CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda item 10 **CX/CF 25/18/10**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

Eighteenth Session
23-27 June 2025
Bangkok, Thailand

PROVISIONAL AGENDA

DISCUSSION PAPER ON PYRROLIZIDINE ALKALOIDS

REVIEW OF THE *CODE OF PRACTICE TO PREVENT AND REDUCE PYRROLIZIDINE ALKALOID CONTAMINATION IN FOOD AND FEED* (CXC 74-2014)

DEVELOPMENT OF GUIDANCE ON SAMPLING AND ANALYSIS PERFORMANCE CHARACTERISTICS FOR THE COLLECTION OF DATA FOR SUBMISSION TO THE GEMS/FOOD DATABASE

(Prepared by the Electronic Working Group chaired by Türkiye
and co-chaired by the United Kingdom and the Netherlands)

Codex members and observers wishing to submit comments on
the recommendations for the revision of the
Code of practice for the reduction to prevent and reduce pyrrolizidine alkaloid contamination in food and feed (CXC 74-2014) and the
Guidance on sampling and analysis performance characteristics for the collection of data for submission to the GEMS/Food database
should do so as instructed in CL 2025/27-CF, available on the Codex webpage[1]

BACKGROUND

1. The 15th Session[2] of the Codex Committee on Contaminants in Foods (CCCF15, 2022) agreed to re-convene the Electronic Working Group (EWG), chaired by the European Union (EU), working in English, to prepare a discussion paper on pyrrolizidine alkaloids (PAs) to look into the feasibility of possible follow-up actions for consideration by CCCF16 following the JECFA evaluation of these compounds.

2. CCCF16 (2023)[3] agreed to issue a circular letter (CL 2023/40-CF) requesting comments on the recommendations in the discussion paper CX/CF 23/16/11[1], and that the EWG, chaired by the EU, would prepare a revised paper based on the comments received in response to the CL for consideration by CCCF17.

3. CCCF17 (2024)[4] considered the recommendations in the discussion paper CX/CF 24/17/10[1] and agreed to:

   (i) further develop the discussion paper on the revision of the *Code of practice to prevent and reduce pyrrolizidine alkaloid contamination in food and feed* (CXC 74-2014), which would also address practices for honey; provide a project document to substantiate the proposal for new work as well as proposal for the revised code of practice (CoP);

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1 Codex webpage/Circular Letters: http://www.fao.org/fao-who-codexalimentarius/resources/circular-letters/en/
Codex webpage/CCCF/Circular Letters: http://www.fao.org/fao-who-codexalimentarius/committees/committee/related-circular-letters/en/?committee=CCCF
CCCF working documents, including the reports, are available on the Codex webpage: https://www.fao.org/fao-who-codexalimentarius/committees/committee/related-meetings/pt/?committee=CCCF

2 REP22/CF15, paras. 220, 224

3 REP23/CF16, para 84

4 REP24/CF17, para 98

(ii) develop guidance on sampling and analysis performance characteristics for the collection of data to be submitted to the GEMS/Food database, which would be incorporated in a JECFA call for data; and

(iii) to establish an EWG chaired by Türkiye, co-chaired by the United Kingdom and the Netherlands, to develop the discussion paper.

WORK PROCESS

4. The discussion paper CX/CF 24/17/10[1] was uploaded onto the Codex Online Forum on 22 December 2024, and the EWG was invited to share information on the following issues:

(i) Publications or other unpublished work (e.g. regional or national CoPs, scientific studies, reports, policy literature, government documents, etc.) on preventing and reducing PAs in food and feed not included in the discussion paper:

(a) New weed control measures for preventing and reducing pyrrolizidine alkaloids (PAs) contamination in food and feed (published post-2014) and the efficacy of existing weed control measures.

(b) Measures for preventing and reducing the risk of PAs in beekeeping and beekeeping products (honey, pollen), including processing.

(c) Measures for preventing and reducing the risk of PAs in food commodities, including herbal infusions, tea, food supplements, culinary herbs, and feed.

(ii) Regulations, guidelines, procedures for sampling and analysis (screening, microscopic (for pollen), semi-quantitative, and confirmation methods), performance characteristics (not included in the discussion paper).

(iii) PAs and their toxicological profiles to be considered in determining their risk in food and feed.

5. The Netherlands, the United Kingdom, and the United States of America (USA) shared information for the CoP, including sampling and analysis methods, risk assessment, and relevant scientific publications.

6. The draft documents prepared under the following headings were shared at the Online Forum on January 19, 2025, to receive suggestions from EWG members and observers:

(I) Discussion Paper: “PYRROLIZIDINE ALKALOIDS”

(II) Project Document: “PROPOSAL FOR A NEW WORK ON

- AN UPDATE OF THE *CODE OF PRACTICE FOR WEED CONTROL TO PREVENT AND REDUCE PYRROLIZIDINE ALKALOID CONTAMINATION IN FOOD AND FEED* (CXS 74-2014)
- DEVELOP GUİDANCE ON SAMPLİNG AND ANALYSİS PERFORMANCE CHARACTERİSTİCS FOR THE COLLECTİON OF DATA TO BE SUBMİTTED TO THE GEMS/FOOD DATABASE

(III) Code of Practice: PROPOSED REVISED *CODE OF PRACTICE TO PREVENT AND REDUCE PYRROLIZIDINE ALKALOID CONTAMINATION IN FOOD AND FEED*

(IV) Sampling and Analysis: PROPOSED GUIDELINES ON SAMPLING AND ANALYSIS PERFORMANCE CHARACTERISTICS FOR THE DETERMINATION OF PYRROLIZIDINE ALKALOIDS LEVELS IN FOOD

7. The draft documents were circulated once, and six members (Japan, New Zealand, Poland, the Netherlands, the United Kingdom, and the USA) and two observers (Food Drink Europe and the International Alliance of Dietary/Food Supplement (IADSA)) submitted comments and suggestions for the documents.

SUMMARY OF KEY POINTS OF DISCUSSION

Recommendations from EWG members and observers, and revision/development of the documents

A - Project document

8. It was suggested that the project document be prepared only for the CoP and that the sections on sampling and analysis methods be removed. They noted that the sampling and analysis methods will be an internal document and that proposing a new study on this topic is premature. In line with the suggestions, the project document was revised to cover only the CoP.

B - Code of Practice

9. Comments were made that general information was unnecessary in the document and that only specific information on preventing PAs contamination was needed. Per the suggestions, the document has been simplified.

10. As with bee products, comments were made on the need for separate chapters in the CoP for food supplements and teas. Creating a separate section for bee products is due to the inappropriateness of the weed control approach in areas where these products are obtained. Other parts of the CoP are already being developed for foods other than bee products. However, separate sections can be created for these products when information on specific recommendations is provided in future studies.

11. As medicines are outside the scope of the Codex Alimentarius Commission (CAC) and the risk of PAs in plant oils is low, it was recommended that these foods be removed from the document. Medicines and plant oils were removed per the recommendations. Similar recommendations have been made for foods of animal origin. However, these foods have been retained in the document as the Joint FAO/WHO Expert Committee on Food Additives (JECFA) has a recommendation for data requirements for these foods.

12. It was noted that some plants that naturally contain PA may be marketed for food and feed, and that the CoP should focus solely on weed control. A sentence was added to the scope section of the CoP stating that placing plants naturally containing PAs as food or feed on the market is not covered.

13. It was suggested that the CoP be clarified as to whether it will be a revised document or a new document, and if it will be a revised document, to ensure that changes can be tracked and referenced. The document has been revised to ensure that changes can be tracked, and references have been added.

C - Sampling and Analysis, including data collection

14. For PAs where reference is made to sampling methods for mycotoxins, it was stated that detailed plans for sampling should be removed from the document on the grounds that this approach is not appropriate. It was suggested that the *General guidelines on sampling* (CXG 50-2004) should be referenced to include basic information on sample handling in outline. The relevant section of the document was revised in line with the suggestions.

15. It was proposed to remove the discussion of general requirements and validation of the method of analysis from the document and specify only specific criteria for the limit of quantification (LOQ), precision, and recovery for data collection. The relevant section of the document was revised in accordance with the suggestions.

16. It was proposed that the individual PAs table in the document should not be mandatory for data calls. The approach of making the individual PAs table mandatory was retained. However, a statement was added to the document that if there is a strong indication that some PAs are not detected in foods from the region of interest, these PAs do not need to be analysed.

17. It was suggested that the approach be clarified, whether the call for data should be for individual PAs or the sum of PAs. A call for data for individual PAs was considered appropriate.

18. It was proposed that the foods for which a data call will be issued should be decided at CCCF18. The table of foods for the data call is kept in the document, but the decision will be made at CCCF18.

19. As medicines are outside the scope of the CAC and the risk of PAs in plant oils is low, it was recommended that these commodities be removed from the document. These commodities were removed in accordance with the recommendations. Similar recommendations have been made for foods of animal origin, but these foods have been retained in the document, as JECFA has a recommendation for data requirements.

Documents prepared according to EWG recommendations

20. The following Appendices are attached to this document:

- Appendix I: The Discussion Paper on Pyrrolizidine Alkaloids.
- Appendix II: The Project Document proposing new work.
- Appendix III: The proposed revised Code of practice to prevent and reduce pyrrolizidine alkaloid contamination in food and feed.
- Appendix IV: The reference documents used to revise the CoP.
- Appendix V: The proposed guidance on sampling and analysis performance characteristics for data collection to be submitted to the GEMS/Food database for pyrrolizidine alkaloids in foods.
- Appendix VI: The list of registered members and observers participating in the EWG.

CONCLUSIONS

25. There is sufficient evidence to support the revision of the CoP and the development of an internal document to complement the CoP and to support the generation of data for possible future evaluation by CCCF.

26. Conversely, it would be appropriate to discuss the new/revised chapters further and explore whether creating specific chapters for foods such as supplements and teas is possible.

RECOMMENDATIONS

27. CCCF is invited to consider the following:

Code of practice

28. If new work should be proposed on a revision of the *Code of practice to prevent and reduce pyrrolizidine alkaloids contamination in food and feed* (CXC 74-2014), and, in the affirmative, to consider the points below:

    (i) Review the project document in Appendix II and make any necessary adjustments to ensure a robust rationale for the revision of the CoP, to forward it to CAC48 (2025) for approval as new work.

    (ii) Review the outline for the proposed revised CoP as presented in Appendix II to guide the EWG in further developing the CoP following approval of new work by CAC48, in particular as to whether the proposed revisions are reasonable and if further improvements could be made, e.g. addition of new sections, further development of revised sections, and whether data/information to support such revisions is available for consideration by the EWG in further developing the CoP.

        Note: Risk management measures for inclusion in Codex codes of practice should be readily available, applicable worldwide, and proven to be effective across different production scales, including small- and medium-sized businesses.

    (iii) Re-establish the EWG to further develop the CoP based on the guidance provided by CCCF, for consideration by CCCF19 (2026).

Guidance on sampling and analysis performance characteristics for the collection of data for submission to the GEMS/Food Database

29. The guidance in Appendix V and:

    (i) Review the provisions and make any recommendations for improvements as/if necessary.

    (ii) Confirm that the document is to guide data collection for their submission to the GEMS/Food database to support possible future work by CCCF on PAs.

APPENDIX I

DISCUSSION PAPER ON PYRROLIZIDINE ALKALOIDS

(For information)

BACKGROUND

Pyrrolizidine alkaloids (PAs)

1. Pyrrolizidine alkaloids (PAs) are toxins produced by an estimated 6000 plant species. More than 600 different PAs, mainly 1,2-unsaturated PAs, including their associated nitrogen oxides (N-oxides), are known, and new PAs continue to be identified in both new and previously studied plant species. The main plant sources are the families *Boraginaceae* (all genera), *Asteraceae* (tribes *Senecioneae* and *Eupatorieae*) and *Fabaceae* (genus Crotalaria). In this discussion paper, the term "PAs" used by itself refers to saturated and 1,2-unsaturated PAs and their associated N-oxides, and the term "1,2-unsaturated PAs" refers to all 1,2-unsaturated PAs and their associated N-oxides.

2. Different plant species in these families produce characteristic mixtures of 1,2-unsaturated PAs, their saturated analogues, and varying amounts of their corresponding N-oxides, resulting in species-specific PAs profiles. The PAs present in these plants are esters of pyrrolizidine diols. The pyrrolizidine moieties are called necines, and the esterifying acids are necic acids. These PAs can be classified as open-chain monoesters, open-chain diesters, and macrocyclic diesters. Hence, the profiles of PAs in food can reveal from which plant family the weed causing the contamination comes from. Additional methods for identification of the PA-containing weed plant include visual inspection in the field or at harvest, microscopic inspection of seeds (e.g. contamination in seed spices), as well as deoxyribonucleic acid (DNA)-metabarcoding[1].

3. Saturated and unsaturated PAs have a typical heterocyclic structure in common, but differ in their potential toxicity, depending on the presence or absence of a double bond between C1 and C2. Fortunately, most plants contain saturated PAs without this double bond and are therefore less toxic for consumption by humans or animals. In a minority of plants, however, PAs with this double bond between C1 and C2 exhibit strong hepatotoxic, genotoxic, cytotoxic, neurotoxic, and tumorigenic potentials. An overview of the structural formulae of the most relevant PAs is provided in Figure 1 of the WHO Food Additives Series: 71–S2[2].

4. PAs may be present in foods through three possible routes:

   a. as an inherent component of the PA-containing edible plants (the direct and deliberate use of PA-containing plant species as herbal teas or herbal supplements);

   b. through contamination of a food with PA-containing plant material (e.g. cereals and cereal products, teas[3], herbal and pollen-based food supplements, salad plants, culinary herbs and spices); and

   c. transfer of PAs from plant material consumed by animals into foods of animal origin (e.g. milk and dairy products, eggs, meat, honey, pollen).

   The available occurrence data indicate that teas, herbal and pollen-based food supplements, salad plants, culinary herbs, spices, honey, and pollen are the greatest concern foods regarding the frequency and level of PAs detection.

JECFA assessment

5. PAs were assessed by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), at its 18th meeting, which took place in Rome, Italy, from 16 to 25 June 2015. Details of the assessment can be found in WHO Food Additives Series: 71-S2, Safety evaluation of certain food additives and contaminants, Supplement 2: Pyrrolizidine alkaloids[2].

