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Health Physics Vol. 57, No. 2 (August), pp. 299–307, 1989
Printed in the U.S.A.

0017-9078/89 $3.00 + .00
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● *Behavior and Effects of Technetium in Plants*

TECHNETIUM-99 CYCLING IN MAPLE TREES: CHARACTERIZATION OF CHANGES IN CHEMICAL FORM

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***Abstract*—Prior field studies near an old radioactive waste disposal site at Oak Ridge, TN, indicated that following root uptake, metabolism by deciduous trees rendered ^{99}Tc less biogeochemically mobile than expected, based on chemistry of the pertechnetate (TcO_4^-) anion. Subsequently, the form of technetium (Tc) in maple tree (*Acer* sp.) sap, leaves, wood and forest leaf litter was characterized using one or more of the following methods: dialysis, physical fractionation, chemical extraction, gel permeation chromatography, enzymatic extraction, or thin layer chromatography (TLC) on silica gel. Chromatography (Sephadex G-25) of TcO_4^- incubated *in vitro* with tree sap showed it to behave similar to TcO_4^- anion. When labeled wood and leaf tissues were processed using a tissue homogenizer, 15% and 40%, respectively, of the Tc was solubilized into phosphate buffer. Most (65% to 80%) of the solubilized Tc passing a 0.45-micron filter also passed through an ultrafiltration membrane with a nominal molecular weight cutoff of 10,000 atomic mass units (amu). A majority (72% to 80%) of the Tc in wood could be chemically removed by successive extractions with ethanol, water and weak mineral acid. These same extractants removed only 23% to 31% of the Tc from maple leaves or forest floor leaf litter. Most of the Tc in leaves and leaf litter was removed only by strongly alkaline reagents typically used to release structural polysaccharides (hemicelluloses) from plant tissues. Chromatography (Sephadex G-25) of the ethanol–water extract from wood and the alkaline extract from leaves demonstrated that Tc in these extracts was not principally TcO_4^- but was complexed with molecules > 1000 amu. Incubations of leaf and wood homogenates with protease approximately doubled the amount of Tc released from contaminated tissues. Ultrafiltration of protease-solubilized Tc from leaves and wood showed that 40% and 93%, respectively, of the Tc was <10,000 amu. TLC of the <10,000 amu fraction indicated the presence of TcO_4^- in wood but not in leaves. In the leaf, TcO_4^- is converted to less soluble forms apparently associated with structural components of leaf cell walls. This conversion explains why ^{99}Tc is not easily leached by rainfall from tree foliage and why ^{99}Tc appears to accumulate in forest floor leaf litter layers at the Oak Ridge study site.**

INTRODUCTION

A PRIOR report (Garten et al. 1986a) described the uptake of ^{99}Tc by a stand of trees, principally red maple (*Acer rubrum* L.), intercepting contaminated ground water from a radioactive waste storage site. The chemical form of Tc in ground water and soil was characterized, and the recycling behavior of ^{99}Tc from aboveground biomass to the forest floor was quantified. A companion paper (Bondietti and Garten 1986) presented details on studies of the chemical form and behavior of ^{99}Tc in the study site soil, specifically associations with organic matter and the interaction between soil-associated, reduced forms of Tc and the very water soluble TcO_4^- anion.

In field studies, ^{99}Tc was found migrating through soil as TcO_4^- anion (Garten et al. 1986a). However, the distribution and fate of ^{99}Tc in trees indicated that metabolism following root uptake rendered it less biogeochemically mobile than expected, based on the chemistry of TcO_4^-. Following characterization of the distribution of ^{99}Tc in various components of the contaminated forest (Garten et al. 1986a), studies were undertaken to examine the changes in chemical form as Tc moved from ground water, to tree wood, to leaves, and back to the forest floor in leaf litter (Fig. 1). This paper presents evidence for the modification of Tc during its metabolism by maple trees and discusses the significance of these changes in chemical form to its environmental cycling.

MATERIALS AND METHODS

Tc *labeling of trees*

When possible, samples from a study area adjacent to a former waste disposal site were used (see Garten et al. 1986a for a description of the study area). Technetium-99 has been cycling in the terrestrial environment for at least a decade at the former waste site. Wood and leaf samples were collected during summer from red maple trees that were >20 cm in diameter at breast height. Using an electric drill, samples (5- to 7-cm deep) were removed from the trunks of red maple trees. The trees were growing in soil containing ≈28 Bq ^{99}Tc g^{-1} dry weight (dw) (quadrant B8, Table 2 in Garten et al. 1986a). Wood chips were frozen until analyzed

