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Cadmium dietary intake and biomarker data in French high seafood consumers

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Seafood and especially mollusks are known to be a rich source of cadmium (Cd), but little data are available concerning French seafood contamination and Cd exposure of French populations. The objective was then to assess food intake and biological level of Cd in high consumers of seafood, and to determine the impact of the consumption of self-fished mollusks on urinary Cd. Seafood consumption levels of 80 products were assessed for 1011 high consumers aged 18 and over in four French coastal areas, thanks to a validated food frequency questionnaire (FFQ). According to a total diet study approach, seafood samples were collected taking into account preservation methods and supply habits. Food samples were analyzed for Cd. Exposure was assessed by crossing consumption data with contamination data. Total blood and urine samples were collected from 380 subjects of the cohort and analyzed for Cd. The impact of the self-collected mollusks consumption on the Cd biological level adjusted for creatinine was assessed by a multivariate linear regression model. The mean dietary intake of Cd is $2.44 \pm 3.34\,\mu g/kg\,bw/wk$ and the mean urinary Cd (U-Cd) level is $0.65 \pm 0.45\,\mu g/g$ creatinine, and is significantly higher in women than in men ($P < 0.05$). The consumption of self-fished mollusks is significantly negatively associated with U-Cd ($r = -0.11$ [-0.185, -0.009], $P = 0.03$). The results of this study indicate that the biological Cd levels remain below the standards, and also suggest a protective effect of self-fishing, which inspires confidence about the high consumer health safety in terms of Cd exposure.
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Introduction

Cadmium (Cd) is a contaminant found in the environment and in particular in the soil, due to natural erosion but also human and agricultural activities. It thereby enters the food chain. In non-smoking individuals and non-professionally exposed individuals, the main source of Cd exposure is food. In France the most highly contaminated foods are mollusks, offals, leaf vegetables and cereals (DGS, 1992; Decloître, 1998). Digestive absorption of Cd is low (about 5–10%). On the other hand, Cd is a cumulative toxin whose biological half-life is very long (estimated to be 20–30 years in humans). The International Agency for Research on Cancer (IARC) classifies Cd as "carcinogenic for man" (category 1) (IARC, 1993).

Cd has numerous toxic effects, but the main impact of prolonged exposure on the organism is on renal function in both man and animals. The nephrotoxic effects are characterized by degeneration of the proximal tubules and proteinuria (JECFA, 1989; Nogawa et al., 1989; Staessen et al., 1996; Jarup et al., 2000). The risk associated with this degeneration starts to increase when the urinary excretion of Cd exceeds $2.5\,\mu g/g$ creatinine. The JECFA considers this to be the value for which there is an absence of prevalence of renal tubular malfunction (JECFA, 2001). In man, these alterations of the renal function can be accompanied by bone damage, with osteomalacia and demineralization (ATSDR, 1999; Noël et al., 2004). Additionally, relationships exist in man and animals between Cd exposure and retarded fetal growth (Frery et al., 1993); reduced fertility in males has also been reported (Xu et al., 1993; Telisman et al., 2000). But there is no confirmed relationship between Cd dietary intake and arterial hypertension or cancer.

The JECFA established a provisional tolerable weekly intake (PTWI) of $7\,\mu g/kg\,bw/wk$ (bw = body weight; wk = week), using a theoretical prediction model estimating the relationships between the dietary intake of Cd, urinary excretion and associated prevalence of renal tubular malfunction (JECFA, 2001). Recent studies, especially studies including biomarkers in Japan, Europe and the United States, have shown a link between urinary Cd and changes in renal function, and bone/calcium metabolism. Moreover Cd-linked kidney toxicity seems to occur in subjects whose dietary intake remains below the current PTWI (Satarug and Moore, 2004). Nevertheless, the Committee concluded that the new data did not provide a sufficient basis for revising the PTWI (JECFA, 2004).

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In France, the Cd average daily intake was estimated to be 19.6 μg/day for adults in 1998 (Decloître, 1998), and 17 μg/day in 2000 (Leblanc et al., 2000). Following the first French total diet study (TDS) (Leblanc et al., 2005a), the latest estimations in 2005 indicate an average daily intake of 2.7 μg/day in people over 15 years old (Leblanc et al., 2005a), which represents about 4% of the PTWI. Seafood represents 8–25% of dietary intake of Cd (Tressou et al., 2004). Vegetables, potatoes and similar products, due to their importance in human diets, are also major vectors of dietary exposure (23.7% and 21.2%, respectively) in the general population (Leblanc et al., 2000).

Here, the first objective was to assess the Cd dietary intake of French high fish and seafood consumers via an ''indirect'' measure using consumption data and levels of contamination in food, and via a ''direct'' measure using biomarkers associated with Cd exposure. The second objective was to determine the dietary and individual components, especially the consumption of locally fished mollusks, having an impact on the usually-used biomarker of exposure, the urinary Cd (U-Cd).

