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1 **Interspecific comparison of Cd bioaccumulation in European Pectinidae (*Chlamys***  
2 ***varia* and *Pecten maximus*)**

3

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26 ABSTRACT

27 The uptake and loss kinetics of Cd were determined in two species of scallops from the European coasts,  
28 the variegated scallop *Chlamys varia* and the king scallop *Pecten maximus*, following exposures via  
29 seawater, phytoplankton and sediment using highly sensitive radiotracer techniques ( $^{109}\text{Cd}$ ). Results  
30 indicate that, for seawater and dietary pathways, *C. varia* displays higher bioaccumulation capacities in  
31 terms of uptake rate from water and fraction absorbed from ingested food (assimilation efficiency) than  
32 *Pecten maximus*. Regarding sediment exposure, *P. maximus* displayed low steady-state Cd transfer factor  
33 ( $\text{TF}_{\text{SS}} < 1$ ); however, once incorporated, a very large part of Cd transferred from sediment (92 %) was  
34 strongly retained within *P. maximus* tissues.

35 Both species showed a high retention capacity for Cd (biological half-life,  $T_{b/2} > 4$  months), suggesting  
36 efficient mechanisms of detoxification and storage in both species. The digestive gland was found to be  
37 the main storage organ of Cd in the two scallops regardless of the exposure pathway. However, Cd was  
38 stored differently within this organ according to the species considered: 40 % of the total Cd was found in  
39 the soluble cellular fraction in *C. varia* whereas this soluble fraction reached 80 % for *P. maximus*. This  
40 suggests that the two species displayed different Cd detoxification/storage mechanisms.

41 Finally, the present study has determined the relative contribution of the different exposure pathways to  
42 global Cd bioaccumulation for the two scallop species. Results clearly show that for both species, food  
43 constitutes the major accumulation pathway, contributing for > 99 % and 84 % of the global Cd  
44 bioaccumulation in *C. varia* and *P. maximus*, respectively. This work confirms the previous assumption,  
45 derived from a bibliographic overview, that dietary pathway plays a prevalent role in metal  
46 bioaccumulation in Pectinidae.

47

48 KEYWORDS: Cadmium, Metal, Kinetics, Subcellular Distribution, Scallops, Bivalves

49 INTRODUCTION

50 Bivalves usually concentrate efficiently Cd from the surrounded environment (e.g. Eisler 1985). Among  
51 them, Pectinidae can display very high concentrations of this non essential metal that is considered as one  
52 of the most toxic ones. High levels of Cd in scallop tissues have been reported even for species from  
53 pristine and low-contaminated areas such as the Antarctic Ocean or the sub-polar Atlantic Ocean (Mauri  
54 et al. 1990, Viarengo et al. 1993, Bustamante & Miramand 2004), suggesting that scallops have evolved a  
55 natural capacity to accumulate, detoxify and store this metal in their tissues. Investigations carried out in  
56 the field and in the laboratory have revealed the involvement of very efficient detoxification mechanisms.  
57 Indeed, the binding of Cd to high-affinity cytosolic proteins, lysosomes, and mineral concretions is well  
58 known to result in efficient Cd sequestration in Pectinidae (Carmichael & Fowler 1981, Ballan-  
59 Dufrançais et al. 1985, Stone et al. 1986).

60 Even though field investigations have shown that Cd levels are influenced by various factors such as  
61 geographical origin, season, size and sexual maturity (Bryan 1973, Evtushenko et al. 1990, Mauri et al.  
62 1990, Bustamante & Miramand 2004, 2005a), very little is known on the dynamics of Cd  
63 bioaccumulation and retention in this family. To the best of our knowledge, no study has described the Cd  
64 accumulation in Pectinidae exposed via different pathways and its depuration using environmentally  
65 realistic metal levels. For example the earlier study by Eisler et al. (1972) exposed *Aquipecten irradians*  
66 to 10 ppm Cd, a concentration with toxic consequences (Gould et al. 1988) and therefore unlikely to  
67 produce a typical accumulation pattern for Cd. In natural conditions, scallops are exposed to metal  
68 through seawater and food pathways, sediment potentially contributing to either or both. It is therefore  
69 necessary to investigate separately these different exposure pathways to understand their relative  
70 contribution in the global accumulation of the metal (Fowler 1982).

71 Seawater has been often considered as the main source of metal intake for marine organisms (e.g.,  
72 Janssen & Scholz 1979, Borchardt 1983, Riisgard et al. 1987); however the role of the particulate phase,  
73 mainly food, is now recognized to be of primary importance for a large range of taxa (e.g., Warnau et al.

74 1996, 1999, Reinfelder et al. 1998, Wang & Fisher 1999). In the case of Pectinidae, it has been suggested  
75 that food could be the major route of Cd intake on the basis of elevated metal concentrations found in the  
76 digestive gland (Palmer & Rand 1977, Uthe & Chou 1987, Bustamante & Miramand 2005a). However, it  
77 appears necessary to confirm this assumption as the contribution of the dissolved phase could also lead to  
78 high metal concentrations in the storage and detoxification organs (e.g., Borchardt 1983).  
79 Therefore, the present work investigated uptake and loss kinetics of Cd in two species of scallops,  
80 *Chlamys varia* and *Pecten maximus* exposed through seawater, food and/or sediment, depending of their  
81 different living habitats - only seawater and food for *C. varia* and all pathways for *P. maximus* which is  
82 living buried in the bottom sediment and is able to ingest large particles (Mikulich & Tsikhon-Lukamina  
83 1981, Shumway et al. 1987). The use of highly sensitive radiotracer techniques allowed studying  
84 bioaccumulation mechanisms at realistic Cd levels encountered in the field. Three levels of biological  
85 organization were considered in this study, the whole individual, the different organs and the subcellular  
86 fractions of the digestive gland cells, in order to evaluate the biokinetic parameters of the accumulation,  
87 the distribution among the body compartments and the cellular forms of storage in the digestive gland,  
88 respectively. Finally, we used a bioaccumulation model to determine the relative contribution of the  
89 different exposure pathways of Cd for both species.

