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D. CHAKRAVARTI, G. B. LEWIS, Prof. R. F. PALUMBO

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ANALYSES OF RADIONUCLIDES OF BIOLOGICAL INTEREST IN PACIFIC WATERS

By D. CHAKRAVARTI, G. B. LEWIS, R. F. PALUMBO
and A. H. SEYMOUR

Laboratory of Radiation Biology, University of Washington,
Seattle

INVESTIGATIONS of the distribution of radionuclides in marine environments have been made by the Laboratory of Radiation Biology, University of Washington, since 1946. These investigations have included radiochemical analyses of marine samples from the western and central Pacific, the north-eastern Pacific and the Arctic Ocean (Chukchi Sea). The analyses discussed here are those of samples collected recently in the Pacific Ocean. One group of samples was collected in the vicinity of Christmas Island during the 1962 tests of nuclear devices by the United States; another group was taken from near the mouth of the Columbia River where the effluent, carrying radioactive wastes from the Hanford reactors, mixes with the Pacific Ocean; and one sample was taken 250 miles off the Oregon coast.

Radionuclides from both natural and artificial sources occur in these waters. The most abundant and easily detected naturally occurring radionuclide is potassium-40. Other natural radionuclides to be expected in sea-water are radium, uranium, thorium and their decay products. Most of the artificial radionuclides generally present in the oceans are added as fall-out from nuclear detonations. The fall-out nuclides of biological interest include the fission products: strontium-89, 90, zirconium-95-niobium-95, ruthenium-103, 106, caesium-137 and cerium-141, 144; and the induced radionuclides: manganese-54, iron-55, 59, cobalt-57, 58, 60 and zinc-65. The induced radionuclides phosphorus-32, chromium-51 and zinc-65, carried to the sea by the Columbia River, are produced by neutron activation of elements in the water used to cool the Hanford reactors.

Radionuclides in sea-water may be concentrated many thousand-fold by plankton. However, plankton constitutes only a minute fraction of the total water mass, hence the amount of radioactivity in the total water mass is greater than in the total plankton.

The concentration of radioactivity by plankton provides a convenient method for determining the presence, but not the amounts, of radionuclides in sea-water. A quantitative measurement can be made only by water

analysis. The measurement of radionuclides in sea-water is difficult because of the necessity of separating and concentrating atom quantities of the nuclides from gram quantities of sea salts. β -emitting radionuclides, such as phosphorus-32 and strontium-90, require complete separation of the nuclides from the salts to reduce the absorption of β -particles by the sample. Photons from γ -emitting radionuclides, such as chromium-51, zinc-65 and zirconium-95-niobium-95, are not absorbed in the sample; but the amount of salt in a sample large enough to contain a measurable quantity of a radionuclide usually is so great that the sample geometry is reduced to an unacceptable value. Therefore, radionuclides for γ -spectrum analysis also are separated from the salt.

Our purpose here is to discuss methods used at this Laboratory for the analyses of radionuclides in sea-water and to present the results of these analyses. The methods and results of analyses of Christmas Island samples have been reported by Welander and Palumbo¹, and those of the Columbia River samples are reported by Seymour and Lewis²; however, they are reviewed here to present a summary of the Laboratory's methods and results. In addition, values for strontium-90, as determined by other investigators, have been included to indicate the world-wide distribution of this radionuclide in the surface waters of the oceans.

Collection and preparation procedures. Surface sea-water samples were collected from three areas in the Pacific Ocean: in the vicinity of Christmas Island (approximately 2° S.-2° N. latitude and 153° W.-168° W. longitude); within 20 miles of the Columbia River (approximately 46° N. and 124° W.); and 250 miles off the Oregon Coast (approximately 45° N. and 130° W.).

The Christmas Island samples were collected during the summer of 1962 and the samples taken near the Columbia River were collected during January 1961-June 1962. 10 l. of sea-water were filtered using 47-mm HA-type 'Millipore' filters with 0.45 μ pore size. After filtering and chemical separation, the samples were analysed for γ -emitting radionuclides. The Christmas Island samples were analysed also for strontium-90.

On March 20, 1963, a 100-l. sample was collected off the Oregon coast. This sample was divided into 50-l. aliquots which were analysed by different methods for strontium-90. The particulate matter was separated from the water by centrifugation, rather than by filtration, using an SS-4 Servall enclosed superspeed centrifuge with the Szent-Györgyi continuous-flow system. At 17,000 r.p.m., the flow-rate was 200 ml./min. This system was about 30 per cent as effective as the 'Millipore' filter in removing the particulate matter.

