



Food safety concerns regarding pyrrolizidine alkaloid contamination in tea and honey from Italy: exposure levels and health risk implications

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ABSTRACT

This study evaluated the presence and distribution of pyrrolizidine alkaloids (PAs) and their *N*-oxides (PANOs) in black and green tea (*Camellia sinensis* L.) ($n = 79$) and monofloral and multifloral honey ($n = 439$) samples from Italy. Additionally, it aimed to estimate dietary exposure and assess potential health risks from chronic PA/PANO intake among Italian consumers. Three exposure scenarios were considered: 1) tea consumption alone, 2) honey consumption alone, and 3) tea sweetened with honey. PAs/PANOs were detected in 91 % of tea samples, with 28 % exceeding the European regulatory limit of 150 $\mu\text{g}/\text{kg}$. The highest concentration (1345 $\mu\text{g}/\text{kg}$) was found in a black tea sample and lycopsamine, lycopsamine *N*-oxide, and echinatine *N*-oxide were the predominant contaminants. In honey, 25 % of samples contained quantifiable PA/PANO levels, with a mean concentration of 2.00 $\mu\text{g}/\text{kg}$. Thyme honey showed the highest levels (50.0 $\mu\text{g}/\text{kg}$), followed by multifloral and thistle honeys (up to 47.0 $\mu\text{g}/\text{kg}$). Echimidine and echimidine *N*-oxide were the most frequently detected analytes. Risk characterization revealed that the consumption of tea or tea sweetened with honey containing high PA/PANO concentrations can pose health risks for heavy consumers across all age groups, with margins of exposure (MoEs) falling near or below the 10,000 safety threshold. In contrast, honey alone posed minimal risk (MoEs >10,000), except for young consumers of highly contaminated honey. Overall, these findings underscore the need for ongoing PAs/PANOs monitoring and proactive risk management measures, including the potential implementation of regulatory limits for foods like honey not yet covered under European legislation.

1. Introduction

Pyrrolizidine alkaloids (PAs) are a group of naturally occurring chemical compounds produced as secondary metabolites primarily by angiosperms such as Senecio and Eupatorium (Asteraceae), Echium (Boraginaceae), and Crotalaria (Fabaceae) (Hartmann & Ober, 2000). These compounds serve as natural toxins, enabling the plant to protect itself against herbivores and other potential threats. Notably, more than 600 different PAs and their *N*-oxides (*N*-ox, PANOs) have been identified, with 1,2-unsaturated compounds raising particular concern for human health due to their presence within the food chain and their association with the onset of acute and chronic toxicity. Acute and

subacute exposure to PAs can indeed cause severe liver diseases such as fibrosis, cirrhosis, and hepatic veno-occlusive disease (EFSA Panel on Contaminants in the Food Chain [CONTAM] et al., 2017). On the other hand, chronic toxic effects, which are mainly attributed to the formation of reactive pyrroles after metabolic activation, include the onset of lung diseases as well as genotoxicity and carcinogenicity (EFSA CONTAM Panel et al., 2017).

PA poisoning in humans has been well-documented historically, with severe cases of liver disease linked to the consumption of food containing by PA-contaminated seeds. Incidents were reported in the 1920s and 1950s, as well as during major outbreaks in Afghanistan in the 1970s and between 1999 and 2001 (Kakar et al., 2010). Despite

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long-standing awareness of PAs in the food chain and efforts to reduce their concentration in food, their presence continues to be reported. This trend is evidenced by the increasing number of alerts notified by the Rapid Alert System for Food and Feed (RASFF), which highlight tea and honey as particular sources of concern (RASFF, 2024; Fernández-Pintor et al., 2023). The tea plant (*Camellia sinensis* L.), although not a natural source of PAs, is highly susceptible to cross-contamination from PA-producing weeds during cultivation or harvesting (Kaltner et al., 2020). Alternatively, recent studies suggest that tea contamination may result from the natural horizontal transfer in soil, where PAs released by certain plants are absorbed by neighboring crops through their roots (Nowak et al., 2016; Selmar et al., 2015, 2019). On the other side, honey contamination primarily occurs when bees ingest PA-rich pollen and subsequently transfer these compounds into the final product (Kowalczyk et al., 2018).

The European Food Safety Authority (EFSA) assessed dietary exposure to PAs/PANOs in Europe, finding uncertain but potentially concerning levels across various age groups (EFSA, 2016). Toddlers exhibited the highest chronic exposure levels associated to the consumption of tea and herbal infusions, with high consumers reaching up to 153.8–214.0 ng/kg bw/day. Honey contributed to lower, though still notable, PA exposure levels, with adult intake averaging 0.1–7.4 ng/kg bw/day, and high consumers reaching values up to 17.6 ng/kg bw/day. Children consuming substantial amounts of honey had significantly higher exposures, reaching up to 31.1 ng/kg bw/day. When compared to the benchmark dose lower confidence limit for a 10 % excess cancer risk (BMDL₁₀) equal to 237 µg/kg bw and established by EFSA for PAs/PANOs, these exposure levels resulted in highly variable margin of exposure (MoE) values, some of which fell below the safety threshold of 10,000 (EFSA CONTAM Panel et al., 2017). This variability and the associated risk uncertainty were particularly noticeable in younger individuals, who showed MoE values for tea, herbal infusion, and honey consumption ranging from over 10,000,000 to approximately 5000 (EFSA CONTAM Panel et al., 2017).

To manage the risk of genotoxicity and carcinogenicity, it is crucial to minimize the presence of these contaminants in food to the lowest reasonably achievable level. For this reason, maximum permissible levels for the sum of 35 PAs/PANOs have been established in Europe through Regulation (EU) 2023/915 (European Commission, 2023a). These limits apply to various food items, including teas, teas for infants, herbal infusions, food supplements, dried herbs, borage leaves, and pollen products, but notably still exclude honey.

Despite current knowledge identifying tea and honey as significant contributors to dietary PA exposure in the European population, the variability in food contamination and human exposure levels observed in previous assessments highlights the need for updated, region-specific data that may help to better assess the risks posed by these contaminants, particularly in the context of rapidly changing dietary habits and climate conditions.

In light of these issues, the present study focuses on an Italian context with the primary objective of investigating the presence and distribution of 35 PA/PANO analytes in 79 samples of black and green tea (*Camellia sinensis*, L.) and 439 samples of monofloral and multifloral honey collected from various regions. Additionally, the study aimed to estimate the dietary exposure levels to PAs/PANOs among Italian consumers and assess the associated chronic health risks linked to the consumption of these food items.

2. Materials and methods

2.1. Sample collection

Both tea samples commercially available in Italy and honey samples of multiple Italian origins were considered.

The tea samples included 74 black teas and 5 green teas, all derived from *Camellia sinensis* L. in accordance with the definitions provided by

ISO 3720:2011, section 3.1, and ISO 11287:2011, section 3.1, respectively (International Organization for Standardization (ISO) 2011a & 2011b). To ensure an adequate sample size and capture the inherent variability for robust analysis, the samples were collected from the market over the period from 2017 to 2024 and were part of the national official control plan on natural toxins in foods. Each sample unit consisted of a commercial package containing 25 sachets, with 1.4 g of dried and ground tea leaves per sachet.

Honey samples (n = 439) were provided by the National Honey Observatory (www.informamiele.it) and originated from 7 Italian regions located in the northern, central, southern, and insular Italy (namely Friuli-Venezia Giulia, Veneto, Emilia-Romagna, Marche, Basilicata, Calabria, and Sardinia). Samples were sourced directly from local producers between 2017 and 2024. Of the total honey samples, 146 (33 %) were labeled as multifloral, while 293 (67 %) as monofloral, originating from 32 different botanical sources. A detailed list of multifloral and monofloral honey samples showing the number of samples and their geographical and botanical origins is provided in Table 1.

