Infusion of Radionuclides throughout pregnancy; an alternative to single injection studies.

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Abstract. The report of the Independent Advisory Committee (The Black Committee (1984) on the increased rate of childhood cancer in West Cumbria added scientific weight to the need for research into its causes in the area surrounding the Sellafield reprocessing plant. Such levels of childhood leukaemia might be accounted for by i). paternal gonadal irradiation (Gardner et al. 1990) or ii). by unexpected radioactive environmental contamination and a sensitive period in-utero to high LET irradiation or iii). an underestimate of foetal risk. To investigate these possibilities Black made a number of recommendations for research. Some of that recommended research using animals is reported here.

This work is part of a long term study to examine the cancer incidence in the offspring of mice exposed to ^{239}Pu (High LET) or ^{147}Pm(Low LET) throughout pregnancy. The need to model the human intake scenario and the possibility of a critical period during uterine development necessitates constant availability of radionuclides throughout pregnancy. Various methods (multiple daily injections, infusion by external cannula and infusion by indwelling osmotic pump) have been examined and osmotic infusion pumps chosen. These pumps result in a near constant blood concentration for up to 21 days.

Part of the study is the estimation of dose to the critical haemopoietic tissues of the pup from a knowledge of the radionuclide distribution and kinetics. At present the distribution has been followed from birth to 180 days. Activity in the suckling pups at 7 days old is around 1% of the infused activity, though most of this is accounted for by the contents of the stomach and gastrointestinal tract. The liver and femur account for around 0.025% and 0.012% respectively per pup. Activity increases in both liver and femur during lactation after which both concentration and activity fall with time.

Long term studies with the pups of dams exposed to a range of ^{239}Pu concentrations between 0-70kBq/kg are underway. Correlation of average organ dose with tumour incidence will be determined at completion of the life-span study.

Introduction. The concern of the Independant Advisory Committee under the chairmanship of Sir Douglas Black (Black, 1984) and more recently of the Committee on the Medical Effects of Radioactivity in the Environment (COMARE 1986,

1988, 1989) has been whether the developing human is a special risk category for the development of radiation induced childhood leukaemia. Concern centres on in-utero exposure to alpha particle emitting radionuclides e.g. ^{239}Pu, during foetal, neonatal and juvenile stages. Such radionuclides have been reported in the environment surrounding nuclear reprocessing plants (Stather et al, 1986) and have been implicated in the causation of the excess childhood leukaemia incidence seen near Sellafield and Dounreay.

In the report of the NRPB to the Black Committee (Stather et al. 1984) it was pointed out that no specific neonatal or foetal models were available for the calculation of dose from either plutonium or fission products. However, age specific differences in retention, translocation, bone growth rates and remodelling may be vital if the dose to the critical haemopoietic organs are to be assessed with any confidence.

At a recent CEIR (MRC Committee on the Effects of Ionizing Radiation) Forum on "Radionuclides and External Irradiation: Implications for the Embryo and Foetus" (London, 1990) it was concluded that there was a continuing lack of pertinent experimental data on which to base kinetic models relevant to the embryo, foetus and neonate.

Nevertheless, studies in adult mice have demonstrated that alpha emitting radionuclides may induce or alter the temporal distribution of leukaemia. Svoboda (1981) found adult ICR-SPF mice treated with 13kBq^{239}Pu/kg showed no increase in myeloid leukaemia above controls (24%) but that the disease was induced significantly earlier. In another series of experiments (Svoboda 1990) using the same strain injected with between 0 and 12kBq^{239}Pu/kg, a peak in myeloid leukaemia incidence occurred at around 6kBq/kg. This is in agreement with the work of Humphries et al (1987) who found a maximum induction of myeloid leukaemia in adult CBA mice injected at 5kBq/kg. This strain exhibits no spontaneous leukaemia of this type (Mole 1958).

Published studies following contamination of pregnant (Finkel 1947, Finkel and Kisieleski 1976, Weiss et al. 1980, Mason et al. 1991) or pre-pregnant (Green et al. 1979, Taylor 1980) animals with plutonium have all involved the use of single injection regimes, usually as the citrate. Finkel (1947), and Wilkinson and Hoecher (1953) examined the transfer of plutonium from dam to offspring in rats and mice but provided no data on the retention in specific organs considered to be most at risk in either the dam or foetus. Sikov and Mahlum (1968), Green et al. (1979), Weiss and Wallberg (1978) have all studied the transfer of plutonium from the dam to the neonatal and suckling offspring of rats or mice but none have chronically exposed the dam throughout pregnancy. Finkel (1947) first demonstrated the proportion of activity taken up

by the foetus/placenta was inversely proportional to the administered activity injected (in the range 0.59-2.2 MBq/kg). Weiss and Walburg (1978) found a similar effect in the range 90-990 kBq/kg and concluded that realistic estimates of transplacental movement are dependent on extrapolation from low dose data only because of this mass effect. This is especially important at doses that may be pertinent in the environmental situation.

The present study describes the advantages and disadvantages of some of the methods available to produce continuous exposure during pregnancy and gives biokinetic data using ^{241}Pu as a surrogate tracer for ^{239}Pu. CBA/Ca mice were chronically exposed to ^{241}Pu-citrate using subcutaneously implanted osmotic pumps. The activity in the dam, at weaning, and in the neonatal, juvenile, and young adult pups was measured. The resultant concentration-time data were used to estimate the retention parameters in the pups and thus the expected doses in the offspring of dams exposed similarly with a range of ^{239}Pu-citrate concentrations for a life-span experiment.

Materials and Methods.

Animals. With the exception of external infusion experiments, for which Balb/c females were used, proven female CBA/Ca mice (About 17-20 weeks old) mean weight 25.2±0.2 gm were used throughout. They were brought into oestrus by caging on soiled male litter. Three days later the animals were mated in a male/female ratio of 2:1. The females were examined twice daily for the presence of a vaginal plug which was taken to indicate conception (Day 0).

Radiochemical Solutions. Stock ^{241}Pu solution was obtained in 2M HCl. This was converted to the citrate by evaporation of a small aliquot and subsequent resuspension in 0.01M HNO_3:Tri-Sodium Citrate solution (pH 5.5). The resultant solution was purified by ultra-filtration through a 0.02 µm filter (Anotop) and assayed by counting in a liquid scintillation mixture (Keough and Powers,1970) in a Canberra 2200CA liquid scintillation spectrometer. The final concentrations used were:-

Multiple injection regime.....	1.10 kBq/ml.
External Infusion regime.....	1.48 kBq/ml.
Osmotic pump, Internal Infusion Regime.(Pregnant Animals).....	100 kBq/ml.
(Stock Animals).....	128 kBq/ml.

Single Injection Regime. Single i.p. injections (0.3ml) were made daily for five days into stock animals to assay blood parameters. In pregnant animals injections were also made daily for the duration of pregnancy. Pregnant animals were housed two per cage for the duration of pregnancy.

External Infusion Regime. The construction of the co-axial cannula and protective spring were as described elsewhere (Lambert, 1986). Briefly, 6-9 days after detection of a

vaginal plug animals were anaesthetised using Enflurane in a $N_2O:O_2$ mixture. The inner cannula (0.6mm OD) was inserted under the skin of the tail about 2cm from the base and pushed under the skin into the lower abdomen. The end lying in the musculature of the lower abdomen. The outer (1mm OD) cannula was tied to the tail with stainless steel sutures and a protective steel spring covered the tail and cannula. The co-axial cannula passed to a low interia swivel (Orion Research, Cambridge, Mass. USA) enabling unhindered movement of the animal. A further cannula passed to a motorised syringe driver (Harvard Apparatus, Millis, Mass. USA) pumping 0.3 ml/day. Up to nine animals could be infused at any one time. Animals were housed singly in large vessels, and had access to food and water ad-libitum.

