquid nitrogen and placed into a Virtis Freezemobile 3 where they were freeze-dried for twenty-four hours to eliminate as much water from the samples as possible. Next the samples were weighed, followed by heat sealing the sample vials to prevent leaking. Heat sealing is required to

prevent the sample vial lids from opening during the activation due to heat expansion.

The use of dry samples has two advantages. First, dry weight is more consistent than wet weight. Second, dry samples require only single encapsulation for neutron activation. Liquid samples require double encapsulation to provide an extra barrier against leakage of activated liquid. Leaking samples result in greater error, as well as contaminating the pneumatic transfer system and the delayed neutron counting system. Also, double encapsulation greatly reduces the amount of tissue that will fit inside the vial.

The uranium content in the samples was determined by delayed neutron counting. The samples were activated in the Oregon State University TRIGA Reactor. The sample vials were placed in polyethylene pneumatic transfer system specimen capsules with screw lids commonly called "rabbits". These rabbits were then placed in a pneumatic transfer system and shuttled into the reactor core. Here the samples were bombarded by neutrons for one minute. They were then shuttled back from the reactor, removed from the rabbits remotely (with screwdriver and tongs) and placed into the delayed neutron counting assembly. After a twenty second delay from end of bombardment, the samples were counted for one minute. This delay allowed for the transfer and for

the decay of ^{17}N and ^{16}N, produced by the reactions [$^{17}O(n,p)^{17}N$] and [$^{16}O(n,p)^{16}N$]. The half lives of ^{17}N and ^{16}N are 4.1 s and 7.1 s, respectively. A reactor power level of 300 kW was used for skulls. All other bones were activated at 500 kW except right femurs, which along with all other samples were activated at 1 MW.

The delayed neutron counting system has two major components, the detector assembly and the associated electronics. The detector assembly is housed in a 55 gallon drum which contains two layers of paraffin separated by a layer of cadmium, and an annular array of BF_3 neutron detectors inside the inner layer of paraffin. The outer layer of paraffin and the cadmium layer served to greatly reduce the number of external background neutrons reaching the BF_3 tubes. Sample vials are dropped down a plastic tube axially in the center of the drum and come to a rest in the middle of the ring of BF_3 tubes. Fast neutrons from the samples are thermalized by the inner layer of paraffin. The twelve BF_3 tubes are arranged in two staggered rings so as to maximize detector efficiency. The cadmium layer serves a second purpose in shielding the experimenters from neutrons emitted by the samples during counting.

The electronic components consist of a high voltage supply, an electronic junction box, a preamplifier, an

amplifier, a single channel analyzer, a scaler, a delay timer, and a counting timer. The discriminator was set to eliminate background counts from gamma rays. Details of this delayed neutron counting system are discussed thoroughly by Nelson (Ne84).

Two types of standards were prepared. A nominal standard was made from an aliquant of each gavage solution. The nominal standards were activated with each batch of samples in order to adjust for daily variations in the electronic instrumentation, pneumatic transfer timer, and neutron flux. Each nominal solution was standardized against an aliquot from a solution of uranyl nitrate made from U_3O_8 obtained from the National Bureau of Standards (NBS). The standard solutions were made by dissolving the U_3O_8 (1.0037% ^{235}U) from NBS in nitric acid and diluting with distilled water. Since the standards were liquid, they were doubly encapsulated to provide a multiple barrier against leakage during activation.

Two background components were determined. First, an instrumental background was taken to insure proper working order of the delayed neutron counting system. Second, tissue blanks from control rats were activated to determine the amount of uranium normally present in the tissues and any undecayed ^{17}N from oxygen in the sample vials.

The data from this experiment were entered on a computer disk using the Lotus 1-2-3 software package and an IBM PC microcomputer. The built in functions "@SQRT, @AVG, @COUNT, @VAR, AND @SUM" were used in calculations needing the square root, average of a list, number in a list, variance of a list, and sum of a list respectively. The "@STD" function was not used for determination of standard deviation because that function assumes a large sample size. Instead, I used the formula

"@SQRT (@COUNT(LIST) / (@COUNT(LIST)-1) *
@VAR(LIST))"

for sample standard deviation. The standard error of the mean was simply the sample standard deviation of the list divided by the square root of the number in the list. Other statistical comparisons were made using the Number Cruncher Statistical System software.

RESULTS

Sensitivity of DNC

The sensitivity of the delayed neutron counting system (DNC) was measured at three reactor power levels using the 1.0037% enriched ^{235}U NBS solution (Table 2). The data show a linear relation between the DNC sensitivity and power level (Figure 1). The sensitivity values for natural uranium (0.72% ^{235}U) and uranium enriched to 3.34% ^{235}U at 1 MW were calculated from the value measured using the NBS solution as 1.9 $\pm$ 0.2 cpm/ng U and 8.9 $\pm$ 0.8 cpm/ng U, respectfully. These were used in calculating the minimum level of detection (MLD) for samples at the respective enrichments.

Minimum Level of Detection

The MLD was determined for blood, liver, femur, and kidney for both natural uranium and enriched uranium. The MLD is lower for the enriched uranium by approximately a factor of four to five (Table 3). The MLD was found by the formula:

$$MLD = L_D/\text{sensitivity}$$

where $L_D = (2-\sqrt{2})\ (k)\ (\text{sigma}_b) + k^2$ (Cu68)

Table 2: The sensitivity of the delayed neutron counting system for 1.0037% uranium-235 at three reactor power levels.

1 MW (cpm/ng U) ±standard deviation	500 kW (cpm/ng U) ±standard deviation	300 kW (cpm/ng U) ±standard deviation
2.7 ± 0.3 (N=9)	1.5 ± 0.1 (N=8)	1.0 ± 0.2 (N=4)

Figure 1: Delayed neutron counting system sensitivity for 1.0037% ^{235}U enriched uranium in the OSU TRIGA reactor. (N=4-9)

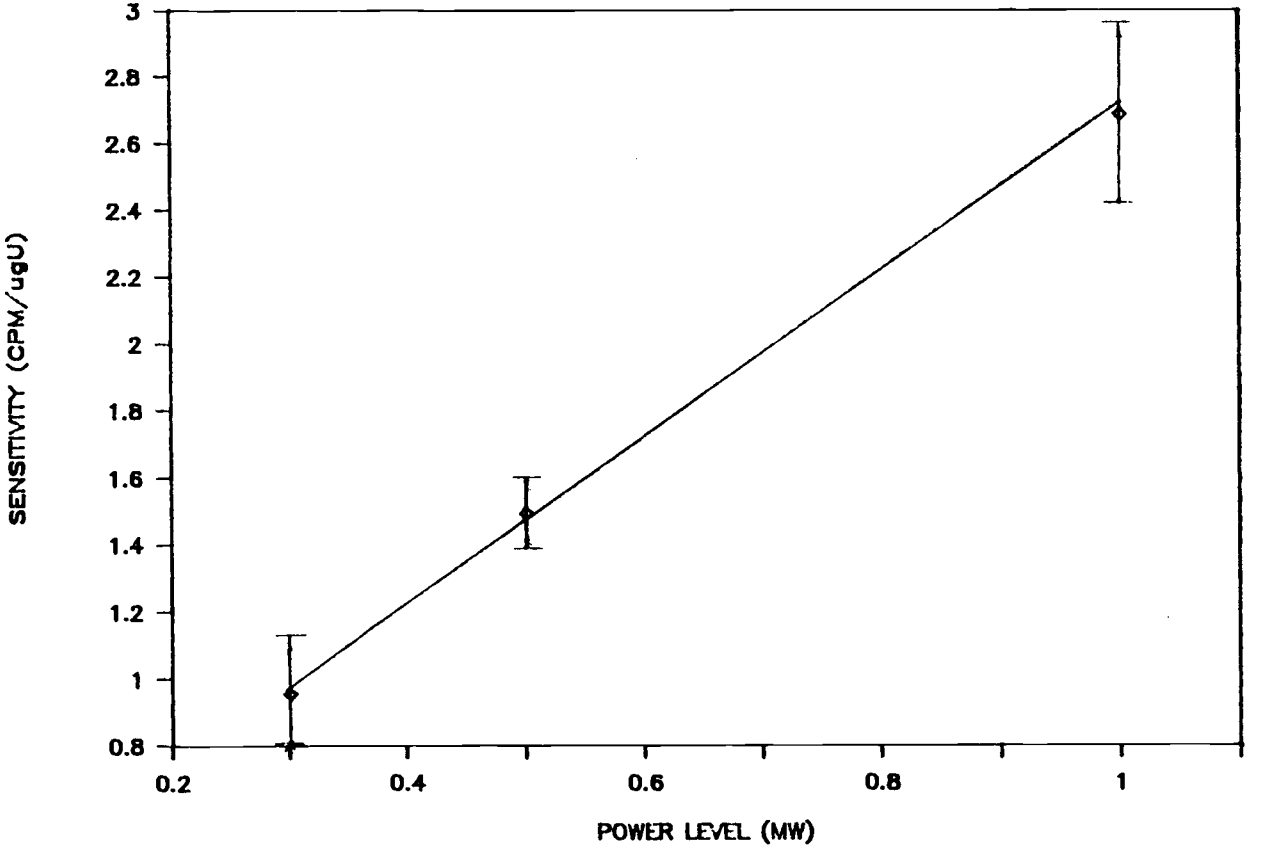


Table 3: The minimum level of detection (95% confidence level) for natural and enriched uranium in blood, liver, kidney, and femur from female Wistar rats, using the delayed neutron counting system. (N = 6)

Tissue	**NATURAL U** (0.72% uranium-235) (ng U)	**ENRICHED U** (3.34% uranium-235) (ng U)
Blood	46	10
Liver	21	5
Kidney	27	6
Femur	21	5

The coefficient ($2\sqrt{2}$) was empirically derived by Currie (Cu68). The confidence level of the MLD depends on the value of k. A value of k = 1 gives an 84.2% confidence level; k = 3 gives a 99% confidence level. I chose k = 2 for a 95% confidence level. $Sigma_b$ is the standard deviation of the total background count rate. This experiment had two components contributing to the overall background: the net counts from the control tissues and the instrumental background. The total background was calculated using the sum of the variances.

$$sigma^2_{total} = sigma^2_{tissue} + sigma^2_{instrument}$$ (Cu68)

The $sigma_{instrument}$ was found to be 4.1, based upon 22 one minute counts.