2.2. Chemicals, reagents and standards

The chemicals and reagents used for sample preparation and the subsequent analysis included: methanol (99 %) obtained from VWR International (Rosny-sous-Bois-cedex, France); reagents for QhEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) extraction (containing 4 g magnesium sulphate, 1 g sodium chloride, 1 g sodium citrate tribasic dihydrate and 0.5 g sodium citrate dibasic sesquihydrate) purchased from QuE-Lab (Castellana Grotte, Italy, cod. Q19B14F091); acetonitrile and formic acid (99 %), both purchased from Carlo Erba Reagents (Val de Reuil Cedex, France); ammonium formate obtained from Sigma-Aldrich (St. Louis, USA). Ultra-pure water for the analysis was prepared in-house from an ion exchange system purchased from Evoqua Water Technologies (Pittsburg, PA, USA). Solid phase extraction (SPE) was performed using Oasis MCX solid-phase extraction cartridges (Mixed-Mode Cation eXchange sorbent, 60 mg/3 mL, Waters Corporation, Milford, MA, USA).

Analytical standards (>98 % purity) were all purchased from Phytolab (Vestenbergsgreuth, Germany) and included: echinidine (Em), echinidine-*N*-ox (EmN), echinatine (En), echinatine-*N*-ox (EnN), europine (Eu), europine-*N*-ox (EuN), heliosupine (Hs), heliosupine-*N*-ox (HsN), heliotrine (Ht), heliotrine-*N*-ox (HtN), indicine (Id), indicine-*N*-ox (IdN), integerrimine (Ir), integerrimine-*N*-ox (IrN), intermedine (Im), intermedine-*N*-ox (ImN), lasiocarpine (Lc), lasiocarpine-*N*-ox (LcN), lycopsamine (Ly), lycopsamine-*N*-ox (LyN), retrorsine (Rt), retrorsine-*N*-ox (RtN), rinderine (Rn), rinderine-*N*-ox (RnN), senecionine (Sn), senecionine-*N*-ox (SnN), seneciophylline (Sp), seneciophylline-*N*-ox (SpN), senecivernine (Sv), senecivernine-*N*-ox (SvN), senkirkine (Sk), spartioidine (St), spartioidine-*N*-ox (StN), usaramine (Us), and usaramine-*N*-ox (UsN). For calibration, a 100 ng/mL standard solution was prepared by diluting 1:100 individual PA/PANO analytical standards with a 95:5 (v/v) water/methanol solution. Additionally, a 10 ng/mL working solution was prepared by performing a 1:10 dilution of the 100 ng/mL standard solution using a 95:5 (v/v) water/methanol mixture.

2.3. Sample preparation and PA extraction

2.3.1. Tea

Preparation of tea samples and extraction of PAs/PANOs were performed using SPE. This included an initial homogenization step, which involved grinding all the sachets included in a single tea package. Then, a 1.0 ± 0.1 g aliquot of grinded sample was processed as follows: 25 mL of 0.2 % formic acid solution was added, the mixture was vortexed and placed on a horizontal shaker for 45 min. After that, the samples were ultracentrifuged at 20,000 rpm for 10 min at room temperature. Finally, 10 mL of the solution (corresponding to 0.4 g of the original sample) was loaded into a SPE cartridge, which was previously activated with 3 mL

Table 1

List of multifloral and monofloral honey samples of different botanical origin sourced from different Italian regions and analyzed for PA/PANO concentrations.

Honey Type	Italian region							Total per type
	Friuli-Venezia Giulia	Veneto	Emilia-Romagna	Marche	Basilicata	Calabria	Sardinia	
Multifloral	14	16	48	11	34	9	14	146
Monofloral								
Acacia (<i>Robinia pseudoacacia</i> L.)	5	20	13	3		17		58
False indigo (<i>Amorpha fruticosa</i> L.)	1	1	2					4
Arbutus							5	5
Asphodelus							17	17
Betonica (<i>Stachys officinalis</i> L.)				1				1
Camedrio maro (<i>Teucrium marum</i>)							2	2
Carob							1	1
Cherry Tree	1	1	2		1			5
Chestnut	6	12	10	2	4	8		42
Citrus					5	7	1	13
Cilantro		1	3		3			7
Clover				1	8			9
Dandelion	1	8	2					11
Eucalyptus					6	2	25	33
Ferula							3	3
Heather carnicina	1	1				1		3
Honeydew			4	3				7
Ivy							1	1
Lavander		3					5	8
Linden	6	2	8					16
Lucerne (<i>Medicago sativa</i>)			5					5
Maple		1						1
Mountain ash	1							1
Mustard				1				1
Rapeseed	1	1	1	3				6
Radish			1					1
Rhododendron		5						5
Soy		1						1
Sunflower		1	1	14				16
Sulla coronaria						2	1	3
Thistle							4	4
Thymus							3	3
Total per region	37	74	100	39	61	46	82	439

methanol and 3 mL 0.2 % formic acid solution. When the percolation of the samples was completed, the cartridges were washed with 3 mL of 0.2 % formic acid solution and 3 mL of methanol. Then, the samples were eluted twice with 2.5 mL of 2.5 % ammonia solution in methanol and the extracts dried under nitrogen at 40 °C. The dry extract obtained was dissolved in 1 mL of water/methanol solution (95:5, v/v), transferred into microcentrifuge tubes, and ultracentrifuged at 13,000 rpm for 10 min at room temperature. Following this, the extract was transferred into vials for liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. Quality control samples (i.e., spiked sample at limit of quantification (LOQ)) were assessed at every batch analysis. All results were corrected for quality control sample recovery.

To simulate as correctly as possible the typical “pattern of use” of tea and obtain information related to the degree of transfer of PAs following infusion, tea infusions tests were also performed. This step was essential for subsequently calculating the dietary exposure rate, enabling a more precise estimation of the amount of PAs potentially ingested by individuals who routinely consume tea prepared as a water-based infusion. To prepare the infusion, 1.4 g of dried product (i.e., one teabag) from three different tea packages was dissolved in 100 mL of boiling water for 3 or 6 min to simulate different usage patterns. The infusion parameters used were adapted from ISO 3103:2019 (International Organization for Standardization (ISO, 2019) but, while the ISO standard considers a portion of 2.0 g of the bulk product, this study adjusted the sample weight to align with the 1.4 g present in one teabag.

After obtaining the infusions, an aliquot of 30 mL (corresponding to 0.42 g of dried product) was acidified with 60 µL of formic acid, loaded into the SPE cartridge, and then processed as described earlier for dried tea samples.

2.3.2. Honey

For the extraction of PAs/PANOs from honey, a 2.5 ± 0.1 g aliquot of manually homogenized sample was processed as follows: 15 mL of 0.2 % formic acid solution was added, the mixture was vortexed and then placed in a horizontal shaker for 45 min. Next, 15 mL of acetonitrile and QuEChERS extraction reagents were added, followed by horizontal shaking for 30 min and centrifugation at 3000 rpm at room temperature for 10 min. An aliquot of 6 mL of the supernatant extract was dried under nitrogen at 40 °C. The dry extract was then resuspended in 1 mL of a water/methanol (95:5, v/v) solution and ultracentrifuged at 13,000 rpm for 10 min at room temperature. The extract was transferred into vial for LC-MS/MS analysis. Quality control samples (i.e., spiked samples at the LOQ) were prepared and assessed at every batch analysis.

