

Dietary exposure assessment to nickel through the consumption of poultry, beef, and pork meat for different age groups in the Italian population

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Abstract

Dietary risk assessment for toxic elements focuses on those listed by Commission Regulation (EU) 2023/915. However, new toxicological evidence suggests expanding research to other elements, including nickel. Classified as carcinogenic by the International Agency for Research on Cancer, nickel exposure mainly occurs through food and water. In individuals with hypersensitivity, oral exposure to this element may trigger symptoms ranging from dermatitis to systemic nickel allergy syndrome. Based on this evidence, the European Food Safety Authority set a tolerable daily intake (TDI) of 13 µg/kg bw/day, recommending further data collection to establish maximum levels in food. In this study, nickel occurrence was evaluated in 809 muscle meat samples (poultry, beef, and pork). Statistical analysis was conducted to identify differences in mean concentrations among the different meat types. Moreover, contamination levels of nickel were used to assess the dietary exposure of different age groups of Italian consumers through meat consumption, providing a comprehensive risk characterization. Toddlers were the most exposed age group, while the elderly were the least exposed. Across all age groups, exposure levels followed the pattern: pork > poultry > beef. Generally, meat consumption contributed less than 1% of the nickel TDI for all the age groups. In particular, the highest contribution to the TDI, equal to 0.86%, was associated with the consumption of pork by toddlers. Therefore, data from this study suggest that nickel contamination in poultry, beef, and pork has a minimal impact on human exposure, posing a negligible risk to public health.

Introduction

The assessment of dietary exposure to nickel currently focuses on foodstuffs listed in the Commission Regulation (EU) 2024/1987 (European Commission, 2024). However, this Regulation does not include meat and meat products, and no maximum levels have been established for these categories.

The International Agency for Research on Cancer (2012) classified nickel compounds as human carcinogens, placing them in Group 1. In contrast, metallic nickel was identified as a possible human carcinogen and categorized in Group 2B.

For occupationally exposed individuals, the primary routes of nickel exposure are through the skin and respiratory tract. Notably,

inhalation of nickel compounds has been linked to an increased risk of developing cancers of the lung, nasal cavity, and paranasal sinuses (Genchi *et al.*, 2020; Prueitt *et al.*, 2020). The carcinogenic mechanisms of nickel are still under investigation. However, nickel can interfere with DNA synthesis, hinder DNA repair processes, and cause the loss of DNA sequences (Mania *et al.*, 2019).

In the general population, nickel exposure primarily occurs through the consumption of food and water. The nickel content in food is influenced by factors such as environmental contamination and the specific type of food; in fact, it is known that plant-based foods tend to have higher nickel levels than animal-based foods (Sharma, 2007; Babaahmadifooladi *et al.*, 2020). Foods commonly associated with high content of this contaminant include cocoa or cocoa-based products, beans, seeds, nuts, and grains (Ščančar *et al.*, 2013; Mania *et al.*, 2019; Schuler *et al.*, 2023).

Currently, the prevalence of nickel allergy in the general population of Europe ranges from approximately 8% to 19% among adults and 8% to 10% among children and adolescents, with a significantly higher occurrence in females (Ahlström *et al.*, 2019). The most common manifestation of nickel allergy is contact dermatitis, which occurs after repeated skin exposure to a specific antigen in sensitized individuals (Ahlström *et al.*, 2019; Bechara *et al.*, 2019; Lombardi *et al.*, 2020). In these individuals, the ingestion of nickel-containing compounds can also lead to gastrointestinal symptoms, including abdominal pain, diarrhea, nausea, and constipation (Lombardi *et al.*, 2020). This condition, known as systemic nickel allergy syndrome, is characterized by immune system dysregulation, with a significant infiltration of pro-inflammatory CD4⁺ T lymphocytes into the duodenal lamina propria and epithelium (Lombardi *et al.*, 2020). Additionally, Th2-type cytokines, such as interleukin (IL)-5 and IL-13, are involved in this process (Minelli *et al.*, 2010). Systemic absorption of nickel can induce widespread reactions, manifesting as eczematous, vasculitic, mucosal, respiratory, urticarial, and gastrointestinal symptoms (Lombardi *et al.*, 2020).