Internal (Osmotic) Pump Implantation. Animals were anaesthetised using Enflurane in a $N_2O:O_2$ mixture. The dorsal fur was trimmed using scissors and depilated using a proprietary cream (VEET, Reckitt and Coleman, UK). A small incision was made and a pump (Alza Corp. Palo Alto, USA) inserted under the skin, delivery tube distal to the wound site. The wound was closed using surgical clips under sterile conditions. After recovery animals were returned two per cage for the remainder of the gestation period and until weaning. The average activity per pump for the non-mated stock animals was 27.1 ± 0.4 kBq and for mated animal infusions was 20.9 kBq/pump, equivalent to 831 kBq ^{241}Pu/kg mouse. The pumping rate was 12µl/day.

Tissue Sampling and Analysis:

i). Multiple Single Injection. Non-mated animals were divided into eight groups. In each group blood was sampled every other day for the five days of the injection regime. Liver, spleen, left femur and blood were taken at sacrifice at various times up to 30 days after the first injection. Mated animals were sampled at 19 days post conception (p.c.).

ii). External Infusion. Samples of maternal organs and pups were taken at sacrifice on day 19 p.c.

iii). Internal Infusion. Non-mated animals were sampled at various times during a period equivalent to that of gestation. Organs sampled were as for mated animals. Mated animals were observed throughout pregnancy and litter size noted at birth (20 days p.c.). At weaning, or 41 days p.c., dams were killed by cervical dislocation. Cardiac blood was taken along with liver, spleen, kidneys, breast tissue and the left femur. Exhausted osmotic pumps were taken and dissolved in methylene dichloride. They were then processed as for biological tissues, to assess total infused activity (by difference).

Pups sampled at various times up to 180 days old were weighed and then killed by cervical dislocation. Samples of blood, spleen, kidneys, stomach, stomach contents, remainder of the intestinal tract (G.I.), liver, heart, left femur and remaining carcass were taken.

Radiochemistry. Plutonium content was measured by solvent extraction after washing using a method modified from Keough and Powers (1970). Samples were ashed at 450°C for 12 hours. The ash was dissolved in 16M HNO_3 and evaporated to dryness, this was repeated until a carbon free white ash was obtained. This was dissolved in 6M HNO_3:HF and evaporated to dryness twice and once with 8M HNO_3 before being dissolved in 9.5ml 2M HNO_3 with 0.5ml 0.2M Boric Acid, 0.05ml 4M Urea and 4ml of scintillant extractant. Scintillant extractant was prepared by the addition of 200ml di-2-ethyl-hexyl-phospate (D2EHP) to 800ml toluene containing 5g p-terphenyl and 0.05g POPOP. Vials were capped and mixed using a vortex mixer and allowed to stand until the two phases separated. Mixing and separation four times results in complete extraction of plutonium into the organic phase.

Calculation of gross organ dose (Gy). The organs of the pups were sampled in order to be able to estimate the dose to various organs in ^{239}Pu exposed animals in the long term study. Dose was estimated by linear regression between the sampling points of the concentration time curve and calculation of the area under the curves which is proportional to dose. It is assumed that the concentration in the pups would be proportional to infused activity and that no mass effect would occur.

Results. Figure 1 shows the concentration (Bq/g(wet tissue)) of ^{241}Pu in circulating blood (Means$\pm$95% Limits) of stock females during the injection regime. Figure 2 shows the log of concentration in blood liver and femur for a period up to 30 days after the first injection (Means$\pm$Standard Error). Exponential changes in concentration caused by physical and biological processes can be described by straight lines fitted to the data (Table 1). Changes in blood concentration can be characterised in the initial period by a line with half time ~5 Hours. By two days after the last injection the concentration falls with a half time of ~22 days, resulting from the concentration halving between days 6 and 30; i.e. between 1/60 and 1/100th of the peak concentration during injection. The concentration in liver may be fitted by a single curve with half time ~9 days, though a better fit may be obtained by two exponentials of half times ~4.3 days and ~22 days. The femur can be fitted by a single curve of half time ~36 days. Table 1 also shows the rates of clearance of activity (Bq) from the liver and femur with half times of 3.9 and 4.9 days respectively.

Figure 3a, shows the concentration of ^{241}Pu expressed as concentration (Bq/g, wet) for liver, femur, kidneys, spleen and blood in stock female animals infused by osmotic pumps over a period of 20 days. This activity is shown in Figure 3b, and can be seen to change linearly with time over the sampling

period. All organs including blood increase in concentration, though that increase in less in blood than other organs.

Because physical disruption of pregnancy was thought to be an important feature of these experiments and influenced the choice of administration technique, the breeding success was recorded. Table 2. summarises the breeding results (% Viviparity), the reproductive success (Pups/dam with vaginal plug). It can be seen that 93% of dams in the multiple injection regime had a successful pregnancy (a figure insignificantly different from stock animals).

The resultant activity concentrations in the maternal liver, femur, breast and blood, also in whole pups and placentae are shown in Table 3. As expected, in all regimes the liver and femur proved to be the major sites of deposition (Finkel, 1947). Multiple injection resulted in ~20%IA/g deposition in the liver, 25%IA/g in the femur and 1.4%IA/g in the breast, at 19 days p.c. The whole pups sampled were about 0.7%IA/g and placentae 14.2%IA/g, a placenta to pup concentration ratio of around 40:1. External infusion resulted in lower concentration percentages in both the liver and femur, 6 and 11.5%IA/g respectively. The average breast measurement was 3%IA/g but was subject to wide variability and was not significantly different to the multiple injection regime. Whole pups contained 0.3%IA/g and the one placenta sampled 5%IA/g, a placenta to pup ratio of around 16:1.

Figure 4, shows the total activity, concentration, percentage of infused activity and the mass of the pups of mothers infused with 20.9 kBq ^{241}Pu-citrate. The top panel (open semicircles) shows the total including activity contained within the stomach contants and the gastrointestinal tract. The line below exludes this activity (closed semicircles). It is interesting to note the rise in activity between day 7 and weaning on day 21 as a consequence of lactation. After weaning both lines coincide and the total activity within the pups declines with a half time of around 233 days. The second panel shows the decline in average concentration between weaning and 180 days. Concentration can be seen to fall an order of magnitude during this period.

Figure 5, shows the same parameters for the pup liver. Once again the increase in activity during the period of lactation can be seen; in this case an increase of 18%. This pattern is reflected in other organs; spleen 20%, heart 42%, femur 111% and remainder 19.5%. In all organs, as expected, the activity and concentrations decline after weaning. When plotted semilogarithmically activity in all organs (represented here by Figure 4), except the liver (Figure 5), fall linearly with time indicating exponential clearance. Table 4 shows the half times of clearance of activity from the pups between weaning and 180 days for the organs sampled. For the liver two lines were fitted, between 21 and 91 days and 91-180 days.

95% confidence limits are quoted save where there is no measureable clearance when the physical half-time is assumed.

Figure Six shows the projected average organ doses to the liver, femur, all other organs and total dose during the neonatal and young adult period for exposure to between 208 and 1660 Bq ^{239}Pu to the mother in-utero calculated using the biokinetic parameters dtermined here using ^{241}Pu. The dose to the liver is between 0.4mGy and 3.3mGy, Group 1 and 4 respectively. The femur dose is between 0.91mGy to 7.2mGy and the total dose between 3.89mGy and 31.1mGy, respectively.

