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PLUTONIUM CONTENT IN MARROW AND MINERALISED BONE IN AN OCCUPATIONALLY EXPOSED PERSON

J. F. McInroy† and R. L. Kathren‡
†Los Alamos National Laboratory
PO Box 1663, MS K-484, Los Alamos, NM 87545, USA
‡United States Transuranium Registry
Hanford Environmental Health Foundation
PO Box 100, Richland, WA 99352, USA

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Abstract — Organic and mineral components of bone from the vertebral bodies of an individual with occupational exposure to plutonium were separated by each of four techniques: (1) chemical separation with ethylenediamine, (2) mechanical separation by a stream of water, (3) mechanical separation by ultrasonic agitation, and (4) thermal separation by heating in an autoclave: all samples were subjected to radiochemical analysis for $^{239+240}$Pu. Nearly all of the total skeletal plutonium was associated with the mineral bone. Concentrations of plutonium in the organic fraction were only a few per cent of those in the mineralised bone. Approximately 3% of the total skeletal plutonium was estimated to be resident in the marrow, with the concentration in the red marrow 2–5 times greater than the concentration in the yellow marrow. This suggests that the dose to the mineralised portion of the bone and closely associated cells, including those of the periosteum and endosteum, may be an order of magnitude or more greater than the dose to the red marrow, resulting in a several fold greater risk from bone tumorigenesis than leukaemias when the relative risk per unit dose is factored in.

INTRODUCTION

Studies in animals and man have clearly established plutonium as a bone seeker initially depositing on bone surfaces. The spatial distribution from plutonium deposited in the skeleton is thus not uniform. The primary carcinogenic risk from deposition of plutonium in the skeleton is associated with the haematopoietic stem cells of the marrow and osteoblasts close to the bone surfaces, namely the cells of the endosteum and periosteum[1,2]. Estimation of the dose equivalent to these cells, and hence the carcinogenic risk, can be determined by application of the ICRP dosimetric model for bone[3,4]. Such estimates require knowledge of the distribution and fate of plutonium within the various tissues of the bone.

There are important spatial–temporal distribution differences as well as similarities in skeletal plutonium distribution in man and animals. In the earliest animal studies it was incorrectly assumed that plutonium tended to concentrate in the non-calcified tissues (i.e. organic component) of the bone, particularly the endosteum and periosteum[5,6]. In subsequent human studies with injected plutonium citrate, Langham and his co-workers[7] observed that bone marrow had the strongest affinity of any tissue for plutonium, accounting for 56% of the injected dose. Langham *et al* did not, however, separate the marrow from the mineral bone samples analysed radiochemically, and further assumed that vertebra, sternum and rib were representative of the skeleton as a whole in making their extrapolation for the total skeletal deposition. In a few studies with animals (beagle, rabbit, mouse) the presence of plutonium in marrow was reported irrespective of the route of entry and led the investigators to call attention to the possible leukemogenic risk[8–13]. The problem is further complicated by the observation in animals that the concentration of plutonium in the marrow is not constant with time[9,14,15]. Some of these animal studies involved the injection of polymeric or colloidal plutonium, which was readily phagocytised by reticuloendothelial (RES) cells in the marrow.

Observations of leukaemia in experimental animals are rare and limited to rodents given large doses of transuranics[16]. More recently, however, it has been demonstrated that myeloid leukaemia as well as osteosarcomas can be induced in mice by ^{239}Pu in bone given appropriate exposure conditions[17], further underscoring the importance of knowing the relative distribution of plutonium in human marrow and mineral bone to determine the dose and potential risk to man. A higher marrow concentration of plutonium may increase the risk of leukaemia above that calculated from the ICRP bone model[4]. If, on the other hand, Pu is concentrated in the endosteum and periosteum or at the interface of the endosteum/periosteum and mineral, as appears to be the case with earliest studies[5–7], or there is a cell sterilisation effect as has been proposed by Thorne and Vennart[18], then the

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leukaemogenic risk will be proportionately less relative to the risk from osteogenic sarcoma.

This paper attempts to provide an estimate of the relative plutonium content and doses to the various components of the skeleton through differential removal or separation of the various constituents of bone by four different methods.