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1 Targeted LC-MS/MS combined with multilocus DNA metabarcoding as a combinatory approach to determine the amount and the source of pyrrolizidine alkaloids contamination in popular cooking herbs, seeds, spices and leafy vegetables, Food Additives & Contaminants: Part A, https://doi.org/10.1080/19440049.2021.1889043
Sorting out the plants responsible for a contamination with pyrrolizidine alkaloids in spice seeds by means of LC-MS/MS and DNA barcoding: Proof of principle with cumin and anise spice seeds. Food Chemistry: Molecular Sciences 4 (2022) 100070 https://doi.org/10.1016/j.fochms.2021.100070

2 WHO Food Additives Series: 71-S2, Safety evaluation of certain food additives and contaminants, Supplement 2: Pyrrolizidine alkaloids Available at https://apps.who.int/iris/rest/bitstreams/1318952/retrieve

3 Teas means tea and other herbal teas

6. JECFA noted that most studies of toxicity and the occurrence of PAs in food were focused on the 1,2-unsaturated PAs. The Committee concluded that while the saturated PAs could not elicit toxicity via the same mechanism as 1,2-unsaturated PAs, their toxicity in humans could not be excluded, but there were insufficient studies for evaluation. JECFA therefore decided to focus the evaluation on the 1,2-unsaturated PAs. Studies performed using extracts or material from PA-containing plants, which did not specify PA content did not allow the toxicity to be related to a dose of a specific PA and were of limited relevance to the evaluation.

7. Exposure to 1,2-unsaturated PAs has been associated with a wide range of effects, with rats being the most sensitive species studied. In vitro studies on metabolic activation indicate that humans are also likely to be sensitive. Laboratory studies have identified the liver as the most sensitive organ in rats, following both short-term and long-term administration of a number of PAs. The 1,2-unsaturated PAs tested form DNA adducts in vitro and in vivo and are mutagenic. Based upon an understanding of their chemistry and metabolism, it is concluded that this property is common to all 1,2-unsaturated PAs, albeit with differing potencies, and is relevant to humans. PAs appear to be antimitotic in hepatocytes. A number of 1,2-unsaturated PAs have been shown to be carcinogenic in rodents, primarily causing haemangiosarcomas in the liver, i.e. originating in the endothelial cells rather than the hepatocytes. Considering the results from animal studies and the relevant mode of action, it is probable that PA exposure resulting in toxicity in humans could also lead to carcinogenicity.

8. JECFA considered that the derivation of a health-based guidance value for PAs was inappropriate in view of the genotoxic mode of action. From the carcinogenicity data in rats, a benchmark-dose $BMDL_{10}$ of 182 µg/kg bw per day for liver haemangiosarcoma in female rats from the USA National Toxicological Program (NTP) study on riddelliine, conducted in 2003, was calculated as the point of departure for use in a margin of exposure (MOE) approach.

9. JECFA considered whether it was possible to identify relative potency factors (RPF) for different 1,2-unsaturated PAs. In addition to the carcinogenicity studies on lasiocarpine and riddelliine, carcinogenicity studies on other PAs were conducted with non-standard protocols, and these do not allow comparison of carcinogenic potency. Based on short-term toxicity and genotoxicity, it appears that the potency is broadly in the order: macrocyclic esters > diesters > monoesters. However, there may also be differences depending on the type of necine base and the stereochemistry. The two PAs tested for carcinogenicity, lasiocarpine and riddelliine, are among the more potent, and it is likely that many of the PAs present in food, such as lycopsamine, are less potent. Ingested PA N-oxides are efficiently reduced to PA free bases in the digestive tract, and to a lesser extent in the liver. JECFA concluded that the data were insufficient to make assumptions about the potency of the N-oxides relative to the parent PA and adopted the conservative approach of assuming equal potency.

10. JECFA calculated MOEs between the BMDL of 182 µg/kg bw per day and mean and high-percentile (90th, 95th or 97.5th, depending on the study) chronic exposure estimates for children and adults from consumption of honey and tea, separately. As several national estimates of dietary exposure were available for each food, MOEs were calculated using a range from the lowest lower-bound mean or high-percentile dietary exposure to the highest upper-bound mean or high-percentile dietary exposure. This range takes into account the uncertainty in measurements of 1,2-unsaturated PAs and their N-oxides and the variability in their concentrations and national estimates of food consumption.

11. For adult consumption of honey, mean and high-percentile chronic dietary exposures to 1,2-unsaturated PAs are in the range 0.00002 to 0.0039 µg/kg bw per day and 0.005 to 0.026 µg/kg bw per day, respectively. These dietary exposures equate to MOEs in the range 46 000 to 9 million for mean exposures and 6900 to 36 000 for high-percentile exposures. For children consuming honey, the ranges of mean and high-percentile chronic dietary exposures to 1,2-unsaturated PAs are 0.00001 to 0.013 µg/kg bw per day and 0.006–0.082 µg/ kg bw per day, equating to MOEs in the range 14 000 to 18 million for mean exposure and 2200 to 30 000 for high-percentile exposure.

12. For adult tea consumption, mean and high-percentile chronic dietary exposures to 1,2-unsaturated PAs are in the range 0.0013 to 0.13 µg/kg bw per day and 0.01 to 0.26 µg/kg bw per day, respectively. These dietary exposures equate to MOEs in the range 1400 to 140 000 for mean exposure and 700 to 18 000 for high-percentile exposure. For children consuming tea, the range of mean and high-percentile chronic dietary exposures to 1,2-unsaturated PAs are 0.005 to 0.018 µg/kg bw per day and 0.027 – 0.076 µg/kg bw per day, respectively. These dietary exposures equate to MOEs in the range 10 000 to 36 000 for mean exposure and 2400 to 6700 for high-percentile exposure. JECFA noted that estimates of dietary exposure to 1,2-unsaturated PAs and their N-oxides from tea consumption are likely to be overestimates, as concentration data from herbal teas have been combined with information on total tea consumption.

13. JECFA also noted insufficient information to determine MOEs for other food types or the total diet.

14. JECFA noted that a broad range of PAs has been reported in animal feed, but the data were not adequate to assess whether transfer to products of animal origin, such as milk, meat, and eggs, could make a major contribution to dietary exposure.

15. The data were insufficient to identify a point of departure for use in calculating MOEs for acute exposure. However, the Committee noted that the estimates of mean and high-percentile acute exposure to 1,2-unsaturated PAs for children and adults were up to 0.784 µg/kg bw per day, which is 23-fold lower than the lowest reported exposure of 18 µg/kg bw per day associated with human disease following 6 weeks of exposure.

16. Based on limited occurrence data, JECFA noted that the calculated MOEs for honey (high consumers) and tea (mean and high consumers) indicated a potential concern. It should be noted that PAs measured in these commodities might not be representative of all food groups and all regions. However, it provided a conservative risk estimate as it was compared to the $BMDL_{10}$ for the potent PA riddelliine, and most of the PAs commonly found in food are likely to be less potent than riddelliine.

17. JECFA considered it concerning that exposure to a single food product could result in such low MOEs. The Committee noted that exposure to PAs resulted from other food items, and animal products such as milk might contribute to the total exposure due to the presence of PAs in feed. A first indication of total exposure could be obtained from a small duplicate-diet study, from which an MOE of 140 000 could be derived, but it was unclear how representative these data were.

18. The comparison of estimates of acute dietary exposure to PAs from honey and tea with the lowest reported dose causing human disease did not indicate a concern. There was insufficient information to conclude on food or beverages other than honey and tea.

Risk assessment by other scientific bodies

19. On 8 November 2011, the Scientific Panel on Contaminants in the Food Chain (CONTAM Panel) of the European Food Safety Authority (EFSA) published a scientific opinion on the risks to public health related to the presence of PAs in food and feed[4]. The CONTAM Panel concluded that 1,2-unsaturated PAs may act as human genotoxic carcinogens. A benchmark dose lower confidence limit for a 10% excess cancer risk ($BMDL_{10}$) of 70 µg/kg bw per day for induction of liver haemangiosarcomas by lasiocarpine in male rats was calculated as the reference point for comparison with the estimated dietary exposure, for the application of the Margin of Exposure (MOE) approach. Based on occurrence data limited to honey, the CONTAM Panel concluded that there was a possible health concern for those toddlers and children who are high honey consumers.

20. On 27 July 2017, EFSA published the statement on the risks for human health related to the presence of PAs in honey, tea, herbal infusions, and food supplements[5]. The CONTAM Panel established a new reference point of 237 µg/kg body weight per day to assess the carcinogenic risks of pyrrolizidine alkaloids. The CONTAM Panel concluded that there is a possible concern for human health related to the exposure to PAs, in particular for frequent and high consumers of tea and herbal infusions. The Panel noted that consumption of food supplements based on PA-producing plants could result in exposure levels too close (i.e., less than 100 times lower) to the range of doses known to cause severe acute/short-term toxicity.

21. Türkiye used in their scientific opinion on the results of the monitoring program for PAs and the risk to public health, the $BMDL_{10}$ value of 237 µg/kg body weight per day for the application of the MOE approach to assess the carcinogenic risks of PAs[6]. It has been evaluated that exposure amounts to PAs through thyme consumption may cause low health concerns in all age groups compared to the average per capita consumption amount (0.1 g/day). Although the risk of health concerns varies according to different age groups and consumption scenarios, there is a possible risk of health concerns, especially in consumption scenarios with high amounts (2 and 4 g per day).

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4 EFSA CONTAM Panel, 2011. Scientific Opinion on Pyrrolizidine alkaloids in food and feed. EFSA Journal 2011; 9(11):2406. [134 pp.], https://doi.org/10.2903/j.efsa.2011.2406.

5 EFSA CONTAM Panel, 2017. Statement on the risks for human health related to the presence of pyrrolizidine alkaloids in honey, tea, herbal infusions and food supplements. EFSA Journal 2017;15(7):4908, 34 pp. https://doi.org/10.2903/j.efsa.2017.4908.

6 Available at (In Turkish): https://www.tarimorman.gov.tr/GKGM/Belgeler/DB_Risk_Degerlendirme/BilimselGorus/Kekikte_PAlar_Bilimsel_Gorus.pdf.

22. Characterization and lifetime dietary risk assessment of eighteen 1,2-unsaturated PAs in honey by New Zealand[7]. The lowest $BMDL_{10}$ value for riddelliine, derived with the two-stage model, was 182 µg/kg bw per day, or 0.182 mg/kg bw per day. To enable risk characterization of exposures to PAs through New Zealand honey, the JECFA $BMDL_{10}$ value was adopted as the point of departure. Mean, median, and 95th percentile exposure assessment results were characterized using an MOE approach. In New Zealand, risk management for genotoxic carcinogens aims for an MOE of 10 000 or higher to identify if the exposure is of low concern for public health. Assessment of lifetime exposures from the retail honey survey dataset identifies no immediate cause for concern for general consumers with the prevalence of PAs in New Zealand honey. However, uncertainty over high consumption habits limits the characterization of this risk for sensitive populations.

JECFA Recommendations

23. JECFA noted that several gaps still exist in the overall PAs database, from toxicological and epidemiological aspects to methods of analysis and occurrence levels in different food products, among others. As the missing information has precluded a more definitive assessment, to fill these data gaps, JECFA recommended the following:

    a. To establish internationally agreed-upon high-quality standards and certified reference materials that would allow accurate analytical determination and quantification of the different PAs.

    b. To further study the effects of processing on the occurrence of PAs, taking into account possible metabolites formed during processing.

    c. To generate occurrence data from areas other than the European Union (EU) and on food products other than honey, particularly foods of animal origin, to improve dietary exposure estimates for PAs across the range of potentially PA-containing foods and from different geographical regions.

    d. To conduct additional toxicological investigations to establish:

        i. the relative potency of PAs, taking into account toxicokinetics and genotoxicity; and

        ii. a point of departure to be used in risk assessment of acute dietary exposure to PAs;

    e. To carry out epidemiological studies on long-term follow-ups of incidents of PA contamination and assess the carcinogenic potential of PAs in humans.

    f. To generate more information on:

        i. toxicity and occurrence of saturated PAs, as most available data are on the 1,2-unsaturated PAs, and also because the saturated PAs elicit toxicity by a different mode of action; and

        ii. transfer from feed to food, to estimate whether PA concentrations in food resulting from PAs in feed could be of concern for human health.

Methods of analysis

24. The specific analytical issues associated with the screening and quantification of PAs – saturated and unsaturated PAs and their N-oxides – in various foods and feeds, include:

    a. wide variations in PA concentrations in food and feed samples;

    b. variation in PA profiles between plants in various regions of the world;

    c. the stability of PAs during storage; and

    d. the issue of whether to quantify individual PAs or total necines.

25. PAs are usually extracted from plants and food samples with hot or cold methanol or ethanol, or dilute aqueous acid in quantitative methods. The alcoholic or aqueous acid extracts are then applied to prepared strong cation exchange solid-phase extraction (SPE) cartridges, followed by washing of the cartridges with water and methanol to remove non-adsorbed impurities, and then elution of the PAs and N-oxide components using a small volume of ammoniated methanol. Subsequent evaporation and reconstitution of the residue in methanol or another suitable solvent produce samples ready for analysis of PAs.

7 Available at: https://www.mdpi.com/2072-6651/13/12/843

26. Several screening methods are available, including thin-layer chromatography (TLC), electrophoresis, nuclear magnetic resonance (NMR), and immunological methods. TLC with colorimetric detection of 1,2-unsaturated PAs is inexpensive, but the results are qualitative rather than quantitative. NMR has been used to determine the total alkaloid content, but it probably lacks the sensitivity required for food safety risk assessment purposes. Enzyme-linked immunosorbent assay (ELISA) -based screening methods for 1,2-unsaturated PAs and their N-oxides have been developed but are currently limited by a lack of antibodies that specifically bind all of the 1,2-unsaturated PAs and their N-oxides with comparable affinity. At the same time, antibodies developed for specific 1,2-unsaturated PAs or their N-oxides seem to lack specificity for other 1,2-unsaturated PAs or their N-oxides. Developing sensitive ELISAs for quantifying necines could be useful in summation analysis methods for quantifying total 1,2-unsaturated PAs and their N-oxides based on hydrolysis. However, results from ELISA should always be confirmed using quantitative reference methods, such as gas chromatography-mass spectrometry (GC-MS) and/or high-performance liquid chromatography–tandem mass spectrometry (HPLC-MS/MS), since immunological methods have limitations in selectivity and reproducibility.

27. In recent years, high-resolution mass spectrometry (HRMS) has been increasingly used as a complementary method for analysing trace-level contaminants in food matrices since it allows the simultaneous screening of target, suspect, and untargeted compounds. Moreover, acquiring accurate MS and MS/MS spectra (resolution < 5 ppm) offers the possibility to detect a theoretically unlimited number of molecules without needing a compound-specific tune, carry out retrospective data analysis, and perform structural characterization of unknown or suspected compounds. Recently, studies[8] have been carried out to expand the knowledge on the distribution of PAs in foods, aiming at the rapid and automated screening and identification of large numbers of PAs at trace levels in various food matrices. To achieve this goal, an analytical procedure is established that combines a suitable extraction method (e.g., salting–out assisted liquid–liquid extraction (SALLE) of aqueous extracts) with ultra-high-performance liquid chromatography coupled with high-resolution tandem mass spectrometry (UHPLC-HRMS/MS).

