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Title: RADIOTECHNETIUM AS AN ENVIRONMENTAL CONTAMINANT: METABOLISM BY TWO FRESHWATER SPECIES, THE PEARL MUSSEL (*MARGARITIFERA MARGARITIFERA*) AND THE ROUGH-SKINNED NEWT (*TARICHA GRANULOSA*)

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Dr. David L. Willis

There is an increasing world inventory of radiotechnetium (mainly ^{99}Tc) and it is inevitable that some of this will be released to the environment. However, little is known about the environmental behavior of this element.

This work examined technetium's behavior in two representative freshwater species, the pearl mussel (*Margaritifera margaritifera*) and the rough-skinned newt (*Taricha granulosa*). The organisms were exposed to aqueous solutions of ^{95m}Tc as pertechnetate, under chronic conditions. Uptake of the nuclide was followed for 63 and 51 days, respectively, by whole-body scintillation assay. The final whole-body concentration factors obtained were 0.9 for the mussel and 11.3 for the newt. The subsequent retention of this body burden by the two species was followed for 58 and 30 days, respectively. Both species exhibited two-phase retention patterns with long-lived components

containing 65 and 75% of the initial activity, respectively. The biological half-lives for these long-lived components were calculated as 87 days for the mussel and 37 days for the newt.

Tissue distribution data indicated that the soft tissues of the mussels contained more than twice the concentration of Tc activity as the shell, and that this ratio remained constant throughout both uptake and retention. This seemed to indicate a passive uptake and loss of the nuclide. In the soft tissues of the mussel the site of greatest Tc concentration was the foot-visceral mass, while the adductor muscle contained the least. The distribution of Tc in the tissues of the newt indicated that the major site of localization was the intestine. This activity was evenly distributed between the intestinal tissues and the contents of the gut.

This study showed that, in the freshwater species studied, technetium is neither extensively concentrated nor tenaciously retained. It appears that uptake and loss are passive rather than due to an active metabolic mechanism. However, several unanswered questions were raised during this work which point out the need for continued research into the movement and behavior of technetium in freshwater ecosystems.

Radiotechnetium as an Environmental Contaminant: Its Metabolism by Two Freshwater Species, the Pearl Mussel (*Margaritifera margaritifera*) and the Rough-Skinned Newt (*Taricha granulosa*)

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Mark Hevland

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Professor of Radiation Biology in charge of major

Redacted for Privacy

Chairman of Department of General Science

Redacted for Privacy

Dean of Graduate School

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INTRODUCTION

There is an increasing awareness that the behavior of technetium (Tc) in the environment should be explored more fully. The lack of information that now exists is the result of two major factors: the low levels present in the environment and the difficulty in measuring those levels. Due to these factors, technetium has received only limited attention in the past. However, with the possibility of increased environmental input of Tc, more attention is being focused on its movement and metabolism.

Technetium

The existence of element number 43 (technetium) was predicted by Mendeleev on the basis of his Periodic Law (Spitsyn, *et al.*, 1977). After 70 years of unsuccessful attempts to find technetium in nature, it was artificially produced in 1937. Technetium is the lightest element having no stable isotope. Technetium-97 has the longest half-life of the 21 known isotopes (2×10^6 yr). Therefore, essentially no primordial Tc is present today, and it is unlikely that a significant amount was present when life began on earth (Gearing *et al.*, 1975).

The chemistry of technetium has been the subject of many reviews (Kenna, 1962; Kotegov *et al.*, 1968; Gorski and Koch, 1969; McFadden, 1980). In its chemical reactions, Tc resembles the other members of

group VII B, especially rhenium (Re) and, to a lesser extent, manganese (Mn). Technetium is known to exist in all valence states from +7 to -1, the most stable valences being +7 and +4. However, due to its high vapor pressure as a solid and in solution, Tc_2O_2 and $HTcO_4$ can be quite volatile (Cobble *et al.*, 1953). It appears that, in the presence of organic substances and with a shift to reducing conditions, the valence state of Tc may be markedly influenced possibly leading to less soluble forms or complexes (McFadden, 1980).

Technetium's presence in the environment arises from several sources: naturally occurring Tc, fallout from nuclear weapons testing, releases during the uranium fuel cycle and disposal of radiopharmaceuticals. The most abundant of the isotopes released is ^{99}Tc. This isotope has a physical half-life of 2.13×10^5 years and emits a weak beta particle (E_{max} = 0.292 MeV) on decay.

Small amounts of ^{99}Tc (1×10^{-12} µg/kg in pitchblende) arise naturally by spontaneous fission of ^{238}U (Spitsyn *et al.*, 1977), and a minute contribution comes from slow neutron induced fission of ^{235}U in minerals. Interactions of cosmic neutrons with minerals containing molybdenum, ruthenium, and niobium also contribute to the small amount of naturally produced technetium found in the environment. The amount of naturally occurring Tc is so small that no good estimates of its inventory are available at this time.

Induced fission (thermal neutron) results in the formation of large amounts of ^{99}Tc. For example, the fission yield of ^{99}Tc for ^{235}U is 6.1% and for ^{239}Pu, 6.4%. As a result, most of the Tc found in the environment is the result of fission production.

There is limited information concerning Tc levels in the envi-

ronment as a result of fallout from nuclear weapons testing. Direct measurements of rainfall in Texas have shown ^{99}Tc concentration ranging from 1.8×10^{-3} to 3.6×10^{-2} pCi/liter due mostly to weapons testings (Erhardt and Attrep, 1978). Hardy (1972) estimated the average ^{99}Tc levels in soil (25 cm depth) at 0.35 pCi/kg soil. This estimate was based on calculations from the relative fission yields and the measured levels of ^{90}Sr and ^{137}Cs assuming equivalent retention and deposition of fission products.

Technetium-99, along with other fission products, is produced in the fuel elements of nuclear reactors. A recent estimate placed the ^{99}Tc inventory in the United States at 2.86×10^{6} Ci by the year 2000 (Burkholder *et al.*, 1975). This could be released to the environment during fuel reprocessing. Concentrations as high as 693 μCi/liter are expected in purex reprocessing wastes (Routson and Cataldo, 1978). Therefore, environmental contamination is possible through the release of the volatile Tc compounds to the atmosphere from the liquid waste discharges.

Technetium may also enter the environment from the normal operation of power reactors. Liquid wastes from light water reactors contain ^{99}Mo. Molybdenum-99 is the parent nuclide of ^{99}Tc, i.e. when ^{99}Mo decays, it forms ^{99}Tc. However, one Ci of ^{99}Mo does not give rise to one Ci of ^{99}Tc, but only about 36 nCi. This great difference is due to the different rates of decay for the two nuclides (67 hr vs. 213,000 yr). The actual release of ^{99}Mo from Portland General Electric's Trojan Nuclear Power Plant has been reported (PGE, 1980). About 1.07 mCi of ^{99}Mo were released in the waste water during the first half of 1980 (Jan. 1 - June 30, 1980). That amount would result in a yearly

input of only about 0.08 nCi ^{99}Tc to the environment.

Another pathway that results in technetium entering the environment is the disposal and excretion of Tc-containing radiopharmaceuticals. Technetium-99m (the metastable isomer of ^{99}Tc with a 6 hr half-life) is widely used in nuclear medicine as an imaging agent. For a typical brain scan, 15 mCi of ^{99m}Tc is administered intravenously (Blahd, 1971). Over 70% of that injected dose will be excreted from the body within the first 48 hours (Andros *et al.*, 1965), and likely enter public sewage systems. For example, Sodd *et al.*, (1975) found that on a given working day from 50 to 70 mCi of ^{99m}Tc flowed through the Hamilton Co. (Cincinnati, Ohio) sewage treatment plant. However, that amount will result in only about 50 nCi of ^{99}Tc entering the Ohio River each year from this one source. (The considerable reduction, again, is due to the markedly different half-lives of the two isomers.) Granted, this is a small source, but with radioisotope procedures being performed at most medical centers and their use increasing by 10 to 15 percent per year (Moss, 1973), it contributes an increasing, relatively unmonitored release to the environment (McFadden, 1980).

Technetium in the environment can exist in any of several chemical forms. It appears that the most stable and characteristic oxidation state found in nature is as the pertechnetate ion (TcO_4^-), where the technetium is in the heptavalent state (Kotegov *et al.*, 1968; Nishita *et al.*, 1978; Wildung *et al.*, 1979; Beasley and Gonor, 1980). The pertechnetate ion is highly stable over a broad pH range and is not easily precipitated even in the marine environment. However, a reducing environment may result in the formation of insoluble forms (Wildung *et al.*, 1979).

The amount of ^{99}Tc entering the environment is difficult to evaluate. This is partly due to the very low environmental levels of ^{99}Tc normally present. However, the major problem is the manner by which ^{99}Tc decays. The relatively weak beta particles (E_{max} = 0.292 MeV) make radiological separation from other beta emitters also found in environmental samples nearly impossible. Only recently have "atomic absorption spectrometry" and "isotope dilution mass spectrometry" techniques been established to accurately measure Tc in environmental samples (Kaye *et al.*, 1977; Kaye and Ballou, 1978).

Assessments of human radiation dose from ^{99}Tc have become of concern only recently. Due to medical use of Tc as an imaging agent, the thyroid and G.I. tract are known to be target organs. Knowing this, Till *et al.* (1979) predicted that a release of one Ci/yr ^{99}Tc from a gaseous diffusion enrichment plant would result in a dose commitment of 18 mrem/yr to the G.I. tract and 80 mrem/yr to the thyroid, for those living within a one-mile radius of the facility. The doses were calculated using a soil-to-plant concentration factor of 50. These calculations yielded a dose to the thyroid that exceeded the radiation protection standards promulgated by the U. S. Environmental Protection Agency of 75 mrem/yr in 40 CFR 190 (USEPA, 1976).

Environmental Behavior of Technetium

In the terrestrial environment, the type of soil will determine the amount of TcO_4^- being sorbed to particles. Pertechnetate will be sorbed in significant amounts only in soils with a high content of organic matter and low pH (Gast, 1975; Wildung *et al.*, 1979). Otherwise, a large proportion of the Tc present will remain soluble and potentially available for leaching or uptake by plants.

There is growing evidence that Tc supplied as pertechnetate is accumulated by plants. Several studies utilizing soybeans and wheat (Wildung *et al.*, 1977), and tumbleweed and cheatgrass (Cataldo *et al.*, 1976; Routson and Cataldo, 1978) indicated that Tc was readily taken up by the root and concentrated in the leaves and reproductive tissues. These studies also indicated that if Tc concentrations exceeded 0.1 ppm some plants exhibited a biochemical toxicity to the element (Wildung *et al.*, 1977).

