

COO-2447-4

THE BEHAVIOR OF TECHNETIUM-99 IN SOILS AND PLANTS

Progress Report

Robert G. Gast

Department of Soil Science
University of Minnesota
St. Paul, Minnesota

April 1, 1975 - March 31, 1976

PREPARED FOR THE U.S. ENERGY RESEARCH AND DEVELOPMENT ADMINISTRATION

UNDER CONTRACT NO. AT (11-1) - 2447

NOTICE
This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ABSTRACT

Work described in this report is aimed at establishing the magnitude and mechanisms of Tc-99 sorption by soils and uptake by plants. Studies showed that sorption of Tc-99 by soils was essentially stopped by sterilization of the soil, further indicating that the sorption process is related to microbial activity. Studies also showed that sorption can occur under aerobic as well as anaerobic conditions. However, there was considerable difference in the extent of sorption for different soils which remains to be explained. Tc-99 was shown to be toxic to germinating seeds at low concentrations, but not when added to more mature plants. Initial evidence suggests this is a chemically rather than a radiologically induced toxicity but this remains to be completely resolved as well as whether there is a threshold level of Tc-99 required before toxicity occurs.

LEGAL NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States or the United States Energy Research and Development Administration, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product or process disclosed or represents that its use would not infringe privately owned rights.

I. INTRODUCTION

This technical progress report covers the results of studies dealing with the behavior of technetium-99 in soils and plants for the period of approximately November 1, 1974 through November 15, 1975 under contract AT (11-1) - 2447 with the U.S. Energy Research and Development Administration. Approximately 10 percent of the principal investigators' time was devoted to the project during the reporting period.

This report deals with a continuation of those studies outlined in the first progress report (U.S. ERDA NO. COO-2447-1) involving the sorption of Tc-99 by soils and uptake by plants.

II. REVIEW OF CURRENT LITERATURE

Although there were several new entries in the literature dealing with technetium, most dealt with either animal studies, health physics aspects or analytical procedures for detecting or recovering Tc. The only report dealing with sorption of Tc by soils or uptake by plants was an abstract by Wildung et al. (1975) who reported that Tc-99 uptake by plants resulted in toxicity symptoms in the form of a reduction in internode elongation and an irregular expansion of the leaf blade in plants. They also observed that stems and cotyledons had the greatest Tc accumulation and that it appears that Tc (as pertechnetate) may act as a nutrient analog possibly accounting for the high removal of Tc-99 from soils by plants.

III. MATERIALS AND METHODS

Since most of the basic techniques and procedures involved in both the soil sorption and plant uptake studies were outline in the earlier progress report (U.S. ERDA Report NO. COO-2447-1) only the general procedures used and their application to the specific studies involved will be given here.

A. Technetium-99 source

Technetium-99 was obtained from the International Chemical and Nuclear Corp., Irvine, California. The carrier-free material is supplied in 1 N NH_4OH in the form of ammonium pertechnetate (NH_4TcO_4).

B. Radioassay for Tc-99

Tc-99 was determined using a Packard Tri-Carb liquid scintillation spectrometer (Model 3375) with the discriminators and amplification preset for 14-C counting. The basic technique and scintillant used were the same as outlined in the earlier report.

C. Soil characterization

The soils used were the same as those characterized and used in the first phases of the study.

D. Sorption of Tc-99 by soils

1) Effect of soil sterilization on Tc-99 sorption

Earlier results suggested that sorption of Tc-99 by soils may be related to microbial activity. A soil sterilization experiment was conducted to further pursue this possibility. For this purpose, sterilization by steam without pressure, or tyndallization, (Schmidt, 1975) was recommended over other possible procedures since it results in less change in the treated soil (Parkinson *et al.*, 1971). The soil and Tc-99 were sterilized separately in order to decrease the chances of complexation of technetium by products released from the soil by the sterilization process.

The procedure involved placing two g (oven-dry basis) of Bergland, Arveson or Nicollet surface soil in tared 50 ml polypropylene centrifuge tubes with screw closures. The soil was moistened with 2 or 3 ml of distilled water one day before beginning the sterilization process in order to promote spore germination and to insure better steam penetration. The tubes were covered with aluminum foil and reweighed. The covered tubes and contents were steamed in an autoclave unit (with the steam outlet valve open) for 1 hour each day on days 0, 1, and 3. Between steamings the tubes were stored at room temperature in the laboratory. This split sterilization procedure allows for germination of spores between heat treatments. After the third steaming, the covered tubes were reweighed to determine the amount of water in soil (this was needed to make dilution corrections later). The aqueous Tc-99 solution, tube caps and all glassware used to contain and transfer the solution were autoclaved.

Transfer of the solution (25 ml, 0.0025 μ Ci Tc-99/ml) to the tubes was done with automatic pipeting devices using aseptic techniques in a sterile transfer room. The tubes were capped, reweighted and the necks covered with tape. The sealed tubes were then placed in a 25°C water bath.

Biweekly sampling commenced after 4 weeks and included 3 replicates for each soil at each sampling date. Sampling entailed centrifugation for 10 minutes at 15,000 rpm, filtration of the supernatant through Whatman #42 paper and liquid scintillation counting of 1 ml aliquots of the filtrate.

2) Effect of aeration of soil-water suspensions on sorption of Tc-99

a. Effect of aeration by open-bottle shaking

In order to determine whether previously observed Tc-99 sorption was associated with development of anaerobic conditions in the soil-water system, a similar experiment was conducted in which well-aerated

conditions were maintained.

