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W. H. Adams
J. R. Buchholz
C. W. Christenson
G. L. Johnson
Eric B. Fowler

los alamos
scientific laboratory
of the University of California
LOS ALAMOS, NEW MEXICO 87544

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STUDIES OF PLUTONIUM, AMERICIUM, AND URANIUM IN ENVIRONMENTAL MATRICES

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ABSTRACT

A nitric acid-hydrofluoric acid treatment for dissolution of plutonium oxides in soils has been developed; its adaption to other biological matrices is discussed. Plutonium recoveries of 94 to 99% from 1-g samples of spiked and heated soils are reported. Adaptation of the acid solution to subsequent anion exchange separation of plutonium, followed by coupling to known electroplating techniques, is described.

The uptake of plutonium, americium, and uranium from spiked soils by alfalfa, beans, radishes, lettuce, tomatoes, and barley is reported.

The "apparent" solubility of $^{238}PuO_2$ in tap water was measured, and the deposition of plutonium in fish, algae, and snails in aquaria containing $^{238}PuO_2$ microspheres is reported.

I. INTRODUCTION

Problems other than waste disposal problems are routinely posed to the Industrial Waste Group (H-7) of the Los Alamos Scientific Laboratory (LASL) Health Division. Many of these problems are of a short-term, preliminary, investigative nature, and their study usually is terminated after a few months' time. However, the techniques developed and some of the results have general applications and may be of interest to other workers in comparable areas of investigation. Results from a few of these investigations are set forth in this report. It should be recognized that the results were not obtained from long, detailed research projects, so they are presented merely as possible guides to those confronted with similar problems.

II. DEVELOPMENT AND APPLICATION OF THE SOIL DIGESTION PROCEDURE

A simple, rapid method for dissolution of plutonium in a large number (1500) of soil and vegetation samples was needed to assess the environmental impact of an accidental release of plutonium near Palomares, Spain in 1966. Primary requirements set for the method were minimal equipment and space, acceptable accuracy, and ease of application.

A. Standard Soils

To check different plutonium extraction methods, standard soils containing known amounts of $^{239-240}Pu$ were prepared. One portion of a water-deposited, loamy sand soil obtained from the Los Lunas, New Mexico, Experiment Station was heated at 600°C for 2 h to remove organic matter. Another portion of the same soil was heated at 1050°C for 2 h to destroy all ion exchange sites. Both samples were then spiked with plutonium nitrate solution to a level of 8880 dis/min/g of soil and dried at ambient temperature; each was then blended in a "V" blender for 2 h. The first portion was then heated at 300°C for 2 h, and the second at 900°C for 2 h to convert the plutonium nitrate to the oxide. The 900°C-prepared sample would contain a plutonium oxide, probably as difficult to dissolve as that to be found in the Spanish soil samples.

B. Plutonium Dissolution

Dissolution methods investigated included fusion of the standard soil samples with $KF-K_2S_2O_7$,[1] or Na_2CO_3, followed by an appropriate acidic dissolution of the melt, as well as acid extraction of plutonium using hot nitric acid or nitric-hydrofluoric acid solutions. After dissolution, the plutonium could be separated from other radionuclides and nonradioactive salts in solution by adaptation of known techniques. The fusion methods were too tedious for routine use, so they were used only to check acid-treated residues for plutonium.

The plutonium in standard soil samples heated to 300°C was quantitatively extracted from the soil by hot 8 $\underline{M}$ HNO_3, but only 41% was extracted when this acid was used to remove the plutonium from a standard soil that had been heated to 900°C. Hot 8 $\underline{M}$ HNO_3 extraction was rejected because it was expected that the plutonium in the Spanish soils would be more difficult to extract than that in the 300°C-heated standard soil.

Earlier work[2] had demonstrated that plutonium could be removed from soils by refluxing them with 12 $\underline{M}$ HNO_3-0.1 $\underline{M}$ HF solution; this method was effective in extracting plutonium from a 2-g sample of standard soil that had been heated to 900°C (Table I).

In these experiments, plutonium was extracted by refluxing a 2-g sample of standard soil with 80 ml of 12 $\underline{M}$ HNO_3-0.1 $\underline{M}$ HF for 2 h. The residue was removed by centrifugation, and the supernatant was diluted to 250 ml with distilled water. An aliquot of the solution was pipetted onto a 40-mm-diam dished stainless steel planchet, dried, flamed, and counted in an internal proportional counter (50% efficiency) to check plutonium recovery. Although this method effectively removed plutonium from the soil, the use of reflux condensers and 500-ml boiling flasks was too cumbersome and required extensive hood space.

Therefore, a modification of the procedure was sought whereby less expensive glassware could be used and more samples could be analyzed in the available hood space.

A similar method was tried in which a 2-g soil sample was placed in a 40-ml centrifuge tube; 25 ml of 12 $\underline{M}$ HNO_3-0.1 $\underline{M}$ HF were added, and the mixture was heated at 90°C for 15 to 30 min. The tubes containing the soil and acid were then centrifuged,

TABLE I

PERCENTAGE OF PLUTONIUM RECOVERED FROM HEATED NEW MEXICO SOIL

12 $\underline{M}$ HNO_3-0.1 $\underline{M}$ HF (reflux)

Temp (°C)	Sample No.								
	1	2	3	4	5	6	7	8	Av
300°C	105	102	100	102	102	---	---	---	102
900°C	97	86	93	100	96	98	91	87	94

and the supernatant was decanted. The extraction was repeated twice, and an aliquot of the combined supernatants was counted for gross alpha content. This procedure gave 50 to 60% plutonium recovery from the 900°C-heated spiked soil. The recovery was not so good as that obtained in the 2 h reflux because of HF evaporation during the extraction and attendant reduction in matrix and plutonium dissolution. Therefore, HF was increased to 1.0 M and the centrifuge tube was covered with a small watch glass so the acid solution would reflux; also the soil sample size was reduced to about 1 g.

Refluxing 25 ml of the acid solution with 0.5 to 1.0 g of soil for 15 min gave mean plutonium recoveries of greater than 96% (Table II).

TABLE II

PLUTONIUM RECOVERED FROM NEW MEXICO SOIL HEATED AT 900°C, USING 12 M HNO_3-1 M HF ACID SOLUTION

Replicate No.	% Recovered
1	100.5
2	97.5
3	97.0
4	96.0
5	96.5
6	99.0
7	94.2
8	95.5
9	105.0
10	90.6
11	91.7
12	95.5
13	98.0
14	98.0
15	96.4
16	98.4
x	96.9 ±3.3 (1σ)

The acid digestion procedure was applicable to a wide variety of sample matrices, after the specific predigestion treatments listed below.

1. Soils. Air-dried soil samples of 1 g or less were heated at 600°C for 3 h to remove organic matter. Three hours' time was sufficient because the soils contained small amounts of organic matter; if soils with high organic matter content are to be analyzed, it may be necessary to increase the heating time.

2. Vegetation. A freshly cut sample of vegetation which would yield 1 g or less of plant ash (ash weight of many plants is 1 to 4% of the wet weight) was dried at 110°C for 12 h. The dried plant was then ashed at gradually increasing temperatures beginning at 200°C and increasing 100°C each hour to 600°C. The sample was then ashed at 600°C for an additional 12 to 15 h, at which time the carbon had been converted to the carbonate or volatilized as CO_2 and the ash was ready for the acid treatment. Attempts to wet ash plant samples using HNO_3-H_2O_2 or HNO_3-$HClO_4$ acids were not successful because of the formation of substances that interfered with the plutonium sorption or the washing of the sorbed plutonium in the subsequent anion exchange separation steps.

3. Aqueous Waste Samples. Liquid waste samples from the treatment plants of the Liquid Waste Management Group (H-7) contain a variety of dissolved salts, radionuclides, and various other materials in suspension which are collected from different laboratory buildings in Los Alamos. These samples were adapted to the acid procedure by using a 100- to 500-ml aliquot, adding 10 ml of concentrated HNO_3, and evaporating to dryness under an ir heat lamp. The residue was then subjected to the HNO_3-HF extraction to dissolve the plutonium. (Larger 10- to 15-liter aqueous samples, such as those associated with aquarium experiments, have also been acidified with nitric acid and evaporated to

dryness.) The dry residue containing the plutonium was then treated with HNO_3-HF before the plutonium was separated by anion exchange.