Control Tissues

Control samples were taken from untreated rats to determine the number of net background counts from the tissues. The variance of these tissue background counts is the "$sigma^2_{tissue}$" component of the total background variance. The average cpm for each tissue was normalized to adjust for the varying sample weights (Table 4).

Table 4: The mean net counts per minute in the DNC for tissues from untreated female Wistar rats. (Normalized for sample weight.) (N = 6)

Control Tissue	Mean Weight Adjusted CPM ± Standard Deviation
Blood	21 ± 14
Liver	13 ± 5
Kidney	24 ± 8
Femur	23 ± 5

Skeletal Burden

The skeletal burden was determined by multiplying the femur burden by an empirically determined conversion factor. The 3 mg/kg, 240-hour treatment group was chosen to calculate the conversion factor for two reasons. First, 3 mg/kg was the middle of the three doses. Second, this treatment group corresponded to one of the male treatment groups used to determine the femur to whole skeleton ratio by LaTouche et al. (La86). The mean whole skeletal burden was 0.8442 µg U, and the mean right femur burden was 0.0430 µg U, resulting in a right femur to whole skeleton ratio of 1:19.6 $\pm$ 2.1.

The mean uranium skeletal burden for each treatment group is listed in Table 5. The pattern of uptake of uranium by the skeleton for the 30 mg/kg and 0.3 mg/kg doses increases to a peak between 4 and 24 hours followed by a gradual elimination of uranium. The peak value for the 3 mg/kg treatment group was between 8 and 240 hours following gavage. The maximum skeletal burdens for the 30, 3, and 0.3 mg U/kg treatment groups were 6.6, 3.3, and 0.7 µg U, respectively. The pattern of uptake and elimination of uranium in the skeleton is shown in Figures 2-4.

Table 5: Mean uranium skeletal burden (µg U ± SE) in young adult female Wistar rats for three gavage doses of uranium. (N = 6-9)

Hours post gavage	Uranium gavage dose in mg U/kg body weight		
	30 mg/kg	3 mg/kg	0.3 mg/kg
4	2.66 ± 0.40	1.29 ± 0.36	0.59 ± 0.09
8	6.64 ± 1.06	1.57 ± 0.29	0.65 ± 0.13
24	6.21 ± 0.99	3.12 ± 0.69	0.57 ± 0.08
48	ND	2.06 ± 0.40	0.45 ± 0.07
96	ND	3.29 ± 0.85	0.47 ± 0.09
240	3.97 ± 0.56	0.84 ± 0.12	0.53 ± 0.07

Figure 2: Mean skeletal uranium burden and kidney uranium burden (± SE) for female Wistar rats gavaged with 0.3 mg U/kg body weight. (N=6-9)

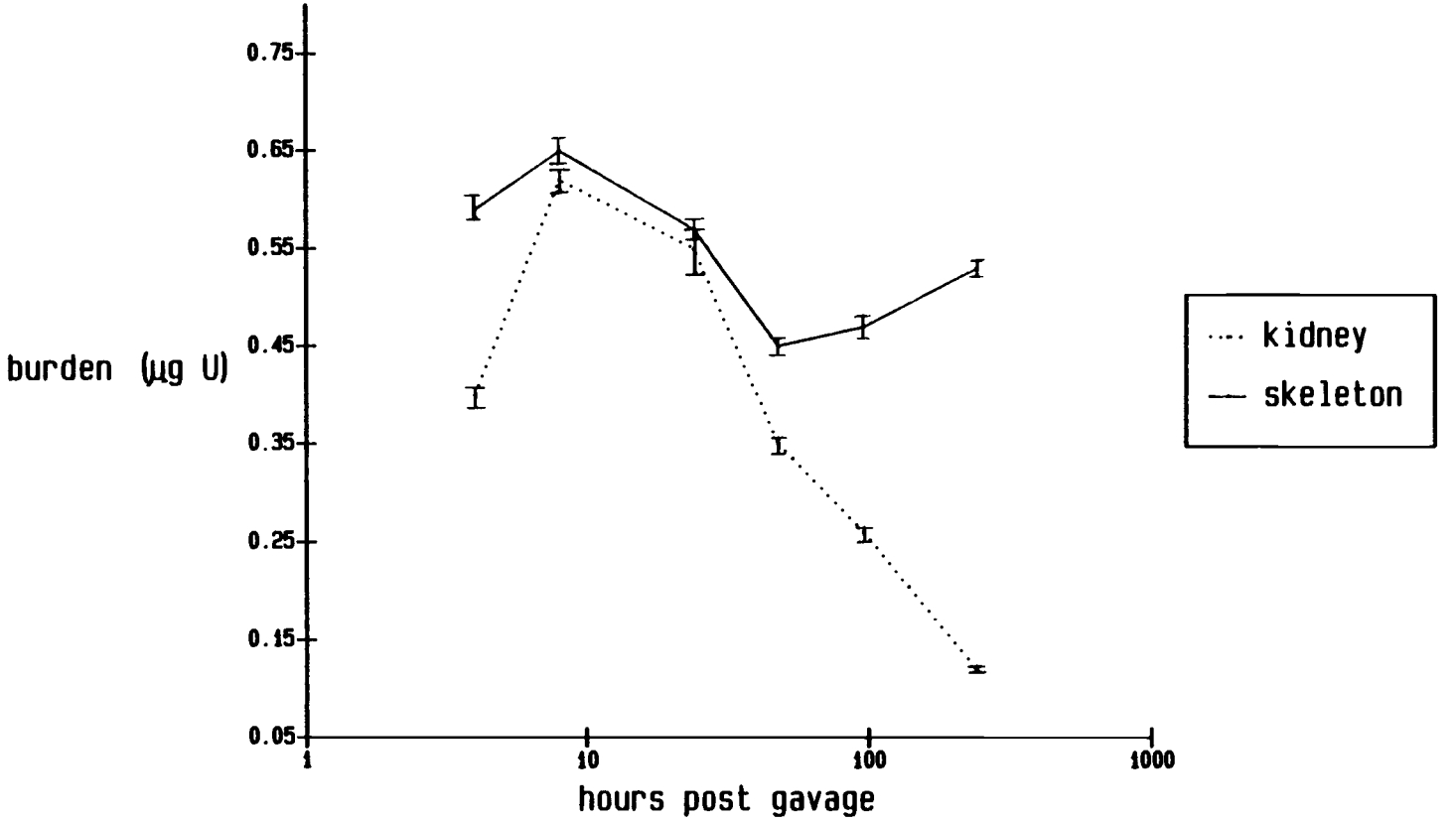


Figure 3: Mean skeletal uranium burden and kidney uranium burden (± SE) for female Wistar rats gavaged with 3 mg U/kg body weight. (N=6-8)

