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THE BIOACCUMULATION AND FOOD CHAIN TRANSFER OF CORROSION PRODUCTS FROM RADIOACTIVE STAINLESS STEEL

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July 1986

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SUMMARY

Two sets of experiments were conducted to determine if corrosion products from radioactive Type 347 stainless steel could be biologically transferred from sediment through a marine food chain, and whether corrosion products dissolved in seawater could be bioaccumulated and then eliminated. Corrosion products containing ^{60}Co and ^{63}Ni from the radioactive stainless steel were introduced into marine sediments. Infaunal polychaete worms exposed to these sediments bioaccumulated the radionuclides. The feeding of these worms to shrimp and fish resulted in a trophic transfer of the radioactive products across a one-step food chain. The magnitude of the transfers are described in terms of transfer factors. Dissolved corrosion products as measured by the radionuclides were also bioaccumulated by shrimp and fish, shrimp concentrating more than fish. Concentration factors were calculated.

CONTENTS

FIGURES

TABLES

INTRODUCTION

In 1979 the Pacific Northwest Laboratory began a series of studies under contract to Knolls Atomic Power Laboratory to investigate the possibility of corrosion products from radioactive stainless steel dissolving in seawater, associating with deep-sea sediments, or being bioaccumulated. Although the experiments were necessarily done in the laboratory, their purpose was to indicate what might happen to the corrosion products if radioactive stainless steel was placed in the deep sea. The experiments used small strips of neutron-activated Type 347 stainless steel which contained 9-13% nickel and <0.8% cobalt. The strips were neutron-activated in 1972, so at the time the studies began most of the short-lived isotopes, such as ^{55}Fe, had decayed away. Release of the longer-lived ^{60}Co (5.27-year half life) was used as a measure of corrosion.

Schmidt (1982) showed that corrosion products bind to deep-sea sediments. Young (1982) found that passing seawater over the stainless steel resulted in the formation of soluble corrosion products that were biologically available to marine animals living both in and out of the sediment, as indicated by the animals' uptake of ^{60}Co. A soft-bodied polychaete (Neanthes virens) living in the sediment accumulated more ^{60}Co than clams or sea urchins, possibly because of the large, absorptive epidermal surface and uptake through the gut by ingestion of contaminated sediment, and possibly because of a higher concentration factor for cobalt. These studies indicated that corrosion products from radioactive stainless steel deposited on the deep-sea floor may be accumulated by benthic fauna.

This study was designed to indicate 1) whether any food-chain transfer of the radioactive corrosion products was possible in the deep sea, and 2) whether animals could take up corrosion products that were simply dissolved and not associated with sediment. The report presents the results of two experiments, again by necessity performed in the laboratory with deep-sea surrogates. In one experiment, infaunal polychaetes (Neanthes virens and Aberenicola pacifica) were exposed to contaminated sediments and fed to shrimp and fish (Pandalus danae and Parophrys vetulus), potential predators

of the worms. In the other, shrimp and fish were exposed to seawater contaminated with corrosion products. In addition to ^{60}Co, the trophic transfer experiment examined ^{63}Ni, another long-lived isotope (half-life 92 years) found in radioactive Type 347 stainless steel.

MATERIALS AND METHODS

FOOD CHAIN TRANSFER

Five neutron-activated strips of Type 347 stainless steel were immersed in a slurry (7.3 liters) of clayey silt from Sequim Bay, Washington. The slurry was bubbled with nitrogen and stirred for 71 days. At the end of this time the sediment was allowed to settle, the overlying water was drained off, and the radioactive sediment was mixed with silty sand which came from natural habitat of the prey animals, the polychaetes Abarenicola pacifica and Neanthes virens. Samples of sediments were lyophilysed for counting.

A recirculating exposure system was constructed as an integrated chain of aquaria to ensure that the radionuclide concentrations available to organisms by separate uptake pathways (water, sediment, and food) were in the same relative proportions (see Figure 1). The aquaria were connected to allow water flow between tanks, and seawater was pumped from one end of the tank chain, through a charcoal filter, into the other end of the chain. The chain included 1) two temperature-controlled tanks for housing the polychaetes, 2) two insulated aquaria that were partitioned with perforated PVC into 16 cells for the predators, 3) a variable speed pump, and 4) an activated charcoal filter. The charcoal filter was added because initial tests showed an excessive buildup of metabolic wastes that jeopardized the health of the animals. The charcoal also removed the soluble radionuclides, eliminating water a s a vector for their bioaccumulation from water. The seawater was oxygenated, and its temperature was maintained at 10°C.

Neanthes and Abarenicola are sediment dwellers that serve as prey for numerous demersal organisms, and both animals have been shown to accumulate the radioactive corrosion products (Young 1982). The predators for the experiment were the shrimp Pandalus danae (approximately 1.2 g dry wt each) and juvenile English sole, Parophrys vetulus (approximately 1.6 g dry wt each). The polychaetes were placed in plastic trays filled with the radioactive sediment mixture, 16 Abarenicola in each of 12 small trays

(5 x 7 x 22.5 cm) and six Neanthes in eight larger trays (5 x 15 x 22.5 cm). The polychaetes remained in the sediment a minimum of 20 days before being used as food for the fish and shrimp. Thirty-two Parophrys and an equal number of Pandalus were placed in the two partitioned aquaria, the fish in one and shrimp in the other, with two animals per cell.