TERMINOLOGY

Since the terminology relating to the various components of the skeleton is sometimes imprecise or may have more than a single meaning, the following definitions have been adopted for the purposes of this paper. These are largely based on the definitions and description of terms provided in ICRP Publication 23[19] but it should be noted that in some instances the definitions or usage of terms herein may deviate from Reference Man or those of some standard anatomy texts and medical dictionaries.

Ash fraction — the residue remaining after wet or dry ashing to remove the water and organic material. The ash fraction is essentially hydroxyapatite.

Bone — an individual discrete or specific anatomically separate unit of the skeleton which consists of both an organic and inorganic (mineral) fraction.

Bone mineral — essentially hydroxyapatite $Ca_{10}[PO_4]_6[OH]_2$, excluding cellular or organic components of the bone matrix.

Endosteum — the tissue lining the medullary cavity of the bone; this is part of the organic fraction.

Marrow — the soft substance filling the medullary cavities of the bone; the marrow is one component of the organic fraction, and consists of fat cells and maturing blood cells, together with suporting connective tissue and blood vessels. The red bone marrow in the adult is a major haematopoietic organ, and is the location of most of the cellular components of the marrow; the yellow marrow is primarily fat, and a few primitive blood cells.

Organic fraction — all bone material other than the bone mineral. The organic component includes the marrow, the periosteum/endosteum and other adherent tissue material including fat, associated water and collagen.

Periosteum — a specialised connective tissue possessing bone-forming potentiality covering the external surfaces of the bone.

Skeleton — the bony framework of the body, including associated cellular components and the red and yellow marrow but specifically excluding the ligaments and cartilage.

METHODS

Four separate methods were used to separate the mineral and non-mineral portions of the bone: (1) extraction with ethylenediamine, (2) mechanical removal by water jet, (3) sonication, and (4) autoclaving. In the water jet procedure, bone is irrigated with a stream of water containing a detergent, washing the marrow from the bone cavity. The sonication method employs ultrasonic agitation in an isotonic solution to separate the marrow from mineral bone[20]. A third method involves heating bone in an autoclave and is used for removing marrow from long bones such as the femur[21]. The ethylenediamine extraction technique was chosen as the standard for comparison purposes as previous studies have shown this method removes all organic material from mineral bone[22]. In addition, ethylenediamine extraction was the only method investigated that involves a direct chemical attack on the organic material although it has been shown that this treatment does not remove Pu from the bone surface during the extraction procedure[23].

Four of the larger vertebrae — the twelfth thoracic vertebra (T-12), and the first, second, and fourth lumbar vertebrae (L-1, L-2 and L-4), were obtained at autopsy from United States Transuranium Registry (USTR) Case 193. This donor was a 62 year old chemical engineer with known occupational exposure to plutonium; most of his exposure was incurred as a result of accidental inhalation exposure some 40 years prior to death from heart disease. This individual had no known history of identifiable bone disease. USTR Case 193 has been described in detail by McInroy *et al*[24].

Adjacent or near adjacent vertebrae were chosen with the expectation that the concentration of Pu would be approximately uniform within a vertebral body and nearly equal between adjacent vertebrae. The intervertebral cartilaginous discs associated with each vertebra were removed with a scalpel, taking care to remove as much as possible of the tightly adherent cartilage without damage to the underlying bone. This effort was not fully successful, as some tightly adherent cartilage could not be removed without damage or removal of the underlying bone, and of necessity, remained attached to vertebral body. The vertebral arches were then removed with a Stryker saw, leaving only the vertebral body.

Each body was divided in half sagittally with a Stryker saw. The right and left halves of each vertebra were identified by the letters 'A' and 'B', respectively, following the specific vertebral identification; for example, L-1-B would refer to the

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Table 1. Weight of bone ash and organic components.

