AN ABSTRACT OF THE THESIS OF

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Title: UPTAKE AND RETENTION OF TECHNETIUM BY TWO FRESHWATER SPECIES, THE CRAYFISH PACIFASTACUS LENIUSCULUS AND THE SNAIL JUGA SILICULA

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The patterns of uptake, retention, and tissue distribution of ^{95m}Tc in two freshwater species, the crayfish Pacifastacus leniusculus and the snail Juga silicula have been investigated. The animals were chronically exposed in water to which sodium pertechnetate had been added. Final whole-body concentration ratios (CR) obtained were 1.6 and 41 for the crayfish and the snail, respectively. Following the uptake study the animals were placed in fresh non-radioactive water for a retention study. Two-phase retention patterns were observed in both species. The long-lived component of the crayfish curve contained 82% of the body burden, while that of the snail contained 90%. Biological half-lives for the long-lived retention components were determined to be 141 days and 120 days for the crayfish and the snail, respectively. Tissue distribution data showed that 79-100% of the crayfish whole-body

activity was shared between the exoskeleton and the digestive gland at all times, whereas the soft tissues of the snail contained 82-96% of the whole-body activity.

Data from these two species are similar to past research results that indicated uptake of Tc is highly variable among different species. The final crayfish CR value obtained is several orders of magnitude below those reported for the taxonomically-related lobster, and is among the lowest values reported for freshwater invertebrates. Uptake by J. silicula indicates a more significant accumulation of Tc, however a high degree of variation between identical animals was observed.

This study suggests that Tc contamination in the two study species would not pose a significant health problem to humans. The crayfish represents a potential direct pathway to man, however the degree to which P. leniusculus concentrated Tc indicates that this would not be a health hazard. The higher degree of concentration by the snail represents a potential indirect hazard. Further studies investigating such factors as variability in individual uptake and the efficiency of trophic transfer of Tc need to be undertaken in order to accurately assess the impact of Tc releases.

Uptake and Retention of Technetuim by Two Freshwater Species, the Crayfish *Pacifastacus leniusculus* and the Snail *Juga silicula*

by

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INTRODUCTION

Knowledge of the behavior of long-lived radionuclides in the environment is of considerable importance when attempting to assess the potential radiological impact of radionuclide releases. The environmental presence of technetium as a result of nuclear weapons testing, the nuclear fuel cycle, and the radiopharmaceutical industry has been suspected for several decades; despite this, suprisingly little research has been undertaken to investigate its environmental and biological behavior. A specific isotope of technetium, ^{99}Tc, is of major concern, due to its relative abundance and long half-life (210,000 yrs). A unique feature of technetium is its virtual absence from the natural environment prior to the nuclear age. This situation has probably been a key reason that study of its biological and environmental behavior has only recently been initiated. This study then, has been undertaken in order to add to the body of knowledge concerning the behavior of technetium in the environment, specifically in freshwater ecosystems.

Technetium

The discovery of technetium (Tc) in 1937 is attributed to Perrier and Segre (1937). The name "technetium" originated from a Greek word meaning "artificial", and was given to the element 10 years after its

discovery (Perrier and Segre, 1947)

All 21 isotopes of technetium are radioactive. They range in mass numbers from 90 to 110, with 9 isomers. The majority of these isotopes may be artificially produced by irradiation of a stable molybdenum target in a cyclotron; however the fission of uranium, thorium or plutonium is the source of virtually all environmental technetium. These fission-produced isotopes are formed either directly, or via "mother" isotopes such as ^{99}Mo.

Technetium is a second row transition element, in the manganese subgroup. Knowledge of the fairly diverse chemical behavior of technetium has developed irregularly over the past 20 years, prompted mainly by the nuclear medicine community (Jones and Davison, 1982). Compounds of technetium have been studied in all oxidation states from -1 to +7, with Tc(+7) being the most thoroughly characterized as well as the most stable state (Anders, 1959). Other common oxidation states of technetium include Tc(+4) and Tc(0).

By far the most common use of technetium today is in the area of nuclear medicine. Technetium-99m, with a physical half-life of 6 hrs, has played an important role in shaping the field of diagnostic nuclear medicine. In the several million nuclear medicine procedures performed worldwide annually, ^{99m}Tc is the predominantly used radionuclide, and its use is still growing exponentially (Richards, Tucker and Srivastava, 1982).

Sources of Technetium in the Environment

Small amounts of technetium are present naturally in uranium ore, typically found in concentrations of 10^{-10} to 10^{-15}g ^{99}Tc

per kg of ore (Kenna and Kuroda, 1964). This is present primarily as a result of the spontaneous fission of ^{238}U, with smaller amounts added from the neutron-induced fission of ^{235}U (Kotegov, Pavlov and Shvedov, 1968). Very small amounts of ^{99}Tc are also present in molybdenum-containing minerals as a result of the capture of cosmic neutrons by ^{98}Mo. These natural sources account for only a minute fraction of the technetium in the environment; in fact, it was not until 1964 that naturally-occuring technetium was first discovered (Kenna and Kuroda, 1964).

Man-made sources account for the vast majority of technetium in the environment. The major artificial source is the induced fissioning of uranium, which results primarily in the production of the long-lived isotope ^{99}Tc. Technetium-99 has an approximate fission yield of 6.1% from ^{235}U and 6.4% from ^{239}Pu. The detonation of nuclear devices has therefore resulted in large quantities of technetium being introduced into the environment. Technetium-99 from fallout has been measured in surface water (Golchert and Sedlet, 1969), as well as in rain samples (Attrep, Enochs, and Broz, 1971; Ehrhardt and Attrep, 1978).

Several steps in the nuclear fuel cycle also pose the potential for release of technetium. Fuel reprocessing facilities may release ^{99}Tc to the atmosphere, as well as to liquid effluents (Till, Hoffman, and Dunning, 1979; Wildung, McFadden and Garland, 1979). Typical waste solutions, such as from the Purex process, contain Tc at the level of 6.5-41 mg/l (Boyd, 1959), and ^{99}Tc has been measured in the environment surrounding reprocessing facilities (Jeanmaire et al., 1981; Garland et al., 1983).

Since ^{99}Tc is found in spent fuel along with other fission products, release may occur from waste repositories storing spent fuel or solidified high-level waste. It has been calculated that a typical canister of solidified high-level waste from a pressurized water reactor contains approximately 27 Ci of ^{99}Tc (NRC, 1983). For comparison, this same canister would contain approximately 0.2 Ci of ^{137}Cs. Technetium-99 has been measured in ground water surrounding repository sites (Brown, 1967; NRC, 1978), and was recently ranked first among radionuclides posing a threat to man during long-term storage of nuclear waste in a basalt repository (Barney and Wood, 1980). This ranking was based primarily on solubility and half-life.

Blaylock and Witherspoon (1975), concluded that ^{99}Tc was the most abundant isotope in the liquid effluent from a uranium enrichment facility, and low levels of ^{99}Tc have recently been measured in soil and plants near such a facility (Hoffman et al.,1982a). Additionally, the formation of volatile Tc compounds during the UF_6 enrichment process may also result in the release of small quantities of technetium to the atmosphere (Till et al., 1979). Finally, the routine operation of nuclear power reactors poses the potential for small releases of technetium to the environment. Molybdenum-99, parent nuclide of ^{99}Tc (in a neutron capture reaction), is contained in the cooling water of water-cooled reactors, and ^{99}Tc is present in fuel elements along with other fission products. Because these radionuclides are normally contained in the primary coolant system, releases of technetium to the environment are anticipated to be very low.

The other source of technetium release to the environment of any significance is via the use of radiopharmaceuticals. The workhorse of this industry is the relatively short-lived ^{99m}Tc, which is used as a body-imaging agent in activities of approximately 10 mCi per investigation (Birks, 1975). The use of ^{99m}Tc in diagnostic nuclear medicine leads to the release of technetium to the environment via patient excreta. Readily detectable levels of ^{99m}Tc have been measured in sewage effluent that included waste water from metropolitan hospitals (Moss, 1973; Sodd, Velten, and Saenger, 1975). In the latter study, it was estimated that as much as 70 mCi of ^{99m}Tc flowed through a sewage treatment plant receiving waste water from a local hospital on any given working day. However, because of the great difference in physical half-lives of ^{99m}Tc and its daughter ^{99}Tc (6.0 hours and 210,000 years, respectively), very little ^{99}Tc activity will result from the decay of ^{99m}Tc. As an example, the 70 mCi of ^{99m}Tc that Sodd *et al.* estimated flowed through a sewage treatment plant each day will decay into approximately 0.23 nCi of ^{99}Tc.

Environmental Behavior of Technetium

The most stable form of technetium in the environment is normally the pertechnetate ion $(TcO_4)^-$ (Wildung *et al.*, 1979; McFadden, 1980; Spitsyn, Kuzina and Oblova, 1977; Sheppard *et al.*, 1983). The pertechnetate ion is also highly soluble, so there is the potential for high mobility of technetium in soils (Brown, 1967). Laboratory experiments show no retardation of flow of pertechnetate through fractured granite (Failor *et al.*, 1982), and laboratory soil

adsorption experiments yield distribution coefficients that indicate the potential for high mobility (Routson, Jansen, and Robinson, 1977; Wildung et al., 1979). In contrast, however, 8 of 11 soils tested in another set of studies sorbed 98% of the added ^{99}Tc (as pertechnetate) within a period of 2 to 5 weeks (Landa, Thorvig, and Gast, 1977). In general, it appears that little or no sorption of pertechnetate occurs in soils with low organic matter content (Sheppard et al., 1983). Other soil conditions that can affect sorption and retention of technetium include the degree of aeration, pH, microbial activity, mineral composition, and organic complexing agents (Landa et al., 1977; Routson et al., 1977; Wildung et al., 1979).

Several studies have demonstrated varying degrees of technetium uptake by plants (Wildung, Garland, and Cataldo, 1977; Routson and Cataldo, 1978; Mousny, Roucoux, and Myttenaere, 1979; Berlyn, Dhillou, and Koslow, 1980; Hoffman et al., 1982a; Hoffman et al., 1982b; Garland et al., 1983). Laboratory experiments have indicated concentration ratio (CR)[1] values for ^{99}Tc ranging from 50 to over 1000 (Routson and Cataldo, 1978; Landa et al., 1977), whereas field studies yield CR values from 1.4 to 36 (Hoffman et al., 1982a). The main reason for the large difference in CR values appears to be the method of determining it (Garland et al., 1983). In field studies

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(1) The term Concentration Ratio (CR), as defined in the Results section, will be used in this paper. Other authors use similar terms, e.g., Concentration Factor (CF) or Bioaccumulation Factor (BF). In laboratory studies where a true equilibrium concentration is not normally reached, the term CR is appropriate. When citing other studies, the term used in the cited work will be quoted so as not to initiate any misunderstanding of the term as originally used.

the technetium is normally concentrated in the 0-2 cm soil layer, whereas laboratory studies typically have used soils with uniformly distributed technetium in solution. Recent field studies using the technetium concentrated in the 0-35 cm soil layer (normal rooting zone), indicate CR values as high as 370 (Garland et al., 1983). Other factors that can affect CR values are soil type, plant species, and other physical, chemical and biological processes.

The uptake of technetium by marine organisms has also been reported. Concentration factors (CF) for phytoplankton generally do not exceed 20 (Gearing, Van Baalen and Parker, 1971; Fisher, 1982); however Gromov reported a CF of 70,000 for a natural plankton community (as cited in Fisher, 1982). Species of the common brown alga Fucus appear to concentrate technetium (Birks, 1975; Pentreath et al., 1980; Jeanmaire et al., 1981), however other species of marine algae tend to accumulate it only minimally (Gearing et al., 1971; Jeanmaire et al., 1981; Beasley, Gonor and Lorz, 1982). Higher marine life also has demonstrated the concentration of technetium as pertechnetate. Reported CF values for marine invertebrates range from 1.5 for the mussel Mytilus californianus (Beasley, Gonor, and Lorz, 1982), to over 1,000 for the lobster Homarus gammarus (Pentreath, 1981). The red abalone Haliotis rufescens, a gastropod, falls midway between these extremes with a range of CF values from 135 to 205 (Spies, 1975). Beasley, Gonor and Lorz (1982) presented a summary of whole body technetium CF values for a variety of marine organisms.

