

Bioaccumulation of  $^{106}\text{Ru}$  by marine Phytoplankton

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## ABSTRACT

The chlorophyte *Dunaliella tertiolecta* and the diatom *Thalassiosira pseudonana* readily accumulate  $^{106}\text{Ru}$  reaching volume concentration factors of roughly  $10^5$  and  $10^6$ , respectively. The uptake process is passive and is most likely related to surface sorption. High uptake by live and dead cells (phytodetritus) indicate that phytoplankton were likely initial vectors in the rapid vertical transport of  $^{106}\text{Ru}$  noted after the arrival of Chernobyl fallout.

## INTRODUCTION

The radioisotopes of ruthenium, particularly  $^{103}\text{Ru}$  and  $^{106}\text{Ru}$ , are considered among the more important radioactive contaminants because of their relatively high yield from fission and their moderately long half-lives. Ruthenium-106 ( $T_{1/2} = 373$  days) has entered the marine environment primarily as fallout from previous nuclear tests and in waste effluents from several nuclear reprocessing plants. Furthermore, the recent accident at Chernobyl resulted in a major input of  $^{106}\text{Ru}$  to marine waters (Fowler et al., 1987).

The behaviour of ruthenium in sea water is complex (IAEA, 1975) and a large fraction of the radionuclide concentration is associated with particulate matter (Coughtrey and Thorne, 1983). The relatively high reactivity of  $^{106}\text{Ru}$  results in its being readily accumulated by a variety of marine organisms including phytoplankton. Data from recent Chernobyl fallout studies (Fowler et al., 1987; Kempe and Nies, 1987) have suggested that phytoplankton is probably the vector by which  $^{106}\text{Ru}$  enters the pelagic food chain and is subsequently transported vertically through the water column. Since information on the mechanisms controlling  $^{106}\text{Ru}$  uptake by phytoplankton species is limited, a series of experiments were undertaken to examine  $^{106}\text{Ru}$  bioenergetics in two common species of phytoplankton.

## METHODS AND MATERIALS

The chlorophyte *Dunaliella tertiolecta* (clone DUN) and the diatom *Thalassiosira pseudonana* (clone 3H) were used in all experiments. Different concentrations of cells in mid to late log phase were exposed to  $^{106}\text{Ru}$  (as  $\text{RuCl}_2$ ) in the light and dark under rigorously controlled conditions similar to those described previously for other radionuclides (Fisher et al., 1983). In addition, heat-killed cells of both species were exposed in parallel with live cultures to examine the effect of metabolism on uptake. All culture conditions, filtration techniques, counting procedures and computation of volume concentration factors were identical to those employed by Fisher et al. (1983) for these species.

## RESULTS AND DISCUSSION

The results of two sets of experiments each carried out in triplicate are presented in Table 1. It is clear that uptake by these two species is primarily a passive adsorptive process since there was virtually no difference in the accumulation pattern between live cells in the dark and dead cells. Furthermore, during the first 24 hours, uptake by live cells in the light was also identical to the other two treatments; however, between days 1 and 3 concentration factors decreased as cell density increased. This is most likely an effect of reduced uptake by biological dilution. Uptake was also noticeably higher in the green alga *Dunaliella* ( $\text{VCF} \approx 10^5$ )

Table 1. Bioaccumulation of  $^{106}\text{Ru}$  (Volume concentration factor  $\times 10^4$ )<sup>†</sup> over time in uptake experiments with *D. tertiolecta* (DUN) and *T. pseudonana* (3H).

A = alive; De = dead; L = light; D = dark

Species	Treatment	1 hr.	1 d.	2 d.	3 d.
(VCF $\times 10^4$ )					
3H	A L	0.174	2.55	1.72	1.10
3H	A D	0.112	5.65	9.85	14.0
3H	De	0.23	4.17	15.3	37.6
DUN	A L	0.95	36.8	25.1	16.8
DUN	A D	0.72	46.7	81.8	122
DUN	De	1.34	47.1	92.7	118

<sup>†</sup>VCF values are averages of two experiments with three replicates each.

than in the diatom *Thalassiosira* ( $\text{VCF} \approx 10^4$ ). This differs from the transuranic elements plutonium and americium with which the reverse is found (Fisher et al., 1983). In any event, the relatively high VCFs for  $^{106}\text{Ru}$  in these two species, which approach those reported for transuranics, indicate that  $^{106}\text{Ru}$  entering the sea would rapidly become associated with phytoplankton which could then be passed on to zooplankton grazing the cells. Thus, contaminated phytodetritus and zooplankton excreta would become prime vectors for rapidly moving ruthenium downward in the water column as was seen to occur immediately following the Chernobyl accident in both the Mediterranean (Fowler et al., 1987) and the North Sea (Kempe and Nies, 1987).

