Agenda Item 10 **CX/CF 24/17/10**

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JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

17th Session
15-19 April 2024
Panama City, Panama

PYRROLIZIDINE ALKALOIDS

(Prepared by Electronic Working Group chaired by European Union)

INTRODUCTION

1. At the 15th session[1] of CCCF (CCCF15, 2022), CCCF 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.

2. At the 16th session[2] of CCCF (CCCF16, 2023), it was agreed to issue a circular letter (CL) requesting comments on the recommendations in the discussion paper CX/CF 23/16/11 and that the EWG, chaired by the EU, working in English only, prepares a revised paper based on the comments received in response the CL for consideration by CCCF17.

3. The CL 2023/40-CF was issued in July 2023 requesting comments by 30 September 2023 on the recommendations for follow-up risk management actions on PAs as described in CX/CF 23/16/11, Appendix I, paragraph 33, points (a) to (d), namely:

   (i) Consider whether the discussion paper in Appendix I of CX/CF 23/16/11 provides sufficient data/information that support the recommendations in paragraph 33 in relation to the update of the *Code of Practice (CoP) for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed* (CXC 74-2014) and, if not, provide general and specific comments on additional points that should be considered in the discussion paper that could guide further work of the EWG.

   (ii) In case the discussion paper provides sufficient grounds to proceed with the new work:

      (a) Review the proposal for new work in Appendix II and provide general/specific comments to improve the project document as appropriate;

      (b) Indicate agreement to establish an EWG to update CXC 74-2014 for consideration by CCCF17 (2024).

   (iii) With regard to the recommendation (a) Codex Members and Observers are invited to provide input on minimum requirements to which occurrence data have to comply with to submit to GEMS/Food database that would enable the EWG to prepare a proposal for consideration by CCCF17.

4. Comments were received from Canada, Chile, Egypt, Iraq, New Zealand, Türkiye and USA. Editorial comments on the discussion paper were received from the Netherlands after the deadline for submission of comments to CL 2023/40-CF.

5. As regards recommendation (i) and (ii), whether the discussion paper in Appendix I of CX/CF 23/16/11 provides sufficient data/information that support the update of the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed*, the following comments were received:

   – Chile, Egypt, Iraq and New Zealand agree to proceed with the new work to update the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed* and did not provide further comments.

[1] REP22/CF15, para 224
[2] REP23/CF16, para 84

– Canada supports the update of the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed*. However, it is unclear if the Code of Practice should be updated or if the new information could be provided as Annex to the existing Code of Practice. The discussion paper should be further elaborated to provide more information on the options to update the Code of Practice or provide the new information as Annex before taking a decision.

– Türkiye provided information on ongoing work on the prevention of the presence of PAs in oregano, cumin and mint and on monitoring of PAs in honey, tea and some herbal infusions. Türkiye supports the work on updating CXC 74-2014. However as regards the presence of PAs in honey, given the nature of beekeeping activity, it is considered that the focus on weed control in the Code of Practice is not that relevant for the presence of PAs and that for PAs in honey a separate Code of Practice should be developed. Therefore, it is appropriate to clarify first the scope and foods to be covered and to start the work on food that are at risk for pyrrolizidine alkaloid contamination due to weed contamination.

– The U.S.A is of the opinion that the discussion paper should be further developed before taking a decision on new work for updating the Code of Practice. In particular, the discussion paper should elaborate more on what information is available on mitigation of PAs in specific foods. Taking into account the outcome of the JECFA assessment, honey and tea should be the highest priority while information on potential mitigation methods for herbal infusions, food supplements, herbs and spices is also of interest.

6. As regards recommendation (iii) on the elaboration of a document defining the minimum requirements to which occurrence data have to comply with for submission to GEMS/Food database, the following comments were received:

– Canada considers that the analytical requirements need to be determined and agreed, that fully validated methods of analysis and high-quality standards should be available before launching a call for data and that data gathering would take several years.

– Türkiye supports the development of a document with minimum analytical requirements for occurrence data but highlights also the importance to provide criteria for the sampling method.

– The U.S.A agrees to the issuance of minimal data requirements but highlights the importance of defining the purpose of gathering new data given that there might be different data requirements for different purposes. Given the challenges related to the analysis of PAs in food, it might be appropriate to consult with CCCMAS on minimum analytical requirements.