The aqueous chemistry of technetium has been studied fairly extensively, and has been reviewed by several authors (Anders, 1959; Boyd, 1959; Kotegov et al., 1968; Spitsyn et al., 1977; Wildung et al.

1979). Technetium(+7) is generally the most stable oxidation state, and the pertechnetate ion $(TcO_4)^-$ is the most stable ion of technetium in aqueous solution (Kotegov et al., 1968). Pertechnetate is highly stable over a wide range of pH values, and this stability is expected to extend to natural waters (Wildung et al., 1979; McFadden, 1980). Several agents are known to reduce pertechnetate in aqueous solution, including Zn and HCl (Anders, 1959); even dust or organic vapors can act as reducing agents when pertechnetate is highly concentrated (Kotegov et al., 1968). Nonetheless, it would seem reasonable to assume that Tc(+7), as pertechnetate, would govern the behavior of technetium in fresh water under aerobic conditions.

With the exception of two previous reports, information on the biological behavior of technetium in freshwater organisms is virtually nonexistent. Blaylock, Frank, and DeAngelis (1982) reported CF values of 11, 75 and 121 for carp (Caprynus carpio), mosquitofish (Gambusia affinis), and snails (Helisoma sp.), respectively. Technetium was present in solution as pertechnetate, and a single, or "acute", application was employed. Using a slightly different experimental protocol, Hevland (1981) reported CF values of 0.9 and 11.3 for the freshwater mussel Margaritifera margaritifera and the rough-skinned newt Taricha granulosa, respectively. Technetium was also present as pertechnetate in this study; however uptake was from water only, and animals were under chronic exposure. Blaylock's study considered the response of an entire pond ecosystem to an acute dose of technetium, and expected steady-state body burdens were then calculated using modeling methods. In Hevland's laboratory study, on the other hand, CF values were calculated from measured activities of animals and

water samples. Because of the differences in these two studies, the CF values might not be directly comparable. Although somewhat dissimilar in methods, the results of both studies showed that the G.I. tract contained the highest concentration of technetium in the vertebrates, and in the two molluscs studied the soft tissue contained more than twice the activity of the shell.

Objectives of the Study

The general objective of this study was to examine the metabolic activity of technetium in the crayfish and the freshwater snail. Items specifically examined in each species included:

- degree and pattern(s) of whole-body technetium accumulation, including final concentration ratio achieved
- time to reach equilibrium activity
- pattern(s) of whole-body technetium elimination
- determination of biological and effective half lives for technetium
- patterns of tissue distribution of technetium during uptake and elimination

Isotope Used in The Study

To permit in vivo measurements of technetium in the animals, the gamma-emitting isotope ^{95m}Tc was used. Technetium-95m has a physical half life of 61 days, which is appropriate for a study of this type. The isotope was present in solution as pertechnetate, at a constant activity concentration.

Animals Used in This Study

The crayfish Pacifastacus leniusculus and the snail Juga silicula were chosen as experimental animals for several reasons: both are common inhabitants of freshwater streams in the Pacific Northwest; they are easily collected locally; both are important in the trophic organization of the stream community, and previous technetium studies with taxonomically-related marine species raised interest in the comparison of uptake patterns in these organisms. The red abalone Haliotis rufescens was reported to have CF values for technetium of up to 205 (Spies, 1975), and a CF of over 1,000 was reported for the lobster Homarus gammarus (Pentreath, 1981). The relatively high CF values reported for these marine animals prompted the selection of the snail and the crayfish for this study.

Summary

Because of the scarcity of information on Tc behavior in freshwater organisms, the investigation into the patterns of uptake and subsequent retention of Tc by two freshwater invertebrates was undertaken. The crayfish Pacifastacus leniusculus and the snail Juga silicula were chosen as representative of the crustacean and gastropod populations, respectively. This study was undertaken in order to add to the limited body of information concerning the environmental and biological behavior of technetium in freshwater ecosystems. It is hoped that with additional knowledge of this type, the significance of releases of technetium to the environment may be more accurately assessed.

MATERIALS AND METHODS

Radionuclide Used

Because of its above-mentioned physical characteristics, the isotope ^{95m}Tc was used as a tracer in this study. The isotope was originally obtained from New England Nuclear Corporation (NENC) as sodium pertechnetate in distilled water, pH 4. An aliquot of sodium pertechnetate was generously supplied to this laboratory by Dr. T.M. Beasley, OSU School of Oceanography. Dr. Beasley had been obtaining pertechnetate from NENC for several years, and had previously determined the radiochemical purity of this and previous batches to be excellent (Beasley, Gonor and Lorz, 1982). Isotopic purity was confirmed by identifying the major gamma-ray transitions at 204, 587, and 837 keV, and by measurement of the physical half life. The isotope was obtained as carrier free, and the activity concentration of the stock solution at the time of receipt was 7.74 μCi/ml.

Test solutions were prepared by diluting appropriate quantities of ^{95m}Tc stock solution with water to achieve an activity concentration of 0.4 μCi/l. These solutions were changed frequently during the experiments; at each change, a fresh dilution was made from the stock solution.

Radiation Detection Systems

For the detection of ^{95m}Tc gamma emissions, two different solid scintillation detection systems were used. A 25 cm x 30 cm NaI(Tl) crystal coupled to a photomultiplier tube, surrounded by 20 cm

of lead shielding, was used for all crayfish whole-body counting and most crayfish tissue sample counting. All activities were recorded as gross counts by a Davidson 1024-channel analyzer and a digital printer. This system was calibrated to 5 keV/channel. The counting window used was from channel 123 to channel 238, which corresponded to an energy range of 0.615 to 1.190 Mev. Counting efficiency of the system was approximately 57% with an average background count rate of 570 cpm. This equipment was located two blocks from the animal holding facility.

The second detection system was a Packard Auto-Gamma Spectrometer system, used for counting all whole snails and tissues, as well as some of the crayfish tissues. The detector was a 7.6 cm x 7.6 cm NaI(Tl) well-crystal coupled to a photomultiplier assembly, with an automatic sample changer, a dual-channel analyzer, and a digital printer. This system was used with a detector gain of 30% and a counting "window" of 50-420 arbitrary units, which corresponded approximately to an energy range of 150 to 900 keV. These optimal detector gain and window settings were experimentally determined. Average counting efficiency for this system was 72%, and the average background count rate was 230 cpm. This system was located adjacent to the crayfish holding facility.

Relative counting standards were prepared for each detection system. Pieces of a cellulose sponge were cut to the approximate dimensions of the samples to be counted on each system. Then a small volume of ^{95m}Tc stock solution was pipetted into a small volume of water, and this was then absorbed on to the sponge. The activity deposited on each sponge was calculated by multiplying the volume of

stock solution used by the activity concentration of the stock solution. An 8 cm x 2 cm x 1.5 cm piece of sponge was cut to approximate a medium-sized crayfish, and was used with the large crystal system; a 2 cm x 0.5 cm x 0.5 cm piece of sponge was used with the Auto-Gamma system, to approximate the dimensions of the snails used. Sponges were wrapped in plastic film, then sealed in counting tubes and appropriately labeled. The standards were prepared in this way to minimize counting errors due to differences in geometry between counting standards and the experimental animals.

Animals Used and Experimental Procedures

Although the experimental protocol for each species was essentially the same, some procedures were slightly different. Therefore, the general biology, collection and maintenance, and experimental procedure for each animal will be discussed separately.

The Crayfish *Pacifastacus leniusculus*

This crayfish is abundant in streams west of the Cascade mountains, from northern California northward to British Columbia. In Oregon, *P. leniusculus* is primarily an inhabitant of streams, but is also found in ponds and lakes (Mason, 1963)

P. leniusculus is a relatively large, orange/brown crayfish, often reaching 15 cm in length. These crayfish are the largest and longest-lived members of the freshwater crustacea in this region, and constitute an important food source for many fish (Miller, 1960). As omnivores, they occupy a unique niche in freshwater ecosystems. As mentioned previously, *P. leniusculus* was selected as an experimental

animal because of its local abundance and its trophic status in stream ecosystems. An additional factor was interest in comparing crayfish data with the high Tc uptake reported for the taxonomically-related lobster (Pentreath, 1981).

Collection and Maintenance

All crayfish were collected from Rock Creek, Benton County, OR, approximately 2 km upstream from State Highway 34. They were collected by dip net or trap, during August and November of 1982. Intermolt males were most readily collected, therefore they were used in all subsequent studies. Crayfish were identified as P. leniusculus from Hobbs (1972). After collection the crayfish were transported to the laboratory in a styrofoam cooler; travel time to the laboratory was approximately 30 minutes.

Crayfish were maintained in the laboratory in groups of 10-30 in plexiglass or glass aquaria. Water used in all studies was from Rock Creek, kept at 15 $\pm$ 1°C to simulate conditions at the time of collection. Aquaria were cooled by placing them in fiberglass-lined troughs through which refrigerated tap water circulated. A Westinghouse cooler was used to cool the circulating tap water. Aquarium water was filtered through glass wool and charcoal, and aerated with airstones. A 13/11 h light/dark photoperiod was used throughout acclimation, maintenance and during all experiments.

Crayfish were fed every two weeks during maintenance, and weekly during the experiments. The maintenance diet consisted of beef liver gelatin, which was prepared by blending 62 g beef liver with 1 l tap water, heating to a boil, and stirring in 120 g gelatin powder. This mixture was then poured into shallow trays and allowed to cool. Beef

liver gelatin was used because it could be fed directly to the group-held crayfish without fouling the water. Raw beef liver was fed to the crayfish during all studies, when the crayfish were fed individually in separate dishes from the experimental pans.

Uptake Study

Uptake during chronic exposure to pertechnetate solution was followed for 64 days. Eleven crayfish, ranging in weight from 15 to 25 g, were initially placed in 11 l of test solution in a 46 cm x 30 cm x 15 cm plastic pan with plexiglass dividers inserted to keep the crayfish separated. The pan was cooled in the water trough as previously described for the aquaria. The test solution was changed every two weeks, and was reconstitued as necessary with additional stock solution to keep the activity concentration of the water at 0.4 μCi/l.

For whole-body assay, crayfish were first removed from the test solution, rinsed, weighed, and placed in 12 cm x 6 cm x 6 cm plastic dishes with 200 ml of cooled water for transport to the counting facility. A styrofoam cooler was used to hand-carry the crayfish to the counting facility, where they were held in a cold room (temperature = 16°C). For counting, crayfish were removed from the transport dishes, rinsed with tap water and placed individually in 60 dram prescription vials with 75 ml fresh water. The vial was then capped, placed in a plastic bag, and positioned in the counting chamber. A sufficient number of counts was accumulated to achieve a statistical counting error of less than 1%. It was determined that ^{95m}Tc was not contaminating the vials, so they were simply rinsed with tap water between counting of each crayfish and reused. A count

of an empty vial was made prior to counting the crayfish and was used as a background count. Eight of the original 11 crayfish survived to the end of the 64 day uptake study.

Retention Study

To determine the pattern of retention/elimination of the accumulated ^{95m}Tc, the 8 surviving crayfish from the uptake study were assayed for whole-body activity and placed in fresh water, in the same container used for the uptake study. This initial activity measurement represented the initial body burden of ^{95m}Tc for each crayfish, to be used in retention study calculations. Except for the absence of ^{95m}Tc, all conditions were the same as during the uptake study. Water in the pan was changed frequently to minimize reabsorption of excreted ^{95m}Tc.

As in the uptake study, crayfish were periodically removed and assayed for whole-body activity. The percent of initial body burden retained was calculated each counting day by dividing measured whole-body activity, corrected for decay, by the initial activity. Counting procedures were identical to those used during the uptake study, except the crayfish were not weighed at each count; only occasional weighings were made to monitor weight loss. After 52 days of elimination the remaining crayfish were sacrificed and dissected for tissue distribution data. Five crayfish survived to the end of the 52 day retention study.