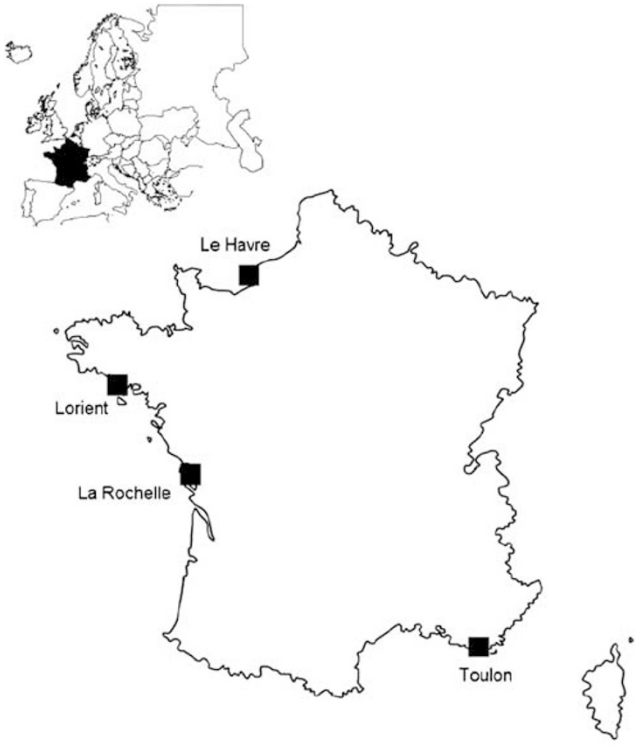

Figure 1. Location of the 4 French coastal study areas.

Subjects and methods

Subjects and Consumption Survey

A total of 1011 high fish and seafood consumers (at least two meals a week) aged 18 and over were recruited in four French coastal areas (Figure 1): Le Havre in Normandy/Baie de Seine, Lorient in south Brittany, La Rochelle in Gironde/south Charente Maritime and Toulon in Mediterranean/Var; within a radius of 20–25 km around those points. The distribution of individuals questioned within each region was calculated on the basis of the French census population survey carried out by the French national Institute for statistic and economical surveys (INSEE, 1999). A representative consumer population sample was ensured using the so-called ''random route'' method, that is, choosing at random the first address and then by door-to-door, canvassing every five doors. The response rate was 43%. The detailed results for acceptability of the consumption survey are described elsewhere (Leblanc, 2006).

Consumptions for 80 fish and seafood were assessed using a food frequency questionnaire (FFQ) first validated by a pilot survey in two coastal regions using a consumption diary and a FFQ (AFSSA, 2003). The FFQ included questions on the quantity consumed and on the purchase place frequency for each product (Leblanc, 2006; Bemrah et al., submitted).

Biological Analysis

All participants in the food consumption survey were invited to participate in this biological part, in which blood and urine were sampled. Blood and morning urine samples were taken from a subsample of 380 volunteers (38%). Reasons why some refused and exclusion criteria are described elsewhere (Leblanc, 2006). The level of Cd was determined by inductively coupled plasma-mass spectrometry (ICP-MS). To ensure optimal reliability of the blood analysis results, blood Cd was measured by two different laboratories on all the samples taken (LOD = 0.03–0.3 μg/l, LOQ = 0.1–1.0 μg/l). Cd was also measured in the urine samples (LOD = 0.1 μg/l, LOQ = 0.5 μg/l).

Blood was collected in trace element-free heparin tubes with controlled Cd-level (S-Monovette for Trace Metal Analysis). For the first laboratory, samples were unfrozen and sonicated for 20 min. A 250-μl volume was sampled for analysis. A 1-ml volume of 70% nitric acid and 0.25 ml of hydrogen peroxide were added. Samples were sonicated for 90 min, then left overnight at room temperature. An 8.5-ml volume of ultrapure water was added before analysis by ICP-MS Thermo X7 (Thermo Electron). Quantification was by standard addition method on pooled samples (addition of known levels of Cd in the tested samples). The method was validated by the analysis of a certified reference material (CRM) for the determination of total concentrations (SERONORM I, Sero AS). Twenty replicates were analyzed (three by analysis sequence). The certified concentration in Cd of this CRM was 0.74 ± 0.06 μg/l and the experimental concentration was 0.63 ± 0.20 μg/l. The accuracy of the analysis was 88%.

For the second laboratory, Cd level analysis was performed by ICP-MS using an Agilent 7500a instrument.

Whole blood samples were first diluted 1:2 with a solution of Triton X-100 at 0.2% in water (i.e., a dilution 1:40).

Urine was collected in 10-ml polypropylene tubes, which were analyzed for Cd to compensate for contamination. Cd determination in urine was performed by ICP-MS using an Agilent 7500a instrument. Urine samples were diluted 1:20 with 1% nitric acid containing 10 μg/l of europium, 100 μg/l of gold and 10 g/l of glycerol (Nixon and Moyer, 1996; Schramel et al., 1997; White and Sabbioni, 1998; Komaromy-Hiller et al., 2000; Wilhelm et al., 2004; Heitland and Köster, 2006). Europium was used as an internal standard. Cd and europium were measured at m/z 111 and 153, respectively. The linearity was tested up to 20 μg/l. The urine quality control material used was Lyphochek urine metals control, level 1, lot 69091 (BioRad) diluted with deionised water (1:2). The certified concentration in Cd of this Lyphochek urine metals control was 8.20 (6.98–9.44 μg/l), and the experimental concentration obtained after a 1:2 dilution was 3.60 ± 0.10 μg/l ($n = 40$).