7. The discussion paper in Appendix I has been updated taking into account the comments received to CL 2023/40-CF. It contains in particular updates as regards methods of analysis and sampling procedures and on the content of the different Codes of Practice /guidance on the prevention of PAs in food.

8. The EWG (the list of participants is in Appendix IV) was requested to particularly examine and provide comments on:

– the proposed minimum analytical requirements for the analysis of PAs in food (§ 32 of Appendix I)

– the proposed principles for guidance on sampling procedure (§ 38 of Appendix I)

– the proposed options for updating the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed* and the possible need for elaboration of a separate Code of Practice for prevention and reduction of pyrrolizidine alkaloid contamination in honey (§ 46 of Appendix I)

– the proposed approach for the elaboration of a document providing a guidance for sampling and defining the minimum analytical requirements to which occurrence data have to comply with for submission to the GEMS/Food database (§ 47 of Appendix I).

– the proposed recommendations for agreement by CCCF17

9. Comments were received from Belgium, The Netherlands and the U.S.A. Divergent views were expressed as regards the proposed recommendations for agreement by CCCF17 (see § 11) in particular on

– the need to address the prevention and reduction presence of pyrrolizidine alkaloids in honey in a separate Code of Practice or as a separate Annex to the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed* (CXC 74-2014) (see § 11 (b)).

– the need to define minimum analytical minimum requirements for the occurrence data to be submitted to GEMS/Food database following a call for data. Reference is made to the discussions on the guidance on data analysis for development of maximum levels and for improved data collection that establishing analytical requirements in a call for data would restrict the time span or geographical range of data by not allowing data submitted before the analytical requirements would be in place or data not compliant with the requirements for use in the data analysis. It might further discourage data submission from regions /countries where the analytical equipment to meet the requirements is not available (see § 11 (c)).

– the need to define the purpose of the new data before launching a call for data, i.e. if the data are requested to support the review of the Code of Practice or if the data are also requested for a possible future development of maximum levels (without necessarily committing to accept in the future new work setting of MLs) (see § 11 (c)).

RECOMMENDATIONS TO CCCF

10. CCCF is requested to consider the following recommendations:

a. agree on new work to update the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed* (CXC 74-2014) eventually by complementing the Code with specific Annexes for tea, herbs and herbal infusions, food supplements and spices (see § 46 of Appendix I) and to agree on the proposal for new work as outlined in Appendix II;

b. discuss the need for a separate code of practice for the prevention and reduction of the presence of PAs in honey (see for justification § 46 of Appendix I). In case it is agreed that such separate code of practice is appropriate, a discussion paper is to be prepared by an EWG for consideration by CCCF18;

c. agree to elaborate a document (see § 46 of Appendix I) providing a guidance for sampling (based on the principles outlined in § 38 of Appendix I) and defining the minimum analytical requirements (based on the criteria outlined in § 32 of Appendix I and consult with CCMAS) to which occurrence data have to comply with for submission to the GEMS/Food database. In case it is agreed that such document should be elaborated, the document should be prepared for consideration by CCCF18 in view of issuing a future call for data on the presence of PAs in food and feed;

d. re-establish the EWG to work on an update of the *Code of Practice for Weed Control to Prevent and Reduce Pyrrolizidine Alkaloid Contamination in Food and Feed* (CXC 74-2014) and to prepare a document providing a guidance for sampling and defining the minimum analytical requirements to which occurrence data have to comply with for submission to the GEMS/Food database for consideration by CCCF18.

APPENDIX I

DISCUSSION PAPER ON PYRROLIZIDINE ALKALOIDS

(For consideration by CCCF)

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*).

2. Different plant species in these families produce characteristic mixtures of 1,2-unsaturated PAs and their saturated analogues and varying amounts of their corresponding *N*-oxides. The PAs present in these plants are esters of pyrrolizidine diols. The pyrrolizidine moieties are referred to as necines, and the esterifying acids involved 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 on the field or at harvest, microscopic inspection of seeds (e.g. contamination in seed spices), as well as DNA-metabarcoding[3].

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 not 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. 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. An overview of the structural formulae of the most relevant PAs in provided in figure 1 of the WHO Food Additives Series: 71 – S2[4]

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

   a. as an inherent component of the food;

   b. through contamination of a food, with PA-containing plant material (e.g. contamination of food with PA-containing weeds); and

   c. transfer of PAs from plant material consumed by animals into foods of animal origin.