If soil conditions do not encourage sorbtion, deep percolation of pertechnetate can occur. For example, Brown (1967) reported that Tc was detected in the ground-water beneath the deep sediments underlying the Hanford (WA) radioactive waste storage facilities. The Hanford site was chosen as one of the nation's largest nuclear facilities because of its remote location and the depth of the sediments above the underlying ground water. However, the arid organically poor soil may be facilitating the migration of Tc wastes from the waste cribs down through the sediments and into the ground-water below. Brown (1967) has reported concentrations of ^{99}Tc in the ground-water approximately equal to that in the original waste stream when it was discharged into the percolation cribs.

The uptake of Tc has also been reported for marine biota. Brinks (1975) reported concentration factors in excess of 10^4 for the marine algae *Fucus serratus* when the isotope was suppled as TcO_4^-. Pentreath *et al.* (1980) also noted that *Fucus vesiculosus* appears to preferentially accumulate ^{99}Tc, but studies of other marine algae did not support such concentration of Tc (Gearing *et al.*, 1975; Beasley and Gonor, 1980; Pentreath *et al.*, 1980; Jeanmaire *et al.*, 1981). These

reports will be discussed in detail later but one conclusion is apparent, namely ". . . it is not possible to make generalizations concerning the bioavailability of TcO_4^- to marine organisms." (Beasley and Gonor, 1980).

Differences in absorption were studied in two mammals after ingestion of incorporated or unincorporated Tc. Sullivan *et al.* (1978) using guinea pigs and rats studied Tc absorption in the gut. The Tc was supplied (1) as incorporated in soybean plants (plants grown in the presence of TcO_4^-), (2) as pertechnetate in spiked soybean tissues (unincorporated), and (3) as TcO_4^- in aqueous solution to be gavaged. The results showed that 90% of the administered dose was excreted within 2 days regardless of the form of administration. However, the incorporated Tc (1) was excreted almost exclusively in the feces, whereas the unincorporated Tc (2,3) was excreted almost equally between the urine and the feces. This suggests that the incorporated Tc was not absorbed through the gut wall, indicating perhaps that a chemical change took place during the incorporation of the Tc into the soybean tissues that altered its availability to the animal.

As of this date only one study of Tc uptake in freshwater organisms has come to my attention (Blaylock *et al.*, 1980). In this work, concentration factors for two fish (carp and mosquito fish) and a snail were reported to be 11, 75, and 121, respectively. While the concentration factor for the carp was less than the value recommended in the U. S. Nuclear Regulatory Commission's Regulatory Guide 1.109 (USNRC, 1977) for calculating radiation doses to man, the factors for the mosquito fish and the snail exceeded the recommendations by 5 and 25 times.

Our present understanding of technetium's movement and behavior in the environment is inadequate. More information is necessary to gain an understanding of the metabolism of this element by the biota and to identify pathways that could lead to man. One such pathway is through the freshwater ecosystem. With increasing amounts of Tc being released, a more precise evaluation of the effects of these inputs into freshwater is needed.

Technetium-95m

To follow technetium's movement and metabolism in freshwater organisms for several months required a radioisotope that had an appropriate physical half-life (T_p). Another characteristic that was required for this type of study was a decay method that would permit *in vivo* measurement of the Tc in the experimental animal. Of the isotopes of technetium commercially available, only ^{95m}Tc had these characteristics. It has a T_p of 61 days, so the loss of activity due to physical decay would not present a problem in studies lasting as long as four months. Its gamma ray emissions permitted whole-body scintillation counting of the amount of Tc present within the living organisms without harming them.

Experimental Animals

To follow the metabolism of ^{95m}Tc, two distinct freshwater species were selected as experimental animals. One, the pearl mussel *Margaritifera margaritifera*, was representative of mollusks found in freshwater streams and lakes. The other, the rough-skinned new *Taricha granulosa*, represented the amphibian population that is often an important member of freshwater ecosystems.

Margaritifera margaritifera

The use of a bivalve mollusk as a means of assessing pollutants in marine and estuarine ecosystems has wide acceptance, as noted by the "Mussel Watch" program (Goldberg *et al.*, 1978). Therefore, it was deemed appropriate to use the freshwater bivalve *M. margaritifera* to study the movement and behavior of technetium in fresh water.

The freshwater pearl mussel is a ubiquitous bivalve mollusk. Its geographical range includes parts of western and eastern North America, northeastern parts of Asia, and throughout central and northern Europe (Hendelberg, 1960). It is essentially a river inhabitant. Boycott (1936) speaks of *M. margaritifera* as being found in a "well defined river habitat . . . most of its habitats are places where fishermen would expect to get trout and hope for salmon. . . ." These distribution characteristics make this mussel useful as a biological indicator.

Not only does this species have a wide geographical distribution but it also has a long life span. Age is determined by counting the annual layers in the ligaments. By using this method researchers have found mussels as old as 116 years (Hendelberg, 1960). This longevity makes *M. margaritifera* an excellent indicator of long term exposure to contaminants.

These mussels can range in size from 5 to 15 cm (Hendelberg, 1960), and weigh as much as 260 g. They have been reported to have seasonal distribution patterns, being found in the shallow water near the bank in the spring and distributed over the entire width of the river in mid to late summer (Roscoe and Redelings, 1964). They were easily collected. They are found partly buried in gravel stream beds.

Their black shell makes them look somewhat like a small stone on the river bottom. These mussels are easily maintained in the laboratory for long periods, and they have a past history of use in studies of this type (Short *et al.*, 1969; Mellinger, 1972; Terhaar *et al.*, 1977). It was therefore felt that *M. margaritifera,* the pearl mussel, would make an excellent study species, and that the data collected could be compared with findings reported for similar work performed with marine organisms.

Taricha granulosa

Studies detailing technetium's behavior in aquatic ecosystems have thus far focused on various invertebrates and fishes. Amphibians have not been considered. Amphibians can be a major component of the biomass in a freshwater system. They also represent a means of transporting radioactivity or other contaminants from the aquatic environment to the surrounding terrestrial habitats.

The rough-skinned newt *Taricha granulosa* is a representative amphibian of this region. It has a geographical distribution from northwestern California through western Oregon, Washington and British Columbia to the southeastern tip of Alaska (Bishop, 1943). Large populations can be found in lakes, streams and ponds during the breeding season, while at other times it is found on land.

The newt's size varies according to its sex and the season of the year. The adult male weighs 15 g on the average in late spring and summer and about 12 g in the winter. Its length is about 18 cm. The female of the species weighs about three grams less and is about 4 cm shorter on the average (Valett, 1970).

This newt can be identified by its brownish red dorsal color

and its yellowish underside. The animal has a relatively long life expectancy of about 11 years (Twitty, 1966). It is abundant because of a chemical toxin that is secreted onto its skin that is a repellant to its predators. The newt may be captured easily while rising to the surface of a pond to fill its lungs or while sunning on the aquatic vegetation.

Taricha granulosa was chosen for this work to represent an amphibian species in a freshwater ecosystem. Due to the lack of amphibian data, technetium's behavior in the newt will have to be compared with other aquatic vertebrate organisms.

Objectives

The purpose of this work was to investigate the patterns of uptake, retention and tissue distribution of technetium by the two freshwater organisms described above. Technetium-95m was used as a tracer because of its favorable decay characteristics and 61-day physical half-life. This laboratory study was designed to follow the uptake of technetium from freshwater, and every attempt was made to eliminate uptake from other sources. This was done to avoid variations due to differential feeding characteristics of the animals involved. Exposure to the isotope was in a chronic, static setting.

This work was undertaken to help build a foundation of information concerning technetium's behavior in the freshwater ecosystem. With this information and information from marine and terrestrial environments, potential problems that might arise from continued or increased releases of technetium might be identified and quantified.

METHODS AND MATERIALS

Radiation Measurements

The majority of the energy released during the decay of ^{95m}Tc is through gamma emissions, therefore, solid scintillation detection was used for all radioassays throughout these studies. Two detection systems were used. The first consisted of a 25 X 30 cm NaI (Tl) crystal coupled to a photocathode assembly (Bicron, Newbury, Ohio). The crystal assembly was surrounded by 20 cm of lead shielding. The data were analyzed and displayed using a 1056 channel analyzer linked to a digital printer. This entire system was housed in a temperature-controlled environment, maintained at 17±3°C. This "large" crystal unit was used for all whole body counting and some of the tissue samples. The system was located in a facility two blocks from the animal holding facility.

The second counting unit was a Packard Auto-Gamma system. This consisted of a 7.6 X 7.6 cm NaI (Tl) well crystal-photocathode assembly with an automated sample changer. Data were analyzed by a dual channel analyzer coupled to a digital printer. This system was used to assay most of the tissue samples from individual organisms, and was located adjacent to the animal holding facility.

Both instruments were calibrated to detect the gamma energies of technetium-95m with maximum efficiency and minimum background. The large crystal system was calibrated to 5 keV/channel, and an integrated

count from channels 123 through 238 (0.165 - 1.190 MeV) was displayed. The Auto-Gamma system was set with a detector gain of 50% and a counting "window" of 90 to 700 units (arbitrary units). With the instruments calibrated in this manner the detector efficiencies were 50 and 64% respectively with average background count rates of 580 and 240 counts per minute. When the same samples were counted on both systems the results agreed to within ± 1%.

Counting Standards and Sample Position

The ^{95m}Tc was produced commercially (New England Nuclear Corporation, Boston, Mass.) by cyclotron irradiation of a stable Mo target. Following chemical purification, the isotope was supplied as an essentially carrier-free solution of pertechnetate ($^{95m}TcO_4^-$) in distilled water (Beasley and Gonor, 1980).

No ^{95m}Tc standard source was available so nominal "counting standards" were prepared. These consisted of known volumes of the stock solution distributed on small pieces of household sponge cut in a configuration similar to the whole animal or tissue to be studied. The technetium-containing sponge was then sealed in a polypropylene tube and labeled. The radioactivity of these counting standards at the time of preparation was calculated by correcting the original activity per unit volume of the stock solution (supplied by New England Nuclear Corp.) for physical decay, and multiplying it by the volume distributed on the sponge. It was subsequently determined that detector inefficiencies were unaffected by the configuration of the standard, when the total sample was contained in the bottom two-thirds of the well. Therefore, sample position was not a cricitical problem with

the detection systems utilized.

Count rate data from the counting standards and samples were collected at each assay. All data were background corrected to obtain net counts per minute (cpm). These cpm data were converted to disintegration rate (dpm) by dividing the cpm value by the detector efficiency. Then these dpm values were corrected for decay since the date of initial technetium exposure. For sample data these two steps were combined and the cpm values were converted to dpm at the time of initial ^{95m}Tc exposure by the following:

$$\text{Sample dpm at } t_0 = \text{sample cpm at } t_1 \times \frac{\text{Ctg Std. dpm at } t_0}{\text{Ctg Std. cpm at } t_1}$$

t_0 = time at initial Tc exposure

t_1 = time at assay

For whole body counting, plastic vials (6.4 cm diameter X 9 cm high) were used. It was determined that the vials did not absorb the isotope and they were used repeatedly, with only tap water rinsing between uses. The test organisms were placed in the same orientation and position for each assay, although, as mentioned above, sample orientation and position did not significantly affect the assay results. The vials were placed in small plastic bags just prior to counting to avoid contaminating the detector. When using the Auto-Gamma system, the tissue samples were secured in plastic film, placed in 15 X 130 mm polypropylene counting tubes and loaded on the automated sample turntable.