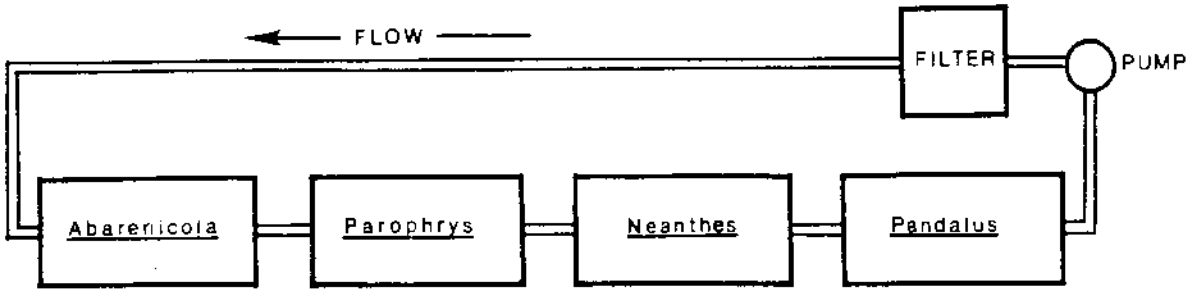

FIGURE 1. Recirculating Exposure System for the Food Chain Transfer Experiment. Tanks containing Abarenicola and Neanthes were temperature-controlled. Tanks containing Parophrys and Pandalus were partitioned into sixteen cells with perforated PVC. Each cell contained two animals

Sixteen each of the fish and shrimp were fed worms from the contaminated sediments. The remaining predators were given worms exposed to clean sediments. All worms were allowed to clear their guts before they were used as food. The predators were fed two trays of either Abarenicola or Neanthes every 2 days over a 1-month period for a total of 15 feedings. Worms were replaced in the first several trays, and these were cycled sequentially after the last original tray was used. All worms were diced

and equal amounts of food were delivered to each cell. The approximate dry weights of Abarenicola and Neanthes fed each predator per ration were 0.06 g and 0.4 g, respectively. Any food not consumed after 6 hours was removed; nearly all food, however, was eaten. During most of the feedings, when it was decided that an extra worm was available, a sample was saved for radionuclide counting.

During the course of the experiment, a few of the shrimp and fish died. These were removed and frozen, but in the end were not used for analysis. Two days after the last feeding, all remaining predators were sacrificed, frozen, and lyophilysed. Eight each of the control and experimental fish and shrimp were ground and prepared for whole animal analysis. The remaining individuals were dissected for analysis of the tissue. All samples were then ground, placed in 3-ml counting tubes, weighed, and counted at least 1000 minutes for ^{60}Co using a Ge(Li) detector coupled to a computer-interfaced Canberra 8180 multichannel analyser. The samples were background and decay corrected to the date of initiation of the experiment. Concentrations of ^{60}Co were expressed in units of picocuries (pCi) per gram (g) dry wt of material. Detection limits varied with sample size and counting time.

Nickel-63, a weak beta emitter (0.0659 MeV) was counted by liquid scintillation spectroscopy after selective radiochemical separation. Samples were prepared in the following manner. Sediment samples were dried at 80°C for 24 hours and ground into a fine powder. Two-gram aliquots of sediment were placed in beakers with the addition of 2 ml of Ni-carrier solution (1 mg Ni/ml), 25 ml of 6N hydrochloric acid (HCl) and 10 ml of distilled water). Samples were placed on a hot plate at 110° to 115°C for 1 hour, then evaporated to approximately 15 ml. The leachate was filtered and washed to give approximately 30 ml of filtrate.

Individual tissue samples were homogenized in a blender, dried at 80°C for 24 hours, and ground to a fine powder. Two-gram aliquots (or less) were placed in pre-ashed, pre-weighed porcelain crucibles and ashed in a muffle furnace at 550°C for 36 hours. To the crucibles were added 2 ml of Ni-carrier solution (1 mg Ni/ml), 10 ml of 6N HCl and 5 ml of distilled water. The resulting solution was heated at 110° to 115°C for 15 min, filtered, and rinsed to give approximately 30 ml. The ^{63}Ni was extracted

from these preparations using a phase separation technique (Appendix A), and the resulting purification was counted with a Beckman® LS 7000 scintillation counter.

Correlation analysis was used to examine the relative magnitudes of ^{63}Ni and ^{60}Co bioaccumulation.

ACCUMULATION OF CORROSION PRODUCTS FROM THE WATER BY FISH AND SHRIMP

Three experiments were performed to determine the bioaccumulation of corrosion products dissolved in seawater. In experiment 1, five strips of radioactive Type 347 stainless steel were suspended in a lead-shielded plastic container, and seawater was pumped from a 90-liter Instant Ocean Aquarium past the stainless steel at a rate of 200 ml per minute and returned to the aquarium. This system ran at room temperature for 71 days. It was then cooled to 10°C, and 65 Pandalus danae were placed in the aquarium. Five at a time were removed and frozen at intervals of 5, 10, 18, and 30 days. The remaining shrimp were fed minced clams every few days. Sometime just before the 53rd day, all the remaining shrimp died, probably because of a critical metabolite buildup, so five of these were also removed and frozen. All samples were processed and counted for ^{60}Co and ^{63}Ni, as described above.

