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Case reports of tropane alkaloid contamination in spinach from Italy and its potential implications for consumer health

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ABSTRACT

This study reports cases of intoxication that occurred in various regions of Italy in October 2022. These incidents were linked to the consumption of leafy vegetables contaminated with tropane alkaloids (TAs), likely due to suspected cross-contamination with toxic plants from the *Datura* genus. Although official controls were unable to identify the specific weed responsible for the contamination, chemical analysis of the remaining spinach and spinach-based foods consumed by the affected patients revealed concentrations of atropine and scopolamine up to 4642 μg/kg and 8158 μg/kg, respectively. While European regulations currently lack maximum limits for TAs in leafy vegetables, the concentrations detected in 4 out of 5 samples exceeded the maximum limits currently established for cereal-based foods, infant cereal-based foods, and herbal infusions.

To assess the acute dietary exposure to atropine and scopolamine, estimated daily intakes of the Italian population were calculated using the TA concentrations measured in spinach samples or assuming the presence of 1 % of the weight of one *Datura stramonium* leaf in a 500 g spinach pouch. Both exposure scenarios resulted in concerning exposure levels, far exceeding the acute reference dose of 0.016 μg/kg bw by 2–1200 times. Toddlers exhibited the highest mean and 95th percentile acute dietary exposures compared to adults and the elderly. These findings emphasize the necessity of intensifying monitoring efforts and enacting regulatory measures to minimize exposure to TAs from dietary sources that are less commonly associated with contamination, especially for mitigating potential health risks for vulnerable population groups.

1. Introduction

Tropane alkaloids (TAs) are secondary metabolites produced by various plant species, mainly belonging to the Brassicaceae, Solanaceae, and Erythroxylaceae families (González-Gómez, Morante-Zarcero, Pérez-Quintanilla, & Sierra, 2022), González-Gómez, Morante-Zarcero, Pereira, et al., 2022s well as other families such as Rhizophoraceae, Proteaceae, Euphorbiaceae, and Convolvulaceae (Gonçalves et al., 2017). These compounds act as a defense mechanism against other organisms and have allelopathic effects on other plant species (Croteau et al., 2000; Jank & Rath, 2021). Until recently, a complete understanding of the biosynthetic mechanisms of these compounds has been lacking (Huang et al., 2021).

Due to their bioactive properties, TAs have been used in ancient times for various purposes, such as magic potions, recreational and shamanic poisons, sedatives, and hallucinogens (Dey et al., 2020; EFSA, 2018; Romera-Torres et al., 2018). Nowadays, they are employed in contemporary medicine, specifically in fields such as ophthalmology, cardiology, and gastroenterology (Dey et al., 2020; Romera-Torres et al., 2018). More than 200 different TAs have been described so far, but the most widely studied ones are (−)-hyoscyamine, scopolamine, and atropine (i.e., the racemic mixture of (±)-hyoscyamine) (EFSA, 2008). The biological activity of TAs involves competitive antagonism of muscarinic acetylcholine receptors, resulting in the inhibition of acetylcholine binding (EFSA, 2008). The anticholinergic activity causes a variety of symptoms such as dry mouth, decreased secretions,

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difficulty in speaking and swallowing, tachycardia, drowsiness, dizziness, hallucinations, and delirium. Higher doses of TAs may result in depression of the central nervous system, circulatory and respiratory failure, coma, and death (Debnath et al., 2018; EFSA, 2022; Fatur & Kreft, 2020; Khoshnam-Rad et al., 2022; Kohnen-Johannsen & Kayser, 2019).

Human exposure to TAs is primarily associated with the dietary consumption of some plants belonging to the Solanaceae family, which are frequently responsible for cross-contamination of crops during harvesting (EFSA, 2018; Fatur & Kreft, 2020; González-Gómez et al., 2023). Indeed, due to the invasive nature of TA-producing plants and the presence of TAs in all plant parts, there is a significant likelihood of unintentional mixing with edible crops during both harvesting and processing stages (Adamse et al., 2014; EFSA, 2013; González-Gómez et al., 2023). Cereals, pseudo-cereals, legumes, and grains have been found to be the most commonly contaminated crops, as evidenced by numerous alerts reported on the Rapid Alert System for Food and Feed (RASFF) portal over the past 28 years (RASFF, 2023). Contamination episodes have resulted in several significant poisoning outbreaks, the most notable being the 2019 Uganda outbreak, which involved the contamination of a fortified blended product distributed by the World Food Programme (WFP, 2019), known as "Super Cereal" (Abia et al., 2020; Adamse et al., 2014; de Nijs et al., 2023; EFSA, 2022; González-Gómez, Morante-Zarcero, Pérez-Quintanilla, & Sierra, 2022; Haughey et al., 2021).

To assess the permissible dietary exposure levels to TAs, the European Food Safety Authority (EFSA) established an acute reference dose (ARfD) of 0.016 μg/kg bw for the sum of (−)-hyoscyamine and (−)-scopolamine as a health-based guidance value. The derivation of this value was based on findings from a study of healthy young male adults, which identified effects such as a decrease in heart rate and the central nervous system symptoms described above (EFSA, 2013; Perharič, Juvan, & Stanovnik, 2013; Perharič, Kozelj, et al., 2013). Moreover, the Commission Recommendation (EU) 2015/976 requires Member States to implement monitoring plans for the presence of TAs in high-risk food items, and the Regulation (EU) 2023/915 establishes maximum levels for atropine and scopolamine for both processed and unprocessed cereal-based foods, as well as herbal infusions and cereal-based foods for infants and young children (European Commission, 2023).

These regulatory measures are a direct outcome of the large-scale acute exposure assessment conducted by EFSA in 2018 (EFSA, 2018). Over the span of seven years (2009–2016), EFSA collected and analyzed data on TAs occurrence from nearly forty-thousand food samples. By employing a probabilistic approach, it was observed that cereal-based foods and tea/herbal infusions were the main contributors to the exposure to the sum of atropine and scopolamine across all the age groups and various food categories considered. The highest mean acute dietary exposure levels, ranging from 1 to 21 ng/kg bw per day, were observed in the young population (infants, toddlers, and other children), even though the ARfD for the sum of atropine and scopolamine was exceeded by high consumers of all age groups (EFSA, 2018). Similarly, in the context of the 2020 joint expert meeting of the Food and Agriculture Organization (FAO) and the World Health Organization (WHO), a probabilistic approach was employed to assess the dietary exposure to TAs across various population groups part of the WFP. Rice, corn, and sorghum were identified as the primary contributors to dietary exposure across the investigated countries, with mean levels below 1 ng/kg bw per day for all population groups, except in the Republic of Zambia (FAO/WHO, 2020).

Currently, no defined maximum limits of TAs are in place for leafy vegetable products such as spinach and spinach-based infant food, despite compelling evidence from multiple studies demonstrating considerable levels of TAs in these products (Adamse et al., 2014; Castilla-Fernandez et al., 2021; Cornara et al., 2018; González-Gómez, Morante-Zarcero, Pereira, et al., 2022; Mulder et al., 2016). Contamination of leafy vegetables by atropine and scopolamine is typically associated with seeds from poisonous plants of the *Datura* genus, while cases of contamination caused by toxic leaves of these plants are possible but rare. Indeed, leaves of *Datura* species (including *Datura innoxia, Datura stramonium*, and *Datura metel*) can be responsible for TA cross-contamination of different vegetable food products because of their similarity in morphological and botanical traits to other frequently consumed vegetables (especially young leaves of borage and spinach), their global distribution, and their invasiveness (Abia et al., 2020; Cornara et al., 2018; de Nijs et al., 2023). Furthermore, food products originating from organic agriculture appear to have a higher susceptibility to TA contamination. This is attributed to the implementation of distinct weed management practices in crop fields, which, if not appropriately carried out, can elevate the risk of unintentionally co-harvesting with *Datura* plants (Cirlini et al., 2018). Within this context, a recent incident of TA poisoning occurred in France, where a family experienced severe neurological symptoms such as agitation and hallucinations after consuming spinach purchased from the market which were found to be contaminated with atropine and scopolamine. Similarly, in Athens, seven people were hospitalized with anticholinergic syndrome after consuming boiled blites that were contaminated with *Datura innoxia*. Additionally, in Slovakia in 2021, over a hundred cases of intoxication were reported due to the consumption of deep-frozen spinach puree contaminated with 3446 μg/kg of atropine and 3860 μg/kg of scopolamine, resulting from the accidental presence of *Datura* plant residues (Plackova et al., 2022; RASFF, 2023).