Based on this evidence, the European Food Safety Authority (EFSA) assessed the health risks associated with dietary exposure to nickel and identified an increased incidence of post-implantation loss in rats, observed in one- and two-generation studies, as the critical effect for chronic nickel exposure. To assess the risk, a benchmark dose approach was applied, based on which a Benchmark dose lower confidence limit of 10% of 1.3 mg of nickel per kilogram of body weight (bw) per day was selected. This value served as the reference point for establishing the tolerable daily intake (TDI), which was set at 13 µg/kg bw by applying a default uncertainty factor of 100 to account for both intra- and inter-species variability. In addition, EFSA recommended the collection of new data on the presence of nickel in food to define relevant maximum levels in food matrices (European Food Safety Authority, 2020). Therefore, this study aimed to evaluate the dietary exposure of Italian consumers, categorized by age group, to nickel through the consumption of poultry, beef, and pork meat, providing the basis for a comprehensive risk characterization related to these food matrices.

Materials and Methods

Sampling

Between 2011 and 2023, a total of 156 poultry, 306 bovine, and 347 pig muscle tissue samples were collected as part of official

control activities carried out by the competent veterinary authorities in the Emilia-Romagna and Lombardy regions. The samples were subsequently analyzed by the Food Chemistry Department in Bologna, part of the *Istituto Zooprofilattico Sperimentale* of Lombardy and Emilia-Romagna “B. Ubertini”, a public health institute within the Italian National Health Service that provides services in animal health, food safety, and zoonoses.

Sample preparation and inductively coupled plasma-mass spectrometry analysis

Muscle tissue samples were analyzed for nickel content using inductively coupled plasma mass spectrometry. A 3.0±0.5 g portion of each sample was weighed in 50 mL polypropylene tubes (Digi-Tubes SCP Science, QuantAnalitica SRL, Osnago, Italy) and 10 mL of nitric acid (Carlo Erba, Cornaredo, Milan) was added. The samples were digested overnight in a temperature-controlled mineralizer (Digi-Prep SCP-Science, QuantAnalitica SRL, Osnago, Italy) at 75±10°C and then allowed to cool down to room temperature. Each digested sample was made up to a final volume of 20 mL with ultrapure water (Evoqua Water Technologies, Pittsburgh, PA, USA) and subsequently diluted 10-fold with 2% (v/v) nitric acid and 0.5% (v/v) hydrochloric acid mixture (Carlo Erba, Cornaredo, Milan). The quantification of nickel was carried out using a single quadrupole inductively coupled plasma-mass spectrometer (ICP-MS 7700 Series Agilent Technologies Inc., Santa Clara, CA, USA) with an ASX-500 CETAC autosampler (Cetac Technologies, Omaha, NE, USA).

The analytical method was validated and accredited according to the international standard UNI CEI EN ISO/IEC 17025:2018 (ISO, 2018). The instrumental parameters were optimized during the start-up phase (*Supplementary Table 1*). The quantification of nickel was performed using external calibration, employing a solvent calibration curve constructed by injecting a reference standard multi-element solution containing 26 elements, including nickel (CPA Chem Ltd., Bogomilovo, Bulgaria), at concentrations ranging from 0.01 to 100 µg/L.

An internal standard solution, containing bismuth (Bi), germanium (Ge), indium (In), lithium (Li), lutetium (Lu), rhodium (Rh), scandium (Sc) and terbium (Tb) in 10% nitric acid (CPA Chem Ltd., Bogomilovo, Bulgaria) at 1 mg/L was continuously infused during the analysis to compensate for instrumental variability.

The isotope selected for nickel quantification was ⁶⁰Ni, as it has a good natural abundance and is less affected by spectral interferences compared to the more abundant ⁵⁸Ni. The instrument was operated in “No Gas” mode, with no helium flow in the collision cell, to maximize sensitivity, since interferences at 60 m/z are minimal in these matrices, making the use of collision gas unnecessary. The detection and quantification limits were 3 µg/kg (limit of detection) and 5 µg/kg (limit of quantification), respectively. To ensure the validity of the results, a blank sample was mineralized and processed in the same manner as the test samples for each analytical series. The accuracy of the method was confirmed using certified reference materials in each analytical batch (BCR 679 white cabbage and DLA 58/2016 green lipped mussels).

Data analysis

For descriptive statistics, the concentration of nickel was expressed in mg/kg, considering the dilution factor applied during the extraction procedure and the exact sample weight. Mean concentrations, median, standard deviation, minimum, and maximum values were calculated for each of the three types of meat matrices. Finally, the analysis of variance and Tukey's *post-hoc* test (p≤0.05)

were used to compare the mean nickel concentrations across the different meat matrices.