In experiment 2, the seawater from experiment 1 was replaced with clean seawater, and four of the stainless strips were exposed as described in experiment 1. Type 347 stainless steel, however, is highly resistant to corrosion by seawater. To obtain sufficient soluble corrosion products in a shorter period of time, the surface area of one strip was increased by tumbling it in a container of seawater and quartz sand for 42 days. This seawater was then passed through a 0.2-μm filter, and about one third of the filtrate was added to the 90 liters of seawater in the aquarium. Eighty Pandalus danae were then placed in the aquarium, five individuals were removed for the determination of ^{60}Co accumulation at days 1, 3, 7, 14, 22 and 27. Those remaining in the water during this time were again fed minced

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clam every few days. After 2 hours any uneaten clam was removed. On the 27th day, the remaining shrimp were taken out of the radioactive water and placed in a clean, continuous-flow aquarium to examine ^{60}Co elimination. These individuals were sampled at 1, 3, 7, 14 and 26 days.

English sole were treated similarly in experiment 3. The remaining two-thirds of the corrosion product concentrate was added to another 90 liters of seawater at 10°C, and 80 fish were added. These also were sampled for measurements of ^{60}Co uptake (1, 3, 8, 15 and 28 days) and elimination (1, 4, 8, 15, 22 and 29 days).

All fish and shrimp samples were frozen, lyophilysed, ground in a ball mill, placed in tared counting tubes, weighed, and counted for ^{60}Co with a Packard® automated gamma counter equipped with a NaI well detector.

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RESULTS

FOOD CHAIN TRANSFER OF RADIOACTIVE CORROSION PRODUCTS

The total ^{60}Co and ^{63}Ni activities of the Sequim Bay clayey silt after the 71-day exposure of the radioactive stainless steel were 268 pCi/g and 110 pCi/g, respectively. This preparation was mixed with the sandy silt from the haabitat of the prey animals. The mean activities for ^{60}Co and ^{63}Ni were 14.8 and 5.17 pCi/g, respectively (see Table 1.).

TABLE 1. Cobalt-60 and ^{63}Ni in Sediment

Sediment	Sample Number	^{63}Ni (pCi/g)	^{60}Co (pCi/g)
Sequim Bay Clayey-silt	1	120	272
	2	100	263
Clayey-silt/ sandy/silt mixture	1	8.58	19.3
	2	5.74	13.0
	3	<3.58	13.3
	4	2.76	13.5

Abarenicola accumulated generally more of the radioactive corrosion product than Neanthes (see Table 2); however, a statistical comparison is inappropriate because the exposure times were not necessarily identical. There is a moderate correlation in the magnitudes of accumulation of the two nuclides in the worms (Abarenicola, $r = 0.83$; Neanthes, $r = 0.73$). Because there was no detectable activity in the water, the only kind of concentration factor that can be expressed is a factor for the transfer of radioactivity from sediment to animal:

$$TF_s = \frac{\text{Activity at trophic level (x) (pCi/g)}}{\text{Activity of the sediment (pCi/g)}}$$

With the exception of one anomalous value, the TS_s for Neanthes ranged from 1.2 to 5.5 for ^{60}Co and 3.0 to 15.7 for ^{63}Ni (Table 2). The higher TF_s values for ^{63}Ni indicate that this nuclide was more readily bioaccumulated from the sediment than was ^{60}Co.

Whole body and tissue accumulations of the two radionuclides in Parophrys vetulus and Pandalus danae after being fed the radioactive worms are shown in Tables 3 and 4, respectively. Pandalus accumulated more ^{60}Co and, in general, more ^{63}Ni than Parophrys. Most of the ^{60}Co was concentrated in the hepatonpancreas of the shrimp and the least in the muscle. Likewise, the viscera of at least two of the English sole had high levels of the nuclide, where it was probably concentrated in the liver. Nickel-63 was concentrated mainly in the hepatonpancreas and exoskeleton of the shrimp and skin and viscera of the fish. There were no good correlations between the relative accumulations of the two nuclides. The TF_s values for Parophrys and Pandalus, unlike those for the worms, were generally less than unity (Tables 3 and 4). There was no measurable activity in any shrimp or fish fed nonradioactive food.

TABLE 2. Cobalt-60 Activity and Tissue-sediment Concentration Ratios in the Polychaetes

Species	Sample Number	^{60}Co (pCi/g)	^{60}Co TF_s	^{63}Ni (pCi/g)	^{63}Ni TF_s
Neanthes virens	1	22.8	1.5	8.56	1.7
	2	81.0	5.5	40.9	7.9
	3	18.4	1.2	15.6	3.0
	4	55.7	3.8	18.0	3.5
	5	2.68	0.18	2.98	0.58
	6	25.1	1.7	15.8	3.1
	7	35.1	2.4	20.1	3.9
	8	65.1	4.4	81.3	15.7
	9	30.8 37.4 (25.0)[a]	2.1	26.8 25.6 (23.5)[a]	5.2
Abarenicola pacifica	1	115	7.8	91.9	17.8
	2	117	7.9	112	21.7
	3	89.0	6.0	103	19.9
	4	30.3	2.0	15.5	3.0
	5	45.7	3.1	39.4	7.6
	6	64.2	4.3	99.4	19.2
	7	74.3 76.5 (33.0)[a]	5.0	94.1 79.3 (36.7)[a]	18.2

[a] Mean and standard deviation

The radioactivity in the shrimp and fish was lower than that in the worms, but there was still a transfer of the nuclide from prey to predator. This can be expressed as a factor of the transfer of the radioactivity from food (Amiard and Amiard-Triquet 1977);

$$TF_f = \frac{\text{Activity at trophic level (x) (pCi/g)}}{\text{Activity at trophic level (x-1) (pCi/g)}}$$

