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PRELIMINARY EXPERIMENTS WITH Ni-62 AS A TRACER OF THE  
CORROSION PRODUCTS OF NICKEL IN  
MARINE SYSTEMS



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PRELIMINARY EXPERIMENTS WITH Ni-62 AS A TRACER OF THE  
CORROSION PRODUCTS OF NICKEL IN  
MARINE SYSTEMS<sup>1</sup>

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INTRODUCTION

In 1979, the Bigelow Laboratory for Ocean Sciences began a program of research for the Knolls Atomic Power Laboratory. The general purpose of this research was, and remains, to provide information on the pathways and rates at which low level radioactive wastes may enter the biological system of the deep-sea. If such pathways were found, the possibility exists for radioactive material to be passed upwards through the food web and eventually be ingested by humans. Information on the rate of any such tranference is required for evaluating the deep-sea floor as a potential disposal site for low level radioactive materials.

The initial step is to determine the degree to which marine organisms with deep-sea affinities can take up metallic corrosion products in a simple aquarium system. Appreciable uptake would establish the beginning of a potential pathway and indicate that further, more sophisticated, experiments are required. The fundamental nature of radioactive substances is, however, a major impediment to the design and execution of such experiments. Ideally, the basic research program could be greatly accelerated if a substance was available which mimicked the behavior of the radioisotope under consideration, but which was not commonly found in nature. This situation would allow rather complex experiments to be done safely and efficiently.

Nickel, one of the elements of interest, occurs as a number of isotopes which have nearly identical chemical properties. Ni-63 is radioactive and is a specific isotope of interest to the deep-sea disposal program. Another is Ni-62, which is stable and occurs in nature as only a small percentage of total nickel. Furthermore, Ni-62 can be detected by sensitive mass spectrometric techniques available at Knolls Atomic Power Laboratory. Hence, it seems feasible to conduct experiments using stable Ni-62 to give information on the uptake of radioactive Ni-63. This document describes the preliminary experiments to establish the use of Ni-62 as a reliable tracer in the uptake of nickel by deep-sea organisms.

#### Aquarium System

The initial experiments were performed in two 208 liter glass aquaria with a bottom surface measuring 46 x 91 cm. Sediment from a local mudflat was frozen at  $-18^{\circ}\text{C}$  for several days to render it abiotic. After thawing, the mud was added to each aquarium to a depth of 8-10 cm. Sand-filtered Boothbay Harbor seawater was added to each tank and adjusted to 34 ‰ using synthetic sea salts. Each tank was insulated on the top and sides with 5 cm thick styrofoam, and the water was chilled to  $7-10^{\circ}\text{C}$  using a single refrigeration unit (FTS liquid cooler, Model MLC-40) in each tank. The water was filtered with standard charcoal and fiberglass aquarium filters, and the filter material was changed at least once a week. Water lost due to evaporation and filter changes was replenished using filtered seawater and synthetic sea salts. The tanks were checked for proper functioning at least once a day.

Temperature was recorded daily and dissolved oxygen, pH, and salinity were measured at appropriate intervals.

#### Specimen Collection

The mudstar *Ctenodiscus crispatus* was selected as the experimental species. This species had several apparent advantages over the other candidates, which included *Ophiopholis aculeata* and *Astropecten americanus*. This deposit feeder burrows through the upper layers of sediment and hence is intimately associated with the interface where corrosion products might be expected to accumulate. It has a wide depth range and is found at depths greater than 1800 m. Geographically, it is circumboreal in distribution, ranging from the Arctic to South America. Experimentally, it has wide applications. It is moderate in size, so it can be conveniently monitored in the lab, yet it is still small enough so several can be kept in limited aquarium space. More importantly, *Ctenodiscus* was recovered in better conditions than the other echinoderms, and we knew of dense patches of them in shallower water where we could sample with more refined gear.

In mid-September 1979, a large quantity of *Ctenodiscus* was collected in the Gulf of Maine south of Monhegan Island using a 40' otter trawl fitted with a fine liner. The animals were returned to the laboratory and held in flowing water tanks at the Maine Department of Marine Resources. A second collection was made on March 3, 1980.