28. Quantitative analysis of PAs is based on the determination of individual PAs, using LC-MS/MS, or a sum parameter method based on analysis of common necine groups, using GC-MS detection. In all cases, pre-concentration and sample clean-up before analysis are required. Some issues of concern are related to N-oxide instability during sample preparation and analysis. Multiple variants of Liquid chromatography Tandem mass spectrometry (LC-MS/MS) methods exist. LC-MS/MS methods offer low detection limits of approximately 1 µg/kg or less and the ability to analyse PAs and PA-N-oxides simultaneously in one run as the main advantages. Challenges common to all analytical methods are the lack of high-quality standards, internal standards, and certified reference materials.

29. For the analysis of pyrrolizidine alkaloids in food, feed and plant materials, several described methods of analysis are available, such as EN 17683:2023 Animal feeding stuffs - Methods of sampling and analysis - Determination of PAs in animal feeding stuff by LC-MS/MS, the method “Determination of PAs in plant-based food and feed materials, including (herbal) teas, herbal food supplements, fodder and feedstuffs by LC-MS/MS” developed by the EU Reference Laboratory for mycotoxins and plant toxins in food and feed (EURL-MP)[9] and the method “Determination of PAs in plant material by SPE-LC-MS/MS” developed by the Bundesinstitut für Risikobewerting from Germany[10]. In Türkiye, the control of the presence of PAs in honey and herbal teas is performed with UHPLC/MS/MS[11].

30. EN 17683:2023 Animal feeding stuffs - Methods of sampling and analysis - Determination of PAs in animal feeding stuff by LC-MS/MS is a standardized method for the quantitative determination of PAs in complete and supplementary feed and forages by liquid chromatography tandem mass spectrometry (LC-MS/MS) after solid phase extraction (SPE) clean-up. The method has been successfully validated in a collaborative trial for the matrices complete feed for horses, complementary feed for horses, complementary feed for rodents, hay, alfalfa, and grass silage. Validation was carried out for the PA, and the validation data are available; and the concentration ranges validated are listed in Table 1:

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8 An analytical platform for the screening and identification of pyrrolizidine alkaloids in food matrices with high risk of contamination. https://doi.org/10.1016/j.foodchem.2022.135058

9 Available at: https://www.wur.nl/nl/show/eurl-mp-method_002-pyrrolizidine-alkaloids-by-lc-msms-vs.htm.

10 Available at: https://www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-pa-in-plant-material.pdf.

11 Available at: https://www.academia.edu/64360579/Quantitation_of_Pyrrolizidine_Alkaloids_in_Honey_and_Herbal_Teas_by_UHPLC_MS_MS_Application_Note?uc-sb-sw=2393958.

Table 1 — Summary of concentration ranges per PA tested in the collaborative trial

Tested pyrrolizidine alkaloid (PA)	Abbreviation	Tested concentration range[a] (µg/kg)	
		From	To
Echimidine	Em	20	435
Echimidine-N-oxide	EmN	5	30
Erucifoline	Er	20	245
Erucifoline-N-oxide	ErN	20	370
Europine	Eu	15	330
Europine-N-oxide	EuN	25	285
Heliotrine	Hn	25	280
Heliotrine-N-oxide	HnN	25	245
Jacobine	Jb	20	230
Jacobine-N-oxide	JbN	20	215
Lasiocarpine	Lc	20	350
Lasiocarpine-N-oxide	LcN	5	250
Intermedine	Lm	25	560
Intermedine-N-oxide	LmN	5	395
Lycopsamine	La	25	500
Lycopsamine-N-oxide	LaN	20	280
Monocrotaline	Mc	20	360
Monocrotaline-N-oxide	McN	20	365
Retrorsine	Re	250	375
Retrorsine-N-oxide	ReN	5	285
Senecionine[b]	Sc	25	205
Senecionine-N-oxide[b]	ScN	5	300
Senecivernine[b]	Sv	20	205
Senecivernine-N-oxide[b]	SvN	5	165
Senkirkine	Sk	20	275
Seneciphylline	Sp	25	225
Seneciphylline-N-oxide	SpN	5	225
Trichodesmine	Td	5	250
Intermedine + Lycopsamine	Lm+La	50	890
Intermedine-N-oxide + Lycopsamine-N-oxide	LmN+LaN	5	645
Senecivernine + Senecionine	Sy+Sc	30	280
Senecivernine-N-oxide + Senecionine-N-oxide	SvN+ScN	10	380

[a] Rounded figures

[b] Individual PA of the isomeric pairs Sv+Sc and SvN+ScN were not evaluated statistically due to insufficient chromatographic separation

31. It was demonstrated that the PA isomeric pairs senecivernine and senecionine, as well as senecivernine-N-oxide and senecionine-N-oxide, cannot be determined individually due to insufficient chromatographic separation. However, the sums of the individual PA of the isomeric pairs were quantified with sufficient reproducibility. Co-elution of other PA-isomers not included in the scope of the method shall be taken into account. A list of potentially co-eluting isomers is presented in Table 2:

Table 2 — List of potentially co-eluting pyrrolizidine alkaloid (PA) isomers

PA validated in the collaborative trial (columns indicate potentially co-eluting isomers)		Potentially co-eluting PA isomers not included in the collaborative trial
echimidine		heliosupine
echimidine-N-oxide		heliosupine-N-oxide
Intermedine	lycopsamine	indicine, echinatine, rinderine
intermedine-N-oxide	lycopsamine-N-oxide	indicine-N-oxide, echinatine-N-oxide, rinderine-N-oxide
retrorsine		usaramine
retrorsine-N-oxide		usaramine-N-oxide
senecionine	senecivernine	integerrimine
senecionine-N-oxide	senecivernine-N-oxide	integerrimine-N-oxide
seneciphylline		spartioidine
seneciphylline-N-oxide		spartioidine-N-oxide

32. Although the calibration range of the method protocol is specified from 10 µg/kg to 300 µg/kg, the results of the collaborative study showed that the dilution of sample extracts with blank sample extracts enables the quantitation of concentrations exceeding the calibration range. Satisfactory reproducibility was achieved when quantifying up to 1428 µg/kg for individual PA and up to 887 µg/kg for the sum of isomeric pairs.

33. The method of analysis "Determination of PAs in plant-based food and feed materials, including (herbal) teas, herbal food supplements, fodder and feedstuffs by LC-MS/MS"[12] developed by the EU Reference Laboratory for mycotoxins and plant toxins in food and feed (EURL-MP) confirms and quantifies (using standard addition to the sample) the following pyrrolizidine alkaloids: echimidine, echimidine-N-oxide, echinatine, echinatine-N-oxide, erucifoline, erucifoline-N-oxide, europine, europine-N-oxide, heliosupine, heliosupine-N-oxide, heliotrine, heliotrine-N-oxide, indicine, indicine-N-oxide, integerrimine, integerrimine-N-oxide, intermedine, intermedine-N-oxide, jacobine, jacobine-N-oxide, jacoline, jaconine, lasiocarpine, lasiocarpine-N-oxide, lycopsamine, lycopsamine-N-oxide, monocrotaline, monocrotaline-N-oxide, retrorsine, retrorsine-N-oxide, rinderine, rinderine-N-oxide, senecionine, senecionine-N-oxide, seneciphylline, seneciphylline-N-oxide, senecivernine, senecivernine-N-oxide, senkirkine, spartioidine, spartioidine-N-oxide, trichodesmine, usaramine and usaramine-N-oxide. The method is applicable for plant-based materials in the concentration range of 0 to 500 µg/kg. The limit of quantification (LOQ) for the individual PAs is 5 µg/kg. The method is available to analyse 44 PAs in food and feed matrices. The method has been in-house validated for teas, dried herbs, herbal infusions, cumin, food supplements containing botanical preparations or pollen, pollen products, and feed materials of plant origin. The underlying validation data are not publicly available but could be obtained upon request. High-quality standards are commercially available from at least two suppliers for the quantification of the PAs within the scope of the method.

34. The method "Determination of pyrrolizidine alkaloids (PA) in plant material by SPE-LC-MS/MS" developed by the Bundesinstitut für Risikobewerting from Germany[13] analyses the following PA in plant material: echimidine (Em), echimidine-N-oxide (EmN), erucifoline (Er), erucifoline-N-oxide (ErN), europine (Eu), europine-N-oxide (EuN), heliotrine (Hn), heliotrine-N-oxide (HnN), intermedine (Im), intermedine-N-oxide (ImN), jacobine (Jb), jacobine-N-oxide (JbN), lasiocarpine (Lc), lasiocarpine-N-oxide (LcN), lycopsamine (La), lycopsamine-N-oxide (LaN), monocrotaline (Mc), monocrotaline-N-oxide (McN), retrorsine (Re), retrorsine-N-oxide (ReN), senecionine (Sc), senecionine-N-oxide (ScN), seneciphylline (Sp), seneciphylline-N-oxide (SpN), senecivernine (Sv), senecivernine-N-oxide (SvN), senkirkine (Sk), trichodesmine (Td). The LOQ for the different PAs is between 1.7 and 6.4 µg/kg. High-quality standards of the PAs within the scope of the method of analysis are commercially available.

12 Available at : https://www.wur.nl/nl/show/eurl-mp-method_002-pyrrolizidine-alkaloids-by-lc-msms-vs.htm.

13 Available at : https://www.bfr.bund.de/cm/349/determination-of-pyrrolizidine-alkaloids-pa-in-plant-material.pdf.

35. The method of analysis for PAs in honey and herbal teas with UHPLC/MS/MS[14], used for official control in Türkiye is a sensitive analytical method for the quantitation of 28 PAs (i.e. echimidine (Em), echimidine-N-oxide (EmN), erucifoline (Er), erucifoline-N-oxide (ErN), europine (Eu), europine-N-oxide (EuN), heliotrine (Hn), heliotrine-N-oxide (HnN), intermedine (Im), intermedine-N-oxide (ImN), jacobine (Jb), jacobine-N-oxide (JbN), lasiocarpine (Lc), lasiocarpine-N-oxide (LcN), lycopsamine (La), lycopsamine-N-oxide (LaN), monocrotaline (Mc), monocrotaline-N-oxide (McN), retrorsine (Re), retrorsine-N-oxide (ReN), senecionine (Sc), senecionine-N-oxide (ScN), seneciphylline (Sp), seneciphylline-N-oxide (SpN), senecivernine (Sv), senecivernine-N-oxide (SvN), senkirkine (Sk), trichodesmine (Td)) in honey and herbal tea. The method comprises an acidic extraction and a cleanup by solid phase extraction (SPE) using a strong cation exchange material. The method was successfully validated for honey and herbal teas. The LOQ for the individual PAs is between 2 and 3 µg/kg. Extraction recoveries for most PAs are in the range of 80 to 120% in the honey samples and between 70 and 85% in the herbal tea samples.

36. Analytical methods for pyrrolizidine alkaloids are summarized in a review[15]. Triple-Quadrupole (QqQ) is an MS analyser widely used to analyse PAs in the studies carried out over the last few years. It has high sensitivity and selectivity and can detect trace amounts present in the matrices, which is also due to its ability to perform multiple reaction monitoring (MRM). In conjunction with QqQ, Ion Trap was also used to analyse PAs. Like QqQ, Ion Trap can also detect low levels of PAs. High-resolution mass spectroscopy (HRMS) has been used more recently and allows a more thorough identification by distinguishing compounds of equal molecular weight but different elemental composition. Time-of-Flight (ToF) and Orbitrap are HRMS. ToF-MS is acknowledged for its excellent mass resolution, accuracy, and sensitivity, making it ideal for the characterization and quantification of complex molecules like PAs. ToF's high resolution allows for precise measurement of molecular masses and fragmentation patterns, differentiating various isomers. Orbitrap, similar to ToF, is a high-resolution mass spectrometry technique noted for its high mass accuracy, resolving power, and sensitivity, which allows for the precise measurement of molecular compounds at low concentrations.

37. In the quantitative analysis of 118 pyrrolizidine alkaloids in food supplements, herbal infusions, honey, and teas by the target screening method[16], the LOQ for individual PAs was calculated as 0.1-2.1 mg/kg in honey and food supplements and 1-12 mg/kg in teas. Precision (expressed as intra-day repeatability, RSD) was assessed at two contamination levels: low (2 µg L−1) and high (100 µg L−1), and the range 63–117% was obtained for the 28 reference PAs in all the studied matrices.

38. The EU Regulation[17] sets out the following specific criteria for the confirmatory analysis method for PAs in food.

    Recovery: The average recovery should be between 70 and 120%.

    The average recovery is the average value from replicates obtained during validation when determining the precision parameters RSDr and RSDwR. The criterion applies to all concentrations and individual toxins.

    In exceptional cases, average recoveries outside the above range can be acceptable, but they shall lie within 50-130% only when the precision criteria for RSDr and RSDwR are met.

    Precision

    RSDr shall be ≤ 20%

    RSDwR shall be ≤ 20%

    RSDR should be ≤ 25%

    These criteria apply to all concentrations.

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14 Available at: https://www.academia.edu/64360579/Quantitation_of_Pyrrolizidine_Alkaloids_in_Honey_and_Herbal_Teas_by_UHPLC_MS_MS_Application_Note?uc-sb-sw=2393958.

15 Pyrrolizidine Alkaloids in Foods, Herbal Drugs, and Food Supplements: Chemistry, Metabolism, Toxicological Significance, Analytical Methods, Occurrence, and Challenges for Future. https://doi.org/10.3390/toxins16020079.

16 Target screening method for the quantitative determination of 118 pyrrolizidine alkaloids in food supplements, herbal infusions, honey and teas by liquid chromatography coupled to quadrupole orbitrap mass spectrometry. https://www.sciencedirect.com/science/article/pii/S030881462300924X?via%3Dihub

17 Commission Implementing Regulation (EU) 2023/2783 of 14 December 2023 laying down the methods of sampling and analysis for the control of the levels of plant toxins in food and repealing Regulation (EU) 2015/705 OJ L, 2023/2783, 15.12.2023 ELI: http://data.europa.eu/eli/reg_impl/2023/2783/oj.

If a laboratory provides evidence that the RSDwR criterion is complied with, there is no need to provide that evidence for the RSDr criterion, as compliance with the RSDwR guarantees compliance with the RSDr criterion.

The criteria for precision apply to both the sum and the individual toxins.

Limit of quantification (LOQ)

LOQ requirement for individual pyrrolizidine alkaloids

in dried product: ≤ 10 µg/kg

in liquid product: ≤ 0.15 µg/kg

39. The Codex Alimentarius Commission (CAC) has issued a guideline[18] for laboratories involved in the testing of foods for import/export, which recommends that such laboratories should:

- use internal quality control procedures, such as those described in the "Harmonized Guidelines for Internal Quality Control in Analytical Chemistry Laboratories;"
- participate in appropriate proficiency testing schemes for food analysis which conform to the requirement laid out in "The International Harmonized Protocol for Proficiency Testing of (Chemical) Analytical Laboratories (Pure Appl. Chem., vol 78, No. 1, pp.145-186, 2006);" and
- whenever available, use methods validated according to principles provided by the CAC.