Creatinine was determined by a colorimetric method based on the Jaffe reaction. Analyses were carried out using a Cobas Mira instrument and reagents from ABX (France).

''Total Diet Study'' and Food Analysis

A local sampling covering the most consumed fish and seafood (88–100% of the total consumption) was conducted, based on the standardized methodology developed in the TDS of the French population (Leblanc et al., 2005b) and recommended by international Committees (US FDA/WHO 1999; WHO/ANFZA/FAO, 2002; WHO/INRA, 2004). A total of 824 products were sampled, including 690 fresh and frozen products and 134 canned products, smoked fish or prepared seafood-based dishes, making 138 samples for fresh and frozen fish, mollusks and crustaceans, and 21 samples for canned products, smoked fish or prepared seafood-based dishes (Sirot et al., 2007).

Cd in the food matrices was measured by ICP-MS Elan 6000 (Perkin Elmer Sciex) (LOD = 0.0004 μg/g fresh weight, LOQ = 0.001 μg/g fresh weight). About 250 mg of dry sample were weighted, then 6 ml of 70% nitric acid were added. Samples were left at room temperature overnight, then 2 ml of hydrogen peroxide were added. Samples were heated at 85°C for 3 h. The extract volume was adjusted to 50 ml with ultrapure water. The method was validated on a CRM (DORM-2, NRCC Canada) for the determination of concentrations, and 16 replicas were analyzed. The certified concentration in Cd of this CRM was 0.043 ± 0.008 μg/g and the experimental concentration was 0.045 ± 0.08 μg/g. The accuracy of the analysis was 104%.

Cd Exposure

The studied population was divided in three groups as follows: adult men (18–64 years old), adult women (18–64 years old) and older subjects (65 and over). Only 996 subjects of the 1011 were taken into account. Fifteen have been eliminated because of their incoherent self-declared consumptions. Two methodological approaches were used to assess the nutrient intakes. For the ''external exposure'' approach, the intakes of Cd were calculated by crossing the individual average consumption data from the food consumption survey with the average contamination data obtained by the analysis of representative food samples in each study zone. Cd levels that were undetectable (< LOD) or unquantified (< LOQ) in food were taken to be equal to half these limits, in line with international recommendations (WHO, 1995). Concerning the ''internal exposure'' or ''biomarker approach'', levels that were undetectable or unquantified were taken to be equal to half these limits, but to determine the diet components responsible for the U-Cd levels, the real theoretical levels (results between LOD and LOQ) have been used.

All results are presented as mean $\pm$ SD. The results of blood analysis are the means of the results of the two laboratories. U-Cd levels have been adjusted for creatinine. Pearson's correlations were calculated between the calculated indirect intake and the direct exposure in order to validate the selected markers from a methodological point of view.

To explain the U-Cd by function of the diet components and adjustment variables, a bivariate analysis was first performed. For quantitative variables, Spearman correlation was calculated. For qualitative variables, a t-test for bivariate variables or a Kruskall–Wallis test for more than two modality variables was used. Two multivariate regression linear models as follows were built: a minimal model implying the statistically significantly linked variables with the U-Cd in the bivariate analyses was compared to a maximal model implying all the variables selected. The main analysis was performed for these two models, with interest variables of mollusks and crustaceans consumption coded in a quantitative way. In a secondary analysis these variables were binarized as follows: ''more than 50% of the consumed mollusks are self-fished'' and ''more than 50% of the consumed crustaceans are self-fished''. The interactions between both and the corresponding consumption were introduced in the model.

Partial correlation coefficients were calculated for variables of interest in the main analysis and confidence intervals were calculated by the bootstrap non-parametric method (Efron, 1979). Interactions were tested by the False Discovery Rate method (Benjamini and Hochberg, 1995). A regression diagnosis was performed and the robustness of the models was evaluated. The significance level was chosen to $\alpha = 0.05$.

All data analyses were performed with the use of SAS version 8.02 (SAS Institute Inc., Cary, NC, USA) and R version 2.3.0 (http://cran.r-project.org/).

Results

Fish and Seafood Consumption by High Consumers

Consumptions of the 1011 high seafood consumers of the Calipso study have been described elsewhere (Bemrah et al., submitted) Particularly fish and seafood consumption of the 380 subjects participating to the biological part of the study is not statistically significantly different from consumption of the 1011 subjects. People who participated in the biological part are also considered to be representative of the population studied according to the sex ratio rate, age and body weight distributions.

Cd Contamination of Fish and Seafood

A total of 45.3% of data was censured (<LOD). Among fish (Table 1), some species have Cd levels exceeding 0.30 or 0.05 μg/g, the maximum limits authorized species to species (EC No 1881/2006): saithe (0.07 μg/g), swordfish (0.07 μg/g), and catshark with the highest observed Cd level (0.42 μg/g). These high mean values are due to the exceptional contamination of the composite sample from Le Havre (1.65 μg/g); the concentrations of the composite samples of the three other zones do not exceed the maximal authorized level.