Hence, there exists a potential risk of leafy vegetables being contaminated by plants that produce TAs. Despite the growing awareness of this potential risk, the existing scientific literature offers limited insights into the extent and magnitude of human dietary exposure levels to TAs deriving from the consumption of these food products. In light of these gaps, the primary objective of this study was to present recent cases of TA intoxications resulting from the consumption of spinach and spinach-based foods that occurred in various regions of Italy during October 2022. The study includes the presentation of results from quantification analyses of atropine and scopolamine conducted on leftover samples consumed by the affected individuals, as well as the outcomes of an investigation into the atropine and scopolamine profiles of *Datura* plant leaves. This investigation was carried out for comparative purposes since these leaves were suspected of being responsible for accidental contamination. Furthermore, a secondary objective of this research was to hypothesize the potential levels of TAs to which Italian toddlers, adults, and elderly may be exposed through the consumption of contaminated spinach and spinach-based foods. The aim was to highlight the potential risk these population groups could face if such TA-contaminated food samples are distributed more widely among the population.

2. Materials and methods

2.1. Samples collection

2.1.1. Poisoning cases and food samples

Four cases of suspected anticholinergic intoxication were reported in three regions of Italy (as summarized in Fig. 1) in October 2022 following the consumption of spinach and spinach-based food.

- Case 1: In the Campania region (southern Italy), eight individuals from three different families were hospitalized in Pozzuoli (Naples) with anticholinergic symptoms after consuming spinach potentially mixed with TA-producing plants. Thirty-two vegetable baskets from the same batch were seized by the competent authorities and analyzed. No food residues from the meal were available for analysis.
- Case 2: In the Campania region (southern Italy), two individuals from the same family, exhibiting symptoms of TAs intoxication were reported to the competent authority of Naples by their primary care

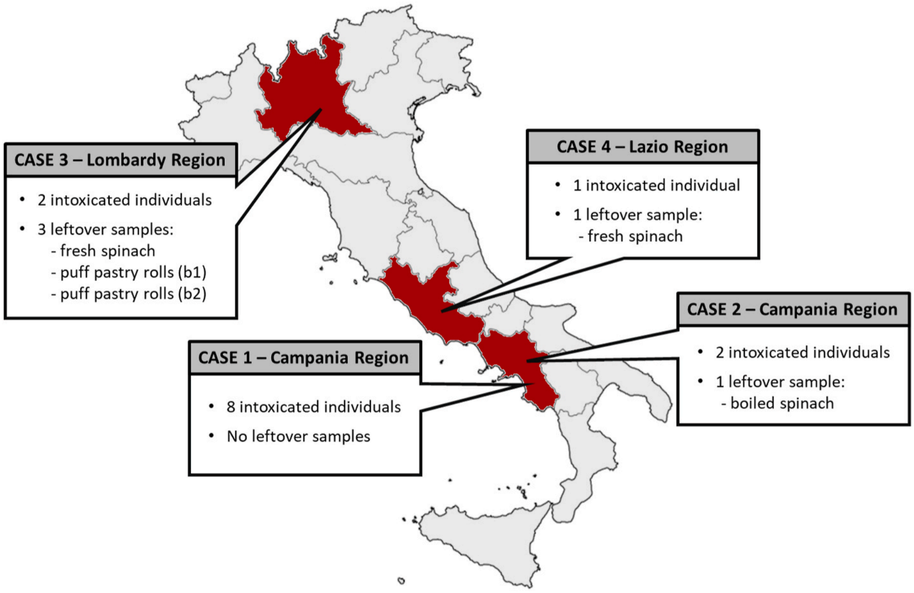


Fig. 1. Summary of intoxication cases due to dietary exposure to tropane alkaloid that occurred in Italy in October 2022.

doctor. Food residues consisting of boiled spinach were recovered from the patients' home and analyzed. The producer was identified, and a hundred baskets of spinach were seized.

- Case 3: In the Lombardy region (northern Italy), two individuals from the same family were hospitalized in Milan after experiencing symptoms indicative of parasympatholytic and spasmolytic poisoning, which were attributed to eating spinach-stuffed puff pastry rolls. The authorities collected a pouch containing spinach residues from the rubbish (a) and two leftover puff pastry rolls (b1, b2), which were destined for analysis.
- Case 4: In the Lazio region (central Italy), one suspected case of TA poisoning due to the consumption of boiled spinach potentially contaminated with TA-producing plants was reported by a hospital in Rome. A pouch containing spinach residues was collected for analysis. No samples from the same batch were available for analysis since they had already been sold and consumed.

Except for Case 4, all the batches of spinach involved in the intoxication cases and seized from the producer companies were visually inspected by botanical experts from the Emilia-Romagna, Campania, and Lombardy regions to identify the potential botanical varieties that could have caused intoxication symptoms. However, no plants typically producing TAs were identified during these inspection controls.

2.1.2. *Datura plant samples*

Due to the significant association of *Datura* spp., particularly *Datura stramonium*, with TA production and food contamination (EFSA, 2008), it was deemed relevant to acquire information on the atropine and scopolamine profiles of this weed. To this end, leaves of *Datura stramonium* and *Datura metel* were collected from the Botanical Garden of the University of Bologna. In addition, two whole plants of spontaneous *Datura stramonium*, measuring 70 cm and 20 cm in height, were obtained from the Ravenna province (Emilia-Romagna region). An illustration of the sampled plant species is reported in Fig. 2.

Only one leaf was selected from each collected sample and their weights were recorded. Every leaf was individually finely ground and destined for analysis, which was conducted on the same day the samples arrived at the National Reference Laboratory for Plant Toxins in Food, Food Chemical Department, of the Istituto Zooprofilattico Sperimentale della Lombardia e dell'Emilia-Romagna (IZSLER). *Datura* leaves have been selected casually and visually following the criteria of being alike

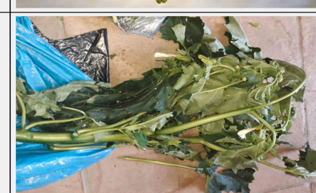
PLANT TYPE	SOURCE	PLANT IMAGE
Datura Stamonium Spontaneous – 20 cm high	Ravenna province – Emilia-Romagna region	
Datura Stamonium Spontaneous – 70 cm high	Ravenna province – Emilia-Romagna region	
Datura Stamonium	Botanical Garden of the University of Bologna – Emilia-Romagna region	
Datura Metel	Botanical Garden of the University of Bologna – Emilia-Romagna region	

Fig. 2. The four samples of Datura species considered in the current study, from which one leaf was selected and designated for the quantification of tropane alkaloid concentrations.

spinach leaves (by dimensions and morphology).

2.2. *Chemicals and solvents*

Methanol, acetonitrile, and formic acid (99 %) for LC-MS were purchased from Carlo Erba Reagents (Val de Reuil Cedex, France);

Oasis® MCX 6 cc (150 mg) LP extraction cartridges were purchased from Waters Corporation (Milford, MA, USA), ammonia solution (30 %) from Carlo Erba Reagents (Val de Reuil Cedex, France), and methanol for extraction and elution from VWR International (Rue d'Aurion, France). Ultra-pure water for the analysis was prepared in-house from an ion exchange system purchased from Evoqua Water Technologies (Pittsburg, PA, USA).

Atropine (from Supelco, Bellefonte, PA, USA), scopolamine, atropine D-3, and scopolamine ^{13}C D-3 (all from A2S - Analytical Standard Solutions, Saint Jean d'Illac, France) 98 % purity were used as calibration and internal standards (ISs).