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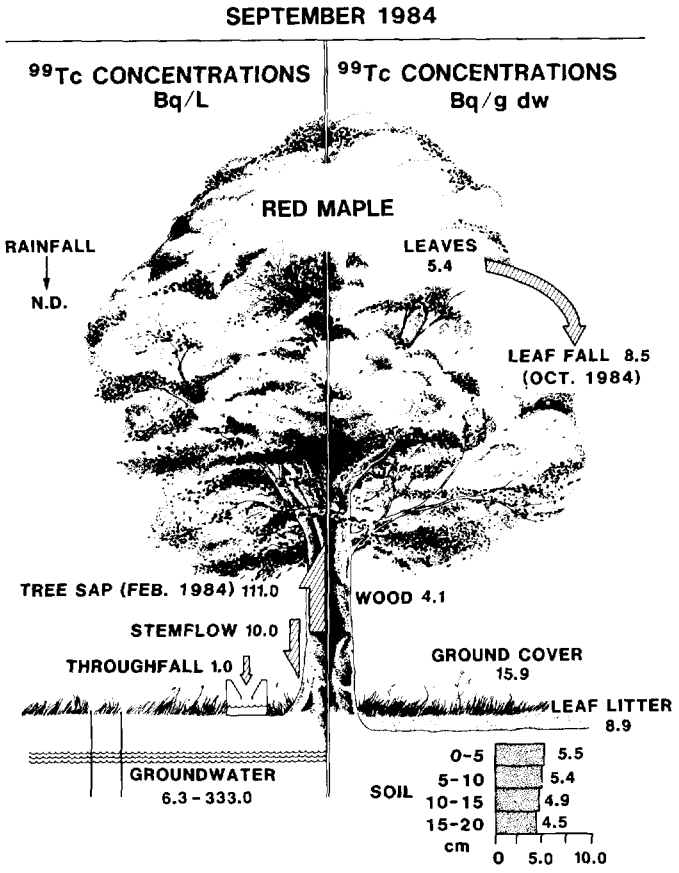

Fig. 1. On the left, concentrations (Bq L^{-1}) of ^{99}Tc in tree sap, stemflow, throughfall and ground water at the Tc study site. On the right, concentrations (Bq g^{-1} dw) of ^{99}Tc in leaves, leaf fall, wood, ground cover, leaf litter and the soil profile (top 20 cm).

Leaf litter was collected from the forest floor at the technetium study area. Because of lower-than-desired concentrations of ^{99}Tc in tree leaves, leaves were also labeled by allowing a small sugar maple tree (*Acer saccharum*) to absorb $^{95m}TcO_4^-$ from an aqueous solution. The tree was excavated from the field and transported to a greenhouse. The root system was cut from the stem underwater, and the stem was put into a 50-mL test tube. All transfers were accomplished while the stem was submerged to prevent disruption to the xylem flow. Technetium-95m (5920 Bq of $^{95m}TcO_4^-$) was added to the test tube and taken up by the tree over a 2-d period. The ^{95m}Tc-labeled leaves were removed from the tree and frozen. Leaves from both the study area trees and the labeled sugar maple were fully formed, mature leaves. Technetium-95m is a γ emitter with a radioactive half-life of 61 d.

Experiments with tree sap

Sap was collected from red maple trees at the Tc study area during February of two successive years (1984 and 1985) by inserting plastic tubing into holes drilled 4–5 cm deep in each trunk. The tubing ran into 8-L plastic bottles which were covered to keep out insects and debris. In the laboratory, the sap was stored in a freezer until analyzed.

The binding of Tc with constituents in tree sap was tested *in vitro* using equilibrium dialysis experiments with ^{95m}Tc. In two experiments, each lasting 24 h, a solution of aqueous TcO_4^- was dialyzed against filtered (0.45-micron) tree sap using a dialysis membrane with a nominal molecular weight (MW) cutoff of 3,500 amu. Upon completion of the dialysis, the concentration of ^{95m}Tc was checked on both sides of the dialysis membrane (i.e., in both the sap and the distilled water). Departure from a 1:1 concentration ratio was expected if ^{95m}Tc was binding to sap constituents.

In vitro binding of TcO_4^- to small molecules (<5,000 amu) in sap was also examined using gel permeation chromatography. Samples of filtered (0.45-micron) sap were incubated for 2 h and for 72 h at room temperature with $\approx$100 Bq of $^{95m}TcO_4^-$. Each sample was chromatographed on Sephadex G-25 gel* which has a nominal MW fractionation range of $\approx$1,000 to 5,000 amu. The column was 25 cm long, with a 2.5-cm diameter and had

* Sephadex G-25 gel, Pharmacia Fine Chemicals, Inc., 800 Centennial Ave., Piscataway, NJ 08854.

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a flow rate of ≈3.5 mL min^{-1}. The eluant, 0.3 M NaCl, was collected in 3.8-mL fractions. The results of the chromatography were compared with a separate, and otherwise identical, chromatography run with $^{95m}TcO_4^-$ in distilled water.

Physical fractionation of Tc *in wood and leaves*

The scheme for physical fractionation of Tc in wood and leaves was as follows: Tissues were disrupted using a tissue homogenizer for 5 min in a cold 0.1 M phosphate buffer and filtered through a coarse stainless steel screen. The unhomogenized residue was analyzed for Tc. The filtrate was centrifuged (10,000 × g, 25 min) to remove unbroken cells, cell debris and larger organelles. The supernate was successively passed through a 0.45-micron filter and ultrafiltration membranes with nominal MW cutoff limits of 10,000 amu and 1,000 amu. A control sample containing $^{95m}TcO_4^-$ in phosphate buffer was passed through an identical set of filters to measure TcO_4^- sorption by the membranes. Ultrafiltrations were performed under N_2 (4.5×10^5 Pa) at room temperature. Filters and the final filtrate were analyzed for Tc.

