received 03 Feb 1444

701377

Health Physics Vol. 48, No. 1 (January), pp. 7–17, 1985
Printed in the U.S.A.

0017-9078/85 $3.00 + .00
© 1985 Health Physics Society
Pergamon Press Ltd.

PLUTONIUM IN GONADS: A SUMMARY OF THE CURRENT STATUS

R. G. THOMAS,† J. W. HEALY and J. F. McINROY
P.O. Box 1663, HSE-DO, MS P228, Los Alamos National Laboratory, Los Alamos, NM 87545

(*Received* 19 *September* 1983; *accepted* 23 *July* 1984)

Abstract—A review of the literature was carried out to investigate the following: the fraction of Pu in the body that was in the gonads; the necessity for a localization factor in calculating the genetic dose to humans; and the possibility that an unexpectedly high relative biological effectiveness (RBE) causing genetic effects could occur for α particles. Human autopsy data from both occupationally exposed persons and the public were relied on to determine the amount of Pu in the gonads. It was found that an average fraction of 3×10^{-3} of the Pu in the body was in the ovaries and testes but there was a wide variation among individuals. While a localization factor over the average of 2.5–4 is needed to calculate the dose to the sensitive cells for rodents, no such factor is required for the human. Information on the RBE for various genetic effects shows RBEs for Pu α's about as would be expected from neutron irradiation. The usual quality factor applied in calculating dose equivalent is appropriate and may be conservative, particularly for females.

I. INTRODUCTION

THE POTENTIAL for genetic damage, resulting in harm to future descendants of those exposed, is one of the major reasons for the control of radiation exposure. Until the last 10 or so years, the gonads were not considered in deriving radiation protection limits for Pu because of the higher quantities deposited in other organs and the relatively small quantities in the gonads. Recently, however, radiation doses to the gonads have been considered in deriving limits (ICRP79).

Richmond and Thomas discussed this topic in a previous report (Ri75). However, with the increase in information available it was considered desirable to provide a review including this information. At the same time, questions have been asked about the influence of distribution within the gonads on the dose to genetically sensitive tissue. Thus, a review is included of the information available on this as well as on the question whether the α radiation could have an unusual RBE for genetic effects.

II. DEPOSITION IN THE GONADS

Data on the quantity of Pu in the gonads and other organs of six species were obtained from the literature. These included data for the mouse (As78; Be75; Gr75; Gr77; Ru79; Se76; Sm79); Chinese hamster (Br79); rat (Pr77; Ta77); monkey (Br80); dog (Ba66; Jo69; Pa69); and human (La80; Fo80; Mc79). These data included several methods of administration and ways of presenting the results that required interpretation to estimate the quantity of Pu in the gonads as related to the body content. For intravenous administration, data on the analysis of other body organs was frequently missing. In this case, the administered dose was used as the body burden. This will provide an overestimate of the body content because excretion is not accounted for but it was judged that this would not be serious in view of the many other uncertainties. In other cases, the content of various organs was given as estimated by measurements at death. Here, the total body burden

† Present address: Health Effects Research Division, Office of Health & Environmental Research, U.S. Department of Energy, Washington, DC 20545.

0005597

LANL

was estimated by summing the organ contents. If the administration was by inhalation, the Pu in the pulmonary system and associated lymph nodes was not included so that the result is a "systemic body burden".

Most of the human data were from two autopsy series. The first was a study of tissues from members of the public from several regions of the United States (Mc79; Fo80). The source of the Pu in this series was primarily from the inhalation of fallout. The second was unpublished autopsy data from workers in Pu-handling facilities who were exposed in the course of their work. Compilations used gave the tissue concentration and contained all measured values including those below the minimum detectable level (negative values and zero amounts included). In these autopsy series, the marrow was included in the bone sample analyzed. The vertebral sample was chosen to represent the skeleton because it was analyzed in more of the cases than any other skeletal sample. Concentrations in the vertebrae were multiplied by a factor of 0.65 to represent the concentration in the total skeleton (Th84). These concentrations were converted to total quantity in the organ by use of the organ weights for Reference Man (ICRP75). The body burden for each individual was taken as the sum of the liver and skeleton. This is a minor error because the great majority of the Pu is in these two organs (Th84).

Table 1 provides the results of these analyses expressed as the fraction of the Pu body burden per gram of gonad. This method of expression was used because it is proportional to the average dose in the organ. Because plots of these values show a reasonable fit to a log normal distribution, both the geometric mean and the geomet-

Table 1. Fraction of plutonium body burden per gram of testes or ovaries

Species	Number subjects	Exposure route*	Arithmetic mean†	Geometric mean	Geometric SD	Exposure timespan
			Testes			
Mouse	92	IV, IP	5.6×10^{-3}	2.9×10^{-3}†	1.16	6h–348 days
Ch. hamster‡	45	IV	1×10^{-3}	1×10^{-3}‡	—	4–700 days
Rat‡	30	IV	5.4×10^{-4}	5.4×10^{-4}‡	—	1–575 days
Monkey‡	2	IV	7.1×10^{-5}	7.1×10^{-5}‡	—	4–45 days
Dog	22	IMP, INH	4.0×10^{-4}	7.5×10^{-5}†	4.9	27d–12 mo
Human§	171 (165)	INH	6.3×10^{-5}	1.5×10^{-5}**	11.5	Continuous
Human¶	3	IV	7×10^{-6}	—	—	5–155 days
Human‖	23	Varied	6.6×10^{-5}	7×10^{-6}**	12.0	Intermittent
			Ovaries			
Mouse	95	IV, IP	5.6×10^{-2}	3.2×10^{-2}†	2.6	1–180 days
Rat‡	33	IV	4.9×10^{-3}	4.9×10^{-3}‡	—	0–146 days
Dog	20	INH	6×10^{-4}	2.9×10^{-4}†	3.4	30–66 days
Human§	47 (45)	INH	3.3×10^{-4}	1.5×10^{-4}**	8.5	Continuous
Human¶	1	IV	9×10^{-6}	9×10^{-6}	—	16 days
Human‖	3	Varied	3×10^{-4}	—	—	Intermittent

