The Loss of Radionuclides in Marine Organisms during Thermal Decomposition

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ABSTRACT

The losses of $^{137}$Cs, $^{106}$Ru-$^{106}$Rh, $^{65}$Zn, $^{60}$Co, $^{54}$Mn, $^{95}$Zr-$^{95}$Nb and $^{144}$Ce from the tissues of bivalves and green algae during dry ashing were investigated under the conditions of the temperature of 450, 550 or 800°C and the ignition time of 12, 24, or 48 hours.

Samples were prepared by rearing the organisms in sea water containing each radionuclide described above.

Biological tissues were decomposed in an electric muffle furnace. Recoveries of individual radionuclides in the tissues were calculated by the radioactivity ratio of pre- and post-ashing of samples. Under the condition of 450°C, ignition was not complete in 24 hours for bivalves and in 12 hours for green algae.

The results of this investigation indicated that $^{137}$Cs, $^{106}$Ru-$^{106}$Rh, $^{65}$Zn and $^{60}$Co showed the loss in some ashing conditions and that the manners of the loss of these radionuclides were different between the organisms. $^{54}$Fe, $^{95}$Zr-$^{95}$Nb and $^{144}$Ce were not significantly lost under any condition used in this experiment. Accordingly, the loss of $^{137}$Cs, $^{106}$Ru-$^{106}$Rh, $^{65}$Zn and $^{60}$Co might be correlated with the biological matrix in which these radionuclides were incorporated, while there seemed to be no effect of biological matrix to the ashing loss of $^{54}$Fe, $^{95}$Zr-$^{95}$Nb and $^{144}$Ce.

The most desirable ashing condition in our investigation was 450°C, 48 hours for $^{137}$Cs and 550°C, 12 hours for other radionuclides in bivalves, and 450°C, 24 hours for $^{137}$Cs, $^{106}$Ru-$^{106}$Rh and $^{65}$Zn, and 550°C, 12 hours for other radionuclides in green algae.

INTRODUCTION

For determination of the trace amount of radionuclides in marine organisms, decomposition of the biological tissues is done commonly. In general, when the...
digestion at relatively low temperature is required to prevent volatilization loss, the wet ashing\textsuperscript{1-4} and low temperature dry ashing\textsuperscript{5,6} methods are commonly used. However, these methods are appropriate for treatment of rather small amounts of samples and not suitable for decomposing large volume of marine organisms. In our present works, more than 10 grams of sample ash are required to determine the concentration of radionuclides by chemical analysis and 20 grams or more of ash for $\gamma$-ray spectrometry. In other words, it is necessary to digest more than 1 kilogram of raw material to obtain enough amounts of ash. Therefore, dry ashing at relatively high temperature has to be applied.

Gorsuch\textsuperscript{7}, Hamilton \textit{et al}\textsuperscript{8} and Green \textit{et al}\textsuperscript{9} reported that recoveries of $^{137}$Cs, $^{65}$Zn, Co and Fe were more than 90\% at the ashing temperature of 450-550°C \textit{in vitro} experiments. However, Strohal \textit{et al}\textsuperscript{4} indicated that recoveries of $^{106}$Ru-$^{106}$Rh, $^{65}$Zn, radioactive Co, $^{54}$Mn and $^{144}$Ce decreased to less than 90\% even at 450°C, \textit{in vivo} experiment using marine biological samples. If this phenomenon is common in any dry ashing condition, the results of the analysis of radionuclides in marine organisms in use of dry ashing method should be corrected for ashing recovery.

In this article, the results of \textit{in vivo} experiment on the loss of radioactive tracers in relation to temperatures and times on ashing are reported. Volatilization of $^{137}$Cs, $^{106}$Ru-$^{106}$Rh, $^{65}$Zn, $^{60}$Co, $^{54}$Fe, $^{54}$Mn, $^{95}$Zr-$^{95}$Nb and $^{144}$Ce from soft tissues of bivalve and green algae during ashing was investigated.

**MATERIALS AND METHODS**

Radionuclides used and their chemical forms were as follows: $^{137}$Cs, $^{65}$Zn, $^{60}$Co, $^{54}$Fe, $^{54}$Mn and $^{144}$Ce as chloride, $^{106}$Ru ($^{106}$Rh) as nitrosylruthenium nitrato complexes and $^{95}$Zr ($^{95}$Nb) as zirconium oxalate.