Chemical fractionation of Tc *in leaves, wood and litter*

Samples of maple leaves, wood and leaf litter were freeze-dried and extracted according to the methods outlined in Fig. 2, which were modified from those described by Bowen et al. (1962). The ^{99}Tc-labeled wood and leaf litter samples were from the field; leaves were obtained from the ^{95m}Tc-labeled maple tree. The plant material was processed using a tissue chopper to break it into small fragments and was then extracted successively, three times each, with the following reagents: 95% ethanol, distilled water, 0.2 N hydrochloric acid (HCl) and 2 N-sodium hydroxide (NaOH). Extractions were performed for 5 min with 50 mL of boiling reagent using a tissue homogenizer. Suspensions were cleared by centrifugation at 15,000 × g for 15 min and the supernates were pooled to yield the various soluble fractions. Replicate extractions were performed on leaves, wood and leaf litter.

Gel permeation chromatography of selected chemical extracts

The ethanol and aqueous fractions from the chemical extraction of maple tree wood were combined and concentrated tenfold under vacuum on a rotary evaporator at 37°C. The NaOH extract from leaves was similarly concentrated. The concentrated fractions were centrifuged at 10,000 × g for 20 min, and 2-mL samples of the clear supernate were chromatographed on a column containing Sephadex G-25 (25 cm long × 2.5 cm in diameter; flow rate ≈ 3.5 mL min^{-1}). Dilute salt solutions (0.1 to 0.3 M NaCl) would not elute the concentrated samples of leaf and wood extracts from the gel; therefore, 0.2 M NaOH was used as an eluant. Fractions from the column were combined in increments of the void volume (V_O). A 2-mL solution of TcO_4^- anion was chromatographed under the same conditions for comparison.

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To check for the *in vitro* sorption of free TcO_4^- to molecules present in the NaOH extract, uncontaminated maple leaves were extracted according to the scheme in Fig. 2. A 2-mL portion of the NaOH extract was incubated for 24 h with $^{95m}TcO_4^-$ and then chromatographed according to the methods described above.

Modified chemical fractionation of Tc *in maple leaves*

Technetium-99 in maple leaves from the contaminated field site was further characterized using a chemical extraction procedure for carbohydrates outlined by Robinson (1980). The extraction scheme is shown in Fig. 3. Leaves were homogenized in 95% ethanol to remove lipids and pigments. The defatted pellet was extracted for 1 h in boiling water and centrifuged to obtain an aqueous fraction containing low MW compounds, sugars, ionic substances, hydrophilic polysaccharides and soluble protein. Pectic substances, polysaccharides and some soluble proteins were later precipitated from the cold aqueous extract by the addition of ethanol (final concentration 80%) and ammonium sulfate.

The water-insoluble material consisting of undissolved cell-wall polysaccharides, structural carbohydrates and noncarbohydrates (Fig. 3) was extracted twice by shaking for 30 min with a solution of 0.2 N sodium bicarbonate-0.3 N sodium carbonate (pH 10.2) and then centrifuged to pellet the extracted hemicelluloses. The

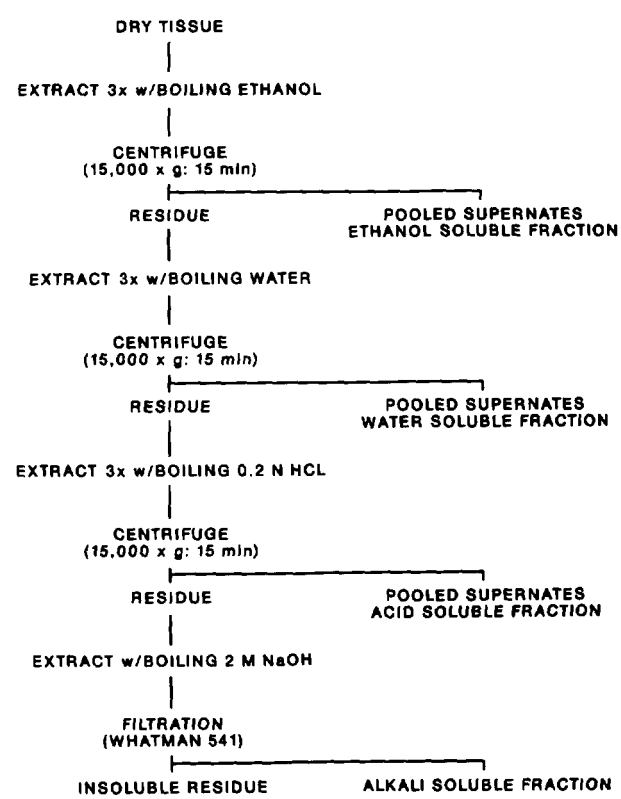

Fig. 2. Method of chemical fractionation of ^{99}Tc in maple tree wood and leaf litter, and fractionation of ^{95m}Tc in maple wood and leaves.