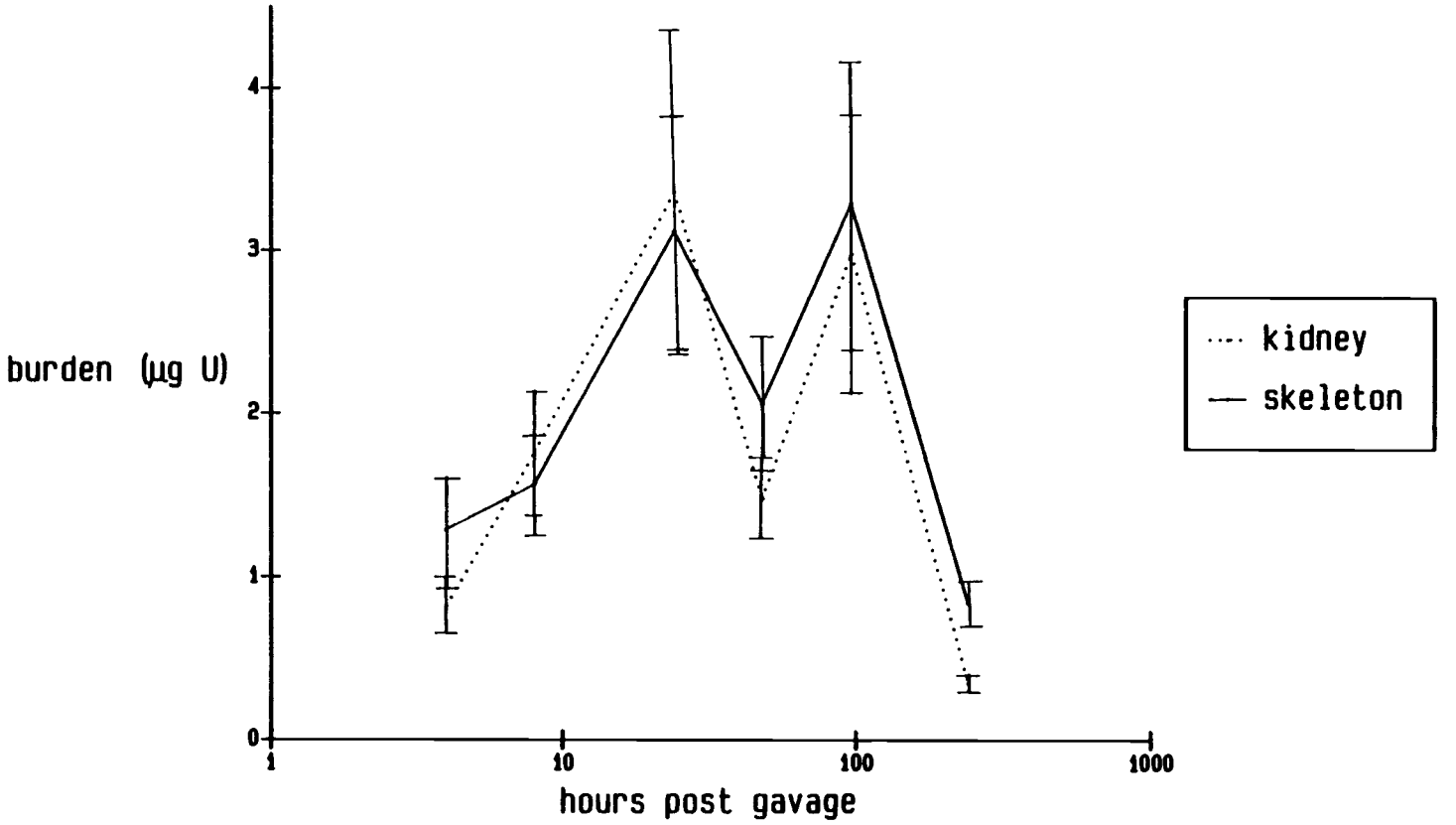
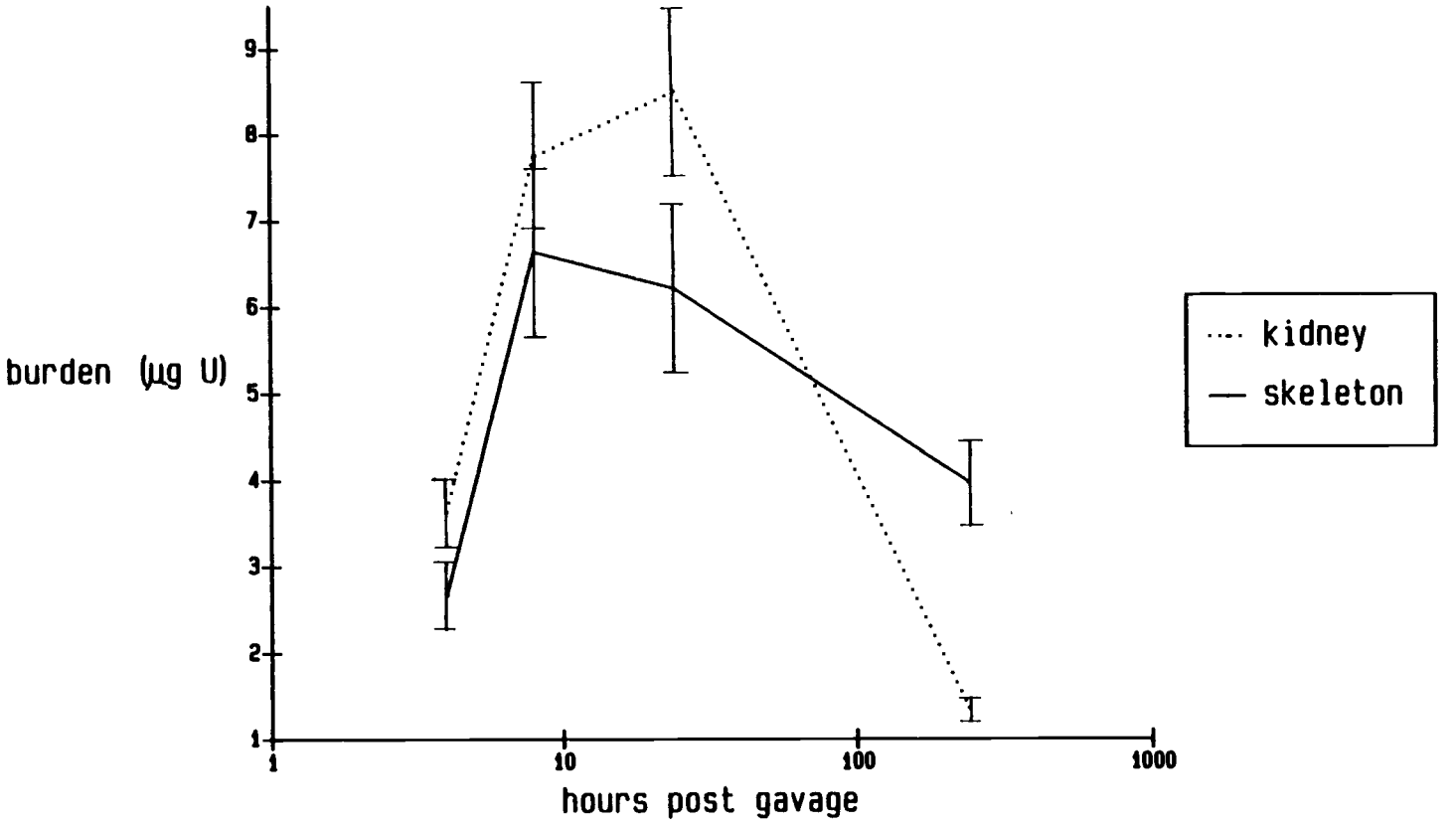


Figure 4: Mean skeletal uranium burden and kidney uranium burden (± SE) for female Wistar rats gavaged with 30 mg U/kg body weight. (N=7-9)



Kidney Burden

The mean uranium kidney burden for each treatment group is listed in table 6. The 30 mg/kg treatment group reached a maximum kidney burden between 8 and 240 hours after gavage. The 3 mg/kg treatment group reached maximum kidney burden between 8 and 48 hours after gavage. The 0.3 mg/kg treatment group, however, reached maximum burden earlier, between 4 and 24 hours after gavage. The maximum kidney burdens for the 30, 3, and 0.3 mg/kg treatment groups were 8.5, 3.4, and 0.6 µg U, respectively. The pattern of uptake and elimination of uranium in the kidneys is shown in Figures 2-4.

Other Tissues

Blood, liver, and ovaries were also assayed. The blood and liver were found to be at or below the MLD for each treatment group. The ovaries were below the MLD for each time period in the highest dose group and were not assayed for the two lower dose groups.

GI Absorption

The extent of GI absorption was determined for each animal and averaged for each treatment group (Table 7). The percent absorption was calculated using the sum of

Table 6: Mean uranium kidney burden (µg U ± SE) in young adult female Wistar rats for three gavage doses of uranium. (N = 6-9)

Hours post gavage	Uranium dose in mg U per kg body weight		
	30 mg/kg	3 mg/kg	0.3 mg/kg
4	3.63 ± 0.40	0.82 ± 0.16	0.40 ± 0.07
8	7.74 ± 0.91	1.76 ± 0.37	0.62 ± 0.12
24	8.50 ± 0.99	3.36 ± 0.92	0.55 ± 0.21
48	ND	1.48 ± 0.24	0.35 ± 0.06
96	ND	2.98 ± 0.85	0.26 ± 0.05
240	1.36 ± 0.14	0.35 ± 0.03	0.12 ± 0.01

Table 7: Sum of kidney and skeletal burdens and calculated percentage GI absorption of uranium for young adult female Wistar rats.

GAVAGE DOSE (mgU/kg)	TIME POST GAVAGE (hours)	KIDNEY + SKELETON BURDEN (µg U ±SE)	MEAN ABSORPTION (dose % ±SE)	N
30	4	6.3 ± 0.7	0.11 ± 0.01	6
30	8	14.4 ± 1.6	0.25 ± 0.03	9
30	24	14.7 ± 1.7	**0.26 ± 0.03**	7
30	240	5.1 ± 0.4	0.09 ± 0.01	7
3	4	1.6 ± 0.3	0.29 ± 0.05	6
3	8	3.6 ± 0.6	0.60 ± 0.11	7
3	24	6.1 ± 1.4	**1.11 ± 0.26**	6
3	48	3.6 ± 0.5	0.58 ± 0.09	8
3	96	6.3 ± 1.6	1.08 ± 0.27	8
3	240	1.2 ± 0.1	0.20 ± 0.02	7
0.3	4	1.0 ± 0.1	1.76 ± 0.22	8
0.3	8	1.2 ± 0.2	**2.09 ± 0.33**	7
0.3	24	1.1 ± 0.1	1.98 ± 0.17	9
0.3	48	0.8 ± 0.1	1.43 ± 0.16	7
0.3	96	0.7 ± 0.1	1.25 ± 0.20	7
0.3	240	0.7 ± 0.1	1.18 ± 0.08	8

the kidney and skeletal burdens divided by the amount of uranium administered.