2.4. LC-MS/MS analysis

The LC-MS/MS system used was an Acquity I class ultra-performance liquid chromatograph (UPLC) coupled to Xevo TQxS mass spectrometer (Waters Corporation, Milford, MA, USA) equipped with TargetLynx software (v.4.1., Waters Corporation, Milford, MA, USA) for system management and data elaboration. The chromatographic column was an Acquity UPLC BEH C8 (100 mm × 2.1 mm, 1.7 µm, Water Corporation, Milford, CT, USA). The mobile phases were composed by 5 mM ammonium formate and 0.1 % formic acid in water (A), and 5 mM ammonium formate and 0.1 % formic acid in methanol (B). The flow rate was set at 0.3 mL/min and the injection volume of the sample was 1 µL. The mobile phase gradient was set as follows: from 5 % to 25 % of B in 10 min; from 25 % to 50 % in 5 min; from 50 % to 100 % in 0.5 min; return to initial condition in 1.5 min and hold for 3 min. Total run time was 20 min. The ESI source was set in positive ionization mode (ESI+)

with a capillary voltage of 1.0 kV, a cone voltage of 35 V, a source temperature of 150 °C, and a desolvation temperature of 600 °C. Ionization and fragmentation conditions were identified and optimized by using a continuous infusion of the tuning solutions and gradually changing the parameters for each monitored analyte.

PAs/PANOs were identified and quantified based on retention time, ion fragments produced, and ion ratio. Two transitions were monitored for each analyte, as detailed in Table S1 of the Supplementary Material. The retention time tolerance window for all the analytes in the samples was set at ± 0.1 min compared to reference peaks in standard solutions. A matrix-matched calibration curve was prepared to quantify the PA/PANO content of samples. Concentrations of the analytes were extrapolated by means of the least squares regression method. Calibration curve concentration levels were 0.5, 1, 2.5, 5, 10, and 25 ng/mL for both honey and tea. The LOQ for all PAs and PANOs was 1.00 µg/kg in honey and 5.00 µg/kg in tea.

Final quantitative results were expressed for the individual analytes as well as the sum (lower bound concentrations) of the quantified compounds (35 PAs/PANOs). For those samples having a response exceeding the linearity concentration range, a further dilution of the final extract was made.

2.4.1. Analytical method validation

During the validation phase, the possible coelution of isomeric alkaloids was evaluated, according to Regulation (EU) 2023/915 (European Commission, 2023a), including: coelution of Ly with Id, En, and Rn; coelution of ImN with IdN; coelution of Sv with Ir; coelution of Em with Hs; coelution of Sp with St; coelution of SpN with StN; coelution of Rt with Us; coelution of RtN with UsN (Table S2, Supplementary Material).

The method developed exhibited linearity for PA/PANO concentrations in the 1.00–50.0 µg/kg range for honey and 5.00–2000 µg/kg range for tea; the coefficient of determination (R^2) was ≥ 0.99 for all the monitored analytes. The method was validated according to the Regulation (EU) 2023/2783 (European Commission, 2023b) and the EURL-MP guidance document on performance criteria for methods of analysis for mycotoxins and plant toxins in food and feed (European Union Reference Laboratory for Mycotoxins and Plant Toxins [EURL-MP], 2024), based on which a recovery rate of 70–120 %, a relative standard deviation of repeatability ($RSD_R\%$) ≤ 20 % and within-laboratory reproducibility ($RSD_R\%$) ≤ 20 % are required. Since the experimental values of all the analytes and all the performance criteria were met, the developed method was considered fit for purpose (Table S3, Supplementary Material).

2.5. Assessment of dietary exposure to PAs and risk characterization

2.5.1. Exposure assessment

Chronic daily intake of the sum of PAs/PANOs from tea, honey, and a combination of both were estimated by multiplying the dietary consumption rates of these food items of the Italian population by the contamination levels experimentally quantified.

Chronic dietary consumption data were retrieved from the EFSA European Comprehensive Food Consumption Database (EFSA, 2024), which incorporates results from the most recent Italian food consumption surveys: IV SCAI CHILD (Turrini et al., 2021) and IV SCAI ADULT (Turrini et al., 2022). These dietary surveys were conducted between 2017 and 2020 (for children) and between 2018 and 2020 (for adults). Food consumption information was collected using food diaries (for children) and 24-h dietary recall (for adults) (Turrini et al., 2021, 2022). In order to formulate different exposure scenarios, both average and 95th percentile (P95) chronic consumption data for “tea beverages” and “honey” were retrieved from the third hierarchical level of FoodEx classification system, focusing on consumers from the age groups of toddlers, children, adolescents, adults, and the elderly. Among different Italian population groups, tea beverages consumption rates used in the

calculations ranged from 2.96 g/kg bw/day to 12.11 g/kg bw/day for average consumers and from 7.50 to 24.30 g/kg bw/day for P95 consumers. Honey consumption rates were in the 0.12–0.45 g/kg bw/day range for average consumers and in the 0.30–1.19 g/kg bw/day range for P95 consumers (EFSA, 2024).

Regarding PA/PANO contamination levels, the concentrations found in honey were used directly. For tea, concentrations were adjusted to account for the transfer rate of contaminants after a 6-min brewing time from dried tea (see Section 2.3.1. Tea), so as to provide more accurate exposure estimates.

To evaluate dietary exposure, multiple scenarios were formulated in order to cover a wide range of possibilities, all considering individuals exposed to the sum of 35 PAs/PANOs. These scenarios included:

- i) Scenario 1: exposure from tea consumption alone. For calculations, both the average PAs/PANOs concentration after water infusion (equal to 1.86 µg/L, Scenario 1 A) and the P95 PAs/PANOs concentration after water infusion (equal to 7.16 µg/L, Scenario 1 B) were considered. Scenario 1 A and Scenario 1 B were each calculated twice, once using the mean dietary consumption data and once using the P95 dietary consumption data of tea beverages.
- ii) Scenario 2: exposure from honey consumption alone. For calculations, both the average PAs/PANOs concentration (equal to 2.00 µg/kg, Scenario 2 A) and the maximum PAs/PANOs concentration (equal to 50.0 µg/kg, Scenario 2 B) found in honey were considered. Scenario 2 A and Scenario 2 B were each calculated twice, once using the mean dietary consumption data and once using the P95 dietary consumption data of honey.
- iii) Scenario 3: combined exposure from tea and honey consumption. For calculations, the common practice of using honey as a sweetener for tea was considered, assuming the addition of two teaspoons (equivalent to 10 g) to the tea. The average PA/PANO contamination levels of tea infusions and honey were combined (equal to 2.06 µg/L, Scenario 3 A), as well as the P95 PA/PANO contamination levels of tea and the maximum PA/PANO contamination level of honey (equal to 12.2 µg/L, Scenario 3 B). Scenario 3 A and Scenario 3 B were each calculated twice, once using the mean dietary consumption data and once using the P95 dietary consumption data of tea beverages.

2.5.2. Risk characterization

Once the daily intake of PAs/PANOs were calculated, they were compared to the relevant toxicological reference point (BMDL₁₀), currently set at 237 µg/kg bw/day (EFSA CONTAM Panel et al., 2017). The MoE values were hence estimated as the ratio between the BMDL₁₀ and the daily intake of contaminants and evaluated as quantitative metrics to assess whether exposure through the consumption of tea, honey, and a combination of both was likely to pose a risk for the population. A MoE threshold of 10,000 was considered relevant, with values higher than 10,000 indicating low concern for genotoxic and carcinogenic risk, and values lower than 10,000 indicating potentially high concern.

2.6. Statistical analysis

Contamination data were summarized using descriptive statistics and presented as mean values, standard deviations, P95, minimum, and maximum concentrations.

PA/PANO concentrations were statistically analyzed to evaluate differences between tea samples (black vs. green teas) using Welch's *t*-test, which accounts for heteroscedasticity and unequal sample sizes (significance level set at $p < 0.05$). Welch's *t*-test was also applied to assess differences in PA/PANO concentrations and individual analytes in the prepared tea beverages subjected to different infusion times ($p < 0.05$). To examine differences in PA/PANO concentrations among honey

samples of different botanical origins, a Welch-ANOVA test coupled to a Games-Howell pairwise comparison test ($p < 0.05$) was applied, excluding honey types with fewer than two samples from the analysis.