* IV—Intravenous; IP—Intraperitoneal; IMP—Implant; INH—Inhalation; Varied (inhalation, wounds, other).

† Geometric means calculated and weighted for number of subjects; Arithmetic means were weighted for number of subjects they represented. Number of subjects often varied between experiments and within experiments.

‡ Only one value given in the reference, representing all subjects.

§ Non-occupationally exposed individuals, primarily to fallout. Arithmetic means calculated on values below two geometric standard deviations above the geometric mean (below 5% limit). Number in parenthesis is number of values arithmetic mean was based on. All values, including negative results, were used in the analysis of the human (non-IV-exposed) data.

¶ Intravenously injected patients.

‖ Occupationally exposed humans.

** Values determined from log-probability plots.

LANL

0005598

ric standard deviation are given along with the arithmetic means.

III. LOCALIZATION WITHIN THE GONADS

A study with male CBA mice by Green *et al.* (Gr75) was carried out following intravenous (IV) injection of 3.2 μCi ^{239}Pu-citrate/kg body mass. Five sets of testes were analyzed at four weeks and six sets at 12 weeks post-injection. Mean testis weights were 73 and 67 mg, respectively, and at sacrifice the average radiation dose rates were calculated to be 0.072 and 0.092 rad/day. Autoradiographs were prepared from 6-μm frozen sections and 12,000 α tracks were counted in more than 700 randomly selected fields. Their studies indicated that most of the Pu was found in the intertubular spaces but that about 40% of the α particles irradiated the spermatogonial cells at the periphery of the seminiferous tubules. At the four- and 12-week sacrifices, the dose rate to the intertubular tissue and to the 10-μm layer of tissue containing the spermatogonial stem cells at the periphery of each tubule was greater than the average throughout the testis. At 12 weeks following injection the authors calculated the average dose to the testes to be 2.5 times lower than to the stem cells and 5.1 times lower than that to the intertubular tissues (Gr75).

Russell and Lindenbaum (Ru79) essentially duplicated the work of Green *et al.* (Gr75) using a different strain ($B6CF_1$) of male mouse with IV injection of 10 μCi ^{239}Pu-citrate/kg body weight. They did autoradiographic and pathologic examination of the testes after sacrifices at 6, 30, 75, 222 and 348 days post-injection. Radiochemical analyses determined that the amount of Pu in the testes remained constant throughout at 0.05% of the injected dose, but the decrease in testes weight by 30% (0.135 g to 0.094 g at 348 days) had a proportionate effect in increasing the Pu concentration. The average dose rate at six days was 0.14 rad/day and at 348 days was 0.20 rad/day. The autoradiographic findings of these authors (Ru79) were such as to increase the 2.5 factor (Gr75) between mean testes radiation dose and dose to stem cells to 4. This increase was the subject of a published letter-to-the-editor exchange between Green *et al.* (Gr80) and Lindenbaum and Russell (Li80).

A study was done by Ash and Parker with male mice (^{238}Pu citrated, 10 μCi/kg injected IV, radiochemical and autoradiographic analyses) (As78). The amount of Pu in the testis did not significantly change from one week to three months of observation post-injection with an average of 0.024% injected dose. The weight of the testis decreased slightly during this period, from an average of 0.074 g at one week to 0.054 g at three months. The initial testicular distribution was diffuse, followed by clustering with time through macrophage uptake of ionic Pu. Ash and Parker (As78) indicate that the accumulation of ^{239}Pu in macrophages may be either from phagocytosis of dead, Pu-containing cells or through a transfer of Pu-contaminated metabolic waste products. The authors speculate that there is a possibility that the ^{239}Pu in the macrophages may be adjacent to seminiferous tubules resulting in an increased irradiation of spermatogonial stem cells.

Priest and Jackson studied the gonads from both sexes of hamster following intravenous injection of 270 μCi ^{241}Pu citrate/kg body weight (^{241}Pu is a β emitter) (Pr78). They found a macrophagic accumulation up to 10 days post-injection in the hamster ovaries and testes. The accumulation was in the follicles of the ovaries and in the interstitial tissue of the testes; the ovarian deposits appeared to migrate with time but the testicular macrophagic deposits remained. The migration of deposits within the ovarian tissue was regarded as likely to lower the effective dose to the viable oocytes. A viable oocyte is defined as a follicular cell which shows no atretic signs such as nuclear pyknosis, chromation deposits, or other evidence of permanent damage resulting in death of the cell. It is assumed that the viable oocyte is either in a state of maturation or not showing signs of not being potentially able to participate in maturation and subsequent ovulation. As in the mouse study above, the Pu in testicular macrophages tended to remain interstitially. The authors concluded that the genetically significant dose rate to viable oocytes was likely to be lower than the average ovary dose rate but may be greater by a factor of 2–2.5 for the spermatogonial cells than the average dose to the testis. Priest indicated that these findings give essentially the same general conclusions as his earlier work with ^{241}Pu injected into hamsters and rats

0000599

LANL

(Pr77). There was no reported decrease in gonadal weight during the short term of these studies.

