Absorption, Transport, and Chemical Fate of Plutonium in Soybean Plants

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Absorption of plutonium (Pu) by soybean plants (*Glycine max* cv. Williams) is limited by Pu solubility in soils. Changes in Pu concentration in different tissues with time to senescence indicate Pu is freely transported through the xylem during growth but not subject to remobilization on flowering. Studies in which the DTPA complex of ^{238}Pu was supplied to the plant suggest a change in chemical form following root absorption. Of the Pu in roots, stems, and leaves at senescence, 28, 54, and 67%, respectively, were soluble. The Pu in the soluble fraction was primarily associated with components of >10 000 equivalent molecular weight in leaves and roots, whereas stems exhibited an equal distribution between components in the >10 000 and <500 molecular weight fractions. Plutonium associated with mature seeds is concentrated in the seed hull (85%) and cotyledons (14%). The Pu associated with the cotyledon was primarily in the insoluble residues and soluble soy whey.

The long half-lives and interest in potential health effects of actinide elements such as plutonium (Pu), arising from the nuclear fuel cycle, have resulted in extensive field and laboratory studies to resolve their environmental behavior. Plants represent a potential route of entry to human food supplies and investigations of the behavior of actinide elements have been summarized in recent reviews (Price, 1973; Mullen and Mosley, 1976; Watters et al., 1980). In general, these studies have shown root absorption of Pu from soil to be relatively low, at least over the short term (Wildung and Garland, 1974), compared with many nutrient species (Cataldo and Wildung, 1978) and certain fission products such as Sr, Cs, and Tc (Routson and Cataldo, 1978a,b).

The complex soil processes leading to the delivery of a soluble Pu species to the root membrane likely represent the rate-limiting step in the ingestion pathway (Wildung et al., 1979). However, the plant root represents the first barrier in the selective accumulation by plants of ions present in the soil solution, and it is critical to determine if Pu discrimination occurs at the root membrane level which would limit Pu uptake by the plant. Of equal importance are the physiological and metabolic processes governing translocation in the plant and the form in edible tissues as these influence gastrointestinal absorption in animals.

The importance of Pu concentration (Wildung and Garland, 1974) and solubility (Lipton and Goldin, 1976) in soil and their effect on plant absorption have been demonstrated. In the latter studies, the presence of synthetic chelators increased Pu absorption in pea plants by a factor of 1000. It is generally held that metal–chelate complexes formed in, or amended to, soils serve to increase the concentration in the root zone of elements normally subjected to hydrolysis and sorption on soil particles. Studies to define the mechanism of metal uptake when supplied as a chelate, i.e., to distinguish between uptake of the metal independent of the metal chelate, have been generally inconclusive (Chaney et al., 1972; Tiffin, 1970; Hill-Cottingham and Lloyd-Jones, 1965). However, if investigations are carefully performed, plant root discrimination can be distinguished from the effects of soil sorption by comparison of Pu uptake in plants grown on hydroponic solutions (in which Pu is maintained in solution as a soluble complex) with plants grown in soil. Furthermore, tissues may be chemically characterized to evaluate the extent of intact chelate uptake and to provide a basis for evaluation of the effects of plant metabolism on chemical form and bioavailability following animal ingestion (Wildung et al., 1979).

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Table I. Properties of Surface Soils Employed in Studies of Plutonium Uptake by Soybean

property	surface soil: Ritzville (silt loam)	Palouse (silty clay loam)	Quillayute (silt loam)
texture class			
% sand	43.6	2.8	10.8
% silt	43.9	75.8	62.2
% clay	12.5	21.4	27.0
cation exchange capacity[a]	14.4	23.8	45.1
pH[b]	6.2	5.6	4.4
% organic carbon	0.7	3.0	12
% pyrophosphate-extractable Fe	0.008	0.066	1.23
% dithionite-extractable Fe	0.14	0.13	0.8
% ammonium oxalate extractable Fe	0.37	0.062	1.41

[a] mequiv/100 g. [b] 0.01 M $CaCl_2$ slurry.

The purpose of this study was to determine the extent to which plant discrimination controls Pu uptake by plants and to provide a general understanding of the physiological processes which influence the fate and behavior of Pu in plants after absorption. Investigations were directed toward describing (1) the absorption by soybean of inorganic Pu from soils and from soils and hydroponic solutions containing the Pu complex of diethylenetriaminepentaacetic acid (DTPA), (2) the chemical form of Pu transported in the xylem following root absorption, (3) the distribution of Pu in the shoot and root with time following absorption, and (4) the chemical form of Pu in leaves, stem, and seeds of soybean plants.