Two g (oven dry basis) of Bergland, Arveson, or Nicollet surface soils were placed in tared 60 ml linear polyethylene bottles and twenty-five ml of an aqueous solution containing 0.0024 μ Ci Tc-99/ml was added to each bottle. The bottles were sealed with their normal polypropylene screw caps which had been modified by drilling a hole through the top. A 4 cm length of 4 mm I.D. glass tubing was inserted into the hole to provide a pathway for gas exchange between the atmosphere and the soil-water suspension. The bottles were shaken continuously on a reciprocating shaker (160 excursions/minute) at room temperature. Evaporative losses from the bottles amounted to about 0.03 ml/day and were replaced by biweekly additions of distilled water.

Biweekly sampling included 3 replicates for each soil at each sampling date. Sampling entailed centrifugation for 10 minutes at 9000 rpm, filtration of the supernatant through Whatman #42 paper and liquid scintillation counting by 1 ml aliquots of the filtrate.

b. Effect of aeration by bubbling

An initial experiment was conducted in which fifty g of Bergland soil (oven-dry basis) was placed in a one liter polyethylene bottle along with a sufficient quantity of 0.0024 μ Ci Tc-99/ml solution to yield a 1:12.5 (w:w) soil:solution ratio as used in the study where the suspensions were shaken in an open container. Air was continuously forced through each of the 3 containers at a rate of about 1200-1500 ml/min by individual aquarium pumps and a length of glass tubing inserted through the mouth to the bottom of the bottle. The mouths of the bottles were covered with aluminum foil and additions

of distilled water were made to the bottles every other day to make up for evaporative losses. Sampling, conducted on an every other day basis for the first 3 weeks and at weekly intervals thereafter, consisted of pipeting off 5 ml of suspension, centrifugation, filtration through Whatman #42 paper and liquid scintillation assay of 1 ml aliquots of the filtrate. This initial experiment was conducted at room temperature ($22 \pm 3^{\circ}\text{C}$).

A second experiment was conducted in the same manner as that described above except that the same three soils used in the open-container shaking experiment were included (Bergland, Arveson, and Nicollet surface) and temperatures were controlled at $15 \pm .02$ and $25 \pm .02^{\circ}\text{C}$.

E. Plant uptake of Tc-99 from incubated soils

Earlier experiments have shown that 1) plants can take up significant quantities of Tc-99 from soils irrigated with the Tc-99 during plant growth and 2) soils can sorb most of the added Tc-99 but only over relatively long periods of time compared to the plant uptake experiments. Studies were in turn conducted to determine whether Tc-99 added to soils followed by moist incubation is equally available for plant uptake. The same soils, were used as in the earlier irrigation experiment. Both unfertilized and fertilized soils were used with three replications on each case. The studies were conducted in a greenhouse with light and temperature conditions maintained as described for the earlier irrigation experiment.

To provide as uniform a distribution of Tc-99 as possible, the soil and Tc-99 were added to the 12 oz. containers in layers, i.e. 100 g soil + 4 ml of $0.5 \mu\text{Ci}/\text{ml}$ Tc-99 solution + deionized water to bring the 100 g soil layer to a 0.1 bar moisture content (taken as field capacity). By this layering process, a total of 6 μCi was applied to 300 g of soil in each pot. The

pots were covered loosely with aluminum foil and the soil maintained near field capacity by weekly additions of deionized water. This moist incubation was carried on for two months in a room maintained at about 25°C.

After the two months of moist incubation, the Tc-99 labeled soils were seeded to Era wheat. A sufficient number of seeds to yield 20 plants per pot (as determined by the germination trials; fifth-day census of emergent plants used as germination index) were placed on the soil surface and covered with 150 g of coarse sand. The pots were covered with Saran Wrap as described above prior to taking to the greenhouse.

Since suboptimal, retarded growth was observed in most of the pots, harvesting of all of the pots on a given date seemed undesirable. Rather plant growth stages were used as a harvest indicator. Since only the seedlings on the Omega soil in the unfertilized pots grew well (>100 mg dry matter), all of the unfertilized pots were harvested when 20% or more of the Omega seedlings were in the 3-leaf stage, i.e. 19 days after planting. Growth on the fertilized soils was better than those not fertilized. In these cases, plants on each of the soils were harvested when 20% or more of the seedlings were in the 3-leaf stage (15 to 26 days after planting). As before, all aerial tissue above the coleoptile was harvested.

F. Effect of Tc-99 on germinating seeds

An initial solution culture experiment was conducted in which wheat seeds were germinated and grown for 10 days in growth pouches containing 50 ml of 1/3 strength Hoagland solution and 0, 0.025, 0.25, 1.0, 6.7 or 20 μ Ci of Tc-99. The solutions were adjusted to pH 7 with four replications per treatment. The pouches were maintained in the dark for the first three days and then placed in a growth chamber under conditions similar to those outlined for earlier experiments. Daily additions of deionized water were made to replace evapotranspiration losses.

On the basis of the results from the initial study, a second experiment was conducted in which corn (Zea mays L.), soybeans (Glycine max L.), radishes (Raphanus sativus L.), oats (Avena sativa L.), barley (Hordeum vulgare L.), and wheat (Triticum aestivum L.) were germinated and grown for ten days under the same conditions and Tc-99 concentrations of 0, 0.025, 0.25, 1.0, 2.5, 5.0, 6.7 and 10.0 μ Ci in 50 ml of solution.

G. Effect of time of addition of Tc-99 toxicity to wheat seedlings

Wheat seedlings were germinated and grown for 10 or 18 days in growth pouches containing 50 ml of 1/2 strength Hoagland solution. Either 1.0 or 5.0 μ Ci Tc-99 was added to the growth pouches on day 0, 2, 4, 6 or 8 following planting of the seeds. Harvesting, sampling preparation and Tc-99 counting was conducted as in previous experiments.

IV. RESULTS AND DISCUSSION

A. Sorption of Tc-99 by soils

1. Effect of sterilization on Tc-99 sorption

Sterilization of Arveson, Bergland and Nicollet surface soils by the tyndallization process essentially eliminated all sorption of Tc-99 over a 70 day period (Fig. 1). All three of these soils had been shown previously to sorb over 98 percent of the Tc-99 in solution in sealed containers under nonsterile conditions. Since alteration of soils by the tyndallization process should be minimal, the results tend to further confirm that previously observed Tc-99 sorption is associated with microbial activity.