90

## 91 MATERIALS AND METHODS

### 92 **Sampling**

93 In spring 2004 and 2005, one hundred variegated scallops *Chlamys varia* and seventy king scallops  
94 *Pecten maximus* were collected on the Atlantic coast (Pertuis Breton, Charente-Maritime) by SCUBA  
95 diving. They were carefully transported to IAEA-MEL premises in Monaco and were acclimatized to  
96 laboratory conditions for 4 weeks (constantly aerated open circuit aquarium; flux: 50 l h<sup>-1</sup>; salinity: 36  
97 p.s.u.; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1; light/dark cycle: 12 h/12 h) prior to experimentations.

98 During this period, scallops were fed daily an algal mixed diet (*Isochrysis galbana*, *Skeletonema*  
99 *costatum*).

100

#### 101 **Radiotracer and counting**

102 Uptake and loss kinetics of  $^{109}\text{Cd}$  in scallop species were determined using a high specific activity  
103 radiotracer purchased from Isotope Product Lab ( $^{109}\text{Cd}$  as  $\text{CdCl}_2$  in 0.1M HCl,  $T_{1/2} = 426.6$  d). The tracer  
104 was counted using a high-resolution  $\gamma$ -spectrometer system composed of three Germanium -N or P type-  
105 detectors (EGNC 33-195-R, Intertechnique) connected to a multichannel analyser (Intergamma,  
106 Intertechnique). The radioactivity was determined by comparison with standards of known activity and of  
107 appropriate geometry. Measurements were corrected for counting efficiency and physical radioactive  
108 decay. The counting time was adjusted to obtain a propagated counting error less than 5 %.

109

#### 110 **Seawater exposure**

111 Twenty three *Chlamys varia* and 23 *Pecten maximus* (average weight  $\pm$  SD:  $30 \pm 7$  g and  $208 \pm 46$  g,  
112 respectively) were placed in a 70-l glass aquarium (constantly aerated closed circuit aquarium; salinity:  
113 36 p.s.u.; temperature:  $17 \pm 0.5^\circ\text{C}$ ; pH:  $8.0 \pm 0.1$ ; light/dark cycle: 12 h/12 h) and exposed for 7 d to  $^{109}\text{Cd}$   
114 dissolved in seawater ( $2 \text{ kBq l}^{-1}$ ). No change in pH was detectable after the tracer addition. Spiked  
115 seawater was renewed twice a day the first two days and then daily in order to keep radioactivity in  
116 seawater constant. Activity of the  $^{109}\text{Cd}$  in seawater was checked before and after each spike renewal,  
117 yielding time-integrated activities of  $2.1 \pm 0.2 \text{ kBq l}^{-1}$ .

118 Nine scallops of each species were collected at different time intervals and were whole-body  
119 radioanalyzed alive (same identified individual each time). At the end of the 7-d exposure period, 5  
120 scallops of each species were sacrificed and dissected. Shell, digestive gland, kidneys, gills, gonad,  
121 mantle, intestine, adductor muscle and the rest of soft tissues were separated and radioanalyzed in order to  
122 assess the  $^{109}\text{Cd}$  body distribution. The remaining scallops were then placed in non contaminating

123 conditions (constantly aerated open circuit; flux: 50 l h<sup>-1</sup>; salinity: 38 p.s.u.; temperature: 17 ± 0.5 °C; pH:  
124 8.0 ± 0.1; light/dark cycle: 12 h/12 h) for 36 d and nine individuals of each species were regularly  
125 radioanalyzed alive in order to follow the loss of <sup>109</sup>Cd from the scallops. Four scallops were collected at  
126 the end of the depuration period and dissected into several body compartments as previously described.

127

#### 128 **Food exposure**

129 The prymnesiophycean *Isochrysis galbana* was used to study <sup>109</sup>Cd transfer to scallops through their diet.  
130 Phytoplankton cells were exposed to 4.8 kBq l<sup>-1</sup> <sup>109</sup>Cd during their growing phase (7 d). After that period,  
131 phytoplankton medium was filtrated (1 µm-mesh size; Osmonic filters), and then resuspended in a 70-l  
132 aquarium (constantly aerated closed-circuit; salinity: 36 p.s.u.; temperature: 17 ± 0.5°C; pH: 8.0 ± 0.1;  
133 light/dark cycle: 12 h/12 h) where six *C. varia* and six *P. maximus* (average weight ± SD: 17 ± 5 g and  
134 127 ± 14 g, respectively) were placed for one week before the feeding experiment. The radioactivity of  
135 the labelled *I. galbana* was γ-counted before and after the filtration. Scallops were allowed to feed on  
136 radiolabelled *I. galbana* for 2 h (cell concentration -5 10<sup>4</sup> cell ml<sup>-1</sup>- was selected to avoid pseudofeces  
137 production). After the feeding period, all scallops were γ-counted and flowing seawater conditions (50 l h<sup>-1</sup>  
138 l) were restored in the aquarium. Individuals were then whole-body γ-counted alive at different time  
139 intervals to follow the loss kinetics of <sup>109</sup>Cd. Four individuals were collected after 16 (*P. maximus*) and 30  
140 d (*C. varia*) of depuration, and dissected to determine the <sup>109</sup>Cd tissue distribution among the different  
141 body compartments (shell, digestive gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and  
142 the rest of soft tissues) and among the subcellular fraction of the digestive gland (see below).

143

#### 144 **Sediment exposure**

145 Since *P. maximus* is living buried into the sediment whereas *C. varia* is fixed on rocks, Cd exposure  
146 through sediment was only assayed for *P. maximus*. Sediment was collected in Wimereux (North-Atlantic  
147 coast of France). Sediment grain size distribution was measured on a Mastersizer micro and the