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 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[5].

6. JECFA noted that most studies of toxicity and of 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. The Committee 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.

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[3] 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

[4] 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

[5] Available at: https://apps.who.int/iris/rest/bitstreams/1318952/retrieve.

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 that have been 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 that it 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. Carcinogenicity has not been investigated in case studies of human poisoning with PAs.

8. JECFA considered that derivation of a health-based guidance value for PAs was not appropriate in view of the genotoxic mode of action. From the carcinogenicity data in rats, a $BMDL_{10}$ 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, although there may also be differences depending on the type of necine base and the stereochemistry. The two PAs that have been 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 not sufficient 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 exposures. 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 consumption of tea, 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 noted also that there is insufficient information to determine MOEs for other food types or for 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 for 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 of concern 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 as a result of 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 reach conclusions 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[6]. The CONTAM Panel concluded that 1,2-unsaturated PAs may act as genotoxic carcinogens in humans. A benchmark dose lower confidence limit for a 10 % excess cancer risk ($BMDL_{10}$) of 70 µg/kg b.w. 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 consumers of honey.

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[7]. 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 reuslts of the monitoring program for PAs and the risk to public health[8] 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. It has been evaluated that exposure amounts to PAs through thyme consumption may cause low health concern 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).

JECFA Recommendations

22. 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, in order to fill these data gaps, JECFA recommended the following:

a. To establish internationally agreed 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 EU and on food products other than honey, particularly foods of animal origin, in order to improve dietary exposure estimates for PAs across the range

[6] 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 .

[7] 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.

[8] Available at: https://www.tarimorman.gov.tr/GKGM/Belgeler/DB_Risk_Degerlendirme/BilimselGorus/Kekikte_PAlar_Bilimsel_Gorus.pdf

of potentially PA-containing foods and from different geographical regions;

d. To conduct additional toxicological investigations in order 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-up of incidents of PA contamination, with the aim to 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;

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

23. 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.

24. PAs are extracted from plants and food samples with hot or cold methanol or ethanol, or dilute aqueous acid. 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.

25. 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. The development of 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.

26. 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 prior to analysis are required. Some issues of concern are related to the instability of N-oxides during sample preparation and analysis. There are multiple variants of Liquid chromatography Tandem mass spectrometry (LC-MS/MS) methods. 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.

27. 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].

28. 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 in 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 concentrations ranges validated are listed in the following Table:

Table — 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	Im	25	560
Intermedine-N-oxide	ImN	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	Im+La	50	890
Intermedine-N-oxide + Lycopsamine-N-oxide	ImN+LaN	5	645
Senecivernine + Senecionine	Sv+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

29. 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 the following table:

[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] Availalble 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

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 der 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

30. 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.

31. 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 (by means of 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 (for the individual PAs is 5 µg/kg. The method is available for the analysis of 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 2 suppliers for quantification of the PAs within the scope of the method.

32. 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 Limit of Quantification 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.

33. 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 limit of quantification for the individual PAs are 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

[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
[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

% in the herbal tea samples.

34. Following specific criteria for the confirmatory method of analysis for PAs in food could be discussed[15]

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 all individual toxins.
In exceptional cases, average recoveries outside the above range can be acceptable but shall lie within 50-130%, and 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.
In case a laboratory provides the 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

Methods of sampling

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

36. 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 that are suitable for different foods and foods at different stages of manufacture Existing sampling protocols for other natural toxins such as mycotoxins should be followed in sampling protocols for PAs in bulk commodities and in consumer products. Sampling protocols for PAs could initially be based on those specified for mycotoxins. Information on sampling for mycotoxins can be obtained from Codex Alimentarius standard CXS 193-1995[16], in which sampling protocols are compiled for several mycotoxins although not for the food and feed commodities of relevance for contamination with PAs. Therefore, specific sampling procedures for the analysis of PAs in tea, herbal infusions, honey, food supplements and spices and as regards feed, the feed materials forages and roughages and compound feed containing herbal products should be developed.

37. Recent research performed by a working group coordinated by the German Federal Institute for Risk Assessment (BfR) aimed to develop a sampling procedure representative for the level of PAs in lots of dried herbs larger than 15 tonnes. The fact that dried herbs, tea and herbal infusions are a matrix with high volume but relative low weight have been taken into account. The results of the research are in publication. The conclusion of the research that for the sampling of batches of dried herbs, teas, herbal infusions and ground spices between 15 and 25 tonnes, an aggregate sample of 4 kg consisting of 50 incremental samples of 80 grammes provides the best balance between representativeness and feasibility.