C. Application of an Acid Treatment Procedure

1. Acid Dissolution Procedures Presently Used at LASL. Almost all of the soil samples and portions of the plant ash samples of vegetation from Palomares, Spain were placed in 50-ml centrifuge tubes and mixed with 25 ml of 12 M HNO_3-1 M HF. The tubes were covered with a 35-mm-diam watch glass and placed in a heated aluminum block that had been drilled to accept 9 to 12 centrifuge tubes. The samples were refluxed for 15 min, cooled, and centrifuged; the supernatant was decanted into a volumetric flask and diluted to volume. A gross alpha count was made on an aliquot taken from the flask. Some of the acid solution samples were processed through a plutonium recovery anion exchange procedure.

Whereas the centrifuge tubes proved satisfactory for small soil samples and portions of plant ash, some biological samples and portions of plant ash, as well as other biological samples such as tomato fruit and leaves and certain grasses, were easier to process if the sample were dried, ashed, and treated with acid in the same appropriate-size beaker.

The acid procedure finally developed, which can be used on most types of soil, vegetation, and liquid waste, is as follows. Twenty-five ml of 12 M HNO_3-1 M HF are added to an appropriate-size beaker containing a 1-g soil or plant ash sample or the residue from an acid evaporation of a liquid waste sample. The beaker is covered with a watch glass, and the mixture is heated on an oscillating hot plate and allowed to reflux for 30 min. The acid mixture is centrifuged to remove fine suspended material, and the supernatant is decanted into a 100-ml beaker. This extraction is repeated, and an additional rinse of 10 to 20 ml of hot 8 M HNO_3 is used. The supernatants are all combined in the 100-ml beaker, 3.5 g of $Al(NO_3)_3 \cdot 9H_2O$ are added to complex the remaining free fluoride, and the solution is then evaporated to dryness under an ir heat lamp.

2. Anion Exchange Recovery of Plutonium. The dry plutonium residue from the acid treatment procedures, Sec. C. 1., is treated with 10 ml of concentrated HNO_3 and 1 ml of 30% H_2O_2, to form tetravalent plutonium, and then evaporated to dryness on a steam bath. The plutonium is next dissolved in 8 M HNO_3 and passed through an ion exchange column loaded with AG-1 x 2,* 50- to 100-mesh, anion exchange resin that has been converted to the nitrate form. The column is a 40-ml reservoir attached to a 1-cm-diam by 7-cm-long column that has been drawn down to a 1-mm-diam drip tip. After sorption of the plutonium, the resin column is washed with 10 to 20 column volumes of 8 M HNO_3 (about 40 to 100 ml total wash solution) to remove salts. The plutonium is then eluted with 25 ml of a solution composed of 0.36 M HCl and 0.01 M HF.[3] The eluate is evaporated to dryness on a steam bath, plutonium is removed from the beaker with an 8 M HNO_3 wash, and the solution is diluted to a known volume.

An appropriate aliquot is pipetted onto a 40-mm-diam stainless steel dished planchet, dried, flamed, and counted in an alpha proportional counter. Smaller aliquots can be placed on a smaller stainless steel planchet, dried, flamed, and counted in an alpha scintillation counter, or the plutonium solution from the column can be subjected to an electrodeposition technique (see Sec. C. 3) to improve resolution in subsequent alpha spectrometer analysis.

An adaptation of Talvitie's[4] method has been successfully used at LASL for determination of plutonium in waste samples. The method consists of taking the residue from

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*A Dowex-1 ion exchange resin that has been purified and sized. Available from Bio-Rad Laboratories, Richmond, CA.

liquid waste samples (see Sec. B.3.), adding 20 ml of 9 M HCl and 0.1 ml of 30% H_2O_2, covering with a watch glass and heating for 1 h on a steam bath, cooling the solution, and passing it through an anion exchange column packed with AG 1 x 2, 50- to 100-mesh resin in the chloride form. The column is washed with 3 to 4 column volumes of 9 M HCl, followed by 10 column volumes of 8 M HNO_3. The plutonium is then eluted from the column with 50 ml of 1.2 M HCl containing 1 ml of 30% H_2O_2. (At this point, the plutonium solution is evaporated to dryness on a steam bath and the plutonium is dissolved in 8 M HNO_3, pipetted onto a 40-mm-diam dished planchet, dried, flamed, and counted.) For electroplating, add 0.5 ml of 18 M H_2SO_4 to the eluate and evaporate until only sulfuric acid remains, then electrodeposit as described by Talvitie.[5]

3. Electrodeposition. The 0.36 M HCl-0.01 M HF eluate from the anion exchange separation of plutonium was evaporated to dryness on a steam bath. The residue was fumed once with 5 ml of concentrated HCl and taken to dryness. One-half ml of 4 M HCl was then added to the residue contained in a 50-ml beaker, 3 ml of 4% ammonium oxalate were added, and the contents was transferred to an electroplating cell. A distilled water rinse of the beaker was added to the cell, and the solution in the cell was diluted to about 10 ml with distilled water. The plutonium was electrodeposited on a 22-mm-diam stainless steel planchet for 5 h at 200-mA current. The solution in the cell was then neutralized with 10% NH_4OH using phenolphthalein indicator just before turning off the electroplating power supply. The planchet was washed with distilled water, dried, flamed, and counted. The recovery of ^{236}Pu (internal standard) added to a soil or plant ash sample that was then processed through acid treatment, anion exchange separation, and electrodeposition was about 75%. The electrodeposition procedure efficiency was about 89% if essentially no iron was present in the plutonium eluted from the anion exchange column.

The procedure now used in the H-7 Laboratory Section is the one reported by Talvitie,[5] and it requires only conversion to a sulfate system, whether a nitrate or a hydrochloric acid anion exchange procedure is used for the plutonium isolation. The plutonium is electrodeposited on a 19-mm-diam polished stainless steel disk cathode from a sulfate system at pH 2 using 1.1 to 1.2 A at 10 to 15 V. The electrodeposition efficiency is essentially 100%; deposition time is reduced to 1 h.

4. Counting. Samples not containing a ^{236}Pu internal standard for yield determination were counted in an alpha proportional counter with 50% efficiency, or on an alpha scintillation counter with 45.5% average counting efficiency. Samples containing a ^{236}Pu tracer were electrodeposited on 19-mm polished stainless steel planchets and counted in an eight-detector 1024-channel alpha spectrometer. Measurements were made with a 300-mm "Ortec" silicon surface barrier detector operated in a vacuum chamber. The average counting efficiency for the eight detectors was 18.4%, and the resolution was 35-KeV FWHM for the 5.5-MeV ^{241}Am alpha peak.

D. Discussion

The 12 M HNO_3-1.0 M HF acid treatment using a single digestion procedure effectively removed plutonium from 1-g samples of Spanish soil. When Na_2CO_3 fusion of residues from an acid treatment followed by anion exchange isolation of plutonium was used, the additional plutonium recovery was not significant. In eight soil samples taken from the device impact area in Palomares, the plutonium recovered from the acid treatment residues by carbonate fusion averaged 0.36% of the total plutonium found. The impact area soil samples averaged 165 000 dis/min/g, and the acid treatment recovered more than 99% of the total plutonium, based on the assumption that the acid digestion plus the carbonate fusion of the acid treatment residue represented total plutonium present. In eight other Palomares soil samples collected in areas other than the impact area and containing an average of 6000 dis/min/g of soil, an average of 5.4% of the total plutonium found was recovered by a Na_2CO_3 fusion of the previously acid-digested residues. Although in this case the average recovery by digestion of the soil with hot acid was only 94.6%, this was still considered satisfactory plutonium recovery.

Hardy and Krey[6] found that Chu's[7] acid treatment procedures using HNO_3-HCl solutions were effective for quantitative plutonium extraction from soils. Hamada[8] also used a HNO_3-HF solution to recover plutonium from 10- to 200-g soil samples. Talvitie[4] used a NHO_3-HF acid treatment followed by an HCl treatment of the residue to dissolve the plutonium in a 1-g soil sample; he reported an average plutonium recovery of 94% from 125 one-g soil samples taken through the entire procedure which included anion exchange separation and electroplating of the plutonium in addition to his acid treatment. Hammond[9] used a HNO_3-HF leach after an initial fuming with concentrated HNO_3; these two acid treatments were then followed by an additional fuming with HNO_3-HCl. A method now used at LASL to dissolve the plutonium in 10-g soil samples from the Nevada Test Site, consisting of a HNO_3-HF acid digestion followed by addition of HCl, is called the LASL-HASL Acid Digestion Method. Plutonium recoveries of 90+% have been attained using the LASL-HASL acid treatment,[10] an anion exchange separation, and electrodeposition of plutonium.