TABLE 3. Cobalt-60 and ^{63}Ni in Parophrys vetulus (pCi/g dry wt)

Sample Number	Whole Fish		Skin		Muscle		Viscera		Remainder		TF_s		TF_f	
	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni
1	1.10	0.658	--	--	--	--	--	--	--	--	0.074	0.127	0.020	0.013
2	1.45	1.16	--	--	--	--	--	--	--	--	0.098	0.224	0.027	0.024
3	3.26	2.36	--	--	--	--	--	--	--	--	0.221	0.457	0.060	0.048
4	0.66	3.49	--	--	--	--	--	--	--	--	0.045	0.675	0.012	0.071
5	1.64	1.63	--	--	--	--	--	--	--	--	0.111	0.315	0.030	0.033
6	0.52	33.6	--	--	--	--	--	--	--	--	0.035	6.50	0.010	0.658
7	2.29	2.57	--	--	--	--	--	--	--	--	0.155	0.497	0.042	0.052
8	1.37	3.90	--	--	--	--	--	--	--	--	0.093	0.755	0.025	0.079
9	--	--	ND	17.9	ND	2.61	26.3	11.4	1.9	2.57	--	--	--	--
10	--	--	ND	25.0	ND	3.14	16.8	12.2	ND	2.66	--	--	--	--
11	--	--	ND	27.2	ND	5.35	ND	< 8.06	0.57	1.47	--	--	--	--
12	--	--	ND	4.90	ND	3.16	ND	28.1	ND	< 6.42	--	--	--	--
13	--	--	ND	< 12.5	ND	<10.6	ND	10.6	ND	< 0.928	--	--	--	--

ND = not detectable
Broken lines indicate not applicable.

TABLE 4. Cobalt-60 and ^{63}Ni in Pandalus danae (pCi/g dry wt)

Sample Number	Whole Shrimp		Exoskeleton		Muscle		Hepato-pancreas		Remainder		TF_s		TF_f	
	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni	^{60}Co	^{63}Ni
1	5.17	4.70	--	--	--	--	--	--	--	--	0.350	0.909	0.095	0.096
2	3.98	4.97	--	--	--	--	--	--	--	--	0.269	0.962	0.073	0.101
3	4.24	6.35	--	--	--	--	--	--	--	--	0.278	1.23	0.078	0.129
4	2.38	4.63	--	--	--	--	--	--	--	--	0.161	0.896	0.044	0.094
5	3.07	2.07	--	--	--	--	--	--	--	--	0.208	0.401	0.056	0.042
6	4.20	3.03	--	--	--	--	--	--	--	--	0.284	0.586	0.077	0.062
7	4.19	2.00	--	--	--	--	--	--	--	--	0.284	0.387	0.077	0.041
8	3.20	1.43	--	--	--	--	--	--	--	--	0.217	0.227	0.059	0.029
9	--	--	3.38	5.05	2.33	5.08	25.4	3.02	7.6	4.46	--	--	--	--
10	--	--	1.91	18.7	ND	<3.98	7.7	<9.18	4.4	5.11	--	--	--	--
11	--	--	2.45	38.7	1.89	10.2	18.3	24.3	7.4	5.18	--	--	--	--
12	--	--	2.09	<0.750	ND	<0.945	16.9	46.8	5.6	1.68	--	--	--	--
13	--	--	3.13	2.65	ND	1.68	23.8	14.8	4.5	5.23	--	--	--	--

ND = not detectable
Broken lines indicate no data

Tables 3 and 4 list TF_f values for each of the whole fish and shrimp. The ^{60}Co and ^{63}Ni activity of each was divided by the mean concentrations of the respective radionuclides of all the measured worms combined. These values are generally less than 0.10.

ACCUMULATION OF CORRDSION PRDDUCTS FROM THE WATER BY FISH AND SHRIMP

Figure 2 shows the results of ^{60}Co and ^{63}Ni accumulation in the shrimp from experiment 1. It can be seen that the initial uptake rate for ^{60}Co was rapid before day 18 and then slowed. Analysis of variance (ANOVA) of log transformed data from day 5 to 53 showed significant differences in the means (see Table 5), and the Student-Newman-Keuls (SNK) multiple comparison test indicates a significant break between days 10 and 18. The curve for the means of ^{63}Ni appears to show rapid uptake for 30 days but then a loss between day 30 and 53. ANOVA again showed significant differences among the means, and the SNK test diagrams show where the differences lie. The relative magnitudes of ^{60}Co and ^{63}Ni tissue accumulations have a fairly high correlation coefficient ($r > 0.87$) in all samples except those from day 30 ($r = 0.45$). Paired t-tests for days 5 through 53 reveal significant differences (0.1D) in the means for the two curves at days 18 and 53. An examination of the cumulative results of the t statistic through time shows a significant difference (0.10) in the uptake rates on days 18, 30, and 53. The loss of ^{63}Ni in the shrimp that died just before 53 days may be a result of different binding sites for the two nuclides in the shrimp, since there was some postmortem decay before the shrimp were sampled.