% absorption =

$$\frac{(100)\ (\text{kidney burden } (\mu g) + \text{ skeletal burden } (\mu g))}{(\text{dose mg/kg})(\text{body weight (kg)})(1000\ \mu g/mg)}$$

The maximum percent of dose absorbed decreased with increasing dose (Figure 5). The kidney and skeletal burdens, however, increased with increasing dose (Figure 6). The 30 mg/kg treatment group reached a maximum percentage absorption between 8 and 240 hours after gavage. The 3 mg/kg treatment group reached a maximum percentage absorption between 8 and 48 hours after gavage. The 0.3 mg/kg treatment group reached a maximum percentage absorption between 4 and 24 hours after gavage and declined only slightly thereafter.

Figure 5: The maximum GI absorption of uranium as percent of dose administered to female Wistar rats.

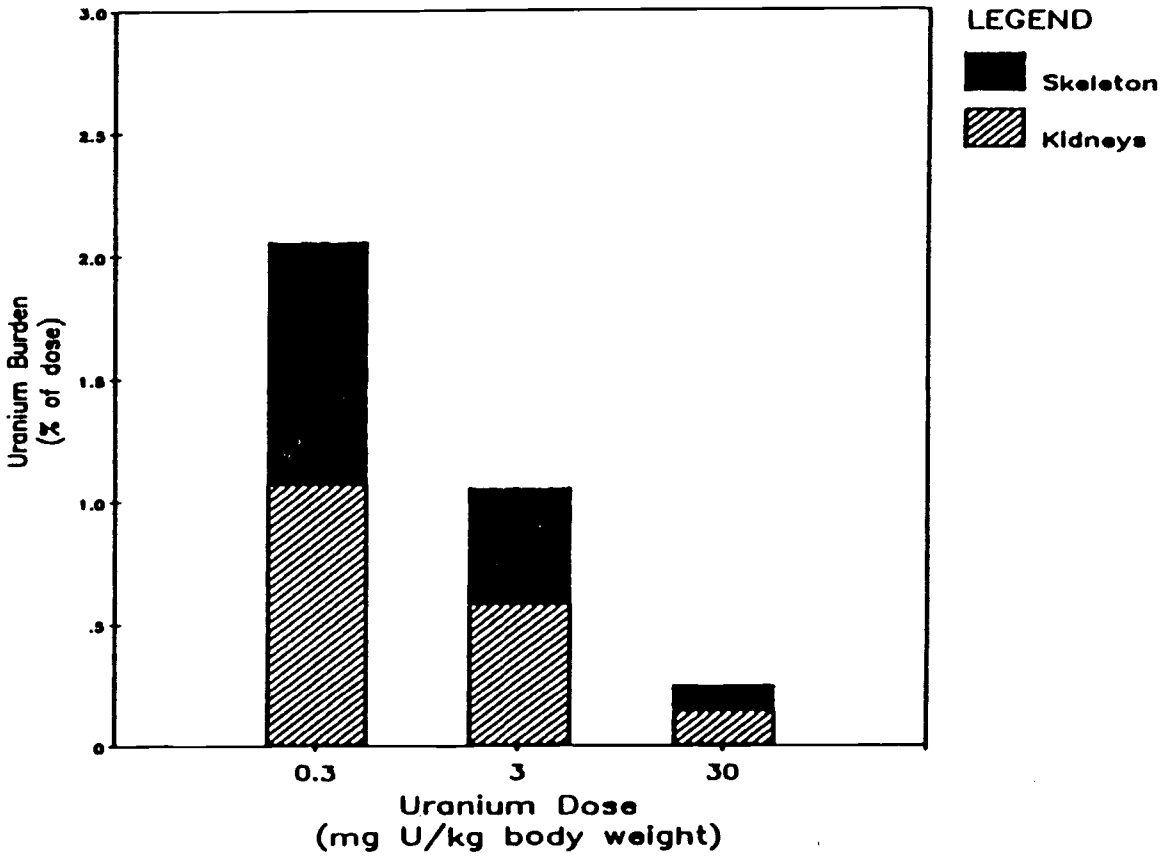
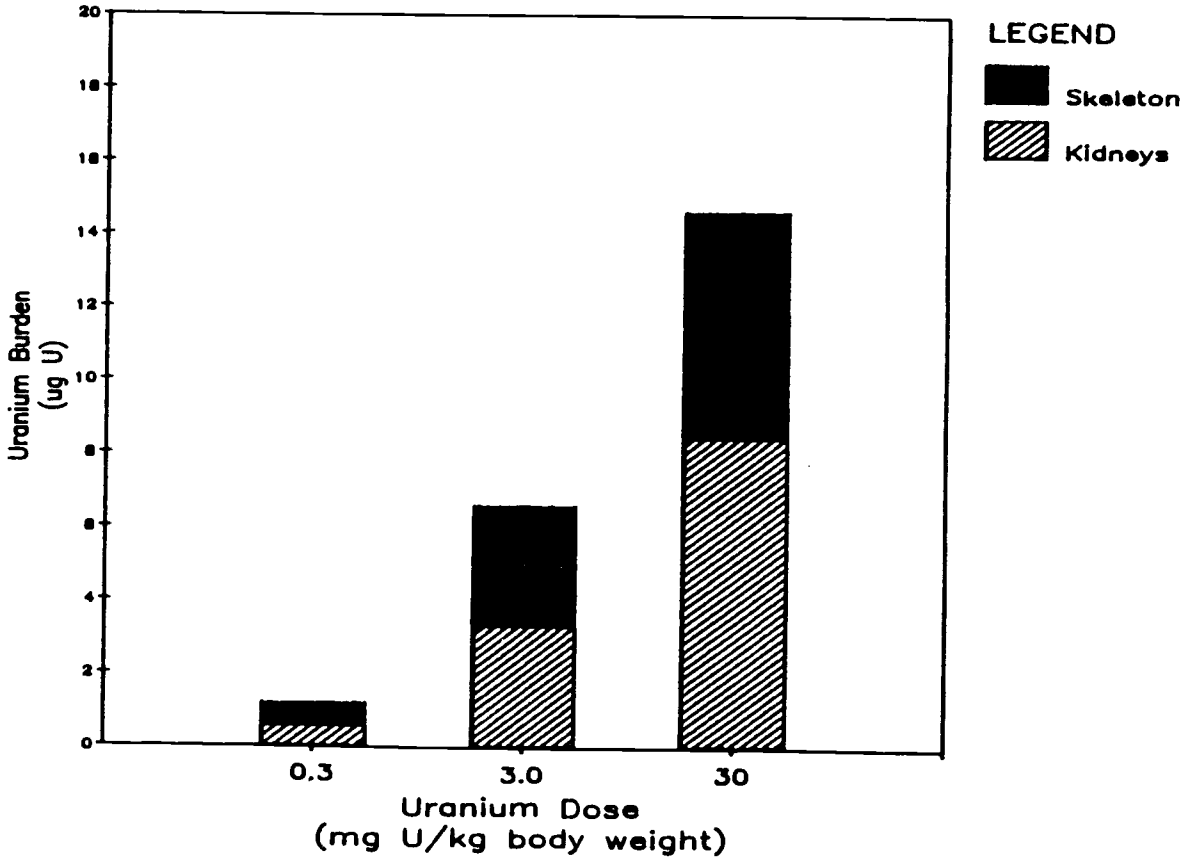


Figure 6: The maximum GI absorption of uranium in female Wistar rats as total kidney and skeletal burden (µg U).



DISCUSSION

The results of this study of uranium biokinetics in young-adult female Wistar rats were congruous with the data from young-adult male Wistar rats (La86). The male data showed blood and liver burdens decreasing sharply with time. After four hours most of the uranium had left the blood and liver. Consistent with the male data, the concentration of uranium in the female blood and liver were below the minimum level of detection four hours after gavage. Because the ovary data were indistinguishable from instrumental background at the highest dosage, ovaries were not sampled at the lower dosages.

A conversion factor was used to determine the whole skeletal burden from the femur burden in both the male and female data. The factor determined for the female rats was 19.6, while for the male skeletal burdens a conversion factor of 18.9 was used. Another investigator has found a skeleton-to-femur ratio of 23 (Su80a). This slight difference in conversion factors would affect the calculated value of the skeletal burden for a given treatment group, but it would not alter the pattern of uptake and elimination.

The Mann-Whitney nonparametric test was used to compare the female and male data. The more common Student's T-test could not be used because it assumes like variances. The female data had larger variances than the male data. A few data points were also considered to be statistical outliers. These values were generally much greater than the remaining more clustered values, and were removed using Chauvenet's criterion. The females were more aggressive and struggled more fiercely during gavage than did the males. This behavior may have introduced additional error to the gavage technique causing the observed larger variances. A small sample size also increases variance. A small sample size (N = 6-9) was used because of the large number of treatment groups to be studied, and because this sample size had been found to be adequate for males.

The general pattern of uptake and elimination of uranium by the skeleton of female rats resembled that of male rats at the highest and lowest dosages (Figures 7, 9). The mean female skeletal burdens at the middle dosage appear to differ from the male skeletal burdens (Figure 8). It should be noted, however, that the female and male 24-, 48-, 96-, and 240-hour time periods were statistically the same ($p > 0.05$) at the middle dosage. The female and male 240-hour time periods at

Figure 7: Mean skeletal uranium burden (± SE) for female and male Wistar rats gavaged with 0.3 mg U/kg body weight.