For plant ash, a single 15-min acid extraction (HNO -HF) of plutonium from 1 g of Artemisia hispanica (Sage) plant ash gave erratic results (Table III), indicating that one-time leaching of plant ash is not reliable. However, plutonium was successfully recovered from a wide variety of plants that had been spiked with plutonium, dried, ashed at 600°C, extracted

TABLE III

PLUTONIUM RECOVERED FROM PLANT ASH USING
12 M HNO_3-1 M HF ACID TREATMENT
FOLLOWED BY Na_2CO_3 FUSION OF THE RESIDUE

Sample[a]	Pu Recovered Acid Digestion (dis/min)	Pu Recovered Na_2CO_3 Fusion (dis/min)	Total Pu dis/min)	Recovered Acid Digestion (%)	Recovered Na_2CO_3 Fusion (%)
G-88-1	91	68	159	57	43
G-88-2	287	68	355	81	19
G-88-3	104	68	177	62	38
G-88-4	276	52	328	84	16
G-88-5	54	152	206	26	74
G-88-6	44	102	146	30	70
G-88-7	336	354	690	49	51
			Av	56	44

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[a] Artemisia hispanica ashed at 600°C

twice with 12 M HNO_3-1.0 M HF, and subjected to the anion exchange recovery procedure (Table IV).

Ninety-two to ninety-nine % of the plutonium added as a spike to 1-g samples of three different types of soils was recovered using the HNO_3-HF acid treatment followed by anion exchange separation of the plutonium (Table IV). Under the conditions of the latter analyses, there were no apparent recovery problems. The samples were not spiked with ^{236}Pu tracer, because the exact amount (essentially a one-isotope source) of plutonium was known and direct recoveries could be determined. Note that use of an internal standard spike of ^{236}Pu in an unknown soil or vegetation sample merely evaluates the chemistry and recovery techniques from the point of addition to completion of the procedure. One does not have complete assurance that added plutonium is in, or will be converted to, the same chemical state as that plutonium already associated with the unknown sample. One should be aware, however, if analyzing for ^{238}Pu, that ^{236}Pu standards do contain from 0.2 to 0.7% ^{238}Pu,[11,12] which could cause problems in determining the correct amount of ^{238}Pu present in the original sample if no corrections were made.

Kressin et al.[13] have proposed use of ^{242}Pu as an internal standard for determining chemical yield in analysis for low levels of ^{238}Pu and ^{239}Pu. Using ^{242}Pu does not eliminate the addition of small amounts of ^{238}Pu as impurities with the standard, because the standard contains nearly 2% ^{238}Pu and more than 1% ^{239}Pu. However, the ^{242}Pu decay products do not have alpha energies in the region of ^{238}Pu or ^{239}Pu, and the ^{242}Pu need not be separated from interfering daughters and recalibrated as does a ^{236}Pu standard.

III. PLANT UPTAKE OF ALPHA ACTIVITY FROM SOILS CONTAINING PLUTONIUM, URANIUM, OR AMERICIUM

A. Soils Spiked with $^{238}PuO_2$ Microspheres

Use of $^{238}PuO_2$ microspheres in Systems for Nuclear Auxiliary Power (SNAP) devices prompted study of the possible effect of microspheres if they were deposited accidentally on plants and soils. Studies were initiated to determine the translocation of plutonium in plants contaminated with $^{238}PuO_2$ microspheres and the plant uptake of plutonium from soils containing $^{238}PuO_2$ microspheres. Translocation of plutonium deposited on the plant leaves could not be determined, because the 100-μm-diam microspheres contained enough

TABLE IV

PLUTONIUM RECOVERED FROM PLANT AND SOIL SAMPLES SPIKED WITH PLUTONIUM
(12 M HNO_3-1 M HF Acid treatment followed by anion exchange)

Sample	Av Wet Wt(g)	Av Dry Wt(g)	Av Ash Wt(g)	Pu Added (dis/min)	Av Pu Recovered (%)	Recovered (Range) (%)	No. of Determinations
Alfalfa	50	11.2	0.877	104	92.4	87 - 102	8
Grass (foxtail)	25	9.8	0.704	104	95.8	90.9 - 99.3	8
Tomato leaves	25	2.8	0.538	5006	90.4	75.8 - 99.8	15
Tomato leaves	25	2.6	0.526	102	96.1	95.1 - 98.0	6
Tomato fruit	112	7.4	0.675	104	94.4	85.1 - 108.8	8
New Mexico Soil	---	0.98	---	104	99.0	93.1 - 105.6	8
Kansas Soil	---	1.00	---	104	94.2	87.5 - 102.4	8
S. Dakota Soil	---	1.01	---	104	92.4	86.2 - 102.4	8

plutonium to burn the plant tissue. The experiment was terminated because of this destruction of plant tissue and the subsequent difficulty in locating and recovering the microspheres.

Thompson[14] likewise found that $^{238}PuO_2$ microspheres burned holes in African violet leaves, causing microspheres to drop through the leaves. Damage to the leaves was noticeable on the second or third day of exposure.

In the plant uptake studies, Los Alamos mountain meadow soil containing 5% organic matter and 0.24% nitrogen was air-dried and screened; the part that did not pass through a 20-mesh screen was discarded. One and two-tenths kg of the sieved, air-dried soil were spiked with 25.6 mg of $^{238}PuO_2$ microspheres and mixed in a large "V" shell blender for 5 h. After 800 to 900 g of unspiked soil was placed in a plastic container 15 cm in diameter and about 13 cm deep, 200 g of the spiked soil was added to the container, followed by another 800 to 900 g of unspiked soil. Six plastic pots were filled in this manner; three were planted to lettuce and three were planted first to oats and later to barley.

The miniature soil plots* were then placed inside a 1.22-m-long, 0.91-m-high, 0.91-m-deep Lucite growth chamber. The chamber was used as a precautionary measure to contain the radioactivity and to keep the plants isolated from other plants grown in the greenhouse. The Lucite box was equipped with two 15-cm-diam portholes with moveable covers. Two exhaust fans mounted to the box exhausted air through a HEPA filter into the greenhouse. The portholes were used as access holes for plant watering and harvesting. Clean surgeon's gloves were used while watering or harvesting the plants. Whenever possible, the plants were cut at such a height that successive cuttings could be obtained from a single planting.

The effect of long exposure of the microspheres to the soil, along with the effect of certain stresses such as freezing and thawing upon the degradation of the microspheres in the soil, was not known. Therefore, the plots of soil were planted, then let lie fallow, then subjected to freezing and thawing cycles to see whether the microspheres would be degraded so as to increase their solubility, as indicated by increased plant uptake of plutonium.

After two lettuce crops and three oat crops were harvested, the soil plots were allowed to lie fallow before being replanted to lettuce and barley on the 474th day after the original soil was spiked. After an additional lettuce and barley crop were harvested, the soil plots lay fallow for 55 days and then were placed in a plastic bag and placed outdoors for the next 6 months (March 21 to September 21, 1967). The plots were then planted to lettuce and barley, and two crops were harvested.

The miniature soil plots were then frozen with dry ice and allowed to thaw at room temperature; this procedure was repeated 10 times before the soil was again planted to lettuce and barley. Five crops were harvested, beginning with the barley harvest on the 890th day after the soil was spiked. The plots then lay fallow for 80 days before being replanted. Two crops were harvested, and another 186 days passed before a planting was made on the 1374th day after the soil was spiked. This crop was harvested 70 days later, or 1444 days from the time the soil was spiked. A final crop of barley planted at about 2730 days was harvested at 2790 days and analyzed for plutonium. This final barley crop concluded a study initiated about 7 1/2 yr earlier.

All of the plants grown in this study were ashed at 600°C as described in Sec. II.B.2. The plant ash, however, was digested with 8 $\underline{M}$ HNO_3 or mixed acids and the

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*These plots contained in 15 cm-diam by 13 cm deep plastic pots are hereafter called plots or miniature soil plots.

plutonium was separated from the dissolved salts by anion exchange. The plutonium was electrodeposited from an HCl-$(NH_4)_2$ C_2O_2 solution .or the modified Talvitie method and counted in an alpha scintillation counter.