Tissue Distribution Study

Crayfish to be used in this study were placed in plastic pans containing test solution with an activity concentration of 0.4 μCi/l. Conditions were similar to the other studies, except there were no

plexiglass dividers in the pans. Instead, short pieces of PVC piping were placed in the pans for the crayfish to use as shelters. As in the other studies, the test solution was reconstituted regularly to maintain an activity level of 0.4 μCi/l. At periodic intervals groups of 3-5 crayfish were removed from the test solution and their whole-body activity was determined as above. Each crayfish was then sacrificed by placing in hot (55°C) tap water for 2 minutes. After weighing, samples of 6 tissues were immediately dissected out. Tissues used were digestive gland, green glands, stomach, exoskeleton, abdominal muscle and blood. Tissue samples were immediately weighed, wrapped in wax paper and placed in counting tubes for subsequent activity assay. Crayfish were sacrificed on days 4, 16, and 52 of uptake.

After 65 days of uptake the remaining crayfish were placed in fresh water for elimination. Water was changed frequently, as in the elimination study, to avoid reabsorption of excreted ^{95m}Tc. At periodic intervals 3-5 crayfish were removed, assayed for whole-body activity, and sacrificed as above. Sacrifice days were days 4, 13, and 29 of elimination; tissue distribution data obtained from the whole-body elimination group for day 52 were also used.

The Freshwater Snail *Juga silicula*

The snail *Juga silicula* (formerly *Oxytrema silicula*), is a common inhabitant of streams in the Pacific Northwest. The distribution of *J. silicula* has been established from southwest Washington south to the Shasta River in northern California, and from the Cascade Mountains west to the Pacific Ocean (Earnest, 1967). The small (1-3

cm length), black shell of J. silicula is easily seen in streams, the snails clinging to rocks or to organic material in the shallower and more rapidly flowing parts of the stream. As Ching (1957) put it, "It is not a snail of still and stagnant water."

J. silicula was thought to be an appropriate experimental animal for many of the same reasons as P. leniusculus: local abundance and ease of collection, importance in the freshwater community, and interest in comparing results with published data on a marine gastropod, the red abalone Haliotis rufescens (Beasley, Lorz, and Gonor, 1982).

Collection and maintenance

Snails were collected from Rock Creek, OR, where State Highway 34 crosses it (approximately 2 km downstream from the collection site of P. leniusculus). Collections were made during April and June of 1983. The snails were hand-picked off the bottom, from rocks or organic debris, and transported to the laboratory in a small plastic bucket. Travel time to the lab was approximately 30 minutes.

The snails were kept in the plastic bucket to acclimate to lab conditions for 2-5 days. Snails were kept in a cold room during acclimation, maintenance, and all studies, where water temperature was maintained at 8.5 $\pm$ 0.5°C , and a 14/10 hour light/dark cycle was used.

Even though J. silicula prefers fast-moving water, maintenance in the laboratory was not extremely difficult. With adequate aeration and circulation of the water, sufficient numbers of snails were kept healthy for over 3 months. Food for the snails consisted at various times of fresh carrot slices, and dried alder and maple leaves.

During the experiments the snails were removed from the test solution and fed in individual containers every 3 weeks. No significant weight loss was noted for any of the snails.

Uptake Study

Fifteen snails, ranging in weight from 0.34 g to 0.70 g, were placed individually in 15 cm x 1.5 cm plastic tubes which had been perforated to allow water circulation. The perforated tubes were placed in a holder inside a 25 cm x 15 cm x 13 cm plexiglass container along with 1.5 l of test solution. The water was aerated, and circulated by means of a paddle suspended in the center of the container, rotated by an overhead electric motor. Water was changed every two weeks, and was reconstituted with additional ^{95m}Tc between changes as necessary to keep the activity concentration of the water at 0.4 μCi/l. Technetium uptake was followed for 51 days, at which time the rate of uptake had slowed significantly.

For whole-body assay, the snails were first removed from the test solution and placed in fresh water for 10 minutes. This 10 minute purge was required to allow any trapped test solution to be released. Snails were then removed from their perforated tubes, rinsed, and put into a counting tube containing 5 ml fresh water. Counting tubes were then individually positioned in the Auto-Gamma counter and counted for a maximum of 5 minutes. A 5 minute limit was used because of an unacceptable rise in the water temperature after more than 5 minutes. All 15 snails survived to the end of the 51 day uptake study.

Retention Study

Near the conclusion of the uptake study the rate of uptake had slowed and was beginning to level off, so the 15 snails were placed in

fresh water for a retention study. Counting procedures were the same as during uptake, except the snails were not weighed every counting day. As with the crayfish, the final uptake activity represented the initial body burden of ^{95m}Tc to be used in the elimination study, and the percent of initial activity retained was likewise calculated each counting day. Elimination was followed for 49 days with no mortalities, at which time all 15 snails were sacrificed for tissue distribution analysis.

Tissue Distribution Study

Sixty-eight snails were placed in 6.8 l of test solution in a plastic pan; activity concentration of the test solution was 0.4 μCi/l. Unlike the uptake and elimination studies, the snails were free to move around in the pan. Water was aerated and circulated in a similar manner as for the other snail studies. There was approximately 40% mortality in this group of snails over the duration of the study, for unknown causes. Only snails that appeared to be healthy were sacrificed and used in the study.

At periodic intervals, 6 snails were removed from the test solution and whole-body activity was determined as above. Snails were then sacrificed by placing them individually in 5 ml of a warm 2% magnesium sulfate solution for 10 minutes. Each snail was then dissected into two tissue components: shell and soft tissue. This was accomplished by sawing and breaking the shell into two or more pieces, and then removing the snail body from the shell halves. Immediately after dissection the shell and soft tissue were weighed, wrapped in wax paper, and placed in separate counting tubes. Counting was done using the Auto-Gamma system, as in the other snail studies. Sacrifice

days were days 2, 9, 23, and 48 of uptake.

After 52 days of exposure to the test solution the remaining snails were placed in fresh water for elimination. At periodic intervals 5-6 snails were removed and sacrificed as above. Sacrifice days were days 1, 5, and 16 of elimination. Data from sacrifice of the elimination group on day 49 were used also.

RESULTS

The Crayfish Pacifastacus leniusculus

Uptake

The uptake of ^{95m}Tc as $(TcO_4)^-$ was followed in the crayfish for 64 days. This was accomplished under chronic, laboratory conditions, and uptake was from water only. The activity concentration of the water was held constant throughout the study.

The mean dpm/g whole-body wet weight was calculated for the eight crayfish that survived the duration of the study. These values are plotted in Figure 1 on a semilog scale as the mean dpm/g wet weight vs. day of exposure to ^{95m}Tc solution. The original data are reported in Appendix Table I.

After a rapid uptake during the first day of exposure the crayfish accumulated ^{95m}Tc at a fairly constant rate over the rest of the study. A linear plot of the data (Figure 2), illustrates the steady rate of uptake. A linear regression of these data from Day 1 to Day 64 shows a strong linear relationship between activity and time ($r^2 = .986$). The slope of the regression line is approximately 17 dpm/g per day.

Concentration ratio (CR), is a measure of the concentration of a radionuclide in an organism relative to the concentration of the radionuclide in the surrounding water. CR, as used in this paper, is defined by Whicker and Schultz (1982):

$$CR = \frac{\mu Ci/g \text{ in compartment of interest}}{\mu Ci/g \text{ in reference compartment}}$$

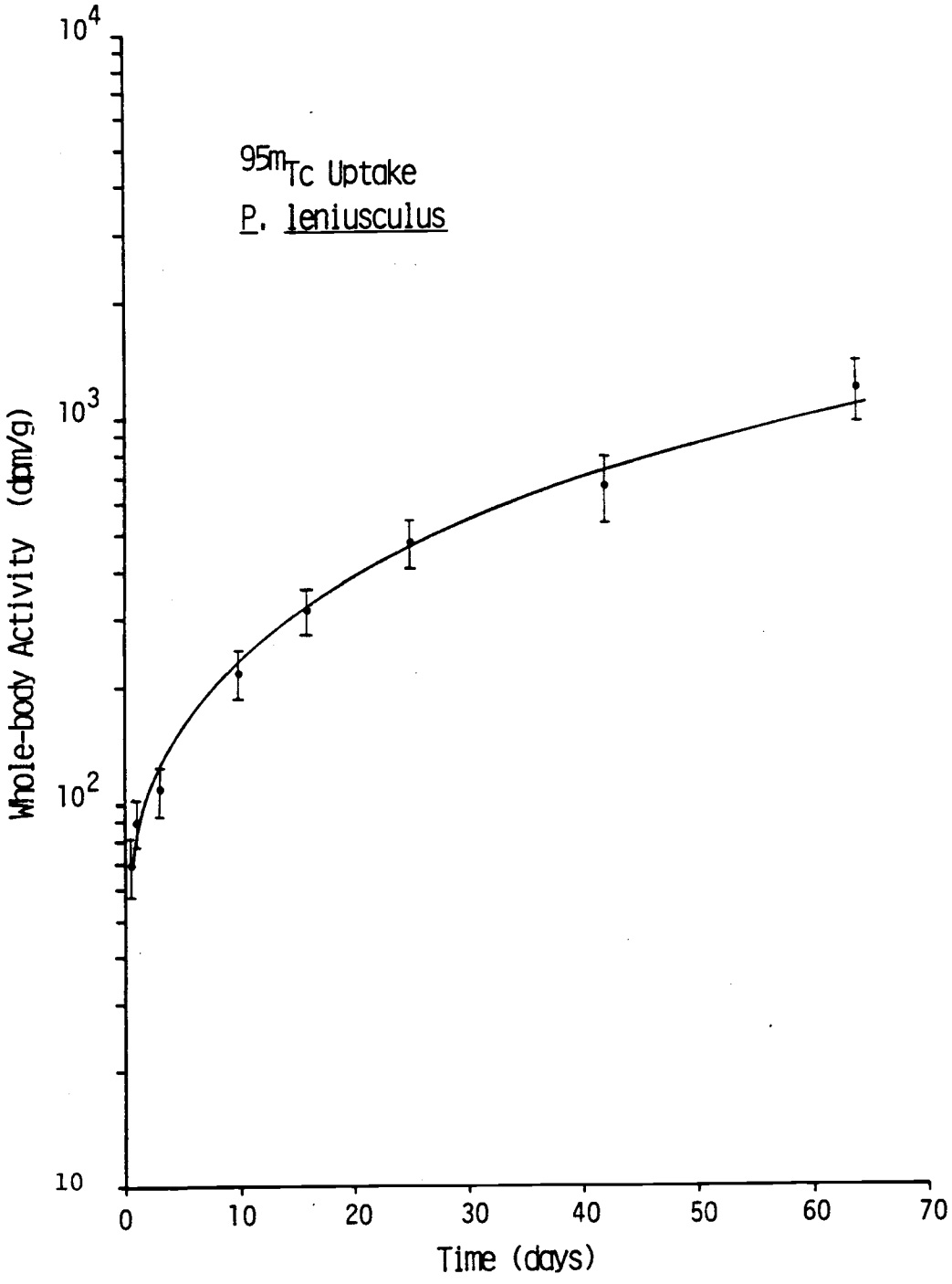

Figure 1. Whole-body uptake of 95mTc by the crayfish Pacifastacus leniusculus during chronic exposure at 15°C. Error bars indicate ± 1 S.D. (n = 8).