2.3. Sample preparation: extraction and purification of TAs via solid-phase extraction (SPE)

Each collected sample (both food and *Datura* samples) was entirely ground with a knife mill grinder (Grindomix GM200, Retsch GmbH, Haan, Germany), and weighed to 2 ± 0.1 g in centrifuge tubes. A total of 20 mL of a methanol/water/formic acid (39:60:1, v/v/v) solution was added to the tubes, which were then vortexed and placed on a shaker stirrer for 1 h. Both food and *Datura* samples (consisting of one previously selected leaf, as detailed in Section 2.1.2. *Datura* plant samples) were subsequently centrifuged at 20,000 rpm for 10 min at +5 °C. Afterward, 5 mL of the supernatants were collected, purified, and pre-concentrated by solid-phase extraction (SPE) using the MCX 150 mg/6 cc cartridges, which were previously conditioned with 6 mL of methanol and 6 mL of the methanol/water/formic acid (39:60:1, v/v/v) solution. The elution was performed with 6 mL of a 3 % ammonia solution in methanol. The remaining solvent was removed under a stream of nitrogen at +40 °C, and then redissolved in 1 mL of methanol/water/formic acid (39:60:1, v/v/v). Following centrifugation at 12,000 rpm for 10 min at room temperature, extracts from food and *Datura* samples were diluted to 1:50 and 1:100,000, respectively, to reduce matrix effects. All the sample extracts were vortexed prior to analysis which was performed in duplicate by using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

2.4. LC-MS/MS analysis

The LC-MS/MS system consisted of an Acquity UPLC I-Class PLUS chromatographic module (Waters Corporation, Milford, MA, USA) coupled with a quadrupole mass spectrometer Xevo® TQ-XS (Waters Corporation, Milford, MA USA), equipped with the managing software MassLynx (Waters Corporation, Milford, MA, USA). The chromatographic separation was performed by using an Acquity UPLC BEH C18 column (130 Å, 1.7 μm, 2.1 mm × 100 mm) from Waters Corporation (Milford, MA, USA), heated at +40 °C. The flow rate and injection volume were set at 0.35 mL/min and 5 μL, respectively. The mobile phase was composed of demineralized water (0.1 % formic acid) (phase A) and acetonitrile (0.1 % formic acid) (phase B). The binary gradient was from 90 % to 10 % of A in 5.5 min, back to initial conditions in 0.5 min, and then kept at the initial composition for 1 min for reconditioning, resulting in a total run time of 7 min.

The ideal ionization and fragmentation conditions for the analytes were identified using a continuous infusion of the tuning solution. Fragmentation was carried out in positive ionization mode (ESI+), with capillary voltage of 0.5 kV, cone voltage of 20 V, source temperature of +150 °C, and desolvation temperature of +600 °C. The identification and quantification of atropine and scopolamine were performed based on retention time, ion fragments produced, and ion ratio. Each analyte was quantified using calibration curves previously built from calibration standards at the following atropine/scopolamine concentrations: 0.5 ng/mL, 0.2 ng/mL, 0.1 ng/mL, 0.05 ng/mL, 0.03 ng/mL, 0.01 ng/mL, 0.0025 ng/mL, containing a concentration of ISs mix of 0.2 ng/mL. The calibration curves were created by plotting the analyte/internal standard peak area ratio versus analyte concentration.

LC-MS/MS parameters are summarized in Table 1. An illustrative example of chromatograms of scopolamine and scopolamine-D3 (IS) originating from leftover puff pastry rolls b1 sample is presented in Fig. 3.

2.4.1. Method performance parameters

Quality assurance/quality control procedures included the assessment of specificity, linearity, recovery rates, repeatability, precision, selectivity, and limits of quantification (LOQs) and limits of detection (LODs) of the method. Specificity was verified, and the presence of interference was checked by analyzing 20 times fresh vegetable samples (spinach, lettuce and broccoli). No significant interfering peaks were detected in the chromatograms of blank extracts. The method exhibited linearity for atropine and scopolamine in the range 0.3–75 μg/kg, with correlation coefficients of the calibration curves higher than 0.99 and a normal distribution of residuals (standard deviation lower than 20 %). Repeatability (expressed as relative standard deviation, RSDr %) and overall recovery were assessed by analyzing blank samples in six replicates spiked with atropine and scopolamine at concentrations of 0.3, 5, 10, 25, 50, and 75 μg/kg. Intermediate precision (RSD_{Ri}) has been calculated on-going considering six data collected in six different days deriving from spiked samples at 5 μg/kg during routine analysis. RSDr and $RSD_{Ri} \leq 20$ % were obtained, in accordance with the guidance document on performance criteria of the European Union Reference Laboratory for Mycotoxins and Plant Toxins (EURL-MP, 2022). The expanded uncertainty of the method (U_{exp} at 95 % confidence level and k = 2) was calculated using a bottom-up approach (two replicates) was 30.1 % and 36.4 % for atropine and scopolamine, respectively. The LOQ of the method was 0.3 μg/kg for both analytes. The LOQ corresponded to the lower tested and validated concentration, ensuring a signal-to-noise ratio of at least 10. The LOD was assessed and set at 0.15 μg/kg where signal-to-noise ratio was at least 5.

The selectivity of the LC–MS/MS method was achieved by monitoring one parent ion and two daughter ions for atropine and scopolamine, together with the relative fragmentation conditions. For atropine D-3 and scopolamine ^{13}C D-3, one parent ion and one daughter ion were monitored, following the identification requirements SANTE/12089/2016 "Guidance document on identification of mycotoxins in food and feed".

Table 1
LC-MS/MS parameters for TAs: chromatography, precursor ion, daughter ions, and fragmentation conditions.

LC conditions	
LC-MS/MS equipment	XEVO TQ-Xs Acquity UPLC I Class Plus Waters
LC Column	UPLC BEH C18 Waters
Mobile Phase	A: water 0.1 % formic acid B: acetonitrile 0.1 % formic acid
Flow (mL/min)	0.35
Injection volume (μL)	5
MS/MS parameters	
Ionization mode	ESI+
Capillary (kV)	0.50
Source Temperature (°C)	150
Desolvation Gas Flow (L/h)	1000
Cone Gas Flow (L/h)	150
Tropane alkaloids detection	

Tropane alkaloids	Precursor ion $[M+H]^+$ (m/z)	Dwell time (s)	Cone (V)	Collision Energy (eV)	Daughter ions (m/z)
Scopolamine	304.2	0.044	42	18	138.2 (Q[a])
			42	18	156.2
Atropine	290.2	0.044	2	20	124.1 (Q[a])
			2	30	93.2
Scopolamine ^{13}C D-3	307.2	0.044	42	18	159.2
Atropine D-3	293.3	0.044	50	22	127.2

[a] Q: quantifier ion.

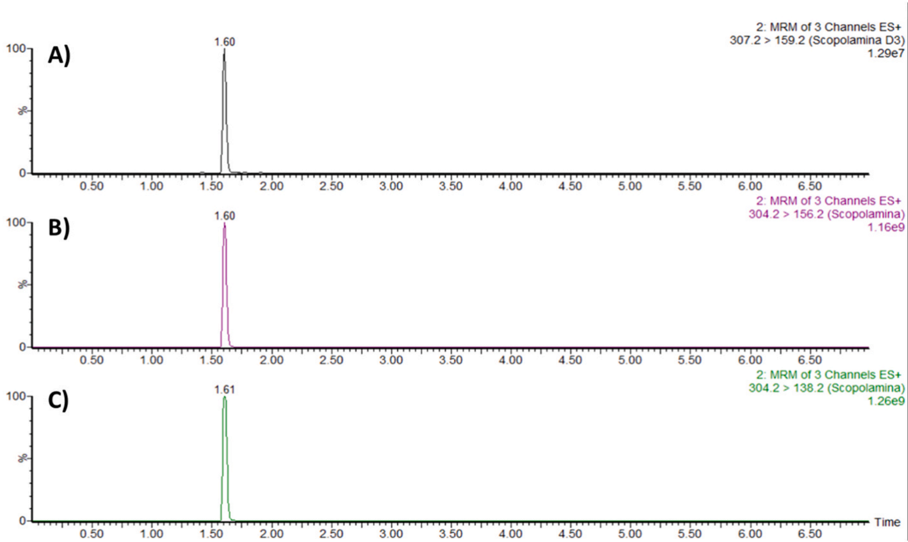


Fig. 3. Representative chromatograms of scopolamine-^{13}C D-3 used as IS (**A**) and scopolamine (m/z 304.2 → m/z 156.2 (**B**), m/z 304.2 → m/z 132.2 for quantifier ion (**C**)) found in the contaminated leftover puff pastry rolls b1.