For all whole body and most tissue samples assayed the counting time and the total accumulated counts assured a counting error of less than ± 1%. For some of the tissue samples a counting error from ± 1

to ± 2% had to be accepted, due to the low ^{95m}Tc activities.

Experimental Water

The water used throughout this study was obtained from a well located at the Oak Creek Fisheries Laboratory, Corvallis, OR. This water was used because assay results indicated that its composition is stable throughout the year. (See Appendix Table 1 for typical results.) This water has been used successfully at Oregon State University for some years to maintain and culture freshwater species, including the two species used in these studies. Unless otherwise stated, the term "water" will refer to well water obtained from this location.

Margaritifera margaritifera

Collection and Maintenance

Nearly 100 mussels (*Margaritifera margaritifera*) were collected during two collection trips in mid-May and at the end of September 1980. The collection site was on the Big Elk River 7.2 kilometers upstream from its confluence with the Yaquina River in Lincoln Co., OR. The mussels were gathered from the gravel bottom by hand, placed in a plastic bucket and transported to the laboratory as soon as possible. Only animals 10 to 20 cm long were taken, most of which were found in mid channel.

Once in the laboratory, the mussels were placed in 55 X 43 X 18 cm polypropylene tanks (25-30 per tank) containing 16 liters of water. The water was maintained at a temperature approximating that of the river at the times of collection (15±0.5°C). The water temperature was held constant by placing the tanks in a 15 X 56 X 180 cm fiber-

glass lined wooden trough containing circulating refrigerated tap water. Refrigeration was provided by a Westinghouse remote cooler. The water in each tank was circulated by means of an all-plastic aquarium pump. This assured continuous mixing and a modest amount of aeration.

After acclimation to the laboratory setting (2 to 5 days) each mussel was scrubbed with a stiff brush to clean the shell surface. An identification number was then painted on the shell with fingernail polish.

The animals were fed at least once each week. A food slurry was produced by adding one gram of fish food (see Appendix Table 2 for composition) to 250 ml of water in a blender and mixing at high speed for one minute. The slurry was then diluted with 16 liters of water and placed in a clean tank to form the feeding solution. The mussels were removed from their holding tanks and placed in the feeding solution for one to three hours, then rinsed and returned to their original tanks. This procedure avoided fouling the holding tanks, and assured that the mussels were not feeding on radioactively labeled food. There was no means of determining how much food was consumed by the mussels, but due to the small number of deaths (two) and the length of the study (six months) it is assumed that they were adequately nourished.

Before experimentation began it was determined that ^{95m}Tc would not be lost from the test solution due to sorption to the sides of the test tanks used (Beasley and Gonor, 1980). Frequent checks on the technetium inventory during all experiments confirmed that no significant loss of technetium occurred.

Uptake Study

A test solution was prepared by adding 5.8 μCi of ^{95m}Tc to 15 liters of well water, to produce a concentration of 0.3 μCi per liter. The test solution was placed in a clean tank equipped as described above.

Twelve mussels were selected and placed in the tank. They were maintained in this solution for the duration of the study. Fresh water was added occasionally to bring the water level up to its original volume, but no additional technetium was added to the system.

To assay the mussels, they were removed from the tank and placed in fresh non-radioactive water or feeding solution for approximately one hour. Each animal was then removed, rinsed in running water and allowed to purge in air for five minutes. Then, it was weighed and placed diagonally in a counting vial with its posterior at the bottom and the ventral side oriented toward the lid. The group of mussels was then transported to the counting facility where the animals were held in their counting vials at 16°C. Only while being transported and assayed (a total of no more than 15 minutes) were they held at ambient temperature. No observable adverse reactions to this procedure were noted.

The mussels were assayed periodically throughout the uptake study. The frequency of assay was directly related to the rate of uptake. Two animals died of unknown causes during the uptake period. Their shells were cleaned of all tissue and subsequently assayed with the live animals for the remainder of the study.

Retention Study

After 63 days of uptake the mussels were still accumulating the

^{95m}Tc at a slow but steady rate. The uptake study was terminated at that time to avoid mussel mortality due to toxicity from their own waste products. The animals were allowed to purge as before, were scrubbed to remove any attached material, and then assayed. These results were used as the initial 100% whole-body activity value for each animal.

The ten living mussels and two shells were then placed in 15 liters of non-radioactive water in a clean tank. The water was changed and the tank cleaned each time the animals were assayed. Assay times were days 1, 2, 4, 8, and weekly thereafter. The results were expressed as percentage of the initial activity remaining corrected for decay. The maintenance and assay procedures were the same as described above.

Tissue Distribution

Seventy mussels were placed in two test tanks (30-40 per tank) containing test solution with a ^{95m}Tc concentration of 0.8 μCi per liter. At regular intervals during uptake, groups of five animals were assayed as before. After the whole body activity had been determined the mussels were shucked and the tissues and shells were assayed separately. The tissues were then dissected into four components: foot-visceral mass, gill, adductor muscle, and mantle-labial palps. Due to the small amounts of tissue involved, the like tissue types from the five animals were pooled for assay.

After a 30-day exposure to the isotope, all remaining mussels were assayed for the initial 100% activity value for each animal. The mussels were then placed in 16 liters of fresh water in a single tank.

The water and tank were changed daily for the first week and subsequently after each assay. At regular intervals five animals were selected and assayed for technetium activity in whole body, shell, tissue and tissue components as described above.

Taricha granulosa

Collection and Maintenance

About 100 rough-skinned newts (*Taricha granulosa*) were collected in early November 1980 from Klickitat Lake located 12.8 kilometers southwest of Harlan, OR (Lincoln Co.). These newts were collected by bringing a small boat into the aquatic vegetation near the shoreline and capturing them with a fine mesh net or by hand. The newts were placed in a bucket of lake water and transported to the laboratory.

Once in the laboratory groups of 20-30 newts were placed in separate polypropylene tanks containing 16 liters of water as described above. A plexiglass lid with air holes was fitted over each tank to prevent escape, and an aquarium pump was used to provide continuous mixing. The water temperature was maintained as described above for the mussels, at a temperature of 15±0.5°C, which was approximately that of the lake water during collection. The newts adjusted readily to these conditions and there was only one death during the study. The newts were acclimated to the laboratory conditions for several days before experimentation began.

For some of the studies the newts were maintained in individual tanks for identification purposes. The individual tanks were 12 X 17 X 6 cm polystyrene containers with perforated lids. These contained approximately 500 ml of water or ^{95m}Tc test solution. Temperature

maintenance was the same as in the larger tanks.

The newts were fed one small manure worm (*Eisnenia* sp.) each week. The worms were placed in the tanks with the newts. The individually housed animals were given five minutes to consume the worm. If not eaten, the worm was removed and the feeding procedure repeated later. The group-housed newts were fed approximately one worm per individual but worms left in the tank uneaten after ten minutes were removed. This procedure was to prevent fouling of the tanks and the possible consumption of technetium-labeled food.

Due to normal skin molting and excretion by the newts the water had to be changed weekly for the individually-housed animals and bimonthly for those housed in groups. Each water change was accompanied by a thorough tank cleaning. The ^{95m}Tc activity was reconstituted to the concentration present just prior to the water change.

Uptake Study

Due to the need to change water frequently, 40 liters of water with a ^{95m}Tc concentration of 0.44 µCi/liter was prepared. This solution was used throughout the uptake study. Five hundred ml of the solution were added to 12 individual tanks and a newt placed in each.

Technetium uptake was determined by successive whole-body assays. The newts were removed from the test solution, rinsed in well water, weighed and placed in counting vials. The remainder of the assay procedure was identical to that used for the mussels. The newts were assayed periodically throughout uptake with no ill effects noted. However, one of the newts appeared bloated during the final stages of uptake. This animal was removed after completion of the uptake study. It subsequently died of unknown causes.

Retention Study

The 11 remaining newts were removed from the technetium solution after 51 days and assayed. These data constituted the initial total whole body activity for each animals that were used to calculate the percent retention as the study progressed.

The newts were then placed in clean individual tanks with 500 ml of water to begin the elimination study. The water was changed daily for the first week and after each assay, subsequently. The animals were assayed using the same procedure described above. The retention study lasted 30 days.

Tissue Distribution

Thirty liters of technetium test solution with a concentration of 0.40 μCi per liter was prepared and divided equally between two test tanks. Thirty newts were placed in each of the tanks. At periodic intervals five newts were removed and assayed as described above. These five were sacrificed by severing the spinal cord at the base of the neck, then dissected. The entire liver and intestine were removed for assay as well as a skin (both brown dorsal and yellow ventral), bone (both rear femurs), and muscle (from the rear leg and tail) sample. The tissue samples were allowed to air dry at room temperature for half an hour, then wrapped in plastic film, placed in counting tubes and assayed using the Auto-Gamma system. The tissue samples were removed after assay, placed on preweighed sections of aluminum foil and dried at 72°C for 48 h for dry weight determinations.

Midway through the study it was apparent that the technetium was localizing in the intestinal samples, but the site of the localization

was unclear. Therefore, the decision was made to separate the intestinal contents from the intestine itself. This was accomplished by flushing the intestine with a small amount of normal saline. The contents and intestines were assayed separately. These results were then added together to obtain the total intestinal value.

After 43 days all remaining newts were assayed. These whole body data were used as the 100% initial activity for the retention study. The newts were then placed in clean individual tanks containing 500 ml of water. The water was changed daily for the first week and weekly thereafter. At regular intervals five newts were removed, assayed and dissected as described above.

The activity (dpm) for skin, bone, and muscle was corrected to show the activity of the total tissue weight. These corrections were made on the basis of skin, bone, and muscle being 8.0%, 5.5%, and 42% of the total body dry weight (Spector, 1956; Scherner, 1967; Valett, 1970). The use of dry tissue weights required that the whole body weight be expressed as dry weight also. Therefore, a wet-to-dry weight ratio of 4.49 was used to convert the wet whole body weight to dry weight (Valette, 1970).

