



Mussels and clams from the Italian fish market. Is there a human exposition risk to metals and arsenic?

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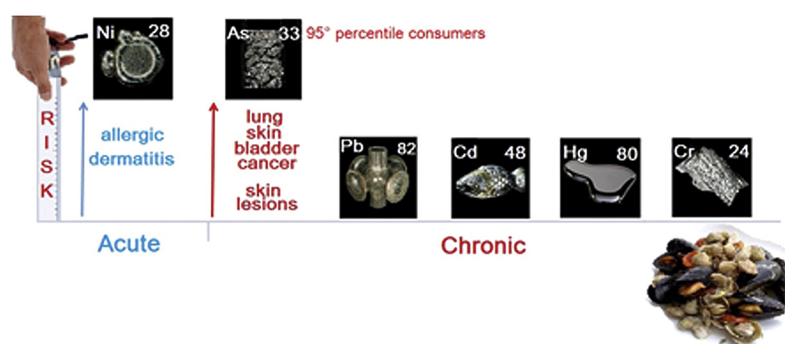
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HIGHLIGHTS

- Mussels and clams are filter feeders that can bioaccumulate various chemicals.
- Seafood metal contamination is a recognized public health risk.
- The presence of metals and arsenic in these molluscs from Italian market was checked.
- A very low risk for the consumer health emerged due to the intake of molluscs.
- Allergic dermatitis in Ni-sensitive subjects may be induced by all analysed samples.

GRAPHICAL ABSTRACT



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ABSTRACT

Seafood is associated with many beneficial effects on human health. However, the overall level of contaminants in biota has increased over the last two centuries and seafood is one of the source of oral exposition to contaminants. Therefore, this work aimed to evaluate cadmium, lead, mercury, arsenic, chromium and nickel presence in mussels and clams, from the Italian market, and the associated risk. The samples were from five different FAO areas. Analyses were carried out using inductively-coupled plasmas-mass spectrometry. The sample concentrations were below the maximum levels stated by Commission Regulation (EC) 1881/2006, except one mussel sample, which was non-compliant for cadmium ($2.13 \pm 0.20 \text{ mg kg}^{-1}$). For arsenic, nickel and chromium, maximum levels are not stated by the European Union. In this study, arsenic ranged from 1.29 to 13.35 mg kg^{-1} and nickel ranged from $<\text{LOQ}$ – 3.98 mg kg^{-1} , except one sample, whose nickel concentration was 21.70 mg kg^{-1} . Chromium was found only in 15 samples, with a maximum concentration of $2.81 \pm 0.27 \text{ mg kg}^{-1}$, in one clam sample. Our results indicate that the average Italian consumption of molluscs, does not pose a risk for consumers, except nickel, which can cause allergic dermatitis in nickel-sensitive individuals. However a particular concern is caused by the exposition to As of the 95th percentile consumers: the Hazard Index for skin

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lesions, was >1, and BMDL₁₀ for lung bladder and skin cancer in all mussel samples was overcome, in the 100% and 25% of mussel and clam samples, respectively.

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1. Introduction

Seafood is an excellent source of nutrients including proteins, vitamins (A, D and B₁₂), minerals and fatty acids, with important human health benefits. Epidemiological and clinical evidence suggests that consumption of high levels of n-3 fatty acids eicosapentaenoic acid and docosahexaenoic acid, from one or two fish-based meals per week, induces protective effects against coronary heart disease, with a 36% decrease in the risk of coronary death and 17% decrease in total mortality. Seafood is also associated with beneficial effects on neurologic development in toddlers and children, such as improved visual acuity, raised mental processing scores and language comprehension (Mozaffarian and Rimm, 2006; EFSA, 2014a). However, over the last two centuries, the level of contaminants, heavy metals and chemical compounds, has increased in the environment and, subsequently, in biota, due to anthropogenic activity, such as industrial activities, mining and agriculture. Some metals, such as lead (Pb) and mercury (Hg) have no biological roles (Sarmiento et al., 2011) and their presence needs to be monitored to prevent and minimise the potential health risks associated with seafood consumption. In many instances, animals near the top of the food chain, such as carnivorous fish species, are most affected by biomagnification of xenobiotics, building up greater and/or more dangerous amounts of toxic materials than animals lower down the food chain. Herbivorous species, like mussels and clams, are filter-feeders, bioaccumulating a wide range of environmental pollutants in their tissues and, thus, can be used as suitable bioindicator organisms to monitor trace metal pollution in marine environments (Langston and Spence, 1995). In the European Union (EU), the breeding of bivalves provides the laying of juvenile bivalves in the open sea and their permanence for about 2 years until adult stage (Baylon, 1990). After collection, the animals are microbiologically controlled, before their marketing. If they do not meet the microbiological criteria after collecting, they must stay in a depuration place, provided with periodically replaced clean water, in order to reduce the microbial load (EU, 2004). In this context, it could be supposed that they can release several chemicals, comprising metals.

