CODEX ALIMENTARIUS COMMISSION E

Food and Agriculture Organization of the United Nations

World Health Organization

Viale delle Terme di Caracalla, 00153 Rome, Italy - Tel: (+39) 06 57051 - Fax: (+39) 06 5705 4593 - E-mail: codex@fao.org - www.codexalimentarius.org

Agenda Item 10 **CX/CF 13/7/10**
February 2013

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

Seventh Session
Moscow, Russian Federation, 8 – 12 April 2013

PROPOSED DRAFT MAXIMUM LEVELS FOR HYDROCYANIC ACID IN CASSAVA AND CASSAVA PRODUCTS

Codex Members and Observers wishing to submit comments on the recommendations put forward in paragraphs 17-23 regarding the revision or establishment of new MLs for cassava and cassava products and in paragraphs 30-31 regarding methods of analysis for determination of total HCN in these products with a view to determine how to proceed further with work on the establishment of MLs for HCN in cassava and cassava products and associated analytical methods should do in writing before **25 March 2013**. Comments should be directed:

to:	with a copy to:
Mrs Tanja Åkesson Codex Contact Point Ministry of Economic Affairs P.O. Box 20401 2500 EK The Hague The Netherlands E-mail: info@codexalimentarius.nl	Secretariat, Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, Viale delle Terme di Caracalla, 00153 Rome, Italy E-mail: codex@fao.org

Note: Supporting information presented in Appendix 1 and its Annexes is not subject to comments.

BACKGROUND

1. At the 3rd meeting of Committee on Contaminants in Foods in 2009, Australia presented a discussion paper on cyanogenic glycosides[1]. The CCCF agreed to request JECFA to re-consider the data available on cyanogenic glycosides and advise on the public health implications of cyanogenic glycosides and their derivatives in food.[2] In addition, and taking into account any assessment by JECFA, the CCCF would consider developing a code of practice for producing, processing and marketing of foods which may contain cyanogenic glycosides or their derivatives.

2. At its 72nd session JECFA conducted a risk assessment of cyanogenic glycosides in foods[3]. Cyanogenic glycosides can cause acute poisoning in humans as well as several chronic diseases associated with under-processed cassava production. JECFA established health-based guidance values (HBGVs) for cyanogenic glycosides; namely, an Acute Reference Dose (ARfD) of 0.09 mg/kg body weight, expressed as cyanide equivalents and a Provisional Maximum Tolerable Daily Intake (PMTDI) of 0.02 mg/kg body weight, as cyanide.

3. Estimates of dietary exposure used conservative estimates (total conversion of cyanogenic glycosides to hydrogen cyanide and without taking account of effects of food preparation or processing in most cases). They indicate possible exceedances of the acute and sub-chronic reference doses in some population groups.

4. Given these possible health impacts, it is important to consider whether existing MLs in commodity standards are protective and whether MLs in other commodities are warranted. It is also appropriate to develop guidance to reduce the concentrations of hydrocyanic acid (HCN) in foods.

5. The 6th session of the CCCF agreed to establish an electronic working group led by Australia and co-chaired by Nigeria to start new work on a code of practice and maximum levels for hydrocyanic acid in cassava and cassava products for comments at Step 3 and consideration by the next session pending approval by the 35th session of the Codex Alimentarius Commission.

---

[1] ALINORM 09/32,41, paras. 105-108
[2] ALINORM 09/32/41, para. 119, ALINORM 10/33/41, para. 100, REP11/CF, para. 92, REP12/CF, para. 40.
[3] http://whqlibdoc.who.int/trs/WHO_TRS_966_eng.pdf

6. In order to carry out this task, the Committee agreed that the working group would:

- undertake a review of the levels and MLs for hydrocyanic acid in existing Codex commodity standards with a view of the possible revision of these MLs and the establishment of new MLs for additional commodities, such as ready-to-eat cassava chips;
- develop a code of practice to reduce the presence of hydrocyanic acid in cassava in which the agricultural aspects and the methods of processing are addressed; and
- identify methods of analysis suitable for analysis of hydrocyanic acid in foods.[4]

7. The 35th session of the Commission noted that the establishment of MLs for HCN in cassava and cassava products would be limited to the section on contaminants, to establish safe levels for this natural toxin in the aforesaid products. It was also noted that different varieties of cassava contained different levels of cyanogenic glycosides from which HCN is formed therefore, this should be taken into account when establishing MLs.[5]

8. This work of the eWG has been progressed as two documents:

- The review of the levels for HCN in bitter and sweet cassava and the MLs for cassava products in existing Codex commodity standards and consideration of MLs for additional cassava commodities (includes identifying methods of analysis of HCN in foods) (CX/CF 13/7/10).
- The code of practice for reduction of HCN in cassava and cassava products (CX/CF 13/7/11).

9. The preparation of the ML review document was led by Australia and the Code of Practice was led by Nigeria. Working group members were Brazil, Canada, China, Columbia, Dominican Republic, European Union (EU), FAO, Federated States of Micronesia, Fiji, Ghana, Indonesia, International Organization of the Flavor Industry, Jamaica, Japan, Malaysia, New Zealand, Nigeria, Papua New Guinea, Philippines, Republic of Korea, Samoa, Solomon Islands, Suriname and Vanuatu (see Appendix 2, List of Participants).

10. The full report providing the supporting information for the conclusions and recommendations on the revision and/or establishment of new MLs for HCN in cassava and cassava products are given in Appendix 1 and its relevant Annexes. The full consideration of the methods of analysis is given in Annex 5 to Appendix 1.

Revision or Establishment of New MLs for HCN in cassava and cassava products

Conclusions

11. The current JECFA risk assessment is conservative because it assumes all hydrogen cyanide present as cyanogenic glycosides in foods will be absorbed by the gastrointestinal tract and, in some cases, is based on an assumption that the concentrations of HCN in foods are at the levels currently used to define sweet cassava or at the ML. JECFA also identified some significant data gaps which made risk characterization difficult, including occurrence data in raw and processed cassava and consumption data from a broader range of countries.

12. Very limited information was provided to the eWG to update the concentrations of HCN found in unprocessed cassava and in processed foods produced from cassava or on dietary consumption patterns. Consequently there remains considerable uncertainty to enable the actual level of human systemic exposure to hydrogen cyanide to be determined.

13. It is feasible that lowering MLs or setting new MLs may have no impact on the actual concentrations of HCN derived from cassava to which people are exposed. In some parts of the world where cassava is a staple component of the diet, ensuring food security may be a higher priority than setting levels for HCN which may in fact not be of a health concern following cassava processing. This is particularly the case in conditions of drought or famine.

14. Other risk management options e.g. education, development and application of a code of practice, are likely to be more effective.

15. There is a need to generate appropriate data for total HCN in cassava and provide information on consumption in order that appropriate MLs can be established (if needed) and risk management advice given as to potential risks.

16. There is an inconsistent approach to express levels / MLs and descriptors for HCN within the standards for sweet and bitter cassava, edible cassava flour and gari. In the case of gari, setting an ML on the basis of “free” HCN does not reflect the potential for the cyanohydrins present to generate HCN *in vivo*.

[4] REP12/CF, paras. 165-166.
[5] REP12/CAC, paras. 140-142 and Appendix VI.

Recommendations

17. It is recommended that a common approach is used for expressing MLs relating to HCN generated from naturally occurring cyanogenic glycosides. The eWG recommends that total HCN should refer to all cyanogenic glycosides, cyanohydrins and "free" HCN in a food as described in the most recent JECFA evaluation of 2012. This would require amending the ML for gari to express it in terms of total HCN, rather than free hydrocyanic acid. It is recommended that the ML for gari be converted to a value reflecting the total HCN level. Since JECFA was not able to characterize the risk from consuming gari, this conversion could be based on the current level, pending generation of further consumption and occurrence data. The CCCF should consider whether new work is proposed on the descriptor or whether this should be deferred until reconsideration of MLs for other cassava products at a later date.

18. Currently there are no MLs for HCN in cassava in the General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995) (GSCTFF). Instead the types of cassava (bitter or sweet) are distinguished by an HCN concentration of 50 mg/kg in their respective standards. It may be appropriate to incorporate MLs for HCN derived from cyanogenic glycosides into the GSCTFF at some point. However it would be more appropriate to make this decision once further information is available to fill the current data gaps.

19. In the absence of a Codex maximum level for hydrogen cyanide for bitter cassava in the GSCTFF the standard for bitter cassava permits the setting of an acceptable maximum level on a safety basis by the national legislation of the importing country pending the outcome of the work of the Committee on Contaminants in Foods on cyanogenic glycosides. It is recommended that this approach is retained until further information is available on the effects of processing and levels in final products derived from bitter cassava.

20. For cassava flour, there are no available estimates of dietary exposure that exceed the ARfD or PMTDI, and therefore there is no need to amend the current ML.

21. For other cassava products at this time new MLs should not be developed because of the conservatism and uncertainty of the risk assessment and the need for further information on concentrations of HCN in cassava-based foods.

22. Other risk management strategies, particularly the development and implementation of a code of practice (CX/CF 13/7/11), should be prioritized. Further data should be collected after the code of practice is in place and its effectiveness should be evaluated before consideration is given to setting new MLs. This should be accompanied by other education and outreach initiatives.

23. Countries should be encouraged to continue to collect data on concentrations of total HCN in cassava and cassava based products, methods of preparation and consumption amounts after implementation of the code of practice. Data are needed on how much cassava and cassava products are consumed and what concentrations of HCN are in the different cassava products eaten in different regions.

Methods of analysis for the determination of HCN in cassava and cassava products

Conclusions

24. While it is feasible to determine total hydrocyanic acid in cassava by determining the individual contributing compounds, no methods have been reported that reliably quantify all potential contributors. Therefore it is preferable that total hydrocyanic acid be determined by a suitable technique which converts all contributors to hydrocyanic acid (acid or enzymatic hydrolysis).

25. Spectrophotometric detection of cyanide (Guignard or König) reaction products or HPLC analysis following pre-column derivatisation appear to be the most common methods currently being used for determining total hydrocyanic acid from cyanogenic plant materials.

26. HPLC methods are likely to be more specific and have reported good sensitivity (e.g. limit of quantification of 2 mg HCN/kg should normally be obtained for EU standard method for animal feed).

27. The picrate paper method appears to be the best current option for a "field" test method and it was developed for this purpose. The colourimetric method of Untang et al. also looks promising as a field tool, but has not been tried on cassava. For basic laboratory analyses the picrate paper or the isonicotinic acid/barbituric acid methods appear suitable. Methods using chromatographic detection of cyanide (e.g. the EU HPLC method for animal feed) are probably currently the "gold standard" due to the specificity of the detection technique.

28. There is very limited information available on the relative performance of different methods. Available comparisons have been carried out within a single laboratory. Definition of analytical method performance is hampered by the lack of certified reference materials and inter-laboratory collaborative trials.

29. Published method limits of detection indicate that test sensitivity is generally adequate to support the standards for edible cassava flour and sweet cassava, but most methods appear to be insufficiently sensitive to test to the levels of the more stringent standard for gari. The EU HPLC method (European Committee for Standardization (CEN), 2012) and the modified picrate method (Bradbury, 2009; Burns et al., 2012) have reported acceptably low limits of detection.

Recommendations

30. A variety of fit-for-purpose analytical methods may be used to determine occurrence levels for total HCN in cassava and its products.

31. Further validation work is required for the analytical methods used to measure total HCN.

Appendix 1

Supporting information on the review of maximum levels (MLs) for hydrocyanic acid (HCN) in existing commodity standards and MLs for additional commodities

Background

Cassava

1. Cassava originated in Latin America and was later introduced into Asia and Africa (FSANZ 2004). Cassava is also known by other common names: manioc, manihot, and yucca.

2. Cassava grows well in a tropical climate and is eaten in Africa, Pacific Island Countries, South America and regions of Asia including Indonesia (Knudsen et al 2005). Cassava is consumed in a number of forms: flour, root slices, grated root (baked, steamed or pan fried), steamed whole root and tapioca pearls made as a pudding (Knudsen et al 2005). It is an important root crop in many countries both for food security and as a cash crop (Nambisan, 2011). It can produce reasonable yields on relatively infertile soil. Furthermore it has a flexible harvest period and has traditionally been used as a reserve in case of natural disasters such as cyclones and droughts. Post-harvest activities including milling and drying are not complicated or capital intensive and thus can be conducted at farm or village level (FAO Regional Programme for Food Security, 2011). Increasingly it is being internationally traded, partly as a result of the migration of people from countries where it is a staple dietary component to other parts of the world where it has not had a traditional history of use.

3. There is a number of varieties of cassava, each of which has varying total HCN concentrations according to the altitude, geographical location and seasonal and production conditions (Oluwole et al 2007). These are arbitrarily considered to be sweet cassava or bitter cassava based on a total HCN content of 50 mg/kg. In drought conditions there is an increased total HCN content due to water stress (Cardoso et al 2005). Thus a variety considered to be “sweet” under one set of conditions may be “bitter” in a different geographical location or climatic conditions. Values from 15-400 mg/kg fresh weight of total HCN in cassava roots have been reported in the literature (FSANZ 2004), although there are reports of even higher levels (Oluwole et al, 2007; Cardoso et al 2005) depending on location of the crops.

Cyanogenic glycosides

4. There are at least 2650 species of plants that produce cyanogenic glycosides (CGs) and usually a corresponding hydrolytic enzyme (beta-glycosidase). The enzyme and CG are brought together when the cell structure of the plant is disrupted with subsequent breakdown to release HCN and an aldehyde or a ketone (Hosel, 1981; Moller and Seigler, 1999). The release of HCN from cyanogenic glycosides can also result from enzymatic hydrolysis by the gut microflora (WHO, 1993; EFSA 2004).
5. Linamarin and to a lesser degree, lotaustralin and linustatin are the cyanogenic glycosides in cassava.