Accordingly, a guideline[19] have been published by the CAC to define and describe the performance criteria, which should be met by methods to analyse pesticide residues in foods and feed. Performance criteria for screening methods and quantitative methods are described, together with a description of the performance parameters of analytical methods. Screening detection limit (SDL) of the screening method is the lowest level at which an analyte has been detected in at least 95% of the samples (e.g. an acceptable false-negative rate of 5%). Acceptable mean recoveries for enforcement purposes of quantitative methods should normally range from 70-120%, with a repeatability (RSD_r) and reproducibility (RSD_R) relative standard deviation ≤20%. For very low concentrations (e.g. < 0.01 mg/kg), some laboratories may accept method performance criteria that fall outside of these criteria (e.g. 60–120% with a RSDr(R) <30%).

40. Methods of analysis should be validated in accordance with the principles for the construction of methods of analysis in the CAC Procedures Manual. Based on the explanations in this section, compliance with the specific criteria given in Table 3 for quantitative methods can be recommended.

Table 3 — Specific criteria for individual 1,2-unsaturated PAs

Specific criteria		Explanations
LOQ for solid foods (≤ µg/kg)	10	
LOQ for liquid foods (≤ µg/L)	0.15	
RSD_r and RSD_R (≤%)	20	For very low concentrations (e.g. < 0.01 mg/kg) some laboratories may accept method performance criteria that fall outside of these criteria (e.g. 60–120% with a RSDr and RSD_R < 30%).
Recovery (%)	70-120	

Methods of sampling

41. PA contamination can be non-homogeneous owing to the uneven distribution of plant parts in a batch of feed or food. Similarly, teas, culinary herbs, spices, salad plants, and pollen granules are likely to be quite heterogeneous regarding contamination. Distribution of PAs in dry teas can be very inhomogeneous owing to variation in the distribution of the plant particles with inherent PA throughout the mix. Relatively more or larger-volume samples will probably be required for such foods than for complex solids, such as meat, and especially liquid foods, such as milk and honey, where more homogeneous contamination within a batch can be expected.

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18 Harmonized IUPAC Guidelines for Single-Laboratory Validation of Methods of Analysis, Pure & Appl. Chem., 74(5), 2002; 835 – 855. https://doi.org/10.1351/pac200274050835.

19 Guidelines on Performance Criteria for Methods of Analysis for the Determination of Pesticide Residues in Food and Feed (CXG 90-2017). Available at : https://www.fao.org/fao-who-codexalimentarius/sh-proxy/jp/?lnk=1&url=https%253A%252F%252Fworkspace.fao.org%252Fsites%252Fcodex%252FStandards%252FCXG%2B90-2017%252FCXG_090e.pdf

42. Proper sampling will, therefore, be critical. Sampling protocols will play a crucial role in the precision with which the levels of are measured in the wide range of foods that are currently known to be subject to contamination. It seems likely that significantly more practical experience will be required before optimum sampling protocols emerge suitable for different foods and foods at different stages of manufacture.

43. In some countries and regions, the legislation[20] on sampling for plant toxins refers to the procedures in place for regulation on mycotoxins[21] and trace elements[22]. In the EU, the same sampling procedures are applied to control plant toxins, including pyrrolizidine alkaloids, and mycotoxins. In Türkiye[23], similar to the EU, the sampling procedures for pyrrolizidine alkaloids in food are applied according to the criteria of the Communiqué published for mycotoxins. These legislations developed for mycotoxin include sampling methods for cereals, dried and powdered spices, milk and milk products, beverages, vegetable oils, food supplements, pollen and pollen products, dried herbs, herbal infusions (dried product), teas (dried product), milk, meat, honey, and eggs. In summary, the following sampling procedure is applied in these regulations.

    - For cereal and cereal products in bulk (> 100 tonnes): 100 incremental samples of 100 g, resulting in an aggregate sample of 10 kg. For smaller lots (≤ 100 tonnes), less incremental samples (3-100) are to be taken.
    - For dried spices with large particle size spices (> 15 tonnes): 100 incremental samples of 200 g, resulting in an aggregate sample of 200 kg. For smaller lots (≤ 15 tonnes), less incremental samples (10-100) are to be taken.
    - For dried spices except those with large particle size and powdered spices (> 15 tonnes): 100 incremental samples of 100 g, resulting in an aggregate sample of 10 kg. For smaller lots (≤ 15 tonnes), less incremental samples (5-100) are to be taken.
    - For milk and milk products, meat and meat products, honey, and eggs: 3-10 incremental samples of at least 100 g, resulting in an aggregate sample of a minimum of 1 kg.
    - For food supplements, pollen, and pollen products (for 1-1000 retail/individual packages): Incremental samples will be taken from 1 to 4 retail/individual packages.
    - For dried herbs, herbal infusions (dried product), teas (dried product), and powdered spices in bulk (> 15 tonnes): 50 incremental samples of 40 g, resulting in an aggregate sample of 2 kg. For smaller lots (≤ 15 tonnes) less incremental samples (3-50) are to be taken.

44. Based on the results of a study on sampling for plant toxins by a working group coordinated by the German Federal Institute for Risk Assessment (BfR), new legislation[24] has been published in the EU. This regulation states that the sampling method for the control of plant toxins in dried herbs, herbal infusions (dried product), teas (dried product), and powdered spices does not guarantee that a sample representative of the lots will be obtained when done according to the sampling method recommended for mycotoxin. According to the result of this determination, sampling for these foods is organized as follows;

    - for dried herbs, herbal infusions (dried product), teas (dried product), and powdered spices in bulk (> 15 tonnes): 50 incremental samples of 80 g, resulting in an aggregate sample of 4 kg. For smaller lots (≤15 tonnes), less incremental samples (3-50) are to be taken.

45. Reflecting current experience, the CoP[25] also stated that it is not always appropriate to sample for PAs in the same way as mycotoxins.

46. Sampling for PA analysis should be further developed in accordance with the Codex General Guide for Sampling developed by CCMAS (CXG 50-2004).

Effects of food and feed processing

47. PAs are generally stable during common food processing, particularly when preparing infusions such as steeping dried teas in hot water, where only partial extraction occurs, and thermal degradation is minimal.

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20 Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32023R2783&qid=1742847347901.

21 Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32023R2782&qid=1742842481402.

22 Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A02007R0333-20240430&qid=1742847423514.

23 Available at (in Turkish): https://www.mevzuat.gov.tr/mevzuat?MevzuatNo=24461&MevzuatTur=9&MevzuatTertip=5.

24 Available at: https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX%3A32024R0885.

25 Available at: https://foodsupplementseurope.org/wp-content/themes/fse-theme/documents/publications-and-guidelines/Pyrrolizidine_Guidelines-June2020.pdf

48. Certain processing methods, such as heat treatment, fermentation, infusion preparation (transfer ratio), and other processes (grinding, washing, and soaking) have shown potential in reducing PA levels in foods. For example, in the cooking of petioles and young ears of Petasites japonicus, a PA-containing plant, both boiling and soaking treatments were found to be important in reducing PAs, with a longer soaking time having a greater effect in reducing PAs. Hydrodistillation[26] in the production of essential oils is effective in PA removal[27].

49. PA N-oxides, which are generally less toxic, can be converted into their more toxic free-base forms during prolonged or high-temperature processes, such as tea manufacturing or drying. For example, senecionine-type PANOs can convert to the free-base forms during manufacturing and brewing. This thermal conversion poses a potential health risk and may offset the benefits of some processing techniques. Therefore, manufacturing processing should be carefully evaluated and controlled to minimize unnecessary thermal conversion of PA N-oxides to PAs[28].

50. Some information is available on the fate of 1,2-unsaturated PAs and their N-oxides during feed production. The occurrence of PAs in animal feed shows that 1,2-unsaturated PAs and their N-oxides are fairly stable during feed production. However, reliable data on the degradation rate and the metabolites that are formed are lacking. In silages with a high-water content, PAs are generally prone to degradation, although the degradation rate varies per condition and PA congener.

51. Filtration of honey results in the reduction of pollen content and reduces the content of 1,2-unsaturated PAs.

52. It is evident that more information is required on the effects of processing on PAs.

Prevention and control

53. Management practices currently focus on minimizing the occurrence of weeds containing 1,2-unsaturated PAs and their N-oxides in feed and food. Management practices to help prevent and reduce the levels of 1,2-unsaturated PAs and their N-oxides in food and feed are established in the *Code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed* (CXC 74-2014). Good agricultural practices, HACCP, and good manufacturing practice strategies must be in place to prevent batches of food contaminated with PAs from entering the food chain and mingling with uncontaminated products.

54. Specific non-Codex Codes of practice and/or guidelines and recommendations to reduce the presence of PAs have been developed for tea, herbal infusions, spices, food supplements, and medicinal products of plant origin. Examples of these are "Code of Practice to prevent and reduce pyrrolizidine alkaloid contamination in raw materials for tea and herbal infusions[29]" developed by Tea and Herbal Infusions Europe, "Guidelines and recommendations to reduce the presence of pyrrolizidine alkaloids in food supplements[30]" developed by Food Supplements Europe, and "Guidelines for Good Agricultural and Wild Collection Practices for Medicinal and Aromatic Plants (GACP-MAP)[31]" developed by EUROPAM, the European Herb Growers Association and the "Code of practice to prevent and reduce pyrrolizidine alkaloid contaminations of medicinal products of plant origin[32]", and the CoP of the European Spice Association[33]. The Food Federation Germany has also developed a Code of Practice[34] for the prevention and reduction of contamination of food with pyrrolizidine alkaloids.

55. Türkiye has developed several Codes of Practice or guidance for the prevention of pyrrolizidine alkaloids:

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26 Giara, D, S. et al. (2022). Quantitative Removal of Pyrrolizidine Alkaloids from Essential Oils by the Hydrodistillation Step in Their Manufacturing Process. Planta Med, 88(07): 538-547.

27 Han, H. et al. (2022). Dissipation pattern and conversion of pyrrolizidine alkaloids (PAs) and pyrrolizidine alkaloid N-oxides (PANOs) during tea manufacturing and brewing. Food Chemistry Volume 390, 133183.

28 Kucukoglu, A. S. et al. (2024). Effects of thermal and nonthermal treatments on microorganisms, pyrrolizidine alkaloids and volatile compounds in oregano (Origanum vulgare L.). Food Chemistry, 440, 138235.

29 Available at: https://thie-online.eu/files/thie/docs/2018-07-12_THIE_Code_of_Practice_PA_in_TEA-HFI_ISSUE_1.pdf

30 Available at: https://foodsupplementseurope.org/wp-content/themes/fse-theme/documents/publications-and-guidelines/Pyrrolizidine_Guidelines-May2021.pdf

31 Available at: https://www.europam.net/wp-content/uploads/2022/11/EUROPAM-GACP-2022.pdf

32 Available at: https://media.journals.elsevier.com/content/files/cop-revision-20090245.pdf

33 European Spice Association - Code of Practice for Weed Management to Prevent and Reduce Pyrrolizidine Alkaloid.

34 Available at (in German): https://www.lebensmittelverband.de/fileadmin/Seiten/Lebensmittel/Sicherheit_und_Recht/Unerwuenschte_Stoffe/Pyrrolizidinalkaloide/code-of-practice-pyrrolizidinalkaloide.pdf

- Code of Practice on Prevention and Mitigation of Pyrrolizidine Alkaloid Contamination in Thyme (Origanum Spp.).[35]
- Implementation Guidance on Prevention and Mitigation of Pyrrolizidine Alkaloid Contamination in Cumin (Cuminum Cyminum L.).[36]
- Implementation Guidance for the Prevention and Mitigation of Pyrrolizidine Alkaloid Contamination in Mint (Mentha Spp.).[37]

56. New Zealand has produced a document[38] entitled "Pyrrolizidine alkaloids in honey: research, implications, and what you can do to reduce them".

57. It can be observed that all Codes of practice or guidance mentioned in paragraphs 53 and 56 reflect the measures referred to in section 7. "Evaluation of the need to proceed to action and in section 8". "Recommended practices (focused on weed control)" of the current Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed (CXC 74-2014). The additional measures to be taken to minimize the presence of PAs in tea, herbal infusions, food supplements, herbs, and spices are limited. However, given the relevance of the presence of PAs in tea, herbal infusions, honey, food supplements, herbs and spices, it is appropriate to update the Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed (CXC 74-2014) as regards the good practices generally applicable to prevent and reduce PAs in food and feed supplemented with good practices for tea, herbal infusions, food supplements, herbs and spices.

58. This could be done by slightly updating sections 7, "Evaluation of the need to proceed to action," and 8, "Recommended practices in the current CoP," providing more concrete examples for tea, herbal infusions, food supplements, herbs, and spices.

    Alternatively, the current Code of Practice, with some minor updates in sections 7 and 8, could be complemented with specific annexes on tea, herbs and herbal infusions, food supplements and spices. A similar approach was followed for *Code of practice for the prevention and reduction of mycotoxin contamination in cereals* (CXC 51-2003), with separate annexes on prevention measures for specific mycotoxins in cereals.

    For the presence of PAs in honey, due to the nature of beekeeping activity and the measures for weed control as mentioned in the current Code of Practice, might not be appropriate for the prevention and reduction of the presence of pyrrolizidine alkaloids in honey. Furthermore, honey has an inherent risk of pyrrolizidine alkaloids, and honey processing might have a higher influence on the presence of pyrrolizidine alkaloids than other foods, affected by contamination with pyrrolizidine alkaloids. Therefore, it might be considered, if needed, to develop a separate Code of Practice for honey.

59. The specific CoPs in paragraphs 53-56 refer to taking measures to prevent and reduce PA contamination at all stages of the food and feed production chain, starting with primary production. These specific CoPs emphasize cooperation between primary production and food and feed establishments. Each should implement appropriate measures within their respective areas of responsibility to mitigate PAs contamination risks.

60. The current CoP could be revised to include measures under the following key headings;

    - Identification of PA-containing plants
    - Knowledge and raising awareness for PA-containing plants
    - Preventing the spread and release of PA-containing plants
    - Integrated weed management (IWM) in cropland
    - Beekeeping based on good colony/hives and, where possible, vegetation management
    - Measures to prevent cross-contamination in all areas
    - Good hygiene and manufacturing practices for food and feed business operators, including primary production
    - The roles of brokers/suppliers/collectors
    - Responsibilities of competent authorities

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35 Available at (In Turkish): https://www.tarimorman.gov.tr/GKGM/Belgeler/DB_Bitki_Sagligi/Kekikte%20PA_Onlenmesi_Kilavuz.pdf

36 Available at (In Turkish): https://www.tarimorman.gov.tr/GKGM/Belgeler/DB_Bitki_Sagligi/Kimyon_Pyrrolizidine_Alkoloid_Kontaminasyonunun_Kilavuz.pdf

37 Available at (In Turkish): https://www.tarimorman.gov.tr/GKGM/Belgeler/DB_Bitki_Sagligi/Nane_Pyrrolizidine_Alkoloid_Kilavuzu.pdf

38 Available at: https://apinz.org.nz/wp-content/uploads/2021/04/PAs-BeeKeeper-April-2021.pdf

Call for data

61. Taking into account the recommendations from JECFA, it is appropriate to issue a future call for data on the presence of pyrrolizidine alkaloids in food and feed. In order to obtain occurrence data that are comparable and reliable, it is important to define the methods of analysis to be used for analysing pyrrolizidine alkaloids and/or to define specific analytical performance criteria with which methods of analysis have to comply with to ensure that data are obtained with methods of analysis with e.g. sufficient sensitivity and precision. In addition, it is important to provide guidance on the method of sampling to ensure that the obtained data are representative for the sampled batch.