One fundamental feature of metals is their ability to persist in the environment (Scientific Committee of Problems of the Environment (SCOPE), 1987). Consequently, they can be taken up by marine organisms due to their presence in water and sediments and they can be biomagnified in superior trophic animals including humans (Kim and Lee, 2010). The metal contamination of seafood is a globally recognised public health risk (Lozano et al., 2010; Squadrone et al., 2016). Long-term exposure and/or high concentrations of metals, can cause adverse effects on human health, such as skin diseases, acute and chronic intoxication, nervous system, blood and gastrointestinal dysfunctions, respiratory problems, as well as mutagenic and cancerogenic effects (Martin and Griswold, 2009).

In particular, Pb can cause severe brain and kidney damage, and in pregnant women, may cause miscarriage. Hg can cause nervous system dysfunctions like tremors, irritability, memory problems, changes in vision and hearing. Cadmium (Cd) can cause kidney diseases and respiratory problems. Inorganic arsenic (As) has been linked to cancer of the skin, lungs and bladder. Chromium (Cr) can

cause breathing problems, cough, asthma, allergic reactions and chronic exposure could cause liver and kidney cancer, particularly linked to Cr(VI) (Martin and Griswold, 2009). Nickel (Ni) could cause allergic reactions and long-term exposure could result in reproductive diseases. Moreover, it is genotoxic, with cancerogenic, immunotoxic, hepatotoxic, neurotoxic, and nephrotoxic effects only after inhalation (EFSA, 2015; Das et al., 2008).

The EC has set maximum levels (MLs) for Cd (1 mg kg⁻¹) and Pb (1.5 mg kg⁻¹) in bivalve molluscs, and for Hg (0.5 mg kg⁻¹) in seafood (EU, 2006). No MLs have yet been established for As, Ni, and Cr by the EU. In 2009, however, the European Food Safety Agency (EFSA, 2009a) concluded that the provisional tolerable weekly intake (PTWI) of As at 15 µg kg⁻¹ body weight, recommended by the Joint Food and Agricultural Organisation/World Health Organisation (FAO/WHO) Expert Committee on Food Additives (JECFA) was no more applicable. Instead, based on epidemiological studies on exposure to As through water and food, the EFSA suggested a range, rather than a single reference point that included the benchmark dose lower confidence limit for a 0.1% increased incidence (BMDL₀₁) of skin lesions and cancer of the lung, skin and bladder, between 0.3 and 8 µg kg⁻¹ body weight day⁻¹ (EFSA, 2009a,b).

Although Cr has no defined MRLs, in 2014, the EFSA suggested a tolerable daily intake (TDI) for Cr(III) of 300 µg kg⁻¹ body weight, which was based on the lack of adverse effects and carcinogenicity in mice and rats, and for the inadequate information about reproductive and developmental toxicity. Cr(VI) is classified by the International Agency for Research on Cancer (IARC) as carcinogenic to humans (Group 1). However, it is scarcely present in food, which is mostly a reductive medium and is largely reduced in the gastrointestinal tract. Therefore, the EFSA concluded that the oral exposure to Cr(VI), for the European population is of low concern for health of all age groups but of potential concern only for high consumers of drinking or bottled water, for the oxidising agents present, in the groups “Infants”, “Toddlers” and “Other children” (EFSA, 2014b).