Health risks of cassava

6. The potential toxicity of a food produced from a cyanogenic plant depends on the likelihood that its preparation will produce a concentration of HCN that is toxic to exposed humans following consumption. If the cyanogenic plant is inadequately detoxified during processing or preparation of the food, the CG in the food may be toxic depending on the concentration of HCN remaining. If the cyanogenic plant is consumed raw or is insufficiently processed, the beta-glycosidase can be released and is active until the low pH of the stomach deactivates the enzyme, releasing at least some HCN from the CG. It is possible that part of the enzyme fraction can be reactivated in the alkaline environment of the gut releasing more HCN from the CG (WHO, 1993).

7. The primary toxicological endpoint of concern for acute HCN exposure is inhibition of mitochondrial oxidation via HCN shutting down the electron transport chain of the inner membrane of the mitochondria (Cheeke, 1989). The cyanide ion inhibits enzymes associated with cellular oxidation and causes a decrease in the utilization of oxygen in the tissues, producing a state of histotoxic anoxia. The clinical symptoms associated with acute toxicity can occur within a few minutes and may include headache, nausea, vomiting, giddiness, palpitations, hyperpnoea then dyspnoea, bradycardia, unconsciousness and violent convulsions, followed by death depending on the dose of HCN (EFSA 2004).

8. Chronic exposure to sub-acute toxic doses of HCN may be involved in the pathogenesis of certain conditions including disturbance of thyroid function in the presence of iodine deficiency and neuropathies. Human cassava-eating populations show ophthalmological and neurological symptoms associated with exposure to HCN, though it is likely that other nutritional or metabolic deficiencies affecting the cyanide detoxification mechanism are also involved (e.g. sulphur-containing amino acids, vitamin B12, sulphate and zinc deficiencies).

9. Several epidemiological studies in cassava-eating populations, which established an association between cyanide exposure and spastic paraparesis, amblyobia ataxia or tropical ataxia neuropathy and possibly goitre, have also been considered. However, the data are highly confounded by other nutritional and environmental factors.

Consideration of cyanogenic glycosides by JECFA

10. The 39th meeting of JECFA considered cyanogenic glycosides and, due to a paucity of data, concluded that no causal relationship could be definitively established between chronic exposure to cyanogenic glycosides and various diseases in humans (WHO, 1993). The Committee also concluded that consumption of cassava flour at a concentration of up to 10 mg/kg HCN, as in the standard for edible cassava flour (CODEX STAN 176-1989), was not associated with acute toxicity. The third session of the CCCF in 2009 requested that JECFA reconsider the available data on cyanogenic glycosides, advise on public health implications of them and their derivatives and decide whether a risk assessment is feasible and appropriate.

11. Consequently the 72nd meeting of JECFA considered the safety of dietary exposure to cyanogenic glycosides from a variety of dietary sources, including cassava and its products. Their updated risk assessment established an ARfD for cyanide equivalents derived from cyanogenic glycosides of 0.9 mg/kg body weight (equivalent to 0.09 mg/kg body weight as cyanide) and a PMTDI of 0.02 mg/kg bw cyanide (WHO, 2012).

12. The conclusions are summarized as follows:

- There were exceedances of the ARfD in adults for raw cassava, apple juice for children, bitter apricot kernels and ready-to-eat cassava chips/crisps.
- There was potential to exceed the PMTDI for populations reliant and not reliant on cassava as a staple food.
- All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI.
- If ready-to-eat cassava chips contained a concentration equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. All mean dietary exposures were below the PMTDI but high-percentile exposures for children were above the PMTDI.
- Application of the level of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing.
- For the ML of 10 mg/kg as HCN for cassava flour, there are no available estimates of dietary exposure that exceed the ARfD or PMTDI.

Current Codex and international standards

13. Currently there are no provisions in the General Standard for Contaminants and Toxins in Food and Feed for HCN levels in cassava and its products.

14. The Codex Alimentarius Commission has developed and published standards for sweet cassava)[6], bitter cassava[7], edible cassava flour[8] and gari[9] (a product obtained from processing cassava tubers) (also spelt as "garri"). The key aspects of these standards are:

- sweet cassava is defined as a raw product containing less than 50 mg/kg of "hydrocyanic acid".
- edible cassava flour is defined as a product suitable for direct human consumption and the level of "total hydrocyanic acid" in the flour must not exceed 10 mg/kg.
- for gari, another product for direct human consumption, the "total hydrocyanic acid" must not exceed 2 mg/kg as free hydrocyanic acid.
- the standard for bitter cassava (300-2010) defines bitter varieties of cassava as those containing more than 50 mg/kg of cyanides expressed as hydrogen cyanide (fresh weight basis). In the absence of a Codex maximum level for hydrogen cyanide for bitter cassava in the General Standard for Contaminants and Toxins in Food and Feed (CODEX STAN 193-1995) it permits the setting of an acceptable maximum level on a safety basis by the national legislation of the importing country pending the outcome of the work of the Committee on Contaminants in Foods on cyanogenic glycosides.

15. labelling provisions in the standard for sweet cassava require a statement that cassava must be peeled and fully cooked before being consumed.

[6] Codex Standard for Sweet Cassava (Codex STAN 238-2003)
[7] Codex Standard for Bitter Cassava (CODEX STAN 300-2010)
[8] Codex Standard for Edible Cassava Flour (CODEX STAN 176-1989)
[9] CODEX Standard for Gari (CODEX STAN 151-1989)

The labelling requirements for bitter cassava to alert consumers to risk of consumption are:

- cassava must not be eaten raw
- cassava shall be peeled, de-pithed, cut into pieces, rinsed and fully cooked before consumption
- cooking or rinsing water must not be consumed or used for other food preparation purposes.

16. MLs for total HCN have been established in a few countries for cassava and cassava derived foods including ready-to-eat cassava chips/crisps and these are for a limited range of substances (**Annex 1**).

Terminology for cyanogenic glycosides and HCN

17. The cyanogenic glycoside content of foods is often reported as mg/kg of HCN in the food. This reflects the "total hydrocyanic acid" content of the food which is often determined by measuring the HCN evolved following enzyme or acid hydrolysis of the cyanogenic glycoside and related cyanohydrins. Some texts refer to "total hydrocyanic acid" as the "cyanogenic potential" of a food or as "bound" or also as "free and combined" hydrocyanic acid.

18. The term "HCN" is the term recommended by the International Union of Pure and Applied Chemists for hydrogen cyanide. The standards for "sweet" and "bitter" cassava refer to levels of "hydrogen cyanide" on a raw food or "fresh weight" basis. However, other Codex standards refer to "total hydrocyanic acid" (edible cassava flour) and "total hydrocyanic acid" "determined as free hydrocyanic acid" (gari). Therefore, even in Codex standards, there does not appear to be a term that is used consistently to describe the total HCN including hydrogen cyanide from all the cyanogenic glycosides.

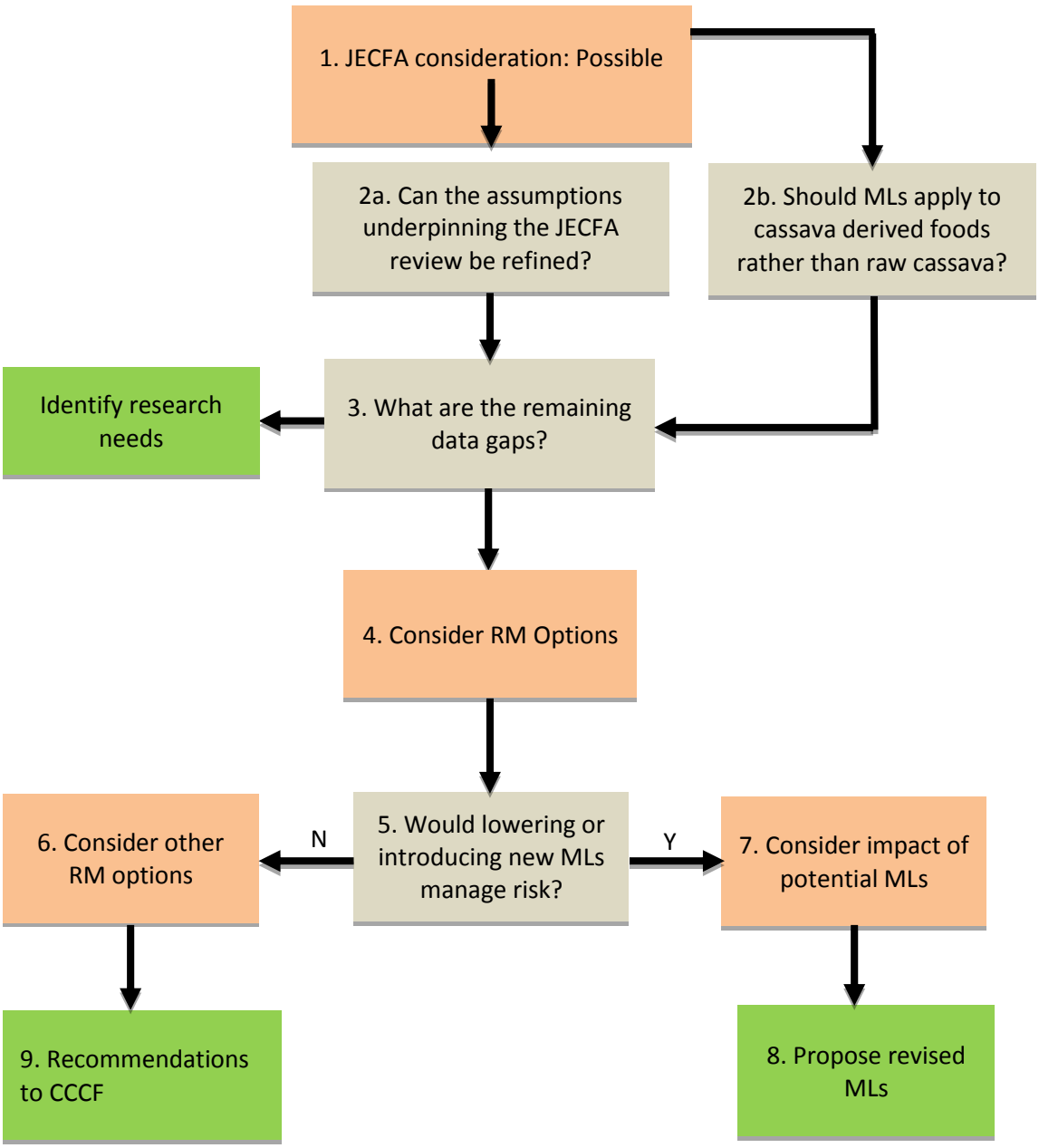
19. In the most recent evaluation by JECFA total HCN was taken to mean all cyanogenic glycosides, cyanohydrins and "free" HCN in a food (WHO, 2012).

Scope

20. This report relates only to consideration of cassava and cassava products and not to other foods where hydrogen cyanide may be produced, and for which JECFA indicated there may be an effect on human health.

Approach to consideration of maximum levels

21. The figure below presents the logic followed by the eWG in determining if MLs need amending or specifying for cassava derived foods. Each step is described below.


1. JECFA consideration: Possible
2a. Can the assumptions underpinning the JECFA review be refined?
2b. Should MLs apply to cassava derived foods rather than raw cassava?
Identify research needs
3. What are the remaining data gaps?
4. Consider RM Options
6. Consider other RM options
N
5. Would lowering or introducing new MLs manage risk?
Y
7. Consider impact of potential MLs
9. Recommendations to CCCF
8. Propose revised MLs

Step 1: JECFA consideration

22. The approaches taken by JECFA for the dietary assessment are summarized in **Annex 2**. Based on the limited data available, JECFA concluded that exceedances of acute and chronic health based guidance values could occur for populations assuming complete conversion of CGs to HCN and for some of the estimates, based on raw cassava with no reduction in levels of HCN due to food processing. Additionally it was estimated that the ARfD and PMTDI would be marginally exceeded by children with a high percentile dietary exposure to ready-to-eat cassava chips. No exceedances were found based on consumption of cassava flour. JECFA noted that more detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

Assumptions relating to concentrations in foods

23. The data used to estimate national exposures to HCN are based on limited data and may overestimate risk since in some cases they are old and predate the introduction of current MLs and subsequent actions taken to reduce HCN concentrations in the raw material and in processed food. The data therefore may not reflect current levels in some foods. For example the Australian data included HCN levels contributed by cassava chips prior to the implementation of an ML for these products.

24. Since the available occurrence data for HCN in cassava and its products were not considered appropriate for determining international estimates of acute and chronic exposure to HCN, these estimates were based on the level for sweet cassava and the ML cassava flour. **Annex 3** contains information on concentrations of HCN found in raw cassava and in processed foods from different regions. Despite the current paucity of data there is evidence that processing of cassava, even varieties high in HCN, can result in low concentrations of HCN in the final product (e.g. baton de manioc and fufu in Cameroon, the fermented akyeke product in Ghana).

Assumptions relating to dietary exposure

25. The dietary exposure data used by JECFA were based on data submitted from Australia and New Zealand and in the literature. Data were available from a small number of countries and are not representative of all countries where cassava products form a considerable part of the diet. Total HCN was used and this represents a conservative analysis, as not all cyanogenic glycosides in the plant material will remain after processing or will be converted to HCN *in vivo*. Due to the insufficient occurrence data for some foods, the absence of adequate concentration data for processed foods and absence of information on consumption, international estimates of chronic and acute dietary exposure were not made.

26. Instead, acute exposure was calculated based on WHO large portion consumption data (97.5th percentile), assuming an HCN concentration of 50 mg/kg for cassava. Large portion consumption data are based on raw commodities only. This resulted in an estimate of 150 µg/kg bw per day for the general population and 330 µg/kg bw per day for children and therefore exceeded the ARfD of 90 µg/kg bw.

27. Chronic dietary exposure was based on the GEMS/Food consumption cluster diets for cassava and assuming the ML of 50 mg/kg was the concentration found in the food as consumed. For diets in clusters A, I, J and K (mainly including African and South American countries) exceedances of the HBGV were estimated. However there was no exceedance estimated for other clusters with lower consumption levels (G, H, L, M). There was no consumption of cassava or cassava flour for a number of clusters, therefore these were not included in the assessment (clusters B, C, D, E and F).