B. Discussion

Although the $^{238}PuO_2$ microspheres placed in the soil were weathered and exposed to other stresses in the soil, very little plutonium was converted to a "soluble" form, as shown by the plants' limited plutonium uptake (Table V). The concentration factor was calculated by dividing the empirically determined plutonium concentration in the plant (expressed as dis/min/g of plant ash) by the calculated concentration of plutonium in the spiked layer of soil (expressed as dis/min/g of soil).

Three kg of fresh lettuce or about 0.9 kg of green barley, grown in soil contaminated with plutonium to the level used in these experiments, would contain the same amount of ^{238}Pu expressed as the maximum permissible concentration of plutonium per liter of drinking water for public use.[15] These plutonium concentrations in lettuce and barley were calculated using the highest plutonium concentrations found in analyses of the different crops (1444-day barley, 332 dis/min/g; 1148-day lettuce, 170 dis/min/g (Table V)) and the values of 2.1 and 4.0% ash (expressed as a percentage of the weight of a freshly cut sample) for lettuce and barley, respectively.

If 10 kg of $^{238}PuO_2$ microspheres were mixed uniformly with soil by plowing to a depth of 15 cm, 4047 m^2 (1 A) of soil would be contaminated with the same concentration of ^{238}Pu used in these experiments. However, experience has shown that the high-altitude loss of a SNAP device did not cause high-level contamination of a relatively small area of soil, but rather an almost uniform distribution of $^{238}PuO_2$ as fallout upon the earth.[16] Such low ^{238}Pu contamination levels would not produce measurable amounts of ^{238}Pu in plants as a result of plant uptake on the basis of results obtained in the experiments described in Sec. III.A. and results summarized in Table V.

C. Soils Spiked with $Pu(NO_3)_4$ Solutions

Experimental minature soil plots similar to those discussed above were set up, using either 1.5 or 5 kg of soil in cylindrical plastic containers. Soils used in these plots were Los Alamos mountain meadow soil, New Mexico water-deposited loamy sand, Kansas loess, South Dakota glacial till, and soil from Palomares, Spain.

The soils were spiked with alpha-emitting radionuclides as follows: Separate samples of Los Alamos mountain meadow soil were spiked with ^{239}Pu, ^{232}U, or ^{241}Am nitrate solution (0.1 M HNO_3) ranging from 4 x 10^4 to 1.4 x 10^6 dis/min/g of dry soil. Spanish soil was spiked with $^{239}Pu\ (NO_3)_4$ solutions (0.1 M HNO_3) to 80 000 to 380 000 dis/min/g of dry soil. Neither soil was heated to convert the plutonium to the oxide. Small portions of New Mexico loamy sand, Kansas loess, and South Dakota glacial till were spiked with $^{239}Pu(NO_3)_4$ solution (0.1 M HNO_3), dried, mixed well, and heated to 300 or 900°C. The spiked and heated soils were then mixed well with unspiked portions of the same soils so that the final soil plots contained 7.4 x 10^5 to 1 x 10^6 dis/min/g of dry soil.

Plots were planted to tomatoes, alfalfa, beans, barley, lettuce, or radishes. When the alfalfa, lettuce, or barley was nearly mature, the plants were cut back to a height that would allow them to reestablish normal growth, permitting several crops from one planting. The harvested plants were dried at 100°C and ashed at 600°C as described in Sec. II.B.2. Radishes were pulled, washed to remove the soil, and separated into bulbs and tops, each part being analyzed for plutonium.

In all cases, the ash from plants or plant parts was treated three times with

TABLE V

PLUTONIUM CONCENTRATION IN PLANT TOPS GROWN IN SOILS SPIKED WITH $^{238}PuO_2$

(Plutonium soil concentration is 6.5×10^8 dis/min/g, calculated.)

Crop	Days Time From Spike To Harvest	Plutonium dis/min/g of Ash	C_p/C_s[a]
Lettuce	74	13	2×10^{-8}
Oats	74	72.7	1.1×10^{-7}
Lettuce	145	80.7	1.2×10^{-7}
Oats	145	82	1.3×10^{-7}
Oats	186	3.5	5.4×10^{-9}
Lettuce[b]	544	Less than control	
Barley[b]	544	9.5	1.5×10^{-8}
Lettuce[c]	811	25.6	3.9×10^{-8}
Barley[c]	811	7.1	1.1×10^{-8}
Lettuce	825	36.4	5.6×10^{-8}
Barley	825	1.6	2.5×10^{-9}
Barley[d]	890	1.5	2.3×10^{-9}
Lettuce	909	31.2	4.8×10^{-8}
Barley	909	5.7	8.7×10^{-8}
Lettuce	949	19.9	3.1×10^{-8}
Barley	949	9.2	1.4×10^{-8}
Lettuce	992	15.5	2.4×10^{-8}
Barley	992	25.2	3.9×10^{-8}
Lettuce	1019	29.5	4.5×10^{-8}
Barley	1019	50.6	7.8×10^{-8}
Lettuce[e]	1148	169.9	2.6×10^{-8}
Barley[e]	1148	50.8	7.8×10^{-8}
Lettuce	1188	88.4	1.4×10^{-7}
Barley	1188	84.4	1.3×10^{-7}
Lettuce[f]	1444	143	2.2×10^{-7}
Barley	1444	332	5.1×10^{-7}
Barley	2790	34	5.2×10^{-8}

	Lettuce	Barley	Oats
Av Conc Factor	6.8×10^{-8}	8.8×10^{-8}	8.2×10^{-8}

---

[a] Concentration factor is dis/min/g of plant ash ÷ dis/min/g of soil.

[b] Soil lay fallow for approximately 9 1/2 months--replanted at about 474 days after soil spiking.

[c] First harvest after 6 months weathering out of doors.

[d] First harvest after 11 freeze-thaw cycles.

[e] First harvest after soil lay fallow for 80 days.

[f] Harvest of crop planted 1374 days after soil lay fallow for 186 days.

hot 12 M HNO_3-1 M HF. The acid solution containing dissolved plutonium was processed through the anion exchange and plutonium electrodeposition procedures outlined in Secs. II.C.2. and 3. The plutonium was thus separated from the salts in solution and other alpha emitters, such as uranium or americium, that might have been present in the Spanish soils.

The acid digestion solutions of ash from plants grown in ^{232}U- and ^{241}Am-spiked soils were analyzed for gross alpha only.

Plutonium concentration factors, C_p/C_s, defined as the dis/min/g of plant ash divided by the dis/min/g of soil, are summarized in Tables VI-VIII. Average observed plutonium concentration factors for alfalfa, beans, and barley grown in soil spiked with solutions of plutonium, uranium, or americium nitrate to three different levels each are summarized in Table VIII.

D. Soils from Palomares, Spain, Accidentally Contaminated with Plutonium Metal and Oxides

Contaminated soil having an average plutonium concentration of 138 000 dis/min/g, from the impact area of the device near Palomares, was also used in similar experimental soil plots. Plants grown in this soil were alfalfa, tomatoes, barley, lettuce, and radishes. The average plutonium concentration factors from analyses of the soil and plants are summarized in Table IX.

E. Discussion of Sections C. and D.

The highest plutonium concentration factors in alfalfa were obtained from those plots to whose soil the plutonium was added in solution without subsequent conversion to the oxide by heating (Table VI). The plutonium concentration factor in alfalfa grown in soil containing 300°C-heated plutonium decreased to about half that in alfalfa grown in spiked, unheated soil. The plutonium concentration factor in alfalfa grown in soil containing 900°C-fired plutonium was only 1/16 that in alfalfa grown in spiked, unheated soil (see the average concentration factor column of Table VI). This reflects the increased refractory nature of PuO_2 as the temperature at which it is heated is increased. The plutonium concentration factors in tomatoes likewise decrease as the temperature increases (Table VII).