Discussion. The concept of "critical periods" during development was defined as early as 1911 from observations on amphibian eggs (Barden, 1911). In 1935 Job et al.(1935) published data showing the teratogenic effects of 90R on day 9 p.c. was hydrocephalus but on day 10-11 was mainly defects of the eye and jaw. Later studies showed that specific responses were dependant on both time and dose (Brent and Jensch, 1967) and confirmed the presence of "stages of maximal sensitivty" in the teratogenic response to radiation (Jacobson, 1969). At lower doses the concerns are damage to the central nervous system (Konermann, 1986) and cancer induction (Stewart and Kneale, 1970). In 1958 Stewart (1958) reported an increased sensitivity of the human foetus to X-irradiation, resulting in increased leukaemia induction following medical irradiation in-utero.

For internal contamination with radionuclides there is no evidence to confirm or refute the presence of a period of increased carcinogenic sensitivity in-utero. It is axiomatic that the effects on the developing foetus depends on the complex interelationship of such factors as, for example, the chemistry of the radionuclide, its transfer kinetics within the dam and more specifically the placenta, the physical half life of the radionuclide, its energy and mode of disintegration and the micro-distribution within the organs of the developing foetus.

a).Methods of Administration. Three modes of exposure of the dam were examined that may meet the basic tennet of constant availablility of radioisotope to the placenta throughout pregnancy. i). Multiple injections are rapidly accomplished with little stress as indicated by viviparity being insignificantly different to controls. Animals remain communally caged and there is no limit on the number that can injected at any time. However, Figure 1 shows the rapid changes in blood concentration observed. The clearance half time of around 5 hours is in broad agreement with that of 8 hours in the rat (Turner and Taylor, 1968) after i.v. injection. This pattern of concentration fails to meet our prime requirement. ii). External infusion via subcutaneous tail cannula, though resulting in constant availability of

radioisotope (unpublished data), was difficult to set up and maintain and was prone to hidden infections in some animals beneath the protective spring. It resulted in the lowest viviparity and fecundity, 30.7% and 2.4 pups/plugged dam respectively. Lambert (1986) reported a 30% failure rate using external infusion on SAS/4 mice. Lemmel and Good (1971) in reviewing the infusion methods then available comment that most are "fragile technically and physiologically". In more recent studies on CBA/Ca mice (unpublished) it has been found that single housing necessitated by the external infusion technique results in pup survival post birth as low as 25% without the added losses associated with the stresses of cannulation and infusion. For these reasons the technique was rejected. iii). Subcutaneous implantation of osmotic pumps was simple and has resulted in between 53% and 95% viviparity and 4-6.6 pups/plugged dam. In other studies viviparity has been found to be more related to environmental (e.g. noise, housing density) than experimental factors (e.g. activity concentration). Sub-cutaneously implanted pumps are capable of producing a near constant blood concentration for a period equal to gestation in the mouse (Figure 3). They do not significantly effect the vivparity or fecundity of the strain used and are technically easy to implant.

b).Distribution of Activity. The distribution and clearance of activity from the liver (Figure 2) following injection was at the low end of the range reported in ICRP 48 (1986), which says liver half times in rats and mice are generally between 4 and 400 days (a wide variation). The second half time observed in the blood (~22 days) is the same as that in the liver. The clearance half time of 36 days in the femur is short in comparison with the general view (ICRP, 48) that skeletal retention half-times may be of the order of the lifespan of the animal, or in the biokinetic model for plutonium in man around 70% of the median lifespan. However the experiment was too short to determine if this was a rapid phase of clearance only.

Mason et al (1991) have reported significant differences in distribution between nulliparous and viviparous dams after injection. Livers of control animals having a higher %IA/g than pregnant animals due to increase in liver mass. A similar pattern is seen in the nulliparous and viviparous animals after infusion though the differences are not significant. A similar pattern is true of the femur. The breast, as expected, has a significantly higher concentration than that of non-suckling dams at 41 days p.c. Concentration of activity in the blood at 41 days p.c. is significantly different between those dams suckling and the nulliparous group, 0.02%IA/g and 0.03%IA/g respectively.

Direct comparison between the samples taken from infusion and injection regimes is difficult, as maternal samples were taken at weaning to validate the pump function and thus the entry of

the pups born into the clearance experiment. It is possible to make some comparison with the other regimes by predicting the concentrations at 19 days p.c. Using the half times found in the multiple injection regime and making the (questionable) assumption that they are not significantly altered by pregnancy, back extrapolation to day 19 p.c. would produce for the liver a predicted value of 16.5-19.5%IA/g (Half time ~9 days) or 21.8%IA/g (Half times 4.3 and 22.4 days), figures comparable with 19.9%IA/g seen in the multiple injection regime. The same exercise undertaken for the femur results in a predicted figure of 12.6-13.2%IA/g, around half the concentration seen in the injection experiment. Figures for one group from our long term infusion experiment are included for comparison. The results from only three dams were available at time of writing and the results shown are broadly comparable with those of the ^{241}Pu infusion experiment. The exception is the concentration in the liver which is lower than expected. Rose and Nelson (1955) reported that the results of single injection and infusion of $^{3}HTdR$ were qualitativly similar over a period of 10 hours. Lambert (1986) report a far greater variability in the distribution of $^{3}HTdR$ following multiple injection than in infused animals over a period of several days. This study shows comparison between injection and infusion regimes depends on the organ chosen for evaluation. The activity concentration in the femur following internal infusion when back extrapolated to 19 days p.c., using the half times from the injection study, produce a concentration similar to the external infusion group but a factor of two different to the injection study. The internal infusion liver concentration, on the other hand, is comparable with the injection regime and threefold higher than the external infusion group. Comparison with the published data in the literature, as summarised by Mason et al.(1991), in Table 5 reveals quantitative differences between single injection and infusion. For example, the %IA/g for the maternal liver in our injection regime is comparable with that of Mason et. al. following single injection on day 13 p.c. The maternal liver, however, contains twice the %IA/g of Masons single injection. ICRP (1979) suggested that initial partitioning of ^{239}Pu activity between the Liver and Skeleton should be 1:1. Assuming the skeleton is a factor of 26x a single femur (Rosenthal et al. 1972), the ratio of plutonium in the liver compared with that in the skeleton is 14:5 in the dam at weaning following infusion. This figure is in agrreement with those shown by McInroy et al (1991) based on human data using ICRP figures (ICRP 30, 1.44:1, ICRP48, 3.47:1).

Previous studies have shown the placenta:foetus concentration ratio to range between 13:1 (Weiss and Walberg, 1978) to 91:1 (Sikov, 1968), with the majority of reported values around 30:1. Both multiple injection and external infusion result in values within this range. Values from internal infusion will be reported in a future paper on deposition and distribution within the foetal period.