All statistical analyses were performed using OriginPro 2023 software (v. 10.0.0.154, Origin Lab Corporation, USA).

3. Results and discussion

3.1. PA concentration levels in dried tea

The analyses conducted on dried tea samples confirmed the presence of PAs in 72 out of 79 (i.e., 91 %) tea samples investigated, with Ly, LyN, and EnN identified as the most prevalent and abundant compounds. This prevalence rate is consistent with earlier literature findings, where a study, in particular, reported detectable levels of at least one specific PA in 91 % of the herbal infusion products tested (Mulder et al., 2018). A lower prevalence was reported in other studies, with rates as low as 30 % for tea samples collected from the Italian market (Martinello et al., 2022) and 39 % for samples from both the Italian and Belgian markets (Rizzo et al., 2023).

Fig. 1A provides a comprehensive visualization of the sum of PA/PANO concentrations measured in the entire tea dataset, including density and key statistical indicators. As observed, the mean value was 151 $\mu\text{g/kg}$, substantially higher than the median value of 50.3 $\mu\text{g/kg}$, indicating a right-skewed distribution of concentrations. The 95th percentile value was nearly four times higher than the mean, reaching 581 $\mu\text{g/kg}$, further emphasizing the presence of a few extreme values in the dataset (Fig. 1A). The maximum recorded value, equal to 1345 $\mu\text{g/kg}$, was over 26 times greater than the mean, highlighting that certain batches of tea contained very high levels of these contaminants. Literature findings globally confirm the trends observed in this study. For instance, Peloso et al. (2023) reported similar concentrations of 158 $\mu\text{g/kg}$ (with RtN as the predominant analyte) after analyzing 35 PA/PANOs in 74 commercial tea samples available in Italy. Chen et al. (2022) investigated the presence of PAs in black tea and green tea, reporting average concentrations of 190 $\mu\text{g/kg}$ and 72.8 $\mu\text{g/kg}$, respectively, and identifying LyN as the most prevalent compound, with an occurrence rate of up to 97 %. Similarly, Edgar et al. (2011), Griffin et al. (2014), and Shimshoni et al. (2015) reported maximum PA concentrations reaching up to 1000, 1733, and 1729 $\mu\text{g/kg}$, respectively. Significantly higher concentrations, reaching up to 47,900 $\mu\text{g/kg}$, were instead reported by Kaltner et al. (2019), further supporting the significant variability in PA levels across the different samples observed in the present study.

When comparing concentrations between black and green teas, black teas were apparently found to be more contaminated, showing mean

concentrations of $156 \pm 250 \mu\text{g/kg}$, compared to green teas, which exhibited a mean concentration of $83.8 \pm 87.4 \mu\text{g/kg}$ (Fig. 1B). However, no statistically significant differences were found between the two sample groups ($p \geq 0.05$). These statistical outcomes are likely to be influenced by the larger number of black tea samples analyzed compared to green tea, which could have reduced the ability to detect true differences. The higher variability in black tea data may have also widened confidence intervals, affecting the overall statistical power of the comparison.

Fig. 1C provides an overview of the distribution of PAs concentrations in tea samples relative to the regulatory limit set by Regulation (EU) 2023/915 (European Commission, 2023a) for this food matrix (150 $\mu\text{g/kg}$) and the LOQ of the method (5.00 $\mu\text{g/kg}$). The majority of the tea samples (72 %) were compliant. However, when considering maximum limit of 75 $\mu\text{g/kg}$ set for tea for infants and young children, the proportion of compliant samples was significantly lower (55.7 %). Approximately 9 % of the tested samples showed PA levels below the LOQ, indicating low or negligible contamination in these cases (Fig. 1C).

3.1.1. Transfer rate of PAs during brewing

The transfer rate of PAs during tea brewing can be influenced by two key factors: i) the migration of analytes from the dried raw material into the infusion, and ii) their potential thermal degradation in hot water (Fernández-Pintor et al., 2023). Table 2 summarizes the results achieved after infusion tests, detailing the percentages of transfer rates of the sum of PAs/PANOs and individual analytes for the three tested tea samples. These rates were calculated by measuring contaminant levels in the dried teabag products (average concentrations of 185, 216, and 640 $\mu\text{g/kg}$) and after 3- and 6-min infusions in boiling water (see Chapter 2.3.1, Tea).

As observed, the average transfer rate for the sum of PAs/PANOs was approximately $85 \pm 19 \%$ after 3 min brewing and $88 \pm 21 \%$ at 6 min brewing, highlighting minimal impact of the brewing time on the overall transfer. Globally, there is a lack of consistent data on how processing factors, such as temperature and brewing duration, influence PA stability and transfer from herbs to infusions. Indeed, the results achieved in the present study agree with those reported by Fernández-Pintor et al. (2023), who found that PAs are stable compounds effectively transferred from dried materials to infusions, being the final concentrations in infusions primarily influenced by the initial contamination levels in the raw material rather than by brewing temperature or duration. On the contrary, the present results strongly contrast with other studies reporting total transfer rates of 30 different PA/PANO analytes into infusions as low as 16–28 % (Picron, Herman et al., 2018). This discrepancy is particularly noteworthy when considering that not only a similar number of compounds were analyzed, but also very similar

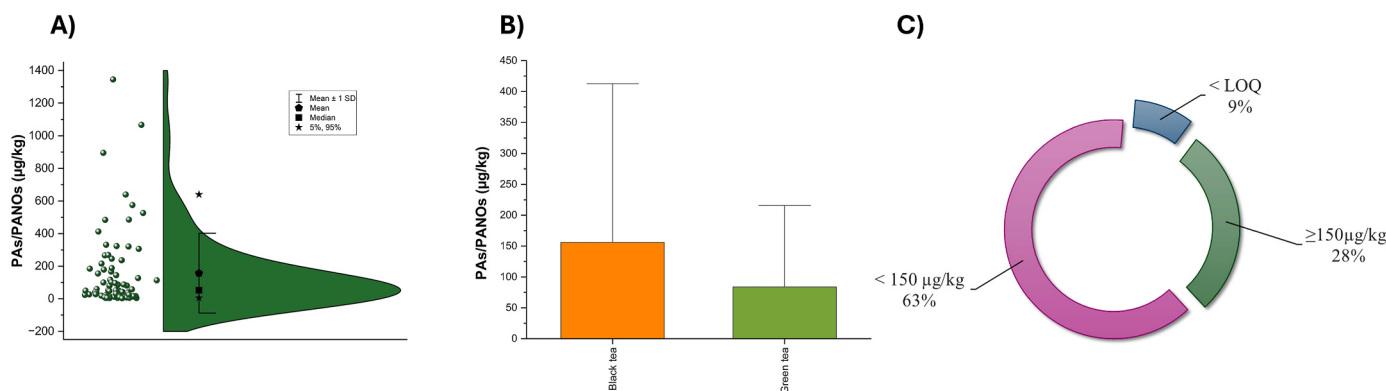


Fig. 1. Representation of PA/PANO concentrations and distributions in the analyzed tea samples. (A) Scatter-violin plot showing concentrations in the 79 tea samples (mean $\pm$ 1 standard deviation: diamond with scatter error bars; median: square; 5th/95th percentiles: stars). (B) Box plot highlighting the mean $\pm$ 1 standard deviation concentrations in black tea and green tea samples. (C) Overall distribution of PA/PANO concentrations in the 79 tea samples, shown in relation to the maximum regulatory limits set by Regulation (EU) 2023/915 (150 $\mu\text{g/kg}$) and the limit of quantification (LOQ) of the method (5 $\mu\text{g/kg}$).

Table 2

Results of infusion experiments showing the percentage transfer rates of the total PAs/PANOs and individual analytes for the three tea samples tested. Missing values indicate analytes that were not measured (i.e., below the limits of quantification).