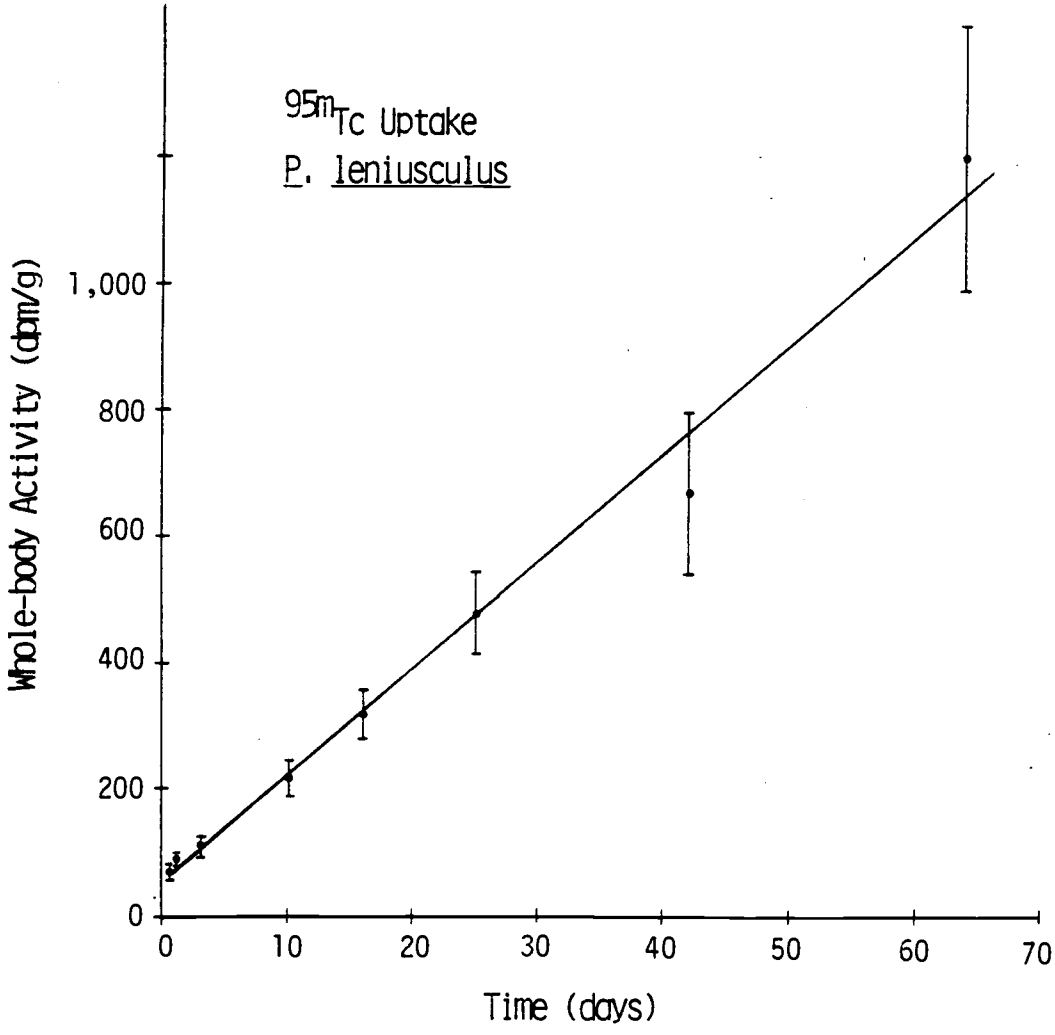

Figure 2. Whole-body uptake of ^{95m}Tc by the crayfish Pacifastacus leniusculus during chronic exposure at 15°C. Error bars indicate ± 1 S.D. (n = 8).

For this aquatic system, the compartment of interest is the animal or tissue, and the reference compartment is the water. The final whole body CR reached by the crayfish ($\pm$ 1 S.D.) was 1.6 $\pm$ 0.3, indicating that after 64 days of exposure to ^{95m}Tc solution they reached a mean whole body activity concentration only 1.6 times that of the water.

Tissue Distribution

The tissue distribution of ^{95m}Tc was followed in the crayfish throughout uptake and elimination. Data were obtained by sacrificing different groups of 3-5 crayfish at various times during the experiments. This differed from the uptake and elimination studies, where the same group of crayfish was measured repeatedly. The 6 tissue groups used were: exoskeleton, muscle, digestive gland, green (antennal) glands, stomach, and blood. The exoskeleton sample was normally taken from the carapace. The muscle sample was from the abdominal (tail) muscle. The original data, expressed as mean dpm/g wet weight in each tissue, are tabulated in Appendix Table II.

The calculated total activity in the three major tissue compartments - digestive gland, exoskeleton, and muscle - is plotted as percent of whole-body activity vs. time in figures 3 and 4 (uptake and retention, respectively). The other tissues each contained less than 5% of the total whole-body activity at all times during uptake and retention.

To determine the total mass of each tissue group in the whole animal, 5 crayfish were completely dissected, and the percent of the total weight in each group was determined. These dissections resulted

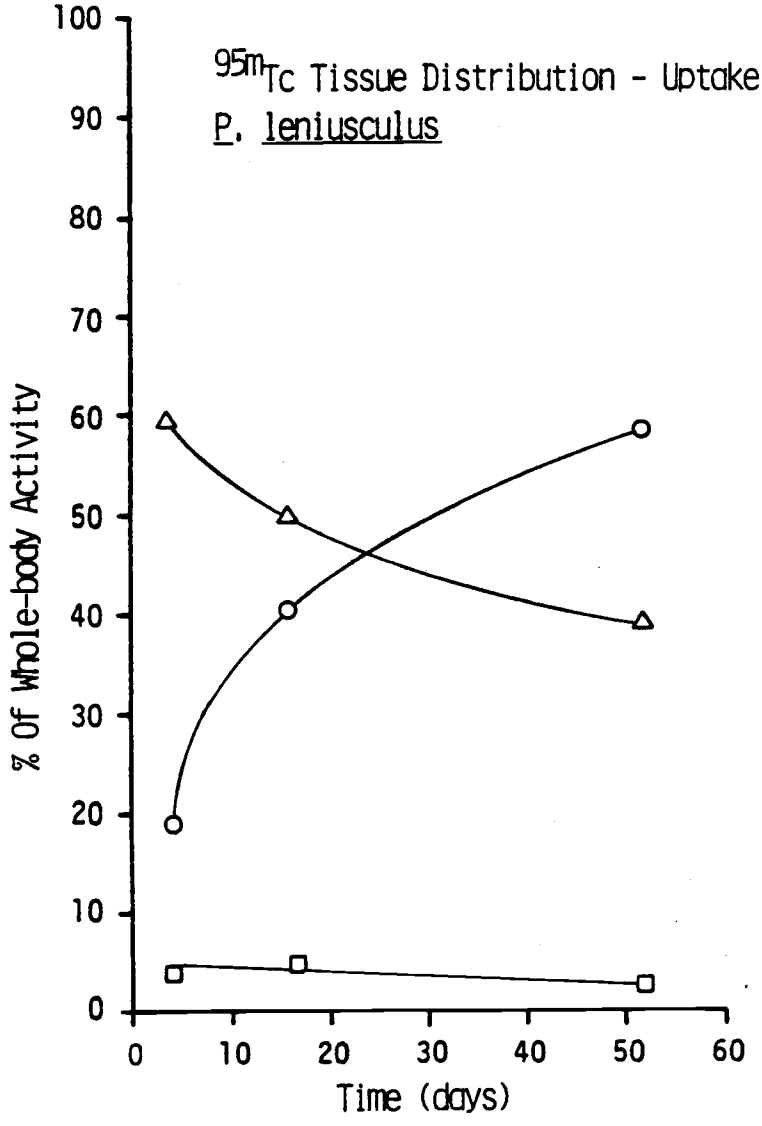

Figure 3. Tissue distribution of ^{95m}Tc in the crayfish Pacifastacus leniusculus during the chronic exposure uptake phase, at 15°C. (O = Digestive gland, Δ = Exoskeleton, □ = Muscle).

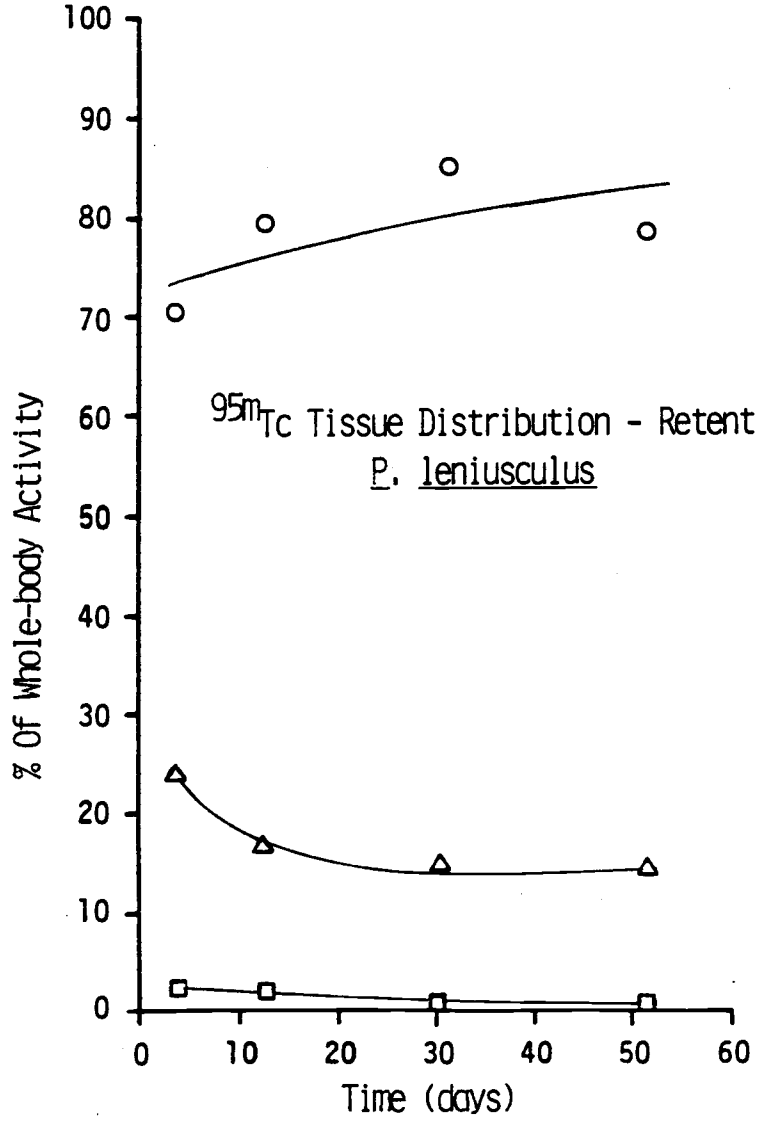

Figure 4. Tissue distribution of ^{95m}Tc in the crayfish Pacifastacus leniusculus during retention phase, at 15°C. (○ = digestive gland, △ = Exoskeleton, □ = Muscle).

in the following percentages of whole-body wet weight (± 1 standard deviation): exoskeleton 66 ± 2%; muscle 22 ± 1%; digestive gland 5.8 ± 0.7%; green glands 0.44 ± 0.09%; stomach (empty) 2.9 ± 0.9%. It was impossible to retrieve all of the blood during the dissections; thus it was assumed that blood comprised the majority of the remaining whole-body weight, or 2%. With this assumption, the sum of these tissues is 99.1%. A sample calculation for values in Fig. 3 follows:

1. Whole-body wet weight : 15.6g
2. Wet weight of exoskeleton : 0.66 x 15.6g = 10.3g
3. Measured activity in exoskeleton sample : 1085 dpm/g
4. Calculated activity of total exoskeleton :

   1085 dpm/g x 10.3g = 11,175 dpm
5. Measured whole-body activity : 25,860 dpm
6. Percent of whole-body activity in exoskeleton :

   11,175 dpm/25,860 dpm = 43%

This value was calculated for each sacrificed crayfish, and the mean values are plotted in Figs. 3 and 4.

As can be seen in Appendix Table II, mean tissue dpm/g values on day 52 of uptake ranged from 150 dpm/g in muscle to 14,000 dpm/g in the digestive gland. In addition to having the highest activity concentration of ^{95m}Tc on a mass basis, the digestive gland also carried the largest fraction of the whole-body activity by day 52. The exoskeleton and the digestive gland together contained 79-100% of the total whole-body activity on all sample days. The exoskeleton took up ^{95m}Tc more rapidly than the digestive gland during uptake, and eliminated it more quickly during the elimination phase. Activity proportions in the other tissues were low and fairly constant

throughout both uptake and elimination. The stomach and green glands tended to retain their accumulated activity longer than the muscle and blood during the retention phase. In general, the lowest activity concentrations were found in muscle and blood during both the uptake and elimination phases.