Sample Calculation:

1) Whole body dry weight = 12 g (wet weight) ÷ 4.49 = 2.67 g
2) Dry weight of total muscle mass = 2.67 g X 42% = 1.12 g
3) ^{95m}Tc activity in muscle sample = 9,000 dpm/g (dry weight)
4) ^{95m}Tc activity of total muscle mass = 9,000 dpm/g X 1.12 g = 10,980 dpm

RESULTS

The uptake, retention and tissue distribution of ^{95m}Tc were investigated in two freshwater species, the pearl mussel *Margaritifera margaritifera* and the rough-skinned newt *Taricha granulosa*. The results of these studies will be discussed separately for each species.

Margaritifera margaritifera

Uptake Study

The uptake of ^{95m}Tc was followed during chronic exposure for 63 days. The results are expressed in dpm/g (whole-body wet weight) and plotted as the mean dpm/g of the eleven animals exposed against time in Figure 1. The original data are presented in Appendix Table 3.

The freshwater mussel accumulated ^{95m}Tc rapidly for the first four days, then the rate slowed. From day 11 through the termination of the uptake study there was a slower, but steady rate of uptake. A simple linear regression of the data for days 11-63 indicated that there was a strong positive correlation (R^2=0.983) between uptake and time. Throughout this period the rate of uptake was about 4 dpm/g per day. It is apparent from Figure 1 that the mussels never reached an equilibrium concentration of the nuclide.

The extent of accumulation of a radionuclide is often reported as the concentration factor (C_f). The C_f is a measure of the concentration capacity of the organism in relation to the radionuclide being studied. Polikarpov (1966) defined C_f as:

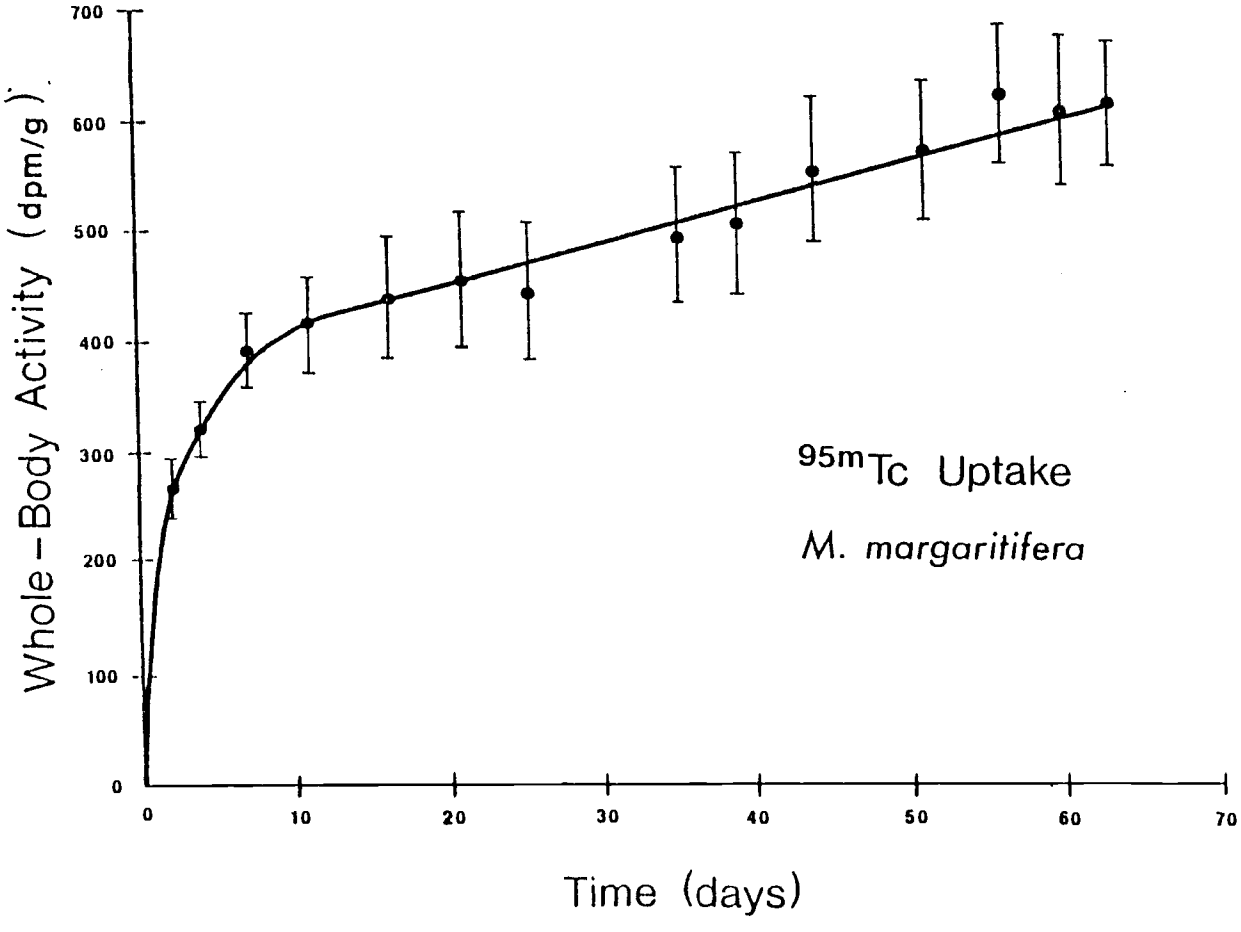

Figure 1. Whole-body uptake of ^{95m}Tc by *Margaritifera margaritifera* during chronic exposure at a temperature of 15°C. (n = 11) Error bars indicate ± 1 standard deviation (SD).

$$C_f = \frac{C}{C'}$$

where C is the concentration of the nuclide in the organism and C' is the concentration in the surrounding medium. The units for C are usually expressed as activity per gram of tissue. The units for C' are activity per equal weight of the surrounding medium. The C_f calculated for the final day of the mussel uptake study was 0.9 ± 0.1 ($\overline{X}$ ± 1 SD). This C_f indicated that the mussels attained an internal concentration approaching that of the external medium.

Retention Study

When an organism is removed from an environment containing a radionuclide, that organism will begin to lose the nuclide from its body. This loss can be expressed alternatively as the amount eliminated or the amount retained. The retention (or excretion) of ^{95m}Tc by the freshwater mussel was followed for 58 days. The data were expressed as the average percent of the initial whole-body Tc activity which remained at various times after being placed in non-radioactive water (Figure 2). The original data are presented in Appendix Table 4.

The retention curve shows two distinct components. The long-lived component contained the majority (65%) of the initial activity, while the short-term one contained the remainder. The long-term component had a biological half-life (T_b) of 87 days. The T_b was determined as follows: (1) A simple linear regression using data from days 16 through 58 was performed and the resulting line was extrapolated back to day 0 (dotted line Figure 2); (2) using the regression equation generated and the initial activity of the long-lived component (day 0 value), the X value that corresponded to one half of the initial

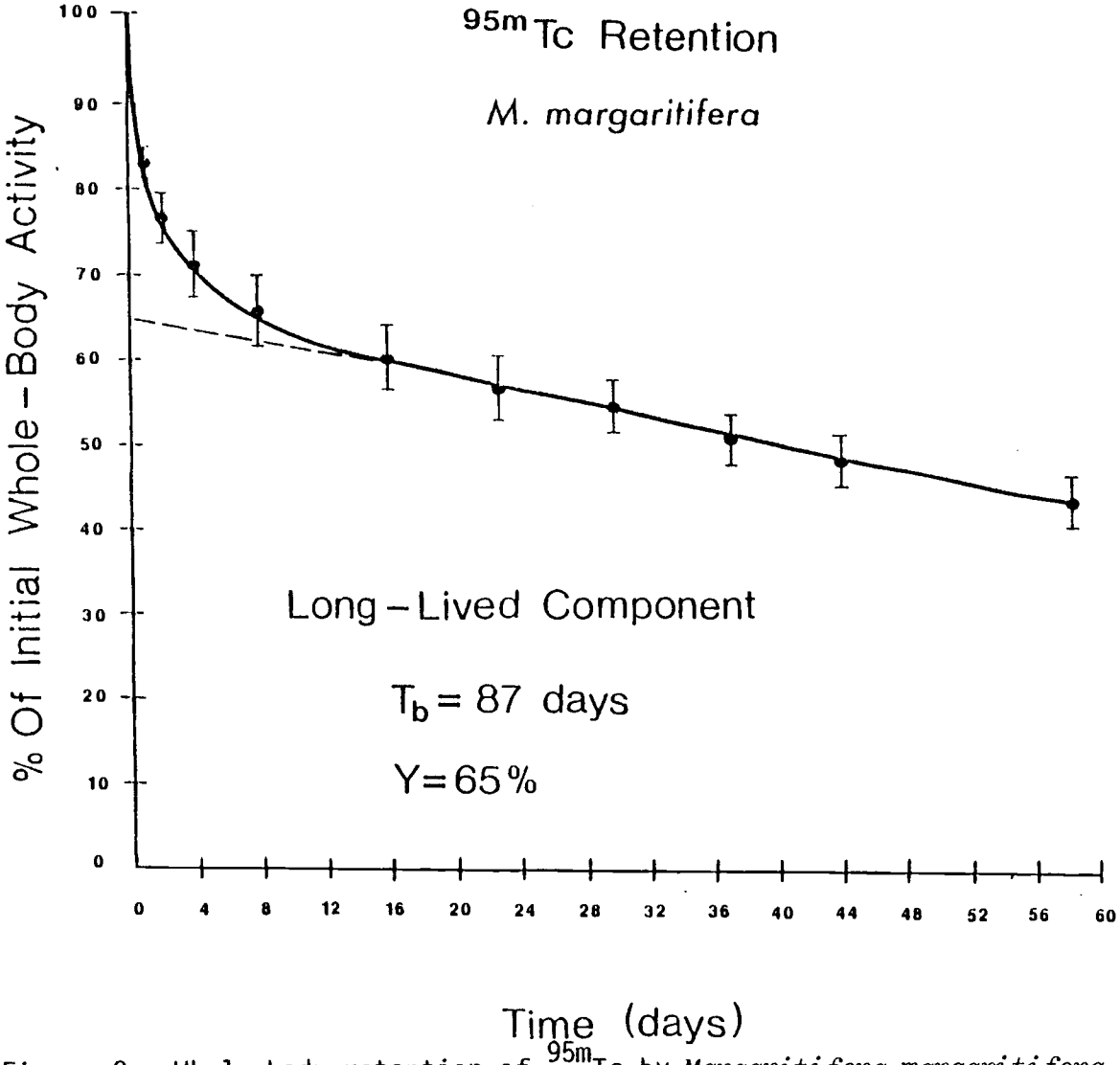

Figure 2. Whole-body retention of ^{95m}Tc by *Margaritifera margaritifera* following chronic exposure at 15°C. (n = 10) Error bars represent ± 1 SD.

activity was calculated, this value being defined as the T_b.

