BIOGEOCHEMICAL STUDIES OF TECHNETIUM IN
MARINE AND ESTUARINE ECOSYSTEMS

MASTER

PROGRESS REPORT

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INTRODUCTION

This report summarizes progress from June 1979 through March 1980 on studies dealing with the biogeochemical behavior of technetium in marine and estuarine ecosystems. Our goal for the first year of this contract was to elaborate the biokinetic behavior of Tc as TcO_4^- in selected marine and estuarine organisms and to determine the affinity of TcO_4^- for different marine sediments under oxygenated conditions. Our first year's results have already led to some conclusions of importance to the Department of Energy. These are as follows:

- 1) Concentration factors for TcO_4^- in bivalve molluscs (oysters and mussels) do not exceed 2 when calculated for whole animals and when uptake is directly from water. The highest concentration factor observed for individual tissues of these organisms is visceral mass (oysters and mussels), byssus (mussels) and gill (oysters and mussels). Of these tissues, visceral mass and byssus show concentration factors at equilibrium of less than 10.
- 2) Direct uptake from water by limpets (archeogastropod) are very much lower than have been reported for red abalone (archeogastropod). Both organisms occupy habitats of the rocky intertidal zone and feed on macroalgae. Concentration factors for the limpet over water do not exceed 10 at equilibrium, while abalone concentration factors over water have been reported to range from 135-205.

- 3) Whole body concentration factors for TcO_4^- in the plaice, Pleuronectes platessa, where uptake is directly from labeled seawater, do not exceed 10 at equilibrium.
- 4) Both the lobster, Homarus gammaris and the polychaete, Nereis diversicolor appear to concentrate Tc efficiently from water labelled initially with TcO_4^- . In the case of lobster, whole body concentration factors may be as high as 500 at equilibrium; similar values are observed for Nereis.
- 5) Both plaice and rays (Raja clavata) fed $^{95\text{m}}\text{Tc}$ labeled Nereis show an initial rapid loss of the isotope for approximately five days. Thereafter, loss is much reduced. Both retention curves appear to be biphasic. In the case of plaice, the long lived component of the loss curve suggests a biological elimination half life of near 30 days. Retention in the ray appears to be approximately twice as long, with a biological elimination halftime of the long component approaching 53 days.
- 6) Shrimp (Palaemon elegans), (Crangon sp.) and Crab (Cancer pagurus) show concentration factors similar to plaice (C. F. is less than 10). Isopods, however, have concentration factors of only 3 following four weeks exposure to labeled seawater.
- 7) Uptake of TcO_4^- by phytoplankton is extremely low, which precludes experiments in which TcO_4^- labeled phytoplankton can be fed to either bivalve molluscs or microzooplankton.
- 8) Sediment distribution coefficients for TcO_4^- are essentially zero and are independent of sediment type in well oxygenated seawater.

- 9) Our experiments to date have shown that it is not possible to make generalizations concerning the bioavailability of TcO_4^- to marine organisms. While Tc appears to be concentrated in certain species of the macroalgae, Fucus, accumulation in phytoplankton essentially does not occur. Of the crustacea so far examined (shrimp, crab, isopods and lobster), only lobster shows an important biomagnification of TcO_4^- . Of the molluscs investigated (mussels, oysters, limpets), none show concentration factors in excess of 5; abalone apparently are the exception. Polychaetes concentrate TcO_4^- from water about as efficiently as they do plutonium. This suggests that the site of deposition is most likely the surficial mucus.

We will address each of these topics in turn throughout this report, but wish first to detail the arrangements which have been made to collaborate in this research with both the International Laboratory of Marine Radioactivity in Monaco and the Fisheries Radiobiological Laboratory at Lowestoft.

COLLABORATION

There was an initial delay between submitting our proposed research on technetium to the Department of Energy and its acceptance for funding of over two years. During that time, our impatience to proceed with the

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- 1) Wildung, R. E., T. R. Garland and D. A. Cataldo (1977). Accumulation of Technetium by Plants. *Hlth. Phys.* 32:314-317.
 - 2) Till, J. E., F. O. Hoffman and D. E. Dunning, Jr. (1979). A new Look at Tc-99 Releases to the Atmosphere. *Hlth. Phys.* 36:21-30.

research was heightened by periodic reports about the uptake of Tc (as TcO_4^-) by certain plants (1) and a continued awareness about the lack of environmental data concerning Tc behavior on which to make reasonable radiological risk assessments (2). During this interim period we had considered ways of accelerating the work without sacrificing the quality of the research in the continued belief that those having the responsibility for assessing the radiological consequences of ocean releases would soon be required to present information on the behavior of Tc. As expansion of activities at the Marine Science Center were precluded because of funds available for the project, it was clear that collaboration with other laboratories involved with similar research was the logical solution.