TABLE VI

PLUTONIUM-239 UPTAKE BY ALFALFA

Soil	Type of Spike	^{239}Pu Conc in Soil (dis/min/g of Dry Soil)	Conc Factor C_p/C_s[a]	Av C_p/C_s All Soils Similar Spike
Los Alamos Mt. Meadow	$^{239}Pu(NO_3)_4$ Soln	50 000	1×10^{-3}	---
Los Alamos Mt. Meadow	$^{239}Pu(NO_3)_4$ Soln	270 000	7×10^{-4}	---
Los Alamos Mt. Meadow	$^{239}Pu(NO_3)_4$ Soln	1 400 000	9×10^{-4}	---
Palomares	$^{239}Pu(NO_3)_4$ Soln	80 000	3×10^{-3}	---
Palomares	$^{239}Pu(NO_3)_4$ Soln	380 000	1×10^{-3}	1.3×10^{-3}
New Mexico Loamy Sand	$^{239}Pu(NO_3)_4$ Soln - 300°C[b]	740 000	1×10^{-3}	---
Kansas Loess	$^{239}Pu(NO_3)_4$ Soln - 300°C[b]	850 000	5×10^{-4}	---
S. Dakota Glacial Till	$^{239}Pu(NO_3)_4$ Soln - 300°C[b]	920 000	7×10^{-4}	7.3×10^{-4}
New Mexico Loamy Sand	$^{239}Pu(NO_3)_4$ Soln - 900°C[b]	970 000	1×10^{-4}	---
Kansas Loess	$^{239}Pu(NO_3)_4$ Soln - 900°C[b]	760 000	8×10^{-5}	---
S. Dakota Glacial Till	$^{239}Pu(NO_3)_4$ Soln - 900°C[b]	1 000 000	7×10^{-5}	8×10^{-5}

---

[a] Concentration factor = dis/min/g of plant ash ÷ dis/min/g of soil.

[b] $^{239}Pu(NO_3)_4$ soln. in 0.1 M HNO_3 added to a small portion of soil, dried, mixed, and fired at temperature indicated.

TABLE VII

PLUTONIUM-239 UPTAKE BY TOMATOES

Soil	Type of Spike[a]	^{239}Pu Conc in Soil (dis/min/g of Dry Soil)	Conc Factor C_p/C_s		Av C_p/C_s	
			Plant	Fruit	Plant	Fruit
New Mexico Loamy Sand	$^{239}Pu(NO_3)_4$ Soln 300°C	740 000	3×10^{-4}	2×10^{-4}	---	---
Kansas Loess	$^{239}Pu(NO_3)_4$ Soln 300°C	850 000	2×10^{-4}	1×10^{-4}	---	---
S. Dakota Glacial Till	$^{239}Pu(NO_3)_4$ Soln 300°C	920 000	7×10^{-5}	2×10^{-5}	1.9×10^{-4}	1.1×10^{-4}
New Mexico Loamy Sand	$^{239}Pu(NO_3)_4$ Soln 900°C	970 000	4×10^{-5}	2×10^{-5}	---	---
Kansas Loess	$^{239}Pu(NO_3)_4$ Soln 900°C	760 000	3×10^{-5}	3×10^{-5}	---	---
S. Dakota Glacial Till	$^{239}Pu(NO_3)_4$ Soln 900°C	1 000 000	2×10^{-5}	1×10^{-5}	3×10^{-5}	2×10^{-5}

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[a] $^{239}Pu(NO_3)_4$soln in 0.1 M HNO_3 added to a small portion of soil, dried, mixed, and fired at temperature indicated.

TABLE VIII

ALPHA UPTAKE BY PLANTS GROWN IN SOIL SPIKED WITH ^{239}Pu, ^{232}U, or ^{241}Am NITRATE SOLUTIONS

(Los Alamos mountain meadow soil--not heated)

Plant	No of Plots	Radio-nuclide	Range of Radionuclide Conc (dis/min/g of Dry Soil)	Av Conc Factor C_p/C_s
Alfalfa	3	^{239}Pu	50 000 - 1 400 000	8.1×10^{-4}
Bean Leaves	3	^{239}Pu	50 000 - 1 400 000	1.3×10^{-3}
Bean Seeds	3	^{239}Pu	50 000 - 1 400 000	1.7×10^{-5}
Alfalfa	3	^{232}U	40 000 - 900 000	5.0×10^{-3}
Barley Plant	3	^{232}U	40 000 - 900 000	1.7×10^{-3}
Barley Grain	1	^{232}U	900 000	1.4×10^{-4}
Alfalfa	3	^{241}Am	40 000 - 1 000 000	2.0×10^{-2}
Barley Plant	3	^{241}Am	40 000 - 1 000 000	6.0×10^{-4}

Plutonium concentration factors (Table IX) in different plants grown in plutonium-contaminated Palomares soil correspond more nearly to the average concentration factors obtained for alfalfa grown in 300°C-heated soil (Table VI). Comparison of the plutonium concentration factors in the plants listed in Tables VI-IX with those for lettuce or barley grown in soil spiked with Batch No. 1 $^{238}PuO_2$ microspheres* (Table V)

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*No other designation could be determined for the two batches of microspheres used. The first batch, noted only as Batch No. 1 was first used at the Los Alamos Scientific Laboratory in July 1965. Batch No. 2 was first used about February 1968. Both batches were prepared at the Mound Laboratory, Miamisburg, OH.

TABLE IX

ALPHA ACTIVITY IN PLANTS GROWN
IN CONTAMINATED SOIL FROM PALOMARES, SPAIN

(Gross alpha activity and ^{239}Pu activity in soils, 139 000 and 138 000 dis/min/g, respectively)

Plant	Conc Factor C_p/C_s	No. of Determinations
Alfalfa[a]	5×10^{-4}	36
Alfalfa[b]	9×10^{-4}	21
Tomato Plant[a]	3×10^{-4}	6
Tomato Fruit[a]	5×10^{-5}	6
Barley[b]	1×10^{-4}	8
Lettuce[b]	1×10^{-4}	6
Radish Top[a]	4×10^{-3}	1
Radish Bulb[a]	3×10^{-2}	1
Radish Bulb, Peeled[a]	4×10^{-4}	1

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[a] 5-kg plot.

[b] 1 1/2-kg plot.

shows that the average concentration factors in the latter are three orders of magnitude lower, probably indicating that the microspheres in this particular batch were less "soluble" than the plutonium oxide formed by heating the plutonium nitrate-spiked soil to 900°C. The $^{239}PuO_2$ particles may also have been smaller than the $^{238}PuO_2$ microspheres and more uniformly distributed in the soil, possibly resulting in increased "solubility" and/or more extensive root contact and, hence, increased plant uptake.

Another phenomenon is noted, (Table VIII) i.e., a mechanism that apparently prevents some plutonium from translocating into bean seeds. The bean seeds contain 200 times less plutonium than do the bean leaves. A similar observation was made when comparing relative amounts of ^{90}Sr in radish seeds and in the rest of the plant.[17] The tomato fruit did not contain significantly less plutonium than the leaves (Table IX); however, the seeds of the fruit were not analyzed separately from the pulp to determine whether a similar process takes place in tomatoes.

The ^{241}Am concentration factors in alfalfa (Table VIII) were 20 to 30 times greater than the plutonium concentration factors in similar experimental plots. This fact may indicate that plants take up trivalent ions more readily than tetravalent ions and that, in time, the ^{241}Am (daughter of ^{241}Pu) content in the soil may increase to the point at which the americium content in the plant is higher than the plutonium content. No similar increased ^{241}Am concentration factor was noted in the few barley plant samples analyzed, indicating that there may be a difference in uptake by different plant species.

Cline[18] reported americium concentration factors 15 to 30 times larger than the plutonium concentration factors in plants grown in similar soils containing only plutonium.

There have been a number of investigations to determine a plant's uptake of plutonium from soils containing fallout plutonium as well as from soils spiked with plutonium. Nishita et al.[19] reported a plutonium concentration factor of 1×10^{-4} in ladino clover grown in soil containing fallout plutonium.

Rediske et al.[20] report a plutonium concentration factor of 9×10^{-4} in barley grown in sandy loam, and plutonium concentration factors of 3×10^{-4}, 2×10^{-4}, 2×10^{-5}, and 2×10^{-4} for bean leaves, tomato leaves, Russian thistle "leaves," and grass blades, respectively, when these plants were grown in a nutrient solution spiked with plutonium.

Barley's plutonium uptake from soils spiked with plutonium,[21] when expressed as the plutonium concentration factor, varies from 4×10^{-5} to 4×10^{-4}, depending on the soil used. The larger value corresponds to the more acidic soil.