Taylor studied the metabolic characteristics of ^{239}Pu in the gonads following IV injection of citrated solution (1.9–3.9 μCi/kg body weight) into rats of both sexes (Ta77). He sacrificed up to 575 days for analysis of testes and 146 days for the females and found no significant loss of Pu from either gonadal tissue during this period. The mean uptake was 0.062% of the injected dose in the testes or 0.054%/g. Mean uptake by the ovaries was 0.032% of the injected ^{239}Pu or 0.53%/g. The author states that about one-third of the Pu α tracks in the testes lie in the region of the spermatogonia and for his study, he estimated an average radiation dose to the whole testis of 34 mrad/day and to the spermatogonal cells of 54 mrad/day. The comparable average dose to the ovaries of the rats was estimated to be 145 mrad/day with autoradiographic data suggesting about 15 mrad/day to the maturing follicles. The high average dose to the ovary results from its smaller size compared to the testis (17 times in the rat). Taylor also adopted the method of Green *et al.* (Gr75) to estimate the dose to spermatogonial cells compared to the average dose and arrived at a factor of 1.6 compared to the 2.5 value that had been reported for the mouse (Gr75).

Green *et al.* injected female CBA mice IV with 5.4 μCi ^{239}Pu/kg body weight as the citrate and studied its fate using chemical and autoradiographic analyses of the ovaries (Gr77). The fraction of injected radioactivity and the daily total radiation dose showed no significant variation with time throughout the 180-day duration of their study. The ovaries had an average weight of 10 mg and an average radiation dose rate of 0.63 rad/day. Early post-injection the α tracks were randomly distributed throughout the tissue, with some concentration occurring in some atretic follicles. By 180 days the random distribution of tracks decreased and there were some discrete clusters. There was no correlation observed between state of the estrous cycle and the pattern of uptake of ^{239}Pu by the ovary. The clustering of Pu into autoradiographically observable tracks was associated with iron-staining cells assumed to be macrophages. At early post-injection times any Pu concentration was associated with non-fertilizable oocytes (atretic) rather than normal maturing follicles. At later times, the concentration of ^{239}Pu in macrophages adjacent to the developing follicles may have resulted in the follicles receiving a somewhat greater dose than that averaged over the entire organ.

Studies from the Inhalation Toxicology Research Institute provide a comparison of experimental animal testicular microanatomy with that of the human (Br79; Br80). Male Chinese hamsters were injected intravenously with 2×10^{-3} or 6×10^{-4} μCi ^{239}Pu citrate/kg body weight and were sacrificed serially at 0, 4, 16, 32, 64, 128, 256, 500 and 700 days (Br79). The activity concentration in the testes (0.1% of the injected dose/g) was relatively constant throughout the experiment, testifying to the very long retention time. Autoradiographs at 500 days showed the radioactivity to be primarily localized in the interstitial tissue. Data on the cynomolgous monkey following inhalation indicated similarly that Pu deposited in the gonads was distributed primarily in the interstitial tissue of the testes (Br80). In conjunction with these studies using ^{239}Pu, they also did non-radioactive testicular morphometric measurements on adult mice and Chinese hamsters as well as humans at various ages (2, 6, 18, 33, 61 and 77 years of age). The relative amounts of tissue in three regions of the testes (intertubule, tubule, stem cell) were measured from projections of tissue slides. From these measurements they were able to use the autoradiographic distribution patterns of ^{239}Pu in the rodents to extrapolate to a projected dosage pattern that would be seen in the human, assuming a microdistribution pattern of Pu similar to that found in the rodent. Their extrapolations projected an α dose to the stem cells that would be in the ratios of approximately 1:0.66:0.49 for the mouse: Chinese hamster: human (all ages). The main reason for these differences is the actual difference in physical size of the testes between species with the interstitial tissue proportionately greater in the larger species. Their results indicate that as the ratio of interstitial tissue to spermatogonia increases, the dose differential between spermatogonia and the whole organ decreases.

LANL

0005600

IV. BIOLOGICAL EFFECTIVENESS

A complete assessment of the potential hazards of Pu must include evidence to answer the question of whether the α particles from Pu produce an unusual degree of damage over that from those heavily ionizing radiations that have been tested. Fortunately, there are several papers in the literature that examine this question for both male and female experimental animals. The following review emphasizes primarily the results and conclusions of the authors in only enough detail to permit an answer to the question. The original papers should be consulted for details and discussion leading to the conclusions.