Chemical separation. A hydroxide precipitation method

was used for the samples taken from near the Columbia River mouth, and a sulphide precipitation method adapted from Sood, Golden and Velten³ was used for the Christmas Island samples. The sulphide method was preferred because it proved to be simpler and equally reliable.

Hydroxide precipitation method. Carriers of manganese, cobalt, iron, zinc and chromium (200 mg each) in their chloride form were added to 10 l. of filtered sea-water sample. Under constant stirring, a predetermined amount of concentrated ammonium hydroxide was added drop-wise and the precipitate was allowed to settle for 15 min. Then a few more drops of ammonium hydroxide were added to check the completeness of the precipitation. The amount of concentrated ammonium hydroxide required for complete precipitation of a 10-l. sample varied with the salinity according to the equation:

$$V = 100 + 3.7S$$

where V is the volume of reagent in ml. and S is the salinity of the sample in parts per thousand. This relationship is based on observations in the range of 10-32 per cent. The precipitate was allowed to settle overnight or longer, after which time most of the clear supernate liquid was siphoned off. The remaining mixture was filtered through a Whatman-42 filter paper under vacuum. The precipitate was wet-ashed with concentrated nitric acid and 30 per cent hydrogen peroxide, dried in a 250-ml. 'Pyrex' beaker and counted. The elements present in greatest quantity in sea-water remain in the supernate. This fraction also was dried and counted. The chemical yields by the hydroxide precipitation method for manganese-54, cobalt-60, iron-59, zinc-65 and chromium-51 ranged from 89 to 100 per cent.

Sulphide precipitation method. Carriers of lanthanum, iron, cobalt, zinc, nickel, zirconium, ruthenium, cerium, caesium and strontium were added to 'Millipore'-filtered sea-water samples, with caesium and strontium serving as hold-back carriers. The sample was acidified to dissolve any suspended matter, heated to 80° C and the pH then adjusted to 11 with ammonium hydroxide. Hydrogen sulphide was bubbled slowly into the solution under constant stirring. After the solution had been saturated with hydrogen sulphide, it was taken off the heat and allowed to settle overnight. After separation from the supernate by filtering, the precipitate, containing ruthenium, cerium, cobalt, chromium, zinc, iron, zirconium and lanthanum, was placed in an eight-ounce plastic jar and counted. The percentage chemical yields by the sulphide precipitation method for zinc-65, cobalt-60, zirconium-95-niobium-95, cerium-144 and ruthenium-103 tags were in agreement with those of Sood *et al.*³ and ranged from 90 to 97 per cent. Strontium was retained in the supernate, as evidenced by less than 1 per cent

strontium-85 activity in the precipitate. As in the hydroxide method, potassium and caesium remained in the supernate with the bulk of the sea salts and no further chemical separation was made.

Strontium-90. The supernate from the foregoing procedure was acidified, hydrogen sulphide was driven off by heat and the pH was adjusted to 5 with ammonium hydroxide and acetic acid. Strontium was co-precipitated with calcium as the oxalate using ammonium oxalate. The precipitate, after filtering, was wet-ashed with nitric acid and hydrogen peroxide. The ash residue was re-precipitated in 80 per cent nitric acid to reduce the calcium-strontium ratio. The strontium nitrate thus obtained was dissolved in 0.2 N hydrochloric acid and the resulting solution was passed on to a 'Dowex 50W X-8' (100-200 mesh) ion-exchange resin column. After rinsing with 0.2 N hydrochloric acid to remove any unadsorbed contaminants, 0.5 per cent oxalic acid was added to remove radioiron. The column was stripped off yttrium-90 as yttrium citrate complex with 5 per cent ammonium citrate at pH 3.5. Storage time for 2 weeks was allowed for the secular equilibrium between strontium-90 and yttrium-90 to be established. The column then was re-eluted with a minimum volume of ammonium citrate, the eluate acidified to break up the yttrium citrate complex and the resulting solution was passed on to a short-stemmed eyedropper column containing a 2-cm resin bed of 'Dowex 50W X-8' (100-200 mesh). When the yttrium-90 was adsorbed to the resin bed the citrate mass was removed with dilute acid (0.2 N nitric acid) wash. The yttrium-90 was stripped off the column with about 5 ml. of 6 N nitric acid on to a stainless steel planchet, dried and the relatively mass-free residue counted. This strontium-90 method was adapted from a procedure developed by Kawabata and Held⁴.