## THE EXPERIMENTS

Experiment 1 - Experiment 1 commenced on November 26, 1979. Seven *Ctenodiscus* were added to a single aquarium in which a 2" x 6" x 0.0005", 873.7 mg Ni-62 foil was suspended (see Figure I). The tank was checked daily as described in the methods section, and dead specimens were removed, labelled, and frozen for shipment to KAPL. During this winter period, the water temperature was held between 5-8°C. The final test specimen died on March 14, 1980 which terminated the experiment.

This experiment was not completely satisfactory. The organisms initially had to be held for over two months in tanks which were not designed for long-term maintenance of temperature-sensitive animals. In spite of this draw-back, the longest-living organisms survived 180 days in captivity and almost four months in the experimental system.

Experiment 2 - Experiment 2 was begun on March 25, 1980. Twenty *Ctenodiscus* were held, since their capture on March 3, at appropriate temperatures in the control tank, increasing their viability. On March 25, they were distributed between the experimental and control tanks, and on March 31, ten were surviving in the control and nine in the experimental tank. These organisms, collected in the late winter, seemed to be healthier than those collected in September, which may be a function of their reproductive cycle or reduced temperature shock. The two tanks were monitored as described above. During April and May, several power outages occurred and the refrigeration units were not able to maintain the designated temperatures during a warm spell in June.

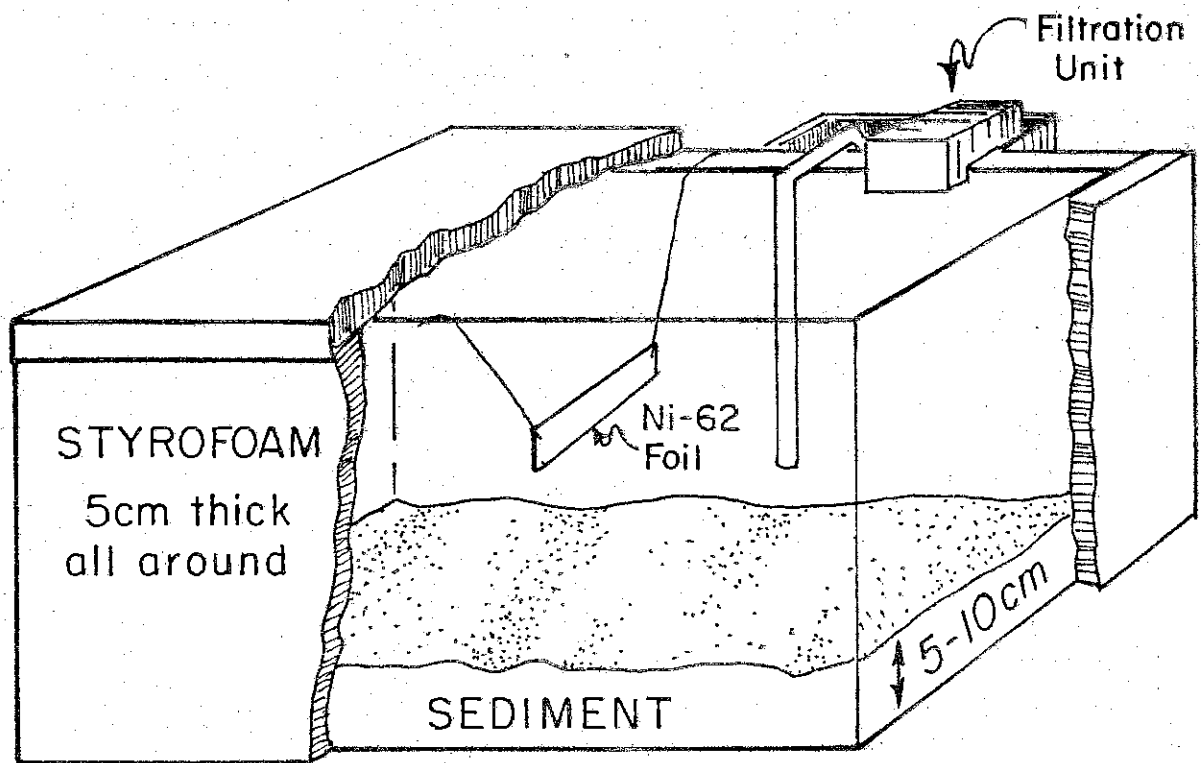


Fig. 1. Experimental aquarium arrangement.