Sample ID	Separation method	Wet wt of bone (g)	Wt of bone ash (g)	Wt of organic material removed (g)	Ash weight / Wet weight
T-12-A	Ethylenediamine	[illegible]	3.43	20.69	0.14
T-12-B	Water jet	[illegible]	4.72	11.51	0.14
L-1-A	Ethylenediamine	[illegible]	7.61	17.33	0.28
L-1-B	Ultrasound	[illegible]	4.35	6.25	0.19
L-2-A	Ethylenediamine	[illegible]	9.57	17.25	0.30
L-2-B	Autoclave	[illegible]	5.55	8.16	0.22
L-4-A	Ethylenediamine	[illegible]	4.92	16.85	0.21
L-4-B	Autoclave	[illegible]	7.08	17.80	0.24

left half of the first lumbar vertebral body. Wet weights were obtained on each vertebral body half after division and are given in Table 1. Ethylenediamine extraction was carried out on the right half ('A') of each of the four vertebral bodies according to the procedure previously described by Miglio and Willis[23]. The extractant was a 20% solution of ethylenediamine in water. Bone samples were subjected to ethylenediamine extraction until the bone material remaining in a cellulose thimble used for the extraction procedure appeared to be white and free of organic matter. This step required a minimum of two eight hour extractions with ethylenediamine. The bone material remaining and the extracted organic material were dried before ashing.

The water jet extraction was performed on the 'B' (i.e. left) half of vertebral body T-12 in the following manner. Sample T-12-B was further divided approximately into halves by cleaving with a large knife. Use of a knife minimised the amount of bone dust and fragments that adhered to either portion of the subdivided vertebral body. Approximately 1 g of Alconox* detergent was added to a 1 litre beaker filled with deionised water. This solution was poured into the reservoir of a commercially available water pump (Water Pik**). Sample T-12-B was placed in a basket fabricated from a stainless steel screen and suspended in a 2 l beaker. A stream of Alconox solution was directed at the material in the marrow spaces of the vertebral body. The bone was washed until no further material appeared to be removed. The wash water and removed organic material were collected in the 2 l beaker. The bone was centrifuged to remove water, and this water was added to the solution in the beaker. The wash solution was evaporated to dryness prior to analysis. The bone material was weighed prior and subsequent to extraction and the weight of the material removed determined from the difference.

The section of bone selected for the ultrasound treatment, L-1-B, was cleaved with a knife into three pieces. This increased the surface area of the exposed marrow cavities and allowed a more complete removal of the organic material from the interior of the bone. The transer medium was 0.8% NaCl solution. Initially, each piece of bone was rinsed with a saline solution, then placed in fresh saline solution and given a short burst of ultrasound. The purpose of the saline rinses and the short burst of ultrasound was to remove any bone dust produced in any of the cutting steps. These saline rinses were combined and analysed for Pu. Each segment of bone from L-1-B was then treated separately by repeated bursts of ultrasonic agitation in 150 ml of saline solution. After the final ultrasonic treatment the remaining bone material was centrifuged to remove saline solution retained in the narrow spaces and then weighed. The saline solution removed by the centrifuge procedure was added to the saline solutuion from the ultrasonic agitation and then analysed for Pu.

Bone segments L-2-B and L-4-B were separately treated in a commercially available pressure cooker used as an autoclave. Segment L-2-B was cut into three pieces with a knife and placed on the convex side of a ribbed watch glass which was, in turn, placed in a tared beaker. This arrangement allowed liquid to drain to the bottom of the beaker. Water was added to the pressure cooker, the top was put in place, and heat was applied until the pressure cap rocked gently. Heating was continued for 30 min, after which the contents were allowed to cool before the top was removed. After cooling, the bone was removed and weighed. The organic material in the beaker was also weighed. Segment L-4-B was not cut into smaller pieces but was otherwise treated in the pressure cooker in a matter identical to L-2-B. After cooling, the beaker containing sample L-4-B

* Alconox, Inc., New York, NY 10003, USA.
** Thermodyne Water Pik, Fort Collins, CO, USA

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was removed from the pressure cooker and placed in an oven for drying at 200°C for 48 h.