148 evaluation of the dry/wet weight ratio was calculated after freeze drying in a LABCONCO Freezone18.  
149 Aerated sediment (9 kg) was placed in plastic bottle, exposed to  $^{109}\text{Cd}$  (516 kBq) for 6 d with constant  
150 agitation, then used to form a homogeneous sediment layer of 4 cm height in a 20-l aquarium. Weakly  
151 bound  $^{109}\text{Cd}$  was allowed to leach overnight under flowing seawater ( $50 \text{ l h}^{-1}$ ) (Warnau et al. 1996). Ten  
152 *P. maximus* (average weight  $\pm$  SD:  $118 \pm 5 \text{ g}$ ) were then placed for 13 d in the aquarium (constantly  
153 aerated open circuit; flux:  $50 \text{ l h}^{-1}$ ; salinity: 36 p.s.u.; temperature:  $17 \pm 0.5^\circ\text{C}$ ; pH:  $8.0 \pm 0.1$ ; light/dark  
154 cycle: 12 h/12 h). Six individuals as well as sediment aliquots were regularly radioanalyzed during the  
155 experiment duration. Activity of  $^{109}\text{Cd}$  in sediment was constant all along the exposure period ( $24.2 \pm 1.9$   
156  $\text{Bq g}^{-1}$  wet wt). At the end of the uptake period, 4 scallops were collected, dissected (shell, digestive  
157 gland, kidneys, gills, gonad, mantle, intestine, adductor muscle and the rest of soft tissues), weighed and  
158  $\gamma$ -counted in order to determine the radiotracer distribution among the body compartments. The remaining  
159 individuals were transferred for 49 d to a new 20-l aquarium containing non contaminated sediment with  
160 flowing seawater and they were regularly radioanalyzed to follow  $^{109}\text{Cd}$  loss kinetics. Also,  $^{109}\text{Cd}$  activity  
161 in sediment was regularly measured in order to ascertain that no contamination of the clean sediment  
162 occurred through  $^{109}\text{Cd}$  recycling (for security, the whole sediment layer was renewed anyway after one  
163 week). At the end of the loss period, 4 scallops were collected and dissected as described above to  
164 determine  $^{109}\text{Cd}$  body distribution and its subcellular distribution in the digestive gland.

165

#### 166 **Subcellular distribution**

167 For all the experiments, the digestive gland of both scallop species were considered to assess the  
168 partitioning of  $^{109}\text{Cd}$  between soluble and insoluble fractions as described by Bustamante & Miramand  
169 (2005b). Briefly, part of digestive gland were homogenized individually with a mortar and pestle on ice  
170 with 10 ml of 0.02 M Tris-HCl buffer, 0.25 M sucrose, 1 mM phenylmethylsulfonylfluoride (PMSF, as  
171 protease inhibitor), at pH 8.6. The homogenates were centrifuged at 80,000 G for 1 h at  $5^\circ\text{C}$  in a Sorvall



172 RC28S ultracentrifuge to separate particle-free supernatant (cytosol; soluble fraction) from the pellet  
173 (insoluble fraction). Homogenate aliquots, cytosols, and pellets were then radioanalyzed.

174

#### 175 **Data analysis**

176 Uptake of the radioisotope was expressed in term of concentration factors (CF: ratio between the  $^{109}\text{Cd}$   
177 activity in scallops –  $\text{Bq g}^{-1}$  wet wt – and time-integrated activity in the seawater –  $\text{Bq g}^{-1}$ ) over time for  
178 the seawater exposure and in term of transfer factors (TF: ratio between the  $^{109}\text{Cd}$  activity in scallops –  $\text{Bq}$   
179  $\text{g}^{-1}$  wet wt – and time-integrated activity in the sediment –  $\text{Bq g}^{-1}$ ) over time for the sediment exposure of  
180 *P. maximus* (Warnau et al. 1996, 1999). Uptake kinetics of  $^{109}\text{Cd}$  in whole-body scallops were fitted using  
181 a simple exponential kinetic model (eq. 1) for the sediment exposure (Statistica<sup>®</sup> 6) and using a linear  
182 model for the seawater exposure (eq. 2):

$$183 \text{CF}_t = \text{CF}_{\text{ss}} (1 - e^{-k_e t}) \text{ (eq. 1)}$$

$$184 \text{CF}_t = k_u t \text{ (eq. 2)}$$

185 where  $\text{CF}_t$  and  $\text{CF}_{\text{ss}}$  ( $\text{CF}_{\text{ss}} = k_u/k_e$ ) are the concentration factors at time  $t$  (d) and at steady state,  
186 respectively;  $k_u$  and  $k_e$  are the uptake and loss rate constants ( $\text{d}^{-1}$ ), respectively (Whicker & Schultz 1982,  
187 Warnau et al. 1996).

188 Depuration of Cd (seawater, food and sediment experiments) was expressed in terms of percentage of  
189 remaining radioactivity (radioactivity at time  $t$  divided by initial radioactivity measured in scallops at the  
190 beginning of the decontamination period \* 100). The percentages of remaining activity were plotted  
191 against time and loss kinetics were described by a double-component exponential model (eq. 3):

$$192 A_t = A_{0s} e^{-k_{es} t} + A_{0l} e^{-k_{el} t} \text{ (eq. 3)}$$

193 where  $A_t$  and  $A_0$  are the remaining activities (%) at time  $t$  (d) and 0, respectively;  $k_e$  is the depuration rate  
194 constant ( $\text{d}^{-1}$ ); 's' and 'l' are the subscripts for the 'short-lived' and 'long-lived' components. For each  
195 exponential component (s and l), a biological half-life can be calculated ( $T_{b/2s}$  and  $T_{b/2l}$ ) from the

196 corresponding depuration rate constant ( $k_{es}$  and  $k_{el}$ , respectively) according to the relation  $T_{b/2} = \ln 2/k_e$   
197 (Warnau et al. 1996). Regarding feeding experiments, the ‘long-lived’ exponential term describes the  
198 fraction of the radiotracer ingested with food that is actually absorbed by the organism (Warnau et al.  
199 1996). The corresponding  $A_{01}$  represents the assimilation efficiency (AE) of the considered radiotracer.  
200 The best fitting regression models were selected according to highest determination coefficient and  
201 examination of residuals. The level of significance for statistical analysis was always set at  $\alpha < 0.05$ .

202

## 203 RESULTS

### 204 **Seawater exposure**

205 Uptake of  $^{109}\text{Cd}$  in whole-body *C. varia* and *P. maximus* displayed linear kinetics ( $r^2 = 0.85$  and  $0.66$ ,  
206 respectively; see Fig. 1). The values estimated for the kinetic parameters and their associated statistics are  
207 presented in Table 1. The concentration factors measured at the end of the uptake period ( $\text{CF}_{7d}$ ) of  $^{109}\text{Cd}$   
208 were  $37 \pm 9$  in *C. varia* and  $18 \pm 7$  in *P. maximus* (Table 2). Calculated  $\text{CF}_{7d}$  for the different organs  
209 indicated that  $^{109}\text{Cd}$  was concentrated selectively in each species, according to the following order:

210 - *C. varia*: kidneys ( $928 \pm 547$ ) > digestive gland ( $322 \pm 175$ )  $\approx$  gills ( $277 \pm 102$ )  $\approx$  foot ( $265 \pm 74$ )  $\approx$  rest  
211 of soft tissues ( $258 \pm 56$ ) > gonad, mantle, intestine and adductor muscle ( $\leq 53 \pm 11$ )

212 - *P. maximus*: kidneys ( $690 \pm 402$ )  $\approx$  digestive gland ( $659 \pm 227$ ) > gills ( $175 \pm 13$ ) > other tissues ( $\leq 78 \pm$   
213 33).