[15] The source of the proposed criteria isCommission 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
[16] Available at : https://www.fao.org/fao-who-codexalimentarius/codex-texts/list-standards/en

38. In the EU, the same sampling procedures are applied for the control of plant toxins, including pyrrolizidine alkaloids, and mycotoxins. The following sampling procedures are applied for the control of PAs in batches of typically 15-25 tonnes of:

- dried herbs, herbal infusions (dried product), teas (dried product) and powdered spices as well food supplements in bulk: 50 incremental samples of 80 g resulting in an aggregate sample of 4 kg[17]. For smaller batches less incremental samples (3-50) are to be taken.
- spices: 100 incremental samples of 100 grams resulting in an aggregate sample of 10 kg[18]. For smaller batches less incremental samples (5-100) are to be taken.
- feed: for roughages and forage with a low specific gravity (e.g. hay), 25 incremental samples resulting in an aggregate sample of minimum 1 kg; for other roughages and forages and compound feed, 25 incremental samples resulting in an aggregate sample of minimum 4 kg[19].

Also for food supplements in retail/individual packages there is a specific sampling procedure for the control of pyrrolizidine alkaloids: 3-25 incremental samples depending on the lot size (number of retail/individual packages) resulting in an aggregate sample weight of 50 – 500 grammes[20].

For the control of PAs in honey, 10 incremental samples of 100 grammes resulting in an aggregate sample of minimum 1 kg.

39. In Türkiye, the sampling method used for the official control of PAs in dried herbs (borage, lovage, majoram, oregano and others) and spices is based on the sampling method for the control of mycotoxins in spices applied in EU and Türkiye, i.e. 100 incremental samples of 100 grams resulting in an aggregate sample of 10 kg in bulk[21]. For smaller batches less incremental samples (5-100) are to be taken resulting in an aggregate sample weight of 0.5 to 10 kg.

However in addition, given the heterogeneity of the presence of pyrrolizidine alkaloids, in case the weight of the aggregate sample is

- 3 kg or less, the aggregate sample is divided into two laboratory samples after mixing;
- more than 3 kg, the aggregate sample is divided into three laboratory samples after mixing The analysis of PAs is performed separately on each laboratory sample and the acceptance of the lot/batch is evaluated based on the average of the laboratory samples.

40. The following principles for the elaboration of a sampling method for typical batch sizes of 15-25 tonnes could be discussed:

- for dried herbs, tea, herbal infusions, powdered spices and food supplements in bulk (high volume, relative low weight): 50 incremental samples of 80 g resulting in an aggregate sample of 4 kg. For smaller batches less incremental samples (3-50) are to be taken.
- for spices, other than powdered spices: 100 incremental samples of 100 grams resulting in an aggregate sample of 10 kg. For smaller batches less incremental samples (5-100) are to be taken.
- for food supplements in retail/individual packages, in case of relevance in international trade, a specific sampling procedure for the control of pyrrolizidine alkaloids could be elaborated based on the number of of retail/individual packages within a batch.
- for honey: 10 incremental samples of 100 grammes resulting in an aggregate sample of minimum 1 kg.
- for feed with a low specific gravity, such as hay and straw, 25 incremental samples resulting in an aggregate sample of minimum 1 kg; for other feed, such as roughages and forages other than those with low specific gravity, and compound feed, 25 incremental samples resulting in an aggregate sample of minimum 4 kg.

Effects of food and feed processing

41. PAs are expected to be stable during most processes applied for food and feed production. The removal of co-harvested seeds and weeds from the raw materials will reduce the content of 1,2-unsaturated PAs and their N-

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17 Available at http://data.europa.eu/eli/reg_impl/2024/885/oj
18 Available at http://data.europa.eu/eli/reg_impl/2023/2782/oj
19 Available at http://data.europa.eu/eli/reg/2009/152/2022-06-28
20 Available at http://data.europa.eu/eli/reg_impl/2023/2782/oj
21 Available at http://data.europa.eu/eli/reg_impl/2023/2782/oj

oxides significantly. The presence of PAs in foods and dietary supplements such as pollen and honey provides a confirmation of the stability of PAs during food processing. However, details on the rate of possible degradation during food processing are not available, with the exception of data on tea infusion. The 1,2-unsaturated PAs and their N-oxides are stable during tea infusion making.