We have been fortunate to establish such a collaborative effort between ourselves and Dr. S. W. Fowler of the IAEA Laboratory in Monaco and Dr. Jan Pentreath of the Fisheries Radiobiological Laboratory at Lowestoft, U. K. We have entered into a coordinated research program with the IAEA on the topic of the biogeochemical behavior of Tc in the oceans and would hope that over the next two years that other selected laboratories will contribute research results as well. This has already begun in a minor way with the participation of the CNEN-EURATOM laboratory in Fiascherino, Italy where Dr. E. H. Schulte has begun studies on the uptake, tissue distribution and loss of TcO_4^- in shrimp.

The advantages which result from this type of collaboration are principally two fold. First the rate of data acquisition is accelerated and unusual findings can be explored more expeditiously. There is a redundancy in choices of certain experimental animals which is in fact

to be encouraged. When slightly different experimental protocols and laboratory and field conditions produce ostensibly the same result in the uptake and loss behavior of Tc in the organisms under investigation, an added measure of confidence can be placed on the interpretation of the data. Secondly, corroboration of data on an international basis is more readily accepted by the international community of scientists concerned with the disposal of radioactivity to the oceans.

For these reasons, we feel that our decision to actively engage in this cooperative approach is in the Department of Energy's best interests and will bring the highest possible return on its invested research funds. We have chosen to report each laboratory's results separately but to highlight both those areas where agreement has been found using organisms of the same species as well as points of disparity. Each laboratory will publish their results separately, but will acknowledge the extent of DOE's support. In the case of Monaco and Lowestoft, this represents the donation of an aliquot of our specially prepared ^{95m}Tc . All other costs of the experimentation have been borne by the individual laboratories.

ISOTOPE PRODUCTION

The ^{95m}Tc we have used for all of the experiments described in this report was produced by New England Nuclear of Boston, Mass. We experienced an unfortunate delay in the anticipated production date owing to difficulties within the processing facilities at NEN. The isotope was not ready for shipment before early December, 1979. Shown in Figure 1 is a spectrum of the ^{95m}Tc as measured in a NaI (Tl) well crystal as well as the decay rate in an aliquot of the stock solution measured over a three

Figure 1. Gamma-ray spectrum and decay curve for ^{95}Tc .

month period. The radiochemical purity of the NEN isotope is excellent and we have confirmed that the chemical form of the Tc is entirely as TcO_4^- . Certain of our experiments to be described later have used the simple diagnostic test to confirm the presence of TcO_4^- versus reduced forms of Tc (TcO_2 or TcCl_6^-) which consists of carrying reduced forms on $\text{Fe}(\text{OH})_3$ in basic solution. We have chemically reduced the TcO_4^- to lower valence states using hydroxylamine hydrochloride in dilute acid and found that it is carried equally well on $\text{Fe}(\text{OH})_3$ or $\text{Mg}(\text{OH})_2$. The latter precipitate has been used to isolate reduced Tc from solution which is subsequently redissolved in a minimum of HCl for re-introduction into seawater for measuring the rate at which reduced forms of Tc are oxidized to TcO_4^- in well oxygenated seawater.

As we stated in our original proposal, the bulk of our first year's results deal with the behavior of Tc in its highest valence state, TcO_4^- . The Monaco Laboratory has done experiments in which direct water uptake of reduced forms of Tc were examined in Nereis, as well as an experiment with shrimp in which both +4 and +7 valence states of Tc were administered to brine shrimp and subsequently fed to large shrimp. At MSC, we have evaluated the distribution coefficients of reduced Tc on marine sediments. One of us (TMB) continues to feel that TcO_4^- may be the most prevalent and most thermodynamically stable state of Tc in seawater as derived from its various sources. The ease with which the lower valence states of Tc are oxidized to TcO_4^- by such oxidizing agents as H_2O_2 , HNO_3 and HNO_2 would argue that Tc discharged in fuel reprocessing wastes would be present as TcO_4^- unless a specific holding reductant were

present in the waste liquors ($\text{NH}_2\text{OH}\cdot\text{HCl}$, for example). Dissolved oxygen itself in seawater appears quite capable of oxidizing reduced Tc to TcO_4^- over time as well. It is reasonable then to place the major emphasis on experiments where Tc is present in this higher valence state.