Beechey *et al.* studied the cytogenic effects of ^{239}Pu α particles in male mice (Be75). Twelve 10-week-old mice were injected with 10 μCi/kg body weight in 1% trisodium citrate. Three litter mates were used as controls. Equal numbers of animals were sacrificed at 6, 12 and 18 weeks. The left testicle was used for Pu analysis and the right was used for chromosome preparations for examination of chromosome abnormalities. Sperm counts and examination of spermatozoa were made on the 18-week animals only. The average dose rate to the testes was about 0.35 rad/day resulting in a decline in weight in 18 weeks to 51% of the controls. Because the epididymal sperm count at this time was 51% of the controls, the authors concluded that much of the weight loss was associated with germ-cell death. Sperm head abnormalities were 9.0% as compared with 4.6% from controls. However, this difference was largely due to one animal with 7.2% abnormalities. Frequencies of autosomal and X–Y chromosome univalents and spermatocytes with fragments were not statistically different from controls although the numerical values were higher for the irradiated animals. The frequency of spermatocytes with quadravalent configurations was significantly above that of the controls. The authors used the data on reciprocal translocations to estimate a rough value for the comparative effectiveness of the α radiation from Pu using data from previous experiments for γ rays and fission neutrons. The rate per rad (average to the testes) for Pu is about 63 times that for γ rays and 2.7 times that for neutrons. The authors conclude that these results are consistent with the assumption that the RBE is about the same as that for fission neutrons with the α dose to spermatogonia 2.5 times the average radiation dose to the testes.

Lüning *et al.* studied intrauterine fetal deaths in female mice mated with males which had previously received 0.01–0.5 μCi ^{239}Pu per animal (Lü76). In the first two series, Pu nitrate was administered with no positive results. Consequently, three more series were run using Pu citrate. Here the males were mated weekly with three females and the females were killed on the seventeenth day for examination of the uterine contents. At the highest dose level (0.5 μCi) the males became sterile between the twelfth and twentieth week. There was also a significant increase in fetal death in all groups after about the fourth week from injection. The effect at the lowest dose (0.05 μCi in this series) seemed to be as great as for the higher doses. In a follow-up experiment, male offspring sired 9–17 weeks after injection were tested. None of the offspring were sterile but a significant increase in both early and late intrauterine deaths was noted. The authors state that the excessive rates of intrauterine deaths were not surprising but the proportion of late deaths in this strain of mice were. More significant, however, was that they had not observed similar effects using x rays, neutrons, ^{90}Sr or ^{45}Ca. They also found some increased intrauterine deaths in offspring of first generation offspring from the initial Pu series injection. The author found these results of sufficient interest to inspire them to plan future experiments.

Searle *et al.* (Se76), in a follow-up of the studies by Beechey *et al.* (Be75), injected 24 male mice 11–17 weeks of age with ^{239}Pu in a 1% trisodium citrate solution. The amounts of Pu were 4 μCi/kg body weight or about 0.1 μCi per mouse. Controls were six litter mates injected with only the citrate solution. This dose of Pu turned out to be too low to give results for comparison with the ^{60}Co part of the study so that their previous Pu work (Be75) had to be used. In the external radiation part of the study, 13 male mice of the same age and hybrid stock were irradiated continuously with ^{60}Co γ rays for 28 weeks at an average dose rate of 5.8 rad/

0005661

LANL

day. These mice were mated once each week for the four weeks before sacrifice. Dose rates in the earlier study with Pu were determined by weighing the left testicle and analyzing it for Pu and they averaged about 0.35 rad/day. The γ irradiation reduced the testes mass to 35% of the controls and the total dose calculated for a time of 28 weeks was 1128 rad. Data from the first study had shown a reduction in testes weight to 58% of control (Be75). The effectiveness of the α particles was perhaps not significantly different (58% of control vs 35% for γ rays) but using these factors they concluded that α particles from Pu were 10–15 times as effective as ^{60}Co γ rays ([5.8 rad/day ÷ 0.35 rad/day] × [35% control mass ÷ 58% control mass] = 10). With regard to sperm count a similar analysis was used by the authors, using the ^{239}Pu injection data from the previous experiments (Be75). For the α-particle dose of 0.35 rad/day the sperm count was reduced to 51% of control whereas in the γ-ray study the 5.8 rad/day reduced the count to 15% of control. The authors concluded that these values for spermocyte count were consistent with the factor of 10–15 they used for effectiveness of α particles over γ rays for the mass reduction even though the result is much lower ([5.8 ÷ 0.35] × [15% sperm count ÷ 51% control sperm count] = 5). For both reciprocal translocations in spermatogonia and fragments in spermatocytes the Pu α particles were about 24 times as effective as the γ radiation. For post-implantation dominant lethals, the estimated rate for Pu was 22 times that of the γ radiation. The authors note that the effectiveness ratio obtained from fragments is the only one based on significant differences from the controls in both the γ-ray and α-particle series. Still, the ratios are similar to Brooks' (Br75) RBE of 15–20 for chromosome damage in liver cells. Although this study combined the results from two different experiments from the same laboratory (Se76; Be75) the comparison is quite effective in showing the difference in the effectiveness of α particles and γ rays.