62. Furthermore, it is important to determine if only occurrence data, whereby PAs are quantified individually, can be accepted or if occurrence data expressed as the sum of alkaloids are analysed. In case of analysing individual PAs, it might be relevant to determine which PAs at least should be analysed. Reporting the results of the analysis as the 'sum of PAs' will facilitate evaluation. However, scientific findings demonstrate that the assumption that all 1,2-unsaturated PAs were equally potent is scientifically incorrect. Recent discussions[39] have been pointed out that the assumption that N-oxides, the dominant form in many plants, are as potent as the major PAs is a conservative approach and may overestimate risk. A food with a higher PA concentration may have less toxicity than another food with a lower PA concentration, depending on the individual PAs detected. In future risk assessments and standards, it could consider defining relative potency factors (RPFs) of 1,2-unsaturated PAs and calculating the sum of 1,2-unsaturated PAs using the RPF approach.

63. The occurrence data for the most frequently detected 1,2-unsaturated PAs in foods, as outlined in Table 4, will facilitate future risk assessment. Therefore, it is recommended to analyse all these 1,2-unsaturated PAs in foods and submit the corresponding data. If suitable analytical methods are available, it is also recommended to analyse the additional 14 PAs given in Table 5, as these are PAs known to co-elute with one or more of the 21 PAs given in Table 4. If available occurrence data strongly suggest that some of the PAs given in these tables are non-detected in regionally relevant foods, these PAs may not need to be analysed. In this case, PAs not analysed should be reported.

Table 4 — Most frequently detected 1,2-unsaturated PAs in food.

Intermedine	Intermedine-N-oxide
Lycopsamine	Lycopsamine-N-oxide
Senecionine	Senecionine-N-oxide
Senecivernine	Senecivernine-N-oxide
Seneciphylline	Seneciphylline-N-oxide
Retrorsine	Retrorsine-N-oxide
Echimidine	Echimidine-N-oxide
Lasiocarpine	Lasiocarpine-N-oxide
Senkirkine	
Europine	Europine-N-oxide
Heliotrine	Heliotrine-N-oxide

Table 5 — PAs to co-elute with PAs given in Table 4

Indicine, echinatine, rinderine (possible co-elution with lycopsamine/intermedine)
Indicine-N-oxide, echinatine-N-oxide, rinderine-N-oxide (possible co-elution with lycopsamine-N-oxide/intermedine-N-oxide)
Integerrimine (possible co-elution with senecivernine/senecionine)
Integerrimine-N-oxide (possible co-elution with senecivernine-N-oxide/senecionine-N-oxide)
Heliosupine (possible co-elution with echimidine)
Heliosupine-N-oxide (possible co-elution with echimidine-N-oxide)
Spartioidine (possible co-elution with seneciphylline)
Spartioidine-N-oxide (possible co-elution with seneciphylline-N-oxide)
Usaramine (possible co-elution with retrorsine)
Usaramine N-oxide (possible co-elution with retrorsine N-oxide)

[39] Available at: https://pubmed.ncbi.nlm.nih.gov/34715696/

64. Data should be collected for individual PAs and not as the sum of the PAs given in Table 4 and Table 5. The results below of the LOQ should be recorded as non-detect. Analytical results should be reported in µg/kg or µg/L.

    It is important to note that Table 4 and Table 5 are not exhaustive. Data for additional 1,2-unsaturated PAs beyond those listed in Table 4 and Table 5 may also be submitted. For example, a certain edible Asteraceae plants contain significant amounts of PAs not listed in these tables, such as petasitenine and neopetasitenine

65. Considering occurrence data and regional representation presented in the JECFA 2020 evaluation and other sources [40], it may be recommended to collect data on the food categories in Table 6. The CCCF18 decides which foods to choose.

Table 6 — Food category and greatest concern foods

Food category[a]	Greatest concern [b] foods
Cereals and cereal products	Grain flour[c], bread[c]
Teas (tea and other herbal teas)	Black tea, green tea, chamomile, rooibos, raspberry leaves, blackberry leaves, nettle, marshmallow, lemon verbena, sage
Culinary herbs	Lovage, oregano, marjoram, parsley, thyme, savory, borage mint, lemon balm, anise, basil, curry, chive, peppermint, fennel
Salad plants	Rocket
Spices	Cumin, anise seed
Food supplements	Herbal supplements, pollen-based supplements
Bee products	Honey, pollen, royal jelly
Foods of animal origin	Eggs[d], milk[d], meat[d]
a) There is a lack of regional data for all food categories. b) Foods were selected based on the frequency or levels of PAs reported in the literature. The available occurrence data indicate that teas, herbal and pollen-based food supplements, salad plants, culinary herbs, spices, honey, and pollen are the greatest concern foods in terms of the frequency and level of detection of PAs. c) There is a lack of data and the frequency of PA detection is low. d) In the JECFA 2020 safety evaluation, it is recommended to provide occurrence data, especially in foods of animal origin. The data show that the frequency and level of PA detection is low.	

66. Sampling for PA analysis should follow the *General Guidelines on Sampling* (CXG 50-2004) developed by the Codex Committee on Methods of Analysis and Sampling (CCMAS).

67. Before launching the call for data, it is appropriate to discuss and agree on the minimum analytical requirements for submitting data to the GEMS/Food database based on the information in this discussion paper (see paragraph 40).

[40] Patrick P. et al. 2018. Occurrence of pyrrolizidine alkaloids in animal- and plant-derived food: result of a survey across Europe. https://doi.org/10.1080/19440049.2017.138272
Florian K. et al. 2020. Occurrence and Risk Assessment of Pyrrolizidine Alkaloids in Spices and Culinary Herbs from Various Geographical Origins. https://doi.org/10.3390/toxins12030155
Picron J,F. et al. 2018. Analytical strategies for the determination of pyrrolizidine alkaloids in plant-based food and examination of the transfer rate during the infusion process.
https://www.sciencedirect.com/science/article/abs/pii/S0308814618310288?via%3Dihub

APPENDIX II

PROPOSAL FOR THE REVISION OF THE
***CODE OF PRACTICE FOR WEED CONTROL TO PREVENT AND REDUCE PYRROLIZIDINE ALKALOID CONTAMINATION IN FOOD AND FEED* (CXS 74-2014)**

PROJECT DOCUMENT

(For consideration by CCCF)

1) Purpose and scope of the project

The proposed revision aims to update the *Code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed (*CXC 74-2014).

The available occurrence data indicate that teas, herbal and pollen-based food supplements, salad plants, culinary herbs, spices, honey, and pollen are the greatest concern foods in terms of the frequency and level of detection of Pyrrolizidine Alkaloids (PAs).

The current Code of Practice (CoP) focuses on weed control to prevent and reduce PAs. Due to the nature of beekeeping activity, the measures for weed control mentioned in the current CoP might not be appropriate for preventing and reducing the presence of PAs in honey. It would also be appropriate to update the CoP to include new approaches to weed control in primary production for other foods and recommendations for food and feed establishments on good manufacturing/hygiene practices.

2) Relevance and timeliness

PAs were assessed by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) at its eightieth meeting, which took place in Rome, Italy, from 16 to 25 June 2015. Details of the assessment can be found in WHO Food Additives Series: 71-S2, Safety evaluation of certain food additives and contaminants, Supplement 2: Pyrrolizidine alkaloids. Available at https://apps.who.int/iris/rest/bitstreams/1318952/retrieve. JECFA concluded that the presence of PAs in certain foods is of concern.

3) Main aspects to be covered

This revision will focus on expanding measures for weed control in primary production, excluding bee products, and developing recommendations for bee products. It will also develop recommendations for food processors, including primary production, testing strategies under good production and hygiene practices.

4) Assessment against the criteria for the establishment of work priorities

(a) **Consumer protection from the point of view of health and fraudulent practices**. To protect consumer health, exposure to PAs should be prevented or reduced. An update of the existing CoP providing recommendations to governments and feed and food business operators will help prevent contaminated food from entering the market.

(b) **Diversification of national legislations and apparent resultant or potential impediments to international trade. Currently, the best practices and legislations.** An update of the existing CoP is needed to ensure that the most recent information on recommended practices for preventing and reducing PAs is available to all member countries. It will also provide the means to enable exporters to ensure reduced levels of PAs and to assist in compliance with any MLs that may be established in the future.

(c) **Scope of work and establishment of priorities between the various sections of the work.** The update of the existing CoP will address all relevant measures for preventing or reducing PAs at the different steps in the food and feed chain: production, harvest, storage, processing, and distribution.

(d) **Work already undertaken by other international organizations in this field.** Codes of practice and/or guidelines and recommendations to reduce the presence of PAs have been developed by sector organisations for specific foods (such as tea and herbal infusions, food supplements, and herbs).

5) Relevance to Codex Strategic Goals

(a) **Goal 1: Address current, emerging, and critical issues in a timely manner. Updating the CoP for prevention or reduction of PAs in food and feed will address the current need for guidance to ensure consumers' health**.

(b) **Goal 2: Develop standards based on science and Codex risk-analysis principles. This work will apply risk analysis principles in updating the CoP by using scientific data and recommendations from FAO/WHO and other recognized expert bodies to support a reduction in consumers' exposure** to PAs.

(c) **Goal 3: Increase impact through the recognition and use of Codex standards.** The proposed update of the CoP ensures that information on recommended practices to prevent and reduce the presence of PAs consists of current best practices and will be available to all member countries, especially those with fewer resources to devote to this topic.

(d) **Goal 4: Facilitate the participation of all Codex Members throughout the standard-setting process.** The update of the CoP will inform all Codex members through the Codex Step process.

(e) **Goal 5: Enhance work management systems and practices that support the efficient and effective achievement of all strategic plan goals.** An update of the CoP will help ensure the development and implementation of effective and efficient work management systems and practices by providing basic guidance for countries and producers to keep foods and feeds highly contaminated with PAs out of the marketplace.

6) Information on the relationship between the proposal and other existing Codex documents

This proposal concerns an update of the existing *Code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed* (CXC 74-2014).

7) Identification of any requirement for any availability of expert scientific advice

PAs were assessed by the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA), at its eightieth meeting, which took place in Rome, Italy, from 16 to 25 June 2015.

8) Identification of any need for technical input to the standard from external bodies

There is no identified need for additional technical input from external bodies.

9) Timeline for completion of the new work

Work will commence following the recommendation by CCCF and approval by CAC in 2025. Completion of work is expected by 2028.

PROPOSED REVISED *CODE OF PRACTICE FOR WEED CONTROL TO PREVENT AND REDUCE PYRROLIZIDINE ALKALOID CONTAMINATION IN FOOD AND FEED* (CXC 74-2014)

(For information)

PROPOSED REVISED CODE OF PRACTICE ~~FOR WEED CONTROL~~ TO PREVENT AND REDUCE PYRROLIZIDINE ALKALOID CONTAMINATION IN FOOD AND FEED

INTRODUCTION

1. ~~Pyrrolizidine alkaloids (PAs) are natural toxins occurring in a wide variety of plants. Over 6 000 plant species throughout the world are expected to contain PAs. PAs are probably the most widely distributed natural toxins that can affect wildlife, livestock and humans.~~ Pyrrolizidine alkaloids (PAs) are toxins produced by an estimated 6000 plant species. More than 600 different PAs, mainly 1,2-unsaturated PAs, including their associated nitrogen oxides (N-oxides) are known, and new PAs continue to be identified in both new and previously studied plant species. In this Code of Practice (CoP), the term "PAs" used by itself refers to saturated and 1,2-unsaturated PAs and their associated N-oxides and the term "1,2-unsaturated PAs" refers to all 1,2-unsaturated PAs and their associated N-oxides. The main plant families known to produce PAs are Boraginaceae (all genera), Asteraceae (tribes *Senecioneae* and *Eupatorieae*) and Fabaceae (genus *Crotalaria*). Various plant species in these and other families produce characteristic mixtures of 1,2-unsaturated PAs and their saturated analogues.

2. ~~PAs have a common toxicity profile with the liver being the main target organ of toxicity. Major signs of toxicity in all animal species include various degrees of progressive liver damage (centrilobular hepatocellular necrosis), and veno-occlusive disease. Furthermore, the International Agency for Research on Cancer (IARC) has classified three PAs, lasiocarpine, monocrotaline and riddelliine, as 'possibly carcinogenic to humans' (Group 2B). PAs may differ in potency, the relative potencies are currently not known due to lack of oral toxicity data on individual PAs, which hampers risk assessment for PAs. 2.~~ The main target organ of PA toxicity is the liver, which may result in various degrees of progressive liver damage and veno-occlusive disease. Furthermore, the International Agency for Research on Cancer (IARC) has classified three PAs, lasiocarpine, monocrotaline and riddelliine, as 'possibly carcinogenic to humans' (Group 2B). Most studies of toxicity and of occurrence of PAs in food, have focused on the 1,2-unsaturated PAs.

3. ~~Risks to humans may arise from the intake of PA contaminated food of vegetable or animal origin and outbreaks of toxicity in farm animals cause economic losses to farmers and rural communities. Direct human cases of poisoning via food are well documented, which in some cases have resulted in deaths. Also, consumption of grain or grain products (flour or bread) contaminated with PA-containing seeds has caused outbreaks of poisoning. Further, plant parts which contain PAs have been identified in foods prepared from agricultural crops, i.e. salad leaves. PAs were also found in products from animal origin, i.e. milk and eggs, indicating transfer of PAs from feed to edible tissues.~~ Humans are exposed to PAs via the consumption of PA contaminated food. Direct human exposure has been reported for 1) the direct and deliberate use of PA-containing plant species as herbal teas or herbal supplements, 2) the consumption of grain or grain products (e.g., flour or bread) and other foods (e.g., culinary herbs) contaminated with PA-containing plant parts, 3) the intake of (drinking) water from wells surrounded with PA-containing plants, 4) consuming honey, 5) intake of products of animal origin contaminated via transfer of PAs from feed. Direct animal poisoning has been reported for animals consuming PA-containing plants due to feed scarcity.

4. ~~Although there are gaps in the information available on the toxicity and relative potency of individual PAs, and the contribution of different foods to overall exposure, dietary exposure to PAs should be as low as possible due to the potential health-threatening effects that can be caused by ingestion of these toxins via feed or food. To achieve this, management practices aimed at the prevention and reduction of contamination of food and feed with PAs must be undertaken.~~ The fact that widely consumed foods such as cereals and cereal products, teas (tea and other herbal teas), milk and dairy products, eggs, meat, honey, herbal and pollen-based food supplements, salad plants, culinary herbs, spices and pollen are at risk makes the prevention and reduction of PA contamination in food and feed very important. Dietary exposure to PAs should be as low as possible due to their potential health-threatening effects. There are PA mitigation measures reported at various stages of the food and feed production process, including pre-harvest, harvest, post-harvest, and manufacturing. These measures applied to both plant-based products (such as cereal and other crops) and animal-derived foods.