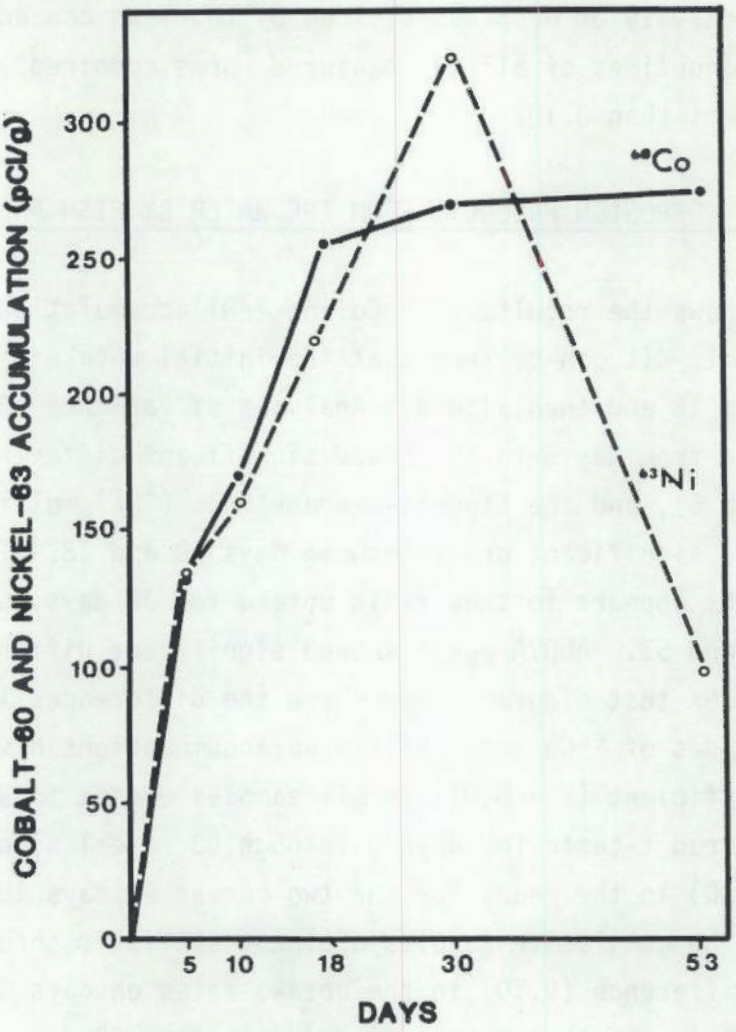

FIGURE 2. Uptake of ^{60}Co (solid line) and ^{63}Ni (broken line) by Pandalus danae.

Figure 3 shows the ^{60}Co uptake and release curves for Pandalus in experiment 2. The radionuclide was not detectable in unexposed control shrimp (day 0). Again, uptake at the beginning of the experiment was rapid but decreased after a few days. There were no statistical differences among the mean accumulations of days 8, 15, 28. The concentration factor

$$CF = \frac{\text{Activity in the organism (pCi/g)}}{\text{Activity in the water (pCi/ml)}}$$

TABLE 5. ANOVA Results and SNK Multiple Comparisons from Experiments on Accumulation of Corrosion Products from Water by Fish and Shrimp

Experiment	Species	Determination	F[(a)]	Critical F value (0.05)[(a)]	SNK multiple comparisons (0.05)[(b)]
1	Pandalus	^{60}Co uptake	7.42	2.90	5 10 18 30 53
		^{63}Ni uptake	4.64	2.90	53 5 10 18 30
2	Pandalus	^{60}Co uptake	54.0	2.87	1 3 8 15 28
		^{60}Co release	3.90	2.45	0[(c)] 4 1 8 15 28 22
3	Parophrys	^{60}Co uptake	36.3	2.62	1 3 7 14 22 27
		^{60}Co release	0.68	2.62	-- no difference in means -

(a) Data was log transformed. If the value for F is greater than the critical F-value (0.05) for the proper degrees of freedom, then there is a 95% probability that there are differences among the means.

(b) Numbers are the days at which a sample of 5 individuals was taken. They are arrayed in ascending order of magnitude of the mean isotope accumulation. The lines represent nonsignificant sets of means.

(c) The day that the shrimp were transferred to noncontaminated seawater.

was 32. When transferred to clean water there was a significant release of ^{60}Co over the 29-day period, but with a large coefficient of variation, (80 to 129% of the mean), during days 15, 22, and 29. This variation was a result of some individuals releasing almost all of their ^{60}Co during this period while others retained much of the isotope.

The results of Parophrys being exposed to dissolved corrosion products are illustrated in Figure 4. Uptake of ^{60}Co was rapid through day 7 but soon reached a plateau. There were no significant differences among the means of days 14, 22, and 27 (Table 5). The level of bioaccumulation was less than in shrimp; the CF was about 1.5. There was no significant release of ^{60}Co during the 26 days in uncontaminated water.