Once the T_b was determined the effective half-life (T_e) could be calculated. The T_e takes into account the elimination of a nuclide by biological means and the loss due to physical decay to give a measure of how long the radionuclide will be effectively present in the organism. The T_e was determined by:

$$T_e = \frac{T_b \times T_p}{T_b + T_p}$$

where, T_e = effective half-life

T_b = biological half-life

T_p = physical half-life

The calculated T_e for ^{95m}Tc was 36 d (T_p = 61 d, T_b = 87 d).

When considering technetium as an environmental contaminant, ^{99}Tc is the isotope of interest. The much longer T_p of ^{99}Tc (2.1 X 10^5 yr) means that only minimal decay will occur during the time the nuclide resides in an animal. This results in a T_e that is essentially equal to the biological half-life of 87 days.

Tissue Distribution

Data for tissue distribution are derived from different groups of five animals each sacrificed and assayed at periodic intervals. This situation is unlike the whole-body uptake and retention studies above where the same group of animals was followed by repeated live assays.

The tissue distribution of ^{95m}Tc was followed throughout both uptake and elimination of the Tc in the shell and soft-tissues. The soft-tissues were further dissected to include four components: foot-

visceral mass (foot), mantle-labial palps (mantle), gills, and adductor muscles (muscle). The results are shown in Tables 1 and 2, with the data expressed as percent of the total whole-body activity in each tissue at the time of sacrifice.

The soft parts generally contained more than twice the amount of Tc as the shell throughout both the uptake and elimination phases. The soft-parts-to-shell ratio remained constant at about 2.3 to 1 indicating that the shell and soft-tissues took up and lost Tc at the same rate. The loss pattern of the nuclide by the two "dead shells" (see Appendix Table 4) was almost identical to that by the live mussels. This reinforces the conclusion that the shell (alive or dead) and tissues lose Tc at the same rate.

During uptake the proportion of Tc found in the adductor muscles, gills, and mantle tissues remained relatively constant through time. However, there was an increase in the proportion of Tc bound in the foot-visceral mass. The data from the retention portion of these studies likewise showed that all four components retained a relatively constant proportion of Tc throughout. The foot, mantle, gills, and muscle contained about 51%, 5%, 6%, and 1.5% of the total whole-body activity respectively.

Concentration factors for the last day of uptake were calculated for each tissue component to assist in comparison of these data with other distribution data reported. At 30 days the C_f ± 1 SD values were as follows: soft-tissues, 1.1 ± 0.2; shell, 0.4 ± 0.1; foot, 2.4; mantle, 1.0; gills, 1.1; muscles, 0.7. The whole-body C_f at this time was calculated to be 0.7 ± 0.1.

Table 1. Distribution of ^{95m}Tc in the tissues of *Margaritifera margaritifera* during uptake at 15°C. Data presented as percent of whole-body activity present at the time of assay (n = 5).

			TISSUE COMPONENTS			
DAY	SHELL[1]	SOFT PARTS[1]	FOOT[2]	MANTLE[2]	GILLS[2]	MUSCLE[2]
1	29.3 ± 2.1	69.6 ± 4.4	11.3	9.4	7.9	3.4
2	30.8 ± 4.1	71.7 ± 11.3	12.9	8.2	8.5	3.8
4	28.7 ± 6.0	74.9 ± 5.4	15.6	8.2	8.7	3.8
8	30.0 ± 5.5	72.6 ± 6.2	23.7	8.2	7.1	2.7
16	30.4 ± 8.0	72.0 ± 8.6	28.2	7.9	7.7	3.4
30	31.7 ± 8.0	70.2 ± 8.3	32.0	8.7	6.4	2.3

1 Data represent the average ± 1 SD.

2 Data represent the pooled samples of the respective tissues: Foot (foot-visceral mass); Mantle (mantle-labial palps); Gills; Muscle (adductor muscles).

Table 2. Distribution of ^{95m}Tc in the tissues of *Margaritifera margaritifera* during retention at 15°C. Data presented as percent of whole-body activity present at the time of assay (n = 5).

			TISSUE COMPONENTS			
DAY	SHELL[1]	SOFT PARTS[1]	FOOT[2]	MANTLE[2]	GILLS[2]	MUSCLE[2]
1	29.9 ± 3.8	70.9 ± 4.5	50.7	4.9	6.0	1.5
2	38.6 ± 3.2	62.3 ± 6.6	43.5	3.9	4.9	1.7
4	34.7 ± 6.6	67.7 ± 6.6	49.0	4.3	6.3	1.9
8	32.4 ± 10.2	72.0 ± 10.3	50.4	5.2	6.0	1.5
16	33.5 ± 9.8	70.1 ± 9.5	52.2	5.7	5.9	1.0
23	26.2 ± 9.7	76.6 ± 9.1	59.6	4.9	7.0	1.3
30	25.1 ± 7.0	78.8 ± 8.4	51.2	5.5	8.0	2.3
42	37.1 ± 15.0	65.9 ± 16.2	49.7	3.9	5.3	1.3

1 Data represent the average ± 1 SD.

2 Data represent the pooled samples of the respective tissues: Foot (foot-visceral mass); Mantle (mantle-labial palps); Gills; Muscle (adductor muscles).

Taricha granulosa

Uptake Study

The uptake of radiotechnetium by the rough-skinned newt was followed for 51 days. Twelve animals were chronically exposed to an aqueous solution of ^{95m}Tc. The results are expressed in dpm/g whole-body weight and plotted as the mean dpm/g against time (Figure 3). The original data are presented in Appendix Table 5.

Initially there was a rapid rate of accumulation of Tc by the newt which began to decrease after about 14 days. However, sometime during day 30 there was a failure in the cooling system. The water temperature, which was usually held at 15°C, reached a high of 22°C before the problem could be corrected. This elevated temperature persisted less than 20 hours.

A higher body temperature would cause an increased metabolic rate in a poikilotherm, such as the newt. This could lead to enhanced ^{95m}Tc uptake. This has been reported for this newt in connection with iodine accumulation (Willis and Valett, 1973). The accidental increase in temperature experienced during this study may have caused an elevated equilibrium uptake value for ^{95m}Tc. The uptake appeared to be slowing before this incident. Subsequently, however, there was a rapid rise from about 7000 dpm/g on day 30 to about 9000 dpm/g on day 33 with very little gain thereafter.

The concentration factor was calculated as described above. It was determined to be 11.2 ± 2.4 ($\bar{X}$ ± 1 SD), after 51 days of chronic exposure to the ^{95m}Tc solution.

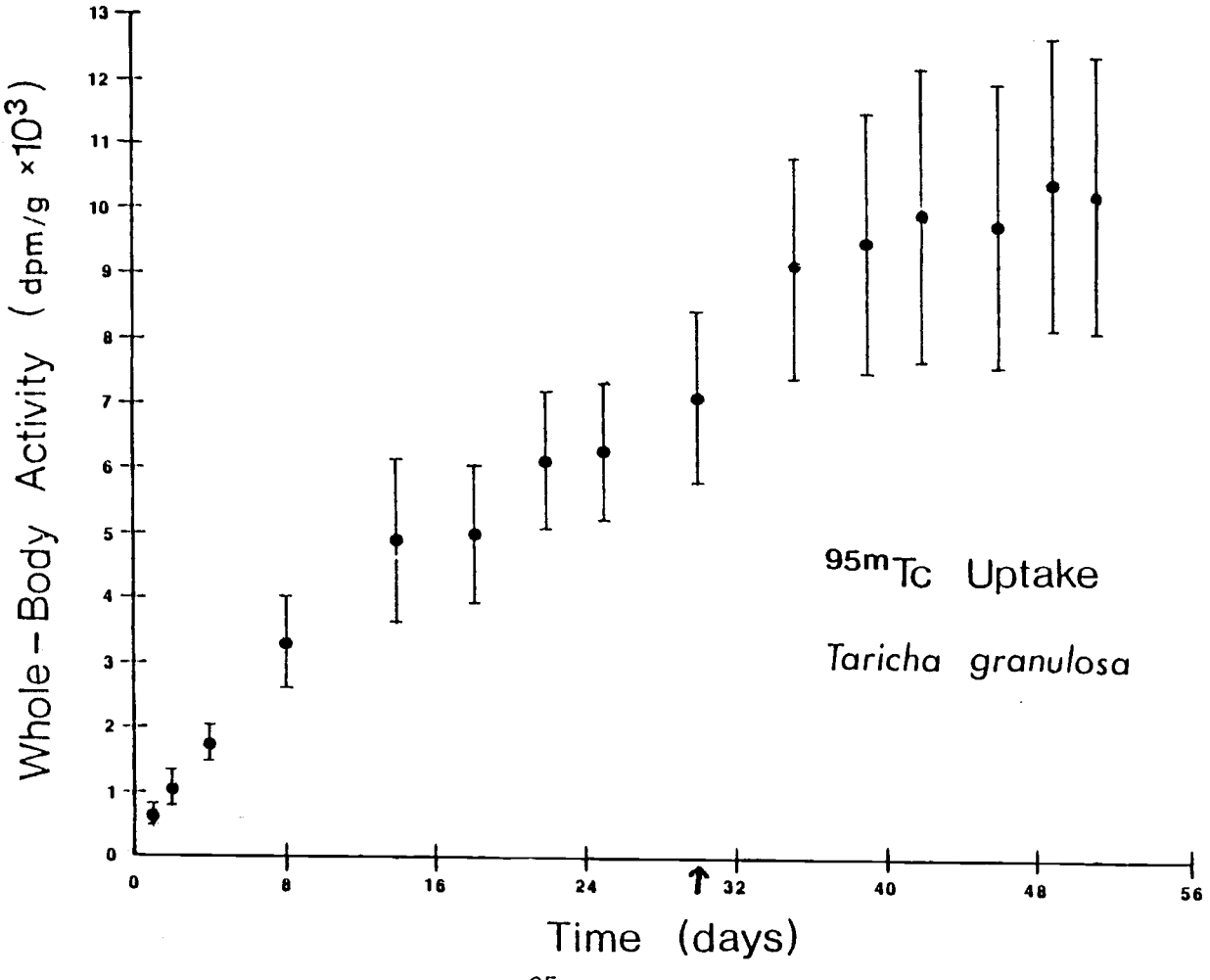

Figure 3. Whole-body uptake of ^{95m}Tc by *Taricha granulosa* during chronic exposure at 15°C. Arrow indicates date of cooling failure with temporary rise in temperature to 22°C. (n = 12) Error bars represent ± 1 SD.

Retention Study

The retention of ^{95m}Tc in the rough-skinned newt was followed for 30 days. The data were expressed as the average percent of the initial whole-body activity plotted against time (Figure 4). The original data are found in Appendix Table 6.