   ~~Management practices to prevent or reduce PA contamination of food and feed can comprise weed management (removal/reduction) practices to reduce exposure of food-producing animals, including livestock and bees, to PA-containing plants, and practices to reduce presence of PAs in raw and processed commodities. This Code of Practice focuses on weed control. Deliberate use of PA-containing plants for foods and feed cannot be justified for any reason without appropriate assessment.~~

~~It should be emphasised that total eradication of PA-containing plants is not feasible or ecologically desirable. Also, grazing animals usually avoid eating most growing plant species containing PAs under normal circumstances. Generally, livestock graze on PA-containing plants when feed gets scarce in conditions of drought or on over-grazed pastures. Livestock may also consume PA-containing plants when they are present in dried form in feed. Therefore, good feeding practice is important besides management through weed control.~~

~~OBJECTIVE~~ SCOPE

5. ~~This Code of Practice aims to provide good management practices for weed control of PA-containing plants to prevent and reduce the contamination of food and feed with PAs. In this regard, this code will cover control measures for the management of the PA-containing plant as well as measures for control of plant release and spread.~~ The scope of this CoP is to provide guidance for good management practices in primary[1] production and food and feed establishments[2] to prevent and reduce PAs contamination. The CoP includes measures on the following key topics:

   - Identification of PA-containing plants
   - Knowledge and raising awareness for PA-containing plants
   - Preventing the spread and release of PA-containing plants
   - Integrated weed management (IWM) in cropland
   - Beekeeping based on good colony/hives and, where possible, vegetation management
   - Measures to prevent cross-contamination in all areas
   - Good hygiene and manufacturing practices for food and feed business operators, including primary production
   - The roles of brokers/suppliers/collectors
   - Responsibilities of competent authorities

   The placing on the market as food or feed of certain plants that naturally contain PA is outside the scope of this CoP. This CoP focuses on weed control for foods other than bee products.

~~3. SCOPE~~

~~The scope of this Code of Practice is to provide guidance to prevent contamination of food and feed with PAs on the one hand and, where contamination cannot be completely avoided, to reduce the PA contamination in food and feed by weed control. This Code of Practice should be read in conjunction with other relevant Codes of Practice for the prevention and reduction of other contaminants in food and feed.~~

~~4. EVALUATION OF COMPLIANCE WITH RELEVANT LEGISLATION~~

~~All management practices presented in this Code of Practice shall be followed in compliance with relevant national or international legislation and standards, including general requirement for consumer and worker protection.~~

LIMITATIONS

6. It should be recognised that the implementation of the management measures described in this ~~Code of Practice~~ CoP may be difficult in ~~a number of~~ some countries. This may be either due to lack of knowledge or resources or due to geographical, environmental or practical limitations, such as ~~the~~ an excessively large land area ~~of land being too large,~~ or the inaccessibility of certain regions ~~for~~ to agricultural machinery. The measures described in this ~~Code of Practice~~ CoP serve therefore as guidance and should be assessed by national authorities or other professional and advisory bodies to determine their suitability and practicality for country-specific conditions. ~~each measure described in this Code of Practice should be assessed by national authorities or other professional and advisory bodies to ensure that it is appropriate and practical for their country-specific conditions.~~

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1 Primary production: Those steps in the food chain up to and including storage and, where appropriate, transport of outputs of farming. This would include growing crops, raising fish and animals, and the harvesting of plants, animals or animal products from a farm or their natural habitat.

2 Establishments mean any unit of a food or feed business.

~~There is currently insufficient information concerning the effectiveness of the various management measures and therefore no full evaluation of the management measures can be conducted. When such information becomes available, an evaluation of the effectiveness of the proposed management measures would be helpful in identifying the most appropriate combination of practices for management of PA-containing plants thereby lowering the chance of PA-contamination of food and feed.~~

~~6. GENERAL PRINCIPLES FOR WEED CONTROL OF PA-CONTAINING PLANTS~~

~~To ensure adequate prevention of the spread of PA-containing plants, and to lower the costs of control measures, early detection and identification of these plants is essential followed by action to prevent contamination of food and feed.~~

~~To achieve an early detection, raising awareness by providing good information to the farmers and local population (including contractors and roadside maintenance staff) is critical. Information could be provided by using materials such as leaflets and website information with an overview and description of the most important PA-containing plants, their ecology, the need to proceed to action and how/where. In this respect, it is important to adapt the type of recommendations to the situation of the person involved, i.e. private persons keeping horses, sheep etc. on a small piece of land need other instructions than professional farmers. Communication with relevant national and local government organisations should also take place.~~

~~Once PA-containing plants are detected, if suitable data are available, the risks for human and animal health must be established in order to identify the need for an integrated weed management plan. In this respect, it must be recognised that the different PA-containing plants may react in a different way to a particular management measure. Therefore, it is always important to keep the ecology of the specific plant in mind. Additionally, influences of weather or climate must be taken into account. When seeking to prevent the spread of the PA-containing plants, all landowners, occupiers and managers must take a collective responsibility to ensure that effective control of the spread is achieved.~~

RECOMMENDED PRACTICES

7. Some plants are known to contain high levels of PAs. For example, Senecio and Heliotropium are reported in the literature to contain 1,000 - 13,000 ppm of individual PA. Even a very small amount of contamination of food or feed with parts of PA-containing plants containing high levels of PA can significantly increase PAs contamination. In this case, standard good management practices such as measures based solely on weed control may be insufficient to adequately prevent and reduce PA contamination from primary production through to the production of the final product. Therefore, further measures should be included in management practices for plants containing high amounts of PA.

8. To prevent and reduce PA contamination, it is important that operators involved in primary production and establishments of food and feed collaborate closely. Each party should implement appropriate measures within their respective responsibilities to mitigate the risk of PAs contamination. Competent authorities should participate in and support the development of management practices and ensure their effectiveness through monitoring and control.

A. GATHERING KNOWLEDGE AND RAISING AWARENESS

9. It is critical to identify PA-containing plants and provide information to relevant parties to prevent their spread and mitigate their presence in the food and feed production chain. This information may be supported with national or regional regulations on the propagation, sale and distribution of PA-containing plants. Competent authorities should develop a database of regionally important PA-containing plants, including information on specific PAs they contain, the food and feed products they may contaminate, photos of the plants at all growth stages, including their seeds and the risk zones. The inclusion of macroscopic-microscopic images of pollen of PA-containing plants in the database can help predict PA contamination in bee products.

10. Competent authorities can share information through websites (e.g., the database) and leaflets summarizing this CoP and regional practices. Trainings should be organized using appropriate materials to raise awareness.

11. Horticulturists and neighbouring should be provided with educational materials. The general public should also be advised on preventing the spread of PA-containing plants from urban areas to agricultural and other lands.

12. Authorities should educate wild harvesters and small-scale distributors about PAs contamination, as even a single contaminated harvest can compromise the safety of an entire batch.

13. Authorities should develop targeted educational materials for beekeepers, highlighting risk zones and PA-containing plants found in beekeeping areas.

B. EVALUATION OF THE NEED ~~TO PROCEED TO~~ FOR ACTION IN PRIMARY PRODUCTION FIELDS

14. ~~Before considering any action, the need to proceed to action~~ The necessity of taking action should be established by identifying the risks posed by the presence of PA-containing plants. This could be done by setting up a tiered risk characterisation approach based on:

- the presence and spread of PA-containing plants in and around land where primary production takes place;
- toxicity of the particular PAs, if known, present in the plant;
- the relevant contributions of the various PA-containing plants to the specific or total PA intake of the livestock or presence in food/feed, if known;
- proximity of the PA-containing plants to ~~arable fields~~ cropland and meadows/pastures/grasslands;
- level of infestation;
- weed management systems in place;
- harvesting methods used and time of harvest;
- post-harvest processing;
- local circumstances;
- climate;
- soil type; and
- vegetation cover of receiving land.

15. The likelihood of PA-containing plants spreading to land used for ~~agricultural practices or~~ grazing ~~and/~~or feed~~/~~ and forage production should serve as a primary factor for assessing risks ~~be the determining factor for assessment of the risk~~.

16. As an example, principles for assessing and managing the risk posed to livestock by ragwort (Jacobaea vulgaris), a common PA-containing plant, have been identified. These have been based on practical considerations of the proximity of the ragwort to pastures for livestock (bullet 4 paragraph 14~~bullet 3 above~~):

- high risk: ragwort is present and flowering/seeding within 50 m of land used for grazing by food-producing animals or land used for feed/forage production;
- medium risk: ragwort is present within 50 m to 100 m of land used for grazing by food-producing animals or land used for feed/forage production;
- low risk: the land on which ragwort is present is more than 100 m from land used for grazing by food-producing animals or land used for feed/forage production.

17. In the example of ragwort control, when a “high risk” situation is identified, the guidance is that immediate action should be taken to control the spread of PA-containing plants using appropriate control techniques taking account of the status of the land. In case of a medium risk, a control policy may be established to ensure that when the situation changes from a medium to a high risk of spread, it is identified and dealt with in a timely manner using appropriate control techniques taking account of the status of the land. In case of a low risk, no immediate action is required.

18. Similar risk assessments and resulting actions could be carried out for other PA-containing plants, ~~but~~ noting that defining risk zones and appropriate actions in other situations, will require ~~the~~ different ecology of the relevant PA-containing plants to be ~~taken into account~~ considered alongside the bullets in paragraph 14~~16~~.

C. ~~RECOMMENDED~~ PRACTICES FOR PRIMARY PRODUCTION

~~8.1. Management of the presence of PA-containing plants~~

~~For managing the presence of PA-containing plants, preferably a combination of non-chemical and chemical methods, i.e. integrated weed management, should be applied to obtain the most effective results.~~

~~The use of an integrated weed management plan could reduce the use of and reliance on herbicides, thereby lowering the chance of herbicide resistance, and allows weed management in most environments. However, it should be noted that in those cases where appropriate herbicides are available, their application alone could be sufficiently effective to manage weed presence.~~

~~Furthermore, an integrated weed management plan should be accompanied with practices to reduce the spread of PA-containing plants thereby preventing infestations to spread.~~

19. ~~It should be kept in mind for the management practices described in this section that their application should not result in harmful consequences for agriculture, the livestock or the pasture. Some methods may be destructive for other plant species (such as the crop) as well as to the target species. Applying these methods must be directed to the eradication of individual plants and done after good planning taking into account possible risks to the environment.~~ It should be emphasised that total eradication of PA-containing plants is not feasible or ecologically desirable. Also, grazing animals usually avoid eating most growing plant species containing PAs under normal circumstances. However, livestock may graze on PA-containing plants when feed gets scarce in conditions of drought or on over-grazed pastures. Additionally, livestock may inadvertently consume PA-containing plants when they are present in dried form in feed.

C.1. Cropland Management

20. For the management practices described in this section it should be kept in mind that their application should not result in harmful consequences for agriculture, the livestock or the pasture. Some methods may be destructive for other plant species (such as the crop) as well as to the target species. Applying these methods must be directed to the eradication of individual plants and done after good planning and considering possible risks to the environment.

21. Weeds that can be mixed with food and feed are classified as foreign matter under the definition of contaminants in national or international legislation and standards, where specific foreign matter levels are established.

22. Good weed management and hygiene practices for weeds will significantly contribute to reducing PA contamination of food and feed.

C.1.1. Weed management

23. Weed management should aim to fully prevent plants with high levels of 1,2-unsaturated PAs from entering the crop at harvest. For this, rational implementation of IWM, including preventative methods (legal and quarantine procedures, and others at the farm level), cultural methods (crop rotation, land preparation, use of cover crops, polyculture farming, mulching, water management, hand or mechanical weeding during the crop's life cycle), chemical methods (use of herbicides), biological methods (classical methods through the introduction of exotic natural enemies and increasing the population of already existing natural enemies), and other non-conventional methods (soil solarization, use of hot water, and others in development) should be used.

24. It is important to maintain prevention practices for PA-containing plants in post-harvest periods or on fallow cropland.

Preventative methods (Control of plant release and spread)

25. Preventive methods apply to the control of plant release and spread not only in cropland but also in other agricultural lands.

26. Various methods are used to prevent the introduction and spread of weeds, but the most important ones are those of a legal or biosecurity nature, which prohibit the movement and/or entry of certain types of imported commodities of plant origin or impose certain restrictions to the entry of certain commodities. The plant quarantine services should conduct risk assessment on the entry of exotic invasive plants and their likelihood to adapt to the new habitat and should compile a list of quarantine weed species based on their assessments.

27. Another way of preventing a weed species from entering an uninfected site from an infected one is by cleaning all tools and implements used. The use of animal manure and crop seeds heavily contaminated by weed seeds should also be avoided. Assure planting of high quality, weed-free crops and weed-free grass seeds. When possible, by national or regional laws and directives, use seed for planting that is not contaminated (e.g., certified seed).

28. Establishing weed-free buffer zones between infested and un-infested lands will help to contain infestation.

~~8.1.1~~ **Mechanical Cultural methods**

29. For crops, sound crop rotations can also minimise weed problems, since it will help to build up soil fertility and structure to produce increasing yields. Increased fertility in its turn will reduce the impact of weeds, and rotating crops can reduce the seeding and germination of weeds. Furthermore, it is recommended to use agricultural methods such as water and nutrient management or mulching. The plant material used for mulching must be free of PA- containing plants and their seeds.

30. PA-containing plants can be controlled by mechanical methods such as pulling, ploughing, milling and slashing. The timing of applying mechanical methods is important. These practices are best applied before flowering of the PA-containing plants to prevent seed production and seed spread. When handling the PA-containing plants, suitable precautions should be taken to protect operators' skin (contact with some plants might cause an allergic reaction) and prevent inhalation of pollen.

31. Effective manual control requires removal of the root crown and all larger roots. Therefore, manual control may only be effective for seedlings and young rosettes in contrast to bigger plants, which normally develop deep roots. In addition, effective hand pulling is useful for small infestations but is not cost-effective for large ones, nor is it suitable for large areas of land. In case of hand pulling, the plants should be handled and transported in a manner that prevents their spread, e.g. in hermetically sealed bags, and destroyed (burned) afterwards. It should be noted that disturbance of the soil may lead to more germination since buried seeds will be exposed to (sun) light.

32. In the case of crops, the best timing of applying mechanical methods is at the start of crop growth. Once the crops are dense, weeds have little chance to grow. In crops such as wheat and millet etc., fields should be weeded prior to planting and periodically during the first six weeks of the growth cycle. A final weeding, about two weeks before harvest, if feasible, could reduce the possibility of contamination of the harvest with toxic plant parts significantly. In fact, in legume crops, mechanical or manual weeding may be the only option if infestation is large. Attention should be paid to areas bordering the crop, as these may constitute a continuous reservoir for the weed infestation.