Extended data on method performance parameters and validation protocols are shown in Table S1 and Table S2 of the Supplementary Material.

2.5. Exposure and health risk assessment

The analysis performed on spinach residues, retrieved from the poisoning incidents described in the previous chapters, confirmed that intoxications were caused by TAs. However, no specific information on the amount of spinach ingested or the TA-producing plant responsible for the intoxication was available. Therefore, in order to estimate the potential dietary intake of TAs resulting from spinach consumption in the event of accidental crop contamination, a deterministic approach was employed to develop hypotheses concerning acute exposure among consumers. Two possible dietary exposure scenarios were formulated: (i) a first scenario, aimed to estimate the intake levels of TAs by individuals consuming the contaminated boiled spinach samples from the Campania region outbreak (i.e., Case 2). In this case, the estimation was based on TA concentrations experimentally quantified in the food residues; (ii) a second scenario, aimed to calculate the potential TA intake levels for intoxicated patients in the event that *Datura stramonium* was indeed present in the consumed spinach. The formulation of this scenario was based on the assumption that a 500 g pouch of spinach (representing a typical market portion) contained 1 % of the total weight of one *Datura stramonium* leaf. In particular, only a small fraction of a whole *Datura stramonium* leaf (i.e., 1 % of the leaf) was considered since, in the reported intoxication cases, botanical experts did not clearly identify distinct leaves, raising suspicion of a potential small contamination portion as a botanical impurity. Additionally, in this second formulated exposure scenario, the contamination levels of *Datura stramonium* were based on those measured in the plant retrieved from the Botanical Garden of the University of Bologna (see *Section 2.1.2.*). This decision was supported by the fact that *Datura stramonium* is associated with higher frequency poisoning incidents compared to other Solanaceae weeds (see *Section 1*. *Introduction*). Specifically, the sum of the concentrations of atropine and scopolamine in the leaf of *Datura stramonium* from the Botanical Garden of the University of Bologna was found to be 289.3 mg/kg (corresponding to an overall amount of 1.17 mg in the whole leaf, which weighted 4.06 g). This concentration was more aligned with the levels reported in the literature (Jakabová et al., 2012; Miraldi et al., 2001; Mroczek et al., 2006), making it the most appropriate sample for an overall assessment of the dietary exposure to TAs and the possible associated health risks for humans. In addition, the decision of formulating the scenario involving adding 1 % of a *Datura stramonium* leaf to a 500 g pouch of spinach was based on the likelihood that such contamination level could be responsible for the observed cases of intoxication. In other words, the concentration of TAs distributed within the entire 500 g of spinach was considered sufficient to potentially induce the observed toxidrome.

For the calculation of the estimated intakes (EIs) of TAs (expressed as μg/kg bw), the Italian population was selected as the target because all the outbreaks mentioned in this article occurred in Italy. Acute dietary consumption data of the food category "spinach-type leaves" (g/kg bw/day) of Italian population were retrieved from the EFSA Comprehensive European Food Consumption Database (EFSA, 2023) at FoodEx2 exposure hierarchy level 3 ("L3"). Only the data from consumption days were used, and it was assumed that the daily consumption rates reported in the database corresponded to the intake of a single meal. Calculations of EIs for both exposure scenarios were done independently for the chosen age groups: toddlers (13–36 months old), adults (18–64 years old), and the elderly (65 years old or over), using both the mean (P50) and 95th percentile (P95) dietary consumption data to evaluate a less pessimistic and the worst-case exposure scenario, respectively.

Finally, to characterize the risk, the obtained EIs were compared with the Acute Reference Dose (ARfD) value of 0.016 μg/kg bw (expressed as the sum of (−)-hyoscyamine and (−)-scopolamine) established by EFSA (EFSA, 2022).

3. Results and discussion

3.1. TA concentrations in leftover food samples

Despite the limited and nonspecific information regarding the possible weed species involved in the contamination of the remaining spinach-based food products seized and sampled by the competent authority, the cooperation among the National Reference Laboratory, the Local Health Authority, and Italian regions with the Italian Ministry of Health enabled the effective management of the emergence of TA poisoning. Thanks to this collaboration, analytical results were presented within a short time, and all the analyzed food residues from the meals (or the spinach from the package used to prepare the dish) were found to contain concentrations of atropine and scopolamine, as listed in Table 2.

As can be observed, the concentrations of TAs were found to be high

Table 2
Average concentrations (μg/kg) of atropine and scopolamine in the analyzed leftover food samples from the TA intoxication cases. Results are displayed with one standard deviation (in brackets).

Case N.	Food sample	Atropine (μg/kg)	Scopolamine (μg/kg)
2	Boiled spinach	982 (33)	1550 (54)
3	a) Spinach residues from the pouch	255[a]	3578[a]
	b) Puff pastry rolls residues b1) Puff pastry roll b2) Puff pastry roll	4290 (470) 4642 (39)	7701 (24) 8158 (753)
4	Spinach residues from the pouch	< LOQ[a]	0.4[a]

[a] Standard deviation not available due to insufficient material. Sample analysis was conducted in a single replicate.

in all the analyzed samples, except for spinach residues from the pouch seized in Case 4, where atropine was below the LOQ, and only small residues of scopolamine were detected. The concentrations of TAs varied from 255 μg/kg (in spinach leaf residues sampled in Case 3) to 4642 μg/kg (in spinach-based puff pastry roll b2 sampled in Case 3). Concentrations of scopolamine were even higher, ranging from 1550 μg/kg (in boiled spinach sampled in Case 2) to 8158 μg/kg (in spinach-based puff pastry roll b2 sampled in Case 3). The most contaminated sample was the puff pastry roll b2 from Case 3, with a sum of atropine and scopolamine of 12,800 μg/kg. Both puff pastry rolls b1 and b2 showed high levels of TAs compared to the other cases, with mean values of 4466 μg/kg and 7930 μg/kg for atropine and scopolamine, respectively (Table 2). Although no maximum limits for spinach and other vegetable foods have been established so far, when comparing our results to the currently available maximum limits set for cereal-based infant foods, other cereal-based and herbal infusions, it became evident that the TA concentrations found in spinach samples from Case 2 and 3 would have been potentially 2–4 orders of magnitude higher (Table 2). Although it is challenging to understand why the levels of TAs in spinach residues from Case 4 were lower than in other cases, the consistently high concentrations of TAs measured in samples make it reasonable to assume that hospitalization might have been necessary for the intoxicated patients. Consequently, these findings support the establishment of a causal relationship between the consumption of contaminated food and the reported symptoms.

In a previous investigation conducted on frozen spinach and spinach-based infant food products, it was found that 24 % of the tested samples contained atropine and scopolamine at levels ranging from 0.02 to 8.19 μg/kg (Castilla-Fernandez et al., 2021). The concentrations in these samples were much lower than those found in the present study and were attributed to accidental mixing with *Datura innoxia* leaves (Castilla-Fernandez et al., 2021). Another recent study by González-Gómez et al. detected the presence of atropine (but not scopolamine) in 3 out of 18 leafy vegetable samples, and the concentrations were also lower than those observed in the current study, ranging from 0.16 to 0.20 μg/kg (González-Gómez, Morante-Zarcero, Pereira, et al., 2022).