The weight of the organic material removed by the ethylenediamine technique was determined by subtracting the weight of the dried mineral bone after extraction from the weight of the wet bone (Table 1). The bone segments from the water jet technique and ultrasound treatment were centrifuged and weighed. The weight after centrifuging was subtracted from the wet weight of the bone to give the weight of organic material. For segment L-2-B the weight of the organic material removed from the bone was the difference between the weight of the bone before the autoclave treatment and the weight of the bone after the autoclave treatment. For segment L-4-B the weight of organic material removed was the difference in weight before autoclave treatment and the weight of the bone after drying in the oven.

Except as noted above for sample L-4-B, which was dried for 48 h, all samples were dried at 200°C for approximately 15 h prior to ashing. Dry ashing was initiated at 250°C and the temperature was increased in increments of 50° until after 9 h a final temperature of 450°C was reached. Samples were maintained at 450° for 48 h. The resulting ash was alternately wet ashed with nitric acid and dry ashed at 350°C until all organic material appeared to be oxidised as indicated by the absence of black carbonaceous residue. Ash weights of bone samples were determined after a subsequent heating at 450°C for 24 h. Residues were dissolved in 9 M HCl and aliquots were taken for analysis according to the method of Boyd *et al*[25] as refined and described by Boyd *et al*[26]. The aliquots were spiked with ^{242}Pu to monitor the chemical recovery. The plutonium was separated by anion exchange on a column of Bio-Rad AG-1X4, 100–200 mesh resin in the chloride form*. The columns were washed with 9 M HCl followed by 7.8 M HNO_3, and the Pu was eluted with 1.2 M HCl–2% H_2O_2. Plutonium was determined by alpha spectroscopy after being electrodeposited onto stainless steel planchets.

Calcium in bone and organic material was determined by titration past the end point with ethylenediaminetetraacetic acid followed by back-titration with a standard zinc solution. Eriochrome Black T was used as the indicator.

Inadvertent removal of any mineralised bone and its associated plutonium by the various extraction procedures represents a potential source of error that would result in an artificially high plutonium content in the organic fraction and a concomitant reduced quantity of plutonium in the ash. The organic fraction extracted by each of the four volunteers.

* Bio-Rad Laboratories, 32nd and Griffin Avenue, Richmond, CA 94804, USA.

methods was analysed for calcium to determine if mineralised bone was also removed by the process. The method used for the determination of Ca was sufficiently sensitive to detect 0.01 g Ca per g of sample. When corrected for an ethylenediamine blank, no Ca was detected in organic material removed by the ethylenediamine extraction technique. No Ca was detected in the organic material removed by the ultrasound or autoclave techniques. The sample treated by the ultrasound technique received an effective preliminary treatment to remove bone dust. The organic material removed from segment T-12-B by the water jet contained 0.12 g of Ca. The likely source of this Ca is bone dust from the cutting operations and from the mechanical action of the water jet which could have broken off and hence removed small bone spicules. No Ca was found in the Alconox detergent at the concentration used and therefore this reagent did not contribute to the Ca observed in the organic material separated by the water jet technique.

RESULTS AND DISCUSSION

The weights of the bone samples before and after extraction, plus the weights of the material extracted are given in Table 1. Examination of these data reveals that in general, the wet weights of the two halves of the vertebral bodies were approximately equal, indicating that the bodies had been more or less equally split. With the exception of the two halves of T-12, the ratio of ash weight to wet weight was typically somewhat greater than the expected range of values for vertebral bodies observed with two previously reported cases[27]. In these cases, the mean ratio of ash weight to wet weight for the approximately corresponding vertebral bodies T-11 through L-5 (N = 10, as not all were analysed) was 0.13 ± 0.02, as compared with a mean of 0.21 ± 0.06 for the eight half vertebral body samples in this case. The reasons for this difference are not readily apparent but could be related to differences in the ashing procedures used, which may have resulted in incomplete ashing in this study. In the earlier study, the complete vertebral body, including the marrow and other organic components, was ashed, while in this study only the bone material remaining after extraction was ashed. Individual variability may account for at least a portion of the difference. It should be noted that the relationship between ash fraction and concentration previously observed by Kathren *et al*[27], was also present in the eight individual samples from this case (Figure 1).