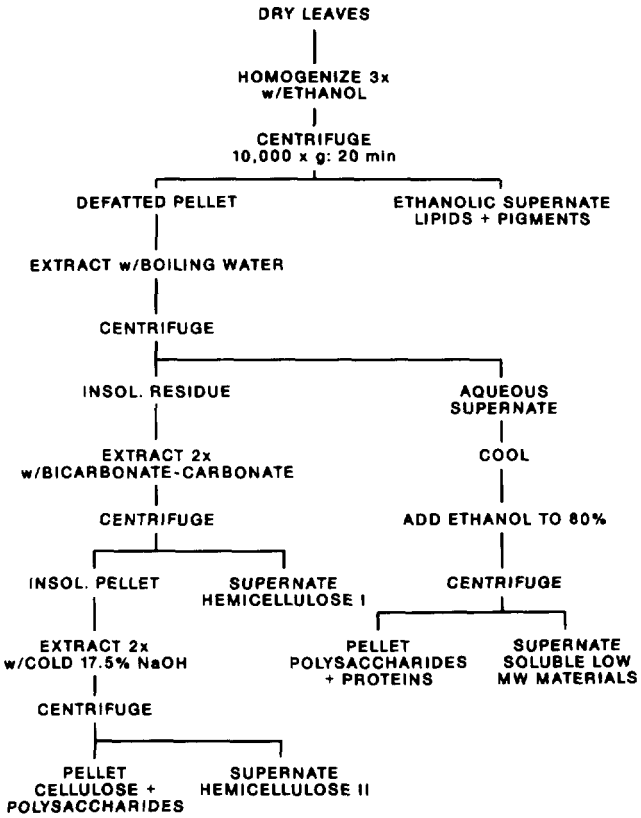

Fig. 3. Modified method for the chemical fractionation of ^{99}Tc in maple tree leaves from the Tc study site; makeup of the various chemical extracts is based on Robinson (1980).

residue was extracted twice by shaking for 15 min with cold 17.5% NaOH to remove a second hemicellulose fraction. The final insoluble residue contained crude cellulose and polysaccharides.

Enzymatic digestion of leaf and wood tissues

Leaf and wood samples collected from the ^{99}Tc study area were each enzymatically digested according to the scheme outlined in Fig. 4. Enzyme extracts were cleared by centrifugation. Tissues were first pulverized dry in a blender and then homogenized in phosphate buffer to break cell walls. Following digestion with protease, lipids were extracted with ethanol. The defatted material was further digested with amylase and cellulase. The final product was extracted with NaOH to remove alkali-soluble hemicelluloses. To determine the importance of the protease treatment to the release of Tc from plant tissues, a control extraction was performed under the same conditions as those outlined in Fig. 4, but excluding protease from the 24-h digestion.

Chromatography and analysis of enzyme extracts

Samples of ^{99}Tc-contaminated leaves (19 g dw) and wood (14 g dw) from the study area were chopped to a fine powder in a blender and then homogenized 5 min in 100 mL 0.01 M phosphate buffer (pH 6.7). Chloramphenicol (5 mg) was added to each homogenate to prevent bacterial growth. Protease (500 mg) and pepsin (1.0 g) were added to digest each homogenate. Homogenates were incubated for 72 h at 37°C in a water bath and centrifuged at 5,400 × g to remove undigested material. Each supernatant was further cleared by centrifugation at 20,000 × g (30 min) and freeze-dried. The freeze-dried and reconstructed extracts were filtered through an ultrafiltration membrane to remove large molecules and proteins (nominal size > 10,000 amu), which were subsequently removed from the membrane by soaking in 25 mL distilled water and analyzed for ^{99}Tc.

Each filtrate (<10,000 amu) was freeze-dried, dissolved in 3–5 mL of distilled water and analyzed for ^{99}Tc. Small amounts (10 μL) of the filtrate that passed an ultrafiltration membrane (<10,000 amu) were subjected to thin-layer chromatography (TLC) on silica gel using a butanol–water–acetic acid (4:5:1) solvent system. TLC plates were developed with 2% ninhydrin solution, cut into 1-cm sections, and the amount of β radioactivity in each section was determined using liquid scintillation counting.

Tc analysis

Extracts and fractions from plant tissues labeled with ^{95m}Tc were analyzed directly using gamma spectrometry. Technetium-99 analyses were performed according to

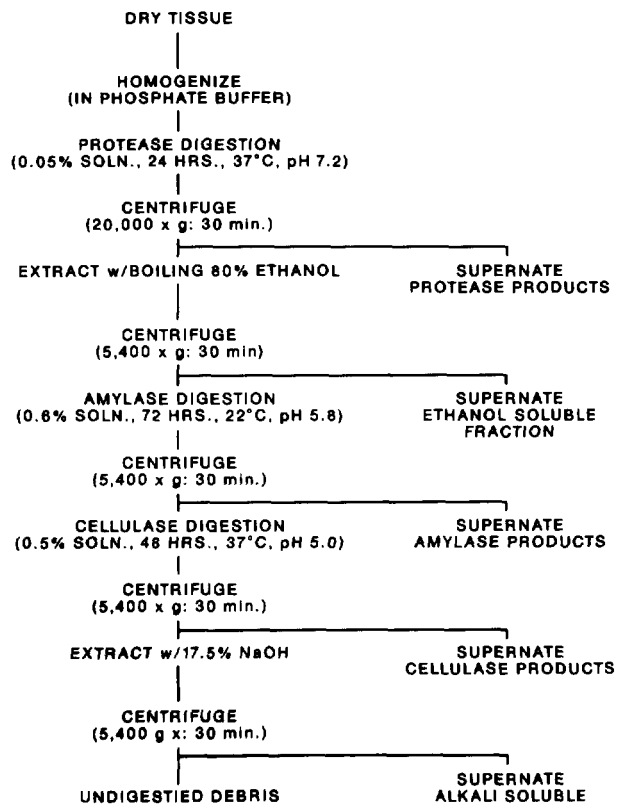

Fig. 4. Scheme for the enzymatic digestion of Tc-labeled leaf and wood tissue.