Interestingly, the raw spinach residue sample from Case 3 had lower concentrations of TAs than the cooked puff pastry rolls made with the same spinach (Table 2). Different potential explanations may underlie the observed variation in TA concentrations. Firstly, it is conceivable that only a minimal fraction of the contaminating plant material accidently remained in the raw spinach residue, while the majority was transferred to the puff pastry roll. Secondly, the presence of different botanical parts with naturally varying TA contamination levels from the same contaminating plant material could have occurred in the spinach samples. It is possible that lower-contaminated botanical parts remained in the residual raw spinach, while higher-contaminated ones were present in the puff pastry rolls. Thirdly, variations in TA concentrations between the raw spinach and the pastry roll could be attributed to the cooking process. However, it is crucial to note that this last hypothesis contradicts findings reported in the literature by other authors investigating the persistence of TAs in food processing. Indeed, raw matrices like buckwheat and millet flour were found to contain higher TA levels compared to heated-processed matrices like bread made from these flours (Kaltner, 2022). While baking was found to be particularly effective in reducing total TA contamination of raw materials by up to 94–100 % (Marín-Sáez et al., 2019a), Marín-Sáez et al., 2019blso boiling was associated to a reduction of TA concentrations in pasta by 24–66 % (Marín-Sáez et al., 2019b). Nevertheless, in this case, the reduction of TA levels was simply due to the transfer of TAs to the cooking water and not because of degradation. Similarly, up to 50 % of the TA concentrations in tea and herbal extracts were found to be transferred to the water infusion (González-Gómez et al., 2022a, 2023; Marín-Sáez et al., 2019b; Mulder et al., 2016). Additional research concerning the fate of TAs during the preservation and processing of food is required to ensure a comprehensive and reliable assessment of risk (Rollo et al., 2023). Furthermore, comprehensive data on the occurrence of TAs in a wider range of food items beyond cereals and herbal infusions should be collected, in order to better elucidate the specific agricultural and climatic conditions that contribute to the presence of these contaminants along the food chain.

3.2. TA concentrations in Datura species leaves

Table 3 presents the results of the analysis of leaves from four invasive TA-producing plant species, including three *Datura stramonium* and one *Datura metel*. The concentrations of both atropine and scopolamine were found to be extremely high in all samples analyzed, with atropine being the most abundant compound in *Datura stramonium*, consistent with previous research records (EFSA, 2013; Jakabovà et al., 2012; Miraldi et al., 2001).

Overall, the concentrations of atropine and scopolamine in *Datura stramonium* leaves were reported to range between 134 and 4710 mg/kg and between 16 and 1790 mg/kg, respectively (EFSA, 2013), while the present study revealed significantly higher values. Notably, the 20 cm high *Datura stramonium* plant showed 5- and 4-fold higher atropine and scopolamine concentrations than the 70 cm high plant (52,078.6 vs. 10,071.5 mg/kg), despite having a smaller leaf surface (Table 3). This outcome aligns with the previous report by Miraldi et al., who suggested that atropine and scopolamine concentrations in *Datura stramonium* plants tend to increase with increasing plant age and decreasing leaf surface, respectively (Miraldi et al., 2001).

Globally, the *Datura stramonium* samples analyzed in this study exhibited higher levels of atropine compared to scopolamine, which contrasts with previous reports indicating higher levels of scopolamine in other *Datura* species such as *Datura innoxia* and *Datura ferox*, where a scopolamine-to-atropine ratio of 2:1 was associated with cases of acute intoxication (EFSA, 2008; Jakabovà et al., 2012; Papoutsis et al., 2010). However, it should be remarked that the overall concentrations and

Table 3
Average concentrations (mg/kg) of atropine and scopolamine in the leaf of each *Datura* species collected.

Datura species	Leaf weight (g)	Atropine (mg/kg)	Scopolamine (mg/kg)
Datura stramonium	4.06	280.7	8.6
Datura stramonium Spontaneous – 70 cm high	10.3	10,071.5 [a]	1797.6[a]
Datura stramonium Spontaneous – 20 cm high	2.93	52,078.6 [a]	6220.3[a]
Datura metel	2.03	332.1	5414.3

[a] = quantification with no internal standard and not corrected for the recovery.

proportions of TAs in *Datura* species were found to vary widely due to factors such as the country of origin, climatic conditions, plant health, organ type, and maturation stage (EFSA, 2008; Jakabovà et al., 2012).

According to prior research, *Datura metel* is usually characterized by higher levels of atropine than scopolamine, although there have been cases where higher levels of scopolamine (3–11 times more) were detected (Hiraoka et al., 1996; Jakabovà et al., 2012; Mroczek et al., 2006). The current study found that the amount of scopolamine in the *Datura metel* sample analyzed was 16 times greater than the amount of atropine (Table 3).

When comparing the levels of atropine and scopolamine in the leaves of *Datura* plants (Table 3) to those found in food samples (Table 2), inconsistencies were observed. Indeed, while higher levels of scopolamine were found in all the food residues from the meals, it was observed that only the leaf of *Datura metel* apparently exhibited a similar TA profile. Nonetheless, while the leaf of *Datura metel* exhibited a scopolamine/atropine ratio of 16, a comparable ratio of approximately 14 was noted exclusively in the residual spinach collected from the pouch responsible for intoxication Case 2. In contrast, the other food items across intoxication cases showed scopolamine levels only twice as high as atropine levels. These incongruities underscore the difficulty of interpreting the results accurately in the absence of a proper weed identification. Indeed, without visual and/or molecular confirmation of the specific *Datura* plant species responsible for the contamination, drawing definitive conclusions based on the levels of atropine and scopolamine detected in food samples and collected plants becomes challenging. The identification of the toxic plant species becomes even more complex in cases where the residue of the consumed meal by intoxicated patients is unavailable for analysis, as in the instance of Case 1. In such scenarios, information regarding the profiles and concentrations of TAs and metabolites in the plasma and urine of affected patients (not available for this study) could offer valuable complementary insights since potentially reflecting those of the ingested contamination sources.

In conclusion, due to the highly invasive nature of *Datura*, the risk of food crop contamination is significant. To mitigate this risk and effectively manage crop contamination by TAs, it is crucial to implement prerequisite programs such as Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs). These programs should encompass proper training and education of farm workers, as well as the cleaning, sieving, storing, and transporting of raw materials.

3.3. Potential health risks associated with tropane alkaloid exposure from the consumption of contaminated spinach

The acute dietary intake estimations of TAs for various age groups of the Italian population are presented in Fig. 4., Table 4, and Table 5. The intakes resulting from the intoxication case that occurred in the Campania region (Case 2) are depicted in Fig. 4A (i.e., exposure scenario 1), while Fig. 4B displays the outcomes of the assumption that 500 g of a spinach pouch was contaminated with 1 % *Datura stramonium* leaf (i.e., exposure scenario 2).

Based on the mean (P50) acute dietary consumption data of spinach-type vegetables of 2.52, 1.36, and 1.39 g/kg bw for toddlers, adults, and the elderly, respectively, and contamination levels of boiled spinach with atropine plus scopolamine of 2532 μg/kg, the calculated EIs of TAs ranged from a minimum of 3.44 μg/kg bw for adults, to 3.51 μg/kg bw for the elderly, up to a maximum of 6.39 μg/kg bw for toddlers (Fig. 4A–Table 4). Using the 95th percentile (P95) acute dietary consumption data of 3.66, 3.96, and 7.58 g/kg bw for adults, the elderly, and toddlers, respectively, the EIs were 9.26 μg/kg bw for the elderly, 10.02 μg/kg bw for adults, up to a maximum of 19.2 μg/kg bw for toddlers (Fig. 4A–Table 4, Table 5). These intake levels align with the occurrence of more severe symptoms such as ataxia and speech disturbance, which have been reported at doses equal to or greater than 4.62 μg/kg bw, as well as mouth dryness, increased heart rate, pupil dilation, which have been reported at doses equal to or greater than 14 μg/kg bw (EFSA, 2013; FAO/WHO, 2020; Perharič, Juvan, & Stanovnik, 2013). Hence, as observed, the EIs for toddlers were significantly higher compared to those estimated for adults and the elderly, which were very close to each other due to similar spinach consumption rates.