Green et al. reports that the activity contained within first day neonates is higher than 18 day p.c. foetae. In their study 30% of the neonatal figure was accounted for by plutonium within the gastro-intestinal tract. In this study, the activity contained within the gastro-intestinal tract of 7 day neonates accounts for ~80% of the total activity. Mason et al. (1991) reporting the results of mid-term contamination states very little activity is transfered to the offspring by lactation compared with the transplacental route. Pre-conception contamination has been shown to transfer very low activities via the placenta and higher amounts via the milk. This results in around a five fold increase in foetal %IA between birth and day 35 in the rat (Taylor, 1980) and birth and day 18 in the mouse (Green et al. 1979). In this study the organs of pups infused throughout pregnancy and exposed to contaminated milk during lactation increase in activity an average of 20% between 7 days and 21 days of age, though the total %IA was still around 0.1% of the total infused.

c).Relevant Calculated Radiation Doses. Using the areas under the concentration time curves, radiation doses to the liver and femur during the neonatal and young adult period resulting from a range of ^{239}Pu exposures have been calculated. The liver, spleen and bone marrow are the major organs at risk for the induction of leukaemia as a result of irradiation in-utero, though the organ at risk depends on time post conception (Moore and Metcalf, 1970). Heamopoiesis begins in the blood islands of the yolk sac around day 7 p.c. The liver contains haemopoietic progenitor cells from day 9 p.c. through to shortly after birth. The marrow and spleen contain stem cells shortly before birth and shortly after birth the haemopoietic function of the liver is finished. Dose to the liver, spleen and marrow in-utero following continuous exposure will be published elsewhere. The total dose in the highest group (1663Bq IA) is calculated to be around 31 mGy, the initial dose rate shortly after birth is around 0.5 mGy/day though this falls rapidly due to growth. Mason et al. (1991) reports that long term haemopoietic damage can be assayed at doses as low as 14mGy. The final calculation of dose to the haemopoietic system awaits the distribution of activity in those pups of the ^{239}Pu exposed animals and the addition of the in-utero doses.

d).On-going Experiments. Long term pups of dams exposed to 0-70 kBq^{239}Pu/kg in-utero have undergone over 30,000 pup days at risk. Humphries has a group of pups exposed to a similar range of plutonium activities by injection mid-term. The estimation of the risk resulting from contamination in-utero to low doses of radionuclides will be undertaken when the tumour incidence and pathology of the last of over 2,000 animals in this cohort is known. Comparison with the data of Humphries may reveal further differences between single injections and continuous exposure regimes.

Summary. This paper has investigated methods to produce a constant availability of radionuclide to the placenta during pregnancy. Single injections result in a very unstable blood concentration. External infusion is fragile technically and physiologically and stresses the animals. Osmotic pumps provide a solution. The concentrations in the pups are consistant with the notion that the later and lower the dose administered the greater the percentage passing via the placenta. They are also consistant with the previous reports of contamination pre-pregnancy and a significant percentage of activity is passed via lactation.

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Table 1: Half Times Exponential Functions Fitted to Concentration and Activity Data Following Multiple Injection Regime

	Concentration (Bq/g(W))			Activity (Bq)		
Phase	Blood	Liver	Femur	Blood	Liver	Femur
Rapid	4.9 Hrs	4.3 days	N/A	N/A	N/A	N/A
Slow	22.1 days	22.1 days	N/A	N/A	N/A	N/A
Composite	N/A	9.1 days	36.1 days	22.4 days	3.9 days	4.9 days

PI = Post Injection
N/A = Not Applicable
r^2 = Correlation Coeff.

Table 2. Breeding Results

Regime	Multiple Injection	External Infusion	Internal Infusion					
			^{241}Pu-STS	^{239}Pu-LT4	^{239}Pu-LT3	^{239}Pu-LT2	^{239}Pu-LT1	LT-Control
Activity	4900 Bq.	5300 Bq.	20100 Bq.	1663 Bq.	832 Bq.	416 Bq.	215 Bq.	
Days of Exposure p.c.	1-19	9-19	0-21	0-21	0-21	0-21	0-21	0-21
Day Sampled p.c.	19	19	41	41	41	41	41	41
% Viviparous	93%	31%	53%	88%	86%	78%	95%	65%
Pups/Plugged Dam	4.9±0.7	2.4±1.3	4.0±0.3	6.1±0.3	6.8±0.5	6.3±0.4	7.0±0.4	6.1±0.5

Key:- STS;Short Term Kinetic Study
LTx; Long Term Lifespan Study

Table 3. Percent of Infused Activity per gram. Post Regime. %IA/g.

Regime	Multiple Injection	External Infusion	Internal Infusion		
			241 Pu-STS	241 Pu-STS	239 Pu-LT1
Activity Bq.	4900	5300	20100	20100	215
Concentration kBq/kg	195	213	832	832	8
Days of Exposure p.c.	1-19	9-19	0-21	0-21	0-21
Day Sampled p.c.	19	19	41	41	41
Organ			Nulliparous	Viviparous	
Maternal Liver	19.9±1.9 (14)	6.0±1.1 (13)	3.9±0.2 (16)	3.3±0.2 (18)	6.5±0.3 (3)
Femur	24.9±4.5 (11)	11.5±2.4 (13)	10.6±0.6 (16)	8.8±0.5 (18)	8.4±0.4 (3)
Breast	1.4±0.1 (14)	3.0±1.6 (4)	0.2±0.1 (2)	1.1±0.1 (11)	0.9±0.2 (3)
Blood	- ± - ( )	- ± - ( )	.03±.01 (13)	.02±.00 (17)	- ± - ( )
Whole Pup	0.7±0.1 (58)	0.3±0.1 (23)	- ± - ( )	.02± .003(12	- ± - ( )
Placenta	14.2±1.7 (27)	5.0± - (1)	- ± - ( )	- ± - ( )	- ± - ( )
Placenta:Pup	20:1	17:1	- : -	- : -	- : -

Table 4. Percentage of Infused Activity at Weaning and Half times of Clearance of Activity between Days 7-180 of age from Pups of Mothers Infused throughout pregnancy.

Organ	%IA @Weaning ± S.E.	Clearance Half-Time	95% Limits
Liver	$22.10 \pm 1.77 \times 10^{-3}$	117 days	(70- 250)
D21-90		50	(- - -)
D90-180		1500	(- - -)
Femur	$4.38 \pm 0.21 \times 10^{-3}$	313	(58- PH)
Spleen	$1.74 \pm 0.29 \times 10^{-3}$	283	(49- PH)
Kidneys	$3.73 \pm 0.85 \times 10^{-3}$	162	(32- PH)
Intestine	$5.80 \pm 1.35 \times 10^{-3}$	70	(30- 450)
Stomach	$1.86 \pm 0.55 \times 10^{-3}$	131	(38- PH)
Heart	$1.40 \pm 0.21 \times 10^{-3}$	1340	(22- PH)
Remainder	$91.00 \pm 5.65 \times 10^{-3}$	261	(115- PH)
Total	$132.01 \pm 0.94 \times 10^{-3}$	233	(125-1272)

PH=Physical Halflife. Upperbound is equal to no biological decay.

Table 5. Comparison of Published Data on Transfer of Plutonium.