214 In terms of body distribution,  $^{109}\text{Cd}$  was mainly found in the digestive gland and in the gills ( $\sim 30$  and  $20$   
215 % of total body load, respectively) for both species. At the end of the uptake experiment, the  $^{109}\text{Cd}$  tissue  
216 distribution shows a similar pattern ( $p_{G\text{-test}} > 0.40$ ) between *C. varia* and *P. maximus*, with the digestive  
217 gland and gills accounting for more than 60 % of the total Cd load (Table 2).

218 After the exposure period, non-contaminating conditions were restored and loss kinetics of  $^{109}\text{Cd}$  were  
219 followed for 36 d. The whole-body loss kinetics of  $^{109}\text{Cd}$  in *C. varia* and *P. maximus* were best described  
220 by a two-component exponential model (Fig. 1 and Table 1). The major part of  $^{109}\text{Cd}$  was efficiently

221 absorbed in *C. varia* and *P. maximus* ( $A_{01} > 77\%$ ). The estimated loss rate constant of the long-lived  
222 components ( $k_{el}$ ) for *C. varia* was low, i.e.  $0.005 \pm 0.001$  and, consequently, the derived biological half-  
223 life reached  $145 \pm 45$  d (Table 1). In the case of *P. maximus*, the loss rate constant was not significantly  
224 different from 0 ( $p > 0.05$ ), and the related  $T_{b/2l}$  of  $^{109}\text{Cd}$  may thus be considered as infinite.

225 After 36 d of depuration, the body distribution of  $^{109}\text{Cd}$  displayed a similar pattern than the one observed  
226 at the end of the exposure period (Table 2). However, it is striking to note that the  $^{109}\text{Cd}$  activity in the  
227 digestive gland of *C. varia* and *P. maximus* remained relatively constant throughout the depuration  
228 duration within the two species, i.e. from  $680 \pm 369$  Bq g<sup>-1</sup> to  $549 \pm 255$  Bq g<sup>-1</sup> for *C. varia* and from  
229  $1,392 \pm 479$  Bq g<sup>-1</sup> to  $1,491 \pm 316$  Bq g<sup>-1</sup> for *P. maximus*, suggesting either a lack of Cd loss from the  
230 digestive gland during this period or a redistribution of the radioisotope from the tissues in contact with  
231 seawater towards this storage organ.

232

### 233 **Dietary exposure**

234 The loss kinetics of  $^{109}\text{Cd}$  ingested with food in both *C. varia* and *P. maximus* were best fitted using a  
235 double exponential model (Fig. 1 and Table 1). *C. varia* displayed a higher assimilation efficiency ( $AE >$   
236  $86\%$ ) than *P. maximus* ( $AE > 80\%$ ). However, in both species, the depuration rate constant,  $k_{el}$ , were not  
237 significantly different from 0 ( $p > 0.39$ ), and therefore the derived  $T_{b/2l}$  were infinite.

238 At the end of the depuration period, the digestive gland contained the main part of  $^{109}\text{Cd}$ , i.e.  $97\%$  for *C.*  
239 *varia* and  $82\%$  for *P. maximus* (Table 2).

240

### 241 **Sediment exposure**

242 Sediment used in the experiment was mainly ( $95.8\%$ ) composed of grains which size ranged from 76 to  
243  $302\ \mu\text{m}$  and its dry/wet wt ratio was 0.80.

244 Whole-body uptake kinetics of sediment-bound  $^{109}\text{Cd}$  in *P. maximus* was best fitted by a single  
245 exponential model (Table 1). TF reached steady-state equilibrium within the 2 weeks of exposure

246 (estimated  $TF_{ss} = 0.034 \pm 0.002$ ). Among the different body compartments, the highest  $TF_{13d}$  was found  
247 in the digestive gland ( $3.35 \pm 1.68$ ; Table 3). This organ also contained the main fraction of the total  $^{109}Cd$   
248 body burden (i.e. 78 %; Table 3). The body compartment containing the second highest proportion was  
249 the mantle (14 % of total  $^{109}Cd$  body burden).

250 The  $^{109}Cd$  whole-body loss kinetics could not be described accurately by the exponential models;  
251 therefore a linear regression ( $Y = aX + b$ ) was applied in order to estimate the radiotracer retention. The  
252 results showed that 92 % of the accumulated  $^{109}Cd$  were efficiently incorporated in *P. maximus* tissues,  
253 with a biological half-life not significantly different from infinite (Table 1). At the end of the depuration  
254 period (31 d) the body distribution of  $^{109}Cd$  was identical to that at the end of the exposure period (Table  
255 3), with the highest proportion of  $^{109}Cd$  located in the digestive gland ( $\approx 80$  %), followed by the mantle ( $\approx$   
256 12 - 14 %). In addition, the  $^{109}Cd$  activities were similar in the two latter tissues at the end of exposure  
257 and depuration periods, viz.  $81 \pm 41$  and  $85 \pm 18$  Bq  $g^{-1}$  in the digestive gland and  $1.4 \pm 0.4$  and  $1.5 \pm 1.4$   
258 Bq  $g^{-1}$  in the mantle.

259

#### 260 **Subcellular distribution**

261 Examination of subcellular distributions indicated that, whatever the contamination pathway (i.e.,  
262 seawater, food or sediment) and the sampling period (i.e., end of uptake or end of loss period), *P.*  
263 *maximus* stored the major part of the cellular  $^{109}Cd$  in the soluble fraction (from 70 to 85 %). In contrast,  
264 the radiotracer was mainly bound to insoluble compounds in *C. varia* (Fig. 2).