~~8.1.2~~ Chemical methods

33. When applied carefully at the recommended dose of the herbicide, chemical spraying with appropriate herbicides may be an effective way of controlling PA-containing plants. Herbicides used should be registered for application in that specific situation. Also, herbicides should preferably be used in combination with other ~~control~~ methods to increase their effectiveness. The choice of herbicide depends on the specific PA-containing plant species, the crop under cultivation and availability of appropriate herbicides.

34. For most PA-containing plants, in general the most effective time to spray herbicides is when the plants are actively growing and commencing flowering, i.e., in the spring before bloom and in the autumn applied to the new rosettes. Some herbicides require other timing due to their mode of action. PA-containing plants should not be sprayed when the plants are stressed either through lack of water, too much water, disease, insect or mechanical damage, as spray effectiveness will diminish.

35. The use of non-selective herbicides may damage the crop species and surrounding crops, pastures, waterways and environment. Hence, it is better to use selective herbicides or limit the use of non-selective herbicides for spray topping the PA-containing plant. Further, some PA-containing plants may develop resistance against a particular herbicide over time. It should be ensured that active substances are registered for the specific purpose in each country. In addition, as these substances are herbicides they may still have an inhibiting effect on crops, so care should be taken in case of possible bordering arable land.

36. In case of established PA-containing perennial plants, it is better to use systemic herbicides. Systemic herbicides are absorbed either by roots or foliar parts of a plant and are then translocated within the plant system to tissues that may be remote from the point of application.

37. In addition, care should be taken that herbicides are applied in suitable weather conditions, since the effective concentration of herbicides could be reduced when applied in unfavourable weather conditions, such as rain falls within 5 hours of application.

~~8.1.3~~ Biological methods

38. Natural enemies of a plant may be used to control PA-containing plants. It may be an economical and effective method. However, efficacy must have been established and the natural enemy must not present an environmental problem itself.

39. Tansy ragwort (Jacobaea vulgaris) densities may for example be reduced by the natural enemies Longitarsus jacobaeae (ragwort flea beetle) and a combination of Longitarsus jacobaeae and Tyria jacobaeae (cinnabar moth). Also Cochylis atricapitana, a ragwort stem and crown boring moth from Europe, was found to reduce the plant height of flowering plants and reduced the size and survival of rosettes. Another biocontrol agent used is Platyptillia isodactyla (ragwort plume moth) which has as common host marsh ragwort (Senecio aquaticus). Deuterocampta quadrijuga (blue heliotrope leaf-beetle) can completely defoliate blue heliotrope (Heliotropium amplexicaule), with both the larvae and adults feeding on the leaves.

40. However, good bio control is currently only feasible for a limited number of species as costs associated with finding, screening and testing potential agents can be very high. As such, successful biological control requires extensive development and establishment phases and costs. For most of the PA-containing plants no effective biological control agent is available. Research has shown that these methods are generally only very effective in the case of non-native plants.

~~8.1.4~~ Other non-conventional methods

41. Soil solarisation, flaming (burning) and use of boiling water are other controlling methods that may be used for small infestations.

42. As there is some evidence that changing soil moisture and nutrient availability may influence the PA content of the roots, leaves and flowers of PA-containing plants, cultivation methods may change the PA content of remaining plants. For example, increasing soil moisture will lead to higher PA-concentrations in the roots. PA concentrations are expected to be higher when nutrient availability is low, i.e. higher concentrations were found in plants grown in sand without nutrients than with nutrients. It is, however, not clear whether the same effect may be expected in flowering plants.

43. Do not transport PA-containing plants unnecessarily and only when stored in hermetically sealed bags or containers.

44. Not all management practices are suitable to be used on every type of land. Therefore, specific management practices to control PA-containing plants are discussed separately hereafter specified by type of land: arable fields, pastures, and areas bordering the crop or pasture.

C.1.2. Food and Feed Hygiene

45. Primary production should, as far as possible, implement measures to control PA-containing plants in accordance with the principles of good food hygiene and good animal feeding to ensure that food and feed are safe and suitable for its intended use.

46. Official control programs should be implemented to monitor and manage weed density in cropland designated for food and feed production. These programs should be particularly intensified as the harvest approaches and should include administrative measures to address non-compliances that may threaten food and feed safety. Example for administrative measures to address non-compliances, 1) if possible, harvesting can be done by hand, 2) optimisation of harvesting technology can help to avoid harvesting weeds with cultivated plants, e.g., by adjusting cutting height, 3) it can be directed to the food sector, which can minimize PA contamination through processing (e.g., extraction).

~~8.1.5 Arable fields~~

~~In the case of crops, the best timing of applying mechanical methods is at the start of crop growth. Once the crops are dense, weeds have little chance to grow. In crops such as wheat and millet etc., fields should be weeded prior to planting and periodically during the first six weeks of the growth cycle. A final weeding, about two weeks before harvest, if feasible, could reduce the possibility of contamination of the harvest with toxic plant parts significantly. In fact, in legume crops, mechanical or manual weeding may be the only option if infestation is large. Attention should be paid to areas bordering the crop, as these may constitute a continuous reservoir for the weed infestation.~~

~~8.1.6~~ C.2. Pastures and areas bordering the crop or pasture Management

47. In pastures and areas bordering the crop or pasture, use alternative plant sources to reduce undesirable growth, i.e., by planting vigorous perennials that will suppress the introduction and growth of PA-containing plants. This can be achieved by 1) sowing winter pasture species; 2) allowing a stand over of summer pasture feed; and 3) growing combinations of winter and summer pastures. Pasture management must also often go along with other forms of weed control, such as herbicides and mechanical means. This should be done in accordance with Good Agricultural Practice, such as appropriate sowing time and depth, adequate fertility and moisture at sowing, which is important to ensure good pasture management.

48. Landowners are generally not legally responsible for the areas bordering the crop or pasture, such as road verges, sides of a ditch and ruderal places. Therefore, for this type of land it is extremely important that all landowners, occupiers, relevant regional councils or agencies and managers take a collective responsibility to ensure ~~that~~ the effective control and prevention of ~~possible~~ the spread of PA containing plants ~~is achieved~~.

49. For large-scale restorations in pastures, mowing and cutting can be more easily applied. Cutting or slashing tansy ragwort (Jacobaea vulgaris) at the start or end of anthesis will reduce the number of flower heads. Therefore, it is recommended to do the first mowing when half of the plants start anthesis, and the second mowing when half of the re-established plants start anthesis again. On the other hand, fireweed (Senecio madagascariensis) should not be slashed in late spring or when more than 25% of the plants are flowering, as the mature plant, that otherwise might have died, may begin re-shooting. However, these mechanical methods are not always effective in killing the plants and may even encourage them to re-shoot as is observed with tansy ragwort (Jacobaea vulgaris) and Paterson's curse (Echium plantagineum). As a consequence, slashing or mowing may need to be executed on a very regular basis and be applied in combination with other control measures as part of an ~~integrated weed management~~ IWM plan. For example, high mowing frequencies can be combined with the use of additional nitrogen that will lead to the promotion of fast-growing grass species which will impair the germination and establishment of PA-containing plants.

~~Attention should be paid to areas bordering the pasture, as these may constitute a continuous reservoir for the weed infestation.~~

50. In pastures, PA-resistant livestock can be quite effectively used in grazing management to reduce PA-containing plants since it may weaken the plants and prevent prolific seeding. Antimethanogenic therapy with bacteria may be used to increase ruminant resistance to PA toxicity. Animals with no previous exposure to PAs are very susceptible to poisoning while animals with prior exposure to PA-containing plants show enhanced rumen detoxifying activity. The bacterium Peptostreptococcus heliotrinreducans most likely plays an important role in this process.

51. In addition, preferably non-food producing animals should be used as PAs may transfer from feed into milk and edible tissues. The best livestock to use are sheep, especially non-pregnant, non-food producing Merino sheep, or goats. If food-producing animals are used, the edible products could potentially contain high levels of PAs, and as a precautionary approach, these edible products must be segregated and not sold for human consumption until it is confirmed that they do not contain PAs. When removing animals from affected areas it is necessary to avoid transfer of seeds on their hooves, coats and digestive tracts, which can infest a new area. That is, livestock can spread seeds by consuming and passing viable seeds through their digestive tract. The seeds that survive the digestive tract are eliminated in the manure, which is rich in nutrients that can increase weed emergence. Thus, for some weed species it may be appropriate to prevent animal grazing when the plants are setting seeds, or the spreading of seeds by livestock can be prevented by placing them into quarantine.

52. Grazing management can be applied on low-level, widespread infestations. However, significant numbers of grazing animals must be available; water and fencing or herding to control movement must be set up and the timing, intensity and duration of grazing must be closely monitored and managed to prevent overgrazing. It must be recognised that overgrazing may lead to loss of the competitive nature of the pasture or of native plants, allowing PA-containing plants to return and spread over the bare soil, which could result in livestock poisoning. Hence, it is recommended to stop grazing during flowering of (a number of) PA-containing plants as their PA-production is then very high.

~~8.2 Control of plant release and spread~~

~~8.2.1 Identify alternative plant sources to reduce undesirable growth~~

~~For crops, sound crop rotations can also minimise weed problems, since it will help to build up soil fertility and structure to produce increasing yields. Increased fertility in its turn will reduce the impact of weeds, and rotating crops can reduce the seeding and germination of weeds. In pastures and areas bordering the crop or pasture, use alternative plant sources to reduce undesirable growth, i.e. by planting vigorous perennials that will suppress the introduction and growth of PA-containing plants. This can be achieved by 1) sowing winter pasture species; 2) allowing a stand over of summer pasture feed; and 3) growing combinations of winter and summer pastures. Pasture management must also often go along with other forms of weed control, such as herbicides and mechanical means. This should be done in accordance with Good Agricultural Practice, such as appropriate sowing time and depth, adequate fertility and moisture at sowing, which is important to ensure good pasture management. Furthermore, it is recommended to use agricultural methods such as water and nutrient management or mulching. The plant material used for mulching must be free of PA-plants and their seeds.~~

~~8.2.2 Control movement of plants/seeds over agricultural zones and pastures~~

~~Assure planting of high quality, weed-free crops and weed-free grass seeds. When possible by national or regional laws and directives, use seed for planting that is not contaminated (e.g. certified seed).~~

~~8.2.3 Control plant seed movement on vehicles and agricultural machinery~~

~~Clean vehicles, machinery and equipment that are used in infested areas to prevent introduction of the PA-containing plant to pastures or other agricultural land by spread of seeds. Weed-free buffer zones between infested and un-infested lands will help to contain any infestation.~~

~~8.2.4 Control plant seed movement on animals~~

53. In case that livestock has grazed in infested areas, place them into quarantine for several days as seed can be carried on the hooves and coats, and in the digestive tracts ~~of livestock~~. Inspect these quarantine areas regularly to assure no PA-containing plants will start infesting those areas.

~~8.2.5 Control of plant and seed movement from urban to agricultural lands and pastures~~

~~Provide educational material to horticulturists and neighbouring property owners to correctly identify PA-containing plants to prevent propagation of unwanted plant species. This information may be supported with national or regional regulations on the propagation, sale and distribution of PA-containing plants. Advise the general public on how to prevent the spread of unwanted, PA-containing plants from urban environments into agricultural and other lands.~~

C.3. Wild Collection

54. Harvesting of many plants in the wild is usually carried out manually, which helps prevent PA contamination. To further reduce the risk of PA contamination, it is critical that wild harvesters have sufficient knowledge to accurately distinguish the target plants from other physically similar and hard-to-differentiate plants.

C.4. Beekeeping

55. FAO has published guidelines for good beekeeping practices for sustainable apiculture, which aim to provide useful information and suggestions for a sustainable management of bees around the world, which can then be applied to project development and implementation. As stated in these guidelines, PA contamination from PA-containing plants can occur in bee products, especially honey and pollen.

56. It would be useful for the competent authorities to implement a general monitoring program for PA in honey and to carry out further studies in areas where honey has high 1,2-unsaturated PAs content to identify the plants responsible for the contamination. Based on this information, competent authorities can create maps based on density information of PA-containing plants and develop good colony and, where possible, vegetation management practices in these areas.

57. Beekeepers should avoid producing bee products from PA-containing plants.

58. Pollen and nectar are the two main sources utilized in the production of bee products. Existing research shows that the concentration of PA in pollen of PA-containing plants is much higher than in nectar. For example, in a study on *Echium vulgare*, Echium-type PAs were detected between 0.3-95.1 mg/kg in nectar and 500-35 000 mg/kg in plant pollen. The intensive transportation of pollen by bees to the hive is essential for worker bees to produce royal jelly, which is used for larval development in the spring. In areas where PA-containing plants are present and especially in areas where vegetation is adversely affected by climatic conditions, the use of traps that prevent pollen from entering the hive may be an option to reduce PA contamination. However, in such case, it may then be necessary to supplement the hive with fermented pollen (bee bread) with low PA concentration.

59. The higher risk of PA contamination in bee products occurs in post-harvest or fallow cropland, as experience indicates that of PA-containing plant density increases. Therefore, it is very important to have IWM practices for PA-containing plants in post-harvest and fallow cropland. Beekeepers should communicate well with farmers and support good management practices in this area.

60. The risk of PA contamination of bee products from beekeeping on cultivated cropland is low. However, since bees fly within a wide radius, the presence of PA-containing plants near hives locations should be monitored. Hives should be placed in alignment with the flowering period of the target crop.

61. With good vegetation and colony management, the risk of PA contamination in natural areas is expected to be low. Efforts to reduce the density of PA-containing plants in pastures and meadows can significantly reduce PA contamination in bee products.

62. When non-PA-containing plants are available, bees are less likely to forage on PA-containing plants. Therefore, the number of hives placed in a region should be carefully managed to avoid exceeding its floral resource capacity. For this purpose, competent authorities may consider implementing hive limitations for specific regions.

63. Beekeepers should prevent PA contamination at all stage of the migratory beekeeping process.

C.5. Prevention of cross-contamination

64. Cross-contamination between PA-contaminated produce and produce harvested following good management practices is a major problem. Therefore, raising awareness among those involved in primary production is essential to prevent cross-contamination. To further mitigate this risk, management practices should align with the principles of good agricultural practices (GAP).

D. PRACTICES FOR FOOD AND FEED ESTABLISHMENTS

65. If applicable, food or feed business operators should identify 1,2 unsaturated PAs as hazards in the food or feed safety systems under good hygiene management. They should also implement control measures check their effectiveness.

66. Based on the data and information collected, a product-specific action plan should be developed, including raw material specifications (e.g., the botanical material or preparations/extracts), action limits and a timeline for improvements.

67. Competent authorities should monitor and enforce compliance with food and feed hygiene principles in food and feed establishments.

D.1. Information on primary production and the role of brokers/suppliers

68. Processing has a limited effect to prevent and reduce PA contamination in food, and may, in some cases, even increase PA concentrations. Therefore, it is crucial to keep PA-containing plants out of the raw material supply.