The specific mechanism or process by which microbial activity is involved in Tc-99 sorption is difficult to resolve, however. It may involve incorporation of Tc-99 into microbial tissues, interaction with microbial metabolites or perhaps effects associated with the depletion of dissolved oxygen by microbial metabolism. Microbial metabolites may reduce Tc(7+)-pertechnetate to a lower valence state species capable of binding to soil organic matter. The reduction of Tc(7+) to Tc(4+) by ascorbic acid, for example, is a commonly used procedure in the preparation of radiopharmaceuticals. Such reduced technetium species can bind irreversibly to proteins, probably by covalent bonding (Dewanjee, 1974).

2. Effect of aeration on Tc-99 sorption

a. Effect of aeration by open-bottle shaking

As indicated previously, the Arveson, Bergland and Nicollet surface soils have been shown to sorb Tc-99 in sealed containers. However these conditions could lead to the development of high microbial respiration rates and subsequent depletion of dissolved oxygen (Avnimelech and Raveh, 1974). As a result, precipitation of technetium as Tc_2S_7 could result

from H_2S production by anaerobic sulfate-reducing bacteria. Also, a drop in Eh could result in mobilization of metals capable of reducing pertechnetate to a species capable of organic matter binding as discussed above. The aeration experiments were conducted to determine whether anaerobic conditions may have existed and been the cause of Tc-99 sorption by soils.

Results of the open bottle, shaking experiment are shown in Fig. 2. In contrast to the sealed-bottle study, the Nicollet surface soil exhibited no sorption of Tc-99 over a 12 week period. The Bergland exhibited an initial high removal of Tc-99 from solution, such as observed in the earlier sealed-bottle studies, but then showed a progressive decrease in sorption ability from the second to the twelfth week. The Arveson soil showed an erratic sorption pattern with from 50 to 80% of the added Tc-99 remaining in solution over the 12 week period.

While one intuitively felt that these suspensions were aerobic, the oxygen status of the suspensions employed in the third sampling of the shaker aeration trials was checked with a YSI model 54 oxygen meter (Yellow Springs Instrument Co., Yellow Springs, Ohio). These suspensions all showed dissolved oxygen levels of between 4.8 and 6.1 ppm O_2 , corresponding to between about 80 to 90% of that measured for unstirred air-saturated water.

b. Effect of aeration by bubbling

The results of the initial bubbling experiment with the Bergland soil are shown in Fig. 3. While sorption appears to be slower than observed in the sealed bottle experiments, the solution concentration of Tc-99 progressively decreased to about 10% of the initial concentration after 2 months.

Essentially the same results were obtained for the Bergland soil where the temperature was controlled at $25 \pm 0.2^{\circ}\text{C}$ (Fig. 4) showing that the results are reproducible. However, there was a decrease in adsorption at 15°C compared to that at 25°C , further indicating that the sorption of Tc-99 is related to microbial activity rather than a pure chemical process; i.e. the amount of sorption by chemical processes should increase as temperature is lowered.

The Arveson soil showed sorption levels intermediate between the Bergland and Nicollet soils at 25°C (Fig. 5) as was found in the open-bottle shaking experiment. It also showed less sorption at 15°C than at 25°C indicating again that a microbial related process is involved. The Nicollet surface soil showed relatively little sorption compared to the Bergland and Arveson soils (Fig. 6). Also, there was greater sorption at 15°C than 25°C indicating that a different process may be involved.

Results of the open-bottle shaker and bubbling experiments show that Tc-99 sorption by soils can occur under aerobic as well as anaerobic conditions. However, a great many unanswered questions remain concerning the mechanisms and conditions under which sorption occurs. For example, while the Bergland and to a lesser extent the Arveson soils continued to sorb Tc-99 in open as well as sealed containers, the Nicollet surface soil did not. These differences are difficult to explain on the basis of the physical and chemical properties of the soils (Table 1). It is also difficult to imagine that these soils would have greatly different microbial populations. However, this possibility will have to be checked along with the possibility that the particular soil samples used are unique or that some particular aspect of storage or pretreatment of the samples may have an influence on the sorption properties.

B. Plant uptake of Tc-99 from incubated soils

Plants germinated and grown in soils which had been moist incubated with Tc-99 for two months generally showed severe growth reduction compared to those for the irrigated soils. This was evidenced by both visual observation and final tissue dry weights (Tables 2 and 3). Even though yields from the incubation and irrigation studies are not directly comparable due to differences in harvest times, this alone cannot account for the large differences in tissue weight. While the seeds had germinated and emerged by the fifth day after planting, the first leaf failed to grow out of the coleoptile on many of the plants. Desiccation and death soon followed on such plants. Leaves of the surviving plants were often very dark green with some yellowing of the tips, and displayed a twisted and stunted growth habit.

Most of the moist incubated soils did show signs of fungal growth at the end of the two month incubation period, and the possibility was considered that the poor plant growth might be associated with a plant pathogen. However, the growth reductions did not occur in plants grown in incubated soils without Tc-99, thus indicating that Tc-99 is toxic to plants. Also, the fact that toxicity appeared in the soil incubation study plants (Tc-99 was present at germination) but not in the soil irrigation or solution culture plants (Tc-99 added 5 and 14 days, respectively after seeding), further suggests that the toxicity is associated with the early stages of embryonic cell division and differentiation.

While plant growth in the incubated soils was poor in most cases, the Tc-99 tissue concentrations are in general quite similar to those found in the irrigation studies (Tables 2 and 3). This might not be expected from the results of early soil sorption experiments unless either (1) sorbed technetium is readily plant-available, or (2) the incubated soil system is not analogous to the earlier soil sorption systems studied and the Tc-99 remains unsorbed.