Upon evaluating the EIs of TAs in relation to the recommended health-based guidance values, it was observed that these intakes exceeded the Acute Reference Dose (ARfD) by approximately 400 times for toddlers and 200 times for adults and the elderly. These ratios were found to be threefold higher in the worst-case (P95) scenario, as summarized in Tables 4 and 5, indicating a potential health concern for individuals across all age groups.

The data derived from the probabilistic acute dietary exposure assessment conducted by EFSA provide a comprehensive overview of

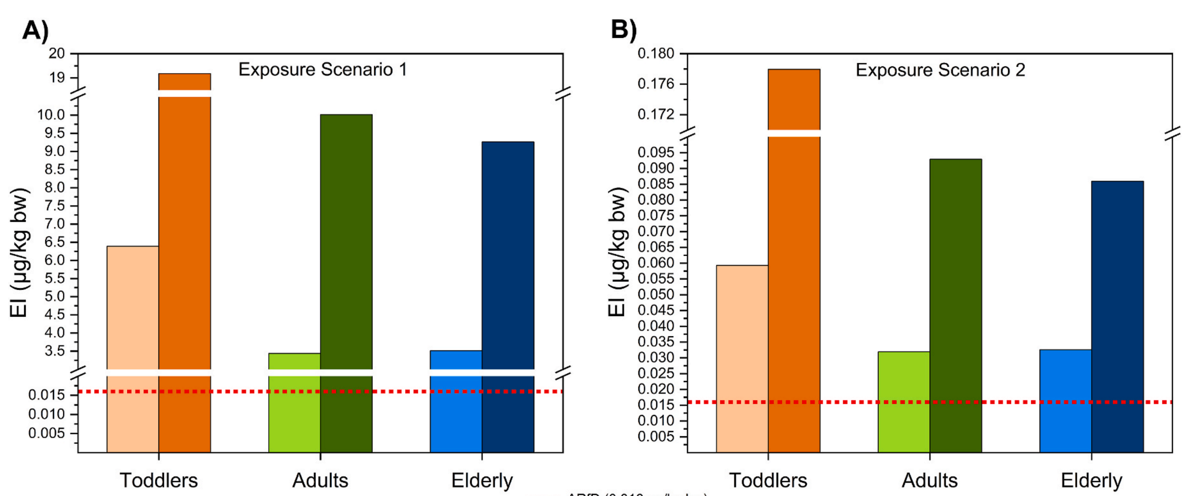


Fig. 4. Estimated intakes (EIs, μg/kg bw) of Italian toddlers, adults, and elderly due to the ingestion of spinach-type vegetables contaminated with (**A**) tropane alkaloid concentrations found in samples responsible for intoxication cases occurred in Campania region (Case 2) and with (**B**) tropane alkaloid concentrations deriving from 1 % *Datura stramonium* leaf contamination. For each population group, the lighter-colored columns represent the EIs of TAs based on the mean (P50) acute dietary consumption data of spinach-type vegetables, while the darker-colored columns represent the EIs of TAs based on the 95th percentile (P95) acute dietary consumption data of spinach-type vegetables. The horizontal dotted line indicates the acute reference dose (ARfD) of 0.016 μg/kg bw established for the sum of (−)-hyoscyamine and (−)-scopolamine.

Table 4
Output of the risk characterization of tropane alkaloids (TAs) presented as ratios between the estimated intakes (EIs) and the acute reference dose (ARfD) of 0.016 μg/kg bw according to the two hypothesized exposure scenarios, different population age groups (toddlers, adults, and elderly), and P50 spinach-type vegetable consumption rates.

		Exposure scenario 1			Exposure scenario 2		
		Boiled spinach from Campania region outbreak			1 % *Datura stramonium* leaf in a 500 g spinach pouch		
Population group	P50 spinach consumption rates (g/kg bw/day)	TA concentrations (μg/kg)	EI (μg/kg bw/day)	EI/ARfD	TA concentrations (μg/kg)	EI (μg/kg bw/day)	EI/ARfD
Toddlers	2.52	2532	6.39	399	23.5	0.059	3.71
Adults	1.36	2532	3.44	215	23.5	0.032	1.99
Elderly	1.39	2532	3.51	220	23.5	0.033	2.04

Table 5
Output of the risk characterization of tropane alkaloids (TAs) presented as ratios between the estimated intakes (EIs) and the acute reference dose (ARfD) of 0.016 μg/kg bw according to the two hypothesized exposure scenarios, different population age groups (toddlers, adults, and elderly), and P95 spinach-type vegetable consumption rates.

		Exposure scenario 1			Exposure scenario 2		
		Boiled spinach from Campania region outbreak			1 % *Datura stramonium* leaf in a 500 g spinach pouch		
Population Group	P95 Spinach consumption rates (g/kg bw/day)	TA concentrations (μg/kg)	EI (μg/kg bw/day)	EI/ARfD	TA concentrations (μg/kg)	EI (μg/kg bw/day)	EI/ARfD
Toddlers	7.58	2532	19.2	1199	23.5	0.178	11.1
Adults	3.96	2532	10.0	626	23.5	0.093	5.81
Elderly	3.66	2532	9.26	579	23.5	0.086	5.37

TAs intake across a broad population base, encompassing 13 different EU countries (EFSA, 2018). Mean acute exposure values ranging from 0.001 to 0.019 μg/kg bw/day and P95 acute exposure values ranging from 0.0005 to 0.053 μg/kg bw/day, based on different age groups within the population, where reported (EFSA, 2018). Within this assessment, bread and cereal-based foods were identified as the main dietary contributors to the total atropine plus scopolamine exposure (EFSA, 2018). In contrast, despite concerns about contamination in herbal teas, infusions, and herbal supplements, the intake of TAs from these specific food items appears to be relatively low. Indeed, TAs intake levels from these products were reported to contribute only 2–8 % of the ARfD (Cirlini et al., 2019).

In 2020, a joint study conducted by FAO/WHO employed a probabilistic approach to assess the acute dietary exposure to TAs from a general diet for populations in countries such as Bangladesh, Bolivia, Laos, the Philippines, Uganda, and Zambia (FAO/WHO 2020). The findings revealed that the mean acute dietary exposure to TAs across the studied countries and population subgroups was below 0.001 μg/kg bw, except for Zambia, where the mean exposure for young children and women was 0.018 μg/kg bw and 0.0046 μg/kg bw, respectively. The P95 exposures ranged from 0.0025 to 0.0035 μg/kg bw, while for the population in Zambia, it was 0.0038 μg/kg bw for young children and 0.01 μg/kg bw for women (FAO/WHO 2020). The results obtained by the FAO differ significantly from those calculated in the current study, with both the mean and P95 intake values being considerably lower. However, it should be noted that the FAO/WHO was unable to utilize occurrence data from the mentioned countries, probably due to the absence of food surveillance and analysis specifically focused on detecting the presence of TAs in food samples (FAO/WHO 2020).

In the formulated exposure scenario 2 (where it was hypothesized the presence of 1 % of a leaf of *Datura stramonium* having 289.3 mg/kg of TAs in a 500 g spinach pouch), the assessment yielded EI values ranging from 0.032 μg/kg bw and 0.033 μg/kg bw for adults and elderly individuals, respectively, up to 0.059 μg/kg bw for toddlers when using the P50 acute consumption data (Fig. 4B–Table 4). Conversely, using the P95 acute dietary consumption data, the calculations for EI yielded values of 0.086 μg/kg bw, 0.093 μg/kg bw, and 0.18 μg/kg bw for adults, elderly individuals, and toddlers, respectively (Fig. 4B–Table 5). Although this second simulated exposure scenario resulted in lower EI values compared to the first scenario, it is evident that toddlers were the most vulnerable population group, with EIs approximately 4 and 11 times higher than the ARfD for P50 and P95 consumers, respectively, as presented in Tables 4 and 5. However, the situation remains concerning for adults and elderly individuals as well, with EIs approximately 2 times higher than the ARfD, even for P50 consumers (Table 4). Healthy toddlers may experience mild to moderately serious health problems at the given exposure levels, but the probability of severe health problems is still low. However, if the ARfD limit is exceeded, particularly by individuals with pre-existing cardiovascular conditions, there is a risk of severe health complications, regardless of age (EFSA, 2013). Moreover, considering the toxic effects of TAs, it is essential to emphasize that the elderly population may be particularly susceptible to the adverse effects of these contaminants due to a higher prevalence of specific conditions such as urinary retention, constipation, paralytic ileus, and glaucoma, which are more common with age (EFSA, 2013).