EXPERIMENTAL SECTION

Plant Culture. Soybean plants (*Glycine Max* cv. Williams) were grown either on Ritzville silt loam by using a soil–solution culture system or on hydroponics and maintained in controlled-environment chambers with a 16/8 h light cycle (~500 $\mu E\ m^2\ s^{-1}$, PAR, at leaf surface), a day/night temperature cycle of 26/22 °C, and 50% relative humidity. The soil–solution system, used to evaluate the time course of Pu uptake and distribution between tissues, employed soils (400 g) amended with $^{238}Pu(NO_3)_4$ at levels of 10 $\mu Ci/g$ dry weight of soil as described previously (Wildung and Garland, 1974). Properties of soils used in this study are given in Table I. Detailed mineralogy was reported previously (Routson

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et al., 1975). Studies of the form of Pu transported and Pu chemical fate in roots, stems, and leaves employed hydroponically grown plants. In the latter studies, seeds were germinated on moist filter paper and individual seedlings transferred to beakers (600 mL) containing aerated nutrient solution (500 mL) 3 days following germination.

The nutrient solution contained 150 mg of KCl, 120 mg of $MgSO_4$, 946 mg of $Ca(NO_3)_2 \cdot 4H_2O$, 68 mg of KH_2PO_4, 0.06 mg of $ZnSO_4 \cdot 7H_2O$, 0.69 mg of H_3BO_3, 0.017 mg of $CuCl_2 \cdot 2H_2O$, 0.024 mg of $Na_2MoO_4 \cdot 2H_2O$, 0.022 mg of $MnCl_2 \cdot 4H_2O$, and 0.6 mg of Fe^{3+} (as $FeCl_3$) per L. The pH was adjusted to 5.8, and the solution changed 3 times a week.

Plutonium Uptake. Hydroponic studies employed either 27- or 65-day-old soybean plants. These were transferred to fresh nutrient solutions containing 0.25 μCi/mL $^{238}Pu_2DTPA_3$ for evaluation of the chemical fate of Pu in roots, stems, leaves, and seeds. Plants of 18 days of age were used for evaluation of the fate of PuDTPA in xylem exudates; to assure stability of the Pu_2DTPA_3 complex, we held the molecular ratio of DTPA/Pu at ~6.0.

Xylem Exudate Collection and Analyses. Plants were placed in nutrient solutions containing $^{238}Pu_2DTPA_3$ for 1 h to allow time for root absorption and partial transfer to the xylem and were decapitated below the primary leaf node. The stem was then fitted with a short piece (2 cm) of gum rubber tubing, the open end of which was fitted with a 1-mm (o.d.) polyethylene tube. Exudate was collected from 0 to 6, 6 to 10, 10 to 15, and 15 to 24 h in cooled vials and assayed for ^{238}Pu activity. Thin-layer electrophoresis of exudates was performed on 20 × 20 cm cellulose plates (MN 300, 0.1-mm thickness), using 0.1 M Hepes buffer (Sigma Chemical Co.). Electrophoresis was performed at constant voltage (400 V) for 40 min.

Fractionation of Tissues. Root, stem, and leaf tissues were cut into ~5-mm sections, placed into 0.02 M ammonium acetate (NH_4OAc) buffer at pH 6.9 (7.5 mL/g of tissue), and homogenized 3 times for 45 s each time with a Sorvall Omni-Mixer (setting 10). The homogenate was centrifuged at 25000g for 15 min, the insoluble pellet was washed once, and the second supernate containing <10% of the total solubles was combined with the first supernatant solution (soluble fraction). The insolubles were acid digested and analyzed for radioactivity. The soluble fraction was subjected to ultrafiltration using Amicon Diaflo UM10, YM5, and UM05 membranes and resulted in fractionation of the solubles into >10000, 10000–5000, 5000–500, and <500 equivalent molecular weight (M_r) fractions (referenced to globular proteins). It should be noted that due to the rejection characteristics of the Diaflo UM10 membrane, the >10000 M_r fractions contain a significant fraction of 10000–1000 M_r material which occurs due to retentate volumes above the filters and rejection efficiencies for particular components. The >10000 M_r fraction was further fractionated on a Sephadex G-100 column. Sephadex fractionation was performed at 6–8 °C on a 2.5 × 85 cm column using 0.02 M NH_4OAc, pH 6.8, as an elution buffer. Flow rate was 30 mL/h. Fractions were collected in 3.0-mL increments, and ^{238}Pu was analyzed in 0.5-mL aliquots. Total dissolved organic carbon was determined after lyophilization (2×) and reconstitution in distilled water. The determination was made by direct injection and combustion, with measurement of the resulting CO_2 by IR analysis on an Oceanographics Total Carbon analyzer.