PRELIMINARY EXPERIMENTS

Prior to the commencement of the animal labeling experiments, it was necessary for each laboratory to determine whether or not wall loss of TcO_4^- in the experimental aquaria to be used would be a significant factor in the experimental design. Quite obviously this has a direct bearing on the frequency with which water must be changed and in the logistics of maintaining a constant activity level in the aquaria. No laboratory experienced wall loss of TcO_4^- over time periods equal to those chosen for periodic water changes. In the case of the Marine Science Center (MSC), we have chosen to routinely replace labeling solutions every six days to prevent the accumulation of unwanted metabolic by-products voided by the experimental animals. The volumes used for holding our specimens has been 30 liters. Lowestoft changes water at essentially the same frequency (7 days) and uses large volumes of solution (90 liters) owing to the size of the experimental animals used. Monaco has used smaller labeling volumes in their experiments and employed more frequent water changes but did, in fact, confirm that wall losses to both glass and plastic were insignificant. Prior to the sediment distribution experiments, wall loss of TcO_4^- to glass from seawater was followed for a full month at MSC. No reduction of activity in the seawater solution was observed other than simple radioactive decay. Periodically, aliquots of seawater were filtered through two 0.1 μ millipore filters to determine whether or not particle formation was

occurring over time. No activity difference was observed between top or bottom filters over the course of the experiment. Periodic samples were also removed to test for valence changes over time. No activity was carried from the seawater solutions on $\text{Fe}(\text{OH})_3$ from basic solutions confirming that the Tc remained as TcO_4^- over the observation time.

The results of these tests confirm that the $^{95\text{m}}\text{Tc}$ used in our experiments remains as TcO_4^- in seawater and that the concentration factor data reported here relate to the direct uptake of this species as a soluble entity.

$^{95\text{m}}\text{Tc}$ uptake by Bivalve Molluscs

Both MSC and Monaco have determined the uptake, tissue distribution and subsequent loss of TcO_4^- from water by mussels and MSC has, in addition, investigated oysters. Monaco has used *Mytilus galloprovincialis* in its experiments while MSC has employed *Mytilus californianus* and the oyster *Crassostrea gigas*. Shown in Figures 2 and 3 are the uptake curves which have been observed in these comparative experiments. There is a remarkable similarity in the equilibrium values obtained for the $^{95\text{m}}\text{Tc}$ concentration factor, even though differences did exist in the experimental protocol employed. At MSC, we had determined early on that uptake of $^{95\text{m}}\text{Tc}$ as TcO_4^- by phytoplankton was most likely an inefficient process. We had intended to do a food and water uptake experiment with both mussels and oysters and had performed an uptake experiment with the naked flagellate *Pseudoisochrysis paradoxa* which is cultured at MSC for other molluscan research projects. Following a two day exposure period, several milligrams of the phytoplankton were isolated by filtration onto glass fiber filters (0.7μ) by suction at 0.1 atm. We discerned no

Figure 2. Pertechetate uptake by *Mytilus galloprovincialis*.

Figure 3. Pertchnetate uptake by *Mytilus californianus* and *Crassostrea gigas*.

difference in the activity retained by the filter containing the phytoplankton and that used to back this filter. Repeating the experiment produced identical results. We therefore decided to periodically feed both oysters and mussels unlabeled phytoplankton at three day intervals to try and maintain the animals in a healthy state. They were removed for periods of one hour each third day and were placed in unlabelled, 0.45 μ millipore filtered seawater containing Pseudoisochrysis cells. Generally, the mussels cleared some 75-90% of the cells within that period of time. The oysters were more susceptible to handling and remained closed for longer periods of time, thereby clearing less of the cell suspension. Following feeding, the animals were re-introduced into the experimental containers for a further three days, at which time the water was changed. The Monaco Laboratory has also observed a lack of uptake of TcO_4^- by a mixed phytoplankton culture and has observed no additional uptake by M. galloprovincialis whether uptake was from seawater alone or from a food-water matrix.

Prior to counting, the animals at MSC were allowed to depurate for either 30 minutes (mussels) or 60 minutes (oysters) in order to clear any labelling solution from the animals that would be entrained at shell closing. In addition, the MSC experiments contained cleaned shells of both mussel and oyster in order to determine the direct shell uptake and loss kinetics of TcO_4^- .