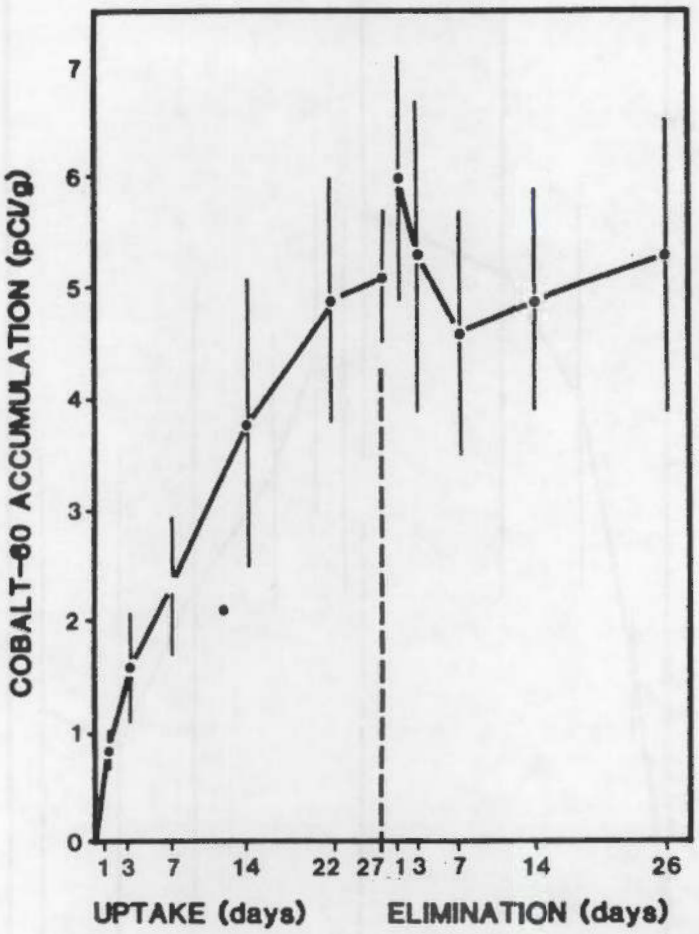

FIGURE 3. Uptake and Elimination of ^{60}Co by Pandalus danae. Vertical bars indicate plus or minus one standard deviation. Broken line indicates when shrimp were removed from seawater containing corrosion products and placed in uncontaminated seawater.

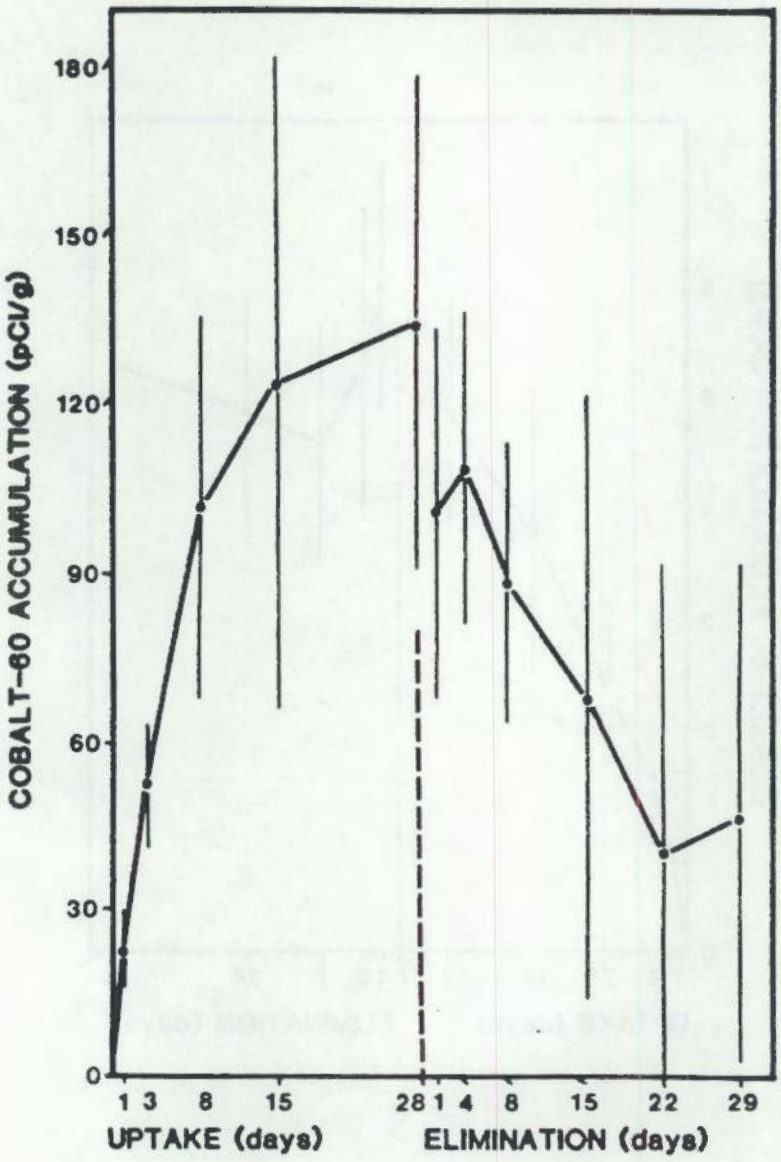

FIGURE 4. Uptake and Elimination of ^{60}Co by Parophyrs vetulus. Vertical bars indicate plus or minus one standard deviation. Broken line indicates when shrimp were removed from seawater containing corrosion products and placed in uncontaminated seawater.

DISCUSSION

Renfro et al. (1975) encouraged the use of an integrated exposure system to ensure that the radionuclide concentrations available to organisms by the separate uptake pathways of food and water are available in the same relative proportions. This system better simulates the natural environment than separate exposure systems for each pathway. The original intention of the present experiment was to examine ^{60}Co accumulation from water as well as from food in the same experimental system. However, a preliminary trial indicated that the buildup of waste metabolites in the water affected the health of the predators, so that an activated-charcoal filter was required to remove the waste products. However, this filter also absorbed any soluble ^{60}Co, so that the water was effectively free of the radionuclide. Even so, the system better approached natural conditions with sediment, prey, and predators in the same exposure water than a system with separate exposures, and variability in the treatment of each animal was reduced.