Seeds collected from mature soybean plants (redistribution study) grown in the split root system and harvested at 115 days, were freeze-dried and dehulled, and embryos separated from cotyledons. Dehulled seeds were ground to 40 mesh and fractionated by a modification of the method of Rackis et al. (1961). Freeze-dried, dehulled seeds (3 g) were extracted twice with 15 mL of n-hexane for 2 h at room temperature. The solutions were centrifuged at 20000g to obtain the lipid fraction (supernatant) and defatted meal. Hexane was removed from the latter by vacuum evaporation, and the defatted meal was extracted twice with 15 mL of H_2O (pH 7.0) for 3 h each and centrifuged to yield the insoluble residue and defatted soy milk. The soy milk was acidified with 0.01 N HCl to pH 4.5 and centrifuged at 20000g for 10 min. The pellet (soy curd) was washed once and recentrifuged, and the wash combined with the original supernatant solution (soy whey). The soy curd was resuspended and neutralized to pH 7.0 with KOH, forming soy proteinate.

Radioanalyses. Analyses of total Pu in each tissue or fraction was performed after ashing (450 °C), dissolving the ash residue in concentrated HNO_3, plating, and counting an aliquot in a low-background 2π gas flow proportional counter.

RESULTS AND DISCUSSION

The tendency for Pu(IV) to hydrolyze at environmental pH levels is well documented, and the reported solubility product for $Pu(OH)_4$ of 10^{-56} (Cleveland, 1979) would preclude the formation of soluble Pu required for plant uptake. However, plants do absorb Pu from soil, with concentration ratios (CR = concentration per gram dry tissue divided by weight of soil) ranging from 10^{-2} to 10^{-6} (ERDA, 1975) observed for a number of different plant species. On a total soil concentration basis, this magnitude of CR value is comparable to elements as diverse chemically and physiologically as Al, Ba, Be, Cr, Si, Th, U, V, W, and even Fe (Cataldo and Wildung, 1978; Bowen, 1966). The rate of root absorption for a given element will be dependent on its solubility in soils and more specifically its activity in soil solution, which is governed by physical, chemical, and microbial factors, and plant physiological processes including membrane transport and aspects of root secretion which aid in solubilization and stabilization of elements within the rhizosphere (Cataldo and Wildung, 1978).

Plutonium Uptake from Soils. Soybean plants exhibited a relatively constant accumulation rate of Pu approximating 90 dpm/day in shoot tissues following root absorption from soil (Figure 1). This is equivalent to 0.01 pmol/day from soils containing 2.5 mmol of ^{238}Pu/g dry weight (10 μCi/g dry weight). However, a comparison of the time course of Pu accumulation (dpm), dry weight, and Pu concentration of shoot tissues over 80 days of growth (Figure 1) indicates that while Pu accumulation is relatively linear, at least through 64 days, dry matter production exhibits a sharp increase at flowering (42 days). This disproportionate behavior of dry matter production and Pu accumulation results in a rapid decrease in shoot Pu concentration (dpm/g) with plant age. Concentration ratios for shoot tissues decreased from 1×10^{-4} at 11 days to 9×10^{-6} at 83 days. Seed concentration ratios were substantially lower than shoot ratios at 2×10^{-7}. Analyses of control tissues show shoot concentrations of Ca, Mg, and Fe remain constant or increase with plant age. These data and similar data for Cs and Sr (Routson and Cataldo, 1978a) suggest a dynamic balance between plant growth rate, nutrient demand, and root absorption, where the plant-available component of an element in soil is not