In 2015, the EFSA set a TDI for Ni at 2.8 µg kg⁻¹ body weight, obtained from studies on the dose response curve of the incidence of litters with a post-implantation loss in rats. EFSA, also, considered, the possibility of eczematous and allergic reactions elicited by acute oral exposure and reported a BMDL₁₀ of 1.1 µg Ni kg⁻¹ body weight, with a margin of exposure (MOE) of 10 or higher, accounting for the variability of immune response in nickel-sensitised individuals (EFSA, 2015).

According to the 2016 European Market Observatory for Fisheries and Aquaculture Products (EUMOFA) report, the consumption of seafood products has been constantly increasing in the last few years, with mussels among the major species. In 2014, 1.27 kg mussels per person per year and 0.33 kg clams per person per year, was recorded. In the same year, the EU production of bivalves increased 14%, reaching 609,600 tons, mainly due to an increase in mussels farmed in Spain, the major producer, followed by France and Italy, where bivalve production represents 70% of all fishery national production (EUMOFA, 2016).

In the current study, inductively coupled plasma-mass spectrometry (ICP-MS) was used to quantitate the six mentioned metal contaminants (As, Cd, Cr, Hg, Pb and Ni), in mussels and clams

obtained from the Italian fish market, and evaluate the Italian consumer's risk, on the basis on the MRLs stated by the EC, where available, or, otherwise, based on the tolerable intakes recommended by the EFSA.

2. Materials and methods

2.1. Chemicals and reagents

Nitric acid (HNO₃, 69% Hiperpur Solution) and hydrogen peroxide (H₂O₂, 30% Hiperpur Solution) were from Panreac Quimica SLU (Castellar del Vallés, Barcelona, Spain). Purified water was obtained through a Milli-Q system (Millipore, Merck KGaA, Darmstadt, Germany).

2.2. Standards

Pb, 1000 µg mL⁻¹ (0.10% w/v), (Pb metal in 2% HNO₃); Hg, 1000 µg mL⁻¹ (0.10% w/v), (Hg metal in 2% HNO₃); Cd, 1000 µg mL⁻¹ (0.10% w/v), (Cd metal in 2% HNO₃); As, 1000 µg mL⁻¹ (As metal in 2% HNO₃); Ni, 1000 µg mL⁻¹ (0.10% w/v), (Ni metal in 2% HNO₃); Cr, 1000 µg mL⁻¹ (0.10% w/v), (Cr metal in 2% HNO₃), and the internal standard Yttrium (Y), at 1000 µg mL⁻¹ (0.10% w/v), (Y metal in 2% HNO₃), were all Baker Instra-Analysed® Reagents (JT Baker, Deventer, Holland).

2.3. Sample collection

A total of 50 mussel (*Mytilus galloprovincialis*, *Mytilus edulis*, *Mytilus chilensis*) and 40 clam (*Venerupis philippinarum*, *Perna canaliculus*, *Tapes decussatus*, *Tapes semidecussatus*, *Meretrix meretrix*, *Meretrix lyrata*) samples were collected from June 2016 until February 2017, at the wholesale Milan fish market, which supplies the whole country. Their origin was randomly chosen, in order to simulate the mollusc meal consumption of an Italian consumer. The samples were from five different FAO areas (Chile; Thailand and Vietnam; New Zealand; North Spain, North France and Portugal; and the Mediterranean Sea). Each sample was prepared from 50 or more molluscs, aged approximately 2 years and with sizes ranging from 35 to 60 mm for mussels and 22–25 mm for clams. The edible part was finely dispersed with an Ultraturrax (IKA®-Werke GmbH and Co. KG, Staufen, Germany) at 13500 rpm for 2 min. All samples were stored at –20 °C, until analysis.

2.4. Sample preparation

A 0.5 ± 0.05 g part of each pooled sample was digested with 5 mL HNO₃ (65% aqueous solution) and 0.5 mL H₂O₂ (30% aqueous solution), in closed Teflon vessels. For the mineralisation, the samples were placed in 3M-TFM® vessels and microwave-digested (Milestone Inc., Shelton, CT, USA), at high temperature (200 °C) for 30 min. A blank solution was prepared, adding only the HNO₃/H₂O₂ (5 mL/0.5 mL) mixture, in one vessel. After mineralisation, the samples and the blank solution, were brought to a volume of 50 mL, with ultrapure water. Then, 1 mL of each solution was placed in a 15-mL vial and brought to 10 mL with ultrapure water.

