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# Concentration and distribution of $^{210}$Po in the tissues of the scallop *Chlamys varia* and the mussel *Mytilus edulis* from the coasts of Charente-Maritime (France)

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**Abstract**

Polonium-210 ($^{210}$Po) has been analysed in the soft parts of two bivalves species, the scallop *Chlamys varia* and the common mussel *Mytilus edulis*, from the Bay of La Rochelle and Ré Island, on the French Atlantic coast. Between those sites, the highest $^{210}$Po concentrations have been found in whole scallop soft parts from La Rochelle, reaching $1181 \pm 29$ Bq kg$^{-1}$ dry weight (dwt), a size effect being related to the highest $^{210}$Po concentration in the smallest scallops. The results show a significant difference in concentrations for similar size individuals between species in each site (*C. varia* > *M. edulis*) and between sites for each species (Ré Island > Bay of La Rochelle). Very high $^{210}$Po concentrations have been found in the digestive gland of *C. varia*, ranging 3150–4637 Bq kg$^{-1}$ dwt. Thus, the digestive gland contains up to 60% of the radionuclide. Subcellular investigations have shown that approximately 40% of the $^{210}$Po contained in the digestive gland is in the cytosolic fraction, suggesting a high bioavailability of the $^{210}$Po from this fraction to the trophic upper level. Calculations will show that approximately 4 kg of scallops flesh intake would be necessary to reach the annual incorporation limit of 1 mSv.


*Keywords:* $^{210}$Po; Molluscs; Scallops; Bioaccumulation; Digestive gland; Subcellular fractionation

## 1. Introduction

Polonium-210 ($^{210}$Po), a naturally occurring radionuclide, is a high energy α-particle emitter in the uranium decay chain and is considered as an important source of internal radiation dose to marine organisms (Cherry and Shannon, 1974; Cherry and Heyraud, 1981, 1982). $^{210}$Po is a also responsible for a significant proportion of the radiation exposure of humans to background radiation, particularly through seafood consumption (CEC, 1989).

$^{210}$Po is known to be accumulated by marine organisms and transferred through marine food chains (Heyraud and Cherry, 1979; Carvalho, 1988), with ingestion being the major route of entry (Carvalho and Fowler, 1994). The tissues of some marine organisms, such as digestive gland of crustaceans and molluscs, and fish liver, may contain elevated concentrations of $^{210}$Po (Skwarzec and Falkowski, 1988; Gouvea et al., 1992; Stepnowski and Skwarzec, 2000a). In these tissues, subcellular distribution of $^{210}$Po, particularly in the cell nucleus, may have important implications with respect to the genetic radiotoxic effects of the $^{210}$Po α-particles (Howell et al., 1990).

Among marine bivalves, scallops were reported to highly concentrate trace metals such as cadmium (Bryan, 1973; Mauri et al., 1990; Bustamante, 1998), but also radionuclides such as americium-241 (Miramand et al., 1991). On the French Atlantic coast, three species of scallops are exploited for human consumption and among these species, *Chlamys varia* is very common on the rocky shore and targeted by commercial fishery as well as for leisure activities. $^{210}$Po was investigated in the scallop *C. varia* from the French Atlantic coast in order to determine the levels and distribution among the tissues. Indeed, very little data are available on the $^{210}$Po

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levels in biota from the French coast and, on a global scale, little is known about $^{210}$Po levels in Pectinidae species. Sampling sites were chosen to determine the difference of $^{210}$Po concentrations in molluscs from a potentially contaminated site by uranium-238 ($^{238}$U) (Pallard and Vervialle, 1999) and a clean one. Moreover, this paper focuses particularly on the concentrations and bioavailability of $^{210}$Po in the digestive gland of the scallop as this organ is currently reported to concentrate this natural radionuclide in molluscs. Results obtained with *C. varia* were compared to those of mussels from the same sampling areas. Finally, the doses ingested by several categories of shellfish consumers were calculated.

## 2. Materials and methods

### 2.1. Sampling

Scallops *C. varia* and mussels *Mytilus edulis* were collected in winter between 1996 and 1998 in Charente-Maritime (Atlantic coast of France) in two places: in an anthropogenically contaminated area, i.e. near the harbour of La Rochelle, and in a clean fishing area, i.e. in Saint-Martin en Ré on the Ré island (Fig. 1). 248 scallops were collected by hand and pooled by size classes. Animals were depurated 48 h in clean seawater to eliminate faecal and pseudo-faecal material. 146 scallops were used for analysis and distribution of $^{210}$Po and 102 scallops for subcellular fractionations of the digestive gland cells. The digestive gland was analysed separately from the soft parts. Thus, the remainders included the adductor muscle, the mantle, the gills, the gonad and the kidneys.