Reference	Mode	Dose kBq/kg	Day of Inject	Sampled Day pc	Maternal Liver	Maternal Femur	%IA/g Foetal Body	Foetal Liver	Foetal Membranes	Placenta	Placenta: Foetus Ratio
Finkel	Single	1,110	13	Birth	-	-	0.100	-	-	-	-
(1947) Mice	Injection	1,110	16	Birth	-	-	0.200	-	-	-	-
Sikov	Single	9,250	15	16.00	2.54	0.73	0.010	0.03	1.93	0.40	40.00
(1968) Rats	Injection	9,250	19	20.00	-	-	0.010	0.04	3.20	0.91	91.00
Sikov	Single	740	15	16.00	(2.60 %IA)	(1.40 %IA)	0.020	-	4.00	0.30	15.00
(1976) Rats	Injection	740	19	20.00	-	-	0.040	-	8.00	0.90	22.50
Moskalev	Single	92,500	13	15.00	(25.80 %IA)	(1.56 %IA)	(0.68 %IA)	-	-	(1.20 %IA)	-
(1969) Rats	Injection	92,500	19	21.00	(23.44 %IA)	(0.98 %IA)	(1.16 %IA)	-	-	(7.55 %IA)	-
Weiss	Single	4	15	17.00	-	13.42	0.930	-	-	12.31	13.00
(1978) Mice	Injection	88	15	17.00	-	14.32	0.800	-	-	11.08	13.90
		955	15	17.00	-	7.45	0.250	-	-	5.51	22.00
Green	Single	340	-2	12.00	9.20	>40.00	0.004	-	{... 0.07 .....}		17.50
(1979) Mice	Injection	340	-2	18.00	8.50	>40.00	0.011	-	{... 0.78 .....}		71.00
Taylor	Single	49	-12	Birth	(2.25 %IA)	(1.72 %IA)	(0.01%IA)	-	-	-	-
(1980) Rats	Injection	49	-12	35Days Old	-	-	(0.045%IA)	-	-	-	-
Mason	Single	30	13	14.00	9.16	26.07	0.450	1.27	37.08	8.07	18.00
(1991) Mice	Injection	30	13	16.00	8.16	31.37	0.700	2.41	51.27	6.62	9.45
After Mason, Humphries and Lord. Int.J.Radiat.Biol. 59(2), 1991, pp467-478											
This Study	Multiple	195	0-19	19.00	19.90	24.90	0.70	-	-	14.20	20.50
241-Pu	Injection				(30.7%IA)		(0.83 %IA)			(1.22 %IA)	
241-Pu	External Infusion	213	9-19	19.00	5.98 (9.0%IA)	11.54	0.14 (0.37 %IA)	-	-	4.96 (0.45 %IA)	35.40
241-Pu	Internal Infusion	831	0-21	41.00	3.27 (7.4%IA)	8.78	-	-	-	-	-
239-Pu	Internal Infusion	8	0-21	41.00	6.52 (12.7%IA)	8.37	-	-	-	-	-

All figures are %IA/g except where indicated %IA. - indicates data not available.
{.....} indicates combined sample.

Figure 1. Blood 241-Pu Concentration After Single Daily Injections.

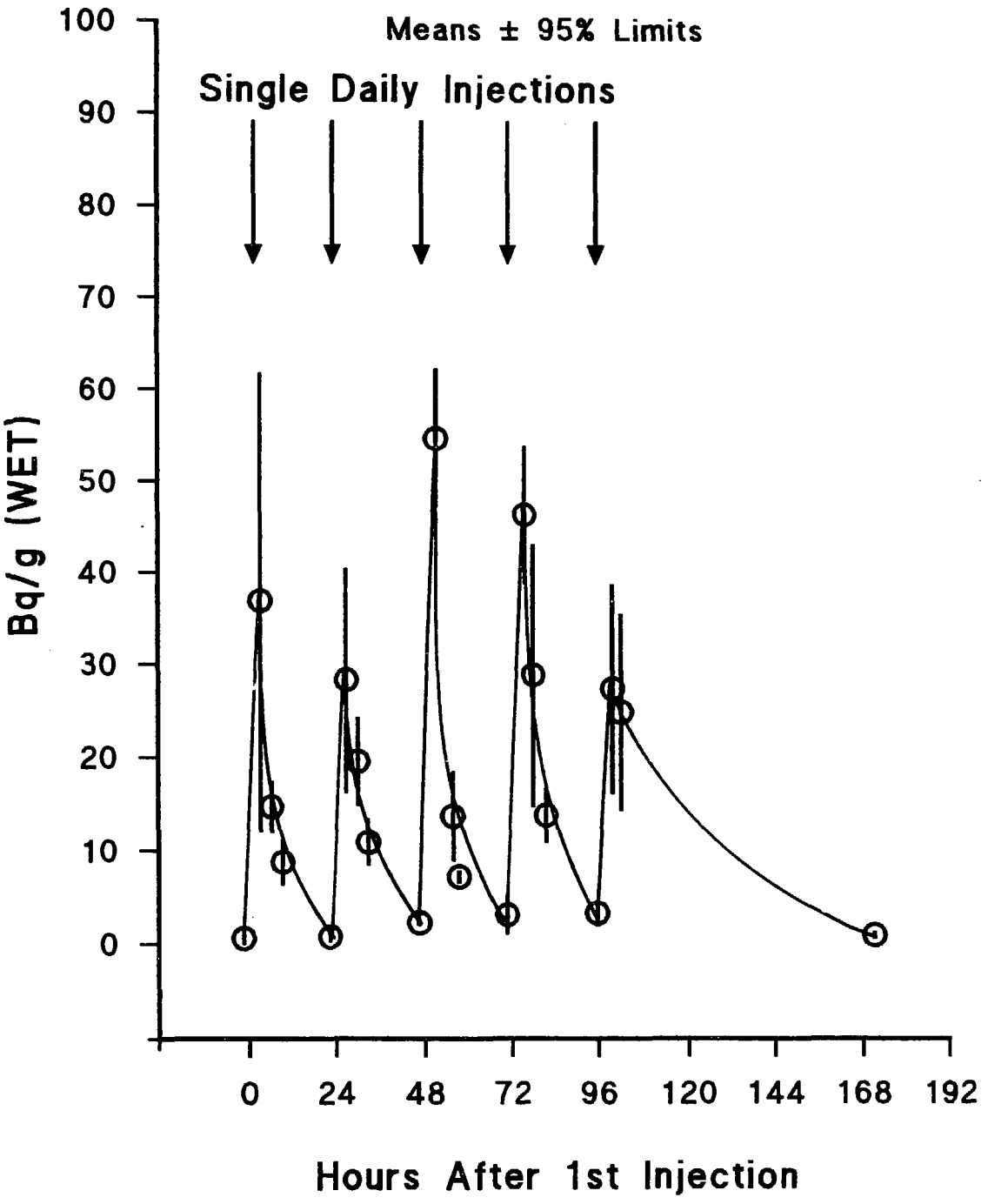


Figure 2. ^{241}Pu Concentration in Blood, Liver and Femur. Following Five Day Single Injection Regime