Concerning mollusks and crustaceans (Table 1), the maximum authorized limits are exceeded by several species, in particular crab (4.1 *versus* 0.5 μg/g), shrimp (1.1 *versus* 0.5 μg/g) and calico scallops (1.1 *versus* 1.0 μg/g). The other bivalve shellfish display lower Cd levels not exceeding 0.04 μg/g. These average contaminations are greater than the mean levels measured in the French monitoring plans: 0.46 μg/g for crab or 0.05 μg/g for shrimp. These differences are due to high contamination of our composite crab sample in Lorient (12 μg/g *versus* less than 1 μg/g in the other sampling zones), and of the composite sample in Le Havre (4 μg/g *versus* less than 0.05 μg/g on the other zones).

Among other seafood, the canned products are the most contaminated by Cd (Table 1). Canned or bottled anchovy and canned sardines have Cd concentrations higher than the authorized limits (0.35 and 0.22 μg/g *versus* 0.1 μg/g). However, these results should be interpreted with care, in view of the homogenization problems encountered during preparations of canned samples. The other products, smoked fish or prepared seafood-based dishes, contain very low levels of Cd.

Despite the deliberate choice of zones contrasted by the existence of old local environmental pollution, no significant differences are found between the Cd contaminations measured in the different study zones (results not presented).

This absence of regional differences can be explained by the low representation of explicitly local origins in the provisioning of seafood products mentioned earlier and by the general respect of local fishing interdictions when these exist.

Table 1. Mean contamination by cadmium of fish and seafood in μg/g wet weight.

Fish	n[a]	Cd	Crustaceans	n[a]	Cd
Anchovy	1	0.0295	Calico scallop	1	1.1391
Angler fish	4	0.0002	Crab	3	4.0954
Cat shark	4	0.4183	Lobster	1	0.4326
Cod	4	0.0004	Scampi	3	0.1077
Common dab	4	0.0002	Shrimp	4	1.0915
Eel	1	0.0033	Spider crab	1	0.4606
Emperor	3	0.0048	Swim crab	2	0.1274
Goatfish	3	0.0005	Mollusks	n[a]	Cd
Grenadier/hoki	4	0.0036	Cockle	2	0.0358
Gurnard	1	0.0002	Common periwinkle	3	0.1890
Haddock	2	0.0036	Cuttle fish	2	0.0559
Hake	4	0.0002	Great scallop	4	0.2695
Halibut	4	0.0335	Mussel	4	0.0329
John dory	2	0.0444	Octopus	1	0.0324
Ling	4	0.0043	Oyster	4	0.0343
Mackerel	4	0.0002	Squid	4	0.0511
Plaice	2	0.0002	Whelk	3	0.7807
Pollack	3	0.0006	Canned seafood	n[a]	Cd
Pout	1	0.0002	Canned anchovy	2	0.3506
Ray	4	0.0388	Canned crab	1	0.1713
Saithe/coalfish	4	0.0719	Canned mackerel	1	0.0446
Salmon	4	0.0002	Canned pilchard	1	0.0126
Sardine	4	0.0019	Canned sardine	1	0.2159
Scorpion fish	1	0.0002	Canned tuna	5	0.0178
Sea bass	4	0.0005	Seafood-based dishes	n[a]	Cd
Sea bream	4	0.0002	Fish soup	2	0.0151
Sole	4	0.0014	Paella	1	0.0128
Swordfish	4	0.0671	Surimi	1	0.0083
Tuna	4	0.0132	Tarama	1	0.0002
Whiting	4	0.0011	Smoked fish	n[a]	Cd
Echinoderm	n[a]	Cd	Smoked haddock	1	0.0002
Sea urchin	1	0.0643	Smoked herring	1	0.0002
			Smoked mackerel	1	0.0027
			Smoked salmon	1	0.0002

Cd, cadmium.

[a]Number of composite samples, each sample is composed of at least five subsamples of the same species, representative of the provisioning methods in each zone (port, market, supermarket, self-fishing…).

Dietary Exposure

The mean dietary intake of Cd is of 2.44 ± 3.34 μg/kg bw/wk. The highest average exposure is noted in Le Havre subjects (4.64 μg/kg bw/wk). Considering the 996 subjects, adult women have a significantly higher Cd intake than adult men ($P<0.05$). But considering only the people who took part in the biological part of the study, there is no significant difference in mean dietary exposure between the subgroups (Table 2). We notice a significant north–south exposure gradient ($P<0.05$), with a minimum mean exposure in Toulon and a maximum in Le Havre, considering all subjects or only the ones participating in the biological assay.