42. 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, although reliable data on the rate of degradation and the metabolites that are formed are lacking.

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

Prevention and control

44. 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 Codex Alimentarius Code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed (CXC 74-2014[22]). Good agricultural practices, HACCP and good manufacturing practice strategies must be in place to prevent batches of food contaminated with PAs entering the food chain and mingling with uncontaminated products.

45. Specific Codes of practice and/or guidelines and recommendations to reduce the presence of PAs have been developed for tea, herbal infusions, 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[23]" developed by Tea and Herbal Infusions Europe, "Guidelines and recommendations to reduce the presence of pyrrolizidine alkaloids in food supplements[24]" developed by Food Supplements Europe, and "Guidelines for Good Agricultural and Wild Collection Practices for Medicinal and Aromatic Plants (GACP-MAP)[25]" 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[26]".

46. Türkiye has developed several Codes of Practice or guidances for the prevention of pyrrolizidine alkaloids:

- Code of Practice on Prevention and Mitigation of Pyrrolizidine Alkaloid Contamination in Thyme (*Origanum* Spp.).[27]
- Implementation Guidance on Prevention and Mitigation of Pyrrolizidine Alkaloid Contamination in Cumin (*Cuminum Cyminum* L.).[28]
- Implementation Guidance for the Prevention and Mitigation of Pyrrolizidine Alkaloid Contamination in Mint (*Mentha* Spp.).[29]

47. It can be observed that all Codes of practice mentioned in §§ 43 and 44 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.

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22 Available at: https://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/en/

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

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

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

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

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

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

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

48. This could be done by slightly updating section 7 Evaluation of the need to proceed to action and section 8. Recommended practices in the current Code, 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[30].

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 presence of pyrrolizidine alkaloids in honey. Furthermore, honey has an inherent risk of pyrrolizidine alkaloids and processing of honey might have a higher influence on the presence of pyrrolizidine alkaloids than the processing of the other foods, affected by contamination with pyrrolizidine alkaloids. Therefore, it might be considered, if needed, to develop a separate Code of Practice for honey.

Call for data

49. 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 (see § 38). Furthermore, it is important to determine if only occurrence data whereby PAs are quantified individually can be accepted or if occurrence data expressed as sum of alkaloids are analysed. In case of analysing individual PAs, it might be relevant to determine which PAs at least should be analysed. Before launching the call for data, it is appropriate to discuss and agree the minimum analytical requirements for submission of data to the GEMS/Food database on the basis of the information contained in this discussion paper (see § 32).

[30] Available at https://www.fao.org/fao-who-codexalimentarius/codex-texts/codes-of-practice/en/

APPENDIX II

PROPOSAL FOR A NEW WORK ON AN UPDATE OF THE CODEX ALIMENTARIUS 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 purpose of the proposed new work is 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 and to supplement the Code with good practices for specific foods and feeds, such as tea, herbal infusions, food supplements, herbs and spices, eventually in separate annexes.

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 are of concern.

3) Main aspects to be covered

This work will address all relevant measures for prevention or reduction of PAs at the different steps in the food and feed chain: production, harvest, storage, processing and distribution. It will also address the evaluation of the need to proceed to action, including the identification of the weeds containing pyrrolizidine alkaloids causing a risk in the crop.

4) Assessment against the criteria for 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 Code of Practice providing recommendations to governments, 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, best practices and legislations. An update of the existing Code of Practice is needed to ensure that the most recent information on recommended practices for preventing and reducing PAs is available to all member countries. It also will 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 Code of Practice will address all relevant measures for prevention or reduction of 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 Code of Practice for prevention or reduction of PAs in food and feed will address the current need for guidance to ensure the health of consumers.

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

(c) **Goal 3 Increase impact through the recognition and use of Codex standards.** The proposed update of the Code of Practice ensures that information on recommended practices to prevent and reduce the presence of PAs consist 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.** Updating the Code of Practice through the Codex Step process will make information on recommended practices to prevent and reduce presence of PAs in food and feed available to all Codex members.

(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 Code of Practice will help ensure 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

Currently, there is no identified need for additional technical input from external bodies.