Similarly, 60 mussels of *M. edulis* were collected in the same places and prepared in the same way as the scallops.

### 2.2. Cellular fractionation

Digestive glands of the other 102 scallops pooled by size (see Table 1) were removed from the soft parts and homogenised in a Dounce potter on ice with 10 volumes of a Tris–HCl buffer 0.02 M sucrose 0.25 M with 1 mM phenylmethylsulfonylfluoride (PMSF, as protease inhibitor) and 5 mM dithiothreitol (DTT, as reducing agent), at pH 8.6. The homogenates were centrifuged at 100 000 G for 1 h at 4 °C in a Beckman LE-70 ultracentrifuge. Particle-free supernatants (cytosols) were separated from the pellet. Aliquots of the homogenates, cytosols and pellets were analysed for $^{210}$Po.

### 2.3. Analytical procedures

Samples were lyophilised then grounded. Aliquots of 500–1000 mg of the homogenates were placed in Teflon bombs along with $^{208}$Po spike. Aliquots were digested using 10 ml of concentrated nitric acid during 48 h at 80 °C. After evaporation to dryness, the residues were dissolved in HCl and the solution made up to 0.3 M (Heyraud and Cherry, 1979; Cherry and Heyraud, 1981). 50 mg of ascorbic acid and 5 ml of hydroxylamine

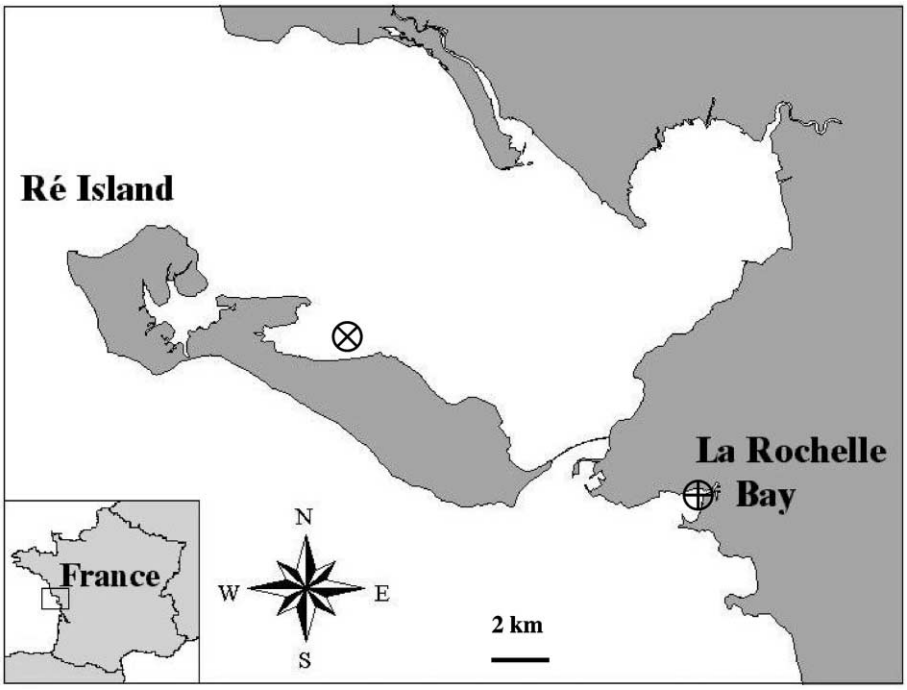


Fig. 1. Sampling location sites for the scallops *C. varia* and the common mussels *M. edulis* (⊗: Saint Martin en Ré; ⊕: La Rochelle Bay).