28. Some dietary exposure estimates used country specific data for cassava consumption. Consumption of cossettes in the Democratic Republic of Congo with analyzed concentrations showed the PMTDI could be exceeded. For Australia and New Zealand, consumption amounts for boiled or baked cassava with analyzed concentrations in the raw tuber, and other foods included in the assessment showed both the acute and chronic HBGVs could be exceeded.

Step 2a: Refinement of assumptions

Total conversion of CGs in food to HCN in gut

29. An assumption that the total HCN content of a food will be converted to free HCN in the gut may over-estimate the risk. The ARfD is based on a developmental toxicity study which administered linamarin by gavage. The ARfD for linamarin was converted to a cyanide-equivalent dose. The PMTDI was based on administration of sodium cyanide via drinking water. In the latter case a value based on sodium cyanide may over-estimate the risk from the consumption of foods with CGs or cyanohydrin as different levels of conversion may occur *in vivo*. As noted by JECFA the results of the dietary exposure based on total HCN is a worst-case scenario. JECFA documented 3 studies of humans exposed to linamarin or HCN. Following consumption of cassava root 28% of ingested linamarin was excreted unmetabolized. In a second study 13% of the cyanide content of gari was absorbed in the gastrointestinal tract. And in a third study, where a cassava-based porridge (ugali) was consumed, approximately 21-23% of ingested linamarin was excreted intact in the urine, mainly within the first 24 hours. Based on the limited data available, JECFA stated in their summary that between 8% and 32% of the cyanogenic glycoside dose was absorbed and excreted unmetabolized (based on all studies of a range of CG containing foods and not just on cassava). The residual fraction of unabsorbed CGs can be converted via microorganisms to ultimately generate HCN. No data appear to be available on the extent to which this conversion occurs for different foods derived from cassava.

Further information on concentrations in foods

30. To address whether the assumptions underpinning the JECFA evaluation could be refined the eWG has considered whether there is additional information available on concentrations in processed foods and considered the effects of processing.

31. Annex 3 contains the information on concentrations in different foods, as documented by JECFA, together with further information from Brazil, the Pacific Islands and the Philippines. A comparison of this compilation with the collation of cassava derived foods which are consumed internationally indicates there appear to be gaps for some products e.g. cassava paste, gethak, growol, tape, tiwul, ugali. Within category groups there are very wide variations e.g. for products derived from cassava leaves. Furthermore where there are reported values for foods which are consumed in different parts of the world they do not represent broad geographical areas and therefore critical factors such as different raw materials and processing, to enable the values to be used more generally.

32. The JECFA conclusion was in-part based on some dietary exposure estimates that were based on the assumption that the food as consumed contained HCN at the ML in the Codex standards. On the basis of the exceedances of the AfRD for children for cassava noted above, calculated using the WHO large portion consumption, processing would need to reduce the HCN level by approximately four-fold to reduce the exposure of high consumers of cassava to below the relevant HBGV.

33. The eWG has considered different food processing of cassava and the evidence base for a reduction in concentrations (**Annex 4**). Further consideration is not given to flour since JECFA indicated there was no risk of exceeding the HBGV if the Codex ML of 10 mg/kg was adhered to.

Further information on consumption of cassava products

34. For the JECFA assessment based on the cluster diets, the consumption data is based on food available for consumption (i.e. all domestic production, minus exports, including imports), not what is actually consumed, therefore would lead to an overestimate of dietary exposure. The highest consumption amount in a cluster was around 280 grams per day (just over one metric measuring cup). Even if the actual consumption was half the level in the cluster diets there would still be an exceedance of the HBGV for the four clusters indicated above if the HCN concentration was at the ML.

35. The EWG did not obtain any additional information on consumption patterns which could be used to refine the dietary exposure estimates with the exception of some information from Brazil.

36. In relation to acute dietary exposure, Brazil stated a high consumer would eat around 500 grams of cassava per day. At a concentration of HCN of 50 mg/kg this would lead to an acute dietary exposure 4.5 times higher than the ARfD. At this level of consumption, the concentration of HCN in the food consumed would need to be 10 mg HCN / kg or less for exposure to remain under the acute reference dose. This level of consumption is higher than the WHO large portion used in the JECFA assessment of around 180 grams/day for adults, which also indicated an exceedance of the ARfD when using the ML of 50 mg/kg.

37. In relation to chronic dietary exposure, Brazil submitted data indicating the consumption of cassava is 5.0 grams/day by urban inhabitants and 11.6 grams/day by rural inhabitants (assumed to be per person per day, not per consumer per day). The cluster diet containing Brazil had a consumption level of 57.7 grams per day for cassava specifically based on food available for consumption. As indicated above this would be an overestimate of actual consumption amounts. Based on the new submitted data and a concentration of 50 mg/kg in the cassava, there would be no exceedance of the PMTDI for either urban or rural inhabitants.

Step 2b: Appropriateness of applying MLs to the commodity (cassava tubers)

38. Currently Codex levels and MLs apply to the cassava tuber and two products derived from it – flour and gari. The types of products consumed in different regions vary considerably.

39. Food preparation methods differ and therefore levels of HCN in the final food will also vary. Therefore consideration needs to be given to whether the control of ML should be at the commodity level or at a generic food type level, based on processing. Codex sets MLs on the basis of the protection of human safety and also to apply common standards to internationally traded goods. Consideration of the need for setting MLs therefore needs to take account of which processed foods are traded, in addition to the trade in raw cassava. Table 1 collates information on types of cassava products and their distribution and use in trade.

Table 1: Types and distribution of cassava products

Type of product	Traded internationally	Country/region	Description
Cassava (sweet) tubers (roots)	No	Australia Central African Republic Democratic Republic of Congo Fiji Indonesia Malawi Mozambique Nigeria Tonga Vanuatu	Varieties of cassava roots that contain less than 50 mg per kg of HCN (fresh weight basis)".
Cassava (Bitter) tubers (roots)	No	Central African Republic Democratic Republic of Congo Fiji Mozambique Nigeria United Republic of Tanzania Malawi Tonga Vanuatu	Bitter varieties of cassava are those that contain more than 50 mg/kg but less than 200 mg/kg HCN (fresh weight basis).
Cassava leaves e.g. *pondu*	No	Brazil Democratic Republic of Congo	Cassava leaves can be eaten as a fresh vegetable, ground fresh and frozen in plastic bags, or dried and ground for sale in plastic bags. Leaves are more nutritionally balanced than the roots and can help to prevent certain deficiency diseases. Leaves, however, may be high in HCN, but the HCN can be reduced to safe levels in most cases when the liquid is squeezed out after grinding and through evaporation during cooking.
Cassava (Dried roots) e.g. Gaplek	Yes	Indonesia	Dried cassava roots are stored or marketed as chips, balls and flour. Chips and balls are milled into flour by pounding with a pestle and mortar in preparation for a meal. There are two broad types of dried cassava roots: fermented and unfermented.
Cassava Flour *Farinha* *Garri* *Casabe*	Yes	Brazil Democratic Republic of Congo Mozambique United Republic of Tanzania Vanuatu	Edible cassava (*Manihot esculenta Crantz*) flour is the product prepared from dried cassava chips or paste by a pounding, grinding or milling process, followed by sifting to separate the fibre from the flour. In case of edible cassava flour prepared from bitter cassava (*Manihot utilissima Pohl*), detoxification is carried out by soaking the tubers in water for a few days, before they undergo drying in the form of whole, pounded tuber (paste) or in small pieces. Toasted flour (Brazil) Toasted flour (West Africa) Flat bread (Caribbean)

Type of product	Traded internationally	Country/region	Description
Cassava chips	Yes	Australia New Zealand Fiji Indonesia The Philippines	Represented as snack foods suitable for consumption in the same state in which they are sold, i.e. with no further preparation and ready for immediate consumption. These foods are often represented as “chips”, “crisps”, “crackers”, “vege crackers” or with other snack food terms. Cassava chips are called “keripik” while cassava crackers are called “krupuk” in Indonesia Cassava chips can be made from cassava slices or extruded, which can have an impact on HCN concentrations in the final product.
Cassava paste (e.g. chickwangue)	Possible	Democratic Republic of Congo	Uncooked and steamed pastes. To prepare the uncooked paste, the roots are soaked in water for three to five days, during which time the roots soften and ferment. The soaked roots are manually crushed and sieved by shaking in a basket in a sack under water, thereby separating the pulp into the sack while collecting the fibre in the basket. Steamed paste is a product that can be stored or marketed in a steamed form. To prepare the paste, fibre is removed by hand from roots fermented by soaking in water. The roots are then stacked in a heap to further ferment. The pulp is ground with a stone or pounded in a mortar. The resulting fine pulp is firmly wrapped in leaves and steamed.
Cassava starch	No	Indonesia	Cassava starch is used directly in different ways or as a raw material for further processing. Special features of cassava starch are its viscosity, resistance to shear stress and resistance to freezing. The main classes of starch-based products are: i) Unmodified or native starch; ii) Modified (physical, chemical, biological) starches for industrial purposes; iii) Sweeteners, including high fructose syrup, glucose (dextrin, monosodium glutamate, pharmaceuticals, etc.).
Cassava (frozen) for frying	Yes	Brazil	Frozen shelled sweet cassava roots pre cooked as a necessary heat treatment before consumption
Attieke	No		Steamed cassava
Akyeke	No	Ghana	Fermented product, steamed
Baton de manioc	No	Cameroon	It is made from the tubers of the cassava (manioc) plant. After the tubers are harvested, they are soaked in water, then boiled, mashed, wrapped in leaves, and then steamed.
Bila	No	Fiji	Sticks of cassava peeled and diced and soaked in water for 3 days. Then mashed, dried, mixed with sugar and coconut, wrapped in a leaf and boiled.
Cossettes	No	Democratic Republic of the Congo	Bitter cassava tubers (whole) are soaked and fermented for 3 days then sun dried.

Type of product	Traded internationally	Country/region	Description
Fufu	No	Cameroon Nigeria	A paste-like meal made from cooked fermented roots or flour
Gari	No	Nigeria	Gari is the finished product obtained by artisanal or industrial processing of cassava tubers. The processing consists of peeling, washing and grating of the tubers. The resulting pulp is put in a porous sack and weighed down with a heavy object for three to four days to express effluent from the pulp while it is fermenting. The de-watered and fermented lump of pulp is pulverized and sieved and the resulting semi-dry fine pulp is toasted in a pan. Gari is presented as flour of variable granule size.
Gethuk	No	Indonesia	Snack food from Indonesia made from peeled and steamed or boiling cassava. After being steamed, it is milled and then polished by sugar.
Growol	No	Indonesia	Indonesian traditional food prepared from cassava. Fresh peeled cassava roots are soaked in water for three to five days, followed by pressing to decrease moisture content. Then, it is steamed to make it “ready to eat”
Ljapu	No	Nigeria	Stepped cassava slices
Maniçoba	No	Brazil	Cassava leaves ground and cooked for 1 week approximately to reduce the HCN content to safe concentrations
Makopa	No	United Republic of Tanzania	Sun-dried tuber pieces
Mocaf (Modified cassava flour)	No	Indonesia	Fresh cassava roots are peeled, washed and shredded. The cassava is then soaked and added with inoculum of lactic acid bacteria (room temperature for 12 hours). The water is removed, followed by pressing, drying, milling and sieving
Multimistura	No	Brazil	Composed of 5% cassava leaves, bran of wheat and rice, corn and wheat flours and other ingredients
Tapioca	Yes	Brazil Nigeria	Cassava is grated and then put in water, pressed and kneaded to release the starch. The starch is permitted to settle at the bottom of the container and the water is drained off. The operation is repeated several times to prepare a high quality product. The damp starch is spread on a pan and toasted in the same way as gari, to form a coarse granular product.

Type of product	Traded internationally	Country/region	Description
Tape	No		Fermented cassava with a sweet and acidic taste and mild alcoholic flavour. Cassava roots are peeled, cut into pieces, washed, steamed until cooked and cooled. Traditionally, the cooked cassava roots are placed in layers on bamboo baskets which are covered with banana leaves. Inoculum (ragi tape) is then sprinkled on each layer. Incubation is carried out at 30ºC for 48-72 hours. During fermentation, the cassava roots soften and develop a sweet/sour slightly alcoholic flavour. The product which is somewhat juicy can be consumed right away.
Tiwul	No	Indonesia	Traditional meal from Java, Indonesia made from dried root cassava flour (tapioca). Water is added and it is steamed for about 20 to 30 minutes.
Tucupi	No	Brazil	A liquid product from a fermentation of bitter cassava pressed mass
Ugali	No	United Republic of Tanzania	Stiff cassava porridge
Vakalavalava	No	Fiji	Grated cassava, wrapped in leaves and boiled or baked.

40. A consideration of the range of products which are produced from cassava, and those which are traded, indicates dried roots, cassava chips, frozen cassava, tapioca and possibly cassava paste, are internationally traded. Notably there are no MLs for several of these. Gari has limited distribution and is not traded, but a standard exists for this product. The eWG could not establish the rationale for this standard being produced although it apparently was developed initially as a regional standard and presumably was to address local health and safety concerns. A number of flour-like products are consumed in different countries but currently Codex standards do not cover these products and JECFA was unable to characterize the risk from consumption of such products due to the absence of consumption data and data on concentrations in food.

41. It might be possible to control for the risk of unacceptably high concentrations of HCN being present in different product types by taking the worst case scenario, and setting the most conservative MLs in the raw commodity. However another approach would be to set different MLs for different food products. This is the approach which has been taken to date in the Codex standards for gari and for flour. This approach has the advantage of providing a more relevant measure to reduce risk than one based on the raw material. However a further consideration is that the total HCN concentration in a food product does not necessarily reflect its relative toxicity since this is dependent on the composition i.e. whether the HCN is present as the CG or as cyanohydrin since they may have different potential to produce HCN in the gut. In the case of gari the higher cyanohydrin concentration, with a consequent higher generation of HCN *in vivo* (WHO, 2012), justifies the lower level.