Each radionuclide was added to an aquarium, containing 8 liters of sea water and equipped with an aeration system. After the level of radioactivity in the sea water reached an equilibrium, 10-20 bivalves (\textit{Tapes japonica}) and 10-20 grams of green algae (\textit{Ulva pertusa}) were cultured without feeding for a week at 20-25°C. Organisms used were decontaminated by keeping them in fresh sea water for a night. Soft tissue of bivalves was separated from their shell and sea water was removed from green algae by filter paper.

One to two grams of bivalve soft tissue (8500-56000 cpm) was put into a plastic test tube. After the measuring the $\gamma$-radioactivity with a well-type, single-channel scintillation counter, the tissue was transferred into a porcelain crucible (30 mL) with cap, and the remained radioactivity in the test tube was counted. Radioactivity before ignition was determined by subtracting the activity remaining in the test tube from the total activity.

The activity of 0.5 gram of green algae (18000-65000 cpm) was counted directly in the crucible with a pulse-height analyzer with 2$\pi$-scintillation detector.

Samples in the crucible were dried at 110°C in an air bath for 12 hours and ashed at 450, 550 or 800°C, for 12, 24 or 48 hours in an electric muffle furnace. The
fluctuation of thermal distributions in the muffle furnace was within ±15°C at each temperature. After ignition, radioactivity of green algae was counted in the same manner as before ashing. The bivalve sample was transferred into the plastic test tube with about 2 ml of concentrated hydrochloric acid and distilled water was added to achieve the same geometry on initial counting and then the radioactivity was determined. The radioactivities remained in the crucible were measured by a radioactivity survey meter, but were under the sensitivity limit (200 cpm) of the survey meter and therefore were negligible in comparison with the experimental error. Ignition experiment under each condition was performed three times and the average of them was shown.

RESULTS

Recoveries (%) of radionuclides after ignition are shown in Table 1. Cs: Recovery of $^{137}$Cs was decreased to under 90% at 550°C, even in 12 hours, and showed extremely low values at 800°C on both organisms. At 450°C it was in 24 hours that green algae had recovery higher than 90%, whereas, the bivalve kept the higher value in 48 hours. Ru: $^{106}$Ru-$^{106}$Rh in both organisms remained quantitatively in 48 hours at 550°C, but was lost considerably at 550°C, except within 12 hours for

| Radionuclide | Temp. | 450°C | 550°C | 800°C |
|--------------|-------|-------|-------|-------|
|              | hr.   | 12    | 24    | 48    | 12    | 24    | 48    | 12    | 24    | 48    |
| $^{137}$Cs   | T.    | 98± 7 | 95± 6 | 92± 1 | 73± 7 | 41± 3 | 34± 3 | 5*    | 4*    | 4*    |
|              | U.    | 94± 9 | 91± 3 | 72± 8 | 78± 6 | 74± 5 | 79± 8 | 0*    | 0*    | 0*    |
| $^{106}$Ru-$^{106}$Rh | T.    | 104± 9 | 95± 9 | 106± 6 | 99± 5 | 80± 4 | 78± 9 | 18± 2 | 21± 2 | 3*    |
|              | U.    | 93±11 | 98±12 | 93± 8 | 72± 6 | 70± 3 | 65± 7 | 35± 4 | 20± 1 | 14± 1 |
| $^{65}$Zn    | T.    | 92± 5 | 100± 6 | 98± 3 | 91± 4 | 93± 9 | 92± 2 | 88± 4 | 71± 7 | 68± 7 |
|              | U.    | 104± 4 | 100± 8 | 93± 8 | 80± 1 | 75± 8 | 87± 3 | 71± 6 | 57± 5 | 61± 8 |
| $^{54}$Co    | T.    | 97± 8 | 94±11 | 92± 7 | 96± 7 | 95± 4 | 88± 2 | 54± 6 | 42± 5 | 38± 4 |
|              | U.    | 91± 9 | 106± 8 | 97± 4 | 97± 7 | 94± 9 | 97±10 | 90± 8 | 96± 7 | 95± 8 |
| $^{59}$Fe    | T.    | 99± 2 | 101± 10 | 97± 6 | 97± 9 | 101± 11 | 90± 2 | 102± 7 | 95± 8 | 100± 9 |
|              | U.    | 100± 3 | 106±14 | 96±10 | 98± 4 | 93± 6 | 101± 8 | 108± 6 | 95± 7 | 90±10 |
| $^{54}$Mn    | T.    | 95± 7 | 96± 5 | 98± 7 | 94± 6 | 95± 10 | 97± 7 | 95± 7 | 93± 4 | 91± 6 |
|              | U.    | 109± 13 | 94± 9 | 95± 3 | 101± 8 | 101± 9 | 92± 3 | 98± 5 | 99± 5 | 98± 6 |
| $^{95}$Zr-$^{95}$Nb | T.    | 93± 8 | 97± 6 | 103± 8 | 91± 5 | 97± 6 | 93±10 | 95±10 | 93±11 | 91± 7 |
|              | U.    | 96±10 | 97±10 | 94± 8 | 99± 9 | 90± 7 | 94± 5 | 90± 6 | 90± 9 | 93± 8 |
| $^{141}$Ce   | T.    | 109±12 | 101± 6 | 98± 6 | 101±11 | 100± 4 | 102± 4 | 97± 4 | 91± 6 | 96± 9 |
|              | U.    | 109±11 | 101± 8 | 98±10 | 96± 8 | 99± 7 | 98± 9 | 94±11 | 94± 7 | 96± 5 |