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published methods (Walker et al. 1980). Solid samples were saturated with ammonium hydroxide and ashed at 500°C. The Tc was solubilized from the ash with a mixture of sulfuric acid and K persulfate. Technetium-99 was extracted from acidified samples (liquids and extracts of ashed material) using tributyl phosphate (TBP). The TBP extract was successively washed with sulfuric acid and HCl, mixed with scintillator, and then counted for β radioactivity on a liquid scintillation counter. Samples of tree sap were acidified and ^{99}Tc was extracted into TBP using similar methods. Based on our experience with these methods, variability due to analytical errors was small; the coefficient of variation for replicate samples was <10% (Garten et al. 1986a).

RESULTS AND DISCUSSION

Tree sap

An analysis of sap from five maple trees, sampled in 1984, revealed ^{99}Tc concentrations ranging from $\approx$16 to 118 Bq L^{-1}. The most highly contaminated sap collected contained <0.2 Bq ^{99}Tc mL^{-1} sample. This concentration was too low to permit characterization of the chemical form of ^{99}Tc in tree sap. Concentration of the sap on a rotary evaporator (under vacuum) produced a sample that was both too viscous for chromatography and unsuitable for radiochemical analysis.

In dialysis experiments, $^{95m}TcO_4^-$ added to an aqueous retentate or an aqueous diffusate came into equilibrium on both sides of the dialysis membrane in the presence of tree sap. This membrane would have retained or excluded organic molecules in the sap with a nominal MW > 3,500 amu. The equilibrium concentrations after prolonged dialysis indicated that TcO_4^- was not reduced by or irreversibly binding to constituents > 3,500 amu that might be present in the sap. Furthermore, $^{95m}TcO_4^-$ incubated with tree sap for 2 or 72 h and then chromatographed on Sephadex G-25 eluted from the chromatography column in a single peak, with a V_E:V_O ratio of 3.6 (replicate runs). Therefore, sap incubated with ^{95m}Tc chromatographed in the same manner as a standard solution of aqueous TcO_4^- (V_E:V_O ratio equaled 3.5).

Technetium (IV) is reported to form chelate structures with many naturally occurring organic ligands, including polycarboxylic acids, hydrocarboxylic acids, polyols, sugars, nucleotides, nucleosides, polysaccharides, and the sulfhydryl-containing amino acids cysteine and methionine (Paquette et al. 1980; Delmotte 1981). Complexation of Tc (IV) with methionine is weak, apparently involving a charge transfer from the unionized amino N (Slifkin and Ellis 1978). Based on the results of dialysis experiments and gel permeation chromatography, it appears that TcO_4^- (Tc VII) is not reduced by or bound to sap constituents. The chemical form of Tc in xylem exudates from soybeans is reported to be TcO_4^- anion (Cataldo et al. 1978; Roucoux 1980). Pertechnetate is also the predominant soluble form of Tc in homogenized spinach roots (Lembrechts and Desmet 1985).

Physical fractionation of Tc *in wood and leaves*

At most, 2% of TcO_4^- in aqueous solution was sorbed by a 0.45-micron filter. The ultrafiltration membrane with a MW cutoff of $\approx$10,000 amu retained only 1% of the Tc. However, there was strong sorption (78%) of TcO_4^- to the ultrafiltration membrane with a MW cutoff of $\approx$1,000 amu. Technetium retained by the latter membrane and that present in the filtrate which passed the membrane was collected into a single fraction and designated <10,000 amu because filtration through the 10,000 amu membrane immediately preceded filtration through the 1,000 amu membrane.

Most of the Tc in wood chips and leaves not solubilized by homogenation was retained in the residue (Table 1). Less than 1% of the Tc solubilized by homogenation of wood chips, and not pelleted by centrifugation at 10,000 × g, was retained by a 0.45-micron filter, while 10% of the Tc from leaves was retained by a 0.45-micron filter. For the wood extract, approximately one-third of the Tc that passed the 0.45-micron filter was retained by an ultrafiltration membrane with a nominal MW cutoff of 10,000 amu. In leaves, only one-fifth of the Tc in the <0.45-micron filtrate was retained by the latter type of membrane. In both wood and leaf extracts, most of the Tc that passed the 0.45-micron filter also passed the ul-

Table 1. Distribution of Tc in various fractions produced by the homogenation of wood or leaf tissue in phosphate buffer, and the amount of solubilized Tc retained by a 0.45-micron filter and an ultrafiltration membrane (nominal MW cutoff 10,000 amu).

	Wood		Leaves	
Fraction	Bq ^{99}Tc	% Total	Bq ^{95m}Tc	% Total
Coarse residue	65.7	83	414	61
Pellet (Cell debris, mitochondria)	1.5	2	[a]	—
Residue > 0.45-micron	<0.2	<1	68	10
Residue > approx. 10,000 amu	4.1	5	38	6
Soluble < approx. 10,000 amu	7.4	9	162	24
Total	79.0	99+	682	101

[a] Sample was lost, but prior experience is that <1% of the Tc can be expected to occur in pellets obtained by this method.