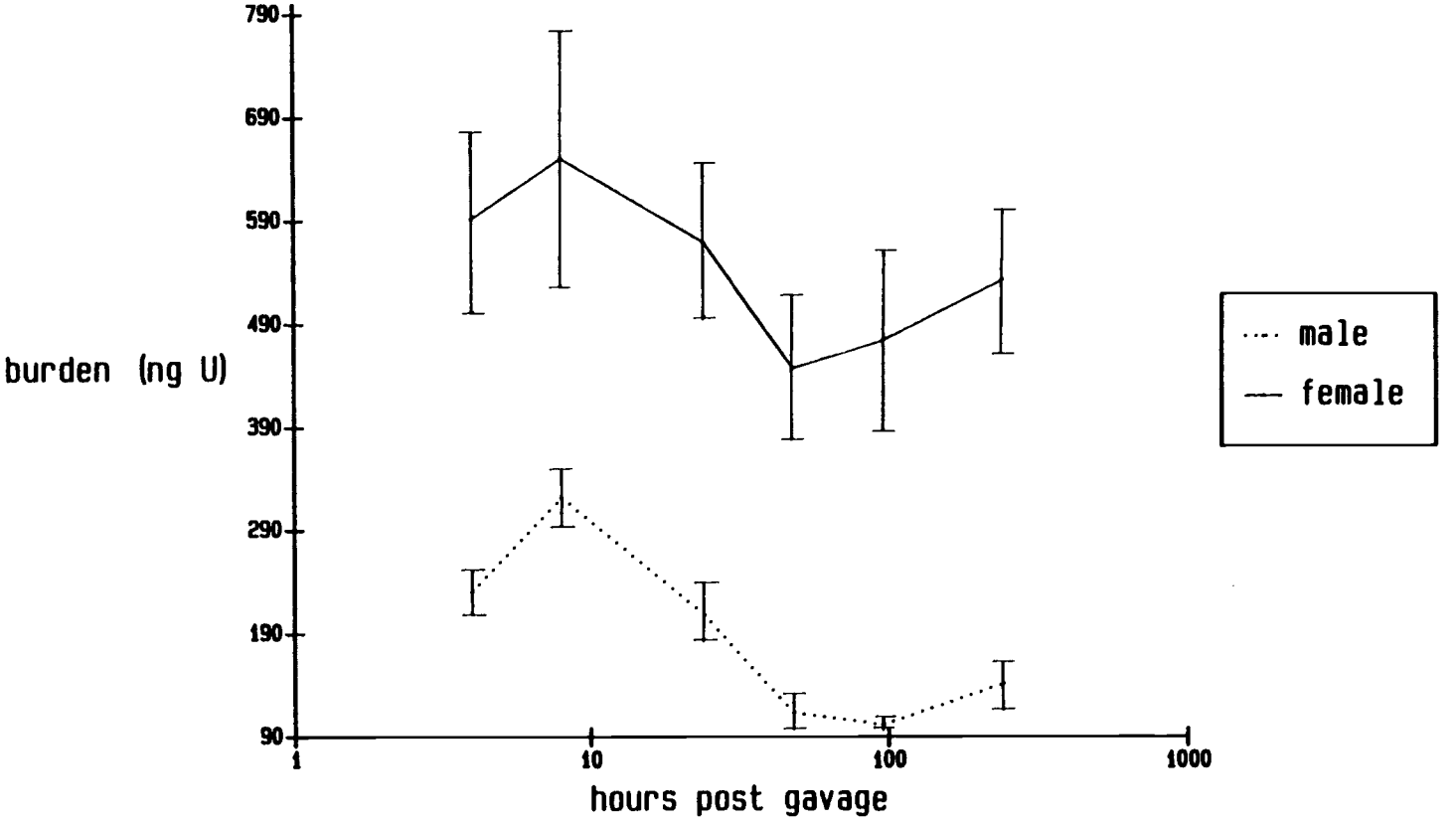


Figure 8: Mean skeletal uranium burden (± SE) for female and male Wistar rats gavaged with 3 mg U/kg body weight.

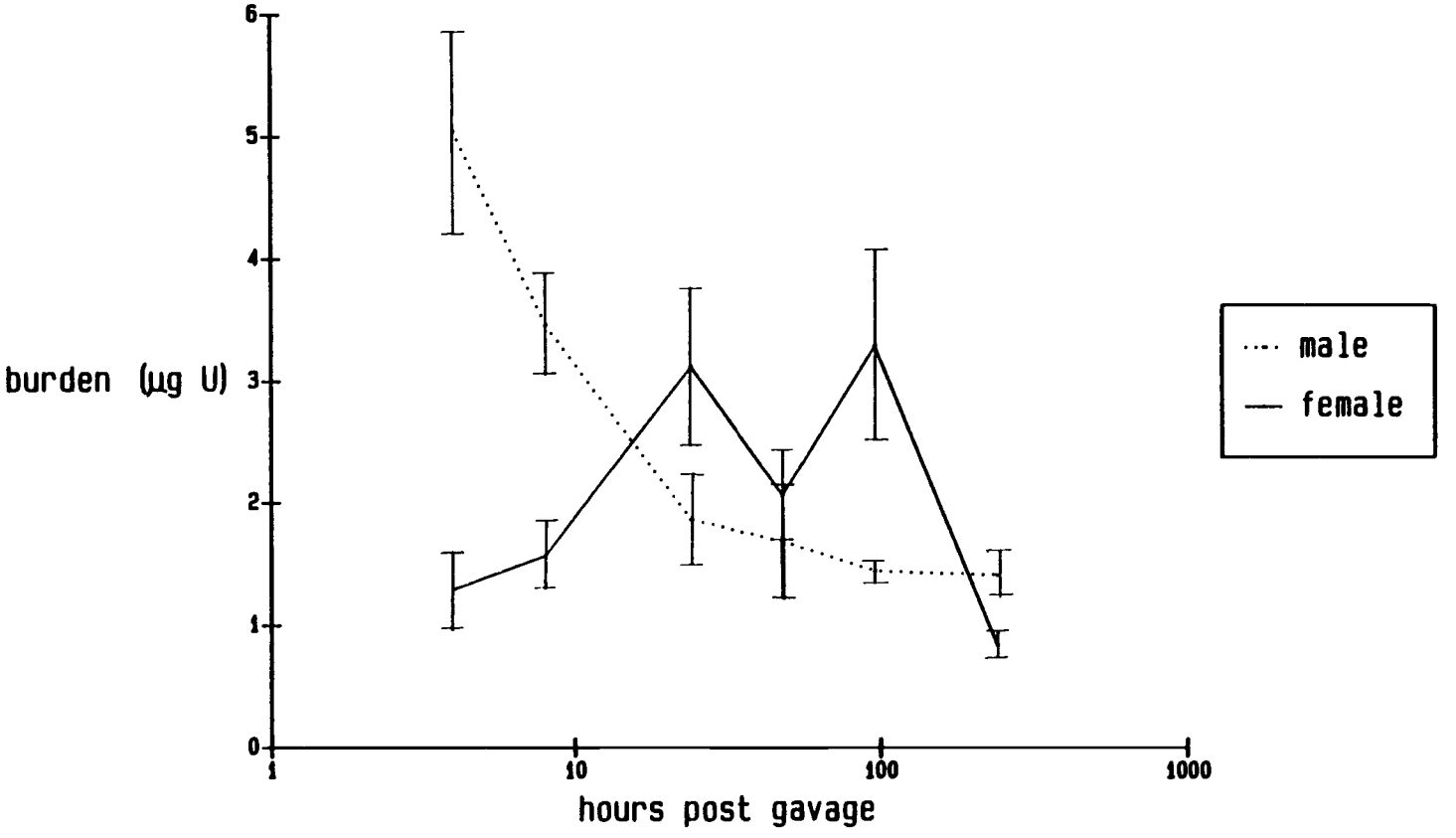
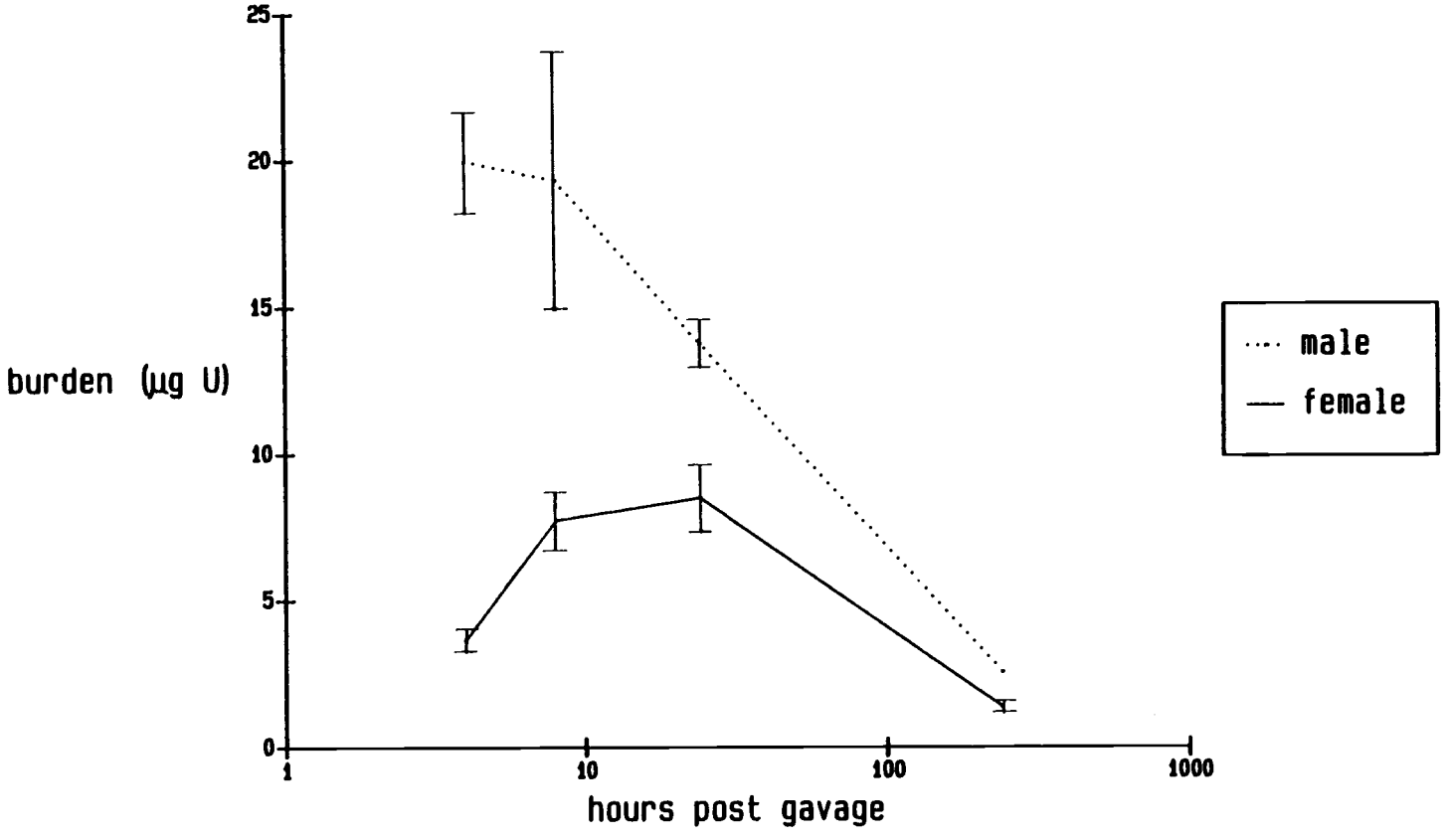