The concentrations of Pu measured in the organic material that was removed from the bone and residual mineral (i.e. ash) fractions of the eight vertebral halves are shown in Table 2. In all cases,

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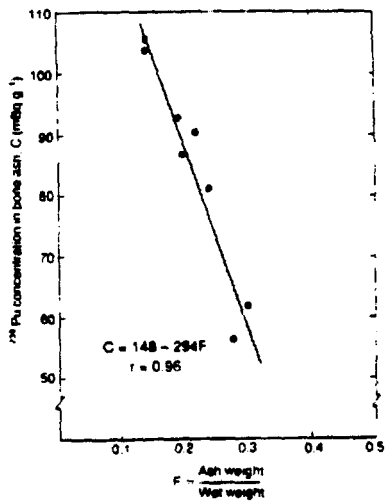

Figure 1. Relationship of bone ash and ^{239}Pu concentrations in vertebral bodies of an occupationally exposed individual (USTR Case No 193). C = 148–294 F. = 0.96.

the concentrations of Pu in the organic components (i.e. the material removed from the bone) are significantly smaller — typically on the order of a few per cent of the concentration in the corresponding inorganic fraction (i.e. ash). This is in substantial agreement with the recent findings of Burkinshaw *et al*[28] who examined plutonium from fall-out in the marrow and mineralised bone fractions of persons from the general population in the United Kingdom. In addition, the two samples treated by autoclaving had significantly lower Pu concentrations in the removed fraction than that observed in any of the other samples. Since the autoclave technique appeared to remove primarily fat and associated water (i.e. yellow marrow, but not the cellular components of the marrow or the periosteum/endosteum), the results suggest that the Pu content of yellow marrow is relatively small, and that what plutonium is in the organic fraction is associated with some other component of the organic material, most likely the cellular components of the marrow and the periosteum/endosteum.

In all cases the total plutonium content of the organic material was significantly less than that in the residual associated inorganic fraction or ash. For the four half-vertebrae treated by ethylenediamine extraction, 12.0 ± 3.8% of the total plutonium in the bone was in the portion that was removed. For the other three methods, this percentage was significantly lower, being lowest for the two autoclaved samples (1.2 and 0.5%), intermediate for the sonication (4.5%) and greatest for the water jet (6.4%). The value for the water jet may be high because of the small amount of bone that was apparently removed by this method, as indicated by the presence of calcium (0.12 g) in the removed fraction.

Although the data shown in Tables 1 and 2 are limited, they can be used to provide at least a crude semiquantitative indication of the plutonium content in the various fractions of bone: i.e. (1) ash or inorganic fraction, (2) red marrow, (3) yellow marrow, (4) periosteum/endosteum. Any such estimates should be considered not only in the light of the limitations of these data, but also with regard to the many uncertainties in the composition of bone and bone marrow, as well as the imprecision in descriptive terminology, both of which have been discussed in some depth by the ICRP in the Reference Man publication[19]. An estimate of the plutonium content of the marrow alone can be made by noting the activity removed along with the

Table 2. Plutonium in bone fractions.

Sample ID	Pu conc. (Bq.kg^{-1})		Conc. in organic / Conc. in ash	Pu content (Bq)			% total Pu in mineral component
	Ash*	Organic*		Ash	Organic	Total	
T-12-A	104 ± 4	3.14 ± 0.14	0.030	0.357	0.065	0.422	84.6
T-12-B	106 ± [illegible]	2.93 ± 0.18	0.028	0.500	0.034	0.534	93.6
L-1-A	56.3 ± 1.9	4.35 ± 0.21	0.077	0.428	0.075	0.503	85.1
L-1-B	93.0 ± 3.2	2.98 ± 0.24	0.032	0.405	0.019	0.424	95.5
L-2-A	62.0 ± 2.0	2.79 ± 0.15	0.045	0.593	0.048	0.641	92.5
L-2-B	90.5 ± 3.0	0.85 ± 0.11	0.009	0.592	0.007	0.599	98.8
L-4-A	86.6 ± 3.1	2.82 ± 0.16	0.033	0.426	0.048	0.474	89.9
L-4-B	81.2 ± 2.7	0.17 ± 0.03	0.002	0.575	0.003	0.578	99.5
				Mean of ethylenediamine treated			88.0±3.8