42. JECFA considered the effect of processing on total HCN for flour. Based on a 97% reduction of total HCN which can arise from the most efficient processing method (crushing and sun drying) they concluded that concentrations of HCN in the source cassava root which were much higher than those specified in the sweet cassava standard (i.e. 330 mg/kg) would not result in an exceedance of the ML of 10 mg/kg in cassava flour. This suggests therefore that ensuring or encouraging efficient food processing could be a more effective risk management measure than the setting of new MLs.

Step 3: What are the remaining data gaps?

43. A number of data gaps were highlighted by JECFA in their risk assessment and are confirmed by this eWG, particularly:

- the lack of information on the amount of cassava consumed
- the concentration of HCN which results in adverse health effects in exposed populations
- metabolism of cyanogenic glycosides from different products to refine the assumption that all cyanide is available for absorption
- concentrations of total HCN in different foods
- how nutritional factors ultimately contribute to the human diseases observed in populations whose diets consist mainly of improperly processed cassava, which involves high cyanide exposure

Step 4: Consider risk management options

44. Revision of MLs could be justified if the estimates of exposure to HCN in food, taking account of further information on effects of processing or concentrations found in different foods in different regions, confirms there is a risk from consumption of cassava products. Furthermore adoption of this option requires evidence that MLs for raw cassava is the critical point for controlling the exposure to HCN for humans

45. Alternatively the effectiveness of options other than amending Codex standards could be considered, such as:

    - development, implementation and evaluation of the efficacy of a code of practice to reduce concentrations to mitigate the risk of exceeding HBGVs.
    - educate vulnerable populations on processing and agricultural practices which can reduce concentrations of HCN.
    - retaining existing MLs and promoting the development of specific MLs within individual regions which take account of concentrations present in the raw commodity and the specific cassava products which are consumed.

Step 5: Evaluation of lowering the MLs or introducing new MLs

46. The effectiveness of reducing MLs can be addressed by looking at the proportion of samples from analytical data that are removed if MLs are lowered, then estimating dietary exposures based on the re-derived mean or other representative concentrations to assess the impact on health. However, there are no distributional data available for CGs to enable JECFA to do that sort of analysis. Only more simple deterministic calculations could be done, and these wouldn't be extensive based on the limited consumption data for different products. Furthermore there appears to be some evidence that even the current MLs are not being complied with in some cases. Setting MLs on the basis of raw cassava may not be the appropriate point of control, given the importance of processing in reducing concentrations and the variability in HCN concentrations in different product types. There is evidence that some products can be produced from cassava tubers above the current level for sweet cassava with concentrations of total HCN which do not pose safety risks. Furthermore the eWG agreed with JECFA's evaluation on the uncertainty of the risk assessment due to the paucity of data. Due to these factors at this stage the lowering of MLs, or development of new ones, does not appear to be an appropriate risk management measure.

Step 6: Evaluation of other risk management options

47. In the absence of generally applicable MLs, setting levels on the basis of individual food types could be considered by individual countries or regions. Such standards would be relevant to their types of food and consumption patterns. This is the approach adopted to date by Australia and New Zealand (cassava crisp snack foods); Brazil (multiminstra) Indonesia (Mocaf) and the Philippines (chips and granules).

48. There appears to be sufficient information to produce a code of practice which, if implemented, could lead to a reduction in exposure to HCN (see Attachment 2). This seems to be a preferred risk management measure at this time.

49. Additionally, there is some evidence that education activities can be an effective measure (Bradbury et al, 2010; Mlingi et al, 2010).

50. Additional research is required to generate more data on concentrations in final foods and consumption to enable more realistic risk assessments with subsequent re-consideration by JECFA.

Step 7: Impact analysis

51. If MLs were to be revised, or new MLs proposed, then an evaluation would need to be made of the impact of these on human health, particularly whether the exceedances of the HBGVs would be removed as a result of the change. Additionally, in recognition of the importance of cassava as a staple diet component in many countries and hence its importance to food security and to trade in developing nations, the potential impact of new MLs on food availability and trade would also need to be addressed. In view of the consideration that amending MLs is not the preferred risk management measure, an impact analysis has not been conducted. However it is noted that due to the importance of cassava as a staple food in times of drought and food scarcity MLs are unlikely to be an effective control measure at times of stress.

Step 8: New MLs

52. Based on the considerations above, the development of new MLs is not currently justified.

REFERENCES

Adindu, Olayemi and Nze-Dike (2003). Cyanogenic potential of some cassava products in Port Harcourt markets in Nigeria. Journal of food Composition and Analysis, 16:21-24.

Agbor-Egbe and Lupe Mbome (2006). The effects of processing techniques in reducing cyanogen levels during production of some Cameroonian cassava foods. Journal of food Composition and Analysis, 19(4): 354-363.

Agostini, M.R. (2006). Produção e utilização de farinha de mandioca comum enriquecida com adição das próprias folhas desidratadas para consumo alimentar. Botucatu – SP: Universidade Estadual Paulista (Master thesis), http://www.pg.fca.unesp.br/Teses/PDFs/Arq0146.pdf

Alonso-Amelot ME, Oliveros A. (2000) A method for the practical quantification and kinetic evaluation of cyanogenesis in plant material. Application to Pteridium aquilinum and Passiflora capsularis. Phytochemical Analysis; 11(5): 309-316.

Amarowicz R, Wanasundara PKJPD, Shahidi F. (1993) TLC Separation of linamarin, linustatin and neolinustatin. Food / Nahrung; 37(1): 88-90.

Bisset FH, Clapp RC, Coburn RA, Ettlinger MG, Long Jr L. (1969) Cyanogenesis in manioc: Concerning lotaustralin. Phytochemistry; 8(11): 2235-2247.

Bradbury HJ (2006). Simple wetting method to reduce cyanogen content of cassava flour. Journal of Food Composition and Analysis, 19, 388-393.

Bradbury JH. (2009) Development of a sensitive picrate method to determine total cyanide and acetone cyanohydrin contents of gari from cassava. Food Chemistry; 113(4): 1329-1333.

Bradbury JH, Bradbury MG, Egan SV. (1994) Comparison of methods of analysis of cyanogens in cassava. Acta Horticulturae; 375: 87-96.

Bradbury JH, Cliff J and Denton IC. (2011) Uptake of wetting method in africa to reduce cyanide poisoning and konzo from cassava. Fd Chem toxicol, 49, 539-542.

Bradbury JH, Egan SV. (1992) Rapid screening assay of cyanide content of cassava. Phytochemical Analysis; 3(2): 91-94.

Bradbury MG, Egan and Bradbury JH(1999). Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. Journal of the Science of Food and Agriculture, 79(4):593-601.

Bradbury JH, Egan SV, Lynch MJ. (1991) Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. Journal of the Science of Food and Agriculture; 55(2): 277-290.

Brimer L, Broegger Christensen S, Moelgaard P, Nartey F. (1983) Determination of cyanogenic compounds by thin-layer chromatography. 1. A densitometric method for quantification of cyanogenic glycosides, employing enzyme preparations (beta-glucuronidase) from *Helix pomatia* and picrate-impregnated ion-exchange sheets. Journal of Agricultural and Food Chemistry; 31(4): 789-793.

Brimer L, Dalgaard L. (1984) Cyanogenic glycosides and cyanohydrins in plant tissues: Qualitative and quantitative determination by enzymatic post-column cleavage and electrochemical detection, after separation by high-performance liquid chromatography. Journal of Chromatography A; 303(0): 77-88.

Brimer L, Rosling H. (1993) Microdiffusion method with solid state detection of cyanogenic glycosides from cassava in human urine. Food and Chemical Toxicology; 31(8): 599-603.

Burns AE, Bradbury JH, Cavagnaro TR, Gleadow RM. (2012) Total cyanide content of cassava food products in Australia. Journal of Food Composition and Analysis; 25(1): 79-82.

Campa C, Schmitt-Kopplin P, Cataldi TRI, Bufo SA, Freitag D, Kettrup A. (2000) Analysis of cyanogenic glycosides by micellar capillary electrophoresis. Journal of Chromatography B: Biomedical Sciences and Applications; 739(1): 95-100.

Cardoso et al (2005). Processing of cassava roots to remove cyanogens. Journal of food Composition and Analysis, 18:451-460.

Carlsson, L., Mlingi, M., Juma, A., Ronquist, G. & Rosling, H. (1999). Metabolic fates in humans of linamarin in cassava flour ingested as stiff porridge. Fd. Chem. Toxicol. 37; 307-312.

Cardoso PA, Mirione E, Ernesto M et al (2005). Processing of cassava roots to remove cyanogens. Journal of Food Composition and Analysis, 18, 451-460.

Chadha RK, Lawrence JF, Ratnayake WMN. (1995) Ion chromatographic determination of cyanide released from flaxseed under autohydrolysis conditions. Food Additives and Contaminants; 12(4): 527-533.

Chiwona-Karltun et al, (2004). Bitter taste in cassava roots correlates with cyanogenic glusoside levels. Journal of the Science of Food and Agriculture, 84(6):581-590.

Cheeke PR. (1989). Toxicants of plant origin. Volume II. Glycosides. CRC Press Inc.

Chiste RC, Cohen KO and Oliveira SS (2005). Determinação de cianeto durante as etapas de processamento da Farinha de mandioca do grupo seca. http://artigocientifico.uol.com.br/uploads/artc_1166151172_39.pdf

Chiste RC, Cohen KO and Oliveira SS (2007). Estudo das propriedades físico-químicas do tucupi - Study of tucupi physicochemical properties. *Ciênc. Tecnol. Aliment.*, 27(3): 437-440, http://www.scielo.br/pdf/cta/v27n3/a02v27n3.pdf

Codex Alimentarius Commission. (1995a) Codex standard for edible cassava flour. Codex Stan 176-1989. Accessed at: http://www.codexalimentarius.org/standards/list-of-standards/en/?no_cache=1. Accessed: 4 December 2012.

Codex Alimentarius Commission. (1995b) Codex standard for gari. Codex Stan 151-1989. Accessed at: http://www.codexalimentarius.org/standards/list-of-standards/en/?no_cache=1. Accessed: 4 December 2012.

Codex Alimentarius Commission. (2011) Codex standard for sweet cassava. Codex Stan 238-2003. Accessed at: http://www.codexalimentarius.org/standards/list-of-standards/en/?no_cache=1. Accessed: 4 December 2012.

Cooke RD. (1978) An enzymatic assay for the total cyanide content of cassava (manihot esculenta crantz). Journal of the Science of Food and Agriculture; 29(4): 345-352.

Cressey P, Saunders D. (2010) Survey of cyanogenic glycosides in plant-based foods. unpublished ESR Client Report FW10037. Christchurch: ESR.

Curtis AJ, Grayless CC, Fall R. (2002) Simultaneous determination of cyanide and carbonyls in cyanogenic plants by gas chromatography-electron capture/photoionization detection. Analyst; 127(11): 1446-1449.

Djazuli and Bradbury (1999). Cyanogen content of cassava roots and flour in Indonesia. Food Chemistry, 65:523-525.

Drochioiu G, Arsene C, Murariu M, Oniscu C. (2008) Analysis of cyanogens with resorcinol and picrate. Food and Chemical Toxicology; 46(11): 3540-3545.

Essers AJA, Alink GM, Speijers GJA, Alexander J, Bouwmeister P-J, van den Brandt PA, Ciere S, Gry J, Herrman J, Kuiper HA, Mortby E, Renwick AG, Shrimpton DH, Vainio H, Vittozzi L, Koeman JH. (1998) Food plant toxicants and safety: Risk assessment and regulation of inherent toxicants in plant foods. Environmental Toxicology and Pharmacology; 5(3): 155-172.

Essers AJA, Bosveld M, van der Grift RM, Voragen AGJ. (1993) Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. Journal of the Science of Food and Agriculture; 63: 287-296.

European Committee for Standardization (CEN). (2012) Animal feeding stuffs - Determination of hydrocyanic acid by HPLC. EN 16160: 2012.

Ernesto et al (2000). Cyanogens in cassava flour and roots and urinary thiocyanate concentration in Mozambique. Journal of Food Composition and Analysis, 13:1-12.

Eyjólfsson R. (1970) Recent advances in the chemistry of cyanogenic glycosides. Fortschritte der Chemie Organischer Naturstoffe; 28: 74-108.

FAO Regional Programme for Food Security (Draft 2011). A study on cyanide levels in cassava and some of its products in selected Pacific Island Countries. Institute of Applied Science, University of the South Pacific.

Feigl F, Anger V. (1966) Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. Analyst; 91(1081): 282-284.

FSANZ (2004). Cyanogenic glycosides in cassava and bamboo shoots. A Human Health Risk Assessment. Technical report Series No. 28, http://www.foodstandards.gov.au/_srcfiles/28_Cyanogenic_glycosides.pdf

FSANZ (2008). Proposal P1002 hydrocyanic acid in ready-to-eat cassava chips. http://www.foodstandards.gov.au/foodstandards/proposals/proposalp1002hydrocy3848.cfm

FSANZ (2009) Final assessment report proposal P1002. Hydrocyanic acid in ready-to-eat cassava chips. First review report. Report Number 2-09. Canberra: FSANZ.

Gomez G and Valdivieso M (1985). Cassava foliage: chemical composition, cyanide content and effect of drying on cyanide elimination. Journal of Food and Agriculture, 36: 433-441.

Haque RM and Bradbury HJ (2002). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry, 77, 107-114.

Hosel, W. (1981). The enzymatic hydrolysis of cyanogenic glucosides. In B. Vennesland, E. E. Conn, C. J. Knowles, J. Westley, & F. Wissing (Eds.), Cyanide in biology (pp. 217-232). London: Academic Press.

Kaminski T.A. et al. (2006). Avaliação dos elementos tóxicos, antinutricionais e patógenos em multimisturas. *Alim Nutr.*, 17(2): 171-179.

Kemdirim OC, Chukwu OA, Achinewhu SC (1995) Effect of traditional processing of cassava on the cyanide content of gari and cassava flour. Plant Foods Hum Nutr. 48(4):335-9.

Keusgen M, Kloock JP, Knobbe DT, Jünger M, Krest I, Goldbach M, Klein W, Schöning MJ. (2004) Direct determination of cyanides by potentiometric biosensors. Sensors and Actuators B: Chemical; 103(1–2): 380-385.