Since 0.01 M CaCl_2 is said to approximate the total electrolyte concentration in the soil solution of non-saline soils at optimum field water content (Peech, 1965), it was used as an extracting agent to test for non-sorbed Tc-99. Overnight shaking of Bearden, Hibbing and Waukegan soil samples, which had sorbed 88% or more of added Tc-99 in previous laboratory experiments, resulted in 11% or less of the sorbed fraction being released to the 0.01 M CaCl_2 solution. Thus, as 0.01 M CaCl_2 is a poor extractant of sorbed Tc-99, it should give some indication of the quantity of free, non-sorbed Tc-99 present in a soil sample.

A single pot of each of the unfertilized, incubated soils was sampled, extracted overnight with CaCl_2 (1:3 soil: CaCl_2 by wt.) and the solution assayed for Tc-99. The results (Table 4) indicate that a large percent of the applied Tc-99 is still "free" after two months of moist incubation. Thus, the high plant Tc-99 tissue concentrations observed are not inconsistent with those from the irrigation study. While the high CaCl_2 extraction figures appear to agree with the high tissue concentrations observed the linear regression correlation coefficient between the two parameters was only 0.58. The reason for the high percentage of non-sorbed Tc-99 after two months of soil contact is not clear, but may be related to aeration effects.

C. Effect of Tc-99 on germinating seeds

Results of the initial experiment in which wheat seeds were germinated and grown for 10 days in growth pouches containing increasing amounts of Tc-99 showed that significant reduction in shoot growth first occurred between Tc-99 additions of 0.25 and 1.0 μCi with continued reduction through the 20 μCi level (Table 5). The results also show relatively little reduction in root growth over the same range although, as will be shown later, this may be an artifact due to inclusion of seed weight in the root yield data.

Measurable effects of Tc-99 toxicity on germinating wheat seedlings then first occurred at initial solution concentration of technetium between 0.3 and 1.2 $\mu\text{g}/\text{ml}$. This corresponds to shoot tissue concentrations between 0.68 and 2.8 $\mu\text{Ci}/\text{g}$. Since the specific activity of Tc-99 is 17 mCi/g , this corresponds to a Tc concentration in the tissue of 40 to 165 ppm.

The effect of Tc-99 on germinating seedlings for the other plant species (including a repeated experiment for wheat seedlings) are given in Tables 6-11. Results for the initial wheat seedling study (Table 5) indicate that there may be a threshold effect or a minimum level of Tc-99 required before toxicity occurs. However, results for the second experiment do not confirm this effect for wheat (Table 6). Results do show that Tc-99 is toxic to germinating seeds for all species studied, with some species showing a threshold effect and others not. Whether there is a threshold level of Tc-99 required for toxicity and what that level might be for a given species remains to be seen.

As indicated above, Tc-99 was toxic to all plant species studied. Wheat, barley, oats and radishes all showed toxicity occurring in the range of 0.025 to 1.0 μCi Tc-99/50 ml (0.03 to 1.2 μg Tc/ml). In contrast toxicity levels for corn and soybeans were somewhat higher; i.e. in the range of 5.0 to 6.7 μCi Tc-99/50 ml or 6.0 - 8.0 μg Tc/ml (Tables 10 and 11). Also, when the seeds are removed before determining root weight, Tc-99 is shown to cause a reduction of root as well as top growth.

The Tc-99 induced toxicity could be either chemically or radiologically induced. Since technetium does not have a stable isotope, there is no direct means of distinguishing between these two possible mechanisms. One could test for possible radiological effects by using a radionuclide with nuclear properties similar to Tc-99 but known not to cause chemical toxicity in the concentration range examined. However, this would require getting the radionuclide into the plant in concentrations, and with macro- and micro-distributions similar to that for Tc-99, and that would be difficult.

Alternately, tissue dose rates were calculated using the following equation (Lapp and Andrews, 1972):

$$\text{Dose rate} = (C_0)(\bar{E}_\beta)(5.92 \times 10^{-4}) \text{ rad sec}^{-1}$$

Where C_0 = radioisotope concentration in tissue, $\mu\text{Ci g}^{-1}$

\bar{E}_β = average beta energy of radioisotope, Mev.

Tc-99 tissue concentrations were expressed on a wet weight basis for these calculations as succulent tissue is being irradiated in the living plants. The average beta energy for Tc-99 was taken as 0.1 Mev.

A tissue dose rate of 2 rads/day at the time of harvest was calculated for the 1.0 $\mu\text{Ci}/50 \text{ ml}$ - treatment, where shoot tissue yield depression was first observed. A dose rate of 16 rads/day was calculated for the shoot tissue at the highest level of added Tc-99 or 20 $\mu\text{Ci}/50 \text{ ml}$. Higher or lower dose rates may have been encountered prior to harvest depending upon the relative rates at which technetium accumulated and new tissue production occurred over the period of the experiment.

These dose rates appear to be quite low when compared with those required in other species to produce growth inhibition (Casarett, 1968). Unfortunately, no reports of studies dealing specifically with seedling growth inhibition in response to chronic irradiation were found in the literature. However, a study of the effect of acute x-irradiation of wheat seedlings by Zirkle and Lampe (1938) showed that exposures of about 400-500 R were required to produce a 20% reduction in shoot growth of wheat seedlings. While Zirkle and Lampe class wheat seedlings as highly radiosensitive, their data makes a 2 rad/day dose rate seem an unlikely cause for the growth reductions observed here.