2.5. ICP-MS analyses

The inductively coupled plasma mass spectrometer (Agilent 7500ce, Agilent Technologies, Santa Clara, CA, USA) used, was equipped with a Cetac ASX-510 auto-sampler (Thermo Fisher Scientific, San Jose, CA, USA). Argon and helium were used pure, at 99.999%.

2.6. Quality control

Quality control, expressed as trueness, was performed by analysing the Certificated Reference Material ERM-CE278k mussel tissue (European Commission's Joint Research Centre, Institute for Reference Materials and Measurements, Geel, Belgium) digested with the same protocol of the samples. The following parameters were also considered, based on Commission Regulation (EC) No 333/2007 (EU, 2007): limit of detection (LOD), defined as three times the standard deviation (SD) of the noise from six different sample blanks; limit of quantification (LOQ), defined as ten times the SD of the noise from six different sample blanks; repeatability, in terms of relative standard deviation (RSD) of measurements made on twelve blank samples spiked with 1 µg kg⁻¹ of each metal, respectively. Uncertainty, expressed as expanded measurement uncertainty (U) was evaluated on repeatability data: unless otherwise stated, results are reported as concentration ± U. Recovery was not calculated, due to the lack of extraction procedures, as stated by the above-mentioned Regulation (EU, 2007).

2.7. Statistical analysis

The descriptive statistics was performed using Microsoft Excel® 2013.

3. Results and discussion

Mussel and clam samples were analysed for the presence of Cd, Pb and Hg, for which the EU set MLs (EU, 2006), and As, Cr and Ni, for which only the EFSA tolerable intakes or threshold doses are reported (EFSA, 2009a, 2014a, 2015). Tables 1 and 2 summarise the data (mg kg⁻¹ wet weight) as the mean ± SD, as well as the 25th, 50th (median), 75th and 100th (maximum concentration) percentiles in mussels and clams, respectively.

Trueness ranged from 102.3 to 105.3%. The LODs were 0.001 mg kg⁻¹, for Cd and Hg, and 0.005 mg kg⁻¹ for the remaining metals. The LOQ was 0.01 mg kg⁻¹, for all metals. These analytical limits allowed us to assess the risk based on the MLs and threshold doses. Repeatability, expressed as RSDs, ranged from 2.5 to 6.2%. Uncertainty, related to a 1.0 µg kg⁻¹ spike of each metal in 12 blank samples, was between 0.05 and 0.12 µg kg⁻¹.

3.1. Metals with MLs: Cd, Hg and Pb

Cd was detected in all samples analysed, except one. The Cd concentration ranged between 0.08 and 2.13 mg kg⁻¹ in mussels and < LOQ–0.90 mg kg⁻¹ in clams. All concentrations were lower than the ML set by EC 1881/2006, of 1 mg kg⁻¹, except one mussel sample from North Spain (2.13 ± 0.10 mg kg⁻¹).

Hg was detected in all samples analysed but was always detected below the ML (0.5 mg kg⁻¹). An overview of mercury studies performed in the Mediterranean Sea region, found that although atmospheric concentrations of Hg species at several coastal sites in the Mediterranean Sea Basin were significantly higher than those recorded at several other coastal sites distributed across North Europe, Hg water levels were comparable to other oceans, reported to represent a geological source for Hg (Kotnik et al., 2014).

Due to its unique chemical-physical properties, Pb persists in the environment for a long time (Orescanin et al., 2006). The concentrations of this metal were, however, lower than the ML stated by the EU (1.5 mg kg⁻¹), both in mussels (<LOQ–0.79 mg kg⁻¹) and in clams (<LOQ–0.93 mg kg⁻¹).

Table 1

Concentrations (mg kg⁻¹), expressed as means±SDs and percentiles of the six analysed metals in mussel samples; n = number of positive samples. Means and SDs are calculated only on positive samples.