Overall, there is a limited amount of literature available on the dietary exposure to TAs, and further research is necessary to compare the presented results and accurately assess the intake of TAs through the whole diet. Considering the potential high exposure of the Italian population revealed by the study, it is crucial to assess the exposure to these toxic compounds within plant-based diets as well. Specifically, vegetarians, due to their distinct consumption patterns, may face a significantly higher risk of TA exposure across all age groups.

4. Conclusions

Despite incidents of poisoning and hospitalization resulting from the consumption of TAs-contaminated spinach-based foods in different regions of Italy in October 2022, inspection procedures were successful in containing the potential outbreak. However, analyses conducted on the remaining spinach-based foods consumed by the intoxicated patients mostly revealed extremely high concentrations of atropine and scopolamine.

Although there are no maximum allowable limits for these alkaloids in leafy vegetables according to European legislation, the concentrations of TAs found in leftover spinach-based food exceeded the maximum limits for certain grains and dried herbs, implying that this could potentially be a cause of health problems and underscoring the urgency to strongly reinforce the monitoring activities of these food matrices as well as the need for regulatory measures.

Based on the evaluation of the dietary exposure of the Italian population to TAs through the consumption of potentially TA-contaminated spinach-like vegetables, it was estimated that toddlers, adults, and the elderly can face a similar risk of being exposed to atropine and scopolamine levels exceeding toxicological health-based guidance values. The risk of acute toxic effects was estimated to be particularly high for toddlers, as even a contamination of a 500 g spinach pouch with just 1 % of a leaf from a *Datura stramonium* sample containing lower levels of TAs could result in exceeding the ARfD by roughly four times for average consumers and up to twelve times for those with high consumption rates. From this viewpoint, the occurrence of unintentional mixing of TA-producing plants such as those belonging to the *Datura* genus with vegetables like spinach because of their similar botanical characteristics emphasizes the importance of strengthening the implementation of GAPs and GMPs, as well as food safety management systems based on HACCP and voluntary standards. These measures play a pivotal role in managing and minimizing the risk by reducing the likelihood of cross-contamination during primary production and food processing.

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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.

Data availability

Data will be made available on request.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.foodcont.2024.110334.

References

Abia, W. A., Montgomery, H., Nugent, A. P., & Elliott, C. T. (2020). Tropane alkaloid contamination of agricultural commodities and food products in relation to consumer health: Learnings from the 2019 Uganda food aid outbreak. *Comprehensive Reviews in Food Science and Food Safety, 20*, 501–525. https://doi.org/10.1111/1541-4337.12664

Adamse, P., van Egmond, H. P., Noordam, M. Y., Mulder, P. P. J., & de Nijs, M. (2014). Tropane alkaloids in food: Poisoning incidents. *Quality Assurance and Safety of Crops & Foods, 6*(1), 15–24. https://doi.org/10.3920/QAS2013.0314

Castilla-Fernandeź, D., Moreno-Gonzaleź, D., García-Reyes, J. F., Ballesteros, E., & Molina- Díaz, A. (2021). Determination of atropine and scopolamine in spinach-based products contaminated with genus *Datura* by UHPLC–MS/MS. *Food Chemistry, 347*(15). https://doi.org/10.1016/j.foodchem.2021.129020

Cirlini, M., Cappucci, V., Galaverna, G., Dall'Asta, C., & Bruni, R. (2019). A sensitive UHPLC-ESI-MS/MS method for the determination of tropane alkaloids in herbal teas and extracts. *Food Control, 105*, 285–291. https://doi.org/10.1016/j.foodcont.2019.05.030

Cirlini, M., Demuth, T. M., Biancardi, A., Rychlik, M., Dall'Asta, C., & Bruni, R. (2018). Are tropane alkaloids present in organic foods? Detection of scopolamine and atropine in organic buckwheat (fagopyron esculentum L.) products by UHPLC–MS/MS. *Food Chemistry, 239*, 141–147. https://doi.org/10.1016/j.foodchem.2017.06.028

Cornara, L., Smeriglio, A., Frigerio, J., Labra, M., Di Gristina, E., Denaro, M., Mora, E., & Trombetta, D. (2018). The problem of misidentification between edible and poisonous wild plants: Reports from the Mediterranean area. *Food and Chemical Toxicology, 119*, 112–121. https://doi.org/10.1016/j.fct.2018.04.066

Croteau, R., Kutchan, T., & Lewis, N. G. (2000). Natural products (secondary metabolites). *Biochemistry and Molecular Biology of Plants, 24*, 1250–1319.

de Nijs, M., Crews, C., Dorgelo, F., MacDonald, S., & Mulder, P. P. J. (2023). Emerging issues on tropane alkaloid contamination of food in Europe. *Toxins, 15*, 98. https://doi.org/10.3390/toxins15020098

Debnath, B., Singh, W. S., Das, M., Goswami, S., Singh, M. K., Maiti, D., & Manna, K. (2018). Role of plant alkaloids on human health: A review of biological activities. *Materials Today Chemistry, 9*, 56–72. https://doi.org/10.1016/j.mtchem.2018.05.001

Dey, P., Kundu, A., Kumar, A., Gupta, M., Lee, M. B., Bhakta, T., Dash, S., & Kim, H. S. (2020). Analysis of alkaloids (indole alkaloids, isoquinoline alkaloids, tropane alkaloids). *Recent Advances in Natural Products Analysis, 15*, 505–567. https://doi.org/10.1016/B978-0-12-816455-6.00015-9

EFSA. (2008). Scientific opinion of the panel on contaminants in the food chain on a request from the European commission on tropane alkaloids (from *datura* sp.) as undesirable substances in animal feed. *EFSA Journal, 691*, 1–55. https://doi.org/10.2903/j.efsa.2008.691

EFSA. (2013). Scientific Opinion on Tropane alkaloids in food and feed. *EFSA Journal, 11*(10), 1–113. https://doi.org/10.2903/j.efsa.2013.3386, 3386.

EFSA. (2018). Scientific report on human acute exposure assessment to tropane alkaloids. *EFSA Journal, 16*(2), 1–29. https://doi.org/10.2903/j.efsa.2018.5160, 5160.

EFSA. (2022). Scientific report on the assessment of the conclusions of the joint FAO/WHO expert meeting on tropane alkaloids. *EFSA Journal, 20*(4), 1–17. https://doi.org/10.2903/j.efsa.2022.7229, 7229.

EFSA. (2023). The EFSA comprehensive food consumption database. Available online: https://www.efsa.europa.eu/en/data-report/food-consumption-data. (Accessed 26 April 2023).

European Commission. (2015). *Commission Recommendation (EU) 2015/976 of 19 June 2015 on the monitoring of the presence of tropane alkaloids in food* (Vol. 157). Official journal of the European Union, L. https://data.europa.eu/eli/reco/2015/976/oj.

European Commission. (2023). *Commission Regulation (EU) 2023/915 of 25 April 2023 on maximum levels for certain contaminants in food* (Vol. 119). Official journal of the European Union, L. https://data.europa.eu/eli/reg/2023/915/oj.

European Union Reference Laboratory for Mycotoxins and Plant Toxins EURL-MP. (2022). In *EURL-MP-guidance doc_003 (version 1.2) Guidance document on performance criteria (draft 29th September 2022)*. Wageningen, The Netherlands: Wageningen Food and Safety Research. Available online: https://www.wur.nl/en/research-results/research-institutes/food-safety-research/referencelaboratory/european-union-reference-laboratory/eurl-mycotoxins-plant-toxins/library-eurl-mp.htm. (Accessed 4 September 2023).