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trafiltration membrane (Table 1), indicating the presence of TcO_4^- or soluble low MW complexes.

The predominance of low MW forms of Tc solubilized from maple wood and leaves by homogenation in phosphate buffer is in agreement with prior studies of the chemical form of Tc in spinach (Lembrechts et al. 1985) and corn leaves (Garten et al. 1986b). In corn leaf homogenates, 80% of the solubilized Tc was found in the cell cytosol obtained by centrifugation at 100,000 × g. Lembrechts et al. (1985) found that 93.5% of the Tc in a homogenate of spinach leaves remained in solution after centrifugation at 12,000 × g. More than 10 organic complexes of Tc were discernible in the supernatant, and between 80% and 90% of these complexes had a MW < 6,000 amu.

Chemical fractionation of Tc *in leaves, wood and litter*

Chemical extraction methods were used to characterize forms of Tc that were not readily solubilized by homogenation in phosphate buffer. In the ^{99}Tc-labeled wood, most (72% to 80%) of the Tc was removed by successive extractions with ethanol, water and weak mineral acid (Table 2). Much of the Tc in wood was removed by aqueous extraction, which was also expected to remove ionic substances, water-soluble carbohydrates and water-soluble protein. Only 20% to 28% of the Tc in wood was extractable by NaOH or was left unextracted in the final insoluble residue.

Results from the chemical extraction of tree leaves and leaf litter were very different from those for wood (Table 2). Less than 10% of the Tc in leaves or leaf litter was removed using ethanol, and somewhat more (17% to 29%) was removed by aqueous and acid extractions. Altogether, only 23% to 31% of the Tc present in leaves or leaf litter was extracted by the first three reagents (Table 2). Most (66 to 71%) of the Tc present in maple leaves or tree leaf litter was insoluble and extractable only by boiling NaOH. This is a relatively large percentage compared with the percentage of insoluble forms previously found in corn leaves (40%, Garten et al. 1986b), soybean leaves (38%, Roucoux 1980), and tomato leaves (16%, Myttenaere et al. 1980). The large release of Tc upon extraction with alkali indicates some association of this element with structural carbohydrates in the maple leaves. Recovery of the initial amount of Tc in leaf tissues through the complete extraction scheme (Table 2) was estimated to be 100%.

Gel permeation chromatography of selected chemical extracts

Since most of the Tc in wood was extractable by successive treatment with ethanol and water, and most of that in leaves was extractable by treatment with alkali, these extracts were examined by gel permeation chromatography. A standard solution of $^{95m}TcO_4^-$ eluted from a Sephadex G-25 column in the third and fourth void volumes (Fig. 5). By comparison, a large portion of the Tc in the ethanol–water extract of maple wood and the NaOH extract of leaves eluted in the second void volume (Fig. 5), indicating that Tc in these extracts was not predominantly TcO_4^- but was Tc complexed with molecules > 1,000 amu. There was a higher percentage of TcO_4^- present in the ethanol–water extract from maple wood than in the NaOH extract from leaves (Fig. 5).

When TcO_4 was incubated for 24 h using the NaOH extract from unlabeled maple leaves and chromatographed on the G-25 column, all radioactivity eluted in a single peak at precisely three void volumes. Therefore, TcO_4^- was not reduced by organic substances extracted from leaves using NaOH. This control demonstrated that Tc was not forming complexes with hydrolysis products or other artifact molecules formed during alkaline extraction of the leaf tissue.

Modified chemical fractionation of Tc *in maple leaves*

A modified chemical fractionation scheme more suited to carbohydrate extraction from plant tissues revealed that ethanol and water-soluble fractions from maple leaves contained only 22% of the total Tc in the leaves (Table 3). As in the first series of chemical extractions with ^{95m}Tc-labeled leaves, most of the ^{99}Tc could be removed from the leaves only with alkali (bicarbonate–carbonate solutions or NaOH). Technetium extractable with the bicarbonate–carbonate solution was designated hemi-

Table 2. Percentage of the Tc in labeled wood, leaves and leaf litter extracted using successive homogenation with ethanol, water, HCl and NaOH. The insoluble residue was that material remaining after extraction with NaOH.

	Percentage of Tc extracted from							
	^{99}Tc-labeled wood		^{95m}Tc-labeled wood		^{95m}Tc-labeled leaves		^{99}Tc-labeled leaf litter	
	Mean	(CV)[a]	Mean	(CV)	Mean	(CV)	Mean	(CV)
Ethanol	10	(0.14)	26	(0.11)	6	(0.03)	2	(0.16)
Water	56	(0.04)	45	(0.11)	12	(0.19)	19	(0.03)
0.2 N HCl	6	(0.90)	9	(0.06)	5	(0.01)	10	(0.20)
2 M NaOH	21	(0.13)	12	(0.35)	71	(0.01)	66	(0.57)
Insoluble residue	7	(0.18)	8	(0.25)	6	(0.39)	3	(0.47)

[a] CV = coefficient of variation for replicate extractions.