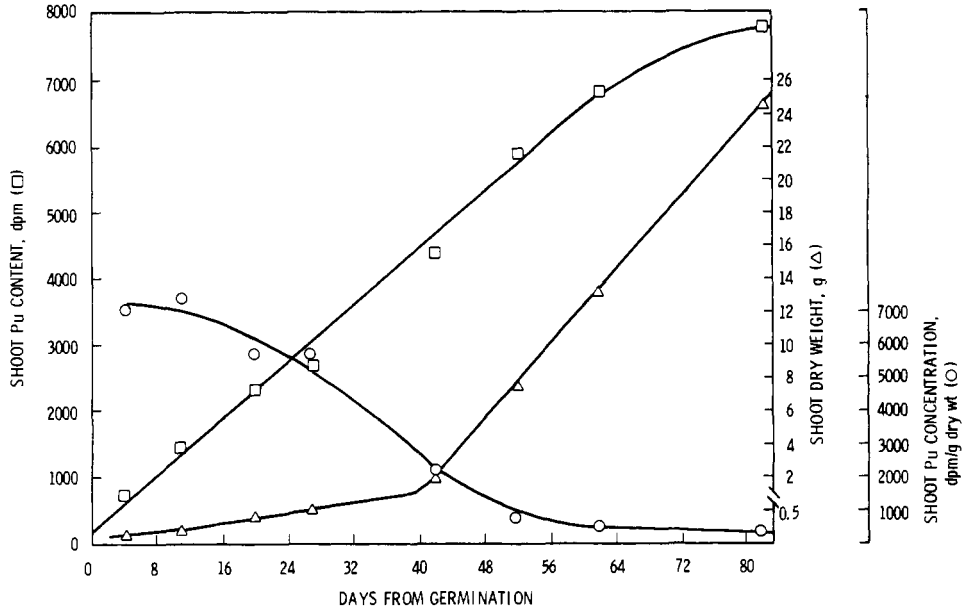

Figure 1. Relationship between shoot content and shoot concentration of plutonium and dry matter production during growth of soybean plants on Ritzville soil amended with $^{238}Pu(NO_3)_4$. Average of three replicate treatments.

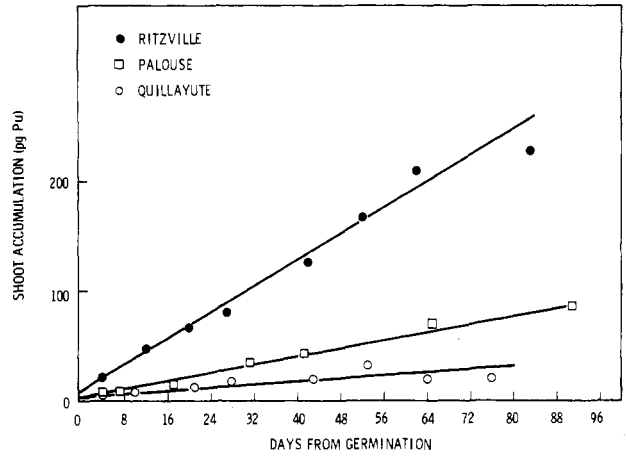

Figure 2. Accumulation of plutonium by whole soybean plants grown in three soils amended with $^{238}Pu(NO_3)_4$. Average of three replicate samples.

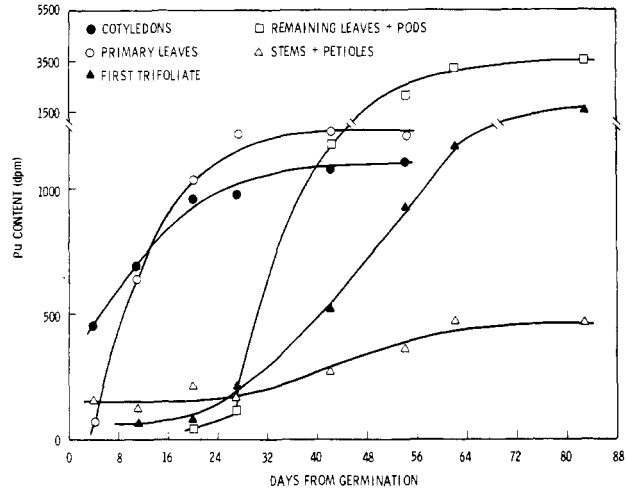

Figure 3. Accumulation of plutonium in shoot tissues of developing soybean plants grown in a soil–solution culture system.

limiting. Thus, it is postulated that available Pu was limiting. In effect, the rate of Pu accumulation in the shoot (~90 dpm/day) may represent the rate at which the Pu in soil solution can be replenished from the soil solid phase for absorption by the plant. Support for the hypothesis of a limited available soil pool is obtained from similar shoot content data for different soils.

The accumulation of Pu is also relatively linear in time for different soil types, but the accumulation rates are a function of soil type. The Pu shoot accumulation values for soybean plants over time for three soils, Ritzville, Palouse and Quillayute (Figure 2), range from 92 to 12 dpm/day (3.1 to 0.4 pg/day). Since the growth characteristics and dry matter production of these plants were similar for the three soils under these growth conditions, it would appear that the rate of replenishment of plant-available Pu to the soil solution is a function of soil properties and controls the concentration of Pu in plants.