Tea sample	Infusion time (min)	Transfer rate (%)									
		PAs/PANOs	Ly	Rt	Sn	Im	LyN	EnN	RtN	ImN	RnN
1	3	76	33				77	112			
1	6	70	33				63	102			
2	3	73	33				83	73		87	
2	6	83	33	19			83	70		120	
3	3	107	82			83	79	156	108		83
3	6	111	78			100	83	163	112		100

infusion and analytical conditions were employed in the present study. Several other factors, often underestimated, may have influenced the tendency of PAs/PANOs to transfer more readily. One such factor can be the leaf particle size, as infusions made from smaller leaf particles can contain concentrations up to four times higher than those prepared with intact leaves (Chen, Wang, et al., 2019).

With regards to individual analytes, not all PAs were found to be effectively transferred following the brewing process (Table 2). For instance, a significant reduction in Ly and Rt concentrations was observed after infusion ($p < 0.05$), suggesting that these compounds may be less readily transferred into a hot water-based medium or more prone to thermal degradation. Moreover, the absence of Rt after a 3-min infusion, coupled with its reduction after 6 min, may suggest a potential delay in the extraction of this compound which resulted in undetectable levels during shorter infusion times, followed by a gradual release from the tea matrix over a longer infusion period (Table 2). Regarding EnN, higher concentrations were observed in the infusion compared to the dried teas ($p < 0.05$), likely due to a non-uniform distribution of the substance in that specific matrix or its possible greater solubility in hot water (Table 2). Moreover, no differences ($p \geq 0.05$) in transfer rates of EnN related to the different infusion time were observed, potentially suggesting that the extraction of this compound is relatively efficient regardless of the duration of infusion.

3.2. PA concentration levels in honey

A summary of PA/PANO contamination levels in all the tested monofloral and multifloral honey samples is presented in Fig. 2A. As observed, the mean and the 95th percentile concentration values were found to be 2.00 $\mu\text{g/kg}$ and 12.4 $\mu\text{g/kg}$, respectively, while the maximum concentration detected was 50.0 $\mu\text{g/kg}$. Globally, a total of 108 samples (25 %) contained PAs/PANOs at concentrations exceeding

the LOQ of 1.00 $\mu\text{g/kg}$ (Fig. 2C). In particular, 19 % of the samples showed PAs values within the 1.00–9.00 $\mu\text{g/kg}$ range, 4 % within 10.0–24.0 $\mu\text{g/kg}$, and 2 % within 25.0–50.0 $\mu\text{g/kg}$ (Fig. 2C). As already observed for tea matrices, these results underscore the substantial variability in PA concentrations across the dataset, with the majority of the honey samples showing relatively low levels, consistent with previous findings (Mulder et al., 2018). In some cases, concentrations as high as 13,019 $\mu\text{g/kg}$ were reported (Kempf et al., 2011), while in other instances, concentrations as low as 2.40–2.70 $\mu\text{g/kg}$ were identified (Mudge et al., 2015). In a study analyzing PAs in 121 samples of monofloral and multifloral honey from three regions across northern, central, and southern Italy, researchers found that 31 % of the samples were contaminated, with an average PA/PANO concentration of 7.11 ± 8.25 $\mu\text{g/kg}$, higher than those found in the present study (Roncadi et al., 2023). The highest contamination levels were observed in honey from southern Italy, reaching up to 33.1 $\mu\text{g/kg}$ in a multifloral sample (Roncadi et al., 2023). Slightly lower occurrence rates of PA/PANOs (17 %), along with average concentration levels of 4.70 ± 11.1 $\mu\text{g/kg}$, were reported in multifloral honey samples from northern Italy (Lucatello et al., 2016). In contrast, a separate study investigating multiple food matrices found honey to be the most contaminated foodstuff, with PAs detected in 78 % of the analyzed samples at concentration ranging from 0.20 to 129 $\mu\text{g/kg}$ (Rizzo et al., 2023).

To further explore the variability in PA concentrations, the botanical origin of the monofloral honey was considered. As shown in Fig. 2B, high levels were found in thistle honey (25.2 ± 16.6 $\mu\text{g/kg}$), followed by thymus (16.7 ± 23.6 $\mu\text{g/kg}$), ferula (13.3 ± 3.87 $\mu\text{g/kg}$), sulla (10.7 ± 6.01 $\mu\text{g/kg}$), and chestnut (2.89 ± 6.72 $\mu\text{g/kg}$) honeys. PA/PANO concentrations in thistle and thymus honeys were significantly higher than those recorded in all other analyzed samples ($p < 0.05$). Supporting these results, a chestnut honey sample from the southern Italian region of Calabria was previously reported as one of the most heavily

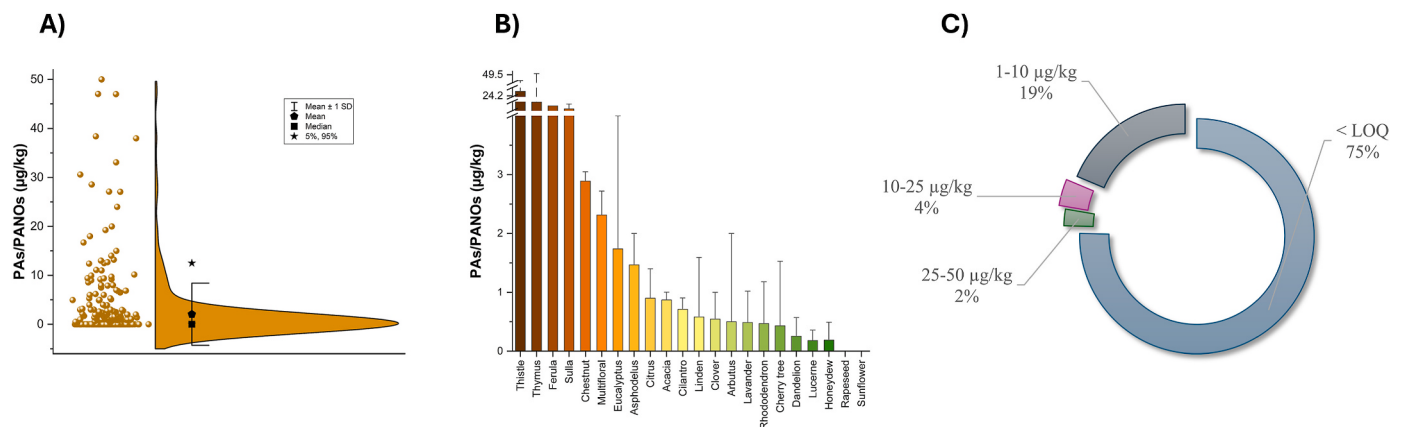


Fig. 2. Representation of PA/PANO concentrations and distributions in the analyzed honey samples. (A) Scatter-violin plot showing concentrations in the 439 tea samples (mean ± 1 standard deviation: diamond with scatter error bars; median: square; 5th/95th percentiles: stars). (B) Box plot highlighting the mean ± 1 standard deviation concentrations in the various monofloral honey samples. (C) Overall distribution of PA/PANO concentrations in the 439 honey samples, shown in relation to the limit of quantification (LOQ) of the method (1 $\mu\text{g/kg}$) and three concentration levels up to the maximum observed.

contaminated, reaching a PA concentration of 33.1 µg/kg (Roncada et al., 2023).

Since thymus is a not PA-producing plant, it is likely that the presence of high contamination levels resulted from the transport of nectar and pollen collected by bees from PA-producing plants typical of the Mediterranean region, such as *Echium* spp., *Senecio* spp., *Borago officinalis*, and *Eupatorium* spp. Similar results have been previously reported by Reinhard et al. (2009), Kempf et al. (2008), and Moreira et al. (2018), who noted that bees tend to be attracted from PA-producing plants, resulting in the contamination of the honey.