9) Timeline for completion of the new work

Work will commence following recommendation by CCCF and approval by CAC in 2024. Completion of work is expected by 2028 or earlier.

APPENDIX III

ORIGINAL LANGUAGE ONLY

Comments in reply to CL 2023/40-CF
submitted by
Canada, Chile, Egypt, Iraq, New Zealand, Türkiye, USA

Background

1. This Appendix compiles comments received through the Codex Online Commenting System (OCS) in response to CL 2023/40-CF issued in September 2023. Under the OCS, comments are compiled in the following order: general comments are listed first, followed by comments on specific sections.

Explanatory notes on the Annex

2. The comments submitted through the OCS are hereby attached as **Annex I** and presented in table format.

Annex I

GENERAL COMMENTS

COMMENT	MEMBER / OBSERVER
Taking into account the recommendations from JECFA, it is appropriate to issue a call for data on the presence of pyrrolizidine alkaloids in food and feed. Canada considers that before a call for data can be issued, the analytical requirements discussed in sentences 2-4 need to be determined and agreed upon. Canada does not currently have a testing method for pyrrolizidine alkaloids. Canada could support a call for data in the future if: 1) the reference methods identified in the discussion document are provided to all member states with supporting validation data; 2) high-quality standards are commercially available for compounds identified in the methods; 3) the Committee could agree to qualitative analysis of other pyrrolizidine alkaloids. However, it would take a number of years before occurrence data could become available for commercial foods sold in Canada. *Agree to elaborate a document defining the minimum requirements to which occurrence data have to comply with for submission to the GEMS/Food database for consideration by CCCF17 in view of issuing a call for data on the presence of pyrrolizidine alkaloids in food and feed.* Canada believes consensus must be reached on analytical considerations prior to any issuance of a call for data. Canada further supports these considerations being taken into account when data is submitted to the GEMS/Food database. *To consider if new work should be proposed on an update of the Codex Alimentarius Code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed (CXC 74-2014) or if the EWG should revise the discussion paper for consideration by CCCF17.* Canada supports the update of CXC 74-2014 (COP for pyrrolizidine alkaloids), if other members feel such an update is warranted. While Canada notes the reference to a number of Codes of Practice in para. 29, it is unclear how and which sections of the COP would be updated and if new information is available in some of those other COPs, if that information supports updating/re-writing of CXC 74-2014 rather than adding an annex with the new information. The discussion document may benefit in describing how and why the COP should be updated. Canada believes the proposal for new work could better explain how and why the COP requires updating.	**Canada**
Chile agradece la oportunidad de presentar observaciones sobre las recomendaciones relativas a las medidas dirigidas a hacer un seguimiento de la gestión de los riesgos asociados a los alcaloides de pirrolizidina Al respecto, Chile quisiera comentar lo siguiente:	**Chile**

- Respecto a considerar si en el Apéndice I del documento de debate CX/CF 23/16/11 se proporcionan suficientes datos/información que respalden las recomendaciones que figuran en el párrafo 33 relativas a la actualización del Código de prácticas para el control de malezas a fin de prevenir y reducir la contaminación de los alimentos y los piensos con alcaloides de pirrolizidina (CXC 74-2014); Chile considera que existe información suficiente y robusta como para proceder con lo propuesto, es decir, actualizar el Código de Prácticas mencionado. Esta actualización sin duda proporcionará los medios que permitirán a países exportadores como Chile garantizar niveles reducidos de alcaloides de pirrolizidina. Respecto a la propuesta relativa al nuevo trabajo del Apéndice II, Chile no tiene observaciones que compartir. - Chile está de acuerdo con establecer un GTE que actualice el Código de prácticas para el control de malezas a fin de prevenir y reducir la contaminación de los alimentos y los piensos con alcaloides de pirrolizidina (CXC 74-2014) con miras a su examen por el CCCF, en su 17.ª reunión (2024).	
Egypt agrees on discussion paper in Appendix I of CX/CF◦23/16/11 Egypt agrees to establish EWG to update the cop In case the discussion paper provides sufficient grounds to proceed with the new work	**Egypt**
Agree with no comments.	**Iraq**
New Zealand would like to thank the Chair of the EWG for drafting the discussion paper and the project document on pyrrolizidine alkaloids. New Zealand does not have any comments on the discussion paper and the proposal for new work. Furthermore New Zealand supports the establishment of an EWG to update the CoP for weed control to prevent and reduce PAs contamination in food and feed (CXC 74-2014) for consideration by CCCF17 (2024).	**New Zealand**
Türkiye would like to thank the EWG members that contributed to the preparation of the discussion paper on pyrrolizidine alkaloids (PAs) for their contributions Türkiye produces and exports many foods that are at risk of PAs. Türkiye ranks first in the production and export of cultivated Oregano (Origanum onites), which is likely to contain PAs due to weed contamination. Türkiye also ranks in the top three in the world in the number of beehives and the amount of honey production and produces other beekeeping products such as pollen. Due to its rich vegetation, various plants used as ingredients in the production of many foods, especially food supplements, are cultivated. Tea, which has a high consumption amount, is produced. It has an important place in the world in terms of bovine and ovine livestock and fishery products. In our country, a large number of studies are being carried out starting from primary production with the receipt of feedback on the risk of PAs in food. Some of these are; - A research was conducted to identify and control weeds in oregano fields which culturally produced and reported in 2016. - A project was carried out to determine the frequency of weeds in oregano fields, their interference with the oregano product according to their vegetative development and to determine the PAs levels in weeds and was completed in 2019.	**Türkiye**