The degree of mobility of Pu within the shoot tissues of the plant over an 83-day period is illustrated in Figure 3. The cotyledons and primary leaves attained near maximum Pu content at 27 days and exhibited little change in content through senescence. The Pu content of the first trifoliate and remaining shoot tissues increased gradually to 27 days, followed by a rapid increase (note scale change, Figure 3). The latter corresponds with the rapid increase in dry matter production (Figure 1) at flowering, characteristic of soybean growth. Stem tissue, containing both immobilized (stored and metabolized) and xylem transport forms, exhibited a gradual increase in Pu content over the 83-day period. These data describe several important aspects of Pu behavior in plants. First, Pu in cotyledon, primary leaves, and first trifoliate does not decrease at senescence, suggesting that Pu is not remobilized from these tissues as is the case for Ca (Bukovac and Wittwer, 1957), Ni (Cataldo et al., 1978), and Ag and Cr (Cataldo and Wildung, 1978). This behavior may account for the comparatively low Pu content of seeds. Second, the distribution of Pu in various shoot tissues (exclusive of stems) is characteristic of xylem transport and indicates that the ultimate concentration of Pu in a specific tissue is related to the flux of transpirational water lost through evapotranspiration and the duration of this process for specific tissues. The final concentration in specific tissues, however, will be dependent on the quantity of plant-available Pu in soils and the root absorption rate. Third, and most importantly, the lack of a marked accumulation of Pu in stem tissues would indicate that Pu is

not readily insolubilized or subject to hydrolysis after uptake by the plant. This is further substantiated by the mobility of Pu within the conducting vessels of the plant stem and shoot tissues and suggests the Pu is in a chemical form which stabilizes it against hydrolysis. The ability of plants to maintain the solubility of hydrolyzable elements through the formation of chemically stable organic complexes has been demonstrated for both a nutrient element, Fe, and nonnutrient element, Ni (Cataldo et al., 1978; Tiffin, 1977).

Chemical Form of Mobile Plutonium in the Xylem. Indirect evidence in these investigations indicate that soil type and solubility govern Pu uptake by plants. It has been suggested that soil Pu solubility in turn results from formation of complexes with microbial metabolites (Watters et al., 1980; Wildung et al., 1979).

However, although certain organic ligands stabilize polyvalent cations in solution, there is some question whether the organo–metal complex is absorbed intact by the plant root. The majority of available evidence (Beckett and Anderson, 1973), although indirect, indicates that at physiological concentrations a metal and its predominant ligand are accumulated independently and not absorbed stoichiometrically. Since Pu mobility within the plant, especially in stems (Figure 3), suggests that hydrolysis of Pu is limited following transfer from root to shoot, a series of experiments was undertaken to determine whether Pu_2DTPA_3 supplied to plant roots is absorbed and transported to shoots intact or undergoes recomplexation.

Hydroponically grown, 18-day-old soybean plants were placed in Pu_2DTPA_3 solutions (500 mL) containing 48 ng of Pu/mL, and the concentration of Pu in shoot tissues and xylem exudates determined over a 24-h period. Concentration ratios for shoot tissues increased from 6×10^{-3} after 1 h of uptake to 0.3 after 24 h. Concentration in xylem exudates increased from ~0.03 ng/mL of exudate at 1 h to 1.6 ng/mL at 24 h. When Pu is supplied as $Pu(NO_3)_2$ under these experimental conditions, <1 pg/mL Pu is found in exudates at 24 h, indicating that the apparent discrimination in absorption by plant roots results from a lack of available Pu species which in these studies was alleviated by the use of Pu_2DTPA_3.

For determination of whether the form of Pu supplied to roots of soybean is absorbed intact or subject to a change in chemical form resulting from plant metabolism, plants were supplied with Pu_2DTPA_3, as described above, and decapitated, and xylem exudates were collected at intervals over a 24-h period and analyzed for Pu-containing components. The comparative electrophoretic mobilities of the supplied Pu_2DTPA_3 in in vivo exudates and control exudates spiked with Pu $(NO_3)_4$ are illustrated in Figure 4. Three anionic components (b, c, and e) are found in all in vivo exudates; component d, which has a mobility similar to that of Pu_2DTPA_3, is not present in all in vivo exudate samples. When $Pu(NO_3)_4$ is spiked into control exudates, five Pu-containing components (a, b, c, e, and g) are formed, all of which except component a are found in in vivo exudates following uptake from solutions of Pu_2DTPA_3. These data indicate that Pu is either absorbed by plant roots as Pu^{4+} or the Pu_2DTPA_3 complex does not persist during transfer to the xylem. Relatedly, FeEDDHA supplied to soybean roots is found as iron citrate in xylem exudates (Tiffin, 1970), and exudates spiked with either Ni, Cd, or Zn (D. A. Cataldo, unpublished data) have the capacity to rapidly and efficiently complex these cations, resulting in metal–complexes electrophoretically similar to in vivo forms. Further support for Pu complexation within the plant is derived from animal feeding studies,