Evaluation of dietary exposure to nickel and risk characterization

The following population groups were selected for the evaluation of dietary exposure to nickel: infants (0-11 months), toddlers (12-35 months), children (3-9 years), adolescents (10-17 years), adults (18-64 years), and the elderly (65+ years). To calculate the estimated daily intake (EDI) of nickel (expressed in $\mu\text{g}/\text{kg}$ bw/day) deriving from the consumption of poultry, beef, and pork, the mean nickel concentration found in each of the three types of meat was multiplied by the average dietary consumption rates of poultry, beef, and pork per population group in Italy. Dietary consumption data were retrieved from the EFSA Comprehensive European Food Consumption Database (European Food Safety Authority, 2022). The calculated EDI values were then compared to the TDI of 13 $\mu\text{g}/\text{kg}$ bw established by EFSA to determine whether meat consumption alone constituted a significant source of nickel intake and whether it approached or exceeded the established safety threshold.

Results

Nickel contamination levels in poultry, beef, and pork meat samples

The mean nickel concentrations in poultry, beef, and pork samples were 0.016 ± 0.057 mg/kg, 0.010 ± 0.037 mg/kg, and 0.009 ± 0.015 mg/kg, respectively. Statistical analysis showed no significant difference in nickel concentrations between the three meat types ($p > 0.05$). Overall, approximately 45%, 40%, and 38% of poultry, beef, and pork samples, respectively, showed nickel

concentrations below the limit of quantification (< 5 $\mu\text{g}/\text{kg}$). Maximum concentrations measured were 0.583 mg/kg in a poultry meat sample, 0.606 mg/kg in a beef meat sample, and 0.158 mg/kg in a pork meat sample. The results are summarized in Figure 1 and Table 1.

Nickel dietary exposure assessment and contribution of the consumption of poultry, beef, and pork meat to the tolerable daily intake attainment

Results related to the nickel dietary exposure through the consumption of poultry, beef, and pork meat for different age groups of the Italian population are listed in Table 2. Based on the results of the dietary exposure assessment, the contribution of poultry, beef, and pork consumption to the attainment of the nickel TDI for the different age groups in the Italian population was calculated. The results are shown in Figure 2.

Discussion

The analysis of nickel concentrations in samples of poultry, beef, and pork revealed comparable levels of contamination across the three meat types. Although poultry samples showed the highest average concentration, followed closely by beef and then pork, statistical analysis indicated that these differences were not significant. This suggests that the type of meat did not have a meaningful impact on nickel levels. A considerable proportion of samples in each group had nickel concentrations below the limit of quantification, with poultry slightly more frequently falling below this threshold than beef or pork. Isolated cases of higher contamination were observed, with the highest levels found in individual beef and poultry samples, while pork samples showed lower maximum concentrations overall. These occasional spikes may be attributed to factors such as environmental exposure, feed composition, and

Table 1. Mean concentration, median, standard deviation, minimum and maximum values calculated for each of the three types of meat matrices.

	Poultry	Beef	Pork
Mean (mg/kg)	0.016	0.010	0.009
Median (mg/kg)	0.005	0.006	0.006
SD	0.057	0.037	0.015
Minimum value (mg/kg)	<LOQ	<LOQ	<LOQ
Maximum value (mg/kg)	0.583	0.606	0.158

SD, standard deviation; LOQ, limit of quantification.

Table 2. Mean dietary exposure levels to nickel through the consumption of the three types of meat.

	Ni EDI through the consumption of poultry meat ($\mu\text{g}/\text{kg}$ bw/day)	Ni EDI through the consumption of beef meat ($\mu\text{g}/\text{kg}$ bw/day)	Ni EDI through the consumption of pork meat ($\mu\text{g}/\text{kg}$ bw/day)
Infants	0.053	0.019	0.098
Toddlers	0.054	0.026	0.112
Children	0.042	0.020	0.085
Adolescents	0.028	0.017	0.069
Adults	0.023	0.013	0.051
Elderly	0.019	0.009	0.042

Ni, nickel; bw, body weight; EDI, estimated daily intake.

farming practices affecting certain samples rather than reflecting a broader trend across meat types (European Food Safety Authority, 2015a; Ghidini *et al.*, 2022). However, due to the absence of detailed information on these aspects, it is not possible to reliably associate the observed nickel levels with particular sources of contamination.