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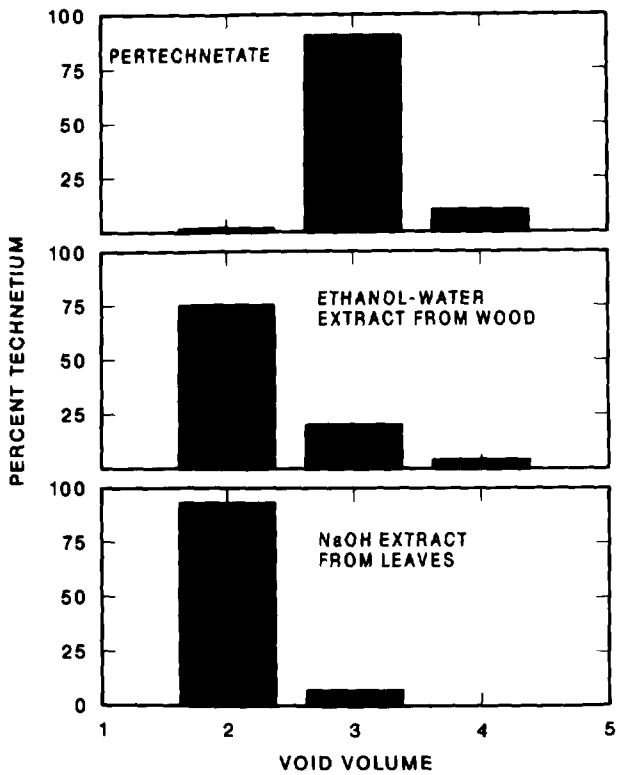

Fig. 5. Chromatograms of ^{95m}Tc (TcO_4^-), the combined ethanol–water extract of ^{99}Tc from maple wood, and the NaOH extract of ^{95m}Tc from maple leaves chromatographed on Sephadex G-25 (column length 25 cm, column diameter 2.5 cm, flow rate 3.5 mL min^{-1}, eluant 0.2 M NaOH). Fractions of eluate were pooled in the first, second, third, fourth and fifth void volumes (V_O).

cellulose I and that extractable with NaOH was designated hemicellulose II. Hemicellulose refers to various poorly defined polysaccharides of the cell wall, exclusive of cellulose, that are soluble in dilute alkali.

Technetium that was soluble in NaOH was further fractionated into low MW and high MW hemicellulose by immediately acidifying the extract to pH 4.5 (Robinson 1980). Less than 5% of the Tc was found in the high MW hemicellulose precipitate. This extraction scheme indicated that Tc was incorporated in or otherwise associated with structural elements of the cell walls in maple leaves.

Enzymatic digestion of maple leaf and wood tissue

Digestion of contaminated leaves and wood with protease increased the release of Tc from plant tissue homogenates relative to control incubations performed in the same manner without protease. When homogenized tissues were incubated without protease, 22% and 9% of the ^{99}Tc was released from wood and leaves, respectively. The inclusion of protease in incubations more than doubled the removal of Tc from wood and from leaves (Table 4). Subsequent extraction of protease-treated leaves with ethanol, amylase, cellulase and alkali demonstrated that almost half of the ^{99}Tc in maple leaves was associated with hemicellulose (NaOH extractable) and undegraded cellulose or other structural polysaccharides (residue) (Table 4).

Analysis and chromatography of the enzyme extracts

In enzyme (protease + pepsin) soluble-extracts from ^{99}Tc-contaminated leaves, 60% of the Tc did not pass an ultrafiltration membrane with a nominal MW cutoff of 10,000 amu. Of the 40% that passed the membrane, 85% of the β radioactivity showed little migration from the origin in TLC (R_f = 0 to 0.08) and was accompanied by a strong stain in response to ninhydrin reagent. In enzyme-soluble extracts from wood, 93% of the ^{99}Tc passed the same type of ultrafiltration membrane. TLC showed that 63% of the β activity in the <10,000 amu fraction migrated with an R_f value of $\approx$0.40 and was not accompanied by a positive reaction to ninhydrin. TLC of TcO_4^- anion alone (standard solution) revealed that 98% of the ^{99}Tc migrated with an R_f value of $\approx$0.48.

CONCLUSION

A data summary detailing evidence for the relative importance of free TcO_4^- and organically bound Tc in

Table 3. Amount of percentage distribution of ^{99}Tc in chemical extracts of maple leaves (see Fig. 3 for the extraction scheme and constituent makeup of the various extracts).

Fraction	Generalized constituents	Bq	%
Ethanolic supernate	Lipids and pigments	7.4	9
Water soluble			
Supernate	Ionic substances, sugars	7.4	9
Pellet	Pectic substances and polysaccharides	3.3	4
Water insoluble			
Bicarbonate soluble	Hemicellulose I	14.8	18
Bicarbonate insoluble			
NaOH soluble	Hemicellulose II	31.1	39
Pellet	Cellulose and polysaccharides	16.7	21

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Table 4. Release of ^{99}Tc from homogenized maple leaves and wood by successive treatments with protease, ethanol, amylase, cellulase and sodium hydroxide (see Fig. 4 for method).

	Percentage removed by treatment with					^{99}Tc in residue
Tissue	Protease	Ethanol	Amylase	Cellulase	NaOH	%
Wood	51	35	1	<1	8	1
Leaves	21	24	1	1	36	13

contaminated leaves and wood indicates that there are multiple forms of Tc in maple trees (Table 5). The high extractability of Tc by successive extractions with ethanol and water showed that Tc incorporated into maple wood occurred in predominantly soluble forms. Chromatography of the ethanol-water-soluble fraction indicated some presence (<10%) of free TcO_4^-, but most of the Tc in this fraction appeared to be organically complexed with molecules > 1,000 amu. If Tc is transported as TcO_4^- in the xylem sap, its metabolism and incorporation into woody tissues apparently involves some association with hydrophilic organic complexes, most of which are smaller than 10,000 amu.