The above arguments suggest that Tc-99 toxicity is chemical rather than radiological in nature. Also, the Tc-99 toxicity symptoms resemble those associated with damage by 2, 4-D and related selectively translocated herbicides. Such herbicides function as weed control agents by accumulating to toxic levels

in the meristematic regions and inducing cell division and enlargement, callus and tumor formation and tissue crushing. This unregulated growth leads to complete disorganization of the vascular tissues, and thus, lacking the ability to translocate water, salts, and metabolites, the plants die. Symptoms of 2, 4-D injury include a twisting or bending of stems and leaves, (resulting from differential growth rates in petioles, pulvini, and elongating regions of the stem) and a cessation of growth followed by death of tissues (Crafts and Robbins, 1962).

If Tc-99 toxicity is chemical in nature, it might be expected that the symptoms would be similar to those for stable analogs of pertechnetate such as iodide whose physiological behavior resembles that of TcO_4^- , at least in animals. Lewis and Powers (1941) found about 20% depression in top tissue yields of barley grown in a nutrient solution containing 0.50 ppm added iodine (tissue iodine content was 160 ppm). At 1.0 ppm added iodine, all plants died. Tissue concentrations as low as 6 ppm have resulted in reduced growth. However, the toxicity symptoms described include a general chlorosis, yellow interenal patches and brown necrotic spots, and are unlike those seen with Tc-99 in wheat seedlings.

D. Effect of time of addition on Tc-99 toxicity to wheat seedlings.

Additions of 1.0 and 5.0 μ Ci of Tc-99 to 50 ml of 1/2 strength Hoagland's solution had a depressing effect on both top and root growth when added as late as the sixth or in some instances the eighth day following the start of germination (Tables 13 and 14). Growth depression was greatest at the earlier dates of application and tended to be greater in tops than roots.

ACKNOWLEDGMENTS

Special recognition is given to the following individuals who have contributed greatly to the overall efforts of the project during the past year.

1. Dr. Edward R. Landa, former Research Assistant. (Current address: Department of Soil Science, Oregon State University, Corvallis, Oregon 97331).
2. Lisa Hart Thorvig, Research Assistant
3. Kathy Gallagher, Laboratory Technician

LITERATURE CITED

Avnimelech, Y. and Raveh, A. (1974). The Control of Nitrate Accumulation in Soils by Induced Denitrification. *Water Research* 8:553-555.

Casarett, A.P. (1968) *Radiation Biology*. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Crafts, A.S. and Robbins, W.W. (1962) *Weed Control*, Third Edition. McGraw-Hill. New York.

Dewanjee, M.K. (1974) Binding of Tc-99m ion to hemoglobin. *J. Nucl. Med.* 15:703-706.

Lapp, R.E. and Andrews, H.L. (1972) *Nuclear Radiation Physics*. Prentice-Hall, Inc. Englewood Cliffs, New Jersey.

Lewis, J.C. and Powers, W.L. (1941) Iodine in Relation to Plant Nutrition. *J. Agric. Res.* 63:623-637.

Parkinson, D., Gray, F.R.G. and Williams, S.F. (1971) Methods for Studying the Ecology of Soil Microorganisms. International Biological Programme Handbook No. 19. Blackwell Scientific Publications, Oxford.

Peech, M (1965) Hydrogen-ion Activity: In C.A. Black, Ed. *Methods of Soil Analysis*. Amer. Soc. Agronomy (Monogr. 9) Madison, Wisc. p. 914-926.

Schmidt, E.L. (1975) Personal communication. Professor of Soil Science and Microbiology. University of Minnesota.

Wildung, R.E., Garland, F.R. and Cataldo, D.A. (1975) Accumulation of Technetium from Soil by Plants--A potential mechanism for uptake and toxicity. *Agromony Abstracts*, 1975 Annual Meetings, American Society of Agronomy, p. 126.

Zirkle, R.E. and Lampe, I. (1938) Differences in the Relative Action of Neutrons and Roentgen Rays on Closely Related Tissues: *Am. J. Roentgenol. Radium Therapy*. 39:613-627.

LIST OF FIGURES

Fig. 1. Effect of steam sterilization pretreatment of soils on sorption of Tc-99 by soils from aqueous solution at 25⁰C.

Fig. 2. Effect of aeration by open-bottle shaking on Tc-99 sorption by soils from aqueous solutions at ambient temperature.

Fig. 3. Effect of aeration by bubbling on Tc-99 sorption by Bergland soil from aqueous solution at ambient temperature.

Fig. 4. Effect of aeration by bubbling on Tc-99 sorption by Bergland soil from aqueous solution at 15 and 25⁰C.

Fig. 5. Effect of aeration by bubbling on Tc-99 sorption by Nicollet surface soil from aqueous solution at 15 and 25⁰C.

Fig. 6. Effect of aeration by bubbling on Tc-99 sorption by Arveson soil from aqueous solution at 15 and 25⁰C.

LIST OF TABLES

Table 1. Chemical and physical properties of Bergland, Arveson, and Nicollet surface soils.

Table 2. Uptake of Tc-99 by aerial parts of wheat seedlings from unfertilized and fertilized soils irrigated with Tc-99 labeled water.

Table 3. Uptake of Tc-99 by aerial parts of wheat seedlings from unfertilized and fertilized soils moist incubated with Tc-99 for two months prior to seeding.

Table 4. Extraction of Tc-99 from moist-incubated soils by 0.01 M CaCl_2 .

Table 5. Tissue yield and Tc-99 concentration of 10-day-old wheat seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 6. Tissue yield and Tc-99 concentration of 10-day-old wheat seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 7. Tissue yield and Tc-99 concentration of 10-day-old barley seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 8. Tissue and Tc-99 concentration of 10-day-old oat seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 9. Tissue yield and Tc-99 concentration of 10-day-old radish seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 10. Tissue yield and Tc-99 concentration of 10-day-old soybean seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 11. Tissue yield and Tc-99 concentration of 10-day-old corn seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99.