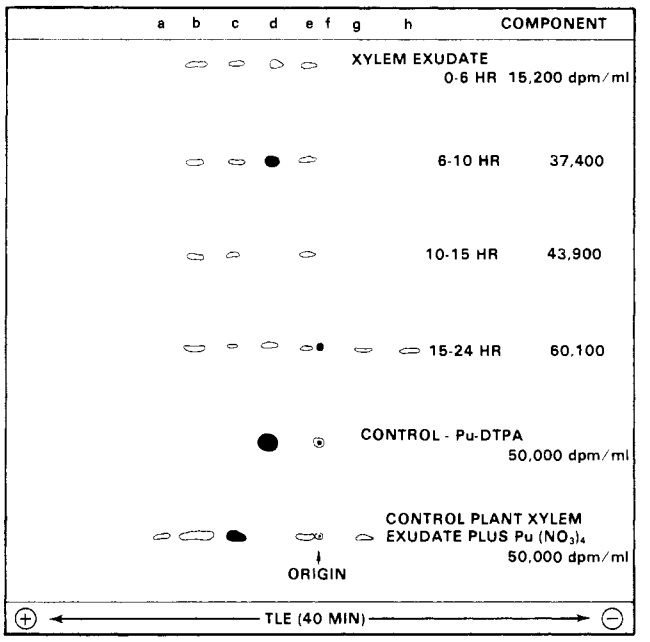

Figure 4. Electrophoretic behavior of ^{238}Pu in xylem exudates from 18-day-old soybean plants following root absorption from solutions containing $^{238}Pu_2DTPA_3$. Comparison of $^{238}Pu_2DTPA_3$ alone, control xylem exudates spiked in vitro with $^{238}Pu(NO_3)_4$, and in vivo xylem exudates following root absorption from solutions containing $^{238}Pu_2DTPA_3$.

where plant-incorporated Pu, resulting from root absorption of Pu supplied to soil or nutrient solution either as $Pu(NO_3)_4$ or Pu_2DTPA_3, is substantially more available to animals than Pu directly gavaged to the animal in these forms (Wildung et al., 1979).

Chemical Fate of Root Absorbed Pu in Roots, Stems, and Leaves. The mobility of Pu within the plant, the presence of Pu complexes with plant ligands in xylem exudates, and indirect evidence relating to gastrointestinal absorption and metal metabolism in plants all suggest that Pu is organically complexed by higher plants. The chemical fate of ^{238}Pu in soybean tissues (27 day old) following root absorption from Pu_2DTPA_3 solutions was determined after 5 days of absorption and metabolism (Table II). After 5 days, the roots are the major repository of Pu, with 0.6 and 15.4% of the Pu accumulated being contained in stem and leaf tissue, respectively. A portion of the Pu associated with the root may be as surface-absorbed hydrolysis products since both inorganic and DTPA complexed Pu (in the absence of excess DTPA) are prone to hydrolysis. Yet after absorption, as suggested by the presence of Pu in the xylem exudates, substantial Pu, especially in stems and leaves, may be defined as "soluble" (not sedimented by centrifugation at 20000*g*). Fractionation by molecular weight shows ~90% of the soluble Pu in leaves and roots was associated with compounds of equivalent M_r >10 000, with less than 6% of the Pu associated with the 5000–500 M_r fraction. The latter M_r fraction should contain any Pu_2DTPA_3 absorbed by the plant. While these data suggest that Pu is chemically stabilized by metabolism and/or incorporation into a relatively high M_r material, fractionation of stem tissues indicate an important difference. Stems, although having 60% of the soluble Pu associated with components of >10 000 M_r, also have 25% of the Pu associated with low molecular weight components (<500 M_r). On the basis of the lack of significant immobilization of Pu in stems and petioles (Figure 3), it can be assumed that this comparatively high proportion of Pu in the <500 M_r fraction represents low molecular weight transport forms. Again,