	Pb	Hg	Cd	As	Ni	Cr
n	48	50	50	50	50	6
Mean ± SD	0.23 ± 21	0.05 ± 0.07	0.29 ± 0.27	5.04 ± 2.34	0.96 ± 0.88	0.051 ± 0.14
25 th percentile	0.09	0.02	0.14	3.45	0.32	0.00
Median	0.17	0.04	0.23	4.76	0.57	0.00
75 th percentile	0.31	0.06	0.38	5.94	1.54	0.00
Maximum concentration	0.79	0.16	2.13	13.36	3.98	0.64

Table 2

Concentrations (mg kg⁻¹), expressed as means±SDs and percentiles of the six analysed metals in clam samples; n = number of positive samples. Means and SDs are calculated only on positive samples.

	Pb	Hg	Cd	As	Ni	Cr
n	40	39	36	39	39	9
Mean ± SD	1.23 ± 2.35	0.05 ± 0.07	0.31 ± 0.27	4.86 ± 2.33	1.23 ± 2.35	0.16 ± 0.48
25 th percentile	0.09	0.019	0.14	3.278	0.33	0.000
Median	0.17	0.03	0.24	4.68	0.62	0.000
75 th percentile	0.31	0.04	0.41	5.81	1.64	0.000
Maximum concentration	0.93	0.53	0.90	12.65	21.70	2.82

3.2. Metals without MLs: As, Cr and Ni

The toxic effects of As, are produced mostly by its inorganic forms. This study analysed the total As i.e. the sum of organic and inorganic As. The concentration ranged from <LOQ–13.36 mg kg⁻¹ in mussels and from <LOQ–12.65 mg kg⁻¹ in clams. The highest concentrations were found in one mussel sample from the Italian coast of the South Adriatic Sea and in one clam sample from Portugal. Even if the EU has not stated MRLs for As, the report titled “Dietary exposure to inorganic As in the European population” (EFSA, 2014a), provides some interesting observations. After withdrawing the PTWI of 15 µg kg⁻¹ body weight, in 2011, the JECFA indicated “a BMDL for a 0.5% increased incidence of lung cancer, of 3.0 µg kg⁻¹ body weight per day” (JECFA, 2011). Previously, the EFSA (2009a) had indicated “a BMDL₀₁ between 0.3 and 8 µg kg⁻¹ body weight per day for an increased risk of 1% of cancer of the lung, skin and bladder, as well as skin lesions”. In the report, the EFSA cared to recommend the speciation of As present in food samples. Yet, although As was detected in 97.4% of almost 104,000 food samples, the data collected to calculate human exposition, only reported the total As concentration (EFSA, 2009a). Moreover, the data from the scientific literature do not allow to accurately calculate the amount of inorganic As from total As in seafood (Francesconi, 2010). This is because the relationship is variable between species and the content of inorganic As is inversely proportional to the total As content. For instance, in 175 Norwegian blue mussel samples, Sloth and Julshamn (2008), found that the inorganic/organic As ratio was usually less than 10%, except in a few mussels, in which inorganic As represented up to 42% of the total. Thus, to assess the risk associated with As present in the analysed molluscs, the current study applied a very conservative approach, by supposing that the normal amount of inorganic As in mussels and clams is the 42% of the total and considering the maximum concentrations detected in mussels and clams, as if they were usual concentrations. The inorganic As concentration should, therefore, be 5.61 mg kg⁻¹ in mussels and 5.31 mg kg⁻¹ in clams. Accounting for the abovementioned annual consumption of molluscs, an adult consumer, with a body weight of 70 kg, should, therefore, consume $(5.61 \times 1.27) \times (365 \times 70)^{-1} = 0.28 \mu\text{g (kg body weight day)}^{-1}$ of As from mussels and $(5.31 \times 0.33) \times (365 \times 70)^{-1} = 0.07 \mu\text{g (kg body weight day)}^{-1}$ As from clams, which are below the BMDL₁₀ value of 0.3 µg kg body weight⁻¹.

Cr was found in 12% of the mussel samples and 22% of the clam samples, with maximum concentrations of 0.64 ± 0.03 and $2.82 \pm 0.13 \text{ mg kg}^{-1}$, respectively. When based on a 300-g fish meal day⁻¹, which, according to the European Agency for the Evaluation of Medicinal Products (EMA), is an “arbitrarily high consumption value to ensure the protection of the majority of the consumers” (EMA, 2001), these concentrations of edible tissue of mussels or clams, would provide a Cr intake of 0.19 and 0.85 mg day⁻¹, respectively. These values are significantly lower (110 and 25 times, respectively) than 21 mg day⁻¹ Cr intake for an adult person of 70 kg, calculated from the TDI value of 300 µg Cr(III) kg⁻¹ body weight per day. As stated by EFSA the toxic relevance of Cr(VI) is negligible in food, therefore was not considered. (EFSA, 2014b).

