Use of Radiotracer Techniques to Study Subcellular Distribution of Metals and Radionuclides in Bivalves from the Noumea Lagoon, New Caledonia

Marc Metian, Laëtitia Hédouin, C. Barbot, Jean-Louis Teyssié, Scott Fowler, Françoise Goudard, Paco Bustamante, Jean-Pierre Durand, Jacques Piéri, Michel Warnau

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M. Metian[1], L. Hédouin[1-3], C. Barbot[4], J.-L. Teyssié[1], S.W. Fowler[1], F. Goudard[4], P.Bustamante[2], J.-P. Durand[4], J. Piéri[4], M. Warnau[1]

[1] International Atomic Energy Agency – Marine Environment Laboratory, 4 Quai Antoine Ier, MC-98000 Principality of Monaco.

[2] Laboratoire Biologie et Environnement Marins, FRE 2727, Université de La Rochelle, 22 avenue Michel Crépeau, F-17042 Cedex, France

[3] IRD-Noumea Center, BPA5, 98848 Nouméa Cedex, New-Caledonia

[4] Groupe SMAB – EA 2160 – Laboratoire GERMETRAD, Université de Nantes, Pôle Mer et Littoral, 2 rue de la Houssinière BP92208, F-44322 Nantes Cedex 3, France

Correspondence to: M. Warnau Tel. : +377 97 97 72 58, Fax : +377 97 97 72 76;
E-mail address : m.warnau@iaea.org

New Caledonia is the third largest producer of nickel in the world, and this small South Pacific island is estimated to contain no less than 20% of the total stock of Ni on the planet (Connell 2003). Metal contamination resulting from the nickel mining industry and related activities constitutes a long lasting threat to the marine ecosystems sheltered by the second largest reef system in the world (Labrosse et al. 2000). However, as almost a rule when it concerns tropical ecotoxicology, available information on metal contamination in New Caledonia waters is extremely scarce and very little is known about the extent of local contamination and possible environmental impacts (Labrosse et al. 2000). Moreover, a new extraction process for Ni ("lixiviation", *viz.* acidic extraction) has recently been tested at the industrial level and should be implemented in the near future (2006-2007). This process will result inevitably in increased discharges of co-occurring metals in Ni ores (e.g. Co and Cr). Thus, basic information on metal metabolism and behaviour is needed in order to assess the possible impact of these additional metal inputs on local ecosystems.

The objective of the present study was to determine the potential toxicity of metals in two species commonly found in the lagoon: the edible clam *Gafrarium tumidum* and the oyster *Isognomon isognomon*. Contaminant partitioning within the cells (soluble vs. insoluble fractions) determines the likelihood of inducing deleterious effects (reaction with cellular components) (Viarengo 1985) as well as to being transferred to higher trophic levels (Reinfelder and Fisher 1991). Therefore, the subcellular distribution of five metals (Cd, Co, Cr, Zn, Ag) and two anthropogenic radionuclides (^{134}Cs, ^{241}Am) was examined in the gills and visceral mass of both species following direct seawater exposure to these elements using highly sensitive radiotracer techniques.

MATERIALS AND METHODS

Both bivalve species were collected in August 2002 by SCUBA diving in Dumbéa bay (*G. tumidum*) and Maa bay (*I. isognomon*) (Nouméa, New Caledonia) and were immediately shipped to the IAEA-MEL premises in Monaco where they were acclimated to laboratory conditions (open circuit aquaria; water renewal 10% hr^{-1}; S: 36 p.s.u.; T: 25±0.5°C) for 6 wk prior to experimentation. The organisms were then experimentally exposed for 28 days to radiotracers of five heavy metals (^{109}Cd, ^{57}Co, ^{51}Cr, ^{65}Zn, ^{110m}Ag) and two radionuclides (^{134}Cs, ^{241}Am) directly via sea water. Periodically during the exposure phase, the bivalves were transferred to unlabelled sea water for a short time (1-2 hrs) where they fed on mixed phytoplankton cultures before being returned to the labelled sea water for further uptake. At the end of the experiment, 6 individuals of each species were collected and dissected. The gills and visceral mass were separated, pooled, and processed for subcellular fractioning, using differential centrifugation (Boisson et al. 2003). Briefly, homogeneized tissues were centrifuged successively:

- at 900 × *g* for 10 min (to sediment nuclei and heavy lysosomes),
- at 12,000 × *g* for 15 min (to sediment lysosomes and mitochondria),
- at 45,000 × *g* for 30 min (to sediment light mitochondria and plasma membranes),
- and finally at 115,000 × *g* for 70 min (to separate microsomes from the cytosolic fraction, the latter constituting the supernatant).