Knudsen I, Søborg I, Eriksen F, Pilegaard K, Pedersen J. (2005). Risk assessment and risk management of novel plant foods. Concept and principles. Tema Nord 2005: 588. pp. 47-49.

Kobaisy M, Oomah BD, Mazza G. (1996) Determination of cyanogenic glycosides in flaxseed by barbituric acid-Pyridine, Pyridine-Pyrazolone, and High-Performance Liquid Chromatography Methods. Journal of Agricultural and Food Chemistry; 44(10): 3178-3181.

Kupchella L, Syty A. (1984) Determination of cyanogenic glycoside in seeds by molecular absorption spectrometry in the gas phase. Journal of the Association of Official Analytical Chemists; 67(1): 188-191.

Lambert JL, Ramasamy J, Paukstelis JV. (1975) Stable reagents for the colorimetric determination of cyanide by modified Koenig reactions. Analytical Chemistry; 47(6): 916-918.

Laurena AC, Revilleza MJR, Mendoza EMT. (1994) Polyphenols, phytate, cyanogenic glycosides, and trypsin inhibitor activity of several Philippine indigenous food legumes. Journal of Food Composition and Analysis; 7(3): 194-202.

Mak KW, Law AC, Tokuda S, Yanase H, Renneberg R. (2005) Application of cyanide hydrolase from Klebsiella sp. in a biosensor system for the detection of low-level cyanide. Applied Microbiology and Biotechnology; 67(5): 631-636.

Mariscal, et al., (2009) Recommended Cassava Varieties in the Philippines. Unpublished Report. Philippine Root Crop Research and Training Center, Visayas State University, Visca, Baybay City, Philippines. (Accessed 22 January 2009).

Mezzete T.F. (2009). Seleção de clones-elite de mandioca de mesa visando a características agronômicas, tecnológicas e químicas. *Bragantia*, 68(3): 601-609.

Mkumbira et al, (2003). Classification of cassava into "bitter" and "cool" in Malawi: from farmers' perception to characterisation by molecular markers. Euphytica, 132(1):7-22.

Miles D, Jansson E, Mai MC, Azer M, Day P, Shadbolt C, Stitt V, Kiermeier A, Szabo E. (2011) A survey of total hydrocyanic acid content in ready-to-eat cassava-based chips obtained in the Australian market in 2008. Journal of Food Protection; 74(6): 980-985.

Mlingi et al (1998). Low cyanide exposure from consumption of cassava in Dar es Salaam, Tanzania. Natural Toxins, 6:67-72.

Mlingi NLV, Nkya S, Tatala SR, Rashid S and Bradbury JH. (2010) Recurrence of konzo in southern Tanzania: rehabilitation and prevention using the wetting method. Fd. Chem. Toxicol. 49, 673-677.

Moller, B.L. and Seigler, D.S. (1999). Biosynthesis of cyanogenic glycosides, cyanolipids and related compounds. In B.K. Singh (Ed.), Plant amino acids biochemistry and biotechnology (pp. 563-609) Marcel Dekker.

Nagashima S. (1978) Spectrophotometric determination of cyanide with sodium isonicotinate and sodium barbiturate. Analytica Chimica Acta; 99(1): 197-201.

Nambisan B (2011). Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. Food abd Chemical Toxicology, 49, 690-693.

Ngudi DD, Kuo Y-H, Lambein F.(2002). Food safety and amino acid balance in processed cassava "cossettes". Journal of Agricultural and Food Chemistry. 50: 3042-3049.

Obile, Tano-Debrah and amoa-Awua (2004). Souring and breakdown of cyanogenic glycosides during the processing of cassava into akyeke. International Journal of food Microbiology, 93(1):115-121.

Oliveira L.A. et al. (2009) Avaliação do conteúdo de carotenóides e compostos cianogênicos em híbridos de mandioca. *Revista Raízes e Amidos Tropicais*, 5: 805-809.

Oluwole et al, (2000). Low prevalence of ataxic polyneuropathy in a community with high exposure to cyanide from cassava foods. Journal of Neurology, 249:1034-1040.

Oluwole OSA, Onabolu AO, Mtunda K and Mlingi N (2007). Characterization of cassava (Manihot esculenta Crantz) varieties in Nigeria and Tanzania and farmers perception of toxicity of cassava. Journal of Food Composition and Analysis, 20, 559-567.

Onabolu AO, Oluwole OS, Bokanga M. (2002) Loss of residual cyanogens in a cassava food during short-term storage. Int J Food Sci Nutr. 53(4):343-9.

Padmaja G. (1995.) Cyanide detoxification in cassava for food and feed use. Critical Reviews in Food Science and Nutrition, 35 (4): 229-339.

Ravi S, Padmaja G.(1997). Mechanism of cyanogen reduction in cassava roots during cooking. Journal of the Science of Food and Agriculture. 75: 427-432.

Rimoldi F. et al. (2005). Teores de amido, de HCN e tempo de cozimento de raízes tuberosas de cultivares de mandioca-de-mesa coletadas no paraná. In: XI Congresso Brasileiro de Mandioca, 2005, Campo Grande - MS. Ciência e Tecnologia para a Raiz do Brasil. Campo Grande - MS, v.1, http://www.cpao.embrapa.br/11cbm/_html/trabalhos/arquivoPDF/pasta49a.PDF

Saka JDK, Mhone ARK, Brimer L. (1998) An improved microdiffusion method with solid-phase detection for the determination of total cyanogens in fresh cassava. Comparison to the method of Cooke (1978). Journal of the Science of Food and Agriculture; 76(3): 334-340.

Sano A, Takimoto N, Takitani S. (1992) High-performance liquid chromatographic determination of cyanide in human red blood cells by pre-column fluorescence derivatization. Journal of Chromatography B: Biomedical Sciences and Applications; 582(1–2): 131-135.

Sant´Ana A.F. and Domene S.M.A. (2008). Teores de glicosídeos cianogênicos em derivados de mandioca determinados por protocolo adaptado ao laboratório de micronutrientes – IC PUC-CAMPINAS. In: *XIII Encontro de Iniciação Científica da PUC-Campinas, Anais., Campinas – SP,* http://www.puc-campinas.edu.br/websist/portal/pesquisa/ic/pic2008/resumos/Resumo/%7B9CFE52AC-6CEA-4567-8530-B9DBE094A65F%7D.pdf

Silva G.G.C. et al. (2004). Toxicidade cianogênica em partes da plante de cultivares de mandioca cultivados em Mossoró-RN. *Revista Ceres*, 51 (293): 57-66.

Sokari and Karibo (1992). Changes in cassava toxicity during processing into gari and ijapi-two fermented food products. Food Additives and Contaminants, 9(4):379-384.

Sornyotha S, Kyu KL, Ratanakhanokchai K. (2007) Purification and detection of linamarin from cassava root cortex by high performance liquid chromatography. Food Chemistry; 104(4): 1750-1754.

Untang M, Shiowatana J, Siripinyanond A. (2010) A simple cyanide test kit for water and fruit juices. Analytical Methods; 2(11): 1698-1701.

WHO (1993). Cyanogenic glycosides. In: Toxicological evaluation of certain food additives and naturally occurring toxicants. Geneva, World Health Organization, 39th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (WHO Food Additives Series 30). Available at http://www.inchem.org/documents/jecfa/jecmono/v30je18.htm.

WHO (2012). Cyanogenic glycosides. In: Safety evaluation of certain food additives and contaminants. Geneva, World Health Organization, 74th Meeting of the Joint FAO/WHO Expert Committee on Food Additives (WHO Food Additives Series 65). Available at: WHO Food Additives Series No. 65, 2012.

Annex 1

National Standards and/or requirements for cassava in food

Country	Food	Concentration of HCN (mg/kg)	Other information
Australia and New Zealand [10]	Ready-to-eat cassava chips Raw sweet cassava	10 (total HCN) <50 (total HCN)	Standard 1.4.1 of the *Australia New Zealand Food Standards Code* sets out the maximum level of 10mg/kg total HCN in cassava chips Standard 1.4.4 – Prohibited the sale of cassava other than "sweet cassava". Consistent with the existing Codex Standard (CODEX STAN 238-2003), sweet cassava is defined in the Code (Standard 1.1.2 – Supplementary Definitions for Foods) as "those varieties of cassava roots grown from *Manihot esculenta Crantz* of the *Euphoribiacae* family that contain less than 50 mg per kg of HCN (fresh weight basis)". Standard 1.2.6 – Directions for Use and Storage includes a requirement for raw sweet cassava to be labelled or accompanied by a statement indicating that sweet cassava should be peeled and fully cooked before being consumed.
Brazil[11]	Multimistura	5	Multimistura is composed of 5% cassava leaves, bran of wheat and rice, corn and wheat flours and other ingredients
Indonesia	Cassava Flour Mocaf (modified cassava flour)	40 10	
The Philippines[12]	Dried cassava chips and granules	10 (total HCN on a dry weight basis)	Refers to total hydrocyanic acid which includes the hydrocyanic acid which maybe enzymatically released from a cyanogenic glycoside as well as any free or unbound hydrocyanic acid in cassava, expressed as milligrams of hydrocyanic acid per kilogram of cassava by-products (mg/kg)
	Sweet cassava	<50	Sweet cassava contains <50 mg hydrogen cyanide per kg of cassava root (fresh weight basis) and must be peeled and fully cooked before being consumed

[10] Australia/New Zealand http://www.comlaw.gov.au/Series/F2008B00618 and http://www.comlaw.gov.au/Series/F2008B00621

[11] Brazil. Resolução RDC n.53 de 15/06/2000. Regulamento Técnico para Fixação de Identidade e Qualidade de Mistura à base de Farelos de Cereais. Aprovado pelo Decreto 3.029, de 16 de abril de 1999. Diário Oficial da União. 2000 19 jun.

[12] Philippine National Standard for dried cassava chips and granules PNS/BAFPS 29:2010
ICS 67.080and Philippine National Standard for sweet cassava PNS/BAFPS 119:2013

Annex 2

JECFA evaluation of Dietary exposure

1. The dietary exposure estimates evaluated by JECFA included those submitted to JECFA for Australia and New Zealand and other information found in the literature (primarily for Africa and Europe). Both acute and chronic estimates of dietary exposure were considered. The estimated dietary exposures were generally expressed as exposure to total HCN, as this was the form recorded in most of the occurrence or analytical data. However, dietary exposure can be to cyanogenic glycosides, cyanohydrins or HCN, depending on the processing of the food. The use of total HCN for the dietary exposure estimates represents the maximum possible exposure to cyanide coming from substances derived from cyanogenic glycosides in foods.

2. Acute dietary exposures to total HCN were estimated using mean, maximum or high-percentile (e.g. 95th percentile) concentrations of total HCN from analytical data. Consumption data used for the estimates were amounts per day of individual foods for consumers with maximum or high consumption at the 97.5th, 95th or 90th percentile, where available.

3. Estimated acute dietary exposures to total HCN for a range of foods from the small number of countries for which information was available ranged between 1 and 1044 µg/kg bw per day, depending on the food and the population groups assessed. Highest exposure estimates of concentrations of HCN from cassava derived foods were for cassava (300 µg/kg bw per day for adults in New Zealand), ready-to-eat cassava chips (up to about 1000 µg/kg bw per day for children and up to 370 µg/kg bw per day for adults in Australia and New Zealand).

4. National estimates of chronic dietary exposure to total HCN[13] were available for Australia, the Democratic Republic of the Congo, Europe, New Zealand, Norway and the United Kingdom. These estimates are based on different food sources, including raw and processed foods and foods in which cyanogenic glycosides occur as a result of flavouring uses.

5. Estimated chronic dietary exposures to total HCN from the countries for which information was available were between less than 1 and 60 µg/kg bw per day for consumers with average exposure and between 2 and 150 µg/kg bw per day for consumers with high exposure.

6. The available occurrence data for cyanogenic glycosides were deemed not to be appropriate for use in determining international estimates of dietary exposure to total HCN in combination with the GEMS/Food consumption cluster diets for chronic dietary exposure or with WHO 97.5th percentile large portion data for acute dietary exposure. There were insufficient occurrence data for some foods or no concentration data for prepared or processed foods, which are more reflective of the concentrations in foods as consumed. In addition, cyanogenic glycosides occur in many processed foods, and consumption data for many of these foods are not included in the consumption cluster diets or large portion data. Therefore, no international estimates of chronic or acute dietary exposure were prepared.

Evaluation of existing MLs in relation to dietary exposure to cyanogenic glycosides

7. MLs for total HCN have been established by Codex and in a number of countries for foods including sweet cassava, cassava flour, gari and ready-to-eat cassava chips/crisps and for many foods containing flavouring agents.

8. Estimates of chronic and acute dietary exposure to total HCN were calculated for Australia and New Zealand, for which analytical survey data for ready-to-eat cassava chips (collected before the FSANZ ML was established) were substituted with the ML of 10 mg/kg. This resulted in mean chronic dietary exposures of 10 µg/kg bw per day for children and 2–11 µg/kg bw per day for adults and 90th percentile exposures of 10–40 µg/kg bw per day for children and 10–12 µg/kg bw per day for adults. These chronic exposure estimates are about 2–5 times lower than estimated dietary exposures based on mean survey values for cassava chips.

9. If all cassava chips were at the ML of 10 mg/kg, the estimated acute dietary exposures to total HCN would be up to a maximum of 100 µg/kg bw per day for children and 25 µg/kg bw per day for adults. These acute estimates are about 4–14 times lower than estimated dietary exposures based on mean survey values for cassava chips.

10. Acute dietary exposures based on WHO large portion consumption data for sweet cassava using an HCN concentration of 50 mg/kg were 150 µg/kg bw per day for the general population and 330 µg/kg bw per day for children. There was no consumption value for cassava flour in the large portion data set, but if it was assumed that the consumption of cassava flour is equivalent to that of cassava, estimated exposure to HCN based on the Codex ML of 10 mg/kg would be 30 µg/kg bw per day for the general population and 70 µg/kg bw per day for children.