Cross et al. (1975) suggest the use of natural food when examining the food chain transfer of radionuclides in the laboratory. There is evidence that retention of a radionuclide by the prey is better after eating labeled natural food than artificial food. The worms used in this experiment form part of the natural diet of English sole and are also likely to be fed upon by Pandalus since all live in the same area. Both species ate the worms readily in the experiment.

There is little information on the accumulation of ^{63}Ni by marine organisms. Nickel-63 was first detected in biological tissue by Beasley and Held (1969). Their measurements of ^{63}Ni in the kidneys of the clam Tridacna sp. from the Pacific nuclear test sites (Bikini and Eniwetok Atols) ranged up to 163 disintegrations per minute (dpm) (73 pCi) per gram. Kidneys from Tridacna some distance from the test sites (Christmas Island and Penrhyn Atoll) contained concentrations of less than 1.0 dpm (0.45 pCi) per gram. Composite shellfish samples from the eastern seaboard of the United States contained 0.D2 dpm (0.009 pCi) per gram. Hardy and Roesijadi (1982) exposed the clam Protothaca staminea to a $NiNO_3$ solution containing carrier-free ^{63}Ni. They measured a CF of four (dry wt basis), but most of the nuclide was accumulated by the shell. Of the soft tissues, the gills had the

highest concentrations. Jenkins (1980) reviewed stable nickel accumulation in aquatic animals. Whole Pandalus montagni contained 25 µg/g wet wt in the skin, liver, and muscle, respectively (Wright 1976). Since no other information on ^{63}Ni bioaccumulation was located, the remaining discussion will dwell primarily on ^{60}Co.

The worms exposed to the radioactive sediment accumulated ^{60}Co, but the TF_s, less than eight, was considered low. The TF_s values for ^{63}Ni were somewhat higher. Amiard-Trique (1975) found that when intertidal sediment-dwelling invertebrates, including Arenicola marina, were exposed to ^{60}Co in either water or sediment alone, water appeared to be the better vector for bioaccumulation. Arenicola had a higher CF and a faster rate of uptake in water than in sediment. Amiard-Trique believed that even though the sediment was a sink for ^{60}Co, the physicochemical form of the radionuclide made it relatively unavailable for bioaccumulation. He also found that Arenicola redistributed radionuclides in the sediment by burrowing and ingestion and excretion of the sediment particles.

The transfer factors from sediment to worms suggested that Abarenicola can accumulate more of the radioactive corrosion product than Neanthes. However, since the duration of exposure of the two species of worms varied and it is not known whether an uptake equilibrium was reached, it cannot be said that the level of accumulation in Neanthes would never catch up to that in Abarenicola. It is possible, though, that equilibrium was reached rather rapidly. Amiard-Triquet (1975) showed that accumulation of ^{60}Co from sediment by Arenecola marina stabilized in less than two weeks. To determine whether the different sediment transfer factors between Abarenicola and Neanthes are real would require further investigation.

The experiments presented in this report showed that both ^{60}Co and ^{63}Ni were transferred through a food chain. Food has proven to be an important pathway of ^{60}Co uptake in some marine animals. During the days of atmospheric testing of nuclear weapons, Folsom and Young (1965) found that albacore accumulated ^{60}Co from eating contaminated squid. Suzuki et al. (1981) showed that uptake and elimination were faster in fish that obtained other hand, were highly variable in the amount of ^{60}Co accumulated, and even after 4 months, it was not obvious whether equilibrium had been attained. Their TF_f was less than 0.005, and it was estimated that assimilated only

0.55% of the ^{60}Co ingested. The isotope concentrated in the liver, kidney, heart, and spleen. When placed in clean water, they lost no activity in 100 days.

These results parallel several aspects of the experiments in this report. Even though English sole in the feeding experiment may not have reached an uptake equilibrium (they appear to have done so during uptake of corrosion products from the water), they contained less ^{60}Co than the shrimp when either food or water was the vector. In the experiments where the predators were transferred to clean water, the sole, like Pleuronectes, lost no ^{60}Co.

Amiard-Trique and Amiard (1975) and Amiard and Amiard-Trique (1975, 1977) also quantified the transfer of ^{60}Co within the following food chain:

Navicular ramosillima ---> Scrobicularia planna
(diatom) (mollusc)

---> Carcinus maenus ---> Rattus rattus
(crustacean) (mammal)

The authors found that 1) the percentage of assimilated ^{60}Co decreased within a trophic level with each successive feeding and approached zero in the rat. In other words, the ratio of the radioactivity of animals at trophic level x to the radioactivity of the water was almost 5.0 between diatoms and molluscs, 1.5 between molluscs and crustaceans, and only 7.1×10^{-4} between crustaceans and the mammal; 2) once accumulation reached equilibrium in the molluscs and crabs, elimination was very slow; and 3) food was a more important route for internal accumulation--primarily the gut and hepatopancreas in the invertebrates, and kidney and liver in the rat--and water led to external contamination. This was three-step food chain. It is expected that if the one-step food chain of worms to fish or shrimp were carried through additional trophic levels, a similar reduction in radionuclide transfer would occur. This reduction essentially means that there is a dilution of the radionuclide as it is passed up a food chain.