The Monaco Laboratory employed a feeding schedule which was slightly longer than those at MSC (5 day intervals), and fed for slightly longer periods of time (1 hour vs. 0.5 hr). The important point to be noted is that the average concentration factors for mussels and oysters from both

experiments never exceeded two. These results are in sharp contrast to the results of Spies' (3) experiments with red abalone as mentioned earlier. This should not be interpreted to mean that we think Spies' work is questionable. As we will describe shortly, individual species of crustacea show markedly different abilities to concentrate TcO_4^- which are quite puzzling. Individual tissue concentration factors for both mussels and oysters have been determined. The results are presented in Tables 1 and 2. There are several points to be emphasized when comparing these data. First, the length of the uptake experiments differ significantly at the time of sacrifice: The Monaco data relate to a 28 day uptake period while the MSC animals were in label for nearly 60 days. Secondly, the sizes of the mussels used at MSC were significantly larger than those at Monaco. Finally, the mussels at MSC were collected with byssus intact and new byssus growth was minimal. MSC also did a coarse dissection into soft parts and shell before further partitioning the tissues. Five animals were dissected at MSC in the coarse dissection; individual tissues thereafter were pooled and the concentration factors reported represent the mean value for those five animals. There are clearly some differences in the individual tissue values for the two mussel data sets, principally for byssus and visceral mass. All other tissues appear reasonably consistent. While the differences in byssus may be due to new thread formation by M. galloprovincialis following introduction into the labeling medium, the differences between the C. F.s observed in visceral mass remains largely unexplained.

Both MSC and Monaco have followed the uptake of $^{95\text{m}}\text{TcO}_4^-$ by mussel tissues over time. In both cases, the combined soft tissue uptake rate

3) Spies, R. B. (1975). Uptake of Technetium from Seawater by the Red Abalone, Haliotis rufescens. Hlth. Phys. 29:695-699.

Table 1. Concentration factors for tissues in the mussel
M. galloprovincialis at equilibrium

Tissues	Uptake from solution	Uptake from solution and food
	Day 29 for uptake	Day 28 for uptake
Byssus	23.55 (22.17-24.93)	7.89 (4.22-11.57)
Visceral mass	17.06 (15.53-18.59)	17.50 (14.78-20.23)
Gills	2.20 (2.13-2.27)	1.95 (1.91-2.00)
Mantle	1.89 (1.77-2.01)	1.72 (1.64-1.81)
Muscles	1.80 (1.75-1.85)	1.39 (1.27-1.50)
Shell	1.20 (1.10-1.31)	2.26 (1.64-2.88)

The values are the mean of two animals. The numbers in parentheses indicate the range.

Table 2. Concentration factors for tissues in the mussel
M. Californianus and the oyster, C. gigas at equilibrium

Exposure time: 59 days

<u>Mussels</u>		
Tissue	Concentration Factors	Range
Soft parts plus byssus	3.9 ± 0.4	3.4 - 4.4
Shell	1.2 ± 0.2	0.95 - 1.42
Visceral Mass	7.8	
Gills	2.9	
Byssus	7.0	
Gonads	2.8	
Mantle	1.8	
Foot	1.8	
Adductor	1.1	

exceeds those measured for shell in live animals. Interestingly, when only cleaned shells are introduced into TcO_4^- labelled seawater, uptake occurs quite readily. Figure 4 shows results for both oyster and mussel shell. The oyster shell used was from an experimental animal that died at day 19 during uptake. It is clear that at that time, the shell had reached near equilibrium values with the water. By contrast, mussel shell showed a general increase in uptake over the entire 50-60 day period in labeled solution. The equilibrium value reached by cleaned shells was essentially identical to that attained by shells of live animals and suggests passive uptake by the shell matrix. The 'dead' shells were thoroughly cleaned to remove epiphyton and debris prior to the experiments and we believe them to be free of organisms likely to concentrate TcO_4^- .

To date, only Monaco has collected data on the loss of $^{95\text{m}}\text{Tc}$ from mussels under laboratory and field conditions. Unfortunately, the animals in the field experiment were lost near day 10 so that long term loss data from that experiment will not be available. Through day 10 however, loss appeared faster for those animals held in the field than for those held in the laboratory. In both cases, the animals were constantly submerged. Inspection of Figure 5 shows early rapid losses of as much as 40% of the original label in as little as one day. The longer component of loss in the laboratory-held mussels suggest biological half lives greater than 100 days. We think it likely that this slow loss is due to removal of $^{95\text{m}}\text{Tc}$ from shell rather than soft tissue, however. Both oysters and mussels are presently in loss at MSC. Seven oysters are in flowing seawater in the laboratory and seven are deployed

Figure 4. Pertechetate uptake by oyster and mussel shells.