The gross alpha activity and plutonium concentrations given in Table IX are averages of 59 and 8 samples, respectively. The 59 gross alpha determinations varied from 46 000 to 401 000 dis/min/g of soil, and the 8 plutonium determinations varied from 45 000 to 405 000 dis/min/g of soil. These numbers indicate that the differences in the replicate analyses for plutonium in Palomares soil are associated with the nonuniform distribution of plutonium oxide particulate matter. This same nonhomogeneity within a soil sample was noted earlier in the determination of plutonium in a large number of soil samples from Palomares, Spain.[22]

As might be expected with root crops such as radishes, most of the plutonium found in the plant is located in the bulb skin (Table IX). The peeled bulb contained 1.33% of the plutonium present in the total bulb, and the radish top contained about 14% of the plutonium per gram of plant ash found in the unpeeled bulb. The leaves and the peeled bulb contain about the same plutonium concentration as do other plants grown in soil plots spiked to comparable levels.

Jacobson and Overstreet[23] also noted that, whereas relatively large amounts (20 to 38%) of plutonium were sorbed on plant roots, very little (about 0.00045 to 0.01%) of that plutonium was translocated to the plant above ground. Rediske and Selders[24] likewise noted the sorption of plutonium IV to the root surfaces from culture solutions but found very small concentrations of plutonium translocated to other parts of the plant.

IV. PLUTONIUM IN AN AQUATIC ENVIRONMENT

A. Plutonium Incorporation by Fish

To determine the effect of $^{238}PuO_2$ microspheres on an aquatic environment in the event of accidental release of plutonium from SNAP devices, microspheres of the radionuclide were placed in aquaria containing goldfish. Two separate studies were made, each with a different batch of $^{238}PuO_2$ microspheres. One study involved microspheres from Batch No. 2 which had shown, in water "solubilization" studies, a tendency to lose activity to the surrounding water. In the second study, microspheres from the Batch No. 1 used in the soil plots described in Sec. III A. were used. The designations Batch No. 1 and Batch No. 2 are made only to indicate two different preparations. All $^{238}PuO_2$ micorspheres are probably not alike, and differences in uptake and "solubility" can occur because of these differences.

In the study using Batch No. 2 microspheres, each of three aquaria was spiked with one $^{238}PuO_2$ microsphere that was about 100 μm in diameter and was calculated to contain about 1.5×10^8 dis/min of total alpha activity. Each microsphere was placed under the sand filter support inside a 50-ml Pyrex beaker, cut so that its side wall height was about 2 to 3 mm. The microsphere was thus easily retrievable and was exposed to the circulating water currents caused by the air bubblers attached to the filter supports. The three aquaria contained six goldfish each; an additional six goldfish, which died before the aquaria were ready for stocking, were analyzed for plutonium and thus served as a plutonium concentration base line for fish not exposed to $^{238}PuO_2$. One fish from aquarium number "one" died after the first 3 days, during which time it had accumulated nearly 200 times more plutonium than was found in the unexposed fish. A fish that died after 6 days' exposure in aquarium number "three" contained 400 times more plutonium than the average unexposed fish (see 1st 6 fish and fish No. 8, Table X).

TABLE X

PLUTONIUM-238 IN FISH AND FISH ORGANS, BATCH NO. 2 MICROSPHERES

Fish No.	Days Exposure to H_2O Contg. $^{238}PuO_2$	Microsphere Activity Found in Whole Fish %	dis/min/g of Total Ashed Fish	dis/min/g of Ashed Organ			
				Gills	Gut and Contents	Flesh	Bones
1st 6	0	---	7.0	---	---	---	---
7	3	4.4×10^{-5}	1 435	---	---	---	---
8	6	1.5×10^{-4}	2 836	---	---	---	---
9	6	1.4×10^{-5}	521	---	---	---	---
10	7	7.7×10^{-6}	302	---	---	---	---
11	9	2.1×10^{-4}	14 935	---	---	---	---
12	10	3.9×10^{-5}	1 566	---	---	---	---
13	14	2.8×10^{-5}	1 561	---	---	---	---
14	15	4.3×10^{-5}	3 512	---	---	---	---
15	17	1.7×10^{-5}	611	---	---	---	---
16	31	2.9×10^{-5}	---	306	7 488	476	309
17	45	7.6×10^{-6}	---	443	39 800	239	138
18	48	2.6×10^{-5}	---	2 935	36 142	292	332
19	49	9.9×10^{-6}	---	246	3 000	376	153
20	104	1.6×10^{-5}	---	21 000	18 000	602	73
21	181	2.0×10^{-5}	---	94	2 818	994	304
22	183	2.3×10^{-4}	---	2 770	32 110	537	119
23	184	7.4×10^{-5}	---	6 750	52 630	320	462
24	185	4.2×10^{-4}	---	2 360	51 940	462	191

Each fish thereafter, from No. 9 through No. 15, whether it died of natural causes or was sacrificed, was analyzed for plutonium as a whole sample. The 16th, 17th, and 18th fish were dissected so that the gills, gut and contents, flesh, and bones could be analyzed for plutonium separately. In all instances, the whole fish or its parts were dried at 110° for about 12 h, then ashed at 600°C. The ash was then leached with 8<u>N</u> HNO_3, and the plutonium was determined by the anion exchange separation method prescribed in Sec. II.C.2.

The water in the aquaria was assayed for plutonium periodically by removing a 1000- ml aliquot that was then acidified with HNO_3 and evaporated to dryness; the residue was treated with 8 <u>M</u> HNO_3 and the resulting acid solution was taken to dryness. The plutonium was converted to the tetravalent nitrate complex by adding concentrated HNO_3 and H_2O_2, after which the sample was taken to dryness on a steam bath. The residue was taken up with 8 <u>M</u> HNO_3, and the plutonium was separated from the salts in solution by anion exchange techniques described in Sec. II.C.2.

The water taken from the aquaria periodically for plutonium analysis was replaced with an equal volume of chlorine-free tap water. Evaporative losses occurring during the studies were compensated for by adding distilled water. At the conclusion of the experiment (6 months' duration), the entire liquid contents of each aquarium was removed and analyzed for plutonium in the same manner as the 1000-ml samples described above. The "solubility" or amount of plutonium lost from the microsphere and found in the aquarium water is presented as a percentage of the microsphere activity in Table XI.

When the aquaria were emptied so that the water could be analyzed, snails that had been placed in the aquaria to control algal growth were removed and analyzed for plutonium (Table XII).

A second study used microspheres from Batch No. 1, the batch used in soils for plant uptake studies. As before, a single

TABLE XI

PLUTONIUM FOUND IN AQUARIUM WATER

(Av of three samples)

Days of Contact with $^{238}PuO_2$	Av Pu dis/min/liter	Total Calculated dis/min in Aquaria	Microsphere Activity Found in Water[a] (%)
9	2.7×10^3	6.9×10^4	4.7×10^{-2}
17	1.2×10^3	3.0×10^4	2.0×10^{-2}
49	4.8×10^2	1.3×10^4	8.8×10^{-2}
72	2.2×10^2	5.7×10^3	3.9×10^{-2}
114	3.6×10^2	9.5×10^3	6.4×10^{-2}
168	3.7×10^2	9.5×10^3	6.4×10^{-2}
185[b]	3.1×10^2	8.2×10^3	5.5×10^{-2}

---

[a] Assuming uniform distribution of plutonium throughout water.

[b] Termination.

TABLE XII

PLUTONIUM FOUND IN SNAILS GROWN IN $^{238}PuO_2$-SPIKED WATER

(Av of all snails found in three aquaria after 185 days)

	dis/min/g (Ash)	dis/min/g (Dry)
Snail Flesh	2791	817
Snail Shell	710	371

microsphere was placed in each aquarium which contained about 26 liters of dechlorinated Los Alamos tap water.

After 2 days and 10 days, one fish from each aquarium was dissected and its parts were analyzed separately for plutonium. After 20 days of exposure, all six fish in one aquarium died. As certain chlorinated organic compounds are toxic, these fish were probably killed by ingested toxic chlorinated organic compounds. The fecal matter from the six relatively large goldfish, along with small amounts of uneaten fish food, began to deplete the oxygen in the water, as was shown by the behavior of the fish. To alleviate this condition, the fish were removed and the water was treated with sodium hypochlorite to oxidize the compounds. Sodium thiosulfate was then added to remove residual chlorine until the water tested chlorine free, at which time the fish were returned to the aquarium. This procedure had been used once before and proved successful. However, this time, toxic compounds such as chloramines may have formed and the six fish shortly died. The water in the affected aquarium was discarded and replaced with fresh dechlorinated tap water.