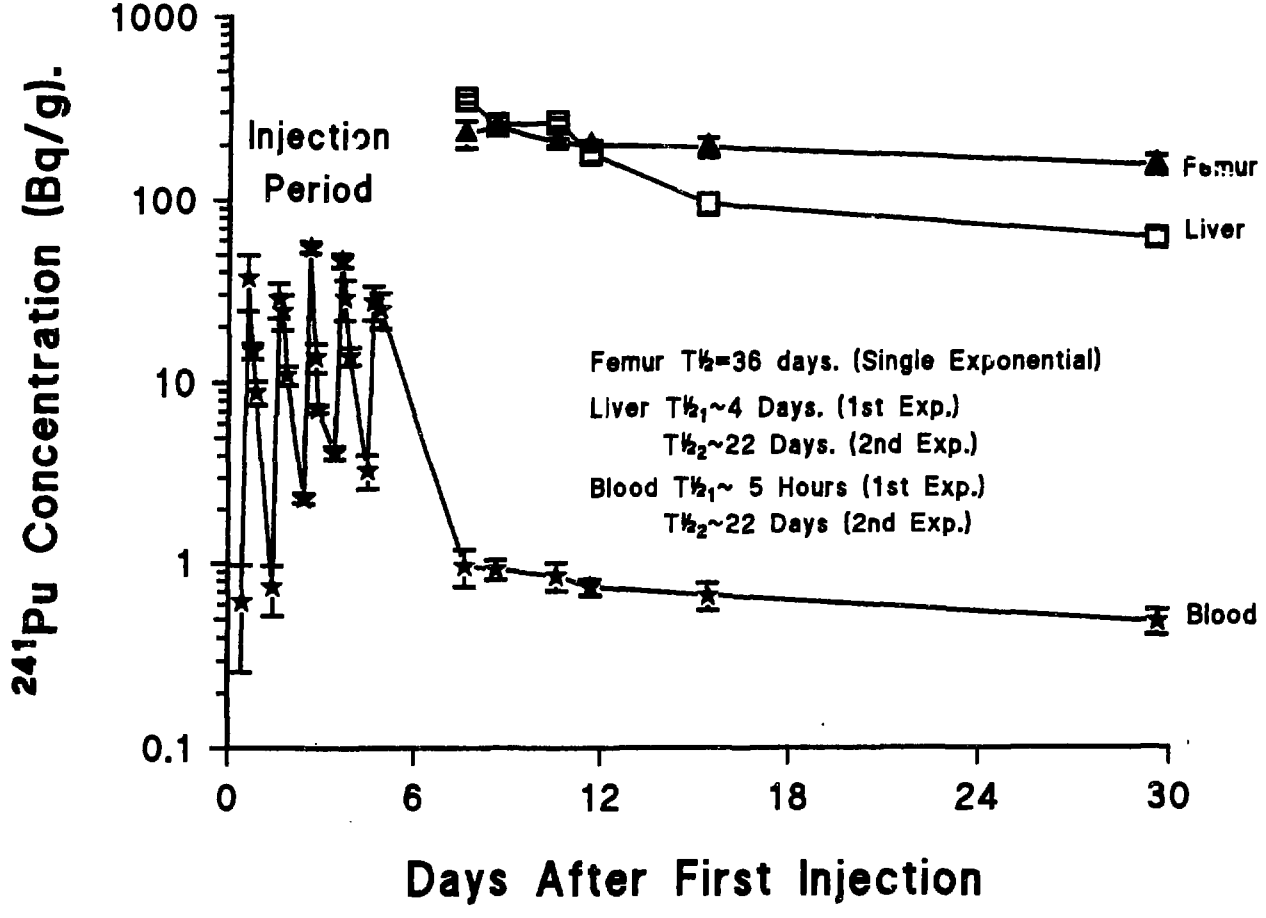


Figure 3a. Organ ^{241}Pu Concentrations in Osmotic Pump Infused Non-pregnant CBA/Ca ♀'s