Figure 5. Tc-95m loss from Mytilus galloprovincialis.

in Yaquina Bay. We have placed five mussels in three loss modes: laboratory, Yaquina Bay and Yaquina Head (a rocky promontory). As with the Monaco experiment, the first two groups of animals are under constant submergence until counted. The Yaquina Head mussels are subject to tidal cycles and can remain out of water for as much as 40% of the time depending upon the time of year. As this is the natural habitat for these animals, it will be of interest to see if loss rates are affected by the animals by being out of water during tidal cycles. Our loss experiments have only been in progress for 10 days, but we have not observed the initial rapid loss of label to the extent seen in the Monaco experiments. Day 1 losses in all animals except those at Yaquina Head amount to less than 20%; those at Yaquina Head had lost about 10% in one day.

Uptake of $^{95m}\text{TcO}_4^-$ by Fishes

Only Lowestoft has conducted water uptake and labeled food experiments with fishes. Dr. Pentreath has utilized the plaice, Pleuronectes platessa, and the thornback ray, Raja clavata, in these experiments. To date, only uptake from water and loss rate data following labelled food intake are available for discussion. Dissection for tissue distribution and turnover rate experiments from the water uptake runs are as yet in progress. Shown in Figure 6 is the accumulation of TcO_4^- from water by the plaice. The plotted data represent the average of four individual fish. Concentration factors even at 55 days exposure are less than 7 and equilibrium seems to be very nearly reached at this time.

Unfortunately, uptake data for TcO_4^- in rays was not obtainable in the first series of experiments. The animals available were small and the repeated handling during counting and water changing caused undue

Figure 6. Pertech-netate uptake from seawater by Pleuronectes platessa.

stress in the animals. It was possible to perform a shorter term experiment on the loss of ^{95m}Tc from the ray following feeding of labeled Nereis. Figures 7 and 8 show the loss curves observed and represent mean values for 6 individual fish in each case. There are clearly differences in the percent retention of the ^{95m}Tc between the two species over time as well as the percent of the original activity absorbed at feeding. Gut clearance in the plaice is very rapid and is estimated to be near 70% of the original label after one day. The percent of the original label absorbed falls between 7-8%. The less extensive data for the ray shows a slower turnover of the original label (by a factor of about 2-2.5) and an initial absorption of perhaps 15% of the ^{95m}Tc from the labeled food.

The long term component of the loss curve for plaice suggests a biological half life of approximately 30 days; the long term component of the loss curve for rays is as yet too scanty for adequate description. These preliminary findings are not unlike those observed for other radionuclide uptake studies using these two species (4). The elasmobranch fish (rays) have been shown to accumulate ^{237}Pu across the gut wall from labeled food while teleosts (plaice) seem less able to do so (5). It will be important to determine where in the fish the Tc has concentrated, both from the feeding experiments and the direct uptake from water experiments.

4) Pentreath, R. J. (1977). Radionuclides in Marine Fish. *Oceanogr. Mar. Biol. Rev.* 15:365-460.

5) Pentreath, R. J. (1978). ^{237}Pu Experiments with the Thornback Ray, Raja clavata. *Mar. Biol.* 48:337-342.

Figure 7. Pertechetate loss from *Pleuronectes platessa* following single feeding.

Figure 8. Pertechetate loss from Raja clavata following single feeding.