Ni was detected in all samples analysed. The concentration range was 0.12–3.98 and 0.097–21.70 mg kg⁻¹ in mussels and clams, respectively. Thus, a consumer, eating the more contaminated mussels or clams, would potentially be exposed to $(3.98 \times 1.27) \times (70 \times 365)^{-1} = 0.2 \mu\text{g (kg body weight day)}^{-1}$ or $(21.70 \times 0.33) \times (70 \times 365)^{-1} = 0.27 \mu\text{g (kg body weight day)}^{-1}$, respectively, amounts of Ni, which are lower than the TDI of 2.8 µg kg⁻¹ body weight.

The acute toxicity of Ni is, however, important, as the most frequent adverse effect in the population is allergic dermatitis. Systemic contact dermatitis, elicited by Ni oral ingestion via food and drugs in nickel-sensitive individuals, consists of generalised eczematous flare-up reactions. A single meal (0.3 kg) of the most contaminated mussels and clams, should provide to a consumer weighing 70 kg, $(3.98 \times 0.3/70) = 17$ and $(21.70 \times 0.3/70) = 93 \mu\text{g kg}^{-1}$ body weight, respectively, an amount much higher than the BMDL₁₀ of 1.1 µg kg⁻¹ body weight. It is of concern, however, that the MOE, when the less contaminated mussels and clams are considered, is lower than the minimum recommended value of 10: a meal should supply 0.51 and 0.42 µg Ni kg⁻¹ body weight, providing MOE values of 2.2 and 2.6 (Yoshihisa and Shimizu, 2012; EFSA, 2015).

3.3. Evaluation of the Hazard Index

The studied elements in mussels and clams are actually a mixture of chemicals that can share some common toxicological

Table 3
Hypothetic target hazard quotients and hazard indexes for the estimated daily exposure to the studied elements via mussels and clams at the highest concentrations detected. All the reference values are by EFSA. TWIs are recalculated and expressed on a daily basis. As is reported as inorganic Arsenic and only Cr(III) is considered.

RfV	Element	Mussels	Clams	Target hazard quotients: MUSSELS-CLAMS (reference values - $\mu\text{g kg}^{-1} \text{ day}^{-1}$)				
		Daily exposure ($\mu\text{g kg}^{-1}$)		Skin	Reproduction/Development	Developmental neurotoxicity	Blood pressure	Kidney
BMDL ₁₀	Pb	0.04	0.01			0.08–0.02 (0.5)	0.03–0.007 (1.5)	0.06–0.016 (0.63)
TWI	Hg	0.01	0.01			0.06–0.06 (0.18)		
TWI	Cd	0.11	0.01					0.29–0.03 (0.38)
BMDL ₁₀	As	0.28	0.07	0.93–0.23 (0.3)				
TDI	Ni	0.20	0.28		0.07–0.10 (2.8)			
TDI	Cr	0.03	0.04		0.0001–0.0001 (300)			
HAZARD INDEX				0.93–0.23	0.0701–0.1001	0.14–0.08		0.35–0.046

Table 4
Hazard indexes for the estimated daily exposure to the studied elements via mussels and clams of the 95° percentile fish consumers.