11. Chronic dietary exposures to HCN from sweet cassava and cassava flour were estimated based on consumption amounts from the GEMS/Food consumption cluster diets and MLs. For sweet cassava at a maximum HCN concentration of 50 mg/kg (Codex level for sweet cassava), estimated dietary exposures ranged between 1 and 235 µg/kg bw per day for the clusters assessed. For cassava flour, based on the Codex ML of 10 mg/kg as total HCN, exposures ranged between less than 0.1 and 14 µg/kg bw per day.

[13] Includes from all food sources, not just cassava products.

Comparison of the dietary exposure to the HBGV

12. From the national acute dietary exposure estimates available to the Committee, the ARfD was exceeded 3-fold for cassava for adults (based on raw samples) and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained 10 mg/kg as HCN, equivalent to the recently established ML in Australia and New Zealand, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

13. Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 0.02 mg/kg body weight as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold for children and between 1- and 2-fold for adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributors to dietary exposure to HCN (84–93%). When the cassava chips contain a concentration equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI.

14. JECFA also reported the application of the level of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded, which is as low as 25 g/day for cassava for chronic exposure.

15. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

16. The level for sweet cassava is for the raw product. If the starting concentration of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give an HCN concentration of 2 mg/kg.

17. The JECFA estimates of exposure were based on limited dietary exposure data, including data from Australia and New Zealand.

Annex 3

Concentrations of HCN in cassava and cassava derived foods

(Extracted from WHO (2012) with the exception of the references for Brazil, the Pacific Islands and the Philippines)

Country	Food	Mean Concentration of HCN or (range) (mg/kg)*	Further information	Reference
Australia	Cassava tubers Cassava chips	5-67 27 55.8 (<10-145)	These reflect the concentrations found before the introduction of an ML in Australia and New Zealand	Bradbury (2006) Hague and Bradbury (2002) FSANZ (2008)
Brazil	Cassava tubers Cassava flour Cassava leaves Cassava flour (raw) Cassava flour (toasted) Tapioca (sweet) Tapioca (sour)[1] Cassava leaves (young) Cassava leaves (old) Cassava leaves	(55 – 76) (26 – 451) (27.8 – 160,1) (43.5 – 300) 32 3.5 (0.24 – 14.45) (0.20 – 26.14) (0.12 – 6.10) (0.08 – 4.16) 239.19 339.81 (112 – 518)	Tapioca fermented to up to 5% of acidity	Rimoldi et al., (2004) Silva et al., (2004) Mezzete et al., (2009) Oliveira et al., (2009) Agostini, (2006) Sant´Ana and Domene, (2008)

Country	Food	Mean Concentration of HCN or (range) (mg/kg)*	Further information	Reference
	Tucupi Multimistura	(55.58 – 157.17) (9.47 – 46.86) <0.3	Total HCN Free HCN	Silva et al., (2004) Chisté et al., (2007) Kaminski et al., (2006)
Cameroon	Cassava tubers Baton de manioc Fufu	197-951 2.5-6.4 13-27.5		Agbor-Egbe and Lupe Mbome (2006)
Democratic Republic of Congo (DRG)	Processed cassava roots (cossettes)	<10	Expressed as total cyanogens, dried weight basis	Ngudi, et al., (2002)
Ghana	Fresh cassava Akyeke	69.3; (100.3 on a dry weight basis) 1.4 to 2.8	 Akyeye – a fermented, steamed product	Obile, et al., (2004)
Indonesia	Cassava tubers Starch and related products Flour, chips, gapek	19 5 (max 19) 54		Djazuli and Bradbury (1999)
Malawi	Cassava tubers: Sweet Bitter Sweet cassava Bitter cassava	 29 (1-123) 153 (22-661) 30 (15-93) 153 (43-251)		Chiwona-Karltun et al, (2004) Mkumbira et al, (2003)
Mozambique	Cassava flour	41 (26-57)		Ernesto et al (2000); Cardoso et al (2005)
Nigeria	Cassava:			Oluwole et al, (2007)

Country	Food	Mean Concentration of HCN or (range) (mg/kg)*	Further information	Reference
	Sweet Bitter Raw cassava Ljapu (stepped cassava slices) Garri Fufu Tapioca	105 (8-1064) 103 (27-543) 76.1 11.8 25.4 (20-30) 20 (10-30) 17.5 (10-20)	Dry weight basis Total cyanogens	 Sokari and Karibo (1992) Adindu, et al., (2003)
Pacific Islands Fiji Tonga Vanuatu Samoa Cook Islands	 Raw cassava Chips Bila Vakalavalava Raw cassava Raw cassava Flour	 35 (13 – 92) 18 (14-21) <0.1 7 (6-8) (boiled) 14 (10-16) (baked) 58 (18 – 151) 47 (26-78)	 Bila sticks of cassava peeled and diced and soaked in water for 3 days. Then mashed, dried, mixed with sugar and coconut, wrapped in a leaf and boiled. Vakalavalava – grated cassava, wrapped in leaves and boiled or baked. [Source and treatment of flour not detailed in the report]	FAO Regional Programme for Food Security (2011)

Country	Food	Mean Concentration of HCN or (range) (mg/kg)*	Further information	Reference
Philippines	Cassava varieties	2.3 to 4 (low) 4.1 to 6.8 (moderate) 8 to 8.2 (high)	The HCN content of cassava in the Philippines is semi-quantitatively classified as low, medium and high based on the rapid picrate assay method used by Centre International de Agriculture Tropical (CIAT) and adopted by plant breeding programs for about 20 years. Recommended cassava varieties are those approved by the Philippine National Seed Industry Council (NSIC) for commercialization based on criteria such as resistance to pest, high yield, etc. Cassava varieties with low HCN content are only those recommended for food and feed. They are also recommended for starch and flour production. Varieties with medium HCN content are recommended for starch, flour and feed production. Varieties with high HCN content are recommended only for highly processed products such as starch and flour. Cassava varieties with high HCN content are likewise recommended for ethanol production since high HCN content generally corresponds to high starch content.	Mariscal, et al., (2009)
United Republic of Tanzania	Sweet cassava bitter Makopa Cassava flour	105 (8-1064) 103 (27-543) 9.4 (0-79) 83.7	 sun-dried tuber pieces 	Oluwole et al, (2000) Mlingi et al (1998) Carlsson et al (1999)

*refers to mean HCN equivalent concentration range on a fresh weight basis, unless otherwise stated

Annex 4

Processing of cassava to reduce total HCN content

1. Proper processing of cyanogenic glycoside-containing foods will reduce the risk to consumers. In regard to cassava, the concentration of total HCN depends on the variety of cassava tuber, the growing conditions and the methods of processing. The relative amount of each cyanogenic component in turn depends on the cyanogenic reaction pathway at the different stages of process, as illustrated in Figure 1. Cyanogenesis is initiated when the plant tissue is damaged. If any of the processing steps does not take place or is interrupted, the final cassava may contain unacceptably high concentrations of total HCN.

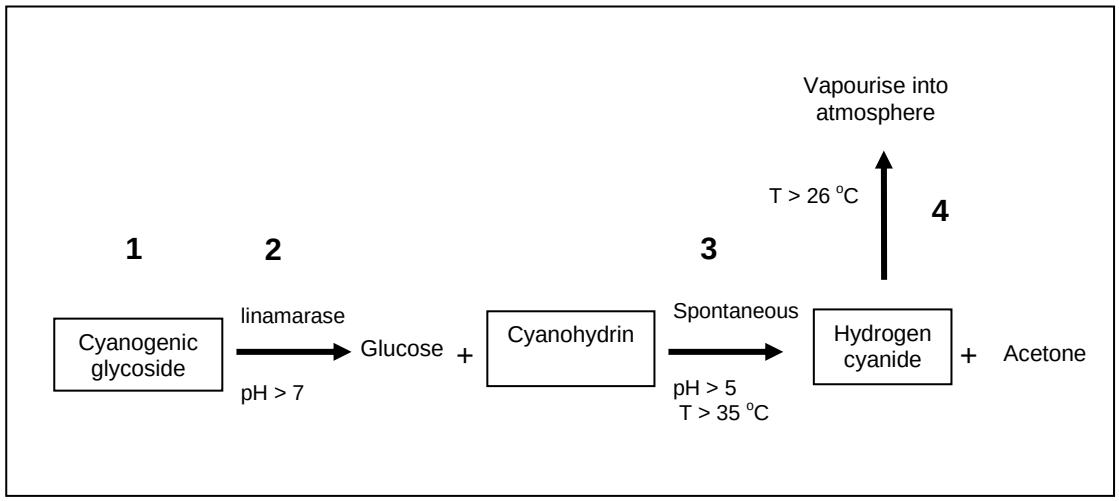


Figure 1: Cyanogenesis reaction pathway and steps in cassava processing. 1: nature of tuber; 2: grating/rasping, soaking, fermentation; 3: sun/oven drying; 4: sun drying, hot manufacturing process (steaming, frying).

2. **Step 1**: Nature of the cassava tuber. High concentrations of cyanogenic glycosides require a greater level of reduction of total HCN to result in an acceptable concentration through the typical cassava product processes.

3. **Step 2**: Grating, soaking and fermenting. The release of enzymes (e.g. linamarase) from the crushed cell walls and the appropriate conditions for the enzymes to react with the cyanogenic glycosides is critical. If this processing step is shortened or modified, for instance in order to prevent microbial growth or browning of the raw cassava, high concentrations of cyanogenic glycosides may remain in the products.

4. Therefore the size of the grated or sliced cassava, the time allowed for the fermentation or soaking to take place and the temperature and pH of the product will each determine how much of the cyanogenic glycoside is reduced. If high heat is used immediately after slicing or grating, for example, in frying of sliced cassava chips or drying in hot ovens, the enzyme would be inactivated and the cooked cassava products would contain high concentrations of cyanogenic glycosides. If low pH preservatives such as acetic acid and sodium metabisulphites are to be used at this stage, it is possible they would affect the conversion of cyanogenic glycosides.

5. **Step 3**: Sun/oven drying. Cassava tubers, once harvested, are usually fermented or dried to inhibit deteriorative physiological changes and microbial growth. The action of enzymes is continued here, as well as the spontaneous breakdown of cyanohydrins to hydrogen cyanide, at pH over 5 and temperature over 35°C. The final product of this cyanogenesis pathway is the volatile hydrogen cyanide, which vapourises at 26°C. Therefore, if the cassava grits/mash/slices are small and spread thinly in the drying step, the hydrogen cyanide can escape more easily to the atmosphere. The use of hot ovens to hasten the drying process, or when sun-drying is not available, may denature the enzyme or trap the enzyme in the dried cassava matrix and prevent the conversion of the cyanogenic glycosides to volatile hydrogen cyanide. Therefore the cassava products that have been dried too quickly would have the cyanogenic, cyanohydrin and cyanide components trapped in the cassava matrix.

6. It has been suggested that sun drying and heap fermentation were inadequate to reduce HCN concentrations in cassava used for flour in the Nampula Province of Mozambique to the WHO level of 10 ppm (Cardoso et al 2005). These authors reported that sun drying and heap fermentation result in up to 30% of the original total HCN remaining in the cassava flour, whereas soaking and crushing prior to sun drying or roasting resulted in only 3% of the original total HCN remaining.

7. The drying process seems to reduce the concentration of cyanide in addition to affecting the enzyme activity. Drying in the sun was more effective at decreasing the concentration of cyanide when compared to oven drying to 60° C (82 to 94% versus 68 to 76%, respectively). It was observed that most of the cyanide present in the foliage drying in the sun was composed of free cyanide (62 to 77%), while in the foliage dried at 60°C there was only 24 to 36% of free cyanide (Gómez and Valdivieso 1985). Another study evaluated the effect of three drying temperatures (45ºC, 60°C and 75°C) on the concentration of cyanide from leaves of five varieties of cassava. It was noted that the lowest concentrations were found in dried leaves to 60ºC, ranging from 7.7 to 15 mg/100 g of dry matter (Padmaja, 1989).

8. **Step 4**: Final food product manufacturing process. If hydrogen cyanide is trapped in the dried cassava products (starch, flour or raw chips), further processing of these products may allow the hydrogen cyanide to escape (if the process temperature is higher than 26°C). If the cyanide is still in the CG form a steaming process at a temperature less than 100°C allows the enzymes (e.g. linamarase) to be reactivated and to hydrolyse the glycosides, freeing hydrogen cyanide. However, if the cassava product (slices or chips) is subjected to high heat in frying, the cyanogenic glycoside will remain in the product.

9. Processing methods generally adopted for cassava include peeling, soaking, fermentation, boiling or cooking, drying, and pounding/millings (Padmaja 1995). However, the different varieties of cassava have been found to have different cyanogen elimination profiles during the cooking of cassava roots. There also appear to be differences in heat stability of β-glucosidase activity of cassava roots which protects the enzyme from total deactivation during cooking (Ravi and Padmaja 1997).

10. "Cossettes" (processed cassava roots), one of the most popular cassava products in the Democratic Republic of Congo, are processed by soaking or immersing fresh bitter cassava roots (whole or peeked) in a stream or stationary water for at least 3 days to allow them to ferment until they become soft. The fermented roots are then taken out, peeled and sundried on racks, roofs of houses which can take from 2-5 days (Hahn; cited in Ngudi et al 2002).

11. Reductions in HCN concentrations in cassava have been achieved by thoroughly mixing cassava flour with water and allowing the cassava to stand in an open vessel for 5 h before cooking, although this method is dependent on there being an adequate amount of linamarase in order for breakdown of linamarin to occur (Bradbury 2006).

12. Typical production of cassava flour or starch, especially the large-scale commercial factories, has ensured that processing steps and parameters are effective in eliminating total HCN from cassava. Cassava starch, also known as tapioca starch is one of the most commonly used starches in food manufacturing and functions as a thickener, emulsifier or confectionery ingredient. Concentrations of total HCN in some modified starches could be as low as 0.01 mg/kg. Mean concentrations of 5 mg/kg were reported by Djazuli and Bradbury (1999).

13. The table below gives a compilation of processing steps and reported concentrations of HCN achieved by them. It would be possible to predict likely concentrations of HCN in different products in the absence of measured values.