Grahn *et al.* compared the effects in male mice from ^{239}Pu, injected as the citrate, with single, weekly and continuous exposures to ^{60}Co γ rays and single and weekly exposures to 0.8-MeV average energy neutrons from a reactor (Gr79). Plutonium administered was 5 and 10 μCi/kg body weight or about 0.14 and 0.28 μCi/mouse. The Pu remained in the testes, with no change during 400 days, at a level of about 0.05% of the initial dose. Because the testes slowly lost weight, the average dose rate increased with time. For their assessments of the data, however, they assumed the dose rate to be constant during the course of the study. These dose rates were 0.15 rad/day for the animals that received 10 μCi/kg body weight and 0.075 rad/day for the 5 μCi/kg animals. This dose is the average to the testes with no correction for localization in or near the more sensitive cells. Data on post-implantation fetal survival indicated that the primary lethal mutation rate is dependent upon the dose rate rather than the total dose. This is because more than 90% of the effect occurs in the post spermatogonial stages and not in the premeiotic stages. The reciprocal chromosome translocations in the spermatogonia of the mice injected with ^{239}Pu did not have a dose-response relationship, although a linear relationship did occur for the γ-ray and neutron exposures. For Pu, the effect was close to the value for neutrons at the lowest dose and then remained about constant with increase in dose. Chromatid fragments were not different between the two groups receiving Pu but they were elevated over the γ-ray values. Abnormal sperm depended upon dose rate for the Pu-injected animals. These abnormalities increased at a rate two to three times greater than for those mice exposed to neutrons. There was no detectable effect on sterility or fertility in the Pu-bearing animals over the first year after injection. However, there was significant somatic injury consisting of bone tumors, anemia, and liver disease. Bone tumors caused about 50% of the deaths in those animals living more than 36 weeks. The authors provide a table giving the relative responses based upon the derived response coefficients per rad for various abnormalities. Those values having to do with Pu are given in Table 2.

From these results, Grahn *et al.* conclude that ". . . Pu does *not* present any unusual or unexpected genetic threat."

Because of the anomalies noted in the cytogenetic damage in previous experiments, Grahn *et al.* (Gr83) repeated the 10-μCi/kg Pu exposure

0005602 LANL

*Table 2. Relative responses of mice to α-particle irradiation from plutonium compared to γ and neutron (η) irradiation**

	α/γ	α/η
Dominant lethals postmeiotic stages	13 ± 3	1.2
Translocations	≤38 ± 5	≤1.0
Fragments	33 ± 5	—
Abnormal sperm	25 ± 8	2.5
Testis weight loss	9.5 ± 4	1.6

* Data in this Table were taken from the reference of Grahn *et al.* (Gr79). Values are the mean relative biological effectiveness ratios ± standard error. No standard error values were given for the ratios.

along with mice exposed weekly to neutrons and ^{60}Co γ rays. Exposures and injections started when the mice were 15 weeks old and continued for 61 weeks. The data on translocation frequencies confirmed the previous data with a concave downward curve of frequency versus dose. For γ rays an essentially linear curve was obtained with, however, an apparent dose rate effect. Neutrons showed a drop-off in effect at the higher doses which was attributed to an effect of cell killing at the higher doses. For the fragments a clear influence of age for the controls and the external radiation groups was shown. The Pu data was, once again, different from the external radiation data. With Pu the frequency of fragments increased for the first 20 weeks and then declined until the final data point at about 60 weeks which did not differ significantly from the controls. The authors speculate that the anomalous pattern from Pu is due to the pattern of deposition in the testes so that there are radiation free areas. In the irradiated areas cell killing occurs and unirradiated cells begin to dominate. If this is the case, they note that Pu given in multiple doses should give a more regular response at a level of about 1/3 to 2/3 of the neutron response.

Searle *et al.* (Se80) examined the effects on breeding in female mice of γ radiation from ^{60}Co and the α radiation from injected ^{239}Pu citrate (Se80). Plutonium levels of 5 μCi/kg and 10 μCi/kg were administered to 16 and 17 females, respectively, with 14 controls receiving citrate only. The females were bred to adult males from a specific locus tester stock. Breeding continued until sterility occurred, usually due to osteosarcoma and consequent weakening. In the ^{60}Co series females were kept in 10 rad/day and 20 rad/day fields continuously except when removed to breed. In both series, several animals were sacrificed at an early stage or when sterility occurred for examination of the ovaries and measurement of Pu distribution. In the Pu series the average dose rates to the ovaries in the Pu series were about 0.5 rad/day for the 5 μCi/kg group and about 1.5 rad/day for the 10 μCi/kg group. It was also noted that all of the Pu animals showed signs of bone damage at death. For onset of sterility the 20 rad/day of γ radiation was earliest at 20 weeks followed by the 10 rad/day γ group, the 10 μCi/kg Pu group and the 5 μCi/kg group, with the 5 μCi/kg group not significantly different from the controls. The litter sizes for radiations were not significantly different than the controls although, after an apparent increase over the first 8–20 weeks, the fall off of litter size was in the same order as onset of sterility. When only litters of more than one animal were considered, postnatal mortality remained low in all series. However, the mortality was significantly increased in the γ series while the Pu series showed a nonsignificant tendency to decrease. One in 936 control offspring and four in 1,443 in the Pu series showed areas of white or cream spotting in portions of the coat. While the difference is not significant, microscopic examination indicated that they might occur from somatic mutations. Rough estimates of the RBE were made from the data. For killing of oocytes the RBE was less than 4.2. Based on the total number of offspring produced to sterility, the RBE was 2.5 for the 10 μCi/kg group and 0.6 for the 5 μCi/kg group respectively. Because the lower number was based on the nonsignificant difference, it was stated that the former number was probably closer to the true value. The conclusion was that the RBE of Pu in the ovary was probably about 3. The lower values are due to nonuniform distribution with the Pu in atretic follicles and later in macrophages.