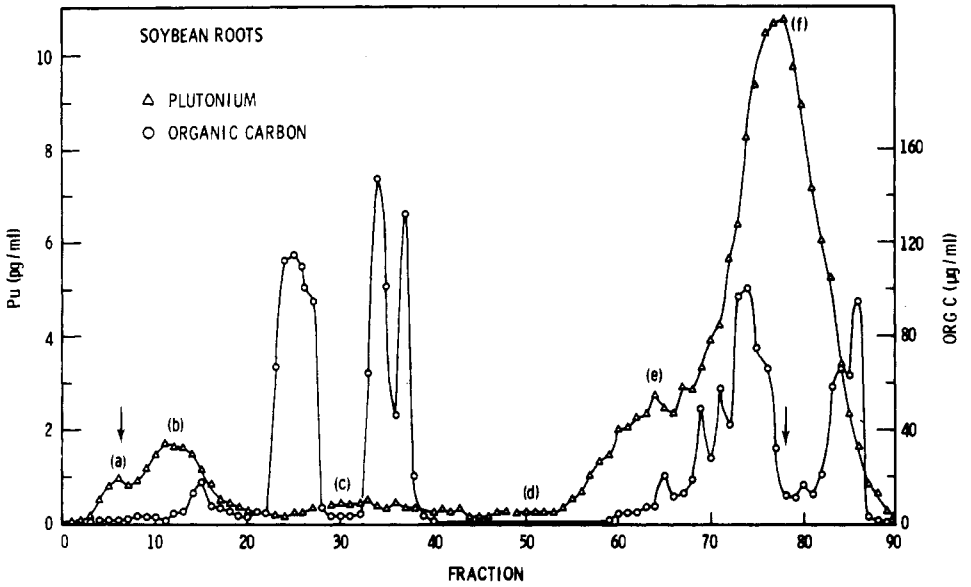

Figure 5. Chromatographic separation of plutonium associated with the >10000 M_r fraction of roots using Sephadex G-100. Twenty-seven day old soybean plants allowed to accumulate and metabolize $^{238}Pu_2DTPA_3$ for 5 days prior to fractionation.

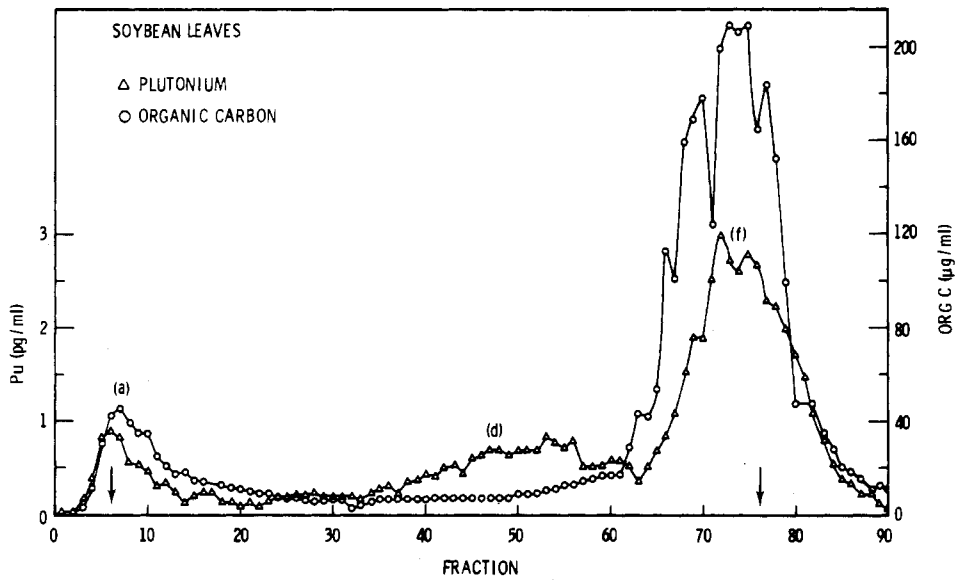

Figure 6. Chromatographic separation of plutonium associated with the >10000 M_r fraction of leaves using Sephadex G-100. Twenty-seven day old soybean plants allowed to accumulate and metabolize $^{238}Pu_2DTPA_3$ for 5 days prior to fractionation.

Pu_2DTPA_3 is not associated with this M_r fraction.

Application of gel permeation chromatography to the >10000 M_r fraction of roots (Figure 5) and leaves (Figure 6) indicates the presence of Pu-containing components ranging in molecular weight from <4000 to >150000 (void volume). At the extremes of the M_r range, there are similarities in Pu distribution in components of roots and leaves. Components a and f contain 2.1 and 70.9% of the soluble Pu in roots and 5.0 and 57.2% of the Pu activity in the leaves, respectively. In the case of roots (Figure 5), the remaining soluble Pu activity is associated with components b and e, which contain 7.4 and 12.3%, respectively. While these two components are not present to any major extent in leaves (Figure 6), an additional component (d) containing 19.9% of the Pu is present in leaves. Both the ultrafiltration (Table II) and gel permeation chromatography data demonstrate conclusively that Pu undergoes a change in chemical form following root absorption. The observed changes in Pu form may be due to metabolism or to nonspecific binding to cell constituents of leaves and roots. Comparison of Pu and organic carbon distribution for leaves and roots (Figures 5 and 6) shows distinct differences in carbon and Pu for resolved components, especially for components a, b, and e for roots and component d in the leaves. While this would suggest that nonspecific binding is limited, the possibility exists that polyvalent Pu may preferentially bind to specific functional groups such as thiols as shown for the divalent cations such as Cd, Cu, and Zn (Underwood, 1977).