Strontium-90 was determined in the sea-water from off the Oregon coast by this method and also by the method of Miyake, Saruhashi and Katsuragi⁵. In the latter method an oxalate precipitation of calcium and strontium is used, followed by EDTA-masking of calcium and recovery of yttrium-90 as yttrium hydroxide after secular equilibrium is reached.

Phosphorus-32. The method of extracting phosphorus-32 from sea-water was suggested by the work of Proctor and Hood⁶ and Murphy and Riley⁷. Sea-water was placed in a separatory funnel and allowed to react with a composite reagent containing ammonium molybdate, sulphuric acid, ascorbic acid and potassium antimony tartrate⁷. Seven parts of *N*-butanol were added to 150 parts of sample, and the solution was made more acidic to favour extraction of the molybdenum blue. After extraction the organic layer was backwashed with dilute sulphuric acid and dried under low heat. The residue was dissolved in a

v in the precipitate. As in the potassium and caesium remained in the bulk of the sea salts and no fraction was made.

supernate from the foregoing procedure hydrogen sulphide was driven off and was adjusted to 5 with ammonium acid. Strontium was co-precipitated as calcium oxalate using ammonium oxalate. The precipitate was washed with nitric acid. The ash residue was re-precipitated with nitric acid to reduce the calcium-strontium nitrate thus obtained with hydrochloric acid and the resulting solution on to a 'Dowex 50W X-8' (100-200 mesh) resin column. After rinsing with hydrochloric acid to remove any unadsorbed calcium oxalic acid was added to remove strontium was stripped off yttrium-90 as a complex with 5 per cent ammonium hydroxide. Storage time for 2 weeks was allowed to reach equilibrium between strontium-90 and yttrium-90 and established. The column then was washed with a minimum volume of ammonium citrate, to break up the yttrium citrate complex solution was passed on to a short column containing a 2-cm resin bed (100-200 mesh). When the yttrium-90 was on the resin bed the citrate mass was washed off with 0.2 N nitric acid wash. The column was stripped off the column with about 5 ml. of 0.2 N nitric acid, dried and counted on a stainless steel planchet, dried and the residue counted. This strontium-90 was determined from a procedure developed by

determined in the sea-water from this method and also by the method of Katsuragi⁵. In the latter method the separation of calcium and strontium is achieved by EDTA-masking of calcium and strontium as yttrium hydroxide after secular equilibrium.

The method of extracting phosphorus-32 as suggested by the work of Proctor and Riley⁷. Sea-water was placed in a beaker and allowed to react with a combining ammonium molybdate, sulphuric acid and potassium antimony tartrate⁷. Ethanol were added to 150 parts of solution was made more acidic to favour phosphorus-32. After extraction the residue was washed with dilute sulphuric acid and dried. The residue was dissolved in a

Table 1. RADIONUCLIDES IN FILTERED SURFACE SEA-WATER FROM TWO AREAS OF THE PACIFIC OCEAN (VALUES IN pc./l.)

Radio-nuclide	Mouth of Columbia River			Christmas Island*		
	N/n†	Average	Maximum	N/n†	Average	Maximum
	January-September 1961					
⁵¹ Cr	10/25	150	770			
⁶⁵ Zn	15/25	21	350			
	November 1961-June 1962			May-July 1962		
⁵¹ Cr	7/9	580	1,300			
⁶⁵ Zn	8/9	5.1	12	4/24	1.3	13
⁹⁰ Zr- ⁹⁰ Nb	7/9	4.5	16	11/24	16	71
¹⁰³ Ru	5/9	0.79	2.5	4/24	3.7	32
^{141,144} Ce	9/9	9.4	27	13/24	10	46
¹³⁷ Cs				3/24	0.62	11
⁶⁰ Co				2/24	0.38	7.0

* From ref. 1.

† N/n, Number of samples with radioactivity greater than background plus the 95 per cent counting error/No. of samples analysed.

minimum amount of aqua regia and again evaporated to dryness. The residue was dissolved in 50 ml. of 15 per cent hydrogen peroxide and made up to 2 N in hydrochloric acid. This mixture was added to a separatory funnel and the molybdate extracted with an equal volume of tri-butyl phosphate. The organic layer was discarded and the aqueous layer extracted again with an equal volume of tri-butyl phosphate. The aqueous layer was then dried on a steel planchet and counted for phosphorus-32. (A more complete description of the method will be reported by Chakravarti, Tuell and Larson⁸.)