It is noteworthy that the European Commission has not yet established maximum permissible levels for PAs in this matrix, leaving a regulatory gap for this food product. As a result, direct comparisons between the PA concentrations found in this study and any regulatory thresholds cannot be made. In addition, the absence of legal standards also complicates the evaluation of contamination levels across different countries or regions, as each may adopt varying measuring criteria or lack sufficient monitoring measures.

When examining each PA/PANO analyte individually, Em and EmN were the most frequently detected compounds, present in 58 honey samples (13 %) at concentrations ranging from 1.00 to 43.0 µg/kg, as consistently reported in the literature by other authors (Lucatello et al., 2016; Roncada et al., 2023). EmN was particularly abundant in honey from Calabria (southern Italy), while Em dominated in honey from Basilicata and Sardinia (southern and insular Italy) (Fig. 3). These two compounds likely originated from plant species endemic to these regions (Fois et al., 2022), suggesting the crucial role of local flora in shaping the PA contamination of honey. Although on a larger scale, the influence of geographical origin on the PA profiles of honey samples was previously demonstrated to be of significant importance. Samples from Mediterranean countries such as France, Greece, Spain, and Turkey showed PA contamination in 90 % of cases, with specific analytes like Eu identified for the first time. In contrast, honey from Belgium showed no contamination, while honey from South America exhibited a high predominance of Em, Ht, Ly, and Im (Picron et al., 2020).

Concentrations and profiles of PAs can undergo significant variations during storage and processing. In particular, PANOs have been reported to degrade over time, while PAs tend to remain relatively stable (Gottschalk et al., 2018; Kaltner et al., 2018). In this study, honey samples collected directly from the production source were analyzed, with varying storage durations at the time of analysis. As a result, definitive conclusions regarding the influence of geographical provenance on the contaminants profile are limited due to these inherent

uncertainties.

3.3. Dietary exposure to PAs from tea and honey consumption

As described in Section 2.5.1 Exposure assessment, the PA/PANO concentrations data, experimentally determined, were used as an input for the calculation of the dietary intake by Italian chronic consumers of tea beverages, honey, and tea beverages sweetened with honey (i.e., exposure scenarios 1, 2, and 3). To create more realistic exposure scenarios for tea consumption, it was decided to use a concentration value in the infusion equal to 88 % (obtained after a 6-min infusion) of the mean concentrations found in dried tea, rather than 85 % (obtained after a 3-min infusion). This adjustment, which provided more conservative and protective exposure estimates, reflects the more common practice among consumers of extending tea infusion times. This approach is still less conservative compared to the methodology adopted by EFSA, which currently assumes a 100 % transfer rate in its exposure assessments to account for uncertainties (EFSA, 2016).

The daily intakes of PAs for both average and P95 consumers according to the six formulated exposure scenarios (i.e., 1 A, 1 B, 2 A, 2 B, 3 A, and 3 B) are summarized in Fig. 4, while detailed calculations are provided in Table S4 of the Supplementary Material.

The calculated daily intakes were characterized by substantial variability, driven both by differences in PA contamination levels and by the significant variation in daily consumption of the food items analyzed. The lowest exposure levels were observed for Scenario 2 A, which reflected honey consumption with average PA contamination levels. In this scenario, the minimum calculated intake was 0.00024 µg/kg bw/day for average adult consumers (Fig. 4B). Conversely, the highest intake levels occurred in Scenario 3 B, simulating the consumption of tea sweetened with honey at P95 and the maximum PA concentrations measured, respectively. In this case, P95 consumers among toddlers were exposed to 0.30 µg/kg bw/day of PAs (Fig. 4C).

With regard to tea beverage consumption, EFSA estimated PA exposure levels for the European population ranging from 0.00003 to 0.0075 µg/kg bw/day for average consumers of younger age groups and 0.00025–0.0101 µg/kg bw/day for P95 consumers in the same category (EFSA CONTAM Panel et al., 2017). For adults, the estimated exposure ranged were in the 0.00001–0.0018 µg/kg bw/day range for average consumers and 0.00003–0.0052 µg/kg bw/day range for P95 consumers (EFSA CONTAM Panel et al., 2017). As estimated from Scenario 1 A and 1 B, the exposure levels for the Italian population, based on the consumption of tea samples analyzed in this study, were approximately one

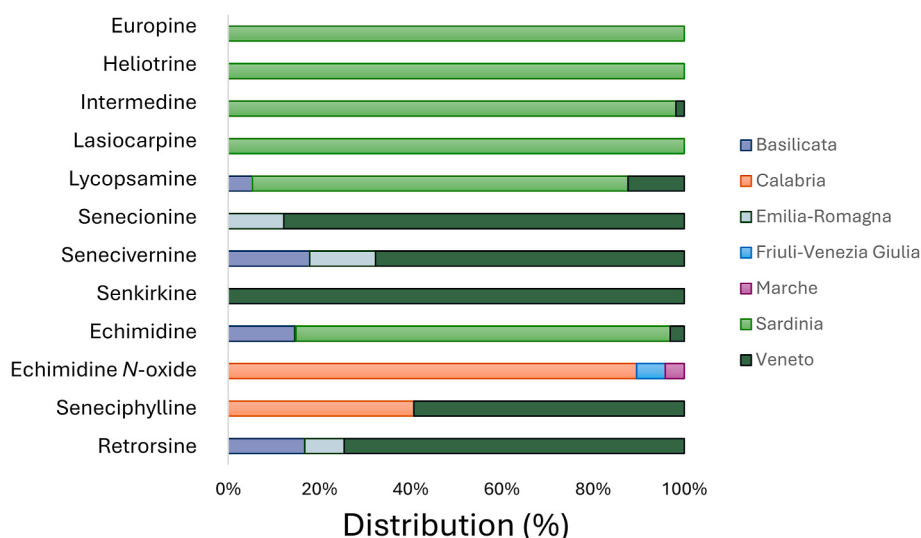
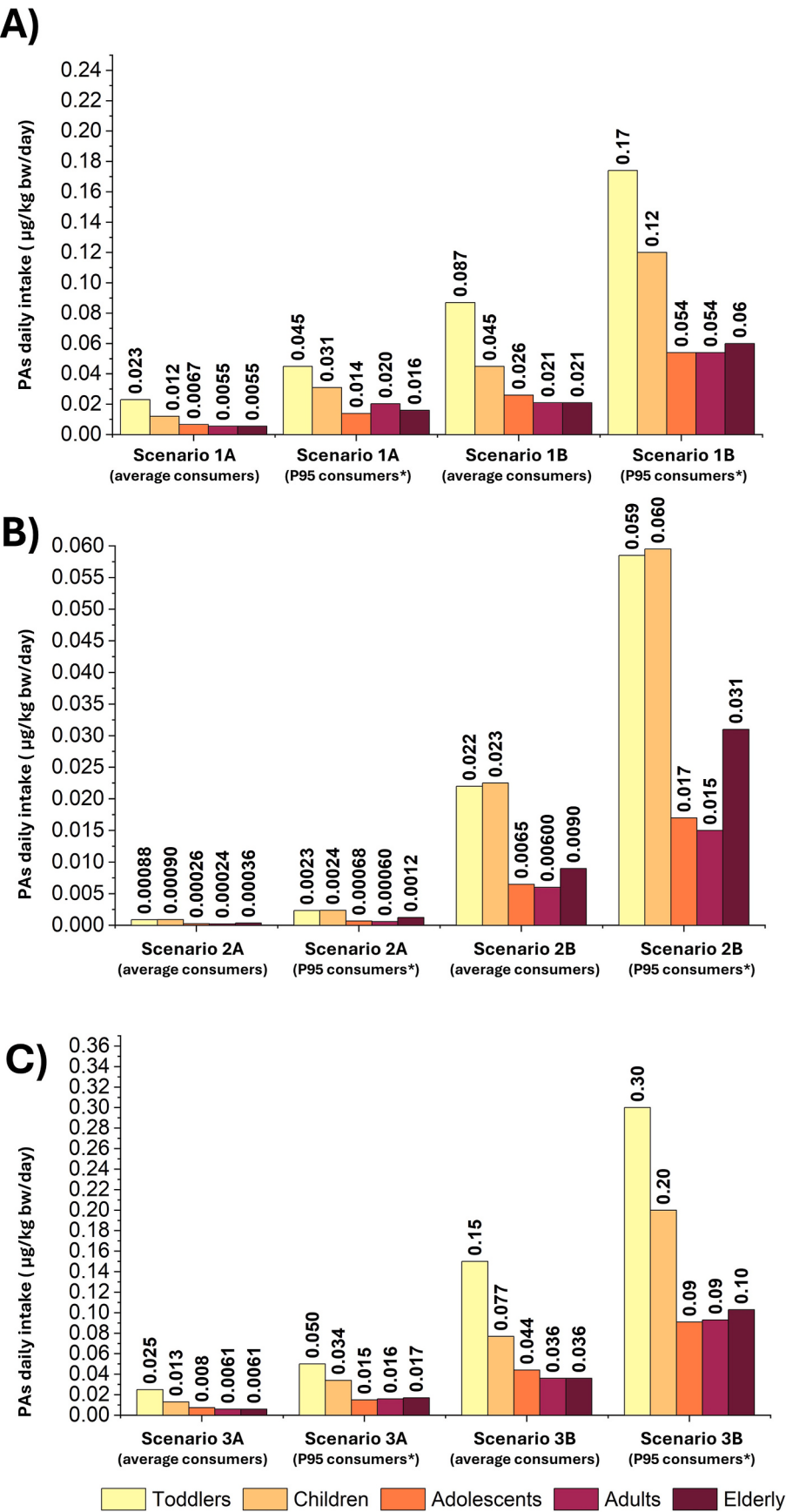