The PTWI of 7 μg/kg bw/wk is exceeded by 8.5% of the subjects through their fish and seafood consumption alone (and 9.5% of subjects for whom biological results are available, among which 9% of adult men, 11% of adult

Table 2. Dietary Cd intake (μg/kg body weight/week) in each subgroup of population.

	n	$\bar{x} \pm$ SD	P10	P50	P90
External exposure (all subjects)					
Adult men (18–64 years)	243	1.94 ± 2.40^{a}	0.20	1.04	4.87
Adult women (18–64 years)	630	2.69 ± 3.70^{b}	0.23	1.44	6.95
Older subjects (>64 years)	123	$2.15 \pm 2.85^{a,b}$	0.28	1.15	5.52
Le Havre	249	4.64 ± 4.63^{a}	0.87	3.40	8.91
Lorient	247	2.28 ± 3.01^{b}	0.34	1.91	6.69
La Rochelle	248	1.52 ± 2.27^{c}	0.21	0.82	3.07
Toulon	252	0.77 ± 0.74^{d}	0.04	0.60	1.62
All	996	2.44 ± 3.34	0.22	1.28	6.23
Biological part (380 subjects)					
Adult men (18–64 years)	91	2.18 ± 2.73^{a}	0.28	1.16	5.26
Adult women (18–64 years)	252	2.57 ± 2.80^{a}	0.28	1.60	7.07
Older subjects (>64 years)	37	1.95 ± 2.42^{a}	0.33	1.29	4.78
Le Havre	81	4.34 ± 3.01^{a}	1.25	3.76	7.97
Lorient	115	2.83 ± 2.85^{b}	0.35	1.83	6.57
La Rochelle	95	1.74 ± 2.49^{c}	0.29	0.99	3.43
Toulon	89	0.84 ± 0.74^{c}	0.12	0.64	1.89
All	380	2.42 ± 2.75	0.28	1.48	6.58

On a same row between the three age/gender subgroups (adult men, women and older subjects), and separately for the for study zones, the values with a different index letter are significantly different $P<0.05$ (Tukey test).

women and 5% of older subjects). Those people consume in average 323 g of shellfish per week (representing 15% of their total consumption of seafood) and 177 g of crustaceans per week (10% of their total consumption), whereas people who do not exceed the PTWI eat in average 122 g of shellfish and 83 g of crustaceans per week (corresponding respectively to 11% and 7% of their total consumption of seafood).

The main contributors to the total exposure (not presented) are fresh and frozen crab (26%), shrimp (25%), whelk and calico scallop (8% each), canned anchovy and great scallop (6% each). However these contributions are not the same in all zones: the main contributors in Le Havre are shrimp (65%), catshark (13%), great scallop (9%) and whelk (8%); in Lorient they are crab (73%), saithe (10%) and canned anchovy (6%); in La Rochelle they are mollusks: calico scallop (43%), whelk (27%) and oyster (7%); in Toulon they are canned anchovy (33%), great scallop (22%), sea urchin (8%), lobster (8%) and canned sardine (6%). For the most exposed individuals in each zone (P90) we find the same main contributors (results not presented). The same species contributed to the PTWI (not presented).

Exposure Biomarker

Depending on the zone, the average Cd blood levels in the range from 0.51 ± 0.41 to 0.70 ± 0.66 μg/l (Table 3). There is neither statistically significant difference between subgroups, nor between different areas.

A concentration exceeding the ''basal'' level of 1 μg/l for non-smokers and 2 μg/l for smokers (INRS, 2005) was found in 18 individuals (5%). This ''basal'' level corresponds to the P95 in the French population that is not professionally exposed. No individuals exceeded the concentration associated with toxicity (20 μg/l).

The U-Cd level is the biomarker usually used. Creatinine is significantly lower in men than in women ($P<0.0001$), and the level of creatinine showed a significant negative correlation with increasing age ($P<0.0001$).

The mean concentration of Cd in urine is of 0.65 ± 0.45 μg/g creatinine, and is not significantly different between zones. Moreover older subjects present a significant higher concentration than adult women, who have a significantly higher concentration than adult men ($P<0.05$). Those measurements indicate that the Cd levels in urine are in the ''basal'' range, less than 2 μg/g creatinine, even for the high percentiles (P90). Only 12 people exceed this value, among which seven are smokers or former smokers with an average age of 52, yet these are not the people having the highest Cd concentrations in the blood.

Determination of the U-Cd

The results of the bivariate analysis (not presented) showed that age, tobacco consumption during life, the length living in the accommodation and quantities of fish and vegetables weekly consumed are significantly positively correlated with the log(U-Cd/creat) ($P<0.05$). Concerning qualitative variables, the gender, professional exposure and use of phosphate fertilizer in one's garden are significantly linked with U-Cd ($P<0.05$) (not presented). The variable concerning the major local fishing of crustaceans is close to significant limit ($P=0.06$).

Table 3. Biological levels of Cd in each subgroup of population.