Concentration ratios for the tissues on the final day of uptake were calculated. After 52 days, the CR values ($\pm$ 1 S.D.) were: digestive gland 16 $\pm$ 5, green glands 11 $\pm$ 9, stomach 1.2 $\pm$ 0.3, exoskeleton 1.0 $\pm$ 0.2, blood 0.5 $\pm$ 0.1, and muscle 0.17 $\pm$ 0.08. The whole-body CR in the sacrificed crayfish at this time was 1.45.

Retention

After 64 days in ^{95m}Tc solution, the eight remaining crayfish were put into fresh water, where whole-body retention was followed for 52 days. Five crayfish survived to the end of the retention study, and only results from these were used. Data are expressed as the percent of initial whole-body activity (Day 0 of elimination) present on each assay day, after correction for decay. A semilog plot of the mean percent of initial activity vs. time is shown in Figure 5. The original data are tabulated in Appendix Table III.

The crayfish retention curve suggests two distinct components, one long-lived and the other short-lived. After analysis it was determined that the long-lived component contained approximately 82% of the initial activity, while the shorter-lived component contained the majority of the remainder. The graphic resolution of the curve was accomplished by a least-squares fit of the exponential portion (days 13-43), and generation of a regression equation (Whicker and

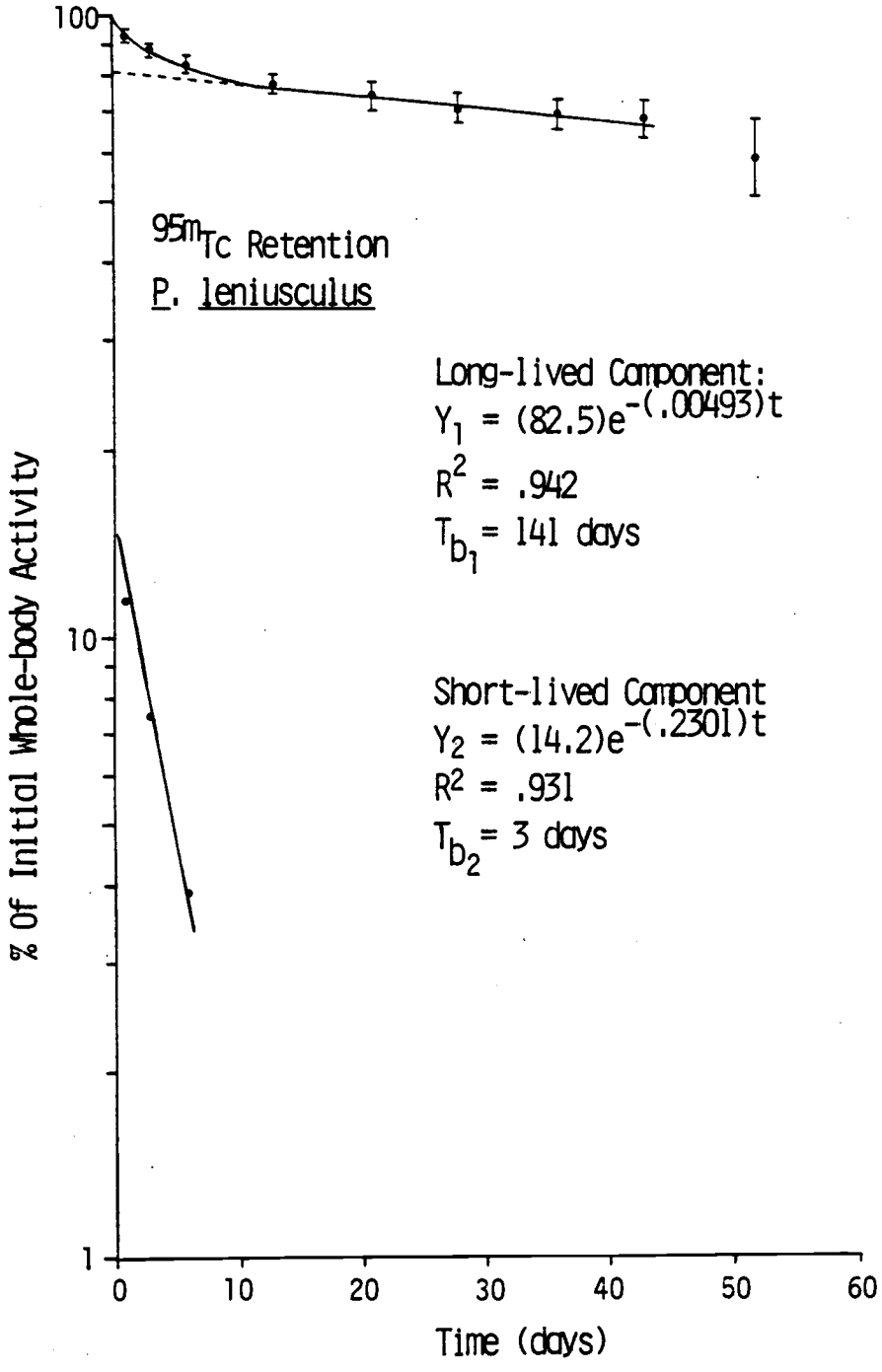

Figure 5. Whole-body retention of ^{95m}Tc by the crayfish Pacifastacus leniusculus following chronic exposure at 15°C. Error bars indicate ± 1 S.D. (n = 5).

Schultz, 1982). The exponential portion was then extrapolated back to Day 0 to estimate the percentage of the initial activity that was contained in the long-lived compartment. The data point for day 52 was omitted from the regression because of an unusually large drop in activity in two of the five remaining crayfish on this measurement. Reasons for the large drop are not known. It is suspected that these crayfish were not healthy at this time.

The biological half-life (T_b) of the long-lived component was determined by calculating the day (x-value) corresponding to an activity level that is one-half of what was initially present in that compartment. The T_b for ^{95m}Tc in the crayfish was calculated to be 141 days. Once the T_b has been determined, the effective half-life (T_e) can be calculated. The T_e is an expression of the overall rate at which activity from a radionuclide diminishes in an organism or compartment, taking both physical decay and biological elimination into account. Effective half-life is calculated as follows:

$$T_e = \frac{(T_p)(T_b)}{T_p + T_b}$$

where T_p, T_b and T_e are the physical, biological, and effective half-lives, respectively. The calculated T_e for ^{95m}Tc in crayfish was 42 days.

As mentioned earlier, the technetium isotope of environmental concern is ^{99}Tc, with a T_p of 210,000 yrs. Assuming that ^{99}Tc and ^{95m}Tc have identical biological half lives, the T_e of ^{99}Tc will be essentially the same as the T_b (141 days). This is because of the large difference between the T_p and

T_b of ^{99}Tc. This simply means that there will be very little decay of ^{99}Tc during the time it resides in the crayfish.

The short-term component in Fig. 5 was generated by subtracting values on the extrapolated line from corresponding measured values, for the period of day 0 to day 9. The resulting differences were then plotted vs. their corresponding days (lower curve in Fig. 5). The ln values of these points exhibit a fairly strong linear relationship with time (r^2 = .931), indicating that this probably represents a single biological compartment in the crayfish. The T_b for this compartment was calculated to be approximately 3 days.

The Freshwater Snail Juga silicula

Uptake

Uptake of ^{95m}Tc by 15 freshwater snails was followed for 51 days. Technetium-95m was present in solution as pertechnetate, and the activity concentration in the water was kept constant. Thus, the snails were chronically exposed, and uptake was from water only. The mean dpm/g whole body wet weight was calculated for the 15 snails, and is plotted vs. time on a semilog scale in Figure 6. The original data are tabulated in Appendix Table IV.

The snails accumulated technetium rapidly, reaching a mean whole-body activity concentration of 2400 dpm/g within 24 hours (a CR of approximately 3). A mean CR of 6 was reached by the third day. By day 25, after reaching a mean CR of 27, the rate of uptake had slowed and was relatively constant. The snails accumulated 77% of their final body burden in the first half of the study (25 days). The final

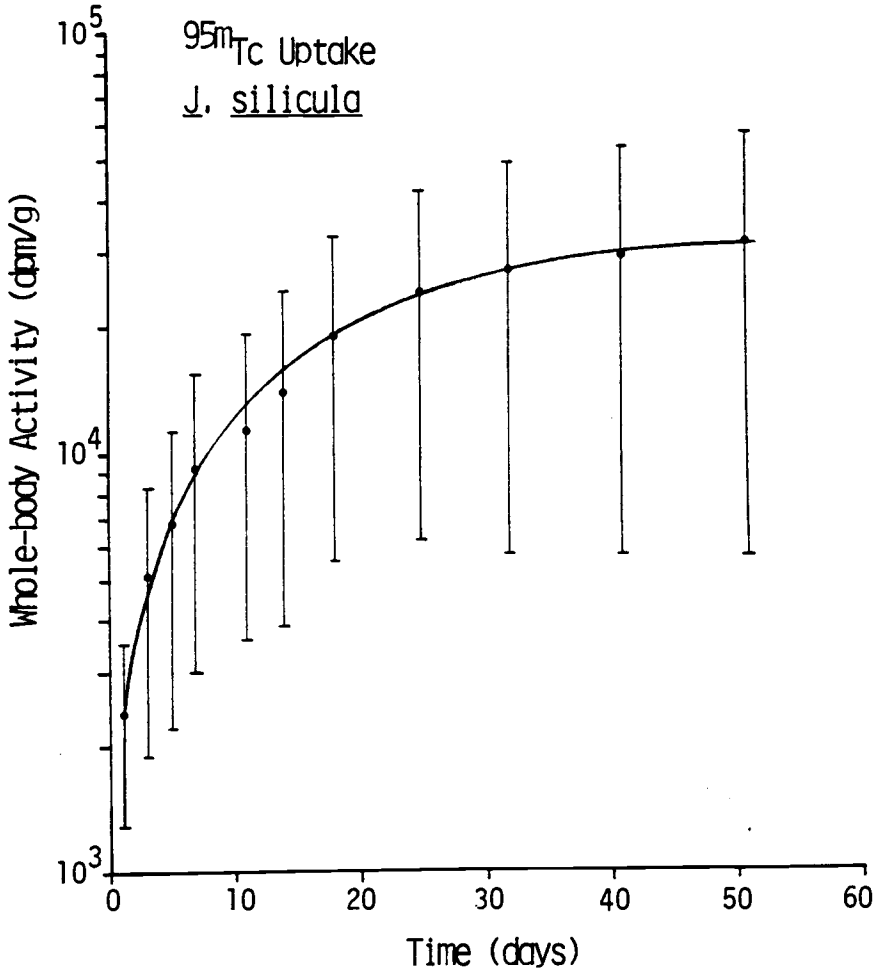

Figure 6. Whole-body uptake of ^{95m}Tc by the snail Juga silicula during chronic exposure at 8.5°C. Error bars indicate ± 1 S.D. (n = 15).

near-equilibrium CR reached by the snails after 51 days was 41 $\pm$ 33.

One feature of the mean activity values of the snails is the large standard deviations (S.D.) associated with them. A large range of individual values resulted in standard deviations that were 70-80% of the means. This could be due to a non-normal distribution of the values; however a calculation of the geometric means, assuming a log-normal distribution, yielded S.D. values just as large.

Tissue Distribution

The distribution of ^{95m}Tc was followed in the shell and soft tissue of the snail. Groups of 5-6 snails were sacrificed periodically during uptake and elimination phases, and were dissected into the two tissue components. Fifteen non-experimental snails were first dissected to determine percentages of weight contained in the shell and soft tissue. The shell comprised 50% of the whole body wet weight, and the soft tissue made up 24%. The remaining fraction of whole-body weight wet was apparently lost due to water evaporation during the dissections. Experimental sacrifice procedures were kept consistent, however, in an attempt to minimize and normalize evaporative and other losses.