Fig. 3. Relative percentage distribution of the main PA/PANO analytes in honey samples according to the Italian region of origin.



(caption on next page)

Fig. 4. Estimated daily intakes of PAs/PANOs ($\mu\text{g/kg bw/day}$) by different Italian population age groups according to various exposure scenarios. (A) Mean (scenario 1 A) and high (P95) (scenario 1 B) consumption of tea. (B) Mean (scenario 2 A) and P95 (scenario 2 B) consumption of honey. (C): Mean (scenario 3 A) and P95 (scenario 3 B) consumption of tea sweetened with honey. *P95 daily intakes for all population age groups (except adults) may lack statistical robustness due to the limited number of consumers.

order of magnitude higher. For example, exposure values for the youngest population segment, even under the less pessimistic Scenario 1 A, were $0.023 \mu\text{g/kg bw/day}$ and $0.012 \mu\text{g/kg bw/day}$ for average toddlers and children, respectively, and $0.045 \mu\text{g/kg bw/day}$ and $0.031 \mu\text{g/kg bw/day}$ for P95 toddlers and children (Fig. 4A). These results were higher compared to those reported for various types of herbal teas, where the consumption of a single cup per day of infusions was estimated to contribute to an intake of $0.00038\text{--}0.00082 \mu\text{g/kg bw/day}$ (Chen et al., 2022). On the contrary, a study conducted in Germany estimated that the intakes of PA/PANO from green and black tea consumption among children were 0.014 and $0.018 \mu\text{g/kg bw/day}$, respectively—closely aligning with the findings of the present study. For P95 children, these values approximately doubled, reaching 0.080 and $0.083 \mu\text{g/kg bw/day}$ (Dusemund et al., 2018).

PA exposure from honey consumption at the European level was estimated to be $0.0001\text{--}0.0074 \mu\text{g/kg bw/day}$ for average consumers in the adult population and $0.0004\text{--}0.0176 \mu\text{g/kg bw/day}$ for P95 consumers in the same group (EFSA CONTAM Panel et al., 2017). These ranges encompass the exposure levels calculated in this study for honey consumption alone by the adult population, which varied from 0.00024 to $0.015 \mu\text{g/kg bw/day}$ (Scenario 2 A, Fig. 4B). For younger populations, European-level estimates were reported to be $0.0003\text{--}0.027 \mu\text{g/kg bw/day}$ for average honey consumers and $0.0007\text{--}0.031 \mu\text{g/kg bw/day}$ for P95 honey consumers (EFSA CONTAM Panel et al., 2017). These values are similar to those estimated in this study, where toddlers were exposed to $0.00088\text{--}0.059 \mu\text{g/kg bw/day}$ and children to $0.00090\text{--}0.060 \mu\text{g/kg bw/day}$ of PAs (Scenario 2 A, Fig. 4B). Comparable results were also reported for Brazilian honey consumers, whose intake ranged from $0.00043 \mu\text{g/kg bw/day}$ for average consumption to $0.0473 \mu\text{g/kg bw/day}$ in a worst-case scenario of high consumption combined with high contamination levels (Bandini & Spisso, 2022). Intakes between 0.002 and $0.007 \mu\text{g/kg bw/day}$ were reported also for German adult honey consumers. However, their upper intake levels were still less than half of those estimated here (Dusemund et al., 2018). On a broader context, the estimates from the current study are also consistent with those reported by Joint FAO/WHO Expert Committees for the adult population ($0.00002\text{--}0.026 \mu\text{g/kg bw/day}$) (Joint FAO/WHO Expert Committee on Food Additives [JECFA], 2020). In contrast, higher exposure levels for average consumers ($0.0046 \mu\text{g/kg bw/day}$) and lower exposure levels for P95 consumers ($0.0054 \mu\text{g/kg}$

bw/day) were recently reported by Pearson and colleagues (Pearson et al., 2021) among population from New Zealand.

Overall, the highest exposure levels calculated for P95 consumers (particularly among younger individuals) warrant clarification due to the limited number of tea and honey consumers from which the food consumption data were retrieved. Although the best available standardized data were used, this highlights a gap in knowledge regarding the robustness of current intake estimates for high consumers and the actual probability of such intake levels occurring, underscoring the need for more refined assessment, ideally using probabilistic approaches.

3.4. Risk characterization

The results from the comparison of the exposure levels with the BMDL₁₀ for PAs/PANOs are summarized in Fig. 5. As observed, complex profiles of potential health risks associated with exposure from tea and honey consumption were identified, varying across different age groups and exposure scenarios.

For tea consumption at average contamination levels (Scenario 1 A), the calculated MoEs ranged from $10,522$ to $43,047$ for mean intake and from 5244 to $16,989$ for P95 intake (Fig. 5). Although these values generally exceeded the critical threshold of $10,000$, indicating low concern, MoEs for P95 toddlers and children fell below this threshold. For tea contaminated with high PA concentrations (Scenario 1 B), MoEs were markedly lower, ranging from 2733 to $11,183$ for mean dietary intake and from 1362 to 4413 for P95 dietary intake in all age groups (Fig. 5). These results may underscore the heightened exposure risk for younger populations, who consume more food relative to their lower body weight compared to adults. Nevertheless, the number of consumers of tea beverages in these subgroups was limited (5.9% for toddlers and 15.3% for children), reflecting inherent constraints in the underlying dietary survey (EFSA, 2024). As such, while a potential concern was identified, the statistical robustness of these high-percentile MOEs cannot be guaranteed and the precise probability of exceeding toxicological thresholds remains uncertain.