* Value ± 1 standard deviation obtained from counting statistics

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organic component by each procedure, and that the ethylenediamine extraction removed not only the marrow and associated fat and water, but also the periosteum/endosteum and collagen. Autoclaving appeared to remove primarily the fat and associated water, and relatively little of the cellular material. The sonication and water jet treatments may thus be presumed to remove the marrow, but not the endosteum/periosteum. However, these procedures may in fact disrupt or rupture cells that are not removed (i.e. cells associated with the endosteum/periosteum or within the mineralised matrix of the bone itself) allowing the plutonium contents, if any, in these cells to leak out and add to what was in the removed component. Similarly, the detergent used with the water jet treatment could remove some plutonium from the bone matrix itself, as well as the cellular and collagenous components. Although the contribution from these sources is unknown, were this in fact to occur it would result in a greater measured activity than was actually in the removed component *per se*, effectively setting an upper limit on the estimates discussed below.

The material removed from Sample L-2-B by autoclaving was essentially all yellow marrow, and contained 1.2% of the total plutonium in the bone (Table 2). The sonication treatment, which removed both the red and yellow marrow but apparently no mineralised bone as indicated by the absence of calcium, contained 4.5% of the total plutonium in the bone sample. The material removed by ethylenediamine extraction contained on the average 12.00% of the total plutonium in the bone. If the weight fractions of red and yellow marrow, periosteum/endosteum, and ash are approximately the same for all the bone samples, then these results suggest that for these samples (which being trabecular bone have a relatively large surface area to weight ratio), a significant fraction of the total plutonium in the bone — of the order of 6–7% — would be found in the periosteum/endosteum. Similarly, of the order of 3% of the total plutonium in the bone in these samples is associated with the red marrow. These estimates of the percentage of plutonium in each of the bone fractions are specific to the samples analysed, and do not readily permit generalisation to the skeleton as a whole.

The relative concentrations in the organic and mineralised fractions, however, (Table 2) can be used to estimate the relative amounts of plutonium in the marrow and in the mineralised portions of the bone of the skeleton as a whole. The organic component extracted from samples treated by sonication (L-1-B) and water jet (T-12-B) was assumed to be most representative of the marrow as a whole, while the material extracted from Sample L-2-B was assumed to be representative of the yellow marrow only. In the former two samples the ratio of the concentration in the organic component relative to the concentration in the ash was 0.032 and 0.028, for a mean of 0.030. In other words, the concentration in the marrow was about 3% of the concentration in the ash of the mineralised fraction. Given that the Reference Man skeleton, by weight has approximately equal amounts of ash (2.8 kg) and marrow (3.0 kg)[19], it seems reasonable to conclude that about 3% of the total plutonium in the skeleton is associated with the marrow, if the samples analysed in this study were representative of the skeleton as a whole. However, since these samples were largely trabecular bone, it is likely that the concentration in these samples was greater than the skeleton as a whole and therefore the estimate of the fraction of plutonium in the marrow in the skeleton as a whole, as calculated above, is too low. The data reported by Kathren *et al*[27] for Case 2 indicate that the Pu concentration in the lumbar vertebral bodies could be as much as 2.2 times the average of the skeleton as a whole; if this were true for the case used in this study, then the marrow could contain as much as 3/(97/2.2 +3) or 6.4% of the total skeletal Pu content. This is probably an upper limit. Interestingly, the small percentage of total skeletal plutonium found in the marrow in this case is essentially identical to that (~4%) seen with ^{241}Am in an individual who incurred a massive acute inhalation exposure 11 years prior to his death[29]. Similarly, Miglio and Willis[23] found about 2.4% of the Pu in the femur shaft to be in the marrow mechanically removed from the lumen and an additional 1.5% in the other extracted organic material.