Other studies have investigated nickel concentrations in these matrices. For instance, a study performed on chicken meat reported that all samples contained nickel concentrations below 0.450 mg/kg (Karaaslan and Yaman, 2018). Yabe *et al.* (2013) found that nickel levels in free-range chicken muscles ranged from 0.057 to 0.106 mg/kg, with an average concentration of 0.075 mg/kg. However, higher values were reported in a study conducted in Turkey, where the maximum nickel concentration in chicken muscle was 2.08 mg/kg (Uluozlu *et al.*, 2009). In addition, according to the EFSA Scientific Opinion, the mean nickel concentration in poultry meat ranged from 0.063-0.099 mg/kg, with 95th percentile values ranging from 0.13-0.21 mg/kg (European Food Safety Authority, 2015b).

Regarding pork, a study carried out in Galicia (north-west Spain) found that nickel content in pork muscle at slaughter was 0.026 mg/kg (López-Alonso *et al.*, 2007). Similarly, Ghidini *et al.* (2022) reported nickel concentrations in pork muscle ranging from 0.0051 to 0.079 mg/kg.

Overall, the nickel concentrations observed in this study, and in other studies investigating its presence in food matrices of animal origin, are relatively low (Yabe *et al.*, 2013; Uluozlu *et al.*, 2009; López-Alonso *et al.*, 2007; Karaaslan and Yaman, 2018; Ghidini *et al.*, 2022). This is particularly evident when compared to the significantly higher mean nickel concentrations reported in plant-based foods such as cocoa beans (9.8 mg/kg), soybeans (5.2 mg/kg), soya products (5.1 mg/kg), walnuts (3.6 mg/kg), peanuts (2.8 mg/kg), and oats (2.3 mg/kg) (Mania *et al.*, 2019).

These low concentrations were found to contribute minimally to the TDI of nickel, set by EFSA at 13 µg/kg bw (European Food Safety Authority, 2020). Indeed, dietary exposure to nickel through meat consumption was consistently below 1% of the TDI across all the consumer sub-categories investigated (Figure 2).

Among the different types of meat, pork was the largest con-

tributor to nickel exposure, whereas beef had the lowest contribution (Figure 2). Between the various age groups, toddlers showed the highest exposure levels, with an EDI of 0.054 µg/kg bw/day from poultry, 0.026 µg/kg bw/day from beef, and 0.112 µg/kg bw/day from pork. Conversely, the elderly had the lowest exposure levels, with an EDI of 0.019 µg/kg bw/day from poultry, 0.009 µg/kg bw/day from beef, and 0.042 µg/kg bw/day from pork (Figure 2). Based on these findings, the group ages of the Italian population can be ranked in decreasing order of risk of nickel exposure from meat consumption as follows: toddlers > infants > children > adolescents > adults > elderly. With regard to meat type, these can be ranked in the following decreasing order of contribution to nickel exposure risk in toddlers, children, adolescents, adults, and the elderly: pork > poultry > beef. In contrast, for toddlers, poultry posed the highest risk, followed by beef and pork, due to distinct dietary consumption patterns specific to this age group.

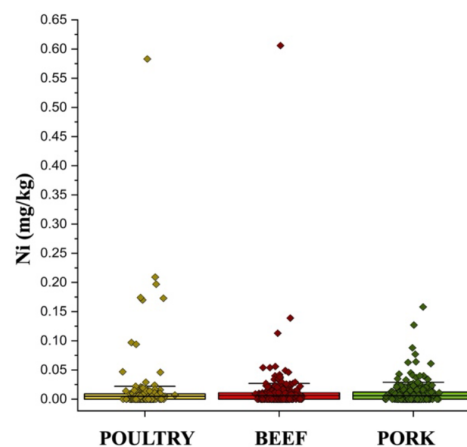


Figure 1. Graph showing the nickel concentration values detected in the samples belonging to the three types of meat matrices.