Uptake of $^{95m}\text{TcO}_4^-$ by Polychaetes

Both Monaco and Lowestoft have obtained data on the direct uptake of TcO_4^- by the polychaete Nereis diversicolor from labelled seawater. In addition to the higher valence state uptake experiments, Monaco performed an uptake with Nereis in which the TcO_4^- was reduced to a lower valence state (+4) by addition of hydrazine sulfate. As yet, the actual form of the reduced state has not been adequately identified. Figures 9 and 10 show the uptake curves for both experiments. It is interesting to note that at 14 days, the C. F.s obtained at Lowestoft are slightly lower than those found at Monaco (250 vs about 350) for TcO_4^- . Individual variation is large, however, and the two sets of data may well overlap. The labeling solution used at Lowestoft contained approximately 1μ Ci/liter, while the Monaco experiment was run at 2μ Ci/liter. For the reduced state of Tc in the Monaco experiment, equilibrium appears to have been attained as early as 8 days, while the higher valence state experiment shows increasing C. F.s even at day 14. The loss rate measurements for all three of these experiments will be very interesting as will measurements to determine the extent to which the label is contained in the surficial mucus of the worms. It has been previously shown that ^{237}Pu shows concentration factors in Nereis diversicolor of about the same order of magnitude as observed for Tc, when uptake is directly from labeled seawater (6). Loss of the ^{237}Pu following labeling was slow ($T_B \text{ } 1/2 = 79$ days) and it will be very interesting to see whether or not Tc rate losses are equally as slow.

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- 6) Fowler, S. W., M. Heyraud and T. M. Beasley (1975). Experimental Studies on Plutonium Kinetics in Marine Biota. IN: Impacts of Nuclear Releases into the Aquatic Environment, IAEA, Vienna, 157-177.

Figure 9. Technetium uptake by Nereis diversicolor using different valence states.

Figure 10. Pertechetate uptake by Nereis diversicolor.

Uptake of $^{95m}\text{TcO}_4^-$ by Crustaceans

As with molluscs, we have observed marked differences in the ability of certain crustaceans to concentrate Tc when the initial chemical form is TcO_4^- . To date, four species of crustacea have been investigated. These include lobster, crab, shrimp and isopods. While crab, shrimp and isopods all show concentration factors at equilibrium of less than 10 over water, lobster have concentration factors greater than 500 after 45 days. Of the four animals studied to date, only lobster, shrimp and isopods have been kept in labeled seawater for time periods greater than two weeks. The crab experiments conducted at Lowestoft were short term and are intended to be repeated with the next batch of isotope. Nevertheless, short term exposures of less than one week indicated concentration factors substantially lower than those for the lobster at comparable times (C. F. at 3 days = 65). Shown in Figures 11 through 14 are the results for the uptake experiments for lobster, shrimp and isopods. The lobster experiment was performed at Lowestoft, the shrimp experiment at Fiascherino and the isopod experiment at Monaco. At this writing, dissection and loss experiments were underway at Lowestoft with lobster and loss experiments are underway with the isopods at Monaco.

The shrimp which have been used at C. N. E. N. at La Spezia are Palaemon elegans. The effect of temperature on the uptake by the shrimp is to increase the rate of uptake of TcO_4^- , yet the equilibrium concentration factor at both 10°C and 20°C are nearly equal. During the experiment, several animals were dissected into eye, exoskeleton, muscle, gill, viscera, hepatopancreas, stomach and heart. Of all the organs measured, only viscera showed easily measurable ^{95m}Tc activity. Further dissection

Figure 11. Pertechetate uptake by Homarus gammarus.

Figure 12. Pertechetate uptake by *Palaemon elegans* (10°C).

Figure 13. Pertechetate uptake by Palaemon elegans (20°C).

Figure 14. Pertechetate uptake by Anilocra mediterranea.

to isolate the hepatopancreas showed that this organ accounted for most of the activity measured in the visceral mass. At days 22-23, concentration factors for hepatopancreas in four experimental animals ranged from 122-184 with a mean value of 150 ± 24 . Interestingly, with five animals which died during experimentation, ^{95m}Tc was measurable in exoskeleton, muscle and gill if the animals were left for several hours in the labeled solution. The mechanism of the uptake has not been elucidated, but it is curious that uptake by these tissues in living shrimp is such an inefficient process. Even with fresh cast molts, concentration factors did not exceed 7.0.

The Monaco Laboratory has studied assimilation and loss of Tc both as Tc (+7) and as Tc (+4) in large shrimp by labeling adult brine shrimp (Artemia salina) in a water and mixed phytoplankton suspension which were then fed to the larger animals. Figure 15 shows the percent retention of the two forms over time after ingesting a single ration of labeled food, followed by cold food feeding ad libitum. The estimated assimilation for Tc (+7) has been placed at 25%; a comparable figure cannot be ascertained for the Tc (+4) experiment as the long-term component of the loss has yet to be established. One of us (TMB) speculates that assimilation for the +4 state will exceed that for Tc (+7) by perhaps a factor of 2. There is clearly a difference in the early rate loss data that argues for greater uptake of the +4 valence state, yet the slope of the percent retention curve between days 4-16 is slightly greater for the +4 valence state experiment than for the +7. Tissue dissections from this experiment will be performed once the long-lived component of the percent retention curve for both experimental groups are well established.