This retention curve had two components indicating that the elimination of Tc from the body involved two physiological compartments. The long-lived component contained 75% of the initial activity with the remainder in the short-lived component. The biological and effective half-lives were calculated for the long-lived component as described above. The T_b was 37 days with an effective half-life for ^{95m}Tc of 25 days. Again the T_e of environmental interest would be that of ^{99}Tc, which was calculated to be 37 days. At the end of the 30 day retention study the newts still contained 45% of their initial body burden of Tc.

Tissue distribution

The tissue distribution of Tc in the newt was followed through both uptake and elimination phases. The results for the tissue components assayed are shown in Tables 3 and 4. The results are expressed as percent of the total whole-body activity in each tissue at the time of assay.

During the uptake portion of the study the muscle mass initially appeared to contain the greatest portion of Tc. This proportion remained at about 27% through day 16 then dropped to below 10% by the end of the 43 day uptake phase. The intestine had a small percent initially (13%) but ultimately contained the greatest proportion of the Tc--nearly 40% of the total activity in the newt by day 43.

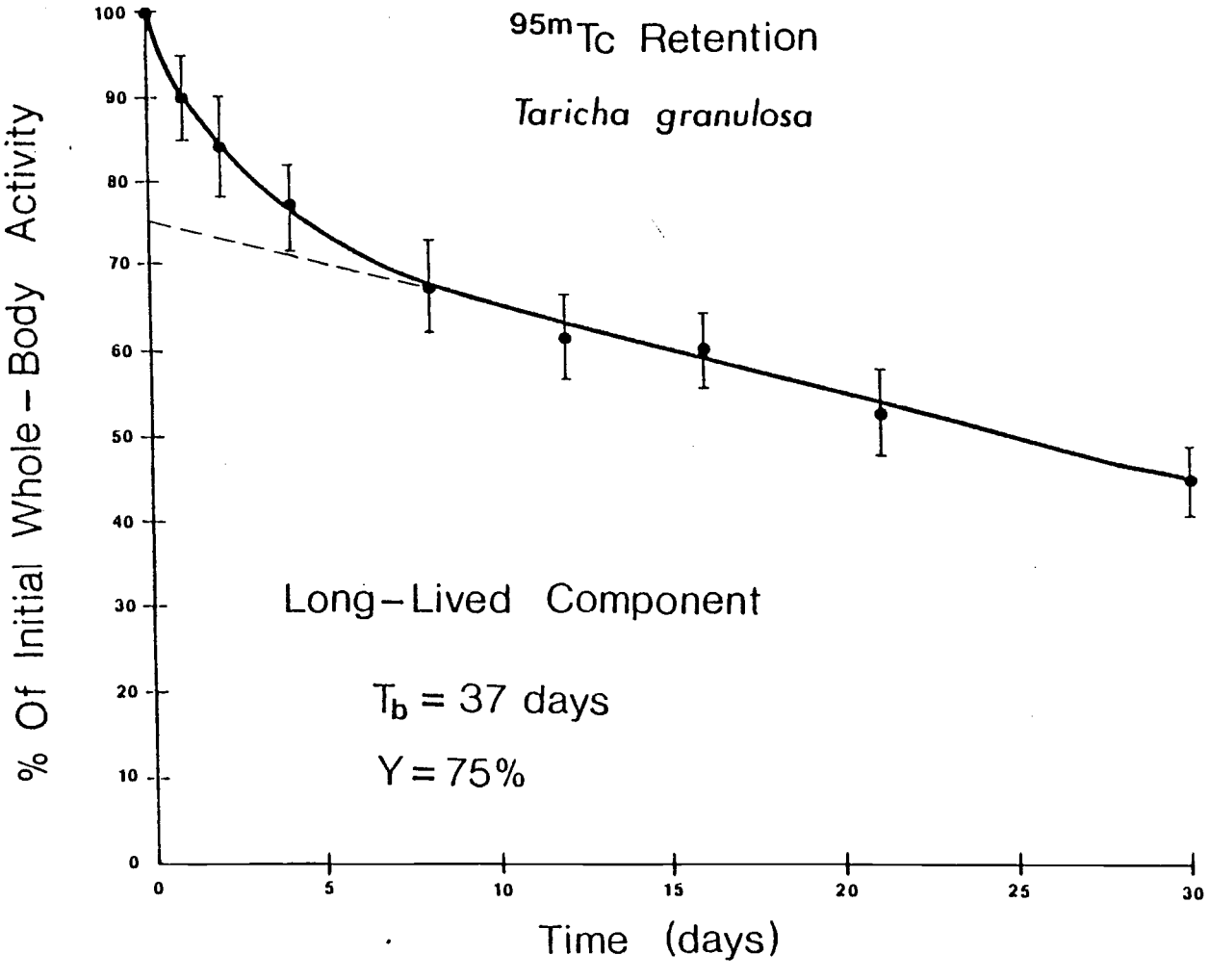

Figure 4. Whole-body retention of ^{95m}Tc by *Taricha granulosa* following chronic exposure at 15°C. (n = 12) Error bars represent ± 1 SD.

Table 3. Distribution of ^{95m}Tc in tissues of *Taricha granulosa* during uptake. Data presented as the percent of the whole-body activity at time of assay ± 1 SD. (n = 5).

TIME	INTESTINE			LIVER	MUSCLE	SKIN	BONE
(Days)	(Contents Only)	(Tissues Only)	(Intact)[1]				
2	-	-	13.3±5.5	3.9±0.6	27.0±11	6.6±0.5	7.6±2.8
9	-	-	24.4±6.3	5.9±2.2	26.3±6.3	6.7±1.5	8.6±1.9
16	-	-	38.9±8.0	6.4±2.6	26.3±9.8	4.9±0.9	4.4±1.8
28	17.9±10	14.4±4.4	32.3±11	9.8±2.5	16.1±7.0	5.6±2.8	5.8±2.0
45	23.9±12	15.5±5.6	39.3±12	9.4±5.4	9.5±3.7	5.5±2.3	6.2±1.3

[1] Intact intestine is the intestinal tissue along with the intestinal contents. When contents only and tissues only values are present, the intact value is the sum of the two components assayed separately.

Table 4. Distribution of ^{95m}Tc in tissues of *Taricha granulosa* during retention. Data presented as percent of the whole-body activity at time of assay ± 1 SD (n = 5).

TIME	INTESTINE			LIVER	MUSCLE	SKIN	BONE
(Days)	(Contents Only)	(Tissues Only)	(Intact)[1]				
2	26.9±11	23.2±6.0	50.1±16	15.0±7.4	9.0±4.3	4.0±1.2	2.7±0.8
6	44.7±18	19.4±4.7	66.5±15	10.7±8.9	3.9±2.0	2.0±1.1	1.1±0.4
12	27.3±10	31.3±12	58.5±6.2	16.3±6.0	3.5±1.6	1.9±0.8	<1
19	29.2±12	14.9±4.3	44.4±14	19.6±6.5	2.5±1.3	<1	<1
28[2]	16.2±13	21.8±10	38.0±2.0	16.8±9.3	<1	<1	<1

[1] Intact intestine is the intestinal tissue along with the intestinal contents. The intact value is the sum of the two components assayed separately.

[2] n = 4

When it became apparent that the intact intestine was a major site of Tc localization, it was deemed important to determine where this localization was occurring. To accomplish this the intact intestine was flushed. This flushing separated the intact intestine into two components, the intestinal tissues and the intestinal contents. The two components were assayed individually and, thereafter, data for the intact intestine were derived by summing these two assays. The flushing was not necessarily quantitative, as some of the intestinal contents usually remained with the tissue component during assay.

The results of the flushing showed that ^{95m}Tc was found in roughly equal amounts in each component. There appeared to be no identifiable trend in the distribution of the Tc in the two components. A comparison between the means of the two components, for each assay date, showed that there was no significant difference between the proportion of the Tc contained in the tissues and that in the contents ($\alpha = 0.1$). However, the large variances and small sample sizes tend to complicate the interpretation of these results.

During the excretion phase, no major redistribution of the nuclide was noted. The bone, skin, and muscle had lost most of their Tc by days 12, 19, and 28, respectively. The radiation detection system used would not permit accurate determination of very small amounts of ^{95m}Tc in the samples. Therefore, once a component contained less than 1% of the total-body burden of ^{95m}Tc the results were expressed as less than 1% (< 1%). The liver samples appeared to maintain a rather constant Tc level throughout the loss phase. The intact intestine was the major site of Tc localization in the newt, with from 38 to 66% of the total body burden. On the last day of the elimination

study, one of the five newts assayed had retained 75% of its initial activity. Upon dissection, a large egg mass was discovered. Due to the egg mass and the abnormally large amount of activity retained, the data obtained from this newt were not included in the "day 28" results. However, the usual tissue samples were assayed along with the egg mass and the results (% of whole-body activity) follow: Egg mass, 37%; liver, 10.1%; intact intestine, 13.2%; intestinal contents, 6.5%; intestinal tissues, 6.7%. It is evident that the presence of the egg mass resulted in a greater portion of the Tc being retained and that the egg mass contained a significant proportion of the total body-burden at the time of sacrifice.

DISCUSSION

The two study species differ markedly, therefore, their results will be discussed separately. The pearl mussel *Margaritifera margaritifera* will be discussed first followed by consideration of the rough-skinned newt's metabolism of Tc.

Margaritifera margaritifera

Uptake Study

The accumulation of Tc by the pearl mussel was unremarkable. The maximum concentration factor for the whole-body only approached one. Even though there was little concentration, equilibrium had not been reached by the end of the 63-day chronic uptake study.

There were no other freshwater bivalve data with which to compare these findings. However, the uptake of ^{95m}Tc by some marine mussels corresponded to that of *M. margaritifera* reported here. Three species of marine mussels (*Mytilus galloprovincialis*, *M. californianus*, and *M. edulis*) were exposed to ^{95m}Tc under similar conditions Beasley *et al.*, 1981; Fowler *et al.*, 1981). The final C_f values were reported as 1.2, 1.5, and 1.7, respectively. The oyster (*Crassostrea gigas*) was also studied (Beasley *et al.*, 1981) and had a final C_f of less than 1.5.

Radiotechnetium's accumulation has been studied in one other freshwater mollusk, the snail *Heliosoma sp.* (Blaylock *et al.*, 1980).

The snail's C_f was reported to be 121 compared to the freshwater mussel's about one. The lack of agreement between these two freshwater species is likely due to the differences in experimental design utilized for the studies. The laboratory study of the freshwater mussels utilized chronic exposure to the nuclide from the aqueous medium only. The snail, on the other hand, was held in an artificial outdoor pond. Its Tc exposure was due not only to the ^{95m}Tc in the water but also from the other members of the community found in the pond on which the snail might have fed. The pond was a flowing system designed to test the effects of a single exposure to the isotope.