- Within the scope of the Integrated Contol Crop Management (ICCM) program, support was given to farmers who had adopted good manafacturing practices in oregano fields, and PA analyses were carried out on samples taken from the production areas of farmers who had successfully completed the programme. The statistical evaluation of the results of the programme, which started in 2023, is ongoing. - Guidance documents for the prevention, minimisation and elimination of PAs risk have been prepared for oregano, cumin and mint. - A monitoring program was started in 2023 covering the entire geography of Türkiye to monitor the current level of PAs risk in honey. - A monitoring program was started in 2023 to monitör the current level of PAs risk in tea and some herbal infusions. - Scientific Opinion on the Results of the Monitoring Program for Pyrrolizidine Alkaloids in Oregano and the Risk to Public Health has been published. Türkiye would be pleased to share its experience, knowledge and data and to take an active role in the implementation of this study. In this context, we hope that our comments on the discussion paper will be useful.	
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 exposures. 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. In other risk assessment studies, different BMDL values are used in MOE calculation. There is a need to explain how the value of 182 µg/kg bw per day used by JECFA was determined. In the risk assessment study conducted on PAs in our country, the BMDL10 value of 237 µg/kg bw per day, which is also used by EFSA, was used. The comparison value used in the interpretation of the calculated MOE values should be clearly stated.	
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 that are suitable for different foods and foods at different stages of manufacture Existing sampling protocols for other natural toxins such as mycotoxins should be followed in sampling protocols for PAs in bulk commodities and in consumer products. Sampling protocols for PAs could initially be based on those specified for mycotoxins. Information on sampling for mycotoxins can be obtained from Codex Alimentarius standard CXS 193-1995[6], in which sampling protocols are compiled for several mycotoxins. We believe that the most important part of this study was the establishment of the sampling method. Although it is considered a correct approach to base sampling method on mycotoxins because of the similarity in heterogeneous distribution, our studies show that the variance may be much higher for PAs. In Codex Standard For Dried Oregano, the level for foreign matter is 0.1%. In our studies, total PAs in a weed was found to be about 2x10^6 µg/kg. If even a small portion of this weed contaminates a food batch, homogenous distribution will not be possible, so even if the batch complies with the standards, the the probability of rejection due to sampling method will be high. Therefore, it would be a better approach to initiate a study on the sampling method for PAs.	
Given the relevance of the presence of pyrrolizidine alkaloids in tea, herbal infusions, honey, food supplements and herbs and spices it is appropriate to update the Codex Code of Practice for weed control to prevent and reduce pyrrolidine alkaloid contamination in food and feed (CXC 74-2014) as regards the good practices generally applicable to prevent and reduce pyrrolizidine alkaloids in food and feed supplemented with good practices for specific foods and feeds.	