Figure 15. Loss of Tc by Lysmata seticaudata from single feeding of different valence states.

Uptake of $^{95m}\text{TcO}_4^-$ by Limpets *Notoacmea scutum*

Our general finding of low uptake of TcO_4^- by bivalve molluscs prompted us to investigate an organism from the Class Gastropoda to see whether or not macroalgae feeders in general have the ability to concentrate TcO_4^- as suggested by Spies' work on abalone. We chose the limpet, *Notoacmea scutum*, which is prevalent on the west coast of the United States. This organism feeds on macroalgae as does abalone and belongs to the same class of organisms. These animals are taken recreationally for food by Asian and Micronesian residents of the west coast. As with our other mollusc experiments, the limpets were enclosed in perforated containers for the labeling experiments and for ease in identification. Unlike our mussel and oyster experiments, the limpets were not fed during uptake in order to more closely parallel the abalone experiment of Spies.

While our experiment is still in progress, it is clear that concentration factors for limpets are at least a factor of 10-20 lower than those of abalone. Figure 16 shows our results to date. The initial uptake rate is faster than we observed for either mussels or oysters and at comparable times (15 days) the limpet concentration factor is about 5X that observed for the bivalve molluscs. The concentration factor is at least a factor of 20 lower than that observed for abalone at 15 days exposure. On day 13 of this experiment one limpet died which afforded the opportunity to dissect the animal to determine tissue distribution. As these animals are small (7-12g wet weight) only three tissues were measured, i.e., shell, viscera and muscle. Surprisingly, we found almost no activity in shell; virtually all of the activity was found in

Figure 16. Pertechetate uptake by Notoacmea scutum.

the viscera, with the remainder being in the muscle tissue. The respective concentration factors for shell, viscera and muscle were 0.07 ± 0.007 , 21 ± 0.2 and 1.9 ± 0.07 . Not only are the concentration factors for whole limpets much lower than those for abalone, but there are sharp differences in the sites of TcO_4^- deposition as well. Abalone shell concentrated significant amounts of TcO_4^- , although isotope did concentrate on organisms that colonized the abalone shell. Spies noted that shell was the third most active tissue of the abalone, yet curiously no concentration factors were reported as such.

We are quite certain that both oyster and mussel shell were not colonized by either boring polychaetes or other organisms as they were thoroughly cleaned and inspected before the start of our experiments. The differences we observe between the shells of various animals must be related more to the crystalline structure of the shell material rather than its chemical composition (calcite and aragonite: CaCO_3) but we prefer to defer discussion of this topic until we have had a chance to more clearly think about this problem. We have determined that the $^{95\text{m}}\text{Tc}$ in mussel shell is very tightly bound. Leaching for one hour in either 0.2N HCl or 0.2N HNO_3 removes less than 20% of the activity. Under these conditions, active evolution of CO_2 occurs and these losses most probably relate to the dissolution of outer layers of shell to which the Tc is bound.

Distribution Coefficients of $^{95\text{m}}\text{Tc}$ for Marine Sediments

We have performed two experiments to determine the distribution coefficients of two forms of $^{95\text{m}}\text{Tc}$ for marine sediments. The sediments were aliquots of cores raised on the cruise of the R/V Knorr in the

southern Pacific Ocean from different water depths. Table 3 lists the pertinent data for the sediments which were preserved at 10°C with seawater until used.

Ten grams of each sediment were suspended in one liter of 0.45 μ millipore filtered seawater containing 5 μ Ci of ^{95m}Tc . In the first case, the isotope was added as TcO_4^- . The mixture was contained in stoppered erlenmeyer flasks and was constantly stirred by means of a teflon covered stirring bar and magnetic stirrer. Aliquots were periodically removed, filtered through pre-weighed millipore filters (0.45 μ) and the filters oven dried at 80°C overnight. We employed a double filter technique to account for the amount of ^{95m}Tc retained by the filter itself. Each backing filter was dried identically to that containing the sediment. A 1 ml aliquot of the filtrate was also retained for counting. Over a 10 day period, we could measure no $^{95m}\text{TcO}_4^-$ uptake by any of the sediments used. Count rates on both filters were identical. The filtered water had the same activity per unit volume when corrected for decay as did the original labeling solution prior to sediment addition. Our findings in this experiment are therefore not unlike those observed by Routson, Jansen and Robinson (7) for soils.