Approximately 88% of the whole-body activity was recovered in the experimental dissections. The 12% loss of whole-body actvity was fairly consistent between sacrificed snails. It was apparently lost with body fluids during dissections. The original data are tabulated in Appendix Table V. The percentages of whole-body activity in the shell and soft tissue are shown in Figures 7 and 8; these values were

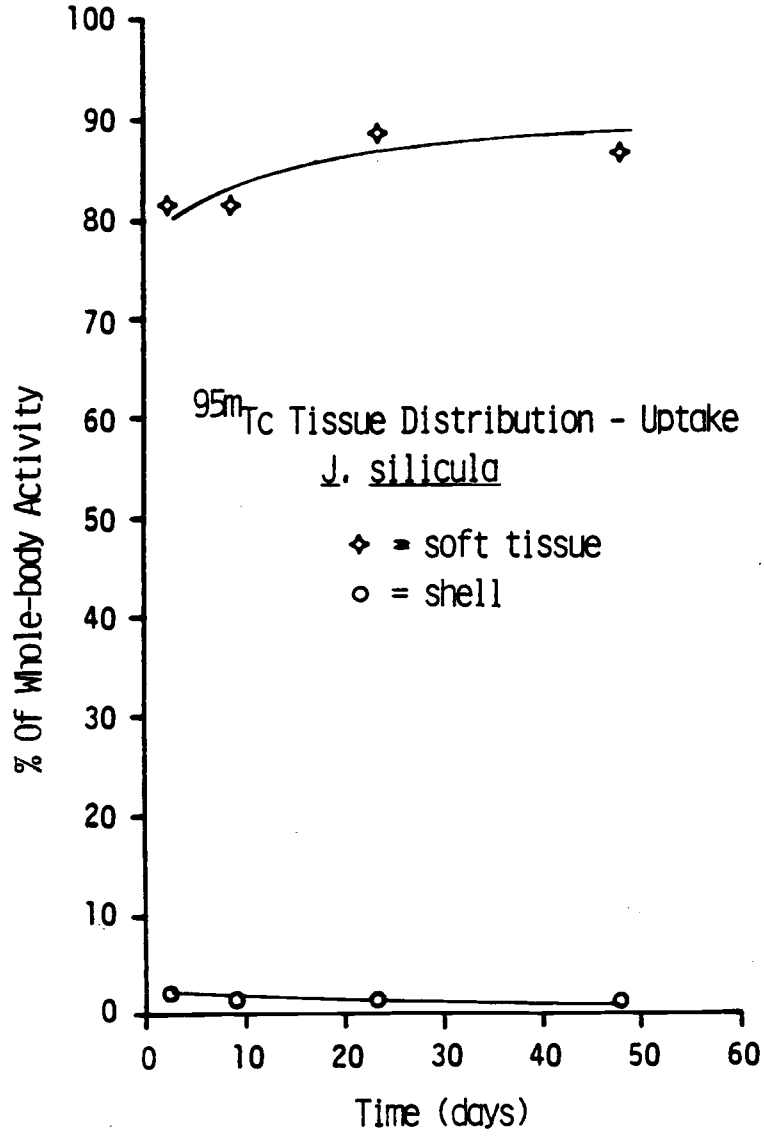

Figure 7. Tissue distribution of ^{95m}Tc in the snail Juga silicula during the chronic exposure uptake phase, at 8.5°C.

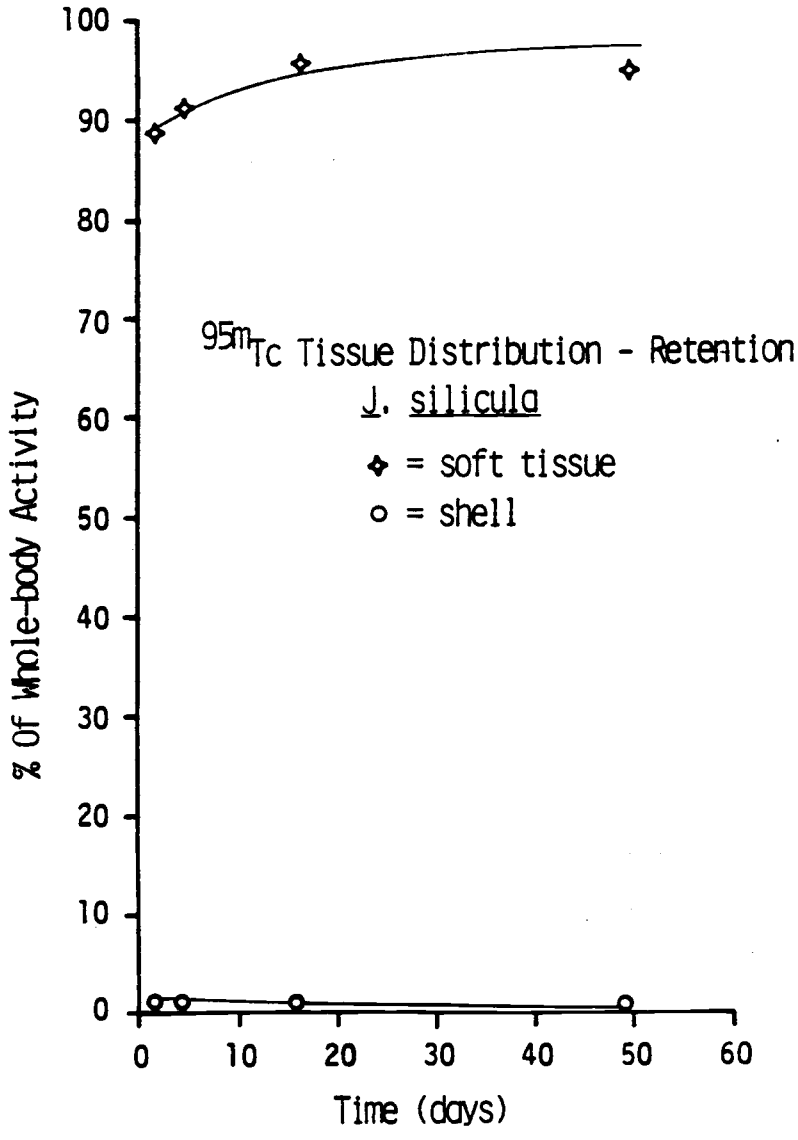

Figure 8. Tissue distribution of ^{95m}Tc in the snail Juga silicula during retention phase, at 8.5°C.

calculated in the same manner as the corresponding crayfish values. By the second day of uptake, the soft tissue contained 82% of the whole-body activity; this increased to 88% by day 23 and essentially remained there for the rest of the uptake phase. During the 49-day elimination study, the soft tissue contained 88-96% of the whole-body activity at all times. The shell held less than 1% of the whole-body activity throughout most of the uptake phase and all of elimination. CR values for shell and soft tissue on the final day of uptake (day 51) were 0.4 $\pm$ 0.1 and 85 $\pm$ 50, respectively. The whole-body CR value on this day was 36 $\pm$ 27.

Retention

After 51 days of uptake, the 15 snails in the uptake study were put into fresh water and whole-body retention of ^{95m}Tc was followed for 49 days. A semilog plot of the mean percent of initial activity retained vs. time is shown in Figure 9. Original data are tabulated in Appendix Table VI.

The snail retention curve suggests at least two components: a long-lived component that initially contained approximately 90% of the accumulated activity, and one or more shorter-lived components. The snails still held 69 $\pm$ 12% of their initial body burden on day 49. Graphic resolution of the curve was accomplished as before, with regression over days 9 - 49. The T_b of the long-lived component in the snail was 120 days. The calculated T_e for ^{95m}Tc in the snail is 40 days; as with the crayfish, the T_e for ^{99}Tc is essentially the same as the T_b.

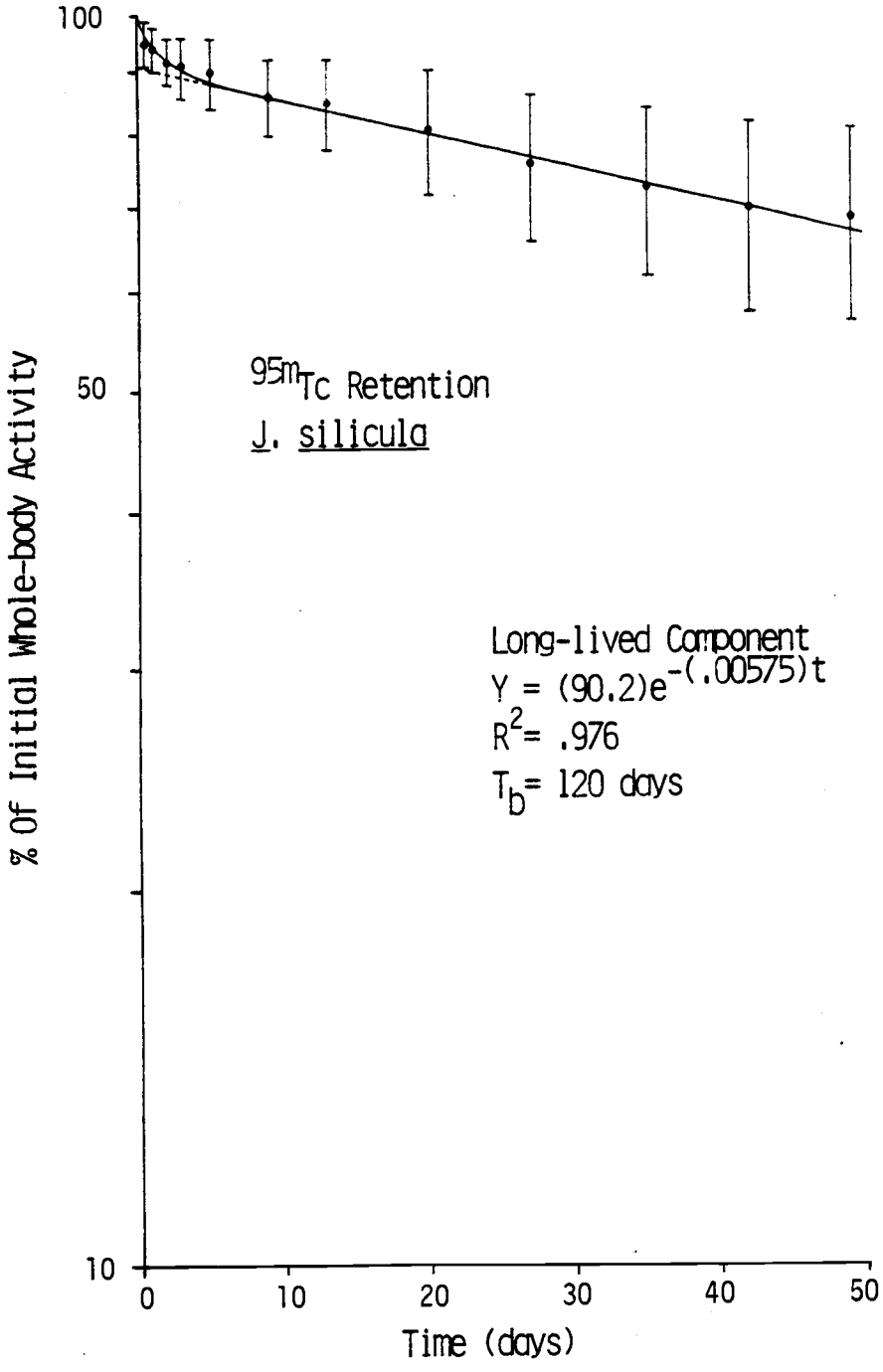

Figure 9. Whole-body retention of ^{95m}Tc by the snail Juga silicula following chronic exposure at 8.5°C. Error bars indicate ± 1 S.D. (n = 15).

DISCUSSION

The Crayfish *Pacifastacus leniusculus*

Uptake

Whole-body accumulation of Tc by the crayfish was very steady over the 64-day uptake period. A CR of 1.6 had been reached by day 64, but equilibrium had not been attained. From the linear graph of whole body uptake (Fig. 2), it is evident that the rate of uptake was essentially constant throughout the study. Because the whole-body activity concentration had clearly not reached equilibrium, it would be useful to have some estimate of the final CR value. Whicker and Schultz (1982) describe equations allowing the calculation of such a value. These calculations require use of a buildup term based on the slope of regression values from the uptake data. However, such a determination is most useful when uptake has reached a nearly steady state. Since the crayfish uptake data had not reached that state and were somewhat scattered between days 25 and 64, such a calculation was not made here.