265

#### 266 **DISCUSSION**

267 Pectinidae are an important marine resource which are both fished and cultured for human consumption  
268 (Ansell et al. 1991, Waller 1991). Hence, the intake of contaminants such as metals by Man through  
269 scallop consumption is a matter of concern. Indeed, Pectinidae are well known for their capacity of  
270 accumulating high levels of metals, and especially Cd, in their tissues (Brooks & Rumsby 1965, Bryan

271 1973, Bustamante & Miramand 2004, 2005b). Interestingly, this high bioaccumulation potential for Cd is  
272 not specific to anthropogenic contamination since scallops from the Antarctic Ocean have high Cd levels  
273 compare to temperate species living in the coastal waters of industrialised countries (Mauri et al. 1990,  
274 Viarengo et al. 1993).

275 Several field studies assumed that food would be the main intake pathway of Cd in scallops as high metal  
276 levels are always found in the digestive gland (Palmer & Rand 1977, Uthe & Chou 1987, Bustamante &  
277 Miramand 2005a). However, the contribution of the dissolved phase is difficult to ascertain in the field as  
278 this route can lead to a significant uptake of Cd and to its redistribution towards storage tissues such as  
279 the digestive gland. Therefore, there is a need to assess the relative importance of dissolved and  
280 particulate Cd pathways in order to better understand their respective contributions, as well as to evaluate  
281 the retention mechanisms leading to the high Cd levels measured in scallop tissues.

282 The experimental exposure of *Chlamys varia* and *Pecten maximus* to <sup>109</sup>Cd via seawater confirmed their  
283 ability to concentrate Cd from the dissolved phase, as previously shown using elevated exposure levels of  
284 stable Cd (Eisler et al. 1972, Carmichael & Fowler 1981). Indeed, after only 7 days of exposure to the  
285 dissolved radiotracer, both scallop species exhibited high whole-body concentration factors (CFs), with  
286  $37 \pm 9$  for *C. varia* and  $18 \pm 7$  for *P. maximus* whole bodies. This difference in CF between the two  
287 species exposed to the same contamination conditions is related (1) to a higher Cd uptake rate (uptake rate  
288 constant: 5.4 vs 2.7) and (2) secondarily, to a higher assimilated fraction (87.8 vs 77.1) in *C. varia*  
289 compared to *P. maximus* (Table 1). However in the specimens collected from the field, *C. varia* displayed  
290 typically lower Cd concentrations than *P. maximus* (Palmer & Rand 1977, Uthe & Chou 1987,  
291 Bustamante & Miramand 2005a). This would suggest that *C. varia* has far more limited capacities of Cd storage  
292 than *P. maximus*.

293 Considering the tissues separately, the organs involved in respiration (i.e. gills), excretion (i.e. kidneys)  
294 and digestion (i.e. digestive gland) displayed higher CFs compared to other body compartments in *P.*  
295 *maximus*, whereas the foot and the compartment “rest of the soft tissues” also showed elevated CFs in *C.*

296 *varia* (see Table 2). However, in terms of distribution among tissues and organs, Cd was mainly located  
297 in the digestive gland, the gills, the kidney and the mantle in both species, the digestive gland containing  
298 more than 30 % of the whole body burden of <sup>109</sup>Cd (Table 2). These results strongly suggest the  
299 occurrence of efficient redistribution mechanisms towards the tissues involved in the storage, excretion  
300 and detoxification processes, i.e. the kidneys and the digestive gland (e.g., Carmichael & Fowler 1981,  
301 Ballan-Dufrançais et al. 1985, Stone et al. 1986). It is also striking to note the difference between both  
302 species concerning the Cd CF in the foot that reached elevated values in *C. varia* (Table 2). In the latter  
303 species, the foot is well developed and contains a byssal gland which main role is to produce the byssus to  
304 stick to rocky substrates whereas *P. maximus* does not produce byssus as it lives buried in the sediment.  
305 Byssus is known to play a role in the elimination of metals from bivalves (Szefer et al. 2006), it is  
306 therefore likely that some metals are transferred from the soft tissues and concentrated in the byssus  
307 rather than merely adsorbed onto its surface from seawater. However, in the case of Cd, previous studies  
308 on mussels suggested that this metal is derived mainly from seawater (Coombs & Keller 1981, Nicholson  
309 & Szefer 2003). The present study was not designed to address this specific issue and our results do allow  
310 supporting internal transfer or waterborne origin of Cd in the byssus. However, further specifically-  
311 designed studies using sensitive radiotracer techniques could bring most interesting information on the  
312 origin of byssal Cd.

313 It is noteworthy that the Cd distribution pattern among the tissues was similar after 7 d of seawater  
314 exposure and after 36 d of depuration for both species (Table 2). Similarly, the subcellular distribution of  
315 Cd was identical at both times for *P. maximus*, with more than 80 % in the soluble fraction of the  
316 digestive gland cells (Fig. 2). Taking into account the relatively long biological half-life of Cd in *P.*  
317 *maximus*, this result indicate that the metal is mainly bound to soluble compounds involved in the storage  
318 of this metal. The implication of metallothionein-like proteins in Cd detoxification and storage in the  
319 digestive gland is well documented in Pectinidae (e.g., Stone et al. 1986, Evtushenko et al. 1990,  
320 Bustamante & Miramand 2005b). However, in *C. varia*, Cd was mainly bound to insoluble compounds

321 (from 59 to 80 %; see Fig. 2), suggesting a time-limited role of the soluble metalloproteins when the  
322 metal enters through the dissolved route (as well as via the food as similar results were found for the  
323 dietary exposure; see Fig. 2). Such a predominant interaction of Cd with the insoluble cellular fraction in  
324 the digestive gland is not a common observation among Pectinidae but has already been shown in some  
325 species (e.g., *Adamussium colbecki*; Viarengo et al. 1993) and would be due to the fact that, among  
326 insoluble cellular components (i.e., organelles, membranes and granules), the lysosomal system can play  
327 a major role in Cd detoxification (by trapping) and excretion (Ballan-Dufrançais et al. 1985, Marigómez  
328 et al. 2002).