The nuclides that are transferred would continue to accumulate in visceral organs that normally concentrate heavy metals. It is also possible that in some animals' elimination would be very slow.

If radioactive stainless steel is deposited in the deep-sea floor, the experimental results indicate that activated corrosion products can be bioaccumulated and transferred along a food chain. However, the corrosion rate for Type 347 stainless is so slow that dilution of the corrosion products outside the sediment should be rapid enough to preclude their bioaccumulation. Inside the sediment where the corrosion products remain more concentrated, infauna may, according to the laboratory experiments, accumulate some radioactivity. The infauna can then transfer this activity to predators.

Actual locations in the deep sea where radioactive corrosion products were released can be found at the sites of two nuclear-powered submarines that were lost at sea during the 1960s, the Thresher and Scorpion. Monitoring of the area adjacent to the debris of these ships has shown small amounts of ^{60}Co and ^{63}Ni in the sediment but none in the water or marine life, including infauna (United States Department of the Navy 1984).

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APPENDIX A

Ni-63 DETERMINATION

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Each 30-ml aliquot of sediment, seawater, or tissue extract was transferred to a 125-ml separatory funnel and carried through the following procedure: Add 5 ml of 10% sodium citrate and mix. Add 1.25 ml of 1% dimethylglyoxime (DMG) in ethyl alcohol and mix. Add concentrated ammonium hydroxide (NH_4OH) dropwise with mixing until the solution turns red, then add 1 ml excess. Extract twice with 40-ml portions of chloroform. Discard the aqueous phase and wash the organic phase with 25 ml of distilled water containing a few drops of concentracted NH_4OH. Back-extract into 10 ml of 8N HCl. Discard organic phase. For sediment samples (which need further purification), add another 1.25 ml of 1% DMG to the back extract and repeat the entire extraction procedure. Transfer the back extract and repeat the entire extraction procedure. Transfer the back extract to a 50-ml beaker and evaporate to near dryness. Take up residue with distilled water and dilute to 1 ml in a volumeric flask. Take out a 50-µl aliquot for Ni recovery determination (procedure follows) and transfer remainder to a 20-ml low-potassium glass scintillation vial. Add 10 ml of Scinterverse II (Fisher Scientific) scintillator solution and shake well.

A blank was carried through the entire procedure for every 10 samples. A standard was prepared by adding 10 ml of scintillator solution to 1 ml of a nickel standard solution (2 mg Ni/ml) and 50 µl of a ^{63}Ni standard reference solution. Samples, blank, and standard were placed in the liquid scintillation spectrometer (Beckman® LS 7000) and allowed to stand for 1 hour before counting. Samples and blanks were counted for 400 min each. The disintegration rates of the samples were then calculated as follows:

$$d/m\ (^{63}Ni) = [c/m\ (sample) - c/m\ (blank)]\ (E)$$

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where

E = efficiency of scintillation spectrometer for Ni-63

$$= \frac{\text{c/m } (^{63}\text{Ni std}) - \text{c/m (backgrd)}}{\text{d/m } (^{63}\text{Ni std})}$$

The following formulas were then used (where applicable) to calculate the final results:

$$\text{Minimum Detectable Count Rate (MDC)} = \left(1.64 \frac{2 \times \text{c/m (backgrd)}}{400 \text{ min}}\right)$$

$$\text{Minimum Detectable Activity (MDA)} = \frac{\text{MDC (c/m) x 1 pCi}}{\text{E (c/d) x 2.22 (d/m)}}$$

$$\text{Corrected MDA} = \frac{\text{MDC (c/m) x 1 pCi}}{\text{E (c/d) x 2.22 (d/m) x Ni recovery}}$$

$$\text{Activity Conc.} = \frac{\text{[sample (c/m) - backgrd (c/m)] x 1 pCi}}{\text{E (c/d) x 2.22 (d/m) x Ni recovery \& dry wt (g)}}$$

Determination of Ni-carrier recovery serves as a measure of the chemical efficiency of the ^{63}Ni extraction procedure. The recovery determination was carried out as follows:

Transfer a 50-µl aliquot of the extracted nickel solution to a 25-ml volumetric flask. Add 2 ml of bromine water and mix. Add just enough NH_4OH (dropwise) to bleach the bromine color. Add four drops of NH_4OH in excess. Cool to room temperature. Add 0.5 ml of 1% DMG in ethanol. Dilute to volume with distilled water. After 30 min, transfer to a 1-cm cuvette and read the optical density (Beckman® 25 spectrophotometer) at 540 nm against a standard curve of optical density (absorbance) versus mg nickel. Prepare standards by transferring 1 ml of the nickel carrier solution (1 mg Ni/ml)

to a 50-ml volumetric flask, adding 10 ml of 8N HCl and diluting to mark with water. From this, transfer 4-, 2-, 1-, 0.5-, and 0-ml aliquots to separate 25-ml volumetric flasks, and proceed as above starting with the addition of bromine water. Obtain the total weight of nickel in 1 ml of sample by reference to the resulting standard curve. The fraction of Ni-carrier recovered is equal to

$$\frac{\text{mg recovered}}{\text{mg added (2 mg)}}$$

where

$$\text{mg recovered} = \frac{\text{mg Ni/ml (from curve)} \times 25 \text{ ml} \times 1 \text{ ml (sample)}}{0.05 \text{ ml aliquot}} .$$

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