Türkiye supports the work on updating CXC 74-2014. However, due to the nature of beekeeping activity, it is considered that the terminology of "weed control" for honey will not be correct and it is considered that it would be appropriate to prepare a separate Code of Practice for honey. In our studies, it is understood that PAs risk is higher in honeys that have high pollen content and consist of many different types of pollen. In honey standards and our national honey legislation, it is stated that pollen is a part of honey and cannot be removed from honey except filtered honey. For this reason, it is considered that the study to be carried out for honey should be handled separately from the study to be carried out for other foods, which will accelerate the study. It is also understood that a Code of Practice will not be prepared for foods with an inherent risk of PAs. Considering that processing is also a part of the Code of Practice, it should be discussed to adopt a new work on the development of CoP for these foods. With this work, it would be appropriate to focus on foods with PAs risk due to weed contamination.	
PARAGRAF 32 In Türkiye, the evaluation is conducted for the total PAs level on the basis of the following PA toxins. In the studies we conducted, certain PAs toxins were found to be present at high levels in weeds, predominantly. — intermedine/lycopsamine, intermedine-N-oxide/lycopsamine-N-oxide, senecionine/senecivernine, senecionine-N-oxide/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 and heliotrine-N-oxide and the following additional 14 pyrrolizidine alkaloids known to co-elute with one or more of the above identified 21 pyrrolizidine alkaloids, making use of certain currently used analytical methods: — 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).	
Agree to elaborate a document defining the minimum requirements to which occurrence data have to comply with for submission to the GEMS/Food database for consideration by CCCF17 in view of issuing a call for data on the presence of pyrrolizidine alkaloids in food and feed. Türkiye support the development of documentation for minimum requirements for occurrence data. However, in our studies, high levels of PAs were detected even in products conforming to standards and harvested from fields where good manafacturing practices are applied due to weeds containing high PAs (2x10^6 µg/kg) and uncertainty in sampling method. In the document to be prepared, in addition to the methods of analysis, it would be appropriate to determine the criteria for sampling method and good manafacturing practices. *To consider if new work should be proposed on an update of the Codex Alimentarius Code of practice for weed control to prevent and reduce pyrrolizidine alkaloid contamination in food and feed (CXC 74-2014) or if the EWG should revise the discussion paper for consideration by CCCF17.* We support the new work on the update of the CXC 74-2014. However, it would be appropriate to clarify the scope of the work and the foods to be covered first. We consider that it would be more efficient to start the work from foods at risk of PAs due to weed contamination.	

We support the establishment of an EWG and would like to share on our experience.	
The U.S. suggests that the EWG further develop the discussion paper for CCCF17. Specifically, the discussion paper should elaborate what information is available on mitigation of pyrrolizidine alkaloids (PAs) in specific foods. Given the JECFA report, honey and tea should be the highest priority; information on potential mitigation methods for herbal infusions, food supplements, and herbs and spices is also of interest. Information that could be presented in the revised Discussion Paper at CCCF17 could include: • Information available from the published literature and other COPs on mitigation methods and their effectiveness. • Steps that can be taken spanning the food production process - - including production, harvesting, storage, processing and distribution - - to reduce or prevent PA contamination. Amending the discussion paper is in line with the conclusion of CCCF16, i.e., “the EWG [will] prepare a revised paper based on the comments received in response to the CL for consideration by CCCF17.” Response to paragraph 2(iii). With regard to the request to provide input on minimum requirements (CL 2023/40-CF), as well as Recommendation 33-d in CX/CF 23/16/12, “to establish an EWG to prepare a document defining the minimum requirements,” the United States agrees that the EWG can consider the issue of minimal data requirements that would be applicable for addressing future work on PAs. Any data requirements proposed by the EWG should be discussed when CCCF17 considers whether to issue a new Call for Data. However, before requesting a Call for Data, the EWG should consider for what purpose new data are being requested; e.g., is this to support the proposed Code of Practice revisions or for new work (which has not yet been discussed in CCCF) such as the establishment of MLs? It is important to note that there may be different data requirements for different purposes, e.g., higher LOQ results may be acceptable for informing work on mitigation in the COP. The Committee should also consider the data requirements in light of the broader discussions of data requirements in Calls for Data in the Guidance on Data Analysis for Development of Maximum Levels for Improved Data Collection EWG (see CX/CF 23/16/12). Given the challenges of analyzing PA data articulated by JECFA (2020), including questions concerning individual versus summation approaches for analysis, stability/extractability of analytes, lack of analytical standards, and lack of validated methods, it may be helpful for CCCF to consult with CCMAS on minimum analytical requirements.	**USA**

APPENDIX IV

List of Participants

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