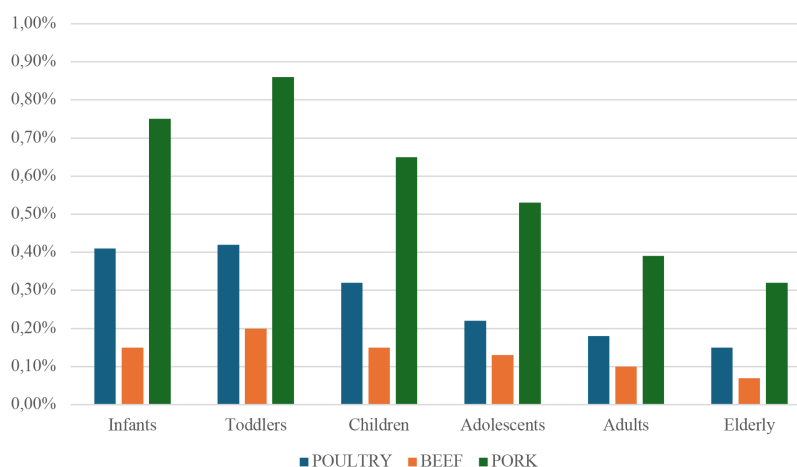


Figure 2. Contribution of the consumption of poultry, beef, and pork meat to nickel tolerable daily intake attainment (13 µg/kg bw) for different age groups in the Italian population. Infants: 0.41%, 0.15%, and 0.75%; toddlers: 0.42%, 0.20%, and 0.86%; children: 0.32%, 0.15%, and 0.65%; adolescents: 0.22%, 0.13%, and 0.53%; adults: 0.18%, 0.10%, and 0.39%; elderly: 0.15%, 0.07%, and 0.32%.

Several studies worldwide evaluated the contribution of meat consumption to nickel dietary exposure. Ghidini *et al.* evaluated the EDI of nickel from pork consumption under various dietary scenarios. In the least concerning scenario, the EDI values for children, adolescents, and adults were 0.0204, 0.0142, and 0.0106 $\mu\text{g}/\text{kg}$ bw/day, respectively. However, in the worst-case scenario, these values increased to 0.0570, 0.0349, and 0.0247 $\mu\text{g}/\text{kg}$ bw/day, highlighting the potential variability in nickel exposure based on dietary habits (Ghidini *et al.*, 2022). A study conducted in Nigeria assessed the impact of cow meat consumption on nickel intake relative to the TDI of 5 $\mu\text{g}/\text{kg}$ bw/day set by the World Health Organization (1993). The findings indicated that cow meat consumption contributed to nickel intake as follows: 10% of the TDI for adult men, 9% for adult women, 8% for pregnant women, 9% for undergraduate students, and 15% for children, remarking the higher relative exposure of younger populations to this contaminant (Ihedioha *et al.*, 2014). Similarly, Zeinali *et al.* (2019) performed a study in Birjand (southeastern Iran), reporting an EDI of nickel at 0.041 $\mu\text{g}/\text{kg}$ bw/day for adults and 0.082 $\mu\text{g}/\text{kg}$ bw/day for children through the consumption of cow meat.

Overall, consistent with our study findings, other research has confirmed that meat contributes minimally to dietary nickel exposure and has demonstrated that younger age groups have higher exposure levels, likely due to their lower bw and specific dietary habits (Ihedioha *et al.*, 2014; Zeinali *et al.*, 2019; Ghidini *et al.*, 2022).

Conclusions

The results of this study show that nickel contamination in poultry, beef, and pork samples contributes minimally to the TDI established by EFSA for this contaminant. While meat may therefore be considered a relatively low-risk food item, it is important to note that many other foods in the overall diet can be significant sources of exposure to nickel. In this context, data gaps still exist regarding nickel intake from animal-derived foods such as milk and eggs, while plant-based foods have been more extensively recognized as primary contributors. This may be relevant in the context of contemporary dietary shifts towards plant-based meat alternatives, raising important questions about the balance between the risks and benefits of traditional meat vs. emerging plant-based alternatives. From a risk management perspective, continuous monitoring of nickel levels in meat is essential to support competent authorities in identifying potentially underestimated dietary exposure sources and implementing preventive measures. This monitoring should continue to encompass meat sampling from multiple geographical regions and over extended time periods to account for the variability in environmental nickel background levels. Furthermore, European legislators could use such data to set a legal threshold for nickel in meat.

Finally, while overall exposure to nickel from meat was found to be low, some consumer groups, such as toddlers, have been identified as more vulnerable. Therefore, it is necessary to prioritize this population by promoting a conscious and informed approach to meat consumption, emphasizing the importance of a balanced and diversified diet to mitigate potential risks.

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Online supplementary material

Supplementary Table 1. Inductively coupled plasma mass spectrometry optimization parameters.