The results reported here for *P. leniusculus* are the first concerning Tc uptake by a freshwater crustacean. However, several marine crustaceans have been studied. Pentreath (1981) reported an equilibrium CF of 1160 for the lobster *Homarus gammarus*, and 14 for the shrimp *Crangon vulgaris*. These values were not actually observed, but were projected from long-term uptake studies. Fowler *et al.* (1981), reported a CF of 8 for the shrimp *Palaemon elegans*. Other marine crustaceans have yielded CF values similar to those of the crab

and the shrimp (tabulated in Beasley, Gonor and Lorz, 1982).

The low CR for the crayfish is interesting when compared to the very high value for the taxonomically-related lobster. The large difference in technetium-concentrating ability of these two species illustrates the wide variation in Tc uptake observed among aquatic organisms. The large difference in CR values might at first be attributed to differences in the respective medium for each animal (e.g., salinity, pH, the presence of other metals or complexing agents). Certainly an important aspect of uptake studies is any interaction between the introduced nuclide and the medium. However, the moderate uptake reported for the shrimp and the crab do not support this as a sole reason for the large difference in concentrating ability.

Other experimental variables that can affect uptake include water temperature and life stage of the experimental animal. The crayfish were kept at a constant temperature throughout the uptake (and elimination) phase, similar to most experimental designs. It is interesting to note that an increase in water temperature increased the rate of uptake of Tc in the shrimp P. elegans, but had no effect on the final concentration of Tc reached (Fowler et al., 1981). The life-stage of the experimental crayfish was unknown, but it was assumed that they were all mature based on their sizes. None of the crayfish gained (or lost) a significant amount of weight during the uptake study, and none of the crayfish molted. It is unknown if the experimental regimen had any effect on their life cycle.

The low accumulation by P. leniusculus suggests a passive uptake mode, whereas the high CF reached by the lobster suggests some sort of

active mediation. The reasons for the large disparity in concentrating ability of these two organisms is unclear; differences in the experimental design of each study may be responsible for some of the disparity. The details of the lobster study are not clear; however, in the same set of studies (Pentreath, 1981), the much lower CF of 14 was reported for the shrimp.

The wide range of CR values for Tc found in laboratory uptake studies, even within similar taxonomic groups, warrants further investigation. The many experimental and biological variables involved make it difficult to generalize concerning Tc uptake among crustaceans. Uptake by the crayfish is among the lowest found in freshwater and marine crustaceans.

Tissue Distribution

The digestive gland and the exoskeleton together contained the majority of the accumulated body burden of Tc in the crayfish. During the initial period of uptake, the exoskeleton contained the largest proportion of Tc, as might be expected. However, a CR of only 0.2 was reached for the exoskeleton during this initial phase. The fraction of whole-body activity contained by the exoskeleton gradually decreased over the uptake period, while simultaneously the proportion held by the digestive gland (hepatopancreas) increased. These two tissues each contained approximately 45% of the total whole-body burden of Tc by day 24 of uptake, and the fractions held by each had changed to approximately 60% and 40% (digestive gland and exoskeleton, respectively), by the end of uptake. All other tissues examined contained less than 5% of the whole-body activity on the final day of

uptake.

The relatively high concentration of Tc found in the digestive gland was not suprising. Bryan (1968) found that the hepatopancreas concentrated zinc and copper more than any other tissues in the crayfish Austropotamobius pallipes. Cobalt has also been found to be concentrated highly in the hepatopancreas of the crayfish (Wiser and Nelson, 1964). Additionally, the digestive glands of several marine crustaceans concentrated Tc to a high degree. Fowler et al. (1981), found that the hepatopancreas of the shrimp P. elegans accumulated ^{95m}Tc to a higher level than any other tissues measured, and he reported a CF of 150 $\pm$ 24 after 22 days of uptake. In addition, Pentreath (1981), found that approximately 50% of the accumulated body burden of ^{95m}Tc was located in the digestive gland of the lobster H. gammarus.

The reason for the concentrating feature of the crayfish digestive gland is not known, but it may lie in the osmoregulating ability of the crayfish (Wiser and Nelson, 1964). As freshwater organisms, crayfish must maintain their body fluids hyperosmotic to their surrounding medium. Thus, they experience a continuous influx of water, which they must excrete in a hypoosmotic urine. Some ions will be excreted along with the excreted water. These excreted ions are replaced by ions taken up from the surrounding water. The digestive gland of the crayfish may absorb pertechnetate from the gut and store it as a replacement ion, as it does many other ions. In any event, the high concentration and relatively long retention period of Tc in the digestive gland indicates that it plays an important role in Tc accumulation by the crayfish.

The green gland of the crayfish also concentrated Tc to a relatively high degree (final CR = 11). However, because of the small mass involved (less than 1% of the total body mass), accumulation by this organ does not result in a significant contribution to the body burden. It is well known that the crayfish green gland acts as an excretory organ. It produces urine apparently via the reabsorption of salts and water, including the efficient reabsorption of sodium and chloride (Pritchard and Kerley, 1970). Pertechnetate is apparently reabsorbed by this organ also. The CR obtained for Tc in the green gland was exceeded only by that of the digestive gland (CR = 16), which tends to suggest reabsorption or retention by the green gland. The low concentration of Tc found in the blood (CR < 1.0) suggests that Tc entering the blood is quickly deposited in the digestive gland or green glands.

Although the exoskeleton contained a significant fraction of the body burden of ^{95m}Tc, the final CR reached was only 1.0. The low concentration of Tc found in the crayfish exoskeleton contrasts with the high levels of other radionuclides and metals often associated with crayfish exoskeletons. Schurr and Stamper (1962), found more than 90% of accumulated ^{85}Sr activity in the exoskeleton of the crayfish Cambarus longulus longerosteris. In the same species, Wiser and Nelson (1964), found that the exoskeleton contained the highest tissue concentration of ^{60}Co, while Reed and Martinedes (1971), also found ^{181}W most highly concentrated in the exoskeleton. Additionally, Anderson and Brower (1978), found relatively high concentrations of Pb in the exoskeleton of the crayfish Orconectes virilis. In addition to crayfish, the exoskeletons of several marine

crustaceans have been found to accumulate Mn, Cr, and Sb (Bryan and Ward, 1965; Tennant and Forster, 1969; van Weers and Louwrier, 1981). However, similar to the crayfish skeleton, Fowler et al. (1981) found ^{95m}Tc CR values of only 1 to 2 in the exoskeleton of the shrimp P. elegans.

The crayfish exoskeleton CR of 1.0 suggests simple adsorption of Tc to the exterior of the crayfish exoskeleton, without significant incorporation of the adsorbed Tc. As far as rates of uptake and retention, the exoskeleton appeared to accumulate Tc faster than the digestive gland during the uptake phase, and to release it faster during the elimination phase. This again suggests a passive mode of uptake by the exoskeleton, whereas metabolic processes may have some control over accumulation and retention by the digestive gland.

Concentration of Tc in tissues of the crayfish considered edible by humans, i.e., the tail muscle, was very low. A final CR of 0.17 was reached by crayfish muscle. This indicates that there would be only a minimal hazard to humans eating crayfish from a contaminated area. However, some crayfish predators (e.g., fish), eat the entire crayfish body, and this presents the potential for transfer of accumulated Tc up the food chain.

Retention

The ^{95m}Tc retention curve obtained for the crayfish is typical of two-compartment retention curves. The pattern observed is similar to those found for the elimination of both tungsten and cobalt from crayfish. Reed and Martinedes (1971), found a two-compartment elimination curve for tungsten, where the long-lived compartment -

suggested to be the exoskeleton - contained approximately 86% of the initial activity. Wiser and Nelson (1964) also found roughly a two-compartment model for the elimination of ^{60}Co. Interestingly, the biological half-life of Tc in the long-lived compartment of the crayfish is considerably longer than that of either tungsten (T_b = 12.2 days) or cobalt (T_b = 37-70 days). As with Tc, tungsten and cobalt are not known to play a role in the metabolism of the crayfish; however, both of these nuclides were concentrated to a higher degree than Tc.

The fairly long T_b for Tc in the crayfish (141 days) is not atypical for the marine crustaceans studied thus far. Pentreath (1981) reported T_b values of 211 days and 339 days for the lobster H. gammarus, and Fowler et al. (1981) reported a T_b of 300 days for the marine isopod Anilocra mediteranea. Of the few freshwater organisms studied prior to the crayfish, the longest T_b reported was for the mussel Margaritifera margaritifera (87 days). Thus the 141-day T_b for the crayfish represents the longest T_b for Tc in freshwater organisms reported to date.

The Snail Juga silicula

Uptake

The whole-body uptake graph (Fig. 6) suggests that the equilibrium whole-body activity concentration was being approached. As indicated for the crayfish, it is possible to calculate the equilibrium CR value from such a set of uptake data (Whicker and Schultz, 1982). However, they indicate that if the data show substantial scatter such a calculation is not feasible. Because of

the wide scatter in the snail data no such calculation was attempted.

Technetium uptake has been studied in only two other freshwater molluscs, the bivalve M. margaritifera, and the gastropod Helisoma sp. A final whole-body CF of 0.9 was reported for M. margaritifera (Hevland, 1981), and a final CF of 121 was predicted for the snail Helisoma (Blaylock et al., 1982). Helisoma is of course the most closely related species to J. silicula; however, the differences in experimental design between the acute dose, outdoor pond study of Blaylock et al. and the current chronic laboratory study make it difficult to compare results of the two studies. As discussed in the Introduction, the differences in experimental technique involved sampling methods, mode of isotope input, determination of the final concentrations factor, and potential mode of Tc uptake. It is simply noted here that the whole-body CF of 121 calculated for Helisoma is a factor of 3 larger than the measured whole-body CF of 41 for J. silicula.

The experimental designs of the uptake studies for the freshwater mussel and the snail were similar, and Tc accumulation by the two differed considerably. In 63 days of chronic exposure, the mussel reached a whole-body CF of only 0.9, significantly lower than the CR of 41 reached by the snail. Although the magnitude of the respective concentrating abilities differed, the general patterns of uptake observed were similar. Both initially accumulated Tc rapidly, followed by a slowing in the rate of uptake within ten days.

The red abalone Haliotis rufescens, a marine mollusc, apparently readily concentrates Tc from water; this gastropod had reported CR values of 100-205 (Spies, 1975; Beasley, Lorz, and Gonor, 1982).

Given the relatively high concentration factors for these three gastropods (J. silicula, Helisoma, and H. rufescens), it might be generalized that gastropods, as a group, tend to concentrate Tc to relatively high levels.

If it is possible to generalize about the gastropods, it might also be possible to generalize about the low Tc uptake exhibited by the other taxonomic group of molluscs studied, the bivalves. In addition to the low CF reported for the freshwater mussel, several marine bivalves studied had reported CF values of 1.3 - 1.5 (Fowler et al., 1981; Beasley, Gonor, and Lorz, 1982). Of the bivalves studied thus far, the highest CR or CF reported is 1.5.

The ability to concentrate Tc may be dependent upon one or more features characteristic of gastropods and not found in bivalves. An obvious difference between the two groups is their feeding methods. Bivalves are generally filter feeders, whereas most gastropods possess a radula and are detritus feeders. However, of the molluscs studied thus far, only the snail Helisoma was purposely exposed to a potentially contaminated food source. In addition, Fowler et al (1981), reported a CF of 1.7 for Mytilus galloprovincialis when uptake was potentially from both food and water. This seems to eliminate feeding characteristics as a sole reason for the differing concentrating abilities of the two groups. Although the two groups of molluscs exhibit distinctly different concentrating abilities, the small number of species studied, as well as the many physical, biological, and experimental factors that can affect uptake of nuclides, make it difficult to support a broad generalization at this point.

Tissue Distribution

The soft tissues contained the majority of the accumulated Tc in the snail (>80%) throughout both the uptake and elimination phases. The relative proportions of whole body activity in the soft tissues and the shell remained relatively constant during both phases, indicating little metabolic redistribution of Tc.