Table 1
$^{210}$Po activity concentrations in soft parts of the scallops and mussels from the coast of Charente-Maritime (Atlantic coast of France), and associated digestive glands and remainders (Bq kg$^{-1}$ dwt ± SD), together with the percentage of the polonium in the digestive gland and the percentage contained in the digestive gland cytosols (% ± SD)

| *Species* | Sample size | Size (mm) | Digestive gland | | | Remainders (activity) | Whole animal (activity) |
|---|---|---|---|---|---|---|---|
| Sampling area and date | | | Activity | % of the soft part | Cytosolic % | | |
| *C. varia* | | | | | | | |
| La Rochelle, 2-1996 | 55 | 33 ± 2 | 4162 ± 406 | 62 ± 6 | 39 ± 1[a] | 643 ± 47 | 1181 ± 29 |
| La Rochelle, 2-1996 | 40 | 37 ± 2 | 3675 ± 88 | 65 ± 1 | 34 ± 1[b] | 434 ± 31 | 896 ± 9 |
| La Rochelle, 2-1996 | 31 | 44 ± 2 | 3150 ± 74 | 63 ± 2 | 42 ± 2[c] | 388 ± 29 | 776 ± 9 |
| Ré Island, 2-1996 | 30 | 44 ± 3 | 4637 ± 584 | 64 ± 10 | NM | 513 ± 41 | 1049 ± 35 |
| *M. edulis* | | | | | | | |
| La Rochelle, 12-1998 | 10 | 47 ± 2 | – | – | – | – | 317 ± 9 |
| La Rochelle, 12-1998 | 10 | 48 ± 1 | – | – | – | – | 289 ± 8 |
| La Rochelle, 12-1998 | 10 | 49 ± 2 | – | – | – | – | 293 ± 9 |
| Ré Island, 12-1998 | 10 | 39 ± 2 | – | – | – | – | 569 ± 15 |
| Ré Island, 12-1998 | 10 | 42 ± 1 | – | – | – | – | 589 ± 15 |
| Ré Island, 12-1998 | 10 | 43 ± 1 | – | – | – | – | 550 ± 14 |

Exponent letters show scallops sampled in La Rochelle site in march 1997: a—$n = 25$, 32 ± 2 mm; b—$n = 7$, 46 ± 3 mm; c—$n = 70$, 51 ± 3 mm and NM—not measured. The fresh/dry weight ratios were 5.7 ± 0.2 for the digestive gland, 7.5 ± 0.1 for the remainders and 7.3 ± 0.1 for the soft parts.

hydrochloride 30% were added to the solution. Finally, the polonium was deposited spontaneously onto a 18 mm diameter disc of silver during agitation at 90 °C for 4 h (Flynn, 1968; Fleer and Bacon, 1984; Skwarzec and Bojanowski, 1988).

Measurements of $^{210}$Po activity were performed on an α-ray spectrophotometer equipped with semiconductor detectors (implanted Si passive junction type; 300 mm$^2$ area) coupled to an analyser (Germain et al., 1995). The extraction yields calculated for the samples fell in the range 80–100%. Analytical blanks, as well as detector background levels, were determined on a regular basis. The results are expressed in terms of sampling date.

## 3. Results

$^{210}$Po concentrations in the whole soft parts of the scallop *C. varia* were calculated from the results obtained for the digestive gland and the remainders. All the results obtained for the scallop *C. varia* and the common mussel *M. edulis* from the Bay of La Rochelle and the Ré Island were expressed in Bq kg$^{-1}$ dry weight (dwt) and are compiled in the Table 1. In the Bay of La Rochelle, small scallops displayed the highest $^{210}$Po concentrations as well as in the whole soft parts than in the remainders and in the digestive gland. Comparison from both sampling sites for scallops of similar size showed that those from the Ré Island exhibit higher $^{210}$Po concentrations than those from La Rochelle Bay. Similar differences also appear for the mussels *M. edulis* (Table 1).

For *C. varia*, highest $^{210}$Po concentrations were found in the digestive gland, ranging from 3150 Bq kg$^{-1}$ in scallops from La Rochelle to 4637 Bq kg$^{-1}$ in scallops from Ré island. The digestive gland contained most of the radionuclide, reaching up 60% of the total soft parts $^{210}$Po (Table 1).

Finally, subcellular investigations have showed that from 34% to 42% of the $^{210}$Po contained in the digestive gland was in the cytosol of the digestive gland cells (Table 1).