Table 12. Tissue yield and Tc-99 concentration of 10-day-old wheat seedlings germinated and grown on 50 ml of 1/2-strength Hoagland solution containing 1.0 or 5.0 μ Ci of Tc-99 added with increasing time after start of germination.

Table 13. Tissue yield and Tc-99 concentration of 18-day-old wheat seedlings germinated and grown on 50 ml of 1/2-strength Hoagland solution containing 1.0 or 5.0 μ Ci of Tc-99 added with increasing time after start of germination.

Table 1. Chemical and physical properties of Bergland, Arveson, and Nicollet surface soils.

<u>Depth sampled (inches)</u>	<u>Bergland</u>	<u>Soil Type</u>	<u>Nicollet</u>
	0-7	Arveson	
% sand	14.0	47.1	27.4
% silt	25.1	24.6	42.9
% clay	60.9	28.3	29.7
pH (H ₂ O)	9.4	7.7	5.91
pH (KCl)	5.5	7.4	5.07
% org. carbon	5.67	2.8	2.39
% Fe ₂ O ₃	2.39	0.21	0.86
CEC (meq/100g)	32.3	14.9	19.3

Table 2. Uptake of Tc-99 by aerial parts of wheat seedlings from unfertilized and fertilized soils irrigated with Tc-99 labeled water. Tabulated values are means of 3 replicates with standard errors of the means indicated in parentheses.

Soil	Tissue dry weight (mg)		% of added Tc-99 in aerial tissue		Tc-99 concentration in plant tissue ⁺⁺ (μ Ci/g)	
	unfert.	fert.	unfert.	fert.	unfert.	fert.
Bearden	231(12)	225(8)	64(2.2)	35(3.4)	16.6 bcd**	9.2 c
Hegne	165(5)	191(6)	61(0.1)	49(1.6)	22.4 ef**	15.2 fg
Hibbing	201(14)	235(9)	62(2.6)	56(1.4)	18.5 cde**	14.3 efg
Nicollet (surface)	169(12)	179(2)	67(0.8)	52(4.7)	24.0 f*	17.5 gh
Nicollet (subsurface)	161(7)	186(3)	57(2.2)	62(3.2)	21.3 ef	19.9 h
Omega	193(6)	211(8)	42(0.1)	31(3.5)	13.1 ab*	8.9 bc
Bergland	195(4)	207(0.1)	62(1.2)	54(0.6)	19.1 de**	16.0 fg
Arveson	191(2)	206(5)	44(2.8)	17(0.6)	13.7 ab**	5.0 a
Waukegan	144(13)	204(6)	56(4.4)	59(5.0)	23.2 f**	17.5 gh
Zimmerman	147(9)	171(5)	52(2.3)	47(1.4)	21.1 ef**	16.5 fgh
peat	275(4)	350(25)	54(3.0)	77(2.3)	11.8 a	13.2 def

+ Concentration factors, $\frac{(\mu\text{Ci/g tissue})}{(\mu\text{Ci/g soil})}$, can be obtained by multiplying ($\mu\text{Ci/g}$) by 50.

‡ Means in same column followed by same letter are not significantly different at the 5% level by Tukey's test (Steel and Torrie, 1960).

*,** Tissue concentration means for unfertilized vs. fertilized treatments (for a given soil) significantly different at the 5% and 1% level of probability respectively.

Table 1. Uptake of Tc-99 by aerial parts of wheat seedlings from unfertilized and fertilized soils moist-incubated with Tc-99 for two months prior to seeding. Tabulated values are means of three replicates with standard errors of the means indicated in parentheses.

Soil	Tissue dry weight (mg)		% of added Tc-99 in aerial tissue		Tc-99 concentration in plant tissue ⁺ (μ Ci/g)	
	unfert.	fert.	unfert.	fert.	unfert.	fert.
Bearden	54(6)	249(11)	17(1.4)	8(2.0)	19.4 bcd	1.9 a
Hegne	30(2)	119(6)	7(0.3)	7(0.9)	14.2 abc	3.8 ab
Hibbing	43(13)	170(5)	15(4.4)	22(3.7)	29.8 e	7.0 abc
Nicollet (surface)	32(2)	96(18)	14(2.0)	22(3.9)	25.5 de	14.1 cd
Nicollet (subsurface)	10(0.4)	26(7)	5(0.6)	11(2.3)	26.2 de	26.1 e
Omega	105(26)	159(7)	12(4.5)	12.4(1.3)	5.9 a	4.7 abc
Bergland	55(6)	141(11)	13(1.2)	23(2.7)	14.4 abc	10.1 abcd
Arveson	72(12)	177(15)	12(3.4)	21(1.6)	10.5 ab	7.0 abc
Waukegan	17(3)	27(5)	7(1.2)	8(1.4)	23.4 cde	17.7 de
Zimmerman	18(3)	121(13)	5(0.3)	23(4.1)	16.1 abcd	11.9 bcd
peat	28(5)	64(8)	7(0.7)	18(2.8)	14.3 abc	17.2 de

⁺ Concentration factors, ($\frac{\mu\text{Ci/g tissue}}{\mu\text{Ci/g soil}}$), can be obtained by multiplying ($\mu\text{Ci/g}$) by 50.

[#] Means in same column followed by same letter are not significantly different at the 5% level by Tukey's test (Steel and Torrie, 1960).

Table 4. Extraction of Tc-99 from moist-incubated soils by 0.01 M CaCl_2 . Tabulated values are means of triplicate samples with standard errors of the means indicated in parentheses.

Soil	Percent Extractable
Bearden	70 (3.8)
Hegne	72 (1.1)
Hibbing	79 (2.4)
Nicollet (surface)	78 (2.7)
Nicollet (subsurface)	101 (2.9)
Omega	31 (1.4)
Bergland	67 (0.3)
Arveson	79 (3.2)
Waukegan	84 (2.2)
Zimmerman	62 (1.9)
Peat	100 (7.9)

Table 5. Tissue yield and Tc-99 concentration of 10-day-old wheat seedlings germinated and grown on 50 ml of 1/3-strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of four replicates with the standard errors of the means indicated in parentheses.