Figure 9: Mean skeletal uranium burden (± SE) for female and male Wistar rats gavaged with 30 mg U/kg body weight.



the lowest dosage were also statistically the same ($p > 0.05$). The remaining treatment groups were statistically different ($p < 0.05$). (No individual animal data were available for the male skeletal and kidney burdens for the 240-hour treatment group at the highest dosage.) The female skeletal burden was greater than the male in the lowest dosage group and less than the male in the highest dosage group. Although statistically different, male and female mean skeletal burdens were not greatly different at the lowest and highest dosages. These results agreed with the findings of Durbin and Wrenn (Du75), that age was more significant than gender in influencing skeletal uptake in rats.

As with the skeleton, the general pattern of uptake and elimination of uranium by the kidneys of female rats resembled that of male rats at the highest and lowest dosages (Figures 10, 12). Although the mean kidney burdens at the middle dosage (Figure 11) appeared to differ, the female and male 24-, 48-, 96-, and 240-hour time periods were statistically the same ($p > 0.05$). The 8-, and 240-hour time periods at the lowest dosage were also statistically the same ($p > 0.05$). (No individual animal data were available for the 240-hour males at the highest dosage.) Similar to the skeletal burdens, the female kidney burden was greater than the

Figure 10: Mean kidney uranium burden (± SE) for female and male Wistar rats gavaged with 0.3 mg U/kg body weight.

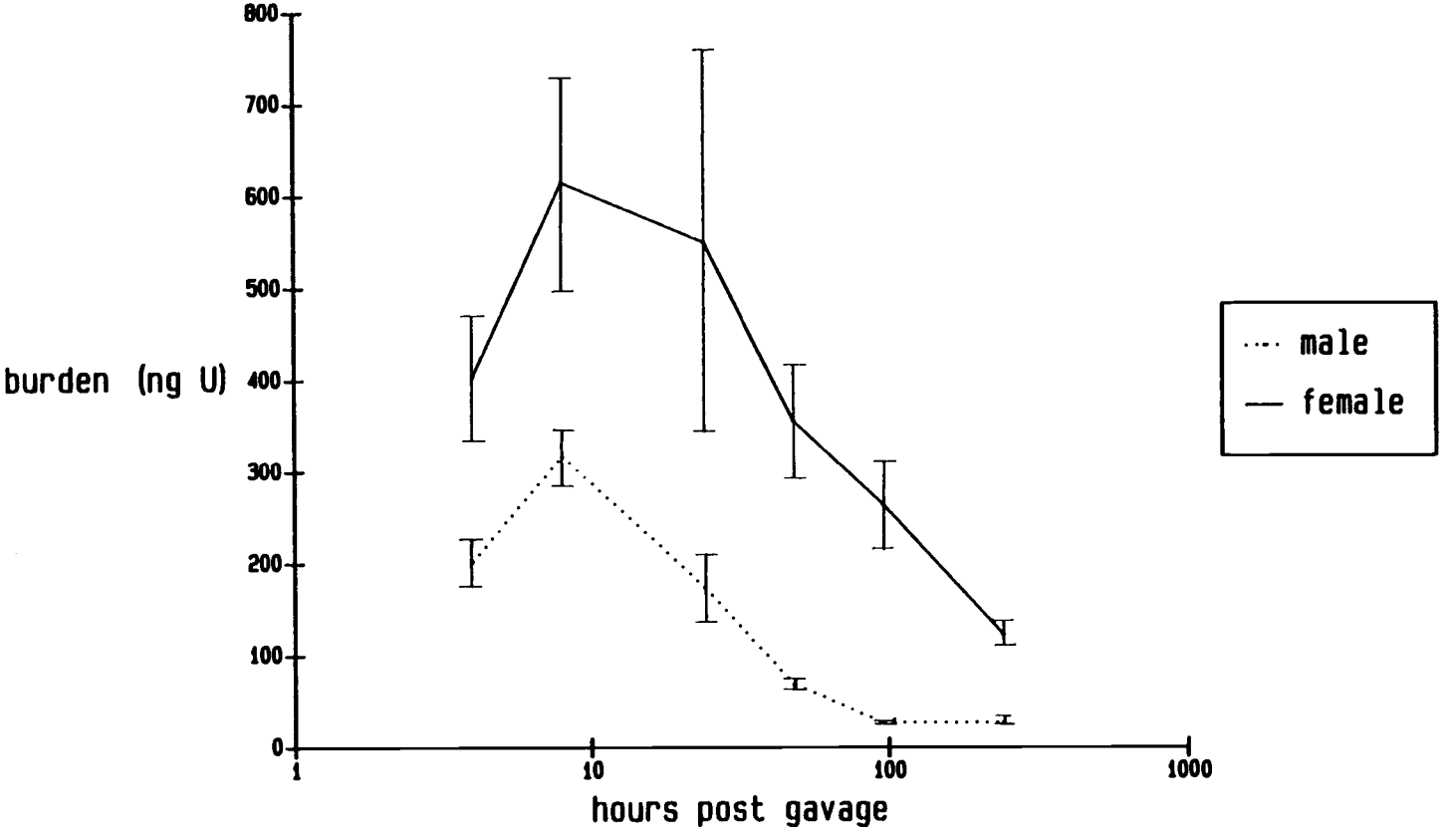


Figure 11: Mean kidney uranium burden (± SE) for female and male Wistar rats gavaged with 3 mg U/kg body weight.