## 4. Discussion

Scallops *C. varia* from the Charente-Maritime coast exhibit very high $^{210}$Po concentrations in their soft parts. As it occurs for stable elements (Boyden, 1977), size influence the $^{210}$Po concentrations in shellfish and Ryan et al. (1999) have noted that at several sampling sites, the smaller mussels from the Irish coast had higher $^{210}$Po concentrations than larger ones. Similarly, small scallops exhibit significantly higher concentrations in the digestive gland and in the remaining tissues, and consequently in the whole animals. Such differences might be explained by the effect of age and the differences in metabolic rates. Because of the size effect, the comparison between $^{210}$Po concentrations in scallops from the Bay of La Rochelle and Ré Island have to be made for individuals of similar size. Surprisingly, the highest $^{210}$Po concentrations were found in the scallops from Ré Island as well as for the mussels whereas there is no evidence of a polonium source in this site remote from industrial activities. On the contrary, in the Bay of La Rochelle, there is evidence for potential polonium source because of the former discharges in the Bay of local industrial wastes containing several thorium isotopes and $^{238}$U (Pallard and Vervialle, 1999). These industrial wastes came from the industrial rare earth extracting plant Rhodia, and could release $^{210}$Po as it is an element in the decay chain of $^{238}$U. In comparison

with Ré Island, high $^{210}$Po concentrations in the scallops from the Bay of La Rochelle seems to be not attributable to the localised ancient discharges.

The $^{210}$Po concentrations in the soft parts of the scallops *C. varia* from both Charente-Maritime sites were 2–3 times higher than those measured in the common mussel *M. edulis* (Table 1). Mussels are considered as sentinel or indicator species for contaminants, but values reported for whole Mytilidae (i.e. *M. edulis*, *M. galloprovincialis* and *M. trossulus*) are always lower than those from *C. varia* (Table 2): for example, *M. edulis* from the French coasts exhibited maximum values of 700 Bq kg$^{-1}$ dwt (Germain et al., 1995) which is a relatively high concentration for this genus (Table 2). This highlight the strong ability of the scallops to concentrate $^{210}$Po as it has been shown several times for both heavy metals and radionuclides (Bryan, 1973; Miramand et al., 1991; Viarengo et al., 1993; Bustamante, 1998). The dissection of the scallop showed that the digestive gland was responsible of such high levels in the whole soft parts. Indeed, for *C. varia*, highest $^{210}$Po concentrations were found in the digestive gland, ranging from 3150 Bq kg$^{-1}$ in scallops from the Bay of La Rochelle to 4637 Bq kg$^{-1}$ in scallops from Ré Island. These polonium concentrations appear to be very high for bivalve molluscs. High $^{210}$Po concentrations have also been measured in the digestive gland of several seashell species such as the mussel *M. trossulus* (1026 ± 107 Bq kg$^{-1}$ dwt), the scallop *Patinopecten yessoensis* (1916 ± 108 Bq kg$^{-1}$ dwt) and the bivalve *Batillus cornutus* (1081 ± 89 Bq kg$^{-1}$ dwt) (Yamamoto et al., 1994; Stepnowski and Skwarzec, 2000a), but the digestive gland of *C. varia* displayed polonium concentrations 2–4 times higher. Nevertheless, highest concentrations have been reported for whole pelagic decapod shrimp, penaeids showing concentrations from 6549 to 27 121 Bq kg$^{-1}$ dwt and carids from 962 to 3404 Bq kg$^{-1}$ dwt (Heyraud et al., 1988). The $^{210}$Po concentrations in the digestive gland of *C. varia* are about 200 times higher than those found in the same tissue from an other scallop species, i.e. *C. islandicus* from the Spitstbergen (Stepnowski and Skwarzec, 2000a). Very low $^{210}$Po concentrations in the digestive gland of this polar mollusc was attributed to particular environmental conditions (e.g. temperature) and a short-time food availability period. In *C. islandicus*, highest $^{210}$Po concentrations were found in the gills that appear to constitute the main polonium entrance for this species. On the contrary, the food way appear to be the major polonium route of entry for *C. varia* as the digestive gland contained concentrations one order of magnitude higher than in the remaining tissues. However, such results for *C. islandicus* are noteworthy as Pectinidae accumulate high levels of trace elements in their digestive gland, even within polar areas. For example, very high levels of Cd were found in the digestive gland of the Antarctic scallop *Adamussium colbecki* in comparison to those from the Mediterranean scallop *Pecten jacobeus* and similar levels of copper and zinc were recorded in both species (Mauri et al., 1990; Viarengo et al., 1993). Moreover, the $^{210}$Po concentrations in the digestive gland of the Japanese scallop *P. yessoensis* are also relatively high (Table 2). In this context, particular attention to $^{210}$Po accumulation in polar organisms would be of great interest.