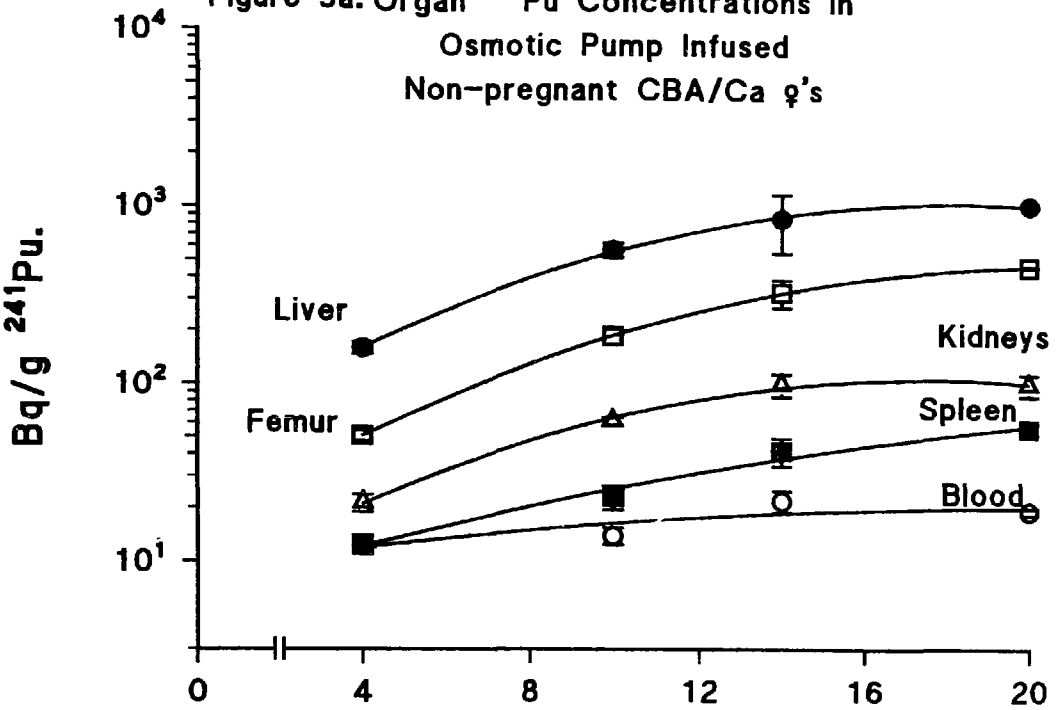


Figure 3b. Cumulative Activity (kBq) Infused From Sub-Cutaneous Osmotic Pumps

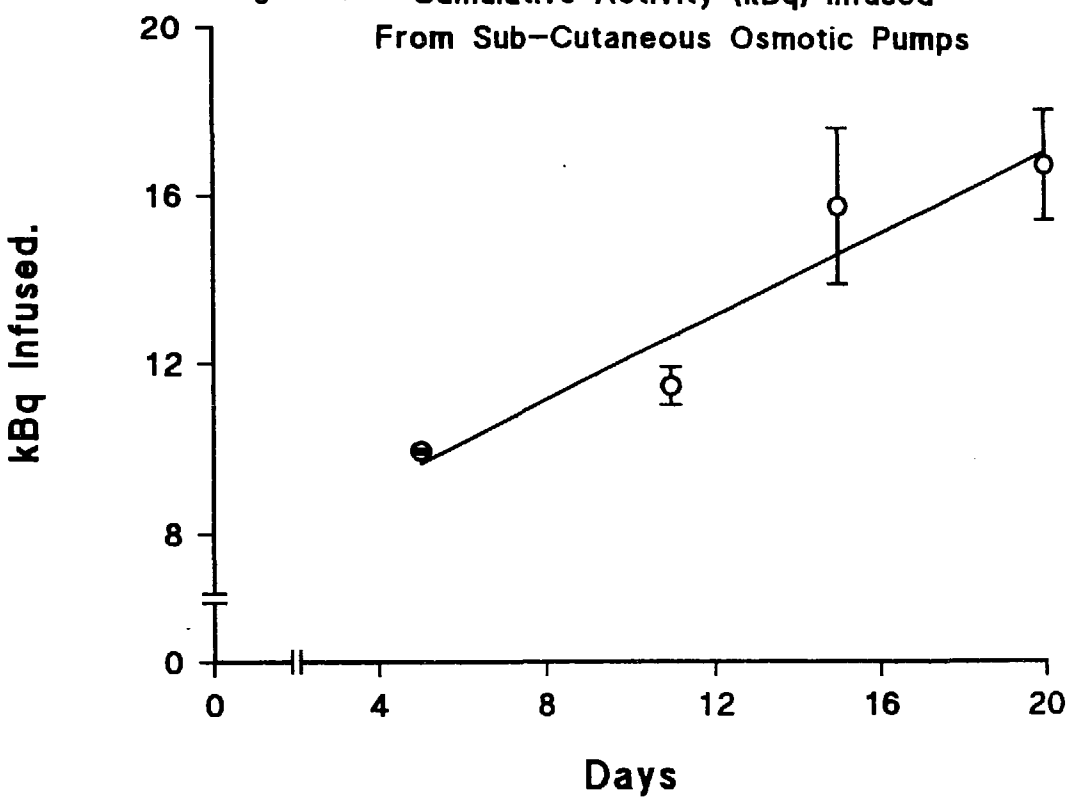
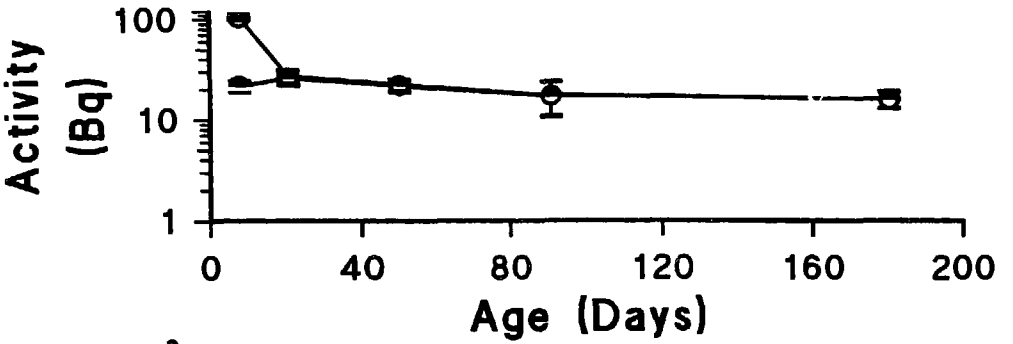
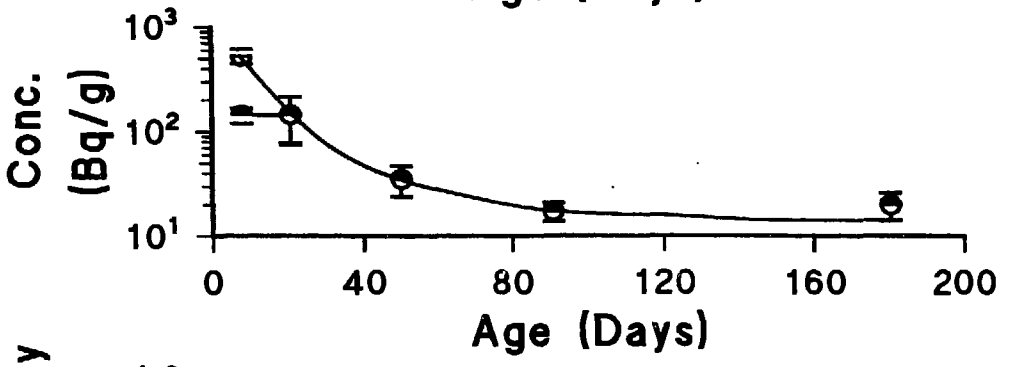
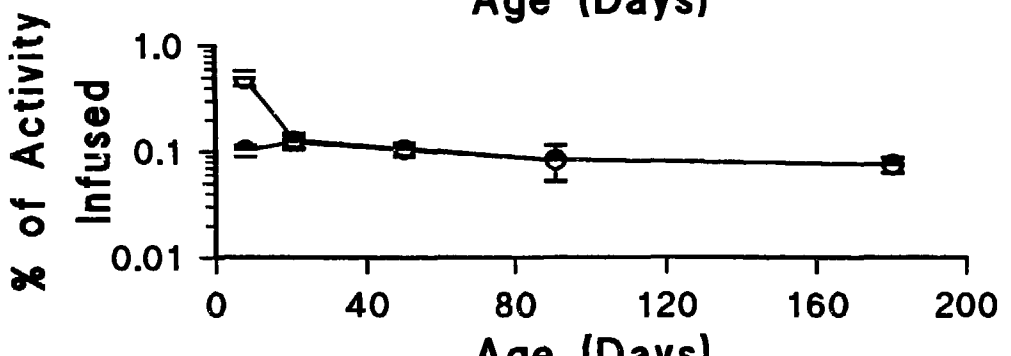
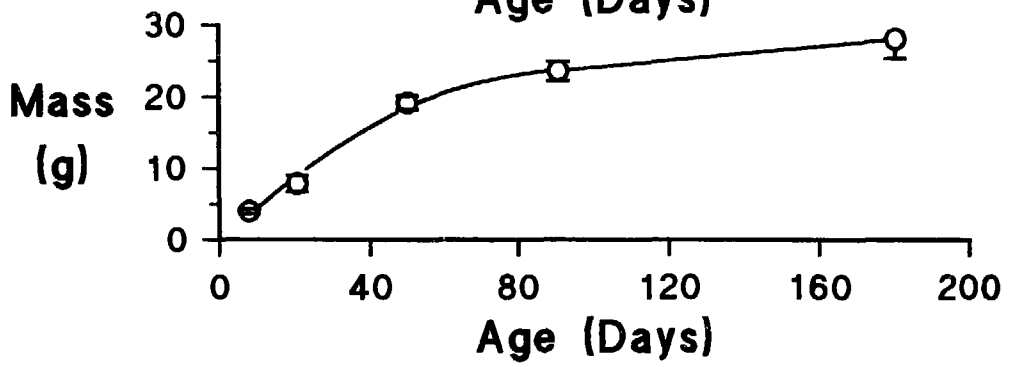


Figure 4. Activity, Concentration, % of Infused Activity and Mass. Sum of all Organs