Both of these factors undoubtedly contributed a degree of mortality. This experiment was terminated as initially planned on July 1, 1980.

#### Analytical Methods

The analysis of total Ni and Ni-62 in the exposed starfish, sediments and water at the Bigelow Laboratory was performed by the Knolls Atomic Power Laboratory. Basically, the analytical technique involves chemical recovery and purification of nickel extracted from a marine sample, followed by an isotopic analysis using conventional isotope dilution mass spectrometry. The following paragraphs are based on the analytical information supplied by KAPL.

The ion microprobe was selected to measure nickel isotope abundance because it has a higher ionizing efficiency for nickel atoms than can be obtained by surface ionization mass spectrometry. The higher efficiency is needed because typical concentrations of nickel in marine organisms are in the ppm range, and only micrograms of nickel in a starfish are available for analysis. The ion microprobe is manufactured by Applied Research Laboratories and has been modified with an electron multiplier detector for pulse-counting single ions.

The concentrations of nickel naturally present in a marine organism, as well as the amount of Ni-62 tracer it has absorbed, is determined by isotope dilution mass spectrometry. In applying this technique, a calibrated amount of highly enriched Ni-61 isotope is added to the chemical solution containing the dissolved marine organism and is homogenized with the solution before the nickel it contains is extracted



chemically. The Ni-61 spike is an internal standard. Because the abundance of the nickel isotopes present in the solution remain fixed throughout the subsequent chemical extraction, each aliquot of solution contains the same composition of nickel isotopes. Hence, analytical results do not depend on the chemical yield of nickel from a marine organism. All that is required is a sample aliquot of sufficient size and purity for analysis in the ion microprobe. In the absence of interfering ions with the same mass as Ni-62, the sensitivity for detecting Ni-62 uptake is limited by the concentration of normal Ni-62 present in a marine organism. For example, when the concentration of normal nickel is approximately 10 ppm, the concentration of Ni-62 uptake that can be measured is about 0.1 ppm.

Nickel can be extracted effectively from a marine organism (starfish) by using a dimethylglyoxime-chloroform extraction. The marine organism is dissolved by acid digestion and then the nickel is isolated by a chloroform extraction of its dimethylglyoximate. The sample is homogenized with a calibrated amount of Ni-61 spike during the acid digestion step. The nickel is readily transferred from the chloroform to an aqueous phase by shaking it after adding dilute hydrochloric acid. The sample is further purified before performing an ion microprobe analysis. This is achieved by repeating at least twice the dimethylglyoxime-chloroform extraction and hydrochloric acid transfer.

A sample mount is prepared as follows for an isotopic analysis in the ion probe. A 5 microliter volume of the chemically extracted nickel solution is pipetted onto a polished and cleaned vitreous carbon disc.

The dried droplet produces a light-colored deposit about 1 mm in diameter. The sample mount is placed in the ion microprobe and ion count-rate data are obtained for the nickel isotopic masses of interest. This is done over a number of selected target areas within the 1 mm diameter dried droplet.

Several corrections have been made to the observed data. These include a reagent blank correction for normal nickel, a small instrumental blank correction and a mass-bias correction determined by analyzing pure normal nickel in the ion microprobe. Unexposed marine organisms (starfish) also have been analyzed for their normal nickel concentrations and to provide an estimate of the minimum amount of excess Ni-62 that can be measured. These corrections place a lower limit of about 0.1 ppm on the concentration of Ni-62 tracer that can be detected in a marine organism that contains a typical normal nickel concentration of 10 ppm.