In the follow-up of their previous work, Searle *et al.* carried out two experiments in which female mice were injected with ^{239}Pu citrate (Se82). In the first experiment a dose of 10 μCi/

0000003

LANL

kg was used with 30 mice with the same number of controls. The mice were bred at six days and dissected 14 days later to study lethality in the embryos. In the second group, 162 animals were injected with 20 μCi/kg with 54 controls. These animals were divided into three groups and mated at 3, 6 or 12 weeks with measurement of dominant lethality as in the other experiment. From previous data, the dose to the ovary in the first study was estimated to be about 10 rad in the period before mating. In the second experiment, Pu in the ovaries was measured and the doses to 3, 6 and 16 weeks were estimated as 110, 245 and 590 rad. There were two major assessments of dominant lethality made, total survival and post-implantation survival. Total survival measured the live embryos as a function of the total number of corpus luteum found (number of living/number of possible embryos). Post-implantation survival was a measure of the live embryos as a function of the total number of impregnations (number of living embryos/total number of implants). In the first experiment, little difference was seen between controls and irradiated animals.

The results from the second study are manifold but generally the authors report as follows: (1) reduction in fraction of pregnancies, (2) pre-implantation lethality, and (3) post-implantation lethality. The pre-implantation lethality was attributed to reduced fertilization as a result of oocyte damage but direct evidence is lacking. The post-implantation lethality was attributed to oocyte killing as reflected in a lesser corpora luteal count and fewer pregnancies. The authors concluded that there was little evidence of genetic damage from the Pu exposures and that the damage was of a fertility nature instead. In the first experiment, little difference was seen between controls and irradiated animals.

In vitro studies by Barnhart and Cox were carried out using ^{238}Pu α particles and Chinese hamster ovary (CHO) cells (Ba79). The CHO cells were grown at 37°C as sub-confluent layers in plastic tissue culture flasks. They were innoculated into plastic dishes for mutagenicity studies at densities of 7.0×10^3 to 1.0×10^5 cells/cm^2 and at 18–212 cells/cm^2 for survival measurements. After 2–3 h, the attached cells were irradiated with the x rays at a rate of 200 rad/min or with α particles from ^{238}Pu electroplated on a metal planchet. A collimator was used to give an approximately parallel beam of α particles, essentially monoenergetic. The survival was measured from colonies in the center of the culture dishes to assure homogeneity. Mutagenesis was measured by allowing a fixed area (3.4-cm-dia circle) of cells to be trypsinized and to be innoculated into dishes for continued culturing. Following irradiation the cells were incubated at 37°C for 6 days, the colonies were fixed in 70% ethanol, and stained with a 1% solution of crystal violet to estimate the survival of colony-forming units. For mutagenesis, plating efficiencies were determined after incubations by plating in 6-thioguanine and the number of cells capable of forming colonies was determined as a measure of lethal mutagenic changes due to the irradiation. Mutation rates of 4.79×10^{-7} and 1.30×10^{-7} mutations per viable cell per rad were found for α particles and x rays, respectively. This indicates an RBE of 3–4 in reasonable agreement with Searle (Se80). However, when mutation rates were plotted against survival a linear relationship was found from which an RBE for both mutation and cell killing could be calculated as a function of survival fraction. RBEs for x rays compared to α particles for the likelihood of producing a mutagenic event in the face of cell killing showed the α particles to be about 0.5–0.7 times as effective as the electromagnetic radiation. The authors state, however, that their results ". . . reflect influences on single cells in culture and do not address the requirement for an additional understanding of genetic expression and an appreciation for modulating effects which may occur in the intact animal."

V. DISCUSSION

This paper was intended to review procedures for calculating meaningful radiation doses to the gonads once Pu is in the body. Points reviewed included the localization near the sensitive cells confirmed in rodents; the possiblity that the RBE may be higher than expected for genetic effects; and an estimate of the quantity passing to the gonads along with some measure of the variability. We will discuss the first two points first.

The work of Green *et al.* (Gr75) has been essentially confirmed by Russell and Linden-

0035004 LANL

baum (Ru79) and other studies so that there is no doubt but that the dose to the sensitive cells in the mouse testes is 2.5–4 times greater than the average dose to this organ. This implies higher genetic effects than would be calculated using the average dose. The careful studies by Brooks show clearly that this localization of dose does not occur in man because of the larger size of the organ and the increased ratio of interstitial tissue to spermatogonia (Br79; Br80). For ovaries, from the studies of Priest and Jackson (Pr78) and those of Taylor (Ta77), it appears that localization near the sensitive cells does not occur and, in fact, the dose to these cells may be lower than the average dose to the ovary.

From these data we conclude that use of the average dose to the gonads is acceptable for radiation protection purposes with no need to require a dose localization factor for the human.

The studies of the effectiveness of the α radiation from Pu in producing various types of genetic damage all used the average dose to the gonads without the localization factor appropriate to the mouse. Even so, the results do not indicate any unusual high effectiveness of the α particles. This is confirmed by the very high doses that were administered to measure any effects; somatic damage from these high doses was significant.

From this it appears that the usual quality factor used in radiation protection work is appropriate and may well be conservative, particularly for the female.

The data on uptake of Pu by the gonads, given in Table 1, show wide variability among species with rodents apparently having a higher deposition per gram although this may be influenced by the method of administration and form of the Pu injected. For purposes of assessing the uptake, the human data is preferable because it eliminates any interspecies difference. The human does, however, suffer from the lack of control found in the animal experiments as is shown by the wide geometric standard deviation. On the other hand, this does represent people taking Pu into their bodies under conditions to be expected in the real world. The agreement in values between workers and the public is encouraging because the exposures are probably to different chemical and physical forms.