		Blood Cd (μg/l)				Total U-Cd (μg/l)				U-Cd/Creat (μg/g)			
	n	$\bar{x} \pm$ SD	P10	P50	P90	$\bar{x} \pm$ SD	P10	P50	P90	$\bar{x} \pm$ SD	P10	P50	P90
Adult men (18–64 years)	91	0.75 ± 0.80^{a}	0.20	0.48	1.51	0.64 ± 0.31^{a}	0.5	0.5	1.0	0.46 ± 0.29^{a}	0.24	0.37	0.81
Adult women (18–64 years)	252	0.58 ± 0.45^{a}	0.21	0.42	1.23	0.63 ± 0.33^{a}	0.5	0.5	1.0	0.67 ± 0.47^{b}	0.29	0.54	1.19
Older subjects (>64 years)	37	0.69 ± 0.77^{a}	0.28	0.48	1.04	0.77 ± 0.50^{a}	0.5	0.5	1.4	0.95 ± 0.50^{c}	0.42	0.83	1.94
Le Havre	81	0.51 ± 0.41^{a}	0.20	0.36	1.14	0.67 ± 0.42^{a}	0.5	0.5	1.1	0.59 ± 0.36^{a}	0.27	0.49	0.98
Lorient	115	0.63 ± 0.59^{a}	0.18	0.43	1.38	0.63 ± 0.26^{a}	0.5	0.5	1.0	0.68 ± 0.43^{a}	0.29	0.57	1.11
La Rochelle	95	0.70 ± 0.66^{a}	0.26	0.51	1.32	0.62 ± 0.31^{a}	0.5	0.5	0.9	0.60 ± 0.49^{a}	0.25	0.45	1.13
Toulon	89	0.67 ± 0.64^{a}	0.24	0.45	1.32	0.69 ± 0.41^{a}	0.5	0.5	1.3	0.72 ± 0.51^{a}	0.31	0.50	1.32
All	380	0.63 ± 0.59	0.22	0.44	1.31	0.65 ± 0.35	0.5	0.5	1.0	0.65 ± 0.45	0.26	0.50	1.19

Creat, creatinine; U-Cd, urinary cadmium.
On a same row between the three age/gender subgroups (adult men, women and older subjects), and separately for the for study zones, the values with a different index letter are significantly different $P<0.05$ (Tukey test).

The minimal model tested was therefore the following: log(U-Cd/creat) = gender + age + zone + tobacco status + tobacco consumed during life + use of phosphate fertilizer + professional exposure + length living in the accommodation + vegetables consumption + fish consumption + fish-based dishes consumption + self-fished mollusk consumption + self-fished crustaceans consumption + bought mollusks consumption + bought crustaceans consumption + ε. The results for the linear regression are presented in Table 4. The introduction in a maximal model of the "bread and cereals consumption + potatoes consumption + offal consumption + smoked fish consumption + canned seafood consumption" variables do not bring any additional relevant information (results not presented).

The variables of gender, age, tobacco status, tobacco consumed during life ($P<0.0001$) and the use of phosphate fertilizer associated with consumption of vegetables from one's garden ($P=0.03$) are significantly linked with U-Cd. Women have a U-Cd significantly higher than men (Table 3) and still have after adjusting with the other variables, with a positive regression coefficient ($P<0.0001$). Age and tobacco consumed during life are positively correlated with U-Cd. The present tobacco status increases the U-Cd compared with a non-smoker. The ex-smoker status seems to decrease the U-Cd compared with a non-smoker, but this effect is not significant ($P=0.34$). And the use of phosphate fertilizer also seems to decrease the U-Cd. The consumption of self-fished mollusks is significantly associated with U-Cd, but this association is negative ($r=-0.11$ [−0.185, −0.009], $P=0.03$), suggesting a protective effect of this fishing practice. After adjusting, there is neither significant effect of the professional exposure, nor of the length of living in one's accommodation, the fish and vegetable consumption. There is no zone effect.

In the secondary analysis, some other variables were introduced in a maximal model: the fact that "more than 50% of the consumed mollusks are self-fished" and that "more than 50% of the consumed crustaceans are self-fished", the interactions between both variables and the corresponding consumptions. The results of this secondary analysis are convergent with those of the main analysis (Table 5). For both models, the conditions of validity were verified and the robustness of the linear regressions performed was assessed to be acceptable. The second model show that the interaction between the consumption of mollusks and the fact that more than 50% of them are self-fished is significantly linked with U-Cd ($P=0.008$). That means that the more a subject eats mollusks and the more he fishes mollusks, the lower is the U-Cd.

Discussion

According to our calculations, 8.5% of the subjects exceed the PTWI of 7 μg/kg bw/wk. But the calculated Cd intake of the high consumers should be interpreted with caution, since it only takes their fish and seafood consumptions into account; the average total diet intake should probably be higher. Moreover, considering the "PTWI" of 3.02 μg/kg bw/wk proposed by Omarova and Phillips in 2007 following a meta-analysis of literature data on Cd toxicity indicators (Omarova and Phillips, 2007), 25% of subjects exceed this value.

Nevertheless the results of the biological analysis indicate that the Cd levels remain below the standards. On one hand, there is a correlation between the U-Cd level and the dietary exposure to Cd from seafood ($r=0.32$, $P<0.0001$). On the other hand, the blood Cd level does not correlate with the exposure to Cd from seafood ($P=0.65$), which is concordant with the bibliography (Berglund et al., 1994). Moreover some studies do not show any correlation between ingested Cd and U-Cd or blood Cd levels (Moon et al., 1999; Olsson et al., 2002; Oskarsson et al., 2004). This phenomenon may be explained by the fact that seafood is not the only food

Table 4. Results of the main covariance analysis.