Reduction of HCN during processing of cassava products

Process step	Mechanism of removal#	% HCN reduction	Reference
Soaking 24h	leaching	13-52%	Agbor-Egbe and Lape Mbone, 2006 Kemdirim et al, 1995
Soaking 48 h	"	73-75%	Agbor-Egbe and Lape Mbone, 2006 Kemdirim et al, 1995
Soaking 72 h	"	90%	Agbor-Egbe and Lape Mbone, 2006 Kemdirim et al, 1995
Mashing and grinding	Disintegration and enzyme action	75%	
Pressing	Leaching and enzyme action	57%	Chiste et al, 2005
Fermentation 4-5d	Disintegration and enzyme action	52-63	Kemdirim et al, 1995; Obilie et al, 2004
Drying, sun	Enzyme	82-86% 80%	Gomez and Valdiviesco, 1985 Nambisan et al, 2011
Drying, oven 60°C	Enzyme	68-76%	Gomez and Valdiviesco, 1985
Storage (gari meal) 4w	Possible enzyme action and evaporation	50-64%	Onabolu et al, 2002
Boiling	Leaching (with initial enzyme activity until inactivated)	96-99% 80%	Obilie et al, 2004 Nambisan, 2011
Baking, frying, steaming	Thermal degradation	20%	Nambisan, 2011
Cooking baton de manioc, fufu		32-55%	Agbor-Egbe and Lape Mbone, 2006
Steaming akyeke		74-80%	Obilie et al, 2004
Garification*		90-93%	Agbor-Egbe and Lape Mbone, 2006
Grating, pounding, sundrying		95%	Nambisan, 2011
Final flour product		92%	

*Fermented, and dried cassava mash, cooked and dried

Based on Nambisan, 2011

Annex 5

METHODS OF ANALYSIS OF TOTAL HYDROCYANIC ACID IN CASSAVA AND CASSAVA PRODUCTS

Background

1. Cyanogenic glycosides present in cassava (*Manihot esculenta*) may be converted to acutely toxic hydrocyanic acid (HCN) during processing or in the gastrointestinal tract by enzymes with β-glucosidase activity. The enzyme may originate from the plant material and come into contact with the glycoside during cutting, grinding or maceration or may be associated with the normal bacterial flora of the gut.
2. Three chemical species are generally recognized in analysis of cyanogenic compounds from cassava; the intact cyanogenic glycosides, linamarin and lotaustralin, the primary products of deglycosylation, acetone cyanohydrin and butanone cyanohydrin, and hydrocyanic acid.
3. Total hydrocyanic acid in cassava products may be defined as "all hydrocyanic acid evolved from linamarin, lotaustralin, acetone cyanohydrin or butanone cyanohydrin during or following enzyme hydrolysis or acid hydrolysis, expressed as milligrams of hydrocyanic acid per kilogram" (FSANZ, 2009).
4. The total cyanogenic potential of cassava or cassava products or total hydrocyanic acid can be assessed by direct analysis of the components of cyanogenic potential (glycosides, cyanohydrins and free hydrocyanic acid) or by analysis of hydrocyanic acid generated under particular experimental conditions. In intact plant material substantially all of the cyanogenic potential will be in the form of intact cyanogenic glycosides, while some processed products may contain a significant amount of cyanohydrin.
5. Currently there are no defined methods of analysis for hydrocyanic acid in Codex standards.

Analysis of compounds contributing to total hydrocyanic acid

6. Individual cassava cyanogenic glycosides have been analyzed by:
    - Gas chromatography (GC) (Bisset *et al.*, 1969);
    - Liquid chromatography (LC) (Brimer and Dalgaard, 1984; Sornyotha *et al.*, 2007); or
    - Thin-layer chromatography (TLC) (Eyjólfsson, 1970; Brimer *et al.*, 1983; Amarowicz *et al.*, 1993).
7. Comparisons of total hydrocyanic acid, calculated from the concentrations of the individual cyanogenic glycosides have been reported to be in good agreement with total hydrocyanic acid, measured by hydrolysis followed by colourimetric determination of cyanide in linseed (Kobaisy *et al.*, 1996). However, calculation of total hydrocyanic acid from determination of the individual cyanogenic glycosides has not gained popularity for the determination of total cyanogenic potential.
8. While some chromatographic methods have reported the ability to detect both cyanogenic glycosides and cyanohydrins, detection of cyanohydrins was only qualitative (Brimer and Dalgaard, 1984). Currently available chromatographic methods measuring the individual components of cyanogenic potential may not be suitable for determining total hydrocyanic acid in processed foods, where cyanohydrins may be present.

Direct analysis of total hydrocyanic acid

9. Methods that determine the total cyanogenic potential by conversion of glycosides and cyanohydrins to hydrocyanic acid can be considered in terms of three key aspects:
    - Extraction or comminution of the plant material;
    - Hydrolysis of the glycosides and cyanohydrins; and
    - Quantification of the resulting cyanide.
10. Methods that determine total hydrocyanic acid in a liquid extract require optimization of extraction of the cyanogenic compounds from the plant matrix to ensure complete extraction. Methods that allow evolution of gaseous hydrogen cyanide often carry out hydrolysis on the whole comminuted plant material. Both approaches have been reported to give similar results (Djazuli and Bradbury, 1999; Haque and Bradbury, 2002).
11. The most common extractant for methods measuring hydrogen cyanide is 0.1 M phosphoric acid, although phosphoric acid concentrations ranging from 0.02 M (European Committee for Standardization (CEN), 2012) to 0.2 M (Drochioiu *et al.*, 2008) have been reported. Hydrochloric acid (0.1 N) has also been used (Laurena *et al.*, 1994). Under these acid conditions linamarase, the native β-glucosidase enzyme that hydrolyses cassava cyanogenic glycosides, is inactive (Bradbury *et al.*, 1994). Acid extraction requires a low acid concentration and low extraction temperatures (<26ºC) to avoid hydrolysis of glycosides and loss of hydrogen cyanide.

Hydrolysis

12. Release of cyanohydrins and hydrocyanic acid from cyanogenic glycosides can be achieved by enzymatic or acid hydrolysis. While some methods have used the endogenous enzyme (linamarase) present in the cassava tissue (Curtis *et al.*, 2002), a process known as autolysis, this is not generally recommended as different cassava cultivars are known to vary considerably in their linamarase activity. Food processing may result in denaturation of endogenous linamarase. Exogenous linamarase allows for greater standardization of the method, but the enzyme is quite expensive when obtained from commercial sources (Cooke, 1978; Essers *et al.*, 1993; Essers *et al.*, 1998; Saka *et al.*, 1998; Bradbury *et al.*, 1999; Haque and Bradbury, 2002; Bradbury, 2009; Miles *et al.*, 2011; Burns *et al.*, 2012). It should be noted that these comments apply to analysis of cassava and cassava products. A commercial enzyme preparation from *Helix pomatia* (the vineyard snail) has been reported to have hydrolytic activity towards a wide range of cyanogenic glycosides, including those from cassava (Brimer *et al.*, 1983).

13. There is considerable variety in reported incubation times used for enzymatic hydrolysis of cassava cyanogenic glycosides. Incubation times of 16-48 hours at temperatures of 21-37ºC have been reported in some studies (Brimer and Rosling, 1993; Bradbury *et al.*, 1994; Saka *et al.*, 1998; Bradbury, 2009; Miles *et al.*, 2011), while other researchers have reported that an incubation time of 15 minutes at 30ºC is adequate (Cooke, 1978; Essers *et al.*, 1993). A recently published EU standard method employs overnight hydrolysis at 38ºC (European Committee for Standardization (CEN), 2012).

14. Acid hydrolysis is comparatively cheap, but requires incubation at elevated temperatures and extreme care must be exercised to avoid concurrent loss of hydrogen cyanide. Hydrolysis is usually carried out in 2 M sulphuric acid at 100ºC (Bradbury *et al.*, 1991; Bradbury *et al.*, 1994; Haque and Bradbury, 2002; Drochioiu *et al.*, 2008).

15. The incubation time required for acid hydrolysis is dependent on the particular cyanogenic glycosides being analyzed (Haque and Bradbury, 2002). Incubation times of 50 minutes have been reported for analysis of cassava (Bradbury *et al.*, 1991). However, a more generalized method, using an extrapolation method to correct for cyanide losses, uses incubation times up to 6 hours (Haque and Bradbury, 2002; Cressey and Saunders, 2010).

Measurement of cyanide – spectrophotometric techniques

16. By far the most common method of measuring hydrogen cyanide evolved from plant material is by spectrophotometry, following reaction of the cyanide to produce a stable chromophore. The most common colour reactions are:

- Guignard reaction. Reaction of the cyanide ion with alkaline picrate to produce a brick-red colour. A variant of this method involves further reaction of the picrate adduct with resorcinol (Drochioiu *et al.*, 2008).
- König reaction. Conversion of cyanide ion to cyanogen halide, usually by addition of chloramine T or N-chlorosuccinimide, followed by reaction with pyridine or a related compound to form a dialdehyde, which is coupled with primary amines or compounds with an active methylene (RH_2) group (Lambert *et al.*, 1975; Bradbury *et al.*, 1994).
- Reaction of cyanide ion with *p*-nitrobenzaldehyde and then *o*-dinitrobenzaldehyde to generate the purple dianion of *o*-nitrophenyl hydroxylamine (Untang *et al.*, 2010).
- Reaction of cyanide ion with benzidine and copper acetate. This method has been largely abandoned due to the carcinogenic nature of benzidine (Bradbury and Egan, 1992) and was replaced by a reaction of cyanide with tetra base (4,4'-methylenebis-(*N, N*-dimethylaniline) and copper acetate (Feigl and Anger, 1966; Bradbury and Egan, 1992).

17. The Guignard reaction may form its distinctive colour product with chemicals other than cyanide (Bradbury *et al.*, 1991; Alonso-Amelot and Oliveros, 2000). Evolution of hydrogen cyanide in a gas stream and trapping of interfering volatile carbonyls in acidic solution of 2,4-dinitrophenylhydrazine has been used (Alonso-Amelot and Oliveros, 2000). The commonly used alkaline picrate paper method involves enzymatic hydrolysis of cyanogenic glycosides in a sealed tube and detection of the evolved hydrogen cyanide by reaction with alkaline picrate impregnated paper suspended above the reaction mixture, to measure gas phase hydrogen cyanide. It is uncertain to what extent the alkaline picrate paper avoids reactions with non-cyanide species. It should be noted that picric acid, used in the preparation of the alkaline picrate paper, is highly explosive and preparation of alkaline picrate paper should only be carried out under controlled laboratory conditions.

18. The König reaction is not specific for cyanide and will also react with thiocyanate (Curtis *et al.*, 2002). A number of reagents can be used with the König reaction. Early studies used a pyridine/pyrazolone reagent (Cooke, 1978). Pyridine/barbituric acid was found to be cheaper and more stable (Bradbury *et al.*, 1991). Pyridine is an unpleasant smelling chemical, with potential adverse human health effects. An alternative colour reagent based on isonicotinic acid/barbituric acid was proposed, which avoids the use of pyridine, without any loss of sensitivity (Nagashima, 1978). This colour reagent has subsequently been employed for analysis of hydrocyanic acid in plant materials (Essers *et al.*, 1993; Haque and Bradbury, 2002; Cressey and Saunders, unpublished). It should be noted that barbituric acid is classified as a narcotic in some countries, which may complicate access to this reagent.

Measurement of cyanide – other techniques

19. Biosensor techniques have been developed to detect cyanide (Keusgen *et al.*, 2004; Mak *et al.*, 2005). These techniques use biosensors to detect the degradation products (formate and ammonia) formed by hydrolysis of cyanide by the enzyme cyanide hydrolase. These techniques were developed for environmental monitoring and do not appear to have been developed into commercial applications.

20. The European Committee for Standardization (CEN) has recently published a standard for the determination of hydrocyanic acid in animal feeding stuffs, including cassava (European Committee for Standardization (CEN), 2012). Hydrocyanic acid is quantified by reversed phase HPLC of taurine/2,3-naphthalene dicarboxy aldehyde (NDA) derivatised hydrocyanic acid. The standard also includes the results of an inter-laboratory collaborative trial. The HPLC component of the method is based on a previously developed method for determining cyanide in red blood cells (Sano *et al.*, 1992).

21. Another HPLC technique has been developed for determination of total hydrocyanic acid from flaxseed (Chadha *et al.*, 1995). The method involves ion chromatography of autolysed plant material with amperometric detection. Samples were cleaned up by passing through a molecular weight filter prior to analysis.

22. Total hydrocyanic acid from seeds has been determined by autolysis followed by distillation into a strongly basic solution. The cyanide was converted to ammonium by excess potassium permanganate and determined by gas phase molecular absorption spectrometry using a modified atomic absorption spectrophotometer (Kupchella and Syty, 1984).

Determination of analytical method performance

23. There is considerable variation in the degree to which different studies have defined the performance of their analytical method.

24. Limits of detection are often not reported and in some cases when they are reported they appear to be equipment limits of detectability (Campa *et al.*, 2000; Curtis *et al.*, 2002; Drochioiu *et al.*, 2008), rather than method limits of detection. Method limits of detection for cassava and cassava products appear to be approximately in the range 1-10 mg/kg wet weight. These limits of detection are adequate to support current standards for edible cassava flour and sweet cassava, with levels for total hydrocyanic acid of 10 and 50 mg/kg, respectively (Codex Alimentarius Commission, 1995a; 2011). However, in many cases they are not adequate to support the current standard for gari, with a level for free hydrocyanic acid of 2 mg/kg (Codex Alimentarius Commission, 1995b).

25. Establishing the accuracy of methods is hampered by the lack of any standardized or certified reference materials for total hydrocyanic acid in cassava or cassava products. One inter-laboratory collaborative trial has been reported, which provides useful information on the performance of a single method (see paragraph 19 above) (European Committee for Standardization (CEN), 2012).