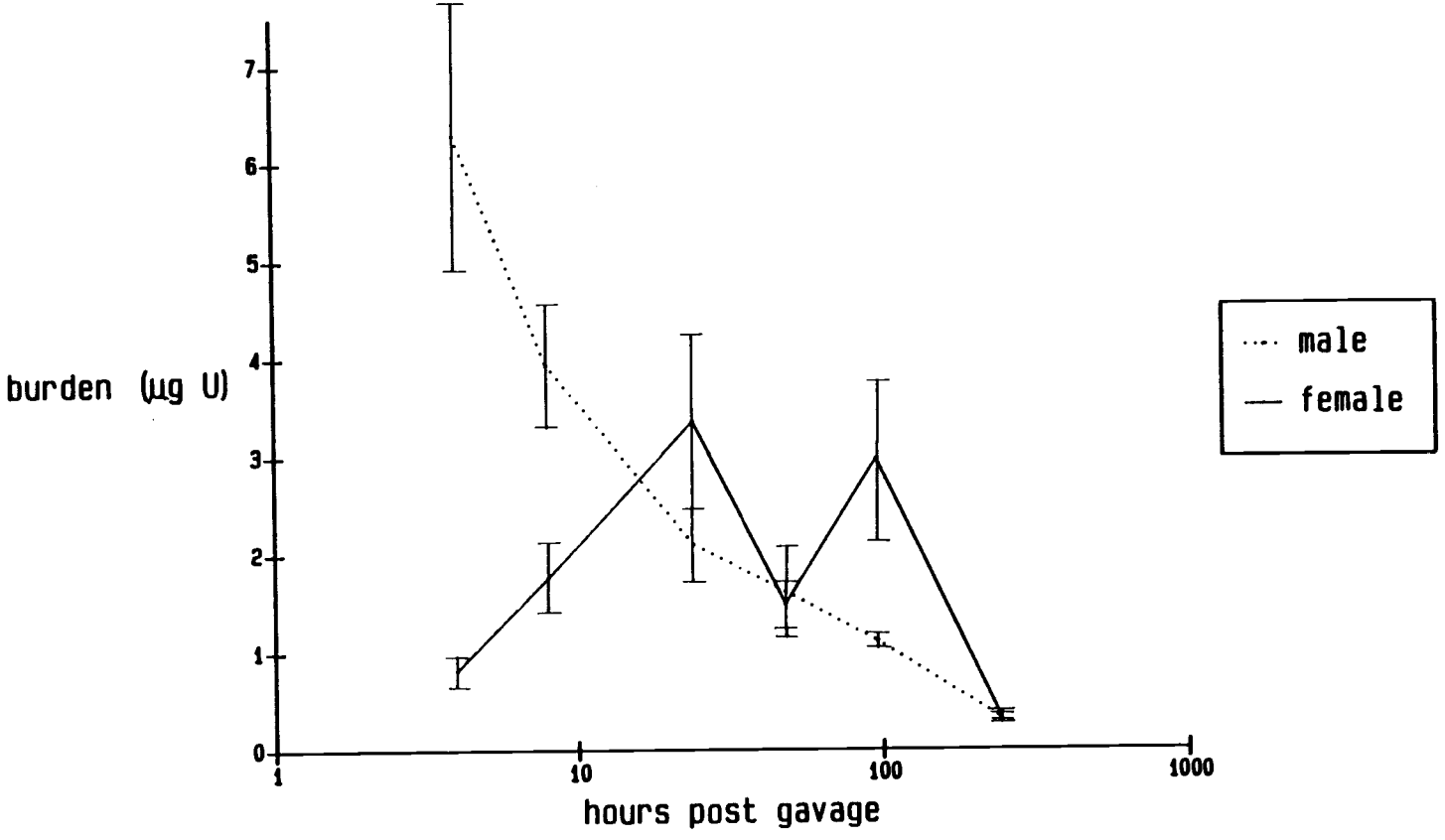
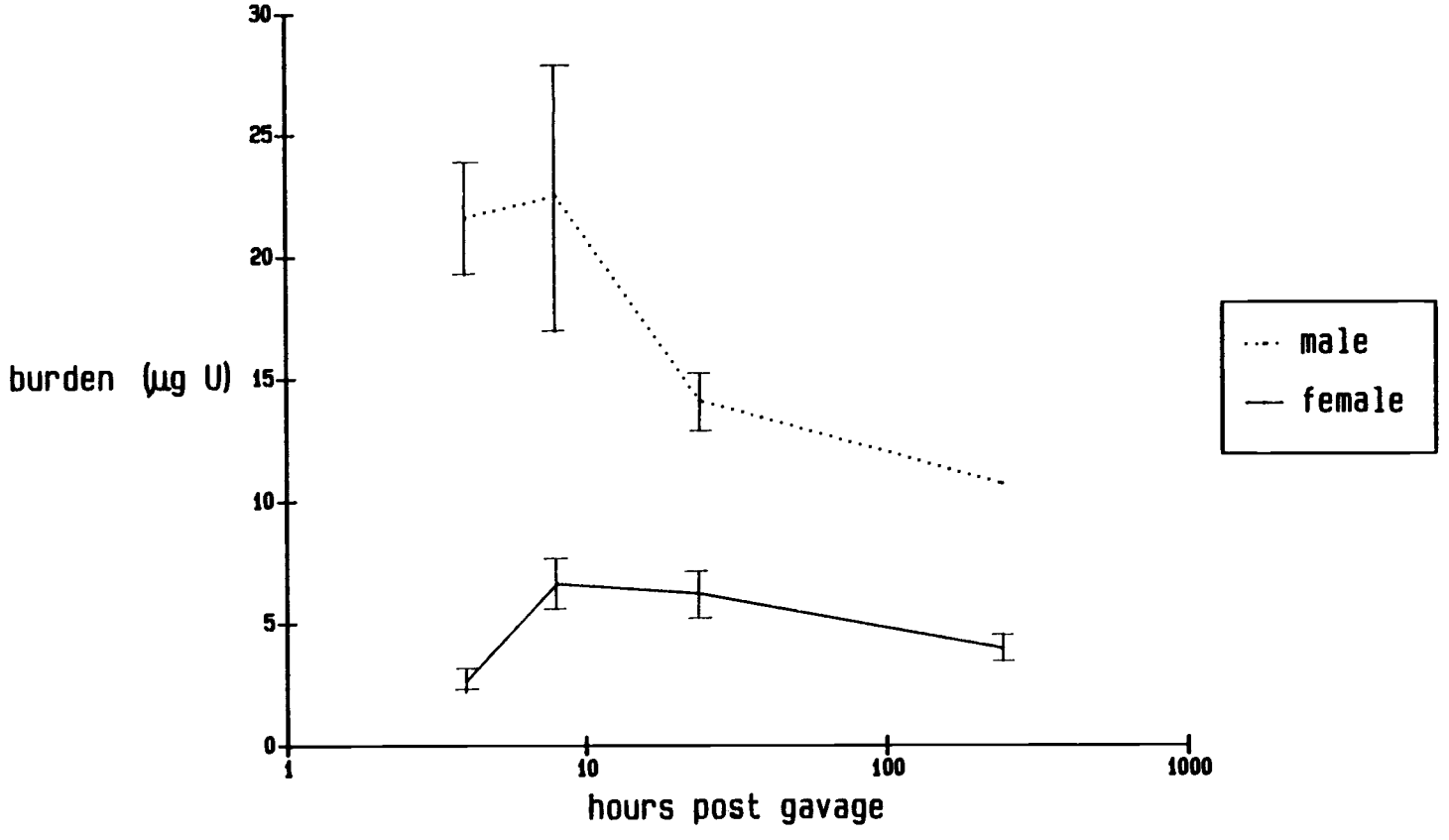


Figure 12: Mean kidney uranium burden (± SE) for female and male Wistar rats gavaged with 30 mg U/kg body weight.



male for the lowest dosage group and less than the male in the highest dosage group. Although most of the treatment groups were statistically different, male and female mean kidney burdens were not greatly different at the lowest and highest dosages, and the latter time periods in the middle dosage were statistically the same.

The patterns of uptake and elimination were generally similar in the kidneys and skeleton for both the female and male rats (Figures 7-12). The highest and lowest dosages appeared to show the kidney eliminating uranium slightly faster than the skeleton. The lowest dosage group even appeared to show a slight increase in skeletal burden after 48 hours. The middle dosage showed a very close relation between the kidney and skeletal burdens. The kidney burden, however, was slightly greater at 8 and 24 hours, and the skeletal burden was slightly greater after 24 hours. The highest dosage showed greater kidney burden from 4 to 24 hours, then a greater skeletal burden at 240 hours. Although the kidney and skeletal burdens resembled each other at each dose, these mild differences suggest a longer retention of uranium in bone as has been noted by others (Du75; Ha48; Wr85; Yu73).

The peak kidney and skeletal burdens appeared to occur near the same time for the female and male rats

(Figures 7-12). In the lowest dosage group, for both females and males, kidney and skeletal burdens peaked between 4 and 24 hours. Furthermore, the female and male mean kidney burdens at 8 hours were statistically the same ($p > 0.05$). In the 3 mg U/kg dosage group, the peak male kidney and skeletal burdens occurred before 8 hours while the female kidney and skeletal burdens appeared to peak after 8 hours. Despite this apparent timing difference, the latter time periods were not statistically different ($p > 0.05$). The highest dosage group had fewer points for comparison, yet the male peak kidney burden appeared to occur slightly earlier than the female burden, and the skeletal peak burdens appeared to occur near the same time.

The values for GI absorption of uranium in female rats were similar to values for male rats (La86). The range of GI absorption values in female rats, was from 0.3 to 2.1% of the uranium dosages from 0.3 to 30 mg U/kg. The range of values for GI absorption in male rats was from 0.6 to 2.8% for uranium dosages of 0.003 to 45 mg U/kg. The male values showed no particular trend with increasing dose. However, the total amount absorbed increased with increasing dosage. The maximum percentage absorption for the female rats decreased with increasing dosage, but, as with the males, the absolute amount of uranium burden increased

with increasing dosage (Figures 5 & 6). From this observation, I would predict that a smaller concentration of uranium in water would result in a greater percent absorption, yet less total uranium would actually be absorbed. Likewise, a larger concentration of uranium would probably result in a smaller percentage absorption with more total uranium being absorbed. Therefore, greater concentrations of uranium in drinking water would be more hazardous because more uranium would enter the body, even though the percentage of GI absorption was less.

This property suggests that the concentration of uranium influences the limited ability of the GI tract to absorb uranium. Well over 90% of the uranium is excreted in the feces. At smaller concentrations, a greater proportion of the available uranium can be absorbed. As the concentration increases, the limited ability to absorb uranium is exceeded. The capacity to absorb uranium is not an absolute threshold level because the total amount absorbed does not reach a plateau level. Rather, the ability to absorb uranium becomes slightly greater, but not to the same degree as the increase in uranium concentration.

The female rat data for GI absorption of uranium were within the range of values reported in previous investigations with animals and humans (Table 8). It

Table 8: Summary of gastrointestinal absorption of uranium in experimental studies of acute exposure in animals and human.

Subject	Uranium % Absorption	Reference
Female rat	0.3	This study
	1.1	
	2.1	
Male rat	0.6	La86
	0.8	
	1.1	
	1.8	
	2.8	
Weanling male rat	2	Nel84
Neonatal rat	6.7	Su80b
Adult rat	0.06	Su80a
Rat	0.35	Ha48
Golden Syrian hamster	0.77	Har81
Mongrel dog	1.55	Fi60
Human	1	Bu58
Human	0.5 – 5	Hu69
Human	1 – 2	Wr85

should be noted, though, that the rats in Sullivan's experiments (Su80a,b), were not starved. Also, no mention of feeding was made by Hamilton (Ha48). Food in the upper GI tract has been shown to greatly decrease GI absorption and increase the variance in absorption (La86). Starved young-adult female, young-adult male, and weanling male rats had a maximum GI absorption values of 2.1%, 2.8% (La86), and 2% (Nel84) respectively, but adult rats given food had a maximum GI absorption of only 0.06% (Su80a). Drinking water with uranium would then be most hazardous when consumed on an empty stomach, such as upon first awakening in the morning.