Due to the very high values of $^{210}$Po concentrations in the digestive gland, this organ contains from 62% to 65% of the total $^{210}$Po in scallops from the Bay of La Rochelle and 64 % in those from Ré Island. Subcellular fractionation of the digestive gland cells of *C. varia* showed that more than 50% of the $^{210}$Po was in the insoluble fraction associated with organites and mem-

Table 2
$^{210}$Po activity concentrations in the digestive gland and whole soft parts of Pectinidae and Mytilidae from different locations sites

| *Species* | $^{210}$Po activity (Bq kg$^{-1}$ dwt) | | References |
|---|---|---|---|
| Sampling area | Digestive gland | Whole animal | |
| *C. islandicus* | | | |
| Spitsbergen | 22.7 ± 1.7 | 82.5 ± 6.7 | Stepnowski and Skwarzec (2000a) |
| *P. yessoensis* | | | |
| Japan | 1916 ± 108[a] | 348 ± 17 | Yamamoto et al. (1994) |
| *M. edulis* | | | |
| English Channel | – | 90–700 | Germain et al. (1995) |
| Baltic Sea | – | 149 ± 55% | Dahlgaard (1996) |
| Irish Sea | 596 ± 8 | 279 ± 4 | McDonald et al. (1986) |
| *M. galloprovincialis* | | | |
| Mediterranean Sea | – | 459 ± 18 | McDonald et al. (1986) |
| *M. trossulus* | | | |
| Baltic sea | 1026 ± 107 | 271.7 ± 27.6 | Stepnowski and Skwarzec (2000a) |

[a] Values recalculated from wet weight.

branes and 34–42% of the total $^{210}$Po was found in the supernatant fraction.

Few results on $^{210}$Po subcellular distribution in storage organs such digestive gland or liver are available in the literature, but they show a great heterogeneity. Thus, in crustaceans, 60% of the radionuclide was found in the cytosolic fraction of the digestive gland from *Saduria entomon* (Stepnowski and Skwarzec, 2000b) while in the South African lobster *Jasus lalandii*, 52% of the $^{210}$Po was found in the microsomal fraction of the digestive cells and only 22% was in the cytosolic fraction (Heyraud et al., 1987). In vertebrates liver homogenates, most of the $^{210}$Po was found in the cytosolic fraction associated with proteins such as metallothionein and ferritine (Aposhian and Bruce, 1991; Durand et al., 1999). Metallothionein levels measured in the scallops *C. varia* from La Rochelle are relatively high, ranging from 3.1 to 4.1 mg g$^{-1}$ wet weight (Bustamante, 1998) but at our knowledge, association of $^{210}$Po with metallothioneins in invertebrates have not been demonstrated.

Trace elements associated to cytosolic ligands are generally considered as highly bioavailable to the trophic upper level (Wallace and Lopez, 1996, 1997). Thus, around 40% of the $^{210}$Po found in the digestive gland of *C. varia* could be considered as highly bioavailable for consumers.

In the sampling area, there is both a professional and sport fishing exploitation of the scallop *C. varia* for human consumption. The catch can represent several thousand tons a year (Letaconnoux and Audouin, 1956). Indeed, this mollusc is historically commonly consumed by local populations for seafood.

Exposure to $^{210}$Po by scallop consumption has been evaluated for several kind of consumers. Indeed, investigations on habits of shellfish consumers from Charente-Maritime have allowed to determine three different categories of consumers (Anonymous, 1994): (1) the mean consumers, eating about 6.5 kg of shellfish per year, (2) the high consumers with 13.2 kg of shellfish per year and (3) the very high consumers with 38.5 kg of shellfish per year. Oysters constitute the main fraction of the shellfish but scallop consumption is not negligible since several kilograms per year are even eaten by the very high consumers. However, a precise value remains difficult to ascertain. For this reason, calculations were limited to an estimation of the scallops mass necessary to reach the $^{210}$Po annual incorporation limit of 1 mSv per year and per public person. Typically, a correcting factor for seafood products is applied to take into account the natural decay of the radionuclide ($^{210}$Po half-life ~138 days). However, in the present study, entire values of the $^{210}$Po activity have been considered since molluscs are normally fresh consumed, between 0 and 2 days, after fishing. Considering that only activities measured in the scallop of commercial size from the fishing area (i.e. Ré Island) have been used and that 55% of the scallop mass is constituted by the shells, approximately 9 kg of scallop *C. varia* per year are necessary to be eaten in order to attain the annual incorporation limit. Our calculations reveal that this limit would not be reached by most of the shellfish consumers from Charente-Maritime.

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