329 After exposure to sediment-bound Cd, *P. maximus* exhibited very low transfer factors (viz.,  $TF_{ss} = 0.034$   
330  $\pm 0.009$ ), indicating that direct contamination due to burying into sediment would represent a minor Cd  
331 uptake pathway in this species. However, at the end of the exposure period, 80 % of the incorporated  
332 metal was found in the digestive gland, which displayed a TF higher than 3 (Table 3). As this organ is not  
333 in direct contact with the sediment, it is suggested that either (1) the radiotracer was progressively  
334 translocated from the tissues in direct contact with sediment and pore water to the digestive gland and/or  
335 (2) *P. maximus* was able to ingest sediment grains. Although sediment grains were never observed in the  
336 valves or in the digestive system in the many dissections carried out during this study, this latter  
337 hypothesis would be plausible as scallops were reported to be able to ingest particles of a wide size range  
338 (particles up to 950  $\mu\text{m}$  have been found in scallop stomachs; Mikulich & Tsikhon-Lukamina 1981,  
339 Shumway et al. 1987). Nevertheless, the assimilated Cd in the digestive gland was efficiently retained and  
340 was mainly bound to cytosolic compounds in the same proportions as in the food experiment, supporting  
341 the hypothesis of ingestion of sediment particles.

342 In the case of dietary exposure, Cd was assimilated to a similar extent in both species, with approx. 80 %  
343 of the radiotracer being incorporated in the scallop tissues. Such a high assimilation efficiency (AE) is  
344 striking as in other bivalve species, lower values were generally reported, e.g. for the tropical clam  
345 *Gafrarium tumidum* (AE = 42 %), the tropical oysters *Isognomon isognomon* and *Malleus regula* (AEs =

346 58 and 51 %, respectively) and the blue mussel *Mytilus edulis* (AE ranging from 8 to 40 %) (e.g., Wang  
347 & Fisher 1997; Hédouin 2006). These results suggest that food would be an important source of Cd for  
348 Pectinidae. However, inter-specific differences in Cd concentrations in scallops from the field (where *C.*  
349 *varia* showed the lowest concentrations) are difficult to explain in regards to the results obtained in our  
350 experiments. Indeed, lower depuration rates resulted in calculated biological half-life exceeding 3 years  
351 (Table 1), meaning that virtually all the assimilated Cd is readily stored in *C. varia* tissues. In contrast, the  
352 biological half-life following food exposure was approx. 4 months for *P. maximus*, indicating a faster  
353 turnover of the metal compared to *C. varia*. It is therefore likely that although living in the same areas, *C.*  
354 *varia* and *P. maximus* do not share the same food in the marine environment. Indeed, different storage  
355 mechanisms in prey can determine Cd bioavailability to higher trophic levels (e.g., Wallace & Lopez  
356 1997, Wallace & Luoma 2003). Moreover, the dissolved and sediment pathways should also have a  
357 strong importance in *P. maximus* (see above). The use of a bioaccumulation model is therefore a  
358 mandatory step to further explore the importance of each exposure pathways (Thomann et al. 1995, Wang  
359 & Fisher 1999). When applying such a model, food appears to be the major route of Cd accumulation in  
360 *C. varia*, with 99.6 % of the metal being accumulated from phytoplankton. In *P. maximus*, it was not  
361 possible to determine accurate data for the model because the kinetic parameters of the post sediment-  
362 exposure loss phase were not significant. Therefore, we only considered food and seawater pathways. In  
363 such conditions, results indicated that food accounted for 84.0 % of the accumulated Cd in *P. maximus*.  
364 Owing to the high assimilation efficiency of sediment-bound Cd ( $A_{01} = 92 \%$ ), it appears necessary to  
365 better delineate the sediment contribution to Cd accumulation in order to consider the three different  
366 pathways (seawater, food and sediment) on the global Cd bioaccumulation by *P. maximus*.

367

## 368 CONCLUSION

369 The present work on the bioaccumulation of Cd in two Pectinidae has confirmed the high Cd  
370 bioaccumulation potential of *C. varia* and *P. maximus*. The organs accumulating Cd to the highest extent



371 in both species are the digestive gland and the kidneys whatever the exposure pathway was. Comparison  
372 of results from laboratory experiments clearly showed that *C. varia* showed higher bioconcentration and  
373 bioaccumulation capacities than *P. maximus*. Since field data have reported higher Cd levels in *P.*  
374 *maximus* than in *C. varia*, it is suggested that Cd should be bioaccumulated by other uptake pathways  
375 than food and seawater. The high assimilation efficiency of Cd ingested through sediment pathway in *P.*  
376 *maximus* indicated that the particulate pathway could play an important role in the global Cd  
377 bioaccumulation process and studies on sediment as well as on suspended particulate matter should be  
378 further investigated to better simulate the different exposure routes of Cd to which Pectinidae are exposed  
379 in the field. Nevertheless, differences between field and laboratory observations could be related to  
380 different detoxification mechanisms in the two species.

381

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388

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477 Table 1. *Chlamys varia* and *Pecten maximus*. Whole-body uptake and loss kinetic parameters of <sup>109</sup>Cd following different exposure experiments:

478 1) 7-d exposure via seawater (n = 9) followed by 36 d of depuration (n = 9);

479 2) 2-hr feeding on radiolabelled *Isochrysis galbana* followed by a depuration period of 16 d (*P. maximus*, n = 6) or 30 d (*C. varia*, n = 6);

480 3) 13-d exposure of *P. maximus* via the sediments (n = 8) followed by 31 d of depuration (n = 8).

481 Uptake parameters: CF<sub>ss</sub> / TF<sub>ss</sub> concentration and transfer factors at steady state; k<sub>u</sub>: uptake rate constant (d<sup>-1</sup>)

482 Depuration parameters: A<sub>0s</sub> and A<sub>0l</sub>: activity (%) lost according to the short- and the long-lived exponential component, respectively; T<sub>b/2</sub> :