Distribution of Pu in Soybean Seeds. While CR values can range from 10^{-3} to 10^{-6} for transfer of inorganic forms of Pu from soils to leaves through root uptake, an additional discrimination (~10–100-fold) has been identified for seeds. This is due to the lack of an obvious remobilization of Pu from roots and senescing tissues during seed filling (Figure 3), which in turn, indicates that the fraction of Pu transported to seeds results from partitioning of xylem water between leaves (evapotranspiration) and seeds (metabolism). So that the lack of remobilization was circumvented and the partitioning of xylem water to pods and seeds was optimized, hydroponically grown soybean plants were provided with a pulse of Pu_2DTPA_3 during pod filling. At maturity, seeds were fractionated to determine Pu distribution. In the mature

Table II. Distribution and Chemical Fate of ^{238}Pu in Soybean Tissues following Five Days of Accumulation from Hydroponic Solutions Containing Pu_2DTPA_3[a]

	% distribution of plutonium in			
tissue fraction	roots	stems	leaves	Pu_2-DTPA$_3$
whole tissue	84.0 ± 1.3	0.6 ± 0.3	15.4 ± 1.0	
fraction[b]				
soluble	27.7 ± 2.9	54.4 ± 1.9	67.4 ± 3.9	
insoluble	72.3 ± 2.9	45.6 ± 1.9	32.6 ± 3.2	
fractionation of solubles[c]				
>10 000	90.3 ± 3.2	60.1 ± 5.1	87.2 ± 7.2	0.1
5000–10 000	5.7 ± 1.1	8.4 ± 6.5	3.1 ± 1.3	0.1
500–5000	2.8 ± 1.9	6.0 ± 1.8	5.8 ± 3.6	92.1
<500	1.2 ± 0.3	25.6 ± 0.4	3.9 ± 2.3	7.9

[a] Mean for two replicate samples; single replicate for Pu_2DTPA_3; 27-day-old plants. [b] Homogenized in 0.02 M NH_4OAc; insolubles (cell walls, organelles, and structural proteins) sedimented at 20000*g*. [c] Fractionation by ultrafiltration.

Table III. Distribution of Pu in Soybean Seed Components and Soy Products

		Pu concn	
components	dry wt, g	fg/g dry wt	%
seed[a]			
seed hull	2.69	86	84.8
embryo	0.82	4.2	1.2
cotyledon	28.88	1.3	14.0
cotyledon fraction[b]			
soy oil	3.56	<0.1	<0.5
defatted meal	21.30	1.8	99.5
soy proteinate	8.24	0.25	5.4
residue	8.54	2.7	62.1
soy whey	4.42	2.8	32.5

[a] Plants grown hydroponically. Plants provided with 72-h pulse of Pu_2DTPA_3 at 65 days from germination, followed by 45 days for seed filling and Pu metabolism. [b] Soy products expressed as percent of cotyledon content; proteinate, residue and soy whey expressed as percent of defatted meal.

seed, the seed hull contains 84.8% of the Pu, with embryo and cotyledon containing the remaining 15% (Table III). The marked accumulation of Pu in seed hulls is substantially higher than the 9% reported for Ni (Cataldo et al., 1978) and 35% for Tl (D. A. Cataldo, unpublished data). The localization of Pu in the seed coat or hull may be due to (1) the lack of a direct vascular connection between the seed and its integuments and/or (2) the chemical instability of the form of Pu being transported between pod and seed tissues prior to seed metabolism. Localization of Pu in the seed hull may be beneficial in that the dose to man might be lower than if Pu was concentrated in the cotyledon which is extensively used in human foodstuffs.

The fate of Pu associated with cotyledons was further studied by using fractionation procedures commercially employed to process soybeans for food products (Table III). Essentially all of the Pu in the cotyledons is associated with the defatted meal following removal of the soy oil. Extraction and precipitation of the defatted meal shows 5, 32, and 62% of the Pu to be associated with soy proteinate, whey, and residue fractions, respectively. Although a sizable fraction (62%) of Pu is associated with the insoluble residue (unextracted protein, carbohydrate, and cell debris), Pu is not found in large quantities in the proteinate fraction (5.4%) as might be concluded from the relatively high concentration of Pu associated with the >10 000 M_r fraction in leaves (Table II). The insoluble residues are generally not directly incorporated into human foodstuffs but are utilized principally as animal feeds; this would indicate that an indirect route to man should be considered for precise dose assessment models. The soluble soy whey (non-acid-precipitable protein, sugars, amino acids, phenolics, and other minor constituents) contain a substantial fraction (32%) of Pu, suggesting some mechanism for solubilization of Pu, since uncomplexed Pu would be expected to hydrolyze and adsorb to seed constituents during fractionation.

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