Table 1. Recoveries (%) of radionuclide in marine organisms after dry ashing.

T: Tapes japonica, U: Ulva pertusa

* standard deviation is less than 1.
bivalves, and resulted in very low recovery at 800°C. Zn: Recovery of $^{65}$Zn was over 90% on both organisms at 450°C, and showed the same results at 550°C for bivalves, but was less than 90% for green algae under any condition except at 450°C. Co: Recovery of $^{60}$Co for ashed bivalves was more than 90% in any ashing period at 450°C, but only in 24 hours at 550°C, and that for green algae was higher than 90% under any condition. Others: Recoveries of $^{59}$Fe, $^{54}$Mn, $^{95}$Zr-$^{95}$Nb and $^{144}$Ce were more than 90% under any ashing condition used in this experiment. Under the condition of 450°C, ignition was not complete in 24 hours for bivalves and in 12 hours for green algae.

Relative standard deviations of the values of recovery ranged between 1.3% and 13%, with average of 8%.

Considering the experimental and counting error, the ashing conditions giving recoveries more than 90% could be satisfactory for practical determination of radioactivity in marine organisms, and are summarized in Fig. 1. Solid lines show the ashing condition with recovery higher than 90%, and dotted lines indicate incomplete ignition, i.e., for bivalves 24 hours and for green algae 12 hours at 450°C.

From the viewpoints of the available recovery and relatively short time of ignition, it is considered that the conditions indicated by open circles (O) in Fig. 1 are satisfactory to digest the marine organisms.

| Radionuclide | Temp | 450°C | 550°C | 800°C |
|--------------|------|-------|-------|-------|
|              | hr.  | 12    | 24    | 48    | 12    | 24    | 48    | 12    | 24    | 48    |
| $^{137}$Cs   | T.   | U.    |       |       |       |       |       |       |       |       |
| $^{106}$Ru-$^{106}$Rh | T. | T. | U.   | U. | U. | U. |
| $^{65}$Zn    | T.   | U.    |       |       |       |       |       |       |       |       |
| $^{60}$Co    | T.   | U.    |       |       |       |       |       |       |       |       |
| $^{59}$Fe    | T.   | U.    |       |       |       |       |       |       |       |       |
| $^{54}$Mn    | T.   | U.    |       |       |       |       |       |       |       |       |
| $^{95}$Zr-$^{95}$Nb | T. | T. | U.   | U. | U. | U. |
| $^{144}$Ce   | T.   | U.    |       |       |       |       |       |       |       |       |

T: *Tapes japonica*  
U: *Ulva pertusa*

Fig. 1. Conditions in which recoveries are more than 90%.
DISCUSSION

From the results of this investigation, it was evident that no \(^{56}\)Fe, \(^{54}\)Mn, \(^{95}\)Zr-\(^{95}\)Nb and \(^{144}\)Ce in marine organisms were lost by ignition at 800°C, 48 hours, but \(^{137}\)Cs, \(^{106}\)Ru-\(^{106}\)Rh, \(^{65}\)Zn and \(^{60}\)Co showed the possibility of loss in some conditions used in this experiment and the aspects of the loss of these radionuclides were different between both organisms.