Tc-99 solution conc. μCi/50 ml	Tissue yield (mg)* shoots	Tissue Tc-99 concentration shoots		Tissue Tc-99 concentration roots	
		μCi/g	μg/g	μCi/g	μg/g
0	0	121 a	147 a	--	--
0.025	0.03	127 a	135 ab	0.062(0.001)	3.7
0.25	0.3	121 a	131 ab	0.68 (0.03)	40
1.0	1.2	85 b	114 ab	2.79 (0.14)	165
2.5	3.0	52 c	101 b	6.90 (0.14)	407
5.0	5.9	38 cd	121 ab	12.11 (0.55)	714
6.7	7.9	31 cd	133 ab	10.93 (0.77)	645
20	23.6	21 d	136 ab	22.51 (1.54)	1328
				7.32 (0.47)	432

* Means in the same column followed by same letter are not significantly different at the 5% level by Tukey's test. (Steel and Torrie, 1960).

Table 6. Tissue yield and Tc-99 concentration of 10-day-old wheat seedlings germinated and grown on 50 ml of 1/3 strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of duplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 Solution Conc. (treatment) μCi/50 ml	Tissue Yield (mg)		Tissue Tc-99 Conc. (μCi/g)		Root/Shoot conc. Ratio
	Shoots	Roots	Shoots	Roots	
Control	135 (11)	94 (7)	---	---	---
0.025	128 (17)	81 (16)	0.0757 (.0012)	0.0159 (.0004)	0.21
0.25	103 (15)	74 (11)	0.960 (.009)	0.181 (.014)	0.19
1.0	63 (5)	50 (.2)	4.929 (.553)	1.326 (.224)	0.27
2.5	38 (3)	40 (.6)	8.966 (.432)	4.640 (.508)	0.52
5.0	34 (4)	50 (5)	13.758 (.394)	6.479 (.802)	0.47
6.7	32 (.4)	49 (2)	25.148 (2.538)	8.022 (1.362)	0.32
10.0	21 (3)	36 (4)	21.447 (2.175)	10.741 (.154)	0.50

Table 7. Tissue yield and Tc-99 concentration of 10-day-old barley seedlings germinated and grown on 50 ml of 1/3 strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of duplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 Solution Conc. (treatment) μCi/50 ml	Tissue Yield (mg)		Tissue Tc-99 Conc. (μCi/g)		Root/Shoot conc. Ratio
	Shoots	Roots	Shoots	Roots	
Control	130 (5)	82 (2)	---	---	---
0.025	142 (6)	85 (2)	0.097 (.004)	0.010 (.001)	0.10
0.25	93 (10)	73 (7)	1.038 (.042)	0.130 (.002)	0.12
1.0	42 (3)	46 (6)	5.323 (.572)	1.532 (.163)	0.29
2.5	33 (3)	43 (4)	11.399 (.613)	3.962 (.363)	0.35
5.0	23 (2)	42 (10)	17.170 (1.806)	9.485 (.249)	0.55
6.7	24 (2)	34 (1)	14.812 (.820)	14.630 (.960)	0.99
10.0	20 (2)	24 (3)	10.653 (2.578)	13.136 (.668)	1.23

*root value for Tc-99 conc. is the mean of 3 reps. from one sample only.

Table 8 issue yield and Tc-99 concentration of 10-day-old oat seedlings germinated and grown on 50 ml of 1/5 strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of duplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 Solution Conc. (treatment) μCi/50 ml	Tissue Yield (mg)		Tissue Tc-99 Conc. (μCi/g)		Root/Shoot conc. Ratio
	Shoots	Roots	Shoots	Roots	
Control	76 (14)	61 (15)	---	---	---
0.025	88 (.3)	63 (7)	0.1285 (.004)	0.0457 (.029)	0.36
0.25	76 (2)	51 (3)	1.120 (.069)	0.231 (.006)	0.21
1.0	33 (7)	25 (2)	5.753 (.180)	1.497 (.000)	0.26*
2.5	23 (2)	21 (4)	---	---	---
5.0	20 (2)	18 (6)	10.122 (.282)	8.632 (1.553)	0.85
6.7	20 (2)	22 (.8)	11.64 (1.261)	9.184 (.787)	0.79
10.0	18 (.8)	18 (.9)	13.674 (1.833)	12.785 (1.352)	0.94

*For root Tc-99 conc. only one rep., therefore: SE - (.000) + shoot value is the mean of 3 reps. from one sample only.

**burnt

Table 9. Tissue yield and Tc-99 concentration of 10-day-old radish seedlings germinated and grown on 50 ml of 1/3 strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of duplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 Solution Conc. (treatment) μCi/50 ml	Tissue Yield (mg)		Tissue Tc-99 Conc. (μCi/g)		Root/Shoot conc. Ratio
	Shoots	Roots	Shoots	Roots	
Control	163 (9)	53 (4)	---	---	---
0.025	156 (11)	45 (3)	0.119 (.004)	0.015 (.003)	0.12
0.25	144 (3)	45 (2)	1.270 (.086)	0.197 (.012)	0.16
1.0	127 (2)	36 (3)	4.563 (.172)	0.545 (.040)	0.12
2.5	67 (8)	16 (4)	19.603 (.852)	2.172 (.416)*	0.11
5.0	64 (2)	10 (.7)	37.751 (1.911)	4.763 (.522)*	0.13
6.7	55 (9)	10 (1)	39.627 (12.036)	4.851 (.435)*	0.12
10.0	52 (2)	7 (.9)	53.955 (1.241)	10.014 (3.381)*	0.19

* less than 3 reps for one or both samples.