Although both the arithmetic and the geometric means are given in Table 1, we prefer to use the arithmetic mean because it represents the average of a large group of people. This permits a realistic average dose to be calculated. From the data in Table 1, it appears that the fractions of the Pu body burdens in the gonads are 6–7 $\times 10^{-5}$ per gram of testes and about 3 $\times 10^{-4}$ per gram of ovary. Using the gonad weights of Standard Man (ICRP75) these values translate to 2–3 $\times 10^{-3}$ for the fraction of Pu body burden uptake in testes and 3 $\times 10^{-3}$ for the ovaries. Within the accuracy of the data it is reasonable to use an uptake of 3 $\times 10^{-3}$ of the Pu body burden for both males and females. Using this uptake factor the dose to the ovaries will be 3–4 times that of the testes. Fortunately, this higher dose to the ovary will be at least, partially, compensated for by the lower RBE for genetic damage in the ovary (Se80). However, using an overall quality factor the dose equivalents to the ovaries will be three to five times greater than that to the testes.

Richmond and Thomas (Ri75) found an uptake in the gonads of 3 $\times 10^{-4}$, about one-tenth the value in this paper. They, however, were limited primarily to animal data and did not have the extensive human information now available.

The ICRP (ICRP79) used the data from the paper by Richmond and Thomas (Ri75) and concluded that the fraction to the testes is 3.5 $\times 10^{-4}$ and to the ovaries 1.1 $\times 10^{-4}$. This resulted in a deposition per gram of 1 $\times 10^{-5}$ for both ovaries and testes. The concentration data in Table 1 indicate that the arithmetic mean measured values in humans are about six times greater for males and 30 times greater for females than the values derived in the ICRP report. Using geometric means these factors reduce to 1.5 and 15, respectively. The fraction in the gonads has a wide variability among individuals as is shown by the geometric standard deviations in Table 1; 11.5 and 12 for males and 8.5 for females. This means that at two standard deviations above the geometric mean the quantities in the gonads of a small fraction of the population could be expected to have at least 3% of the Pu in the body in testes and at least 10% in the ovaries. This variability among individuals seems characteristic of the

0005025 LANL

deposition of Pu (Th84) and perhaps other radionuclides.

It should be recognized that the conclusions drawn in this paper were based upon the best information available and that they may change as future data are accumulated. A review of our conclusions should be made periodically, as was the purpose of this paper, so as to update the input necessary for radiation protection guidelines. There is certainly room for further sophistication in areas concerning dosage localization within the gonads, the relative sensitivity of the various cell types, and perhaps the recommendation of a more quantitatively acceptable value for quality factor. Our primary purpose was to provide a basis from the literature, along with the additional new Pu data from humans, upon which conclusions could more easily be drawn to improve or instill confidence in our current status.

REFERENCES

As78 Ash P. and Parker T., 1978, "The ultrastructure of mouse testicular interstitial tissue containing plutonium-239 and its significance in explaining the observed distribution of plutonium in the testes", *Int. J. Radiat. Biol.* 34(6), 523–536.

Ba66 Bair W. J., Park J. F. and Clarke W. J., 1966, *Long Term Study of Inhaled Plutonium in Dogs*, Air Force Special Weapons Laboratory, Kirtland Air Force Base, NM, Report AFWL-TR-65-214.

Ba79 Barnhart B. J. and Cox S. H., 1979, "Mutagenicity and cytotoxicity of 4.4 MeV α particles emitted by plutonium-238", *Rad. Res.* **80**, 542–548.

Be75 Beechey C. V., Green D., Humphreys E. R. and Searle A. G., 1975, "Cytogenetic effects of plutonium-239 in male mice", *Nature* **256**, 577–589.

Br75 Brooks A. L., 1975, "Chromosome damage in liver cells from low dose rate alpha, beta, and gamma irradiation: derivation of RBE", *Science* **190**, 1090–1092.

Br79 Brooks A. L., Diel J. H. and McClellan R. O., 1979, "The influence of testicular microanatomy on the potential genetic dose from internally deposited ^{239}Pu citrate in Chinese hamster, mouse, and man", *Radiat. Res.* 77, 292–302.

Br80 Brooks A. L., Mewhinney J. W., Redman H. C., Guilmette R. A. and McClellan R. O., 1980, *Distribution, Retention, and Early Cytogenetic Damage in Cynomolgus Monkeys following Inhalation of* ^{239}Pu $(NO_3)_4$, pp. 153–157, Inhalation Toxicology Research Institute, Lovelace Biomedical & Environmental Research Institute, P.O. Box 5890, Albuquerque, NM 87115, Annual Report, LMF-84.

Fo80 Fox T., Tietjen G. L. and McInroy J. F. 1980, "Statistical analysis of a Los Alamos scientific study of plutonium in U.S. autopsy tissue", *Health Phys.* **39**, 877–892.

Gre79 Green D., Howells G. R., Humphreys E. R. and Vennart J. 1979, "Radiation dose to mouse testes from ^{239}Pu", in: *The Health Effects of Plutonium and Radium* (Edited by W. S. S. Jee), pp. 21–29 (Salt Lake City, UT: J. W. Press). Original reference in *Nature* **255**, 77.