26. Studies comparing different analytical techniques have generally reported good agreement for the determination of total hydrocyanic acid (Kobaisy *et al.*, 1996; Djazuli and Bradbury, 1999; Haque and Bradbury, 2002). However, these studies have been performed in a single laboratory and have only made qualitative comparisons between methods, rather than applying statistical tests for difference (e.g. Student's t-test).

Evaluation

27. While it is feasible to determine total hydrocyanic acid in cassava by determining the individual contributing compounds, no methods have been reported that reliably quantify all potential contributors. It is preferable that total hydrocyanic acid be determined by a suitable technique which converts all contributors to hydrocyanic acid.

28. It is not recommended that cyanogenic glycosides be hydrolyzed by autolysis (incubation of samples to utilize endogenous β-glucosidase enzymes), as enzyme levels in foods may be variable or enzymes may have been denatured by food processing.

29. Acid hydrolysis is cheaper than using commercial enzyme preparations and is applicable to any cyanogenic plant material. However, acid hydrolysis is likely to be more technically demanding than enzymatic hydrolysis.

30. Spectrophotometric detection of cyanide (Guignard or König) reaction products or HPLC analysis following pre-column derivatisation appear to be the most common methods currently being used for determining total hydrocyanic acid from cyanogenic plant materials.

31. HPLC methods require an additional clean-up step to prepare samples for chromatography. The capital investment in equipment will also be greater. However, methods using HPLC detection of cyanide are likely to be more specific and have reported good sensitivity (e.g. limit of quantification of 2 mg HCN/kg should normally be obtained for EU standard method for animal feed).

32. The picrate paper method appears to be the best current option for a “field” test method and it was developed for this purpose. The colourimetric method of Untang *et al*. also looks promising as a field tool, but has not been tried on cassava. For basic laboratory analyses the picrate paper or the isonicotinic acid/barbituric acid methods appear suitable and the majority of recent studies on total hydrocyanic acid in cassava or cassava products have used one or other of these methods. Methods using chromatographic detection of cyanide (e.g. the EU HPLC method for animal feed) are probably currently the “gold standard” due to the specificity of the detection technique.

33. There is very limited information available on the relative performance of different methods. Available comparisons have been carried out within a single laboratory.

34. A single inter-laboratory collaborative study has been reported within Europe of the HPLC method mentioned above. No other collaborative studies of total hydrocyanic acid methods were found and no collaborative studies that considered a range of methods were found.

35. Published method limits of detection indicate that test sensitivity is generally adequate to support the Codex standards for edible cassava flour and sweet cassava, but most methods appear to be insufficiently sensitive to test to the levels of the more stringent gari standard. The EU HPLC method (European Committee for Standardization (CEN), 2012) and the modified picrate method (Bradbury, 2009; Burns *et al.*, 2012) have reported acceptably low limits of detection.

36. Definition of analytical method performance for different methods is hampered by the lack of certified reference materials and inter-laboratory collaborative trials. There is also considerable variation in the degree to which different studies have reported analytical method performance (e.g. limit of detection, coefficient of variation, spike recovery).

Appendix 2: List of Participants

Australia

Dr Leigh Henderson (Chair)
Food Standards Australia New Zealand (FSANZ)
PO Box 10559
Wellington
NEW ZEALAND
Email: leigh.henderson@foodstandards.govt.nz

Dr Glenn Stanley
Food Standards Australia New Zealand (FSANZ)
55 Blackall Street
Barton ACT 2600
AUSTRAILA
Email: glenn.stanley@foodstandards.gov.au

Brazil

Lígia Lindner Schreiner
Instituto Nacional de Metrologia, Qualidade e Tecnologia (INMETRO)
Email: ligia.schreiner@anvisa.gov.br

Canada

Ms Roni Bronson
Chemical Health Hazard Assessment Division
Bureau of Chemical Safety, Food Directorate
Health Products and Food Branch
Health Canada
Email: roni.bronson@hc-sc.gc.ca

China

Dr Dawei Chen
Department of Chemical Lab
Key Lab of Food Safety Risk Assessment, Ministry of Health (CFSA)
China National Center for Food Safety Risk Assessment (CFSA)
7 Panjiayuan Nanli, Beijing 10021
Tel: 86-10-67776789
Fax: 86-10-67776789
Email:dila2006@163.com

Ms Shao Yi
National Committee Secretariat for Food Safety Standard
China National Center for Food Safety Risk Assessment (CFSA)
7 Panjiayuan Nanli, Beijing 10021
Tel: 86-10-67776790
Fax: 86-10-67776790
Email: sy1982bb@yahoo.com.cn

Professor Dr Yongning WU
China National Center for Food Safety Risk Assessment (CFSA)
Key Lab of Food Safety Risk Assessment, Ministry of Health (CFSA)
7 Panjiayuan Nanli, Beijing 10021
Tel: 86-10-67776790
Fax: 86-10-67776790
Email: china_cdc@yahoo.cn wuyncdc@yahoo.com.cn

Colombia

Mónica Sofia Cortes Muñoz
Email: monica.cortes@minagricultura.gov.co

Gustavo Alvaro Wills
Email: Gawillsf@unal.edu.co

Sandra Nayibe Vega
Email: svega@ins.gov.co

Dominican Republic

Dr. Matilda Vasquez
Ministry of Public Health
Ministerio de Salud Pública (MSP)
Tel. (Direct): + 809-541-0382
Other Tel. +809-541-3121, ext. 2382
Email: codexsespas@yahoo.com; codexsespas@gmail.com;

European Union

Bernadette Klink-Khachan
European Union Codex Contact Point
European Commission
DG Health and Consumers Directorate-General
Unit G06: Multilateral International Relations
Tel: +32-2-295 79 08
Email: codex@ec.europa.eu

Mr Frans VERSTRAETE
European Commission
Health and Consumers Directorate-General
Tel: +32 - 2 – 295 63 59
Email: frans.verstraete@ec.europa.eu

Fiji

Mr Samuela BOLALAILAI
Ministry of Health
P. O. Box 2223
Government Buildings
Suva, FIJI
Tel: +679 330 6177
Fax: +679 333 1434
Email: samuela.bolalailai@health.gov.fj

Mrs Miliakere NAWAIKULA
Department of Agriculture, Ministry of Primary Industry
Koronivia Research Station, P.O. Box 77
Nausori
FIJI
Tel: +679 347 7738
Fax: +679 347 7546
Email: miliakere.nawaikula@govnet.gov.fj

FSM -Federated States Of Micronesia

Mr Moses PRETRICK
Environmental Health & Preparedness Unit
Division of Health Services
FSM Dept. of Health & Social Affairs
PO Box PS-70
Palikir, Pohnpei FM 96941
Federated States Of Micronesia
Tel: +691 320 8300
Fax: +691 320 8460
Email: mpretrick@fsmhealth.fm

Mr John P. WICHEP
FSM Department of Resources & Development
P. O. Box PS-12
Palikir, Pohnpei FM 96941
Palikir, Pohnpei,
Federated States Of Micronesia
Tel: +691 320 5133 2646
Fax: +691 320 5854
Email: jwichep@fsmrd.fm

Ghana

Mr. Kwamina Van-Ess
Kwamina Van-Ess and Associates
Accra
Ghana
Tel: +233 244 653 167
Email: kwaminav@yahoo.com

Joyce Okoree
Codex Contact Point
Ghana Standards Authority
P. O. Box MB 245
Accra
Ghana
Tel: +233 243 785 375
Email: codex@gsa.gov.gh

Indonesia

Tetty H Sihombing
National Agency of Drug and Food Control
Indonesia
Email: tettyhelfery@yahoo.com, codexbpom@yahoo.com

Jamaica

Ms. Chanoya Kidd
Regulatory Division
Bureau of Standards Jamaica
Email: ckidd@bsj.org.jm

Dr. Dwight Ramdon
Chemistry Department
Bureau of Standards Jamaica
Email: dramdon@bsj.org.jm

Japan

Mr Wataru IIZUKA
Standards and Evaluation Division,
Department of Food Safety,
Ministry of Health, Labour and Welfare
1-2-2 Kasumigaseki, Chiyoda-ku Tokyo 100-8916, Japan
Email: codexj@mhlw.go.jp

Mr Ryo IWASE
Standards and Evaluation Division,
Department of Food Safety,
Ministry of Health, Labour and Welfare
1-2-2 Kasumigaseki, Chiyoda-ku Tokyo 100-8916, Japan
Email: codexj@mhlw.go.jp

Dr Takashi SUZUKI
Standards and Evaluation Division,
Department of Food Safety,
Ministry of Health, Labour and Welfare
1-2-2 Kasumigaseki, Chiyoda-ku Tokyo 100-8916, Japan
Email: codexj@mhlw.go.jp

Dr Tomoaki TSUTSUMI
Division of Foods
National Institute of Health Sciences
1-18-1 Kamiyoga, Setagaya-ku, Tokyo 158-8501, JAPAN
Email: tutumi@nihs.go.jp

Mr. Tetsuo URUSHIYAMA
Food Safety and Consumer Policy Division Ministry of Agriculture, Forestry and Fisheries
1-2-1 Kasumigaseki, Chiyoda-ku, Tokyo, 100-8950 Japan
Email: tetsuo_urushiyama@nm.maff.go.jp,
codex_maff@nm.maff.go.jp

Malaysia

Ms Fauziah Arshad
Standard and Codex Branch
Food Safety and Quality Division
Ministry of Health Malaysia
Tel: +603 8885 0794
Email: fauziaharshad@moh.gov.my

Codex Contact point
Email: ccp_malaysia@moh.gov.my

Ms Maria Afiza Omar
Email: maria.afiza@moh.gov.my

Ms Raizawanis Abdul Rahman
Contaminant Section
Food Safety and Quality Division
Tel: +603 8885 0783
Email: raizawanis@moh.gov.my

New Zealand

Mr John Reeve
Ministry for Primary Industries
Wellington
NEW ZEALAND
Email: john.reeve@mpi.govt.nz

Dr Peter Cressey
ESR (Institute of Environmental Science and Research Ltd)
Christchurch Science Centre
27 Creyke Road
PO Box 29-181
Christchurch 8540
NEW ZEALAND

Nigeria

Dr Abimbola O. ADEGBOYE (Co-Chair)
NAFDAC, Lagos, Nigeria.
Tel: +234 805 317 0810 +234 813 797 9705
Email: bimbostica@yahoo.com

Dr Adeyinka Oludiran
SIDHAS-FCT
Zonal Manager, NC Zonal Office.
Abuja, Nigeria
Email: aoludiran@sidhas.org
adeyinkaoludiran@yahoo.com

Dr Olatunde Oluwatola
Nigeria Institute of Food Science and Technology
NIFST
Email: pcetola@yahoo.com

Prof Oluleye
Registrar
Institute of Public Analysts of Nigeria
Email: dsoluleye@gmail.com

Prof L. O. Sanni
President NIFST
Department of Food Science and Technology
University of Agriculture Abeokuta
lsanni@cgiar.org lateef_2@yahoo.com
Email: nifstoffice@yahoo.com info@nifst.org

Dr E. Okorono
National Root Crops Research Institute
Umudike Abia State Nigeria
Email: ekeokorono@yahoo.com

Dr. O. Fayinminu
Department of Environmental Biology
University of Ibadan
Email: Olorijkb2008@yahoo.com

Mr. M George
Standards Organisation of Nigeria
SON, Abuja, Nigeria
Email: bob_king_george@yahoo.com

Mrs Jane Omojokun
National Agency for Food and Drug Administration and Control
445 Herbert Macaulay Way Yaba Lagos Nigeria
Email: omojokun.j@nafdac.gov.ng

Dr. M. Eimunjeze
National Agency for Food and Drug Administration and Control
445 Herbert Macaulay Way Yaba Lagos Nigeria
Email: eimunjeze.m@nafdac.gov.ng

Dr. M. A. Abubakar
National Agency for Food and Drug Administration and Control
445 Herbert Macaulay Way Yaba Lagos Nigeria
Email: abubakarma62@yahoo.com

Dr O. Oluwole
Federal Institute for Industrial Research Oshodi
FIIRO
Oshodi
Lagos
Email: toyinoluwole2@yahoo.com

Philippines

Mary Grace Gabayoyo
Laboratory Services Division, Food and Drug Administration,
Department of Health - Philippines
Civic Drive, Filinvest Corporate City, Alabang, Muntinlupa City,
Philippines
Tel: +6328571900 local 8201
Email: mggabayoyo@yahoo.com

Karen Kristine Roscom
Standards Development Division, Bureau of Agriculture and
Fisheries Product Standards,
Department of Agriculture - Philippines
BPI Compound, Visayas Ave. Diliman, Quezon City, Philippines
Tel: +6324552858 Telefax no.: +6329206131
Email: kroscom@yahoo.com

Papua New Guinea

Codex Contact Point
Email: codexcontactpoint.png@gmail.com

Republic of Korea

Kil-jin Kang
Korea Food & Drug Administration
Email: catharina@korea.kr; gjgang@korea.kr

Samoa

Codex Contact Point
Email: codex.samoa@mcil.gov.ws

Ms Julia PETELO
Email: iulia.petelo@mcil.gov.ws

Mr Dirk SCHULZ
FAO Sub-Regional Office for the Pacific (SAP)
Apia, SAMOA
Tel: +685 22127
Fax: +685 22 126
Email: dirk.schulz@fao.org

Solomon Islands

Ethel Lano Mapolu
Email: emapolu@moh.gov.sb

Suriname (Republic of Suriname)

Mr. Robert, K.Kross PhD
Email address: robert.kross@uvs.edu
robert_kross@hotmail.com

Vanuatu

Mrs Ruth AMOS - SECONDARY
Food Technology Development Centre & Analytical Unit
Email: ramos@vanuatu.gov.vu

Mrs Tina Soaki-La'au
Food Technology Development Centre & Analytical Unit
Email: tsoaki@vanuatu.gov.vu

Mr Tekon Timothy TUMUKON
National Market Access Coordinator Vanuatu
Email: t.tumukon@phama.com.au

International Organization of the Flavor Industry (IOFI)

Dr T. Cachet
IOFI
6, Avenue des Arts
B-1210 Brussels
BELGIUM
Email: tcachet@iofiorg.org