Table 10. Tissue yield and Tc-99 concentration of 10-day-old soybean seedlings germinated and grown on 50 ml of 1/3 strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of duplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 Solution Conc. (treatment) μCi/50 ml	Tissue Yield (mg)		Tissue Tc-99 Conc. (μCi/g)		Root/Shoot conc. Ratio
	Shoots	Roots	Shoots	Roots	
Controls	315 (112)	122 (29)	---	---	---
0.025	474 (5)	150 (16)	0.025 (.005)	0.037 (.002)	1.45
0.25	332 (51)	132 (26)	0.253 (.096)	0.245 (.018)	0.97
1.0	295 (67)	132 (13)	1.156 (.008)	1.139 (.033)	0.99
2.5	360 (33)	147 (6)	2.305 (.383)	2.444 (.320)	1.06
5.0	302 (62)	134 (8)	5.879 (.043)	4.673 (1.654)	0.79
6.7	159 (15)	75 (18)	6.851 (.012)	5.261 (.505)	0.77
10.0	230 (10)	72 (9)	5.369 (.1762)	7.133 (.420)	1.33

Table 11. Tissue yield and Tc-99 concentration of 10-day-old corn seedlings germinated and grown on 50 ml of 1/3 strength Hoagland solution containing increasing amounts of Tc-99. Tabulated values are means of duplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 Solution Conc. (treatment) μCi/50 ml	Tissue Yield (mg)		Tissue Tc-99 Conc. (μCi/g)		Root/Shoot conc. Ratio
	Shoots	Roots	Shoots	Roots	
*Controls	192 (35)	184 (22)	---	---	---
0.025	152 (33)	295 (29)	0.047 (.002)	0.031 (.014)	0.67
0.25	170 (16)	244 (34)	0.660 (.023)	0.117 (.011)	0.18
1.0	185 (47)	266 (49)	1.728 (.027)	0.46 (.080)	0.27
2.5	221 (8)	338 (35)	3.800 (.058)	1.395 (.063)	0.37
5.0	127 (5)	184 (.5)	8.209 (1.830)	3.202 (.042)	0.39
6.7	57 (7)	107 (8)	9.360 (4.457)	5.233 (1.854)	0.56
10.0	79 (17)	118 (10)	7.196 (2.422)	5.477 (.574)	0.76

* 3 reps. for each control value given

Table 12. Tissue yield and Tc-99 concentration of 10-day-old wheat seedlings germinated and grown on some of 1/2-strength Hoagland solution containing 1.0 or 5.0 μ Ci of Tc-99 added with increasing time after start of germination. Tabulated values are means of triplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 added (μ Ci/50 ml)	Time Tc-99 added* (days)	Shoot tissue yield		Root tissue yield		Tissue Tc-99 conc. (μ Ci/g)	Root/shoot conc. ratio
		(mg)	% of control	(mg)	% of control		
Control	---	138 (4)	---	91 (6)	---	---	---
1.0 μ Ci	0	119 (4)	.86	85 (2)	.93	3.396 (.021)	0.520 (.026)
	2	120 (6)	.87	90 (6)	.99	3.107 (.127)	0.431 (.038)
	4	123 (8)	.89	94 (4)	1.03	3.049 (357)	0.466 (.042)
	6	124 (14)	.90	94 (10)	1.03	2.571 (.060)	0.474 (.053)
	8	131 (7)	.95	95 (5)	1.04	2.096 (.269)	0.517 (.051)
5.0 μ Ci	0	72 (11)	.52	60 (4)	.66	12.624 (1.085)	4.865 (.111)
	2	91 (1.4)	.66	74 (2)	.81	15.914 (1.115)	4.346 (.192)
	4	104 (12)	.75	82 (9)	.90	12.427 (.315)	3.377 (.294)
	6	108 (9)	.78	87 (5)	.96	10.708 (.820)	2.533 (.154)
	8	129 (9)	.93	96 (7)	1.05	7.788 (.517)	2.330 (.256)

* time after start of germination.

Table 13. Tissue yield and Tc-99 concentration of 18-day-old wheat seedlings germinated and grown on 50 ml of 1/2-strength Hoagland solution containing 1.0 and 5.0 μ Ci of Tc-99 added with increasing time after start of germination. Tabulated values are means of triplicate samples with standard errors of the means indicated in parenthesis.

Tc-99 added (μ Ci/50 ml)	Time Tc-99 added* (days)	Shoot tissue yield		Root tissue yield		Tissue Tc-99 conc. (μ Ci/g)		Root/shoot conc. ratio
		(mg)	% of control	(mg)	% of control	Shoots	Roots	
Control	---	336 (18)	---	218 (18)	---	---	---	---
1.0 μ Ci	0	176 (9)	.52	133 (7)	.61	3.058 (.068)	0.280 (.030)	0.09
	2	169 (8)	.50	149 (11)	.68	3.865 (.276)	0.254 (.026)	0.07
	4	244 (20)	.73	206 (11)	.94	2.887 (.136)	0.156 (.007)	0.05
	6	232 (31)	.69	201 (24)	.92	3.185 (.338)	0.171 (.035)	0.05
	8	322 (40)	.96	228 (24)	1.05	2.171 (.040)	0.148 (.004)	0.07
5.0 μ Ci	0	67 (13)	.30	67 (12)	.31	22.632 (1.417)	4.830 (.592)	0.21
	2	110 (12)	.33	91 (13)	.42	22.038 (.057)	3.056 (.365)	0.14
	4	127 (7)	.38	103 (4)	.47	23.849 (1.043)	3.254 (.224)	0.14
	6	158 (9)	.47	146 (16)	.67	19.328 (.496)	1.914 (.209)	0.10
	8	240 (8)	.71	162 (11)	.74	11.761 (.346)	1.260 (.151)	0.11