The concentration of normal nickel and stable Ni-62 tracer uptake in a marine organism are determined as follows. For reference, the isotopic abundances of normal nickel, the Ni-61 calibrated isotope dilution spike and the specially prepared Ni-62 foil are given in Table I. Each sample analyzed in the ion microprobe is comprised of a mixture of different amounts of these three nickel isotopic compositions. At each isotopic mass, the nickel isotope from the three sources add to form the observed isotopic abundances. These abundances, when compared with the calibrated amount of Ni-61 added to a solution containing a dissolved marine organism, allow estimates to be made for the concentrations of

TABLE I  
ISOTOPIC ABUNDANCES OF THREE  
NICKEL SOURCES MEASURED IN THE EXPERIMENT

<u>Source</u>	<u>Abundance in Atomic Percent</u>				
	<u>Ni-58</u>	<u>Ni-60</u>	<u>Ni-61</u>	<u>Ni-62</u>	<u>Ni-64</u>
Normal Nickel <sup>a</sup>	68.3	26.1	1.1	3.6	0.9
Ni-61 Spike <sup>b</sup>	3.45	6.12	88.83	1.40	0.20
Ni-62 Foil <sup>b</sup>	0.38	0.52	0.13	98.70	0.27

<sup>a</sup>Chart of the Nuclides, Knolls Atomic Power Laboratory,  
12th Edition, 1977.

<sup>b</sup>Nickel samples and analysis provided by Oak Ridge National  
Laboratory, 1979.

normal nickel and for the uptake of Ni-62 tracer as a function of exposure time in the controlled aquarium environment.

### Environmental Results

Results are presented in Table II. The experimental data show that a 3-4 month exposure under the conditions of this experiment is not sufficient time to make an accurate measurement of Ni-62 concentration in a starfish. The uptake, if any, is too small to be measured accurately. In Table II, upper limit estimates are put on the Ni-62 concentrations. A review of the experimental measurements and observations will show why only upper limit estimates can be made.

Isotopic abundance measurements using pure nickel reagents show that the ion-counting sensitivity is more than adequate to measure Ni-62 tracer concentrations down to 0.01 ppm. Unfortunately, the nickel samples chemically separated from biological materials in the starfish also contain small amounts of Na, K, Ca, Mg, F, and Cl. So far we have been unable to achieve sufficient purification of the extracted nickel samples to obtain reliable Ni-62 estimates below 0.05 ppm. Although this is excellent sensitivity, it is insufficient to determine what the Ni-62 uptake actually is in these samples. The variability in sample analysis depends on small background peaks at the mass positions 58, 60, 61, and 62. For example, mass 58 ion counts can be affected by the ions  $^{42}\text{Ca}^{16}\text{O}^+$  and  $^{23}\text{Na}^{35}\text{Cl}^+$ , mass 60 by the ions  $^{23}\text{Na}^{37}\text{Cl}^+$ , mass 61 by  $^{24}\text{Mg}^{37}\text{Cl}^+$  and mass 62 by  $^{23}\text{Na}_2^{16}\text{O}^+$  and  $^{39}\text{K}^{23}\text{Na}^+$ . These are only a few of the possible combinations which cause background peaks, but they illustrate the complexity of the problem at low ion-counting rates.

TABLE II

CONCENTRATIONS OF NORMAL NICKEL AND Ni-62 TRACER  
MEASURED IN THE MARINE ORGANISM NICKEL UPTAKE EXPERIMENT

Sample	Exposure Time (days) ((date sampled))	Weight <sup>(1)</sup> (g.)	Normal Ni		Excess Ni-62 (Upper limit)**	
			µg	ppm	µg	ppm
Starfish	21 (12/18/79)	2.03	25*	12.3*	0.12**	0.06**
Starfish	108 (3/14/80)	2.04	27*	13.2*	0.89**	0.43**
Starfish	5 (3/30/80)	2.53	5*	2.0*	0.03*	0.01**
Starfish	13 (4/7/80)	2.11	8*	3.8*	0.49**	0.23**
Starfish	Unexposed control	2.0	6*	3.0*	0.06*	0.03*
Sediment	203 (7/1/80)	0.963	MS 26 IMMA 19	26.8 19.4	0.36** 0.22**	0.37** 0.23**
Sediment	203 (7/1/80)	0.597	#1 5.7 #2 4.8	9.5 8.1	0.06** 0.08**	0.11** 0.13**
Exp. tank water	203 (7/1/80)	19.8 1 (~19,800 g)	1013	0.051	165	0.0083
Respike 500 λ of 25 ml	203 (7/1/80)	19.8 1	1266	0.064	84.5	0.0043
Control tank water	203 (7/1/80)	18.3 1 (~18,300 g)	134	0.0073	---	< 10 <sup>-5</sup>
Respike 5 ml of 25 ml	203	18.3	127	0.0069	---	< 10 <sup>-5</sup>
Foil holder leach	203	---	7.6	---	63.4	---
Reagent blank for water samples	---	---	0.07	---	---	---

(1) Starfish weights are freeze-dried weights. Wet to dry ratio is approximately 1.6.