The six dead fish were dissected, and all like parts were combined to form a single sample; that is, the six heads were analyzed as a single sample, etc. At the same time, a fish from one of the other aquaria was sacrificed, dissected, and analyzed for plutonium. The remaining 11 fish were distributed, 4 each to two aquaria and 3 to one aquarium. With only three or four fish in each aquarium, the air bubblers could supply enough oxygen to the water to sustain them.

Twenty-eight days later (48 days after the study was initiated) a fish from each aquarium that held four fish was sacrificed and analyzed for plutonium. Three additional samplings (one fish from each aquarium) on the 72nd, 113th, and 302nd day of the experiment reduced the population to zero, and the experiment was terminated. The plutonium data from analyses of the fish parts are presented in Table XIII, and the data water analyses data are presented in Table XIV.

B. Discussion

The data in Tables X and XIII show that in each study most of the plutonium activity incorporated in the fish is located in the gills and gut. Up to two orders of magnitude more activity is found in the gut (with contents) and gut organs than in the flesh and bones.

The plutonium concentration in fish No. 21 was calculated to be 17 dis/min/g of wet flesh (the ash content of goldfish flesh was found to be about 1.75% when the flesh was dried then ashed at 600°C). This concentration is about 150% of the concentration guide for plutonium in water for the 168-h week, given in Table II, Column 2 in AEC Manual Appendix 0524, Nov. 1968. The plutonium concentration in the aquarium water, however, doesn't represent that found in natural waters, so the expected plutonium concentration in fish from natural waters would be orders of magnitude lower.

The experiments showed that the fish, along with snails and algae, apparently concentrate the plutonium in aquarium water.

The wet fish gut (Fish No. 24, Table X, 1.4% ash) contained about 730 dis/min/g,

TABLE XIII

PLUTONIUM IN FISH BODY PART IN dis/min/g OF ASH

(Av of number of fish indicated.
Batch No. 1 microspheres.)

Days of Exposure in $^{238}PuO_2$ Water	No. of Fish	Head	Gill	Flesh	Bones	Gut	Gut Organs
2	3	0.46	1.8	0.36	0.72	3.6	0.95
10	3	0.47	2.2	0.28	0.14	61.0	0.75
20	7	0.22	2.2	0.35	0.75	27.0	1.6
48	2	0.93	2.0	0.65	0.36	24.0	4.6
72	3	0.61	2.9	0.65	0.43	36.0	4.9
113[a]	3	0.65	2.4	0.42	0.25	35.0	4.4
302	3	0.41	9.0	0.57	0.44	24.0	13.0

---

[a] Highest microsphere activity found in fish was 4.7 x 10^{-6}%.

whereas the 185-day water (Table XI) contained only 0.31 dis/min/ml, giving a concentration factor of about 2300. The wet flesh of fish No. 21 contained only 17 dis/min/g which represents a concentration factor (fish flesh to water) of about 55 (Fish No. 21, 185-day water). This factor indicates that almost all the plutonium taken into the gut is eliminated and does not find its way into the edible parts of fish.

The fish in a second comparable study with Batch No. 1 microspheres showed little tendency to concentrate plutonium. For example, the 10-day fish sample (Table XIII) had only 60.8 dis/min/g of ashed gut or 0.85 dis/min/g of wet gut which, when compared with water at 10 days (Table XIV), would give a concentration factor of only about 12. That is,

$$\frac{0.85 \text{ dis/min/g of wet gut}}{0.068 \text{ dis/min/ml of water at 10 days}} = 12.$$

Comparison of the plutonium concentration in the 48-day fish sample (Table XIII), based on dis/min/g of wet flesh using an ash content of 1.75%, with the plutonium in water at 42 days (Table XIV), gives a concentration factor of about 0.15. That is,

TABLE XIV

PLUTONIUM CONCENTRATION IN AQUARIUM WATER

(Batch No. 1 microspheres)

Time Exposed to $^{238}PuO_2$	dis/min/liter of Water Av of 3 Samples
1 h	58
24 h	111[a]
48 h	88
3 days	45
4 days	53
9 days	24
10 days	68
16 days	49
19 days	45
25 days	29
42 days	72
145 days	3
302 days	1.4

---

[a] Highest microsphere activity found in water was 1.7 x 10^{-3}%.

$$\frac{0.65\ \text{dis/min/g of ash} \times 0.0175}{0.072\ \text{dis/min/ml of water at 42 days}} = 0.15.$$

The reason for these smaller concentration factors in the second study is not known, but it indicates the differences in batches of microspheres discussed in Sec. IV.A.

If one assumes that commercial food fish concentrate plutonium in the same manner as goldfish, then commercial food fish might pose a problem to man if they lived in water containing $^{238}PuO_2$ microspheres like the Batch No. 2 microspheres at the concentration used in these studies. Experience has shown, however, that the microspheres will probably not be deposited intact on small areas of land or water but will be distributed fairly uniformly like worldwide fallout. At the plutonium concentration levels used in the Batch No. 1 microsphere studies, fish apparently would pose no problem to man when consumed.

A single determination of plutonium in an algal sample in an earlier, preliminary aquarium experiment indicated a concentration factor of about 2000. This factor was calculated using the plutonium concentration in algae expressed as dis/min/g of dry algae and the plutonium concentration in the water sample in dis/min/ml after 24 h (Table XIV). That is,

$$\frac{222\ \text{dis/min/g of dry algae}}{0.111\ \text{dis/min/ml of water}} = 2000.$$

When one compares the plutonium in snails (Table XII) expressed as dis/min/g of dry flesh with the plutonium concentration in water (Table XI, 185-day sample), a concentration factor of about 2600 is found. That is,

$$\frac{\text{dis/min/g dry snail flesh}}{\text{dis/min/ml plutonium in water.}}$$

Alvarez-Ramis[25] did not find a concentrating of plutonium in snails living in soil contaminated with plutonium. However, she did not know the plutonium concentration in the material that passed through the snails' digestive systems, and the plutonium most certainly was not uniformly distributed in the soil but was present as particulate plutonium oxide. These factors make it impossible to determine a meaningful concentration factor as was done in the aquarium experiments.

Pillai et al.[26] also found that marine organisms concentrate plutonium. They report plutonium concentration factors of 1570 for green algae, of 2590 for zooplankton, and of 3 for fish (Bonito), compared with plutonium found in sea water. Wong et al.[27] found that Southern California "seaweeds" concentrate plutonium to 260 to 3500 times its concentration in sea water. Ward[28] reported that flesh from lobsters grown in sea water containing 10^{-2} µCi/liter of ^{239}Pu contained three times as much plutonium as the sea water.

Early work by Newell and Christenson[29] indicated that activated sludge removed 90 to 95% of the plutonium from liquid wastes. This seems to indicate about a tenfold concentration of the plutonium by the organisms or the sludge they produce.

The plutonium concentration factors in algae reported at LASL are certainly within the range that Wong found for "seaweeds." The fish flesh plutonium concentration factor (Batch No. 2 microspheres) was about 15 times higher than that Pillai reported for Bonito fish, perhaps reflecting differences in the species studied.

It is perhaps noteworthy that the microspheres probably are not all alike and may behave differently in water. Therefore, it might be presumptuous to predict actual contamination levels in water and associated animal life on the basis of experimentation with a half-dozen microspheres. Note the vast difference in plutonium concentrations in fish parts (Tables X and XIII) and the difference in plutonium concentrations in water when different batches of microspheres are used (Tables XI and XIV).

C. Solubility of $^{238}PuO_2$ Microspheres in Tap Water

The variation in plutonium analyses of aquarium water (Table XI, Sec. IV.A.) prompted an experimental determination of the "solubility" of $^{238}PuO_2$ microspheres in tap water without fish or algae. A single microsphere was placed in each of two beakers containing 1 liter of Los Alamos tap water. The water in each beaker was stirred continuously with an electric stirring motor, and 10-ml samples of the water were removed periodically and analyzed for gross alpha activity. Exaporative losses were compensated for by adding distilled water, whereas tap water was added in amounts equal to the volume removed for analysis. The gross alpha data are compiled in Table XV.