69. It will be difficult to obtain information on the density of PA-containing plants after harvesting due to crops drying and the heterogeneous distribution of PA-containing plants in bulk lots. Therefore, it is important to gather information on the density and types of PA-containing plants in the production area(s) just before harvest and to adjust supply management accordingly. The density of PA-containing plants prior to harvest can be set as an acceptance criterion, considering account the harvesting method and subsequent food production processes. In case of non-compliance with the acceptance criterion, the primary producer may be advised to remove PA-containing plants before harvesting, and if this is done, the purchase of raw material will be realized.

70. Business officials and brokers involved in the supply chain should have adequate knowledge and awareness of PA-containing plants.

71. Brokers/suppliers should avoid cross-contamination during transportation and storage. Appropriate segregation, packaging and testing should be applied to different lots of primary produce where PA contamination is suspected.

D.2. Preparing raw materials

72. Products with different risk of contamination can be stored or processed separately. For example, if it is known that the harvesting method of a crop in the country is generally done by hand, the risk of PA in the crop is likely to be low. Where possible, it may be worthwhile to store this product in a different location from products with a high PA risk or to separate the production line.

73. Dried raw materials can be treated to remove dust, soil, pollen in an independent area before being put into establishment.

74. If appropriate, physical sorting should ensure that the final product complies with the amount of foreign matter specified in the standards and the design of the production line should aim to make a significant contribution to reducing PA contamination risk. Good physical sorting of the raw material of foods such as grains, culinary herbs, teas and spices must be done to produce unprocessed whole, milled, ground, crushed products.

75. Commercial mixtures of bee pollen often contain high amounts of PAs typical for plants of the genus Echium. These pollen grains are relatively easy to identify visually, as they are of dark purple colour. Technically it should be possible to sort out these dark purple pollen grains, e.g., by the combination of high-speed cameras and pressurized air pulses, which would blow out pollen grains passing by on a conveyor belt. This technique is already used for other foodstuffs. This measure would substantially reduce the PA content of such pollen mixtures. However, as PAs are also contributed by other plants, some PAs might still remain in the product.

76. Although removing pollen from honey is not desirable, it can be filtered to reduce PA contamination. In such cases, it should be labelled as filtered honey.

D.3. Processing

77. PAs are generally stable during common food processing, particularly in the preparation of infusions such as steeping dried teas in hot water, where only partial extraction occurs and thermal degradation is minimal.

78. Certain processing methods, such as heat treatment, fermentation, infusion preparation (transfer ratio) and other processes (grinding, washing and soaking) have shown potential in reducing PA levels in foods. For example, in the cooking of petioles and young ears of Petasites japonicus, a PA-containing plant, both boiling and soaking treatments were found to be important in reducing PAs, with a longer soaking time having a greater effect in reducing PAs. Hydrodistillation in the production of essential oils is effective in PA removal.

79. PA N-oxides—which are generally less toxic—can be converted into their more toxic free-base forms during prolonged or high-temperature processes, such as tea manufacturing or drying. For example, senecionine-type PANOs can convert to the free-base forms during manufacturing and brewing. This thermal conversion poses a potential health risk and may offset the benefits of some processing techniques. Therefore, manufacturing processing should be carefully evaluated and controlled to minimize unnecessary thermal conversion of PA N-oxides to PAs.

80. In silages with a high-water content PAs are generally prone to degradation, although the rate of degradation varies per condition and PA congener.

D.4. Prevention of cross-contamination

81. It is very important to avoid cross-contamination during transportation, storage, sorting and processing. Points of cross-contamination should be identified.

82. Vehicles, warehouses, operating equipment should be cleaned periodically. The initial reception points of raw materials and the storage area where the materials are stored after obtaining information on their PA content should be separated as far as possible.

D.5. Testing strategy

83. It is recommended to implement testing for the presence of PAs, either on the raw materials or at a stage during the processing that can ensure a reliable result that can be representative for the content in the final product.

84. Screening methods can be a good option to identify PAs in food in the broadest possible way without the need for reference standards and to identify PAs that need to be monitored. Using screening methods that allow an unlimited number of target, suspect and non-target PAs to be identified and screened simultaneously will reduce analysis costs, simplify the work and create a unique library for food businesses. Screening methods (e.g., ultra-high performance liquid chromatography coupled with high resolution tandem mass spectrometry) combined with an appropriate extraction technique (e.g., salting-out assisted liquid-liquid extraction) can be used for this purpose. With these methods, quantitative results of target PAs can also be obtained to determine the levels of PAs in foods. These methods can be used to identify PAs that should be monitored in foods of high concern for PA contamination, such as teas, herbal and pollen-based food supplements, salad plants, culinary herbs, spices, honey and pollen.

85. Validated quantitative methods should be used to determine the levels of PAs that should be monitored in foods.

86. In addition to analytical methods for the identification of individual PAs, it is useful to monitor foreign matter (e.g., weeds) in plant-based food and of pollen in honey and bee pollen at any stage during the preparation. In some cases, a strategy focused on controlling foreign matter and pollen may even be sufficient, provided a strong correlation is established and its effectiveness is demonstrated. Macroscopic-microscopic testing techniques can be used to identify plant parts or pollen.

87. For routine checks, a risk-based analysis testing strategy can be applied based on available occurrence data and the following information;

- Where available, primary production information of the raw material (e.g., grower's pre-harvest control, prevalence of PA-containing plants in the region of production)
- Where available, documentation of the raw material supply chain associated with the lot (e.g., field record, analytical reports)
- Risk of PA contamination in food and feed (e.g., high levels of PA can be detected for culinary herbs, teas, honey, salad plants, bee pollen, cereals and spices)
- Part of the plant to be produced (in general, PA are found in higher concentrations in seeds and flowering parts, with lower levels in leaves, stems and roots)
- The growth cycle (annual or perennial)
- The cultivation, harvest and processing conditions (mechanical harvesting, cleaning options)

88. If information on primary production is provided and it is known that mitigation practices for PA risk have been adequately implemented and available occurrence data support this information, periodic or skip testing may be applicable. However, if information on the raw material is insufficient and available occurrence data is variable, periodic testing should be applied.

89. By matching the results of qualitative and quantitative analyses with PA-containing plants as much as possible, providing retrospective information in the food production chain will make a significant contribution to preventing and reducing PA risk.

APPENDIX IV

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APPENDIX V

PROPOSED GUIDANCE ON SAMPLING AND ANALYSIS PERFORMANCE CHARACTERISTICS FOR THE COLLECTION OF DATA TO BE SUBMITTED TO THE GEMS/FOOD DATABASE FOR PYRROLIZIDINE ALKALOIDS IN FOODS

(For consideration by CCCF)

OBJECTIVE

1. The purpose of this guidance is to establish for sampling and analytical method performance criteria used to collect data on pyrrolizidine alkaloids (PAs) in food, intended for submission to the GEMS/Food database.

2. This guidance and the Instructions for Electronic Submission of Data Related to Chemicals in Food and Diet on the GEMS/Food database homepage aim to ensure that data collection is carried out in accordance with its requirements.

PAs WHOSE DATA WILL BE COLLECTED

3. Data should be collected for 1,2-unsaturated PAs (the term "1,2-unsaturated PAs" refers to all 1,2-unsaturated PAs and their associated N-oxides) in foods, as most studies on the toxicity and occurrence of PAs in foods have focused on 1,2-unsaturated PAs.

4. The occurrence data for the most frequently detected 1,2-unsaturated PAs[1] in foods, as outlined in Table 1, will facilitate future risk assessment. Therefore, it is recommended that all these 1,2-unsaturated PAs be analysed in foods and the corresponding data be submitted. If suitable analytical methods are available, it is also recommended to analyse the additional 14 PAs given in Table 2, as these are PAs known to co-elute with one or more of the 21 PAs given in Table 1. If available occurrence data strongly suggest that some of the PAs given in these tables are non-detected in regionally relevant foods, these PAs may not need to be analysed. In this case, PAs not analysed should be reported.

Table 1. Most frequently detected 1,2-unsaturated PAs in food.

Intermedine	Intermedine-N-oxide
Lycopsamine	Lycopsamine-N-oxide
Senecionine	Senecionine-N-oxide
Senecivernine	Senecivernine-N-oxide
Seneciphylline	Seneciphylline-N-oxide
Retrorsine	Retrorsine-N-oxide
Echimidine	Echimidine-N-oxide
Lasiocarpine	Lasiocarpine-N-oxide
Senkirkine	
Europine	Europine-N-oxide
Heliotrine	Heliotrine-N-oxide

Table 2. PAs to co-elute with PAs given in Table 1

-	Indicine, echinatine, rinderine (possible co-elution with lycopsamine/intermedine)
-	Indicine-N-oxide, echinatine-N-oxide, rinderine-N-oxide (possible co-elution with lycopsamine-N-oxide/intermedine-N-oxide)
-	Integerrimine (possible co-elution with senecivernine/senecionine)
-	Integerrimine-N-oxide (possible co-elution with senecivernine-N-oxide/senecionine-N-oxide)
-	Heliosupine (possible co-elution with echimidine)
-	Heliosupine-N-oxide (possible co-elution with echimidine-N-oxide)
-	Spartioidine (possible co-elution with seneciphylline)
-	Spartioidine-N-oxide (possible co-elution with seneciphylline-N-oxide)
-	Usaramine (possible co-elution with retrorsine)
-	Usaramine N-oxide (possible co-elution with retrorsine N-oxide)

[1] JECFA. 2020. Safety evaluation of certain food additives and contaminants, Supplement 2: Pyrrolizidine alkaloids. WHO Food Additives Series: 71-S2.

5. Data should be collected for individual PAs and not as the sum of the PAs given in Table 1 and Table 2. Results below the limit of quantification (LOQ) should be recorded as non-detect. Analytical results should be reported in µg/kg or µg/L.

6. It is important to note that Table 1 and Table 2 are not exhaustive. Data for additional 1,2-unsaturated PAs beyond those listed in Table 1 and Table 2 may also be submitted. For example, a certain edible Asteraceae plants contain significant amounts of PAs not listed in these tables, such as petasitenine and neopetasitenine.

FOODS WHOSE DATA WILL BE COLLECTED

7. Considering occurrence data and regional representation presented in the JECFA 2020 evaluation and other sources[2], it may be recommended to collect data on the food categories in Table 3.

Table 3. Food category and greatest concern foods

Food category[a]	Greatest concern [b] foods
Cereals and cereal products	Grain flour[c], bread[c]
Teas (tea and other herbal teas)	Black tea, green tea, chamomile, rooibos, raspberry leaves, blackberry leaves, nettle, marshmallow, lemon verbena, sage
Culinary herbs	Lovage, oregano, marjoram, parsley, thyme, savory, borage mint, lemon balm, anise, basil, curry, chive, peppermint, fennel
Salad plants	Rocket
Spices	Cumin, anise seed
Food supplements	Herbal supplements, pollen-based supplements
Bee products	Honey, pollen, royal jelly
Foods of animal origin	Eggs[d], milk[d], meat[d]
a) There is a lack of regional data for all food categories. b) Foods were selected based on the frequency or levels of PAs reported in the literature. The available occurrence data indicate that teas, herbal and pollen based-food supplements, salad plants, culinary herbs, spices, honey and pollen are the greatest concern foods in terms of the frequency and level of detection of PAs. c) There is a lack of data, and the frequency of PA detection is low. d) In the JECFA 2020 safety evaluation, it is recommended to provide occurrence data, especially in foods of animal origin. The data show that the frequency and level of PA detection is low.	

SAMPLING AND SAMPLE PREPARATION

8. Sampling for PA analysis should follow the *General guidelines on sampling* (CXG 50-2004) developed by the Codex Committee on Methods of Analysis and Sampling (CCMAS). Contamination due to the presence of PA-producing plants in foods, such as culinary herbs, salad plants, spices, teas, and pollen, occurs sporadically and is therefore non-homogeneous. Therefore, proper sampling is critical.

9. To ensure representative sampling, the number or quantity of incremental samples should be adjusted based on lot size and variability. For large lots that can be physically separated, dividing them into smaller, homogeneous sub-lots is recommended to enhance sample representativeness.

10. As the distribution of PAs is extremely non-homogeneous, samples should be prepared—and especially homogenized—with extreme care. The whole aggregate sample or portions of it should be used for homogenization/grinding to ensure representativeness.

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2 Patrick P. et al. 2018. Occurrence of pyrrolizidine alkaloids in animal- and plant-derived food: result of a survey across Europe. https://doi.org/10.1080/19440049.2017.138272
Florian K. et al. 2020. Occurrence and Risk Assessment of Pyrrolizidine Alkaloids in Spices and Culinary Herbs from Various Geographical Origins. https://doi.org/10.3390/toxins12030155
Picron J,F. et al. 2018. Analytical strategies for the determination of pyrrolizidine alkaloids in plant-based food and examination of the transfer rate during the infusion process.
https://www.sciencedirect.com/science/article/abs/pii/S0308814618310288?via%3Dihub

11. Aggregate sample of liquid foods and solid foods with small particle (particles smaller than 0.85 [3] mm) are homogenized by thorough mixing. The important source of possible PA contamination in honey is pollen. Honey is also a naturally crystallizing food. In such cases, mixing at low temperature (≤ 45°C) can be used to ensure homogenization. The content of encapsulated food supplements should be separated from the capsule with a material suitable for preparing an aggregate sample.

12. Samples of solid foods should be finely ground and thoroughly mixed to achieve the highest possible level of homogenization. Complete homogenization implies that particle size is small particle (particles smaller than 0.85 mm), and the variability associated with sample preparation is minimized. If smaller particle size cannot be achieved, then a larger portion should be taken for analysis to minimize heterogeneity issues.

ANALYSIS OF PERFORMANCE CHARACTERISTICS

13. Data should be obtained using quantitative analysis methods validated in accordance with the principles for the establishment of analysis methods in the CAC Procedural Manual. Analytical methods should meet the specific criteria[1,4] in Table 4.

Table 4. Specific criteria for individual 1,2-unsaturated PAs

Specific criteria		**Explanations**
LOQ for solid foods (≤ µg/kg)	10	
LOQ for liquid foods (≤ µg/L)	0.15	
RSD_r and RSD_R (≤%)	20	For very low concentrations (e.g. < 0.01 mg/kg) some laboratories may accept method performance criteria that fall outside of these criteria (e.g. 60–120% with a RSDr and RSD_R < 30%).
Recovery (%)	70-120	

[3] General standard for contaminants and toxins in food and feed (CXS 193-1995)

[4] Guidelines on performance criteria for methods of analysis for the determination of pesticide residues in food and feed (CXG 90-2017).
COMMISSION IMPLEMENTING REGULATION (EU) 2023/2783 of 14 December 2023 laying down the methods of sampling and analysis for the control of the levels of plant toxins in food and repealing Regulation (EU) 2015/705.
https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=OJ:L_202302783

APPENDIX VI

List of Participants

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