The ICRP used 1% GI absorption in the calculation of the maximum permissible concentration in water (MPC_w) for occupational exposure of uranium in drinking water (Sp73). The data from the female and male rats suggest that 2% GI absorption of uranium may be more appropriate. This would change the MPC_w from 2×10^{-5} to 1×10^{-5} μCi/ml water.

CONCLUSIONS

1 Kidney and skeletal uranium burdens paralleled each other up to ten days following ingestion.

2 Uranium appeared to be eliminated from the kidney more rapidly than from the skeleton.

3 Uranium biokinetics were essentially the same for female and male Wistar rats.

4 The maximum GI absorption for the female rat was 0.3, 1.1, and 2.1% for the 0.3, 3, and 30 mg U/kg body weight dose groups, respectively.

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APPENDICES

APPENDIX A

This appendix contains the individual animal data for the kidney, femur, and skeletal uranium burdens.

Dose mg U/kg	Time hours	Kidney µg U	Femur µg U	Skeleton µg U
0.3	4	0.4027	0.0338	0.6626
0.3	4	0.4419	0.0349	0.6835
0.3	4	0.0766	0.0168	0.3287
0.3	4	0.2312	0.0189	0.3704
0.3	4	0.4459	0.0391	0.7669
0.3	4	0.7348	0.0434	0.8504
0.3	4	0.4751	0.0264	0.5165
0.3	4	0.4192	0.0285	0.5582
3.0	4	0.7432	0.0332	0.6515
3.0	4	0.8107	0.0371	0.7271
3.0	4	0.6746	0.0360	0.7055
3.0	4	ND	0.1610	3.1566
3.0	4	ND	0.1175	2.3035
3.0	4	1.5536	0.0707	1.3857
3.0	4	0.7250	0.0404	0.7918
3.0	4	0.4153	0.0316	0.6191
30.0	4	2.9282	0.1113	2.1816
30.0	4	4.5580	0.1556	3.0489
30.0	4	4.0914	0.1464	2.8700
30.0	4	3.0823	0.1318	2.5839
30.0	4	2.3643	0.0835	1.6362
30.0	4	4.7323	0.1852	3.6300
0.3	8	1.1348	0.0358	0.7014
0.3	8	0.6203	0.0318	0.6225
0.3	8	0.6009	0.0313	0.6126
0.3	8	ND	0.0635	1.2439
0.3	8	0.3064	0.0172	0.3364
0.3	8	0.4993	0.0363	0.7113
0.3	8	0.8945	0.0383	0.7507
0.3	8	0.2498	0.0116	0.2279
3.0	8	0.5142	0.0365	0.7163
3.0	8	1.6226	0.0701	1.3749
3.0	8	1.8066	0.0850	1.6665
3.0	8	2.9591	0.1203	2.3575
3.0	8	1.2193	0.0580	1.1374
3.0	8	0.5555	0.0415	0.8134
3.0	8	1.9956	0.0988	1.9364
3.0	8	3.4428	0.1307	2.5627
30.0	8	4.9294	0.2212	4.3363
30.0	8	10.0239	0.4247	8.3240
30.0	8	6.6291	0.2623	5.1410
30.0	8	8.6791	0.3804	7.4567

Dose mg U/kg	Time hours	Kidney µg U	Femur µg U	Skeleton µg U
30.0	8	5.4886	0.2235	4.3811
30.0	8	13.4821	0.5241	10.2731
30.0	8	5.5717	0.2235	4.3811
30.0	8	6.5374	0.4963	9.7277
30.0	8	8.2758	0.2942	5.7669
0.3	24	0.4089	0.0245	0.4802
0.3	24	0.5989	0.0292	0.5721
0.3	24	0.3385	0.0236	0.4618
0.3	24	0.7838	0.0278	0.5445
0.3	24	0.7674	0.0329	0.6457
0.3	24	0.5140	0.0245	0.4802
0.3	24	0.3798	0.0226	0.4434
0.3	24	0.5159	0.0268	0.5262
0.3	24	0.6388	0.0494	0.9677
3.0	24	7.3936	0.2247	4.4038
3.0	24	0.9798	0.0401	0.7869
3.0	24	ND	0.2828	5.5436
3.0	24	3.0362	0.1140	2.2337
3.0	24	3.2983	0.1676	3.2858
3.0	24	3.8425	0.1878	3.6804
3.0	24	1.6040	0.0983	1.9268
30.0	24	13.0511	0.4722	9.2551
30.0	24	9.1104	0.3739	7.3284
30.0	24	8.7231	0.2836	5.5586
30.0	24	6.3359	0.2147	4.2081
30.0	24	5.7468	0.2466	4.8334
30.0	24	10.2536	0.4037	7.9125
30.0	24	6.2517	0.2248	4.4061
0.3	48	0.1804	0.0169	0.3305
0.3	48	0.5828	0.0256	0.5015
0.3	48	0.4708	0.0306	0.6006
0.3	48	0.1680	0.0169	0.3305
0.3	48	0.4391	0.0201	0.3935
0.3	48	0.3072	0.0182	0.3575
0.3	48	0.3384	0.0316	0.6186
3.0	48	0.6477	0.0311	0.6096
3.0	48	1.9405	0.1082	2.1202
3.0	48	1.9449	0.1076	2.1095
3.0	48	0.8224	0.0512	1.0032
3.0	48	2.0708	0.1885	3.6946
3.0	48	1.7386	0.1348	2.6414
3.0	48	2.1191	0.1207	2.3648

Dose mg U/kg	Time hours	Kidney µg U	Femur µg U	Skeleton µg U
3.0	48	0.5945	0.0989	1.9393
0.3	96	ND	0.0423	0.8281
0.3	96	0.3756	0.0329	0.6458
0.3	96	0.1996	0.0119	0.2335
0.3	96	0.4427	0.0289	0.5665
0.3	96	0.0645	0.0099	0.1939
0.3	96	0.2036	0.0212	0.4159
0.3	96	0.2012	0.0212	0.4159
0.3	96	0.3586	0.0253	0.4951
3.0	96	6.7023	0.3133	6.1400
3.0	96	0.4702	0.0516	1.0105
3.0	96	5.9487	0.3383	6.6315
3.0	96	0.9362	0.0685	1.3435
3.0	96	3.1108	0.2089	4.0945
3.0	96	1.8038	0.1211	2.3741
3.0	96	0.8614	0.0718	1.4069
3.0	96	4.0035	0.1705	3.3414
0.3	240	0.1294	0.0309	0.6061
0.3	240	0.0886	0.0160	0.3128
0.3	240	0.1249	0.0192	0.3762
0.3	240	0.1395	0.0313	0.6141
0.3	240	0.1722	0.0301	0.5903
0.3	240	0.1173	0.0325	0.6379
0.3	240	0.0826	0.0313	0.6141
0.3	240	0.1229	0.0261	0.5110
3.0	240	0.4997	0.0568	1.1140
3.0	240	0.1827	0.0483	0.9467
3.0	240	0.3533	0.0589	1.1538
3.0	240	0.3216	0.0381	0.7476
3.0	240	0.2588	0.0349	0.6838
3.0	240	0.4194	0.0402	0.7874
3.0	240	0.3681	0.0243	0.4767
3.0	240	0.3676	0.0426	0.8352
30.0	240	1.4918	0.1778	3.4842
30.0	240	ND	0.2839	5.5654
30.0	240	0.9416	0.1352	2.6499
30.0	240	0.7659	0.1686	3.3048
30.0	240	1.7637	0.2537	4.9734
30.0	240	1.4950	0.1750	3.4304
30.0	240	1.4488	0.1704	3.3407
30.0	240	1.5961	0.2537	4.9734

APPENDIX B

This appendix contains the individual data for the control samples which were used in determining the minimum level of detection.

Control sample	Net weight (grams)	Net counts (cpm)	Weight adjusted cpm	Average weight adjusted cpm
1 Blood	0.1816	11.30	10.32	20.85
2 Blood	0.1772	6.30	5.90	
3 Blood	0.1995	21.30	17.70	
4 Blood	0.1502	22.30	24.61	
5 Blood	0.1752	49.30	46.64	
6 Blood	0.1107	13.30	19.92	
1 Liver	0.6385	14.30	12.63	12.54
2 Liver	0.5012	16.30	18.35	
3 Liver	0.5650	10.30	10.29	
4 Liver	0.4849	11.30	13.15	
5 Liver	0.5779	17.30	16.89	
6 Liver	0.6163	4.30	3.94	
1 Kidney	0.3385	22.30	22.42	24.42
2 Kidney	0.3341	33.30	33.92	
3 Kidney	0.3360	18.30	18.54	
4 Kidney	0.3327	20.30	20.77	
5 Kidney	0.3753	37.30	33.82	
6 Kidney	0.3250	16.30	17.07	
1 Femur	0.5250	24.30	25.16	23.48
2 Femur	0.5304	21.30	21.83	
3 Femur	0.5241	17.30	17.94	
4 Femur	0.5298	27.30	28.01	
5 Femur	0.6324	21.30	18.31	
6 Femur	0.5192	28.30	29.63	