It should be noted that the organic fraction is not exclusively marrow, but rather includes the periosteum/endosteum, fat, and other organic matter as well as associated interstitial water. Thus, although it is not possible to determine the efficacy of each method with respect to removal of marrow, some inferences can be made regarding the removal of the organic component as such and as distinct from the marrow only. An estimate, admittedly tenuous, can also be made of the relative proportions of plutonium in the red and yellow marrow. For the marrow as a whole, as represented by Samples T-12-B and L-1-B, the concentration of plutonium was about 3.5 times greater than the concentration in the organic material removed from Sample L-2-B, which was assumed to be essentially all fat and water. The percentage of red marrow in vertebrae is approximately 60% in a 62 year old adult male[17]. The remaining 40% can thus be assumed to be yellow marrow with a plutonium concentration of 2/7 of that in the marrow collectively. The concentration of plutonium in the red marrow can be calculated as about 1.4 times the average concentration in the marrow as a whole or

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approximately 5 times that in the yellow marrow and about one seventh that in the mineralised bone.

Another way of estimating the plutonium content of the marrow is to extrapolate the data from this case to the values cited for Reference Man[19]. The Reference Man skeleton has 2.8 kg of ash, and 1.5 kg each of red and yellow marrow. Using the mean concentration of plutonium in the ash of the samples extracted with ethylenediamine, which were thought to be most representative, the calculated Pu content in the ash of a total skeleton of Reference Man size would be 2.8 kg × 77 $Bq.kg^{-1}$ = 216 Bq. Similarly the Pu in red marrow would be 1.5 kg × 2.1 $Bq.kg^{-1}$ = 3.2 Bq and that in yellow marrow, 1.5 kg × 0.85 $Bq.kg^{-1}$ = 1.3 Bq. Hence, the percentage of the total skeletal Pu in the marrow (red + yellow) is (3.2 + 1.3)/(216 + 3.2 + 1.3) × 100 = 2.0%, with about 1.5% in the red marrow alone. If the same adjustment is made as above to correct for non-uniform distribution, about 4.5% of the total skeletal content would be in the marrow, of which about two-thirds is in the red marrow. Note that the concentration in ash (77 $Bq.kg^{-1}$) is the average of the four ethylenediamine results, since these presumably had the least content of organic material. The red marrow value (2.1 $Bq.kg^{-1}$) was the average of the sonication and water jet method results (2.95 $Bq.kg^{-1}$), less the higher of the two yellow marrow values (0.85 $Bq.kg^{-1}$). The lower yellow marrow value was not used for reasons previously discussed.

These estimates of the relative concentrations in red and yellow marrow are clearly tenuous and should be considered only as preliminary first order approximations in that they are based on the assumption that the material extracted by autoclaving was essentially all yellow marrow, while that removed by the sonication and water jet procedures was the entire marrow component. This may or may not be the case. The supporting evidence for the assumptions is relatively weak, and in future work the composition of the removed material will be subjected to morphological examination and other tests to verify and validate the estimates. However, the data in this study do indicate that the mean dose to the mineralised portion of the bone, and presumably to the associated osteoblasts and possibly the periosteum and endosteum, is more than an order of magnitude greater than the dose to the red marrow. This observation generally supports the method of dosimetry put forth in ICRP Publications 26 and 30 which considers the bone surfaces to be a tissue at significant risk. However, given that the cancer induction risk per unit dose equivalent is four-fold greater in the marrow than for bone surfaces, it suggests that the relative risk from skeletal deposition of plutonium, at least at long times after deposition, is at least a factor of two greater to the bone surfaces (i.e. for the development of osteosarcoma) than the risk to the marrow (i.e. leukaemogenesis). Clearly, this contention, if verified by additional studies, portends changes in existing radiation protection recommendations.

CONCLUSIONS

As expected, most of the total skeletal plutonium was associated with the mineralised bone. Concentrations of plutonium were more than an order of magnitude greater in the mineralised portions than in the removed organic fraction. Approximately 3% of the total skeletal plutonium is estimated to be resident in the marrow, with the concentration in the red marrow several times greater than the concentration in the yellow marrow. This suggests that the dose to the mineralised portion of the bone and osteoblasts in the periosteum and endosteum may be an order of magnitude or more than the dose to the red marrow, resulting in several times greater a risk from bone tumours than from leukaemias.

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