Variable	DF	Type III SSQ	*F*	*P*>*F*	Terms	Coeff.	Std	*T*-value	*P*>|*t*|
					Intercept	−1.57116	0.12135	−12.95	<0.0001
Gender	1	1.14964	19.39	<0.0001	Female	0.144345	0.03278	4.40	<0.0001
					Male	0	—	—	—
Zone	3	0.29270	1.65	0.1785	T	0.079927	0.03698	2.16	0.0313
					LR	0.026631	0.03645	0.73	0.4655
					LH	0.016163	0.03647	0.44	0.6579
					L	0	—	—	—
Tobacco status	2	0.59946	5.06	0.0068	Ex-smk	−0.038624	0.04050	−0.95	0.3409
					Smk	0.079751	0.03774	2.11	0.0353
					Non-smk	0	—	—	—
Age	1	8.27439	139.55	<0.0001		0.014090	0.00119	11.81	<0.0001
Professional expo	1	0.06322	1.07	0.3025	No	0.059353	0.05748	1.03	0.3025
					Yes	0	—	—	—
Length living in accommodation	1	0.15075	2.54	0.1117		−0.002199	0.00138	−1.59	0.1117
Tobacco consum/life (g)	1	1.45343	24.51	<0.0001		0.000001	0.00000	4.95	<0.0001
Phosphate fertilizer	1	0.25210	4.25	0.0399	No	0.198072	0.09606	2.06	0.0399
					Yes	0	—	—	—
Fish consum	1	0.03842	0.65	0.4214		0.000025	0.00003	0.80	0.4214
Fish-based dishe consum	1	0.04447	0.75	0.3870		−0.000083	0.000096	−0.87	0.3870
Vegetable consum	1	0.00576	0.10	0.7554		0.000004	0.00001	0.31	0.7554
Self-fished mollusk consum	1	0.26593	4.48	0.0349		−0.000214	0.00010	−2.12	0.0349
Self-fished crust consum	1	0.02768	0.47	0.4949		0.000256	0.00037	0.68	0.4949
Bought mollusk consum	1	0.02702	0.46	0.5000		0.000064	0.00009	0.68	0.5000
Bought crust consum	1	0.00162	0.03	0.8687		0.000030	0.00018	0.17	0.8687

Consum, consumption; crust, crustaceans; Expo, exposure; LH, Le Havre; L, Lorient; LR, La Rochelle; smk, smoker; T, Toulon.

contributing to the Cd exposure. The first French TDS showed that the main food sources of Cd are vegetables and potatoes, far ahead before crustaceans and mollusks, bread, poultry and offal (Leblanc et al., 2005a). In the French general population, fish and seafood represent only 8–25% of the Cd intake via food; and substitutions between consumptions of terrestrial meat products and fish and seafood consumption may explain the absence of a relationship between seafood consumption and blood Cd. Moreover the gastro-intestinal absorption of the Cd (5% on average) vary considerably between individuals (Kikuchi et al., 2003; JECFA, 2004) and seems to be influenced by the composition of the diet and by nutritional factors such as fibers, zinc, calcium and above all iron, as shown by animal models (JECFA, 2004). Some studies have shown that a low blood iron reserve especially in pre-menopausal women could lead to an increase of the Cd intestinal absorption (Flanagan et al., 1978; Kowal, 1988), which can explain the fact that the adult women of our study present a U-Cd statistically higher than men of the same age group. Another factor can explain the absence or the low correlation between the ingested Cd and the biological Cd level. The bioavailability of the ingested Cd differs between diets (Vahter et al., 1996). The authors think that the more the diet is rich in shellfish, the less the Cd is absorbed. The first explanation is that shellfish are rich in iron, which tends to limit the absorption. Increased expression of the metal transporter protein DMT1 has been found in iron deficiency, which would provide subjects with an increased capacity to absorb iron and Cd (Tallkvist et al., 2001; Zoller et al., 2001) The other reason is that in shellfish the Cd is bound to the metallothionein, which might be less accumulated in tissues (Muller et al., 1986; Groten et al., 1991, 1992).

A last parameter to be taken into account when comparing the differences between the biological results and the calculated dietary intakes, is the difficulty in quantifying the real contamination variability of the seafood products consumed. The contribution of beach fishing to the total supply, in particular in Lorient and La Rochelle for mollusks and crustaceans, can induce non-negligible variability in the contamination of the consumed foods. Indeed the IFREMER monitoring plans indicate contaminations levels that can correspond to large differences in concentrations from one point of control to another, which is not the case in Toulon for example. It is therefore possible that the subjects in Toulon are more likely to consume products with trace elements levels close to the average, whereas in La Rochelle and Lorient the food contamination can be much more variable, depending on the provisioning. This may be due to the fact that the ports of Lorient and La Rochelle (and more generally Brittany and the Atlantic coast) commercialize products of more varied origins (fishing zones, foreign boats, etc.) than Mediterranean ports such as Toulon. Consequently, applying an average contamination to products in

Table 5. Results of the secondary covariance analysis.