The failure of the shell to accumulate Tc is not suprising. Hevland (1981), found that the shell of the freshwater mussel reached a final CF of only 0.4 (identical to that reached by the shell of J. silicula). Other investigators have found that the shells of marine molluscs do not concentrate Tc appreciably, either (Beasley, Gonor, and Lorz, 1982; Fowler et al., 1981). Although mollusc shells do not generally concentrate Tc to any great extent, because of their mass, they do normally contain a significant fraction of the accumulated body burden of Tc. The range of values for per cent of body burden held by the shells of molluscs is approximately 30%-50% (Beasley, Gonor, and Lorz, 1982; Hevland, 1981; Spies, 1975; Fowler et al., 1981). J. silicula differed from this general pattern, as the shell never contained more than 18% of the body burden of Tc and generally containined only 5%-15%.

The low degree of uptake by the shell of J. silicula could be due either to adsorption on the surface of the shell, or to active deposition within the shell matrix. Prior to counting, the shells were wiped clean and rinsed in clean water, but they were not scrubbed. More vigorous cleaning may have removed adsorbed Tc from the shell surface. However, Hevland (1981), scrubbed the shells of M.

margaritifera, but found no appreciable difference between assays before and after scrubbing.

Beasley, Gonor, and Lorz (1982), found that dead shells of Mytilus californianus concentrated Tc to the same degree as the shells of living organisms. They also reported that coating either the inner or outer surface of the shells made no difference in either the rate of Tc uptake or the final concentration factor reached.

The low degree of uptake by the shell of J. silicula, together with consideration of the findings of others working with Tc uptake in mussels, tends to suggest that accumulation of Tc by the mollusc shell is a passive physical process rather than an active metabolic one.

Detailed tissue distribution assays were not undertaken in the snail. This was due primarily to the small size of the snail, which made accurate dissections difficult. Furthermore, the distribution of Tc among the soft body parts was not considered important from a radioecological viewpoint, as any foodchain predation on the snail would likely involve consumption of the entire soft tissue portion. Since the majority of Tc accumulated by the snail was found in the soft tissues, food chain transfer of Tc via contaminated snails would be likely.

Retention

The biphasic Tc retention curve of J. silicula showed a rapid loss of a small fraction of the body burden (10%), followed by a slow loss of the remaining large fraction. The long-lived compartment contained approximately 90% of the initial accumulated activity and had a T_b of 120 days.

Blaylock et al. (1982) reported a biological half life for the freshwater snail Helisoma of 39 days. However, as discussed earlier, the design of the Blaylock study differed significantly from this one. In addition to being exposed to potentially contaminated food, Helisoma was put into an elimination phase after only six days of uptake; thus the elimination pattern found may not be comparable to the one found in J. silicula.

The elimination of Tc was also studied in the freshwater mussel, M. margaritifera (Hevland, 1981). Although the biphasic retention curve of the mussels was qualitatively similar to the one for J. silicula, the short-term component of the mussel curve contained a larger fraction of the initial body burden than did the snails' (35% for the mussel vs. 10% for the snails). Thus, the mussels initially and rapidly lost a much larger fraction of their accumulated Tc than the snails did. This may be due to the respective fractions of the body burden held by the shell of each species, as the fractions initially lost are approximately the same as the fractions attributed to the shells of each species during tissue distribution studies.

The T_b of the long lived component of the snail was 120 days, compared to 87 days for the freshwater mussel. The range of experimentally-determined T_b values for Tc in freshwater and marine molluscs is 87 days to 143 days. Thus the 120-day T_b for J. silicula falls midway in this range. Obviously the T_b for the snail is not unusual, but the fraction of the body burden contained in the long-lived component is much higher than that found in any previous studies. The long-lived component of the freshwater mussel M. margaritifera contained 65% of the initial body burden, which is

the highest fraction found for all previous studies reviewed, next to the 90% found for J. silicula. This also can probably be attributed to the relatively low uptake by the shell of J. silicula. The long-term retention of such a significant fraction of accumulated Tc may be an important factor when foodchain transfer is considered. Given the relatively large uptake of Tc by the snail, further studies in this area appear to be warranted.

CONCLUSIONS

The CR values obtained for the crayfish and the snail exemplify the wide range of Tc accumulation values that have been reported for other species. Rather than facilitating any broad generalizations regarding Tc uptake by freshwater organisms, the results obtained here confirm the previously apparent variability. The range of Tc CR values illustrates the probable reason why the NCRP (1984) considers the bioaccumulation factor (or the equivalent CR), to be the largest contributor to uncertainties in aquatic food chain models used to assess the impact of radiological releases.

Uptake of Tc by crayfish is among the lowest reported for crustaceans, whether freshwater or marine. The crayfish CR of 1.6 is also among the lowest reported for aquatic organisms in general. The CR of 41 for the snail is approximately 25 times that of the crayfish, under virtually identical experimental conditions. However, this value is not unusual for the few reported values for gastropods. The source of the variability in individual snail uptake is unknown. Further investigation might result in elucidation of CR inconsistency in this species, as well as CR variability in general.

The mode of uptake by each species is also unclear, and was not investigated in these studies. The low overall uptake by the crayfish, and rapid uptake and elimination of Tc by the crayfish exoskeleton suggests passive uptake in the crayfish. Additionally, there was little or no evidence of redistribution of accumulated Tc in either species. Regardless of the mode of uptake or the degree of

incorporation into tissue, both the crayfish and the snail exhibited rather slow elimination, with biological half lives greater than 100 days.

The chronic exposure used in these studies was intended to mimic an aquatic environment where an equilibrium concentration of Tc existed. This would be the situation if there was a continuous (chronic) low-level release from a nuclear facility. Direct consumption of contaminated crayfish would result in direct ingestion of Tc; however the accumulation of Tc by the crayfish, especially the edible muscle tissue, was very low. Snails are not consumed directly by humans, so the evaluation of potential hazards is more complex. The question of foodchain transfer (e.g., snail --- fish --- human) needs to be investigated with both experimental species before reaching any conclusions. Blaylock et al. (1982) found some evidence that fish accumulated Tc through the foodchain. However, they also suspected that at least 40% of the Tc body burden in these fish was located in the G.I. tract, and thus would not be passed on to humans. These observations indicate that potential doses to humans resulting from Tc releases to the aquatic environment and involving the crayfish and the snail would be low.

Concentration ratios currently used in assessing the release of Tc to aquatic environments are generic values, based on uptake of iodine (Blaylock et al., 1982). A CR of 5 is used for freshwater invertebrates in Regulatory Guide 1.109 (USNRC, 1977). For crayfish, a CR of 5 is reasonable to use in assessments without being overly conservative. A generic CR of 5 used for freshwater snails, however, significantly underestimates the mean CR of 41 reached by J. silicula.

This illustrates the difficulty in using generic CR values. It should be remembered that these CR values were obtained in the laboratory, under conditions where the mode of uptake was strictly limited to water. Actual environmental CR values may be different, due to potential uptake from contaminated food.

The use of generic CR values is increasingly difficult to justify, as the results of more studies are being reported. With the potential for exposure to Tc from nuclear facilities, additional studies on Tc accumulation by freshwater species should be undertaken so that more accurate assessments of the impact of Tc releases may be made.

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APPENDIX

APPENDIX TABLE I. Uptake of ^{95m}Tc from water as pertechnetate by *Pacifastacus leniusculus* at 15°C. Whole body activity values and CR values are ± 1 S.D. (n = 8). Whole-body activity values are plotted against day of uptake in Figures 1 and 2.

Day of Uptake	Whole-Body Activity (dpm/g)	Water Activity Concentration (dpm/ml)	Concentration Ratio (CR)
0.5	70 ± 12	831	.08 ± .01
1	90 ± 12	865	.10 ± .01
3	110 ± 16	810	.14 ± .02
10	220 ± 31	828	.27 ± .04
16	320 ± 42	818	.39 ± .05
25	480 ± 63	778	.62 ± .08
42	670 ± 130	883	.80 ± .14
64	1200 ± 210	781	1.6 ± .3

APPENDIX TABLE II. Tissue activity concentrations of ^{95m}Tc in Pacifastacus leniusculus during uptake and retention phases. Values are mean dpm/mg wet weight ± 1 S.D.

Day	n	Whole-Body	Digestive Gland	Green Gland	Stomach	Exoskeleton	Blood	Muscle
Uptake								
4	5	.14 ± .03	.5 ± .2	.4 ± .1	.13 ± .04	.13 ± .04	.21 ± .01	.032 ± .008
16	4	.35 ± .06	2.4 ± .5	1.4 ± .6	.6 ± .3	.3 ± .1	--	.08 ± .02
52	5	1.3 ± .3	14 ± 4.4	10 ± 8	1.1 ± .3	.8 ± .2	.4 ± .1	.15 ± .08
Retention								
4	5	1.0 ± .4	18 ± 8.2	4 ± 1.4	1.1 ± .5	.43 ± .06	.3 ± .2	.13 ± .08
13	4	.6 ± .2	8.5 ± 2.4	4.5 ± 2.5	1.1 ± .2	.15 ± .06	.08 ± .06	.04 ± .02
29	3	.49 ± .08	7 ± 1.7	3.2 ± .3	.7 ± .2	.12 ± .04	.05 ± .01	.023 ± .004
52	5	.44 ± .08	5.8 ± 1.2	1.9 ± .8	.6 ± .2	.10 ± .01	.025 ± .004	.014 ± .004

APPENDIX TABLE III. Retention of ^{95m}Tc by Pacifastacus leniusculus at 15°C. Values are the mean percent of initial whole-body activity present at the time of assay, corrected for decay, ± 1 S.D. (n = 5). These values are plotted in Figure 5.

Day of Elimination	Initial Activity Remaining (%)
1	93 ± 2
3	88 ± 2
6	84 ± 2
13	78 ± 3
21	74 ± 4
28	71 ± 4
36	69 ± 4
43	68 ± 5
52	59 ± 8

APPENDIX TABLE IV. Uptake of ^{95m}Tc from water as pertechnetate by *Juga silicula* at 8.5°C. Whole-body activity values and CR values are ± 1 S.D. (n = 15). Whole-body activity values are plotted against day of uptake in Figure 6.

Day of Uptake	Whole-Body Activity (dpm/mg)	Water Activity Concentration (dpm/ml)	Concentration Ratio (CR)
1	2.4 ± 1.1	(a)	(a)
3	5.1 ± 3.2	814	6 ± 4
5	6.8 ± 4.6	780	9 ± 6
7	9.2 ± 6.2	720	13 ± 9
10	11.4 ± 7.8	702	16 ± 11
14	14.0 ± 10.0	818	17 ± 12
18	19.0 ± 13.5	775	24 ± 17
25	24.0 ± 17.8	876	27 ± 20
32	27.0 ± 21.3	791	34 ± 27
41	29.0 ± 23.3	698	42 ± 33
51	31.0 ± 25.4	772	41 ± 33

(a) A water sample was not taken on this day.

APPENDIX TABLE V. Tissue activity concentrations of ^{95m}Tc in *Juga silicula* during uptake and retention phases. Values are mean dpm/mg wet weight ± 1 S.D.

Day	n	Whole-Body	Shell	Soft Tissue
Uptake				
2	6	4.6 ± 1.9	.13 ± .06	10.3 ± 5.2
9	6	7.4 ± 2.4	.11 ± .04	19.2 ± 6.1
23	6	20.5 ± 11.0	.15 ± .04	47.3 ± 17.6
48	6	34.0 ± 25.5	.35 ± .09	80.0 ± 47.4
Retention				
1	6	39.1 ± 26.9	.18 ± .05	99.1 ± 61.0
5	5	19.1 ± 12.2	.14 ± .01	42.0 ± 15.4
16	5	28.0 ± 24.0	.22 ± .14	57.4 ± 32.7
49	15	14.0 ± 14.0	.1 ± .1(a)	35.2 ± 26.3

(a) Five of the fifteen shells were less than background, and thus the net dpm/mg for these five was zero.

APPENDIX TABLE VI. Retention of ^{95m}Tc by *Juga silicula* at 8.5°C. Values are the mean percent of initial whole-body activity present at the time of assay, corrected for decay, ± 1 S.D. (n = 15). These values are plotted in Figure 9.

Day of Elimination	Activity Remaining (%)
0.5	95 ± 4
1	94 ± 4
2	92 ± 4
3	91 ± 5
5	90 ± 6
9	86 ± 6
13	85 ± 7
20	81 ± 9
27	76 ± 10
35	73 ± 11
42	70 ± 12
49	69 ± 12