483 biological half-life (d). ASE: asymptotic standard error; r<sup>2</sup>: determination coefficient of the uptake or loss kinetics

| Experiment  | Species           | a. Uptake                                 |                            |                | b. Loss                 |                         |                         |                         |                |
|-------------|-------------------|---|----------------------------|----------------|-------------------------|-------------------------|-------------------------|-------------------------|----------------|
|             |                   | CF <sub>ss</sub> / TF <sub>ss</sub> ± ASE | k <sub>u</sub> ± ASE       | r <sup>2</sup> | A <sub>0s</sub> ± ASE   | T <sub>b/2s</sub> ± ASE | A <sub>0l</sub> ± ASE   | T <sub>b/2l</sub> ± ASE | r <sup>2</sup> |
| 1) Seawater | <i>C. varia</i>   | -   | 5.4 ± 0.2 <sup>d</sup>     | 0.85           | 12.2 ± 3.8 <sup>b</sup> | 0.8                     | 87.8 ± 2.4 <sup>d</sup> | 145 ± 45 <sup>b</sup>   | 0.31           |
|             | <i>P. maximus</i> | -   | 2.7 ± 0.1 <sup>d</sup>     | 0.66           | 23.4 ± 5.7 <sup>c</sup> | 1.1                     | 77.1 ± 4.8 <sup>d</sup> | 913                     | 0.49           |
| 2) Feeding  | <i>C. varia</i>   | -   | -                          | -              | 14.5 ± 4.1 <sup>c</sup> | 0.4                     | 85.8 ± 2.1              | 989                     | 0.21           |
|             | <i>P. maximus</i> | -   | -                          | -              | 20.5 ± 6.1 <sup>b</sup> | 0.02                    | 79.5 ± 3.7 <sup>d</sup> | 138                     | 0.37           |
| 3) Sediment | <i>P. maximus</i> | 0.034 ± 0.002 <sup>d</sup>                | 0.014 ± 0.002 <sup>d</sup> | 0.62           | NC                      | NC                      | 92 <sup>d</sup>         | NC                      | -              |

484

485 Probability of the model adjustment: <sup>a</sup> p < 0.05, <sup>b</sup> p < 0.01, <sup>c</sup> p < 0.001, <sup>d</sup> p < 0.0001; NC: not calculated

486

487 Table 2. *Chlamys varia* and *Pecten maximus*. Concentration Factors (mean CF  $\pm$  SD) and  
 488 tissue distribution (mean %  $\pm$  SD) of  $^{109}\text{Cd}$  during seawater (end of exposure and depuration  
 489 periods) and feeding experiments (16 and 30 d after feeding for *P. maximus* and *C. varia*,  
 490 respectively).

| Species<br>Compartments | Seawater contamination                       |                     | Food contamination                      |                                   |
|-------------------------|--|---------------------|---|-----------------------------------|
|                         | Uptake (7 d, n=5)<br>Concentration<br>Factor | Distribution<br>(%) | Loss (36 d, n=4)<br>Distribution<br>(%) | Loss (n=5)<br>Distribution<br>(%) |
| <i>Chlamys varia</i>    |  |                     |   |                                   |
| Digestive gland         | 322 $\pm$ 175                                | 33 $\pm$ 14         | 41 $\pm$ 18                             | 97 $\pm$ 1                        |
| Gills                   | 277 $\pm$ 102                                | 30 $\pm$ 9          | 23 $\pm$ 6                              | < 1                               |
| Kidneys                 | 928 $\pm$ 547                                | 13 $\pm$ 6          | 15 $\pm$ 8                              | < 1                               |
| Intestine               | 23 $\pm$ 7                                   | < 1                 | 1 $\pm$ 1                               | < 1                               |
| Gonad                   | 45 $\pm$ 65                                  | 1 $\pm$ 1           | 1 $\pm$ 1                               | 1 $\pm$ 0                         |
| Foot                    | 265 $\pm$ 74                                 | 3 $\pm$ 1           | 2 $\pm$ 0                               | < 1                               |
| Mantle                  | 53 $\pm$ 11                                  | 12 $\pm$ 4          | 10 $\pm$ 6                              | < 1                               |
| Adductor muscle         | 21 $\pm$ 6                                   | 4 $\pm$ 1           | 5 $\pm$ 3                               | < 1                               |
| Remaining tissues       | 258 $\pm$ 56                                 | 5 $\pm$ 1           | 2 $\pm$ 0                               | 0 $\pm$ 1                         |
| Whole body              | 37 $\pm$ 9                                   |                     |   |                                   |
| <i>Pecten maximus</i>   |  |                     |   |                                   |
| Digestive gland         | 659 $\pm$ 227                                | 38 $\pm$ 10         | 49 $\pm$ 5                              | 82 $\pm$ 19                       |
| Gills                   | 175 $\pm$ 13                                 | 28 $\pm$ 11         | 19 $\pm$ 2                              | 1 $\pm$ 0                         |
| Kidneys                 | 690 $\pm$ 402                                | 10 $\pm$ 4          | 12 $\pm$ 4                              | 6 $\pm$ 12                        |
| Intestine               | 16 $\pm$ 3                                   | < 1                 | < 1                                     | 1 $\pm$ 1                         |
| Gonad                   | 18 $\pm$ 10                                  | 2 $\pm$ 2           | 2 $\pm$ 1                               | 9 $\pm$ 17                        |
| Foot                    | 13 $\pm$ 5                                   | < 1                 | < 1                                     | 1 $\pm$ 1                         |
| Mantle                  | 28 $\pm$ 5                                   | 11 $\pm$ 2          | 10 $\pm$ 7                              | < 1                               |
| Adductor muscle         | 18 $\pm$ 7                                   | 9 $\pm$ 3           | 7 $\pm$ 1                               | < 1                               |
| Remaining tissues       | 78 $\pm$ 33                                  | 2 $\pm$ 0           | 1 $\pm$ 0                               | 1 $\pm$ 0                         |
| Whole body              | 18 $\pm$ 7                                   |                     |   |                                   |

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494

495 Table 3. *Pecten maximus*. Transfer Factors (mean TF  $\pm$  SD; n = 4) of  $^{109}\text{Cd}$  after a 13-d  
 496 exposure via sediment and tissue distribution (mean %  $\pm$  SD) of  $^{109}\text{Cd}$  at the end of the 13-d  
 497 exposure and 31-d depuration period (n= 5).