Hazard index for 95° percentile consumers		SKIN	Reproduction/development	Developmental neurotoxicity	Blood pressure	Kidney
		As	Ni + Cr	Pb + Hg	Pb	Pb + Cd
MUSSELS	Minimum	1.24	0	0.01	0.00	0.06
	Median	2.86	0.03	0.05	0.03	0.02
	Maximum	8.08	0.26	0.34	0.10	1.14
	Percent of values >	100%	—	—	—	2%
CLAMS	Minimum	0.00	0.00	0.00	0.00	0.00
	Median	0.07	0.03	0.02	0.004	0.05
	Maximum	1.98	0.36	0.17	0.29	0.13
	Percent of values > 1	25%	—	—	—	—

effects, so evoking a dose addition: the Total Dose, i.e. the dose of each toxic agent with similar effects. As far as the cancer risk, oral intake with food has a major role only for arsenic on lung, skin and bladder. Lung cancer is a toxicological effect of exposition only through inhalation of Cd and Ni, similarly to bladder cancer for Cd (EFSA, 2009a,b, 2015). As the cancer risk by mollusc intake is induced only by As, just the non-carcinogenic effects where considered. The Hazard Index (HI) was therefore calculated as $HI = \sum_{i=1}^n \text{Estimated Intake}_i / RfV_i$, where, RfV_i is the Reference Value, representing the human daily intake of the chemical i [e.g., TDI or BMDL] and the *Estimated Intake*, evaluated from the above mentioned annual consumptions, is in same units as the RfV_i (Teuschler, 2013). The considered RfV_i where the reference doses indicated by EFSA (2009a,b, 2010a,b, 2014b, 2015), eventually recalculated on a daily basis. To maintain a very conservative approach, only the highest concentrations of each element in mussels and clams were accounted for. The Target Hazard Quotients (THQ), i.e. the ratio of the potential exposure to the substance and the level at which no adverse effects are expected were then evaluated as $THQ = \text{daily intake} / RfV$ for each compound, for the different end points indicated by EFSA. The results are shown in Table 3. All the HIs were lower than one, indicating that a negligible health hazard could derive from the ingestion of mussels. A study conducted by Leclercq et al. (2009), shows that in 2005–2006 the mean individual daily consumption for Italian adult population was about 40 g day^{-1} of fish and, for the group belonging to the 95th percentile about 145 g day^{-1} . Assuming that the variation between groups in mollusc consumption is similar, the THQ values should be multiplied by $(145/40) = 3.65$. Accounting for this consumption, all mussel samples and the 25% of clam samples showed a $HI > 1$ for skin lesions in high percentiles consumers while all samples (except one) showed a HI

value < 0.1 for Cd and Pb merged dose (Table 4).

4. Discussion

Metal contamination in mussels and clams that are currently present in the Italian market, provide reasons for low concern for average consumers. According to the EU MLs or the EFSA threshold doses, Pb and As do not pose a risk to public health (EU, 2006; EFSA, 2014a). Cr showed concentrations that elicit oral exposure substantially lower than the TDI. (EFSA, 2014b).

Hg was present in only one clam sample, at a concentration higher than the maximum level, but compliant accounting for the uncertainty ($0.53 \pm 0.04 \text{ mg kg}^{-1}$). Considering that the chronic toxicity was evaluated in establishing the MRL, this concentration could not be considered a risk for the consumer. A similar consideration could be made for Cd, detected in one mussel sample, at a concentration of about twice the MRL. In this case, too, because the chronic toxicity studies were accounted for in establishing an MRL, the risk can be considered very low (EU, 2006).

High percentiles consumer are a cause of concern due to chronic oral exposition to As: in fact all mussel samples and 10 clam samples showed a $HI > 1$ for skin lesions. It must be highlighted that this consumer group is furthermore subject to lung, skin and bladder cancer risk, as the BMDL₁₀ value of $0.3 \mu\text{g kg}^{-1}$ is overcome in all the samples (EFSA, 2009a).

Ni demands particular consideration, too. No chronic toxicity risk was indicated by our study. Systemic contact dermatitis, a manifestation of acute Ni toxicity, is the most prevalent effect in the population. Based on our results, the Ni content of all the mollusc samples analysed, could cause the allergic reaction in Ni-sensitive individuals, thus, representing a concern.

5. Conclusion

Finally, this survey on the metal contents in mussels and clams from the Italian market, shows a very low risk for the average consumer health, when the chronic toxicity is considered. High percentile consumers, however, are subject to risk skin lesions, and lung, skin and bladder cancer due to high intake of As. The occurrence of acute toxicity, in the form of allergic dermatitis in sensitive subjects, due to constant Ni presence in the studied molluscs, must, however, be highlighted. Further studies are ongoing to widen the sample number, in order to compare adequate numbers of samples from the different FAO areas.

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