The microspheres, whose diameters were about 190 and 100 μm, were in contact with the water for 294 and 283 days, respectively. Both microspheres were from Batch No. 2, the batch used in the first aquarium studies described in Sec. IV.A. After the microspheres had been in the water for the times indicated above, the water was decanted and the microspheres were recovered. The 1-liter samples of water were analyzed for plutonium using an anion exchange separation procedure after the plutonium was converted to the $Pu(NO_3)_6^{2-}$ complex in 8 N HNO_3.

If all the plutonium found in the water at the completion of the experiment is assumed to be "soluble", the "solubility" of the $^{238}PuO_2$ can be determined and expressed as a percentage of the original activity associated with the microsphere. The original alpha activity of the microspheres was calculated by assuming a sphere and using the approximate diameter of the microspheres (190 and 100 μm), an average density of 10.5 g/cm^3, and the specific activity of ^{238}Pu. The "solubility" data are presented in Table XVI.

In an earlier experiment, the 190-μm microsphere used in this study was placed in contact with tap water for 67 days. At the end of this time, the microsphere was

TABLE XV

ALPHA ACTIVITY FOUND IN WATER CONTAINING A BATCH No. 2 $^{238}PuO_2$ MICROSPHERE

Cumulative Time In Contact with Water, 190-μm-Diam Microsphere	Gross α (dis/min/liter)	Cumulative Time In Contact with Water, 100-μm-Diam Microsphere	Gross α (dis/min/liter)
1 h	280	1 h	5.48×10^3
2 h	1.10×10^4	17 h	5.94×10^3
3 h	440	10 days	7.26×10^3
4 h	280	14 days	8.96×10^3
5 h	420	37 days	5.84×10^4
3 days	600	57 days	1.24×10^4
7 days	920	84 days	1.57×10^4
10 days	1.2×10^3	164 days	2.87×10^4
12 days	1.03×10^3	283 days	5.41×10^4
22 days	2.44×10^5		
26 days	1.06×10^3		
48 days	3×10^3		
68 days	1.76×10^4		
95 days	6.72×10^4		
175 days	1.55×10^5		
294 days	1.73×10^5		

TABLE XVI

SOLUBILITY OF $^{238}PuO_2$ MICROSPHERES IN TAP WATER[a]

Microspheres

190-μm Diameter	≈100-μm Diameter
294 Days Exposure	283 Days Exposure
^{238}Pu Found in Water, 2.88 x 10^5 dis/min/liter	^{238}Pu Found, 1.03 x 10^5dis/min/liter
Solubility, 0.028%	Solubility, 0.07%
Calc. Activity of Microsphere, 1.02 x 10^9 dis/min	Calc. Activity of Microsphere, 1.48 x 10^8dis/min

Los Alamos Tap Water

pH, 8.2
Conductivity, 116 μmho/cm
Phenol. alk, 0.0 mg/liter
Total alk, 60 mg/liter
Na^+, 50 mg/liter
Cl^-, 6.0 mg/liter

F^- , 1.1 mg/liter
Total hardness, 25 mg/liter
Ca^{++}, 9.0 mg/liter
Mg^{++}, 0.5 mg/liter
NO_3^- N, 0.3 mg/liter
Total Solids, 203 mg/liter

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[a] Calculated assuming 80 wt % ^{238}Pu, spherical shape, and 10.5-g/cm^3 av density.

removed and the water was filtered through a 0.22-μm-pore-size Millipore filter that removed about 25% of the alpha activity. The apparent "solubility" of $^{238}PuO_2$ calculated from the plutonium found in the filtrate was 1.34 x 10^{-3}%, whereas that calculated from the alpha activity in the unfiltered water was 1.8 x 10^{-3}%.

The Millipore filter used in the above filtration was quartered and mounted on Nuclear Track Alpha emulsion plates, and the films were exposed from 4 1/2 to 103 h. Using Leary's method to calculate particle size from the number of alpha tracks found on the developed film, 0.033- to 0.16-μm-diam particles were calculated to be present on the Millipore filter. This size range indicates that at least part of the plutonium in the water is particulate matter that should not be considered as being in solution. The particles found on the filter were smaller than the filter's pore size, suggesting that they are attached to larger nonradioactive particulate material.

D. "Solubility" of $^{238}PuO_2$ in Water

Solubility in the classical sense implies formation of ions through loss of electrons. As related to plutonium and probably some other elements, there is a question as to whether the microcolloid or a group of atoms spalled from macroparticles can be considered "soluble." As these "solutions" relate to filtration or transport in a field, they appear to be true solutions; however, the fact that no homogeneous solution is formed is demonstrated by the range of activities determined for aliquots from the same sample.

A question related to plants' low plutonium uptake may be posed. Is the low plutonium uptake observed in green plants a result of true solution in the aqueous soil phase and, hence, more nearly a real indication of the plutonium's solubility? Results from NTA plates lead one to suspect that what has been called "soluble" plutonium may, in fact, be particulate material that would not be evidenced by classical chemistry but can be shown to be present by radiochemical techniques.

The alpha activity, expressed as dis/min/liter (Tables XIV and XV), calculated from the activity found in a 10-ml aliquot, varies considerably. These erratic results could be caused by the spallation of small particles of $^{238}PuO_2$ not uniformly distributed in the water.

Lingren[31] found that of the plutonium "in solution" from $^{238}PuO_2$ microspheres exposed to sea water, 22% migrated to the anode, 23% migrated to the cathode, and 55% did not move in electrochromatographic experiments. In a similar experiment in which plutonium was added as a solution to the sea water, Lingren also found that 30% of the plutonium migrated to the anode, whereas 70% did not move. Lingren suggests the formation of a carbonate or hydroxide complex with a negative charge resulting in movement toward the anode. He also suggested that the 55 to 70% of the plutonium that did not migrate in his two experiments might be a $Pu(OH)_4$ neutral complex. Price[32] mentions plutonium hydrolysis products that are colloidal or exhibit colloidal behavior, and that these colloidal-size particles grow with increased pH. He also suggests that the form of plutonium most likely to occur in natural waters or nuclear processing waste streams is colloidal tetravalent plutonium as $Pu(OH)_4$. Unhydrolyzed plutonium exists in aqueous solutions of 0.1 M hydrogen ion or greater. Precipitation of $Pu(OH)_4$, the end product of hydrolysis, begins at pH 2 or higher and with Pu IV concentrations of 10^{-3} M.

Ockenden and Welch[33] state that during the intermediate stages of hydrolysis there is polymerization of the hydrolyzed forms of Pu IV. This polymerization may occur through oxide or hydroxide bridges with the formation of colloidal aggregates. Lloyd and Haire,[34] however, state that they do not believe that momomeric Pu IV hydroxide species are formed during precipitation of what has been considered to be $Pu(OH)_4$. They do not think that monomeric $Pu(OH)_4$ is involved in the formation of the "so called" Pu IV polymer. They have proposed that the initial precipitate is formed by primary particles that consist of, or readily convert to, very small crystallites of hydrated PuO_2. These crystallites then form clusters of varying sizes which act like ploymers. Rhodes[35] suggests the existence of a polymer or radiocolloid with tracer concentrations of plutonium in solution at a pH greater than 2 and suggests that because the polymer is taken up rapidly by the soil colloid it probably has a positive charge.

Kubose et al.[36] found that in a static system for determination of $^{238}PuO_2$ solubility in sea water, essentially all the plutonium is sorbed on the material settling to the bottom. Although the Los Alamos tap water does not contain large amounts of dissolved salts (Table XVI), some salts did form on the beaker during evaporation. Possibly, some of the plutonium in the solubility studies does sorb on those salts or on the beaker walls.

The solubility (Table XVI) expressed as a percentage of the original activity of the $^{238}PuO_2$ microspheres agrees essentially with that found by Kubose, i.e., about 0.038% at 240 days exposure to sea water.

From a review of all data on "soluvility" of $^{238}PuO_2$, it appears possible that the plutonium in the aquarium and tap water is present as colloidal $Pu(OH)_4$. However, the plutonium could also be present, at least partially, in very small fragments of PuO_2. The chemistry of tracer concentrations of plutonium in waste water needs further study before one can be sure of the chemical or physical state of the plutonium.

V. SUMMARY

Investigations to determine plutonium in a variety of matrices have been discussed; and a basic analytical technique applicable to the matrices has been developed and is reported. Problems peculiar to certain of the matrices have been discussed, questions about the "solubility" of PuO_2 microspheres have been posed, and the need for further work in the area is indicated.

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