\* Observed normal Ni minus a reagent blank of 6.7 µg.

\*\* The amount or concentration of Ni-62 indicated as being present over and above the Ni-62 associated with the natural nickel measured in the sample. Due to possible contributions from interfering ions (see text), the fraction of indicated excess Ni-62 that may actually be due to Ni-62 is not known.

These ions were identified as causing potential background problems by analyzing in the ion probe synthetic sea salt which contains Ca and Mg, and by analyzing a salt mixture which contains KBr and NaCl. The magnitude of this background is variable from sample to sample.

#### DISCUSSION AND CONCLUSIONS

The available data suggests that the uptake of nickel occurred very slowly, if at all. This is in agreement with test data currently being obtained elsewhere (Reference 1), utilizing shallow water species and irradiated stainless steel coupons, wherein measured uptake of Co-60 in marine organisms was equivalent to less than 0.001 ppm after an equivalent 3.5 month exposure. A comparison of the stainless steel and Ni-62 coupon experiments suggests that the three and one-half month Ni-62 uptake may only be of the order of 0.001 ppm.

Available techniques only allow an upper limit to be placed on the amount of Ni-62 uptake in any starfish analyzed in the ion probe. Due to interfering ions at mass positions of interest, one cannot ascertain the amount of actual Ni-62 that may be present. Chemically removing the alkalis, alkaline earths, and halogens should improve attainable detection limits for Ni-62 tracer and allow more definitive statements to be made concerning the amount of Ni-62 that actually may be present. To do this would require an improved technique for separating nickel from starfish. In any event, the data support the conclusion that the uptake and bioutilization of nickel corrosion products by an asteroid similar to those found in the deep ocean proceeds very slowly if at all.

The goal of this project was to demonstrate the utility of Ni-62 as a tracer and the uptake of nickel by the mudstar *Ctenodiscus crispatus*. In both cases, the experiments were not completely conclusive. Some slight nickel uptake may have occurred. The use of Ni-62 as a tracer seems questionable at this time for two reasons: the present difficulty of separating the organismic nickel from other similar mass units; and the necessary time of exposure to the nickel foil. The technical problem of nickel separation can probably be solved for a given amount of effort, but the question of exposure time is more basic.

*Ctenodiscus* is a relatively large, active species, and yet after over 100 days of exposure the present analytical techniques do not allow definitive statements about uptake to be made. An exposure time of a full year or more may be necessary to get accurate results. Many of the species of most interest in the deep-sea are considerably smaller and/or less active. This indicates that an even longer exposure time might be required and a disproportionate amount of manpower would have to be expended in aquarium maintenance to produce little data. Indeed, it is questionable if most deep-sea species can be maintained in an artificial system for more than a short time period without major commitments of financial resources. Furthermore, required exposure time on the order of months approach or surpass the generation time of many species, especially in shallow water forms, and hence any accumulation could be recycled in nature before it is detectable.

It is apparent that the present experimental design is unable to provide satisfactory answers to the research objectives. We suggest a shift

towards the use of the radioactive tracer Ni-63 in laboratory experiments conducted at the Bigelow Laboratory and the use of the stable isotope Ni-62 in *in situ* experiments placed on the Hatteras Plain. Ni-63 (in the foil form) can be used in experiments designed to produce rapid and easily duplicated evidence for nickel uptake by benthic species. The placement of Ni-62 foil at selected locations at abyssal depths (approximately 2000 m) will provide evidence for *in situ* corrosion and uptake of Ni-62 over an extended period of time (6 months or more intervals).



Reference 1 - "Benthic Boundary Layer Program - Interim Report: Biological Fate of Corrosion Products Released from Radioactive Stainless Steel," J. S. Young, Battelle - Marine Research Laboratory, January 1981.