The exposure conditions may also have contributed to the differences between the two freshwater species. The maximum C_f reported for the snail was calculated at a time when the isotope concentration in the water had declined due to removal by the biota and the flushing action of the pond. This contrasts with the ^{95m}Tc concentration in the water used for calculating the C_f of the mussel (Appendix Table 3). It is therefore difficult to compare the ability of these two species to concentrate Tc.

In some cases when Tc uptake has been studied in different species of very similar taxonomic origin the results correspond well. An example is the three species of marine mussels described above whose C_f values ranged from 1.2 to 1.7. Three different crab species (*Carcinus maenas*, *Cancer pagurus* and *Maio squinado*) were also studied and had C_f values of 3, 5, and 7, respectively (Fowler *et al.*, 1981; Pentreath, 1981). In other cases organisms that appear at least somewhat similar accumulate Tc to dramatically different levels. Crabs

and lobsters are both crustaceans. However, the lobster (*Homarus gammarus*) concentrated Tc to 1000 times that found in its surrounding water.

Due to the large reported C_f of the lobster, I attempted uptake studies utilizing a similar freshwater form. Crayfish (*Pacifastacus leniusculus*) were obtained from a local stream and returned to the laboratory. Difficulty was encountered in maintaining a healthy stock of these crustaceans and the experiments were terminated before meaningful data were generated. However, preliminary data suggested that this crayfish did not concentrate the nuclide extensively.

The marine ecosystem has provided another example of variation in the ability to concentrate Tc. Marine mussels and oysters studied were all reported to concentrate the nuclide to about the same level (about 1.5, according to Beasley *et al.*, 1981). The red abalone (*Haliotis rufescens*) was reported to concentrate Tc to a much greater extent, having a C_f of as high as 205 (Spies, 1975). This elevated C_f was confirmed by Beasley (1981). Beasley held the abalone under similar conditions used during his marine mussel studies. His results showed the abalone accumulating Tc up to a level over 100 times that of the surrounding water.

The ability to concentrate Tc may be dependent on the feeding characteristics of the organism being studied. The snail and the abalone are both detritus feeders, while the mussels (both freshwater and marine) and oysters are filter feeders. However, the freshwater mussels were never exposed to a Tc contaminated food source. This was also true for most of the marine organisms studied (Beasley and

Gonor, 1980; Beasley *et al.*, 1981). Spies (1975) eliminated feeding as a possible mode of entry in his abalone study by starving the animals during the length of the study. The design of the experiments, described above, reduced the possibility that feeding was the only mechanism involved in the uptake of Tc. Therefore, feeding characteristics are unlikely to be the only cause of the variation seen in the ability to concentrate Tc by these aquatic species.

The inability of *M. margaritifera* and marine bivalves to concentrate Tc is unexpected. The premise of the "Mussel Watch" program (Goldberg *et al.*, 1978) was that mussels would concentrate environmental pollutants so that they could serve as monitors. This program has been relatively successful for monitoring many contaminants, especially heavy metals. Mellinger (1972), using *M. margaritifera*, reported concentration factors for three heavy metals (Zn, Cd and Hg) at least 20 times greater than that found here for Tc. This indicated that, like the marine mussels, *M. margaritifera* will concentrate many of the common metal pollutants, but not Tc.

The results of this freshwater study point out that the mechanism(s) for the uptake of Tc is (are) simply not well understood at this time. The only conclusion that can be drawn is that the amount of uptake is dependent on the species studied, and the bivalves do not appear to extensively concentrate this potential pollutant.

Tissue Distribution

The distribution of ^{95m}Tc within the tissues of the freshwater mussel during both uptake and elimination of the nuclide indicated little or no active metabolic movement. Throughout these studies

the proportion of Tc in the soft-parts (all tissues and fluids) and the shells remained constant. The soft-parts contained 70% of the total body-burden with the remainder in the shell. If an active metabolic process were involved, this ratio would not have remained as stable as it did. This information suggests a passive mechanism for the uptake and loss of Tc by the pearl mussel.

The Tc associated with the shells could be due to either sorption on the shell or to incorporation in the shell matrix. It is unlikely that it is due solely to sorption. The shells were scrubbed with a stiff brush before each assay to remove sorbed material. Furthermore, when this problem was first considered, several empty shells were assayed, then scrubbed with abrasive on both their inner and outer surfaces, and reassayed. There was no appreciable difference between the two assays. This indicated that the Tc had entered the shell matrix and was not merely sorbed to the exterior surface.

Assays of two "dead" shells during the retention study showed that they lost Tc at the same rate as the live mussels. This further indicated that the loss was not metabolically mediated. Beasley and Gonor (1980) found that the uptake of Tc by dead shells was essentially identical to that attained by the shells of live *Mytilus californianus* supporting a passive mechanism.

Beasley (1981) has continued his work with the incorporation of Tc in the shells of some marine species. He coated either the inner or outer surfaces of mussel shells with wax to determine which surface was responsible for the uptake of Tc. After exposure to ^{95m}Tc he found no real difference in the amount of the isotope associated with

the two treatments. These results also support a passive mechanism of uptake for Tc.

The results of this study indicated that the majority of the Tc in the soft-parts was localized in the foot-visceral mass component. Studies by Beasley *et al.* (1981) with *Mytilus californianus* and *Crassostrea gigas* showed that the highest concentration of ^{95m}Tc was found in the visceral tissues. Spies (1975) also found that the digestive gland of the abalone contained the greatest concentration of Tc.

Of all the tissue components studied, the muscle tissues contained the smallest concentration of Tc. The adductor muscles of *M. margaritifera* never contained more than 1.9% of the total body-burden. In marine mussels and abalone, muscular tissues were also shown to have the smallest concentration of Tc (Spies, 1975; Beasley *et al.*, 1981). Unfortunately, the visceral mass was not separated from the foot tissues in my studies. However, it seems reasonable to assume that, due to the small amount of Tc accumulated in the adductor muscles, the muscular foot would also carry very little Tc. This suggests that the elevated concentration of ^{95m}Tc in the foot-visceral mass of the freshwater mussel was due mostly to the amount contained in the visceral tissues.

Throughout the elimination study each of the tissue components contained a relatively constant proportion of the body burden. This indicated that the rate of elimination was the same in each of the different tissue types. These findings reinforce the conclusion that technetium's movement in the freshwater mussel was due to a passive

rather than an active process.

Retention Study

The retention pattern in *Margaritifera margaritifera* is similar to that in marine mussels and oysters reported by Beasley *et al.* (1981). *M. margaritifera* lost about 30% of the initial body-burden during the first five days with most of the remaining 70% held in a longer lived component. The long-lived component had a T_b of 87 days. A detailed analysis of the short-term component was not undertaken because it contained only a small portion of the initial body-burden. The marine bivalves investigated by Beasley *et al.* (1981) also had a biphasic loss pattern. Their long-lived components contained about 60% of the initial activity and had T_b values ranging from 80 to 134 days.

Mellinger's (1972) study using *M. margaritifera* showed other metals are held more firmly than Tc. He found T_b values for Zn, Cd, and Hg to be 860, 835 and 194 days, respectively, for the long-lived components. These metals were both accumulated more readily and bound more tenaciously than Tc. This may result from these elements either being metabolically essential to the mussel or chemically analogous to other elements that are.

The possibility of Tc being analogous to some biologically important element(s) has been suggested almost since it was first produced in measurable amounts. Technetium has variously been reported to be analogous to manganese and molybdenum (Kenna, 1962; Gearing *et al.*, 1975), iodine as iodate (Andros *et al.*, 1965; Sodd and Jacobs, 1968), and the other group VIIB halogens (Blahd, 1971).

At least for the freshwater mussel it appears that Tc did not behave like any of the elements above. If it had, the C_f would certainly have been greater, perhaps similar to the C_f of 26 for zinc reported by Mellinger (1972) for this species. Beasley *et al.* (1981) reported that the C_f for Tc in the marine mussel *Mytilus californianus* was not reduced by the presence of as much as 20 times the natural concentration of iodate (IO_3^-) in sea water. This suggests that Tc is not analogous to iodine, at least in the marine mussel.

Taricha granulosa

Uptake Study

The results reported here are the first concerning the behavior of Tc in an amphibian species. However, several other aquatic vertebrates have been studied and they will be used for comparison with the newt.

The accumulation of Tc by the rough-skinned newt was uneven, reaching equilibrium after about 45 days. The C_f reached 11.2 at equilibrium. As stated before, this value may have been somewhat elevated due to the cooling system malfunction on day 30 of this study, which probably caused an increased metabolic rate in the newts for about 20 hours. Nevertheless, the 11.2 value is virtually identical to the C_f of 11.0 reported by Blaylock *et al.* (1980) for the carp (*Cyprinus carpio*). Blaylock's results were derived from an acute exposure study, and comparison of his results with this chronic exposure study may not be totally appropriate. The newt's C_f was also similar to the results of a chronic study by Pentreath (1981), where a maximum C_f of 8.7 for a marine fish, the plaice (*Pleuronectes platessa*), was reported.

The lack of information concerning the uptake of Tc by aquatic vertebrates prompted a review of the newt's uptake of other metals. Valett (1970) studied several metabolically active elements, Zn, I, Sr and Co. His results showed a rapid rate of uptake with equilibrium being reached within 10 to 15 days. The different responses by the newt to Tc and the metals studied by Valett may be due to the proposed passive, non-metabolic uptake mechanism(s) for Tc.

Tissue Distribution

The distribution of Tc, during uptake and elimination, was followed in several tissues. It was hoped that the distribution patterns would identify sites of Tc accumulation in the newt, and indicate how firmly the Tc was bound at those sites.

The bone and skin accumulated only a small proportion of the Tc. They never contained more than 10% of the total body-burden. The relatively rapid elimination of the Tc by the bone and skin suggests that the nuclide was not firmly bound there.

The liver contained increasing concentrations of Tc throughout most of the uptake period, and during elimination it maintained a relatively constant proportion of the body-burden. These results suggest that the Tc is bound and held firmly in the liver. However, the mechanism for this remains unclear at this time.

The greatest concentration of Tc was found in the newt's intestine. The intestinal concentration of Tc has been well documented for other vertebrates. Blaylock *et al.* (1980) reported the highest concentration of Tc in the intestines of the fish he studied. Reports from medical uses of Tc (Andros *et al.*, 1965; Harper *et al.*, 1965)

indicated that, in humans, the nuclide was concentrated in the intestine and eliminated through the feces.

When the intestine was identified as a major site of Tc localization, subsequent tissue samples were flushed to separate the contents from the intestinal tissues themselves. The results of assaying these two components showed variable concentrations in each of the two components. However, after statistical analysis, the two components were shown to contain proportions of Tc that were not statistically different from each other throughout the study.