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Transfer of  $^{137}\text{Cs}$  across the Gills epithelial cells of the Crab *Carcinus mediterraneus*

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Apart from their function in osmoregulation and respiration the gills of marine organisms play an absorptive role in distributing a wide range of pollutants into the various tissues and organs. The role of the gills in radioactive caesium transport and distribution in Crustacea has been pointed out by Bryan (1961a,b), but the problem has not yet been solved in detail. Therefore, for a better understanding the transport of radioactive pollutant in epithelial gill cells we studied the transfer of Cs-137 across perfused isolated *Carcinus* gill preparations. Green crabs *Carcinus mediterraneus* Czern., were obtained from the central Adriatic near the island of Dugi Otok. Crabs were acclimated in diluted sea water ( $15 \times 10^{-3}$  salinity), fed and kept at a constant room temperature ( $16^\circ\text{C}$ ). In the experiments described 5 posterior gill pairs were collected for the perfusion technique. Experimental details were described by Lucu and Siebers, 1986; 1987. Radioactive caesium ( $3.7 \text{ kBq Cs-137/ml}$  diluted sea water) was used for experimental purposes. Afferent and efferent blood vessels were connected by polyethylene capillary tubes and preparation was fixed by neoprene block immersed in the bathing solution ( $20 - 40 \text{ ml}$ ). Identical diluted sea water ( $260 \text{ mM Na}^+$ ) to the bathing solution was perfused through the gills by a peristaltic pump. The transbranchial potential (TBP) between the haemolymph oriented gill side and the bathing medium was measured by an Ivell 1111 multimeter device. Cs-137 transport factor ( $\% \times 0.1 \text{ g}^{-1}$  fresh weight of gills) was calculated according to:

$$\text{Transport factor} = \frac{^{137}\text{Cs transported across epithelia}}{^{137}\text{Cs in bathing solution} \times W} \quad (1)$$

where W is the fresh gill weight in grams.

Effects of diuretic amiloride (Merck Sharp Dohme, Munich) on caesium fluxes from the apical (bathing side) to the basolateral haemolymph side (influxes) and in the opposite direction (effluxes) were studied (Table 1). Cs-137 and stable Cs effluxes are greater than influxes, showing that the basolaterally oriented gill side is more permeable to Cs-137 than the apically oriented one. Therefore, the Cs-137 transport factors are larger from the haemolymph side to the bathing side (effluxes) than the fluxes in the opposite direction (Table 1.). In addition, influxes and effluxes of Cs-137 (and stable caesium) was inhibited by amiloride ( $0.1 \text{ mM}$ ), added to the bathing solution. This relatively high concentration of amiloride showed a similar effect on  $\text{Na}^+$  inhibition from the apical site as reported by Lucu and Siebers, 1986. Since Cs have similar physico-chemical behaviour to K (Bryan, 1961) we suggest that Cs competes with K<sup>+</sup> for the K<sup>+</sup>/H<sup>+</sup> exchanger located on the apical membrane side. Since amiloride failed to affect this mechanism at the lower concentrations the presence of an ion channel interaction was precluded.

	Transport factor	TBP	Caesium flux
(% of $^{137}\text{Cs} \times 0.1 \text{ g}^{-1} \text{ h}^{-1}$ )	(mV)	( $\mu\text{mol} \times \text{g}^{-1} \text{ h}^{-1}$ )	
Control	$1.65 \pm 0.20$	$6.49 \pm 1.04$	$-3.1$
Amiloride	$0.93 \pm 0.11$	$4.70 \pm 0.46$	$-7.5$

Table 1. Caesium fluxes through the isolated perfused *Carcinus* gill epithelia. Mean values of 6 observations  $\pm$  S.E. TBP= transbranchial potential (in mV).

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