Variable	DF	Type III SSQ	*F*	*P*>*F*	Terms	Coeff.	Std	*T*-value	*P*>|*t*|
					Intercept	−1.27500	0.07841	−16.26	<0.0001
Gender	1	1.11646	19.04	<0.0001	Female	—	—	—	—
					Male	−0.07112	0.01630	−4.36	<0.0001
Zone	3	0.29506	1.68	0.1715	T	—	—	—	—
					LR	−0.07484	0.02359	−0.317	0.7512
					LH	−0.01006	0.02368	−0.43	0.6712
					L	−0.03176	0.02149	−1.48	0.1404
Tobacco status	2	0.60886	5.19	0.0060	Ex-smk	−0.11930	0.03844	−3.10	0.0021
					Smk	—	—	—	—
					Non-smk	−0.08007	0.03750	−2.14	0.0334
Age	1	8.12346	138.55	<0.0001		0.01409	0.00120	11.77	<0.0001
Professional expo	1	0.06453	1.10	0.3948	No	—	—	—	—
					Yes	−0.03002	0.02861	−1.05	0.1130
Length living in accommodation	1	0.14799	2.52	0.1130		−0.002175	0.00137	−1.59	0.1130
Tobacco consum/life (g)	1	1.40249	23.92	<0.0001		0.00000	0.00000	4.89	<0.0001
Phosphate fertilizer	1	0.25452	4.34	0.0379	No	—	—	—	—
					Yes	−0.09957	0.0478	−2.08	0.0379
Fish consum	1	0.02408	0.41	0.5220		0.00002	0.0000	0.64	0.5220
Fish-based dishe consum	1	0.07467	1.27	0.2598		−0.00011	0.0001	−1.13	0.2598
Vegetable consum	1	0.00433	0.07	0.7859		0.00000	0.0000	0.27	0.7859
Mollusk consum	1	0.03392	0.58	0.4474		−0.00005	0.0000	−0.761	0.4474
Crust consum	1	0.08356	1.43	0.2333		0.00028	0.0002	1.194	0.2333
More than 50% mollusk consum from self-fishing	1	0.10451	1.78	0.1827	No	—	—	—	—
					Yes	0.03686	0.0276	1.34	0.1827
More than 50% crust consum from self-fishing	1	0.21348	3.64	0.0572	No	—	—	—	—
					Yes	−0.05684	0.0298	−1.91	0.0572
Mollusk consum * More than 50% mollusks consum from self-fishing	1	0.41428	7.07	0.0082	No	—	—	—	—
					Yes	−0.00070	0.0000	−2.66	0.0082
Crust consum * More than 50% crust consum from self-fishing	1	0.11985	2.04	0.1537	No	—	—	—	—
					Yes	0.00031	0.0002	1.43	0.1537

consum, consumption; crust, crustaceans; Expo, exposure; LH, Le Havre; L, Lorient; LR, La Rochelle; smk, smoker; T, Toulon.

Toulon is without doubt more in line with the reality than doing so in other zones. This can also explain that the north–south gradient that appeared to be significant with the indirect approach for Cd is therefore not reflected in the biological results. This result was supported by the fact that multivariate analyses do not show any significant link between U-Cd and zones.

Concerning the determination of the U-Cd, our results are concordant with the fact that the age, gender and smoker status strongly influence the U-Cd. Age correlates with U-Cd level, which means that the individuals with the highest levels (>2 μg/g creatinine) are over 50 years old, and which shows the accumulation of Cd in the renal cortex. It is known that renal Cd is associated with age and reaches a plateau at 50 years of age associated with an age-related degeneration of kidney reabsorption function. Recent studies have shown that high biological Cd levels were associated with low bone mineral density, particularly in oldest subjects (>60 years) (Satarug and Moore, 2004).

Concerning smoking status, the number of cigarettes smoked per day correlates strongly with Cd level in the blood ($r=0.62$, $P<0.0001$). Cd oxide generated during the burning of cigarettes is known to be highly bioavailable, 30–40% is absorbed into systemic blood circulation (Satarug and Moore, 2004). Especially for non-smokers, age also correlates with these Cd levels ($r=0.38$, $P<0.0001$), which is normal for an element that accumulates in the body over time. And the use of phosphate fertilizer also seemed to decrease the U-Cd, but this result is not physiologically concordant, suggesting that it may be a bias.

Moreover these results show a protective effect of the consumption of local self-fishing mollusks, with a decrease of

the U-Cd ($r = -0.11$ [-0.185, -0.009], $P = 0.03$). To explain this negative correlation, we suspect specific consumption behaviors. The first hypothesis is that people who self-catch mollusks are well-informed consumers who only fish in healthy zones with a contamination of mollusks lower than the mean. One of the limits of our study for the multivariate analysis is indeed, the absence of details about the local zone of fishing and the dates of this practice that would have been interesting variables to adjust for. An other hypothesis is a modification in dietary behavior linked with the exposure to Cd. The higher exposed people could stop practicing self-fishing because of the awareness campaigns or because of the ban on self-fishing in some contaminated areas. A bias in the response to the questionnaire can also lead to the correlation between self-fishing and low U-Cd, if the people who have the higher U-Cd did not say that they practice the self-fishing because they do it in unhealthy zones, although people who said they are used to self-fishing do it in healthy zones. A last hypothesis is the physiological one. The richer in iron the shellfish are, the lower the absorption of Cd (Vahter et al., 1996). The locally self-fished mollusks may have higher concentrations in iron than the others or less available Cd. Whatever the reason for the negative correlation between the local mollusks self-fishing practice and the U-Cd level, this result leads to an invalidation of the usual hypothetical risk of people with this particular behavior to reach a critical Cd intake, and encourages confidence about health safety of the high consumers in terms of Cd exposure.

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