| Compartments      | Uptake phase    |                  | Loss phase       | 498 |
|-------------------|-----------------|------------------|------------------|-----|
|                   | Transfer Factor | Distribution (%) | Distribution (%) | 499 |
| Digestive gland   | 3.35 $\pm$ 1.68 | 78 $\pm$ 10      | 80 $\pm$ 10      | 500 |
| Gills             | 0.05 $\pm$ 0.04 | 4 $\pm$ 3        | 6 $\pm$ 1        | 501 |
| Kidneys           | 0.12 $\pm$ 0.04 | 1 $\pm$ 1        | < 1              | 502 |
| Intestine         | 0.09 $\pm$ 0.05 | < 1              | < 1              | 503 |
| Gonad             | 0.06 $\pm$ 0.05 | 1 $\pm$ 0        | < 1              | 504 |
| Foot              | 0.03 $\pm$ 0.01 | < 1              | < 1              | 505 |
| Mantle            | 0.06 $\pm$ 0.02 | 14 $\pm$ 8       | 12 $\pm$ 10      | 506 |
| Adductor muscle   | 0.00 $\pm$ 0.00 | 1 $\pm$ 1        | < 1              |     |
| Remaining tissues | 0.06 $\pm$ 0.05 | 1 $\pm$ 1        | < 1              |     |
| Whole body        | 0.04 $\pm$ 0.01 |                  |                  |     |



507 Caption to figures.

508

509

510 Figure 1. *Chlamys varia* and *Pecten maximus*. Uptake and loss kinetics of  $^{109}\text{Cd}$  in scallops  
511 exposed for 7 d via seawater (uptake kinetics A1; Concentration Factors -CF-; mean  $\pm$  SD; n  
512 = 9), then maintained for 36 d in non contaminated conditions (loss kinetics A2; Remaining  
513 activity -%-; mean  $\pm$  SD; n = 9) and after a 2-hr feeding on radiolabelled phytoplankton  
514 *Isochrysis galbana* (loss kinetics B; Remaining activity -%-; mean  $\pm$  SD; n = 6 *C. varia* and n  
515 = 9 *P. maximus*).

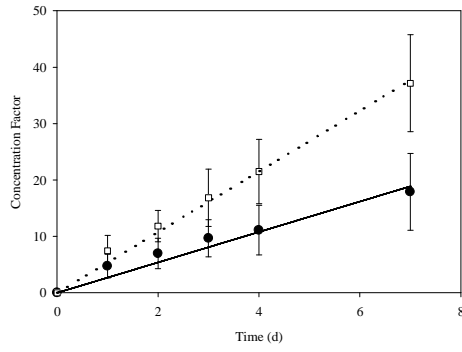
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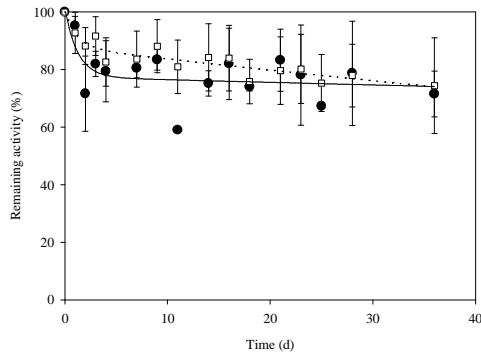
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519 Figure 2. *Chlamys varia* and *Pecten maximus*. Subcellular distribution of  $^{109}\text{Cd}$  in the  
520 digestive gland cells following different exposure experiments: (1) 7-d exposure via seawater  
521 followed by 36 d of depuration; (2) 2-hr feeding on radiolabelled *Isochrysis galbana* followed  
522 by a depuration period of 16 d (*P. maximus*) or 30 d (*C. varia*); (3) 13-d exposure of *P.*  
523 *maximus* via the sediments followed by 31 d of depuration.

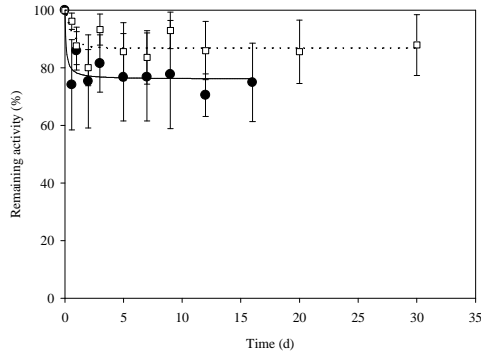
A1. Uptake via seawater





A2. Loss after seawater exposure



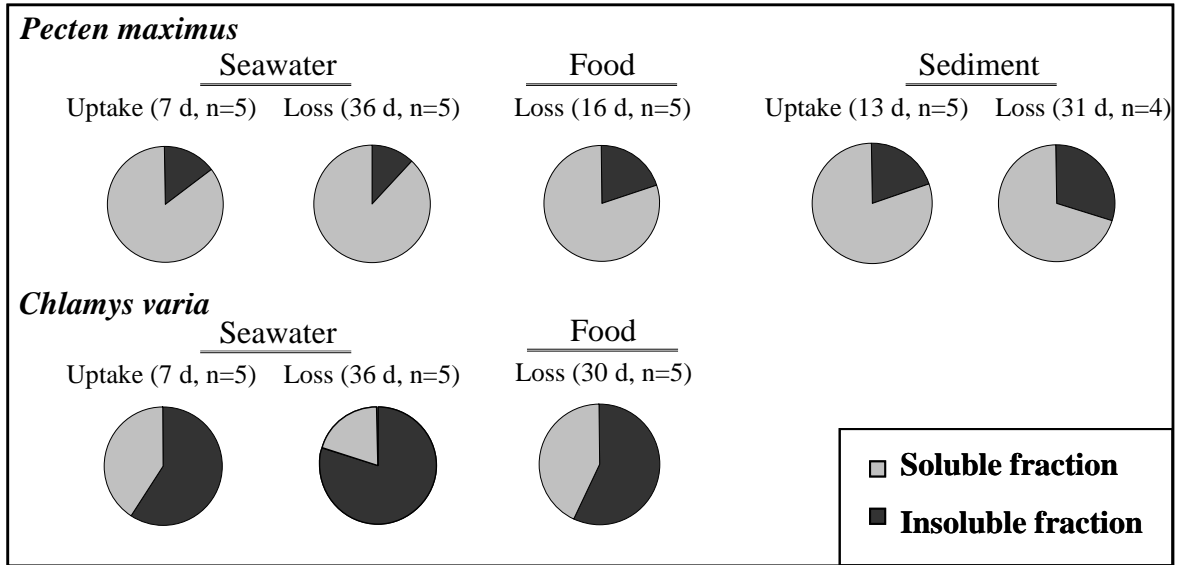
B. Loss after food exposure




*Chlamys varia*

*Pecten maximus*

1  
2  
3  
4  
5

Figure 1.



6

7

8

Figure 2.