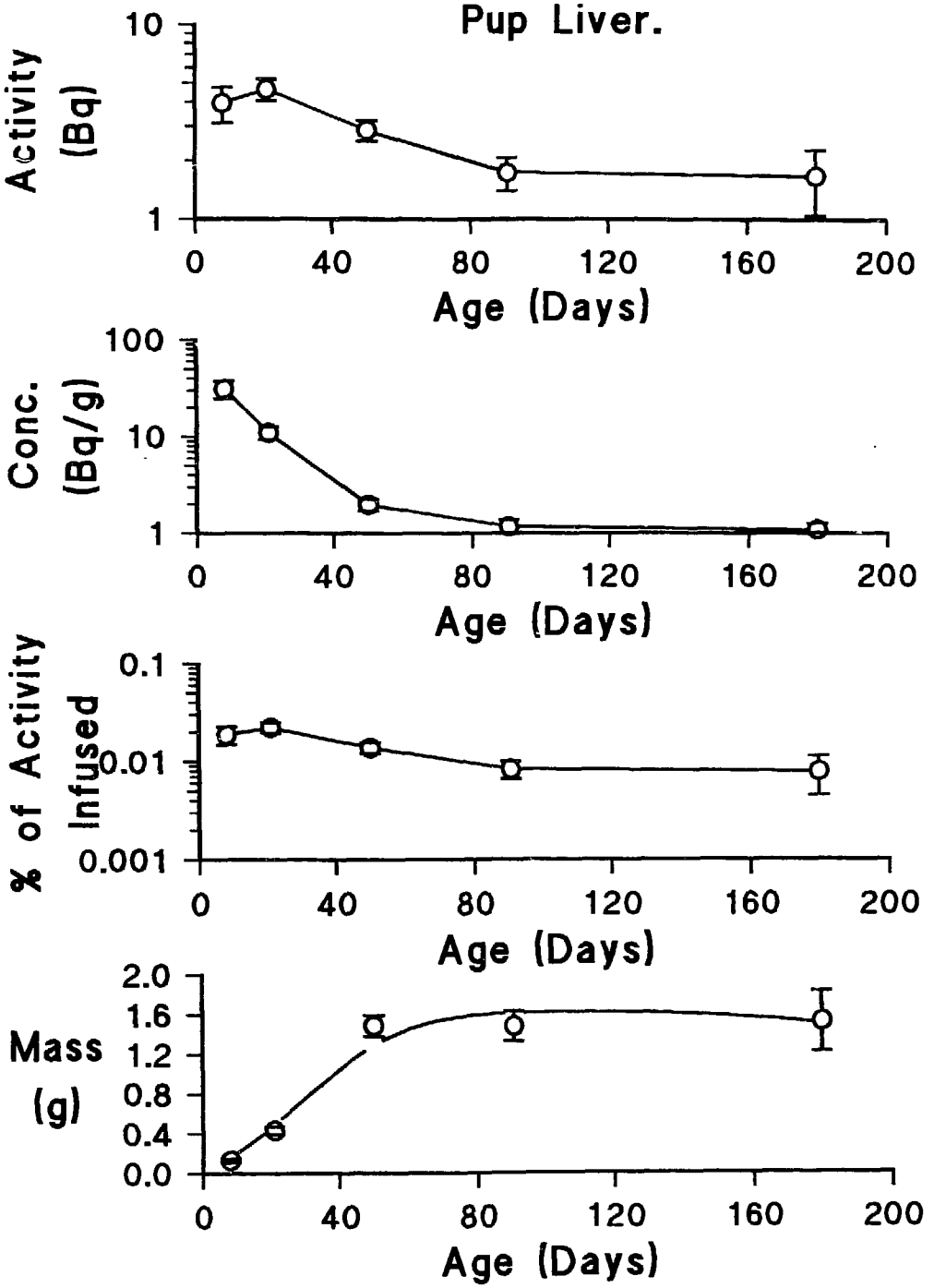




All data are Means ± 95% Confidence Limits

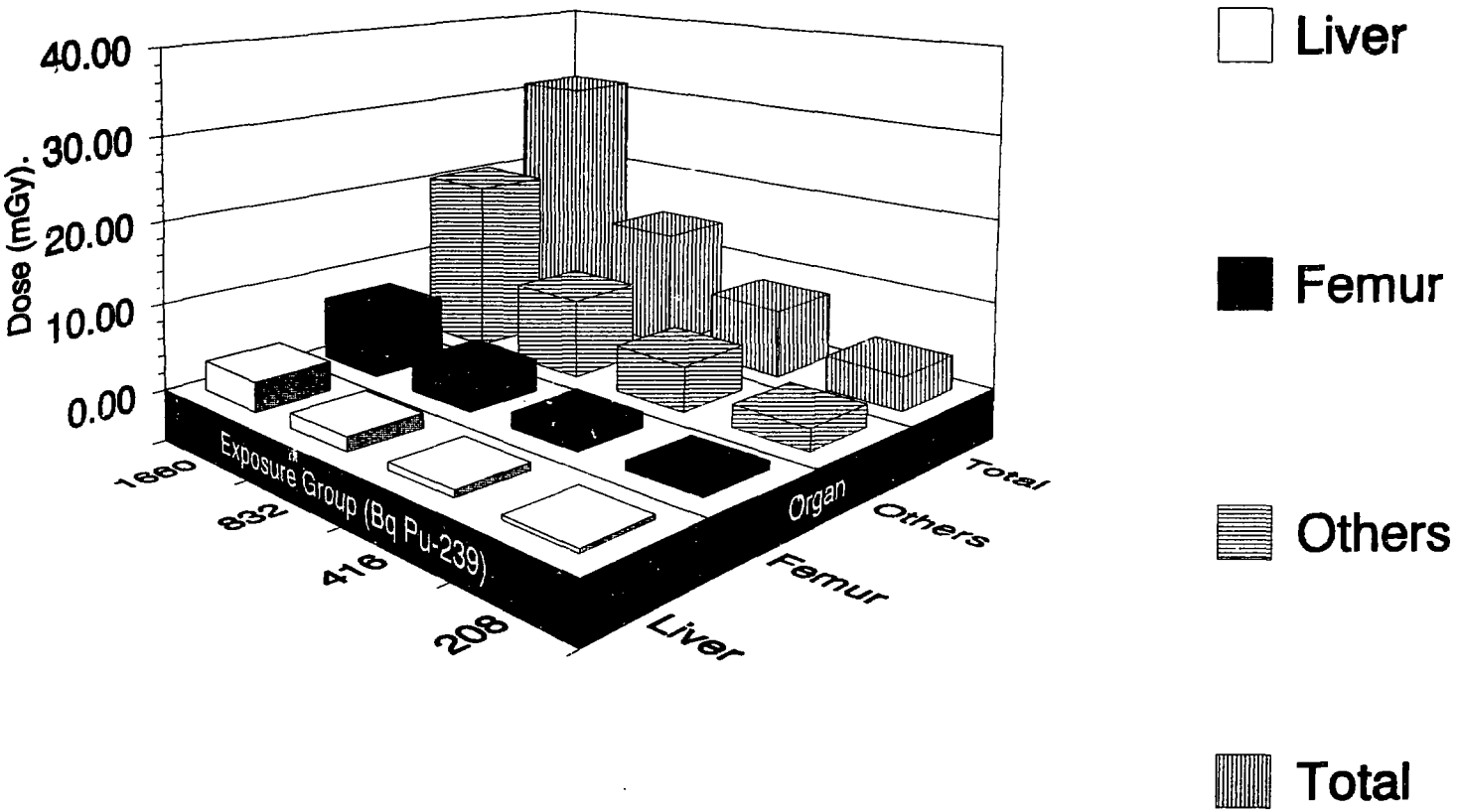
▫: Includes Gastrointestinal Tract, ▪ Not Including G.I.Tract

Figure 5. Activity, Concentration, % of Infused Activity and Mass Pup Liver.



All data are Means ± 95% Confidence Limits

Figure 6. Projected Doses During Neonatal and Young Adult Period (7-180) Days From Exposure In-Utero.



Question and Answer Period for Mountford-Lister

Question: Dr. R. Mole
What was the actual sample that you counted? Bone without marrow or bone with marrow?

Answer: It was bone with marrow. The aim was just to produce a set of ballpark figures to give us some sort of idea of what was going on.

Question: Dr. J. Harrison
What was the variation and duration of pumps with time?

Answer: The pumps are pretty well constant for the 20 days and five-hour warmup period. They pretty well go up in a square wave function for about 20 days. The actual calculated duration is 20.5 days. There is probably a 0.5 day on either side of that. Therefore, you are not talking two or three days error on either side. It is quite tight. I appreciate what you are saying. If some pumps were pumping much later into lactation, then we would be having more of a problem. In the CBACA's, duration of pregnancy is 20 days. We have a few that are born at nineteen days, but very, very few, maybe 5% if that.

Question: Dr. R. McFadden
What is the actual variation in the blood concentration with time?

Answer: Is this the slide you mean? Because it is done in that order, the activity goes up maybe one and a halffold during that period, whereas the liver goes up well over an order of magnitude in that time.

Question: Mr. M. Lupien
For the fetus, do you check some of the organs for early bloodforming functions for the actual patterns and variations?

Answer: We have a group of animals underway at the moment where we are looking at the uterine period and we will be taking animals for those sorts of organs. We have not gone down to that sort of level.

Another set of work done by another worker at St. Bart's involves looking at in vitro and some immunological factors and that might well come under the work she is going to do.

Question: Dr. M. Sikov
Do you have any feelings as to whether the behaviour during lactation is the same as a single injection during pregnancy or the pre-conception period?

Answer: From the literature, there are only two studies that I know of where lactation was considered to be an important pathway. A mid-term contamination gave very low amounts passed by the milk. There is a paper by David Taylor and another one by Green of MRC where they found that lactation was more important. If there was activity present pre-conception, lactation was more important in terms of the uptake rather than the contamination later during development. But the actual amounts passed over were very small.

There actually is one paper that suggests that passage by the milk is probably more related to blood concentration than to amounts actually within the tissue sample of the breast of the mouse -- that is, more related to blood concentration of the dam rather than to the activity just grossly within the breast of the mouse.

Question: Mr. P. Goyette
Have there been any studies to establish blood concentrations due to ingestion?

Answer: No, not that I know of.