The factors, which might affect the loss of radionuclides in the ashing procedure, are temperature, ignition time, chemical state of radionuclides and biological matrix, among which the chemical state in biological materials, and the composition and amount of matrix are generally unknown prior to ashing treatment. Consequently, the results of in vivo experiments are occasionally not agreeable with those of in vitro experiments and in vivo experiment should be necessary to know the loss of radionuclides during ignition\(^{3,8-11}\). Moreover, Martin et al\(^{10}\) referred to that as difference in volatility from in vivo labelled tissue might even occur when the duration of exposure or the route of administration is varied. Therefore, a meaningful study of radionuclide volatility from biological media requires a supply of appropriately labelled tissues, so it might be most desirable that, in sample preparation for this ashing experiment, marine organisms take the radionuclides through the rearing sea water gradually for a week.

Strohal et al\(^{4>}\) suggested that since a radionuclide, such as \(^{65}\)Zn, was not incorporated into the same structural position in different biological tissues, each sample must be examined individually for the ashing loss. However, in another of our ashing experiments using in vivo samples, recovery of \(^{137}\)Cs for carp muscle was 90% in 48 hours at 450°C and 67% in 12 hours at 550°C, therefore, the most desirable ashing condition of \(^{137}\)Cs for the tissue agreed with that for bivalve. On the other hand, for the radionuclides except \(^{56}\)Fe, adequate ashing condition of brown algae was the same as that of green algae, for example, recovery of \(^{137}\)Cs for brown algae was 90% in 24 hours, 57% in 48 hours at 450°C and 76% in 12 hours at 550°C. Accordingly, it seems to be that similar tissues of different organisms show the same aspect of the ashing loss.

On the consideration of the chemical state of radionuclides in the tissue, recoveries of \(^{56}\)Fe and \(^{54}\)Mn were similar to results of our previous report\(^{13}\) and of Gorsuch's in vitro\(^{7}\), and consequently, the loss of these radionuclides appeared to be independent from their chemical state in the tissue. However, recoveries of \(^{137}\)Cs and \(^{106}\)Ru-\(^{106}\)Rh in vivo were higher than those of in vitro experiments\(^{13}\). This discrepancy might be due to the cause indicated by Blincoel\(^{11}\) that cesium loss was caused primarily by the volatility of cesium chloride.

In relation to the matrix of biological tissues, as there were no differences between recoveries of \(^{56}\)Fe, \(^{54}\)Mn, \(^{95}\)Zr-\(^{95}\)Nb and \(^{144}\)Ce from both organisms, recoveries of these radionuclides might be free from the biological matrix\(^{12}\), whereas, there seemed to be the influence of biological matrix to the ashing loss of \(^{137}\)Cs, \(^{106}\)Ru-
$^{106}$Rh, $^{65}$Zn and $^{60}$Co.

It was reported that $^{65}$Zn and Co were hardly lost at a temperature of approximately 550°C and $^{65}$Zn was not also volatiled at 450–550°C in vitro experiments, but these were lost even at 550°C from both organisms in vivo experiment, therefore, as suggested by Strohal et al, "The existence of compounds that vaporize at low temperatures confirms the presence of chelate compounds", might be appropiable from the different results obtained by authors' (in vivo) and formers' (in vitro) experiments. On the contrary, the loss of $^{137}$Cs in vitro experiment in which $^{137}$Cs was spiked to the sample in chloride form, was larger than that in this experiment in vivo, and consequently, it seemed to be that $^{137}$Cs was lost in the inorganic chemical state rather than chelated one, and the loss of $^{106}$Ru–$^{106}$Rh also depended on the formation of inorganic compound. $^{59}$Fe, $^{54}$Mn and $^{144}$Ce could be considered not to be chelated or to be mineralized by heat during ashing procedure even in case of chelated compound formation.

In our investigation, the radionuclide losses by retention on the inside wall of crucibles were negligible in comparison with relative standard deviations. Hamilton et al described that Zn inclined to be retained by the porcelain crucible, but our results on the retention of $^{65}$Zn was comparable with that of Gorsuch's and the retention of $^{137}$Cs was similar to the result by Green et al.

It was reported that variations both of material and the history of the crucibles could alter the amount of lead retention. Although, in our experiments, new porcelain crucibles were used and particular attention was not given to the material and the history of the crucibles, such large retentions were not observed for radio- nuclides used in our experiments.

From the results described above, the authors concluded that biological tissues, such as bivalves and green algae could be ignited by use of the dry ashing method without any loss of radionuclides in these organisms.

In our experiences, even 2 kilograms of raw algae or soft tissues of bivalves were completely ignited under these ashing conditions, nevertheless, position and bulkiness of samples in crucible were varied. Therefore, it might be concluded that these conditions are satisfactory for the ignition of large volume of sample.

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