A review of the relevant literature on tea and herbal infusion consumption revealed a wide variability in calculated MoEs, mainly driven by the type of raw materials and their initial contamination levels. For instance, Wang et al. (2021) reported extremely low MoEs for certain highly contaminated herbal teas, with values as low as 64.8 . Similarly,

Exposure scenario	Toddlers		Children		Adolescents		Adults		Elderly	
	Mean	P95*	Mean	P95*	Mean	P95*	Mean	P95	Mean	P95*
1A	10522	5244	20129	7713	35199	16989	43047	16722	42758	15061
1B	2733	1362	5229	2004	9144	4413	11183	4344	11108	3913
2A	269318	101282	263333	99580	911538	348529	987500	395000	658333	320270
2B	10773	4051	10533	3983	36462	13941	39500	15800	26333	12811
3A	9500	4735	18175	6964	31781	15340	38868	15098	38607	13599
3B	1609	802	3079	1180	5384	2599	6585	2558	6540	2304

Fig. 5. Margins of exposure (MoEs) to the sum of PAs/PANOs by the Italian population associated to the consumption of moderately and highly contaminated tea (scenarios 1 A and 1 B), honey (scenarios 2 A and 2 B), and tea plus honey (scenarios 3 A and 3 B). MoEs are color-coded according to the risk levels: red for MoEs significantly below $10,000$ (high risk), orange for MoEs near the threshold ($10,000 \pm 2000$; moderate risk), and green for MoEs well above $10,000$ (low risk). *P95 MoEs for all population age groups (except adults) may lack statistical robustness due to the limited number of consumers.

Kaltner et al. (2020) highlighted that chronic exposure to PAs through specific herbs could result in MoEs below 10,000, particularly in scenarios involving high contamination levels. Chen, Mulder, et al. (2019) reported MoE values below 10,000 for all five herbal teas they investigated, with borage tea posing the greatest concern. Comparable values were also observed for herbal teas and plant food supplements, suggesting that consuming just one cup of tea per day could result in MoE values falling below the safety threshold (Chen et al., 2017). Conversely, other studies predominantly reported MoE values for tea exceeding the safety threshold of 10,000, except in worst-case scenarios involving highly contaminated teas from specific regions (Jiao et al., 2023), thereby underscoring the influence of regional differences on overall risks.

Exposure to PAs through honey consumption (Scenario 2) varied depending on the contamination level. For average PA concentrations (Scenario 2 A, Fig. 5), MoEs ranged from 99,580 (P95 dietary intakes of children) to 987,500 (mean dietary intakes of adults), suggesting that moderately contaminated honey is unlikely to pose a health risk across all age groups. However, when maximum PA contamination levels were considered (Scenario 2 B, Fig. 5), MoEs for toddlers and children were below or near the threshold of 10,000 regardless of the amount of honey consumed.

The literature concerning risk assessment of PAs in honey similarly highlights considerable variability in MoEs depending on contamination levels. For average consumers, values are typically well above 10,000, indicating low risk under common exposure condition (BfR (Bundesinstitut für Risikobewertung) 2013; EFSA CONTAM Panel et al., 2017; Pearson et al., 2021). However, in other instances, worst-case scenarios involving high consumers of heavily contaminated honey resulted in MoEs falling significantly below the safety threshold. For instance, Bandini and Spisso (2022) calculated lower MoEs under worst-case exposure conditions linked to honey consumption in the Brazilian population, where a daily intake of 20 g of honey per person resulted in an MoE value of 5010. A safer scenario was observed for honey consumers in New Zealand and Germany. In these populations, both adults and younger age groups were generally considered at low risk of critical PA/PANO exposure from honey, as MoE values consistently remained well above 10,000. The only exception occurred in cases of exceptionally high contamination levels (Gottschalk et al., 2020; Pearson et al., 2021). Only one previous study has specifically examined exposure patterns and potential health risks associated with the consumption of locally produced honey by the Italian population (Roncada et al., 2023). However, the risk scenario presented in that study appears more concerning than the findings of the current investigation. Indeed, very low MoEs were estimated for toddlers even at average consumption levels, not only under high-consumption scenarios (Roncada et al., 2023).

The combined tea-and-honey exposure scenario (Scenario 3) presented the highest level of concern. At high PA contamination levels in both matrices, MoEs dropped as low as 1609–6585 even at mean dietary intake levels (Scenario 3 B, Fig. 5), indicating substantial potential health risks across all age groups.

Globally, the findings from the present study underscore several critical aspects related to PA dietary exposure. First, age-dependent vulnerability emerged, with toddlers and children showing consistently lower MoEs across all food matrices. Second, for older population groups, consumption patterns had a more pronounced impact on risk, with notable differences between average and high consumers, emphasizing the importance of considering diverse dietary habits in risk assessments. Furthermore, significant variations in risk profiles were observed based on the PA contamination levels, reinforcing the critical need for rigorous monitoring and control of these contaminants in tea and honey to account for contamination variability in risk assessments.

Despite the valuable insights gained, the results must be interpreted within a broader context that considers inherent limitations and uncertainties. The deterministic method used introduces a conservative bias, as it generates a fixed risk estimate based on assumptions and does

not account for variability in the input data. Additionally, the limited sample size for certain subgroups, especially toddlers and children, arising from the constraints of the national dietary survey, contributes to uncertainty in the reliability of the exposure estimates (particularly at higher percentiles), thereby affecting the overall precision of the risk assessment. All these factors, along with the assumption of equal toxicological potency for all quantified PAs, may have contributed to an overestimation of the risk. On the other hand, the overall risk could also be underestimated due to the limited number of quantified PAs, potentially excluding the cumulative contributions of unquantified analytes. It is also essential to consider that multiple dietary sources of PAs exist, but this study was focused solely on tea and honey, thus overlooking other significant contributors, such as herbal supplements, herbal infusion other than tea, aromatic dried herbs. Finally, the possibility of cumulative or synergistic interactions with other hepatotoxic or carcinogenic compounds was not accounted for in this assessment, representing another important area for future research.

4. Conclusions

The present study revealed a widespread presence of PAs/PANOs in tea (91 % of samples) and a less frequent occurrence in honey (25 % of samples) available in Italy. Notably, some tea samples exceeded regulatory limits. Honey, by contrast, had lower PA levels, though specific varieties (such as thyme, multifloral, and thistle honey) occasionally contained elevated concentrations.

Results from exposure assessment and risk characterization indicated notable health concerns for high consumers of tea and for those combining tea with honey, regardless of age group. Conversely, honey consumption alone posed minimal risk in most cases, except among younger individuals consuming honey with higher PA concentrations.

Since achieving zero tolerance for PAs in food is impractical, implementing robust regulatory measures and preventive strategies is imperative to minimize public health risks. Expanding regulatory limits to currently unregulated foodstuffs like honey, enforcing regular testing of food products, and encouraging innovative agricultural practices to reduce PA contamination at its source are critical actions.

In conclusion, while further research is needed to better understand the long-term health impacts and refine risk assessments, the present study provides crucial and updated insights concerning the exposure of the Italian population to these contaminants. The findings, in particular, underscore the need for a more comprehensive approach to dietary risk assessment, integrating the potential health risks of tea and honey consumption alongside their benefits to provide a more accurate perspective on food safety and public health implications.

CRedit authorship contribution statement

Elisabetta Caprai: Writing – review & editing, Supervision, Project administration, Conceptualization. **Angelica DI Fazio:** Writing – original draft, Resources, Data curation. **Emanuela Zanardi:** Writing – review & editing, Methodology, Formal analysis. **Maria Olga Varrà:** Writing – original draft, Software, Methodology. **Sergio Ghidini:** Writing – review & editing, Supervision, Project administration, Conceptualization. **Gianluca Antonio Romeo:** Visualization, Investigation, Data curation. **Gaetan Minkoumba Sonfack:** Visualization, Formal analysis, Data curation. **Ilaria Prizio:** Visualization, Resources, Investigation. **Stefania Bonan:** Writing – review & editing, Project administration, Conceptualization.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Not applicable.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.foodcont.2025.111388>.

Data availability

Data will be made available on request.

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