Part of the Tc incorporated into maple wood (12% to 21%) was chemically removed using NaOH or remained after alkaline treatment in the insoluble residue (7% to 8%), indicating that nearly one-fourth of the Tc in wood was structurally incorporated into cell walls. Alkali-soluble hemicelluloses, which originate chiefly from cell walls, typically comprise about 30% of the sapwood from deciduous trees (Rogers and Perkins 1968).

Table 5. Data summary indicating the relative importance of organically bound and free TcO_4^- in contaminated maple leaves and wood.

Evidence Consistent with Tc *as* TcO_4^- *in Wood*
- 65% of that solubilized by physical methods was <10,000 amu
- 66% of 71% was chemically extracted using ethanol and water
- 93% of that solubilized by protease digestion was <10,000 amu
- Tc in a protease extract < 10,000 amu migrated like TcO_4^- in TLC

Evidence Consistent with Organically Bound Forms of Tc *in Wood*
- 35% of that solubilized using physical methods was >10,000 amu
- Tc in ethanol-water extracts did not chromatograph like TcO_4^-
- 20% to 28% was alkali extractable or in an insoluble residue
- Protease increased extractability from wood homogenates

Evidence Consistent with Tc *as* TcO_4^- *in Maple Leaves*
- 80% of that solubilized using physical methods was <10,000 amu

Evidence Consistent with Organically Bound Forms of Tc *in Maple Leaves*
- 19% of that solubilized using physical methods was >10,000 amu
- Most (≈70%) was alkali extractable or in an insoluble residue
- Chromatography of Tc in alkaline extracts did not resemble TcO_4^-
- Protease increased extractability from leaf homogenates
- 60% of the protease soluble Tc was >10,000 amu
- Tc in protease extracts < 10,000 amu did not migrate like TcO_4^- in TLC

There was little evidence for the occurrence of large amounts of TcO_4^- in maple leaves; however, most (80%) of the Tc that could be solubilized by physical methods was <10,000 amu in MW. The chemically insoluble nature of Tc in maple leaves and the large amount (70%) extractable with NaOH indicated an association with molecules in the leaf cell walls. Chromatography on Sephadex G-25 showed that Tc in the alkaline extract was not TcO_4^-. Furthermore, leaf Tc solubilized using protease reacted strongly with ninhydrin and, unlike TcO_4^-, showed little mobility in TLC on silica gel.

The *in vitro* formation of methionine and cysteine complexes with Tc, demonstrated by other researchers (Roucoux 1980; Delmotte 1981), implicates structural protein in the cell wall as a potential site for Tc accumulation. Structural models of the primary cell wall in plants, based on suspension-cultured sycamore (*Acer pseudoplatanus*) cells, indicate that a protein layer is sandwiched between hemicellulose layers that are H bonded to cellulose chains (Bonner and Varner 1976). Hence, the cell wall protein is protected by insoluble structural polysaccharides (cellulose and hemicellulose) from attack by treatment with ethanol, water and weak acids. Extraction with NaOH solubilizes the hemicellulose and frees Tc associated with cell wall protein. Protease treatment of maple leaves released only 21% of the leaf Tc, perhaps because protease treatments are ineffective in releasing cell wall protein unless wall polysaccharides are first chemically degraded. Enzyme treatment with cellulase (Table 4) also removed a negligible amount of the leaf Tc, probably because the cell wall protein was still protected by insoluble layers of structural polysaccharides.

Technetium in contaminated ground water appears to be absorbed by tree roots as TcO_4^- and transported in the xylem stream to leaves, where Tc (VII) is reduced to Tc (IV) and organically complexed during metabolism. The organic complexation of Tc in maple leaves (and perhaps wood) has implications for the cycling of ^{99}Tc in forests (Fig. 1). Observations on the probable incorporation of Tc into the structural components of maple leaves explains several observations from field studies (Garten et al. 1986a; Garten 1987), including 1) the progressive accumulation of ^{99}Tc in tree leaves over the growing season, 2) the apparent accumulation of ^{99}Tc in forest floor leaf litter layers, 3) the small amount of ^{99}Tc leaching from tree leaves by rainfall, and 4) the presence of organic soil fractions enriched in ^{99}Tc.

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Chemical extractions of soil from the ^{99}Tc-contaminated study site reveal that between 14% and 23% of the soil Tc was extractable with dilute NaOH (humic substances) (Garten et al. 1986a). Approximately two-thirds of the ^{99}Tc extracted from soil using NaOH will not pass a dialysis membrane with a MW cutoff of 3,500 amu. Many years of ^{99}Tc uptake by tree roots, metabolism and incorporation into structural components of the leaf, and decomposition of seasonal leaf fall has substantially contributed to the presence of Tc-organic matter complexes in the forest soil.

Acknowledgments—This research was sponsored by the Office of Health and Environmental Research, U.S. Department of Energy, under Contract No. DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc., Publication No. 3109, Environmental Sciences Division, Oak Ridge National Laboratory.

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