A second experiment was repeated using the same sediments and the same experimental protocol. Prior to addition of sediment, however, the seawater was made 0.1 M in hydroxylamine hydrochloride. We had previously determined that this reducing agent in seawater at that concentration would reduce TcO_4^- to lower valence states which were carried on both

7) Routson, R. C., G. Jansen, A. V. Robinson (1977). ^{241}Am , ^{237}Np and ^{99}Tc Sorption on Two United States Subsoils from Differing Weathering Intensity Areas. *Health Phys.* 33:311-317.

Table 3. Sediment Description for Kd Experiments

Sediment No.	Latitude Longitude	Water Depth (Meters)	Description
8	67°00.5'S 178°30.1'W	4030	Brown-gray foraminiferous clay and glacial debris. 1% CaCO_3 ; 2-4% opal remainder - Clay
9	68°24.4'S 176°21.9'W	3730	"
11	63°37.9'S 169°19.1'W	2783	Diatomaceous ooze. 3% CaCO_3 ; 60% opal

Mg(OH)_2 and Fe(OH)_3 . The solutions and reducing agents were allowed to equilibrate for 16 hours at which time the sediment was introduced. Table 4 sets out the data for these experiments which are still in progress.

The results of this experiment suggest to us that there is an attraction for the reduced form of Tc and that most probably, this form is TcOCl_4^- . Were the reduced form TcO_2 , we would have expected to see a much higher portion of the Tc occluded with the sediment and retained by the filter.

Oxidation of Reduced Forms of Tc in Seawater

In order to determine the rate at which reduced forms of Tc are reoxidized to TcO_4^- in oxygenated seawater, 1 μCi of $^{95\text{m}}\text{TcO}_4^-$ was added to a solution which was 1 N in HCl and 0.1 N in $\text{NH}_2\text{OH}\cdot\text{HCl}$. The resulting mixture was warmed at 80°C for two hours in a water bath to ensure reduction of the TcO_4^- to lower valence states. The solution was cooled, 10 mg of Mg carrier added and the solution brought to pH 9 by adding concentrated NaOH . The resulting Mg(OH)_2 precipitate was collected by centrifugation and the supernate carefully decanted into a counting tube. The precipitate was washed with doubly distilled water, centrifuged and the supernate discarded. The Mg(OH)_2 precipitate was then dissolved in 0.05 ml of 6 N HCl and transferred to 200 mls of 0.45 μ millipore filtered seawater. This solution was stirred for one minute and a 5ml aliquot removed for counting. Immediately following the activity measurement, the solution was acidified with HCl , 5 mg of Fe carrier was added, and the solution was made basic by the addition of concentrated NH_4OH . Following collection of the precipitate by centrifugation

Table 4. Kd values for reduced Tc species on Marine Sediments

Sediment	Time (days)	Kd
8	1	2.6
9	1	1.1
11	1	0.3
8	4	9.3
9	4	6.9
11	4	3.6
8	10	13.3
9	10	13.6
11	10	7.3

gation, the supernate was discarded and the precipitate washed with distilled water. Five ml of 6N HCl were then added to dissolve the precipitate and the resulting solution was counted for ^{95m}Tc . This procedure was repeated at different time intervals to determine the percent of the activity that was carried by the $\text{Fe}(\text{OH})_3$ precipitate and hence the fraction of the activity still present in solution as reduced forms of Tc. Figure 17 shows the results obtained in this experiment indicating the rapid oxidation of the reduced forms by the dissolved oxygen in the seawater. Within one day, 92% of the reduced forms are reoxidized to TcO_4^- .

We are as yet unsure as to the exact chemical nature of the reduced forms produced in the reduction with $\text{NH}_2\text{OH}\cdot\text{HCl}$. We most probably have a mixture of TcCl_6^{3-} and TcOCl_4^- . We do not think we have produced a purely cationic species of Tc owing to the high concentration of Cl^- present in seawater. The shape of the oxidation curve can be explained by the more rapid oxidation of the TcOCl_4^- species to TcO_4^- coupled to a slower oxidation rate for the purely halogenated form. We are clearly not observing a first order reaction rate for the change of a single species to TcO_4^- due to the curvature in the semi-logarithmic plot.

These data would argue that the principle species, to be expected in well oxygenated seawater would be TcO_4^- and that future animal experiments and analytical isolation techniques should be based on this chemical species.

Figure 17. Oxidation of reduced Tc species in seawater.