Distribution of the radiotracers among the different subcellular fractions was determined using a high-resolution γ-spectrometry system consisting of 4 coaxial Ge (N- or P-type) detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyzer and a computer equipped with a spectra analysis software (Interwinner, Intertechnique). The detectors were calibrated with appropriate standards for the counting geometry used, and all measurements were corrected for background and physical decay of the radiotracers. Counting times were adapted to obtain count rates with relative propagated errors less than 5%.

RESULTS AND DISCUSSION

Measurements of specific enzymatic markers (acid phosphatase for lysosomes, glucose-6-phosphatase for microsomes, 5'-nucléotidase for plasma membranes; (Boisson et al. 2003)) indicated that the purity of the different subcellular fractions was good. Results of the subcellular distribution of the different metal radiotracers and radionuclides in gills and visceral mass are given in Table 1.

Globally, the distributions in both tissues were similar for each bivalve species. The only main departure from this was observed for ^{57}Co in the clam where the cytosolic fraction was much lower in the gills (25%) than in the visceral mass (79%). Cr, Co, Zn, Cd and ^{134}Cs were mainly found in the cytosolic fraction (30-87%) whereas ^{110m}Ag and ^{241}Am were mainly associated with membranes and organelles (65–96%). These results are in agreement with those reported for other

bivalves from temperate waters, e.g., the scallop *Chlamys varia* (Bustamante and Miramand *in press*) and the oyster *Crassostrea gigas* (Milcent et al. 1996).

The predominant distribution of Ag in the insoluble fraction (*viz.* the non-cytosolic fractions) could be due to specific Ag storage/detoxification in these two bivalve species. Indeed, it is well documented that various bivalves are able to

Table 1. Subcellular partitioning (mean %) of radioisotopes in gills and visceral mass of two bivalves.

	Gafrarium tumidum							*Isognomon isognomon*						
Gills	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag	^{134}Cs	^{241}Am	^{51}Cr	^{57}Co	^{65}Zn	^{109}Cd	^{110m}Ag	^{134}Cs	^{241}Am
Nuclei	18	28	28	30	73	20	25	17	16	22	14	23	17	27
Lysosomes + mitochondria	6	7	6	2	6	7	6	10	19	30	15	34	12	36
Membranes	10	17	16	1	6	13	25	19	8	15	10	23	16	10
Microsomes	10	22	19	1	5	13	27	10	5	0	6	7	11	4
Cytosol	57	25	31	67	10	48	17	44	52	33	54	13	45	22
Visceral mass														
Nuclei	28	9	24	10	49	27	42	25	20	28	24	43	26	35
Lysosomes + mitochondria	13	6	10	2	12	11	22	22	10	20	15	27	19	47
Membranes	7	3	15	1	3	6	19	6	2	5	3	19	6	6
Microsomes	6	3	12	1	2	5	13	7	3	6	3	4	7	3
Cytosol	45	79	40	87	35	51	4	39	65	41	54	7	42	9

trap Ag as non-toxic Ag_2S precipitates within their tissues (Berthet et al. 1992; Berthet et al. 1990). This kind of sequestration can inhibit the deleterious effects that could be caused by this highly toxic element, even if present in high concentrations. In addition, preferential distribution in the insoluble subcellular fraction indicates that Ag is not likely to be bioavailable to higher trophic levels (Bustamante and Miramand *in press*; Reinfelder and Fisher 1991).

Preferential distribution of most radioelements in the cytosol suggests that, once incorporated into the cells, a large part of these metals could be toxic, since they are likely to bind with key soluble components of the cells (e.g. proteins, enzymes, DNA). However, in the case of Cd and Zn, a substantial fraction of the cytosolic metal is most probably detoxified as "metal-metalloprotein" complexes, e.g. approximately 40% in the case of Cd in oysters (Boisson et al. 2003). Furthermore, the metals preferentially associated with the cytosolic fraction are likely to be readily bioavailable to higher trophic levels preying on these organisms (Reinfelder and Fisher 1991). This fact is of particular concern here since the clam *G. tumidum* is consumed by local populations, and could therefore be a non-negligible source of human exposure to metals through seafood consumption.

Progress in Ni ore exploitation planned in New Caledonia will result in an increased input of dissolved metals to New Caledonian lagoon waters (Morreton et al. 2004). Such a situation could result in an increased contamination of the local bivalves. Our findings indicate that subcellular partitioning of metals co-occurring in Ni ores will be preferentially cytosolic. Therefore, metal exposure of organisms (including man) preying on these two bivalves could be enhanced as well. Monitoring of metal contamination levels in edible species is therefore recommended following the industrial implementation of the acidic lixiviation process in New Caledonia.

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