FAO & WHO (Food and Agriculture Organization & World Health Organization). (2020). *Joint FAO/WHO expert meeting on tropane alkaloids – 30 march – 3 April 2020* (Vol. 11). Rome: Food Safety and Quality Series. https://doi.org/10.4060/cb1857en

Fatur, K., & Kreft, S. (2020). Common anticholinergic solanaceaous plants of temperate Europe - a review of intoxications from the literature (1966–2018). *Toxicon, 177*, 52–88. https://doi.org/10.1016/j.toxicon.2020.02.005

Gonçalves, C. O., Cubero-Leon, E., Mischke, C., Bratinova, S., & Stroka, J. (2017). *Report on the 2016 proficiency test of the European union reference laboratory for mycotoxins for the network of national reference laboratories: Determination of tropane alkaloids in tea and herbal infusions. JRC Technical Report*, EUR 28662 EN https://data.europa.eu/doi/10.2760/52884.

González-Gómez, L., Ganán, J., Morante-Zarcero, S., Pérez-Quintanilla, D., & Sierra, I. (2023). Atropine and scopolamine occurrence in spices and fennel infusions. *Food Control, 146*. https://doi.org/10.1016/j.foodcont.2022.109555

González-Gómez, L., Morante-Zarcero, S., Pereira, A. M., Camara, J. S., & Sierra, I. (2022). Improved analytical approach for determination of tropane alkaloids in leafy vegetables based on μ-QuEChERS combined with HPLC-MS/MS. *Toxins, 14*, 650. https://doi.org/10.3390/toxins14100650

González-Gómez, L., Morante-Zarcero, S., Pérez-Quintanilla, D., & Sierra, I. (2022). Occurrence and chemistry of tropane alkaloids in foods, with a focus on sample analysis methods: A review on recent trends and technological advances. *Foods, 11*(3), 407. https://doi.org/10.3390/foods11030407

Haughey, S. A., Chevallier, O. P., McVey, C., & Elliott, C. T. (2021). Laboratory investigations into the cause of multiple serious and fatal food poisoning incidents in Uganda during 2019. *Food Control, 121*, Article 107648. https://doi.org/10.1016/j.foodcont.2020.107648

Hiraoka, N., Tashimo, K., Kinoshita, C., & Hiro'oka, M. (1996). Genotypes and alkaloid contents of *datura* metel varieties. *Biological and Pharmaceutical Bulletin, 19*(8), 1086–1089. https://doi.org/10.1248/bpb.19.1086

Huang, J. P., Wang, Y. J., Tian, T., Wang, L., Yan, Y., & Huang, S. X. (2021). Tropane alkaloid biosynthesis: A centennial review. *Natural Product Reports, 38*, 1634–1658. https://doi.org/10.1039/D0NP00076K

Jakabová, S., Vinczec, L., Farkasd, A., Kilára, F., Boros, B., & Felingera, A. (2012). Determination of tropane alkaloids atropine and scopolamine by liquid chromatography–mass spectrometry in plant organs of *Datura* species. *Journal of Chromatography A, 1232*, 295–301. https://doi.org/10.1016/j.chroma.2012.02.036

Jank, B., & Rath, J. (2021). Emerging tropane alkaloid contaminations under climate change. *Trends in Plant Science, 26*(11), 1101–1103. https://doi.org/10.1016/j.tplants.2021.08.001

Kaltner, F. (2022). Fate of food-relevant toxic plant alkaloids during food processing or storing and analytical strategies to unveil potential transformation products. *Journal of Agricultural and Food Chemistry, 70*(20), 5975–5981. https://doi.org/10.1021/acs.jafc.2c01489

Khoshnam-Rad, N., Heydari, M., Mohammadi, K., Mashayekhi, M., Sahraei, Z., & Gholami, K. (2022). *Datura* poisoning in a family: Case series and literature review. *Clinical Case Reports, 10*(7), Article 06091E. https://doi.org/10.1002/ccr3.6091

Kohnen-Johannsen, K. L., & Kayser, O. (2019). Tropane alkaloids: Chemistry, pharmacology, biosynthesis and production. *Molecules, 24*(4), 796. https://doi.org/10.3390/molecules24040796

Marín-Sáez, J., Romero-González, R., & Frenich, A. G. (2019a). Degradation of tropane alkaloids in baked bread samples contaminated with Solanaceae seeds. *Food Research International, 122*, 585–592. https://doi.org/10.1016/j.foodres.2019.01.027

Marín-Sáez, J., Romero-González, R., & Frenich, A. G. (2019b). Effect of tea making and boiling processes on the degradation of tropane T alkaloids in tea and pasta samples contaminated with Solanaceae seeds and coca leaf. *Food Chemistry, 287*, 265–272. https://doi.org/10.1016/j.foodchem.2019.02.091

Miraldi, E., Mastri, A., Ferri, S., & Barni Comparini, I. (2001). Distribution of hyoscyamine and scopolamine in *Datura stramonium*. *Fitoterapia, 72*(6), 644–648. https://doi.org/10.1016/S0367-326X(01)00291-X

Mroczek, T., Głowniak, K., & Kowalsk, J. (2006). Solid-liquid extraction and cation-exchange solid-phase extraction using a mixed-mode polymeric sorbent of *Datura* and related alkaloids. *Journal of Chromatography A, 1107*, 9–18. https://doi.org/10.1016/j.chroma.2005.12.034

Mulder, P. P. J., De Nijs, M., Castellari, M., Hortos, M., MacDonald, S., Crews, C., Hajslova, J., & Stranska, M. (2016). Occurrence of tropane alkaloids in food. *EFSA Supporting publication, 13*(12), 1–200. https://doi.org/10.2903/sp.efsa.2016.EN-1140, 1140E.

Papoutsis, I., Nikolaou, P., Athanaselis, S., Stefanidou, M., Pistos, C., Spiliopoulou, C., & Maravelias, C. (2010). Mass intoxication with *Datura innoxia*—case series and confirmation by analytical toxicology. *Clinical Toxicology, 48*, 143–145. https://doi.org/10.3109/15563650903524134

Perharič, L., Juvan, K. A., & Stanovnik, L. (2013). Acute effects of a low-dose atropine/scopolamine mixture as a food contaminant in human volunteers. *Journal of Applied Toxicology, 33*, 980–990. https://doi.org/10.1002/jat.2797

Perharič, L., Kozelj, G., Druzina, B., & Stanovnik, L. (2013). Risk assessment of buckwheat flour contaminated by thorn-apple (*Datura stramonium* L.) alkaloids: A case study from Slovenia. *Food Additives & Contaminants: Part A, 30*, 321–330. https://doi.org/10.1080/19440049.2012.743189

Plackova, S., Caganova, B., Otrubova, O., Puchon, E., & Batora, I. (2022). Anticholinergic poisoning associated with frozen spinach. *Clinical Toxicology, 60*(1), 30. https://doi.org/10.1080/15563650.2022.2054576

RASFF. (2023). RASFF portal — food and feed safety alerts. Available online: https://webgate.ec.europa.eu/rasff-window/portal/?. (Accessed 22 March 2023).

Rollo, E., Catellani, D., Dall'Asta, C., & Suman, M. (2023). QuEChERS method combined to liquid chromatography high-resolution mass spectrometry for the accurate and sensitive simultaneous determination of pyrrolizidine and tropane alkaloids in cereals and spices. *Journal of Mass Spectrometry*. , Article e4969. https://doi.org/10.1002/jms.4969

Romera-Torres, A., Romero-González, R., Vidal, J. L. M., & Frenich, A. G. (2018). Analytical methods, occurrence and trends of tropane alkaloids and calystegines: An update. *Journal of Chromatography A, 1564*, 1–15. https://doi.org/10.1016/j.chroma.2018.06.004

WFP (World Food Programme). (2019). WFP to dispose of compromised food stocks | World Food Programme. Available online: https://www.wfp.org/news/wfp-dispose-compromised-food-stocks. (Accessed 5 October 2023).