Counting systems. The γ -counting system included a 3-in. solid crystal of sodium iodide, thallium activated, used in conjunction with a 512-Nuclear Data 120 analyser with an X-Y recorder and a digital print-out. Calculations were carried out on an IBM 709 digital computer programmed to analyse for nine isotopes and to express results in picocuries ($\mu\text{c.}$) per g or per l. at time of collection. Only values which were greater than the background plus the 95 per cent counting error were considered significant; all other values were considered to be zero.

Two types of low-background Geiger-Müller counters were used, one for yttrium-90, another for phosphorus-32. The systems included sufficient massive shielding and cosmic-ray guard tubes to reduce the background to approximately 1.5 and 1.0 counts per min. respectively. The counting efficiencies were approximately 25 per cent for yttrium-90 and 40 per cent for phosphorus-32. All counts were standardized and the results are expressed in picocuries per litre (pc./l.) at the time of collection.

The average and maximum values of radionuclides, except those of strontium-90, potassium-40 and phosphorus-32, in sea-water samples are given in Table 1. This Table is a summary of the results of analyses of

Table 2. STRONTIUM-90 IN SURFACE SEA-WATER (VALUES IN pc./l.)

Ref.	Location and dates	No. of samples analysed	Range of values
--	250 miles off Oregon coast, January 1962	1	0.04
1	Christmas Island, January 1962	5	0.03-0.2
10	Atlantic Ocean and Caribbean Sea, 1956-60	60	0.031-0.30
11	Chukchi Sea, 1959-62	7	0.095-0.27
12	Pacific and Indian Oceans, 1960-61		
	6°-40° N	25	0.1-0.9
	0°-40° S	8	0.06-0.1
	60° S (Antarctic)	2	0.01-0.02
9	Western North Pacific Ocean, 1958-60	15	0.6-3.1

samples collected at various times during the period indicated. The appearance of mixed fission products after September 1961 in the samples from the mouth of the Columbia River is the result of fall-out, presumably from the nuclear tests by the U.S.S.R. in October 1961.

Chromium-51, produced in the Hanford reactors, was the most abundant radionuclide in the coastal waters near the mouth of the Columbia River, where it is of interest to the oceanographer as a tag of Columbia River water and sediments. Chromium-51 was neither expected nor detected by visual inspection of the γ -spectra of the Christmas Island water samples.

The two values for strontium-90 from the single sample of sea-water from off the Oregon coast analysed by both the method of Miyako, Saruhashi and Katsuragi⁹ and the modified Kawabata-Held method² described here were 0.037 ± 0.003 (95 per cent counting error) and 0.041 ± 0.009 pc./l., respectively. The latter was the faster of the two methods and produced a sample of lesser mass. The reduction of mass was from several grams to a few milligrams. The values for strontium-90 in surface sea-water from off the Oregon coast and from other areas round the world ranged from 0.01 to 3.1 pc./l. and are presented in Table 2. This radionuclide seems to be ubiquitous in surface waters since no zero values are given in these reports.

Table 3. PHOSPHORUS-32 IN FILTERED SURFACE SEA-WATER FROM NEAR THE MOUTH OF THE COLUMBIA RIVER, JULY 1963 (VALUES IN pc./l. \pm S.D.)

Station	Miles from mouth	Salinity (parts per thousand)	No. of determinations	³² P
63	30	31.76	3	3.57 \pm 0.60
69	24	31.63	1	5.15
74	18	27.80	3	8.03 \pm 0.58
75	9	27.74	3	4.68 \pm 1.63

Values for phosphorus-32 in the sea-water near the Columbia River mouth are given in Table 3. These values ranged from 3.6 to 8.0 pc./l. To our knowledge no other values for phosphorus-32 in sea-water have been reported.

In order to compare the radioactivity from naturally occurring and artificial radionuclides in sea-water, the values for potassium-40 were determined as well as the radionuclides discussed here. For samples collected near the mouth of the Columbia River, these values ranged from 190 to 370 pc./l. and were related to the salinity values. The calculated value for potassium-40 in sea-water of 35 parts per thousand salinity is 330 pc./l.

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