The maintenance of a significant proportion of Tc in the gut contents throughout the study supports two conclusions. First, the isotope is likely being eliminated through the feces, as it is in humans (Beasley *et al.*, 1966; Andros *et al.*, 1965). Unfortunately the fecal material was not assayed during these studies to confirm this assumption. Second, Tc, at least as pertechnetate, can move from the body through the intestinal lining to the gut. Any swallowed Tc that was in the gut would likely be eliminated a few days after the newt was placed in fresh water. Thus, the Tc found in the gut contents during retention is assumed to have come from the movement of Tc across the intestinal boundary.

If Tc can migrate from the body into the gut, the converse might also be true, e.g., Tc present in the gut could move through the intestinal tissues and be absorbed in the body. This would be important for food chain transfer of Tc from one organism to another. However, studies by Sullivan *et al.* (1978) using rats and guinea pigs suggested that Tc incorporated in ingested food did not readily pass

through the intestinal wall.

Retention Study

As found for the freshwater mussel the Tc elimination pattern for the newt was biphasic. For environmental considerations only the long-lived component was of interest. It had a biological half-life of 37 days. The more rapid loss of the isotope by the newt, as compared to the mussel, is likely due to its increased metabolic rate.

Only one other report concerning the retention of Tc by an aquatic vertebrate was found. Pentreath (1981) reported a T_b of 46 days for a marine fish, the plaice. This T_b followed a 62-day chronic exposure to pertechnetate. Pentreath also reported a somewhat shorter T_b of 36.4 days when the fish was exposed to Tc from labeled food. The shorter T_b may have been due to the inability of some of the Tc bound in the food to cross the gut wall (Sullivan *et al.*, 1978). Thus, the newt retention data are quite similar to these marine fish results.

Due to the lack of available data for comparison, the retention of several other elements (Zn, I, Sr, and Co) by the newt were reviewed (Valett, 1970). Those elements had biological half-lives many times greater than that of Tc. The longest was 1233 days for Zn. This supports the contention that Tc does not appear to be metabolically active in the newt.

Retention in an Egg Mass

It was not an objective of this work to evaluate the effects of Tc on the reproductive cycle. However, the discovery of an egg mass in one of the newts on the final day of the retention study led to several interesting points. This egg mass contained nearly 40% of

the Tc body-burden. This was the highest tissue concentration at this time. More study is necessary to determine if this finding was characteristic of the species or merely unique to this individual.

If this finding is not an artifact, several concerns arise. First, the possible detrimental effects of the radiation dose to the developing eggs might be considered. Effects might be either genetic or somatic. Secondly, the eggs of amphibians and fish, when released to the aquatic surroundings, become a food source for other aquatic organisms. If these eggs are contaminated, organisms feeding on them will be exposed to the contaminant, also.

CONCLUSION

Several unresolved questions remain concerning the metabolism of Tc by freshwater species. The mechanism for the accumulation of Tc is not fully understood, but it appears to be passive. Due to the solubility of the pertechnetate ion, binding is possibly associated with a unique cellular environment that results in a change of the ion to a less soluble form.

The results suggest that a single release of Tc into a freshwater system would not pose much of a threat to the biota. The relatively short T_e for ^{99}Tc, in both the newt and the pearl mussel, indicate that once the nuclide was cleared from the water the Tc accumulated in the organisms would not be retained long. The soluble nature of the pertechnetate ion would keep the nuclide in solution and facilitate its rapid movement out of the lake or stream.

Continuous or repeated releases of Tc into a freshwater system might be of concern. If even low levels of ^{99}Tc were repeatedly released over a long period of time, accumulation in the biota would occur. The results of my studies indicated that, with respect to the species studied, accumulation would probably not be significant. However, in other freshwater species, Tc might be concentrated to a much greater extent. The C_f values derived here for the mussel and newt might not be representative of an environmental situation. Many times laboratory studies produce C_f values that are below those obtained

from animals taken from naturally contaminated environments (Polikarpov, 1966).

Nevertheless, it does not appear that low level releases of Tc into a freshwater system will be hazardous. For example, let us assume a C_f of 20, about twice that obtained for the newt, for an edible freshwater fish. Furthermore, assume that this fish is exposed to the maximum permissible concentration of ^{99}Tc (MPC = 3×10^{-4} μCi/ml, according to USNRC, 1980) in the water. At equilibrium the fish would contain about 6×10^{-3} μCi per gram of tissue. Given these conditions a man would have to consume 15 pounds of this fish at one sitting to ingest the maximum permissible body-burden of ^{99}Tc (MPBB = 200 μCi whole-body).

From the tissue distribution results, it is reasonable to assume that at least 35% of the fish's Tc body-burden would be found in the intestine and another 10 to 20% in other non-edible parts. This would result in a man having to consume twice the amount previously stated to obtain the MPBB. These calculations assume that all of the Tc in the ingested material will be absorbed into the body, which is unlikely.

The above example, however unrealistic, indicates that the levels of Tc released to the freshwater environment pose no threat to man. However, a threat to some freshwater organisms may exist. If high enough concentrations of Tc accumulate in certain tissues or species, the possibility of radiation induced damage exists.

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APPENDIX

Appendix Table 1. An analysis of the chemical and physical characteristics of well water from the Oak Creek Fisheries Laboratory 1978. Values are reported in milligrams per liter except for conductivity (mOhms/cm^2) and pH.

	May	February
pH	7.6	7.5
Conductivity (25°C)	262	260
Hardness ($CaCO_3$)	123	121
Bicarbonate (HCO_3)	166	163
Dissolved Solids	165	170
Silica	32	36
Calcium	33	33
Magnesium	9.8	9.4
Sodium	9.2	8.6
Potassium	0.2	0.4
Sulfate	0.2	0.2
Chloride	4.0	3.5
Fluoride	0.1	0.1
Nitrate	0.2	0.1
Iron	0.2	0.2
Phosphate	0.05	0.03

Appendix Table 2. Fishfood composition. The fishfood along with water was used to formulate the "fishfood slurry" used to maintain the freshwater pearl mussel (*Margaritifera margaritifera*) throughout these studies. The fishfood, "Long Life Goldfish Food," was obtained from Sternco Industries, Harrison, N.J.

Protein	≥ 20%
Fat	≥ 2%
Fiber	≥ 5%
Ash	≥ 12%

Appendix Table 3. Uptake of ^{95m}Tc by *M. margaritifera* at 15°C presented as mean dpm/g whole-body wet weight ± 1 SD (n = 12), the ^{95m}Tc concentration in the surrounding water (dpm/ml), and the concentration factor ± 1 SD.

Time (Days)	Whole-body Activity (dpm/g)	Water Concentration (dpm/ml)	Concentration Factor (C_f)
2	269 ± 28	722	0.37 ± .04
4	321 ± 26	732	0.44 ± .04
7	392 ± 33	731	0.54 ± .04
11	415 ± 43	699	0.60 ± .06
16	440 ± 55	705	0.62 ± .08
21	455 ± 61	740	0.62 ± .08
25	446 ± 61	701	0.64 ± .08
35	495 ± 61	706	0.70 ± .09
39	508 ± 63	709	0.71 ± .09
44	553 ± 64	716	0.85 ± .10
51	571 ± 63	654	0.85 ± .10
56	623 ± 62	669	0.89 ± .09
60	609 ± 68	699	0.91 ± .10
63	614 ± 57	670	0.90 ± .09

Appendix Table 4. Retention of ^{95m}Tc by *M. margaritifera* at 15°C presented as mean dpm/g whole-body wet weight ± 1 SD (n = 10), and as percent of the initial whole-body dpm remaining at the time of assay ± 1 SD. Also presented are the mean dpm/g dead shell weight ± 1 SD (n = 2), and the percent of the initial dead shell's total activity remaining ± 1 SD at the time of assay.

Time (Days)	Whole-body (dpm/g)	Whole-body (% remaining)	Shells (dpm/g)	Shells (% remaining)
1	512 ± 58	83.1 ± 2.3	457 ± 120	83.0 ± 2.5
2	472 ± 60	76.6 ± 3.2	439 ± 117	78.0 ± 3.1
4	434 ± 61	70.5 ± 4.1	405 ± 112	73.3 ± 3.3
8	407 ± 60	65.9 ± 4.2	373 ± 114	67.4 ± 5.0
16	364 ± 54	59.0 ± 3.8	332 ± 110	59.7 ± 6.7
23	343 ± 52	55.6 ± 3.9	307 ± 103	55.4 ± 5.8
30	330 ± 47	53.4 ± 3.2	291 ± 98	52.4 ± 5.5
37	306 ± 44	49.6 ± 3.1	278 ± 104	49.8 ± 7.4
44	290 ± 43	47.0 ± 3.3	264 ± 105	47.1 ± 8.1
58	270 ± 40	43.8 ± 3.2	210 ± 83	37.4 ± 6.4

Appendix Table 5. Uptake of ^{95m}Tc by *Taricha granulosa* at 15°C presented as mean dpm/g whole-body wet weight ± 1 SD, the ^{95m}Tc concentration in the surrounding water (dpm/ml), and the concentration factor ± 1 SD (n = 12).

Time (Days)	Whole-body Activity (dpm/g)	Water Concentration (dpm/ml)	Concentration Factor (C_f)
1	622 ± 153	971	0.64 ± 0.16
2	1075 ± 269	965	1. 2 ± 0.28
4	1724 ± 265	946	1.82 ± 0.49
8	3320 ± 720	917	3.62 ± 0.78
14	4891 ± 1265	945	5.17 ± 1.34
18	4973 ± 1056	909	5.46 ± 1.17
22	6103 ± 1312	921	6.63 ± 1.42
25	6260 ± 1090	923	6.78 ± 1.18
30*	7078 ± 1316	924	7.66 ± 1.42
35	9077 ± 1724	895	10.14 ± 1.92
39	9446 ± 2008	871	10.75 ± 2.37
42	9975 ± 2282	923	10.80 ± 2.47
46	9795 ± 2194	927	10.57 ± 2.37
49	10414 ± 2295	930	11.20 ± 2.47
51	10261 ± 2157	920	11.17 ± 2.35

*Cooling failure; temperature reached 25°C.

Appendix Table 6. Retention of ^{95m}Tc by *Taricha granulosa* at 15°C presented as mean whole-body wet weight dpm/g ± 1 SD, and as the percent of the initial whole-body activity remaining at the time of assay ± 1 SD (n = 11).

Time (days)	Whole-body activity (dpm/g wet wt.)	Percent initial activity (%)
0	10,261 ± 2,160	100
1	10,128 ± 2,813	90.0 ± 5.3
2	8,741 ± 2,700	83.7 ± 6.1
4	8,045 ± 2,320	76.9 ± 5.2
8	7,215 ± 1,630	67.6 ± 5.6
12	6,547 ± 1,530	61.6 ± 4.9
16	6,505 ± 1,370	59.5 ± 4.4
21	5,388 ± 1,130	52.5 ± 5.2
30	4,763 ± 1,040	44.8 ± 3.9