Gr77 Green D., Howells G., Vennart J. and Watts R., 1977, "The distribution of plutonium in the mouse ovary", *J. Applied Radiation and Isotopes* **28**, 497–501.

Gr79 Grahn D., Frystak B. H., Lee C. H., Russell J. J. and Lindenbaum A., 1979, *Dominant Lethal Mutations and Chromosome Aberrations Induced in Male Mice by Incorporated* ^{239}Pu *and by External Fission Neutron and Gamma Irradiation*, pp. 163–184, International Atomic Energy Agency Symposium Report, IAEA-SM-237/50 (Vienna: IAEA).

Gr80 Green D., Howells G. and Vennart J., 1980, "Radiation dose to mouse testes from ^{239}Pu", *Health Phys.* **38**, 242–243.

Gr83 Grahn D., Lee C. H. and Farrington B. F., "Interpretation of cytogenetic damage induced in the germ line of male mice exposed for over one year to ^{239}Pu alpha particles, fission neutrons, or ^{60}Co γ-rays", *Radiation Research* (in press).

ICRP72 International Commission on Radiological Protection, 1972, "The RBE for High-LET Radiations with Respect to Mutagenesis", *ICRP Publication* 18 (New York: Pergamon Press).

ICRP75 International Commission on Radiological Protection, 1975, "Report of the Task Group on Reference Man", *ICRP Publication* 23 (New York: Pergamon Press).

ICRP79 International Commission on Radiological Protection, 1979, "Limits for Intakes of Radionuclides by Workers", *ICRP Publication* 30, Annals of the ICRP 2/3-4 (New York: Pergamon Press).

Jo69 Johnson L. J., 1969, *Relative Translocation and Distribution of* Pu *and* Am *from Experimental* PuO_2 *Subcutaneous Implants in Beagles*, U.S. Atomic Energy Commission, Department of Radiology and Radiation Biology, Colorado State University, Fort Collins, CO, Report C00-1787-6. (Available from U.S. Dept. of Energy, Technical Information Center, P.O. Box 62, Oak Ridge, TN 37830.)

La80 Langham W. H., Bassett S. H., Harris P. S.

0005000 LANL

and Carter R. E., 1980, "Distribution and excretion of plutonium administered intravenously to man", *Health Phys.* **38**, 1031–1060.

Li80 Lindenbaum A. and Russell J. J., 1980, "Reply to radiation dose to mouse testes from ^{239}Pu", *Health Phys.* **38**, 243.

Lü76 Lüning K. G., Frolen H. and Nilsson A., 1976, "Genetic effects of ^{239}Pu salt injections in male mice", *Mutat. Res.* **34**, 539.

Mc79 McInroy J. F., Campbell E. E., Moss W. D., Tietjen G. L., Eutsler B. C. and Boyd H. A., 1979, "Plutonium in autopsy tissue: a revision and updating of data reported in LA-4875", *Health Phys.* **37**, 1–136.

Pa69 Park J. F., Howard E. B. and Bair W. J., 1969, *Acute Toxicity of Inhaled* $^{238}PuO_2$ *in Beagle Dogs*, Air Force Weapons Laboratory Report, Air Force Systems Command, Kirtland Air Force Base, NM, AFWL-TR 69–75.

Pr77 Priest N. D., 1977, "The distribution of plutonium-241 in rodents", *Int. J. Radiat. Biol.* **31**(1), 59–78.

Pr78 Priest D. and Jackson S., 1978, "The uptake and redistribution of ^{241}Pu within the gonads", *Int. J. Radiat. Biol.* **34**(1), 49–65.

Ri75 Richmond C. R. and Thomas R. L., 1975, "Plutonium and other actinide elements in gonadal tissue of man and animals", *Health Phys.* **29**, 241–250.

Ru79 Russell J. J. and Lindenbaum A., 1979, "One-year study of nonuniformly distributed plutonium in mouse testis as related to spermatogonial irradiation", *Health Phys.* **36**, 153–157.

Se76 Searle A. G., Beechey C. V., Green D. and Humphreys E. R., 1976, "Cytogenetic effects of protracted exposures to alpha-particles from plutonium-239 and to gamma rays from cobalt-60 compared in male mice", *Mutat. Res.* **41**, 297–310.

Se80 Searle A. G., Beechey C. V., Green D. and Howells G. R., 1980, "Comparative effects of protracted exposures to ^{60}Co γ-radiation and ^{239}Pu α-radiation on breeding performance in female mice", *Int. J. Radiat. Biol.* **37**, 189.

Se82 Searle A. G., Beechey C. V., Green D. and Howells G. R., 1982, "Dominant lethal and ovarian effects of plutonium-239 in female mice", *Int. J. Radiat. Biol.* **42**, 235–244.

Sm79 Smith J. M., Lloyd R. D., Atherton D. R. and Mays C. W., 1979, "The early retention and distribution of monomeric ^{237}Pu (IV) and ^{239}Pu (IV) in C57BL/D mice", in: *The Health Effects of Plutonium and Radium* (Edited by W. S. S. Jee), pp. 7–20 (Salt Lake City, UT: J. W. Press).

Ta77 Taylor D. M., 1977, "The uptake, retention, and distribution of plutonium-239 in rat gonads", *Health Phys.* **32**, 29–31.

Th84 Thomas R. G., Healy J. W. and McInroy J. F., 1984, "Plutonium partitioning among internal organs", *Health Phys.* **46**, 839–844.

LANL

0005607