CODEX ALIMENTARIUS COMMISSION

Food and Agriculture Organization of the United Nations

World Health Organization

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Agenda Item 13 **CX/CF 18/12/13**

JOINT FAO/WHO FOOD STANDARDS PROGRAMME

CODEX COMMITTEE ON CONTAMINANTS IN FOODS

12th Session
Utrecht, The Netherlands, 12 - 16 March 2018

DISCUSSION PAPER ON MAXIMUM LEVEL(S) FOR HYDROCYANIC ACID AND MYCOTOXIN CONTAMINATION IN CASSAVA AND CASSAVA-BASED PRODUCTS

(Prepared by the Electronic Working Group led by Nigeria)

BACKGROUND

1. At CCCF11 (2017), the FAO/WHO Coordinating Committee for Africa (CCAFRICA) requested to know if it is appropriate to extend the existing ML for HCN of 2 mg/kg in gari to fermented cassava products, and whether mycotoxins were of public health concern in these products.
2. Based on the requests of CCAFRICA, CCCF11 recommended[1] and was adopted at the 40th session of CAC that an EWG to be chaired by Nigeria be established to prepare a discussion paper:
   a. On the need and feasibility to establish an ML for HCN in all fermented cassava products and address the issue of harmonizing the expression of HCN levels, i.e. free or total HCN.
   b. Source for data on mycotoxins occurrence in these products that will allow CCCF determine whether mycotoxin contamination would be of public health issue in these products.
3. The EWG carried out its mandate and submitted the following conclusions and recommendations for consideration by CCCF. Information and data in support of the recommendations are presented in Appendix I.

CONCLUSIONSAND RECOMMENDATIONS-

4. The improving global profile of cassava as raw material for human foods, animal feeds and pharmaceutical and confectionary industries has been presented in this discussion paper. Its obvious growing significance in export trade is also highlighted. The health impact of the antinutritional factors in cassava and cassava based products namely hydrocyanic acid and mycotoxins was discussed. These were all done to advise CCCF 12 on the feasibility of setting MLs for HCN in cassava fermented products and elucidate whether mycotoxin contamination is of public health concern. These efforts led to the following recommendations:

(a) The levels of HCN found in three West African cassava fermented food products; *gari, lafun* and *fufu* were above the ML of 2.00 mg/kg free hydrocyanic acid set for gari, which implies that most of them may result in intakes above the recommended PMTDI of 0.02mg/kg body weight.

(b) The discussion paper reveals the presence of aflatoxins, ochratoxinA, deoxynivalenol, 15-acetyl deoxynivalenol, fumonisin B_1 and B_2, zearalenone, α-zearalenol and fusarenon-X in gari, lafun, cassava chips, cassava flour and bread from six African countries namely Nigeria, Cameroon, Uganda, Kenya, Sierra Leone and Rwanda.

(c) About 20 of the 581 cassava food products tested from the six countries had total aflatoxin levels above a hypothetical limit of 10 µg/kg and only 3 of 28 sample analyzed for aflatoxin B_1 from Nigeria had toxin content above the hypothetical limit of 2µg/kg. However, 10 of the 18 samples of gari analyzed for ochratoxin A also from Nigeria contained the toxin above the hypothetical limit of 5µg/kg. Except for these toxins, aflatoxins and ochratoxin A, all toxins occurred at concentrations above the hypothetical limits chosen for the purpose of drawing inferences for discussion.

(d) If the data in “3” above are considered enough to draw inferences, aflatoxins and ochratoxins are the only toxins of public health concern in *gari.*

[1] REP17/CF para paras 14-15

(e) However, the skewedness of the data involved in this work to only Africa and the limited data (18 samples) on ochratoxin A used, would not allow the inferences (1-4) drawn from the work to be representative of the world.

(f) In view of “5” above, we highly recommend that the EWG be given another year to source for data with global representation and therefore submit the discussion paper at CCCF 13 which underscores the need for CAC to use its appropriate platforms to get member countries especially from Africa, Asia, Southern and Central Americas and Caribbean avail information and data on HCN and mycotoxins in cassava based products to the EWG.

APPENDIX I

BACKGROUND INFORMATION
(For information to Codex Members and Observers when considering the conclusions and recommendations)

Consideration of MLs for HCN in CODEX

1. The 30th Session of the Codex Alimentarius Commission (CAC30) (2007) concurred with the recommendation of the 59th Session of the Executive Committee (CCEXEC59) (2007) to adopt the proposed draft Standard for Bitter Cassava, as elaborated by the Committee on Fresh Fruits and Vegetables (CCFFV) at Step 5 and that, as a separate issue, the Committee on Contaminants in Foods (CCCF) should consider the safety levels of hydrogen cyanide (HCN) proposed in the Standard, with a view to a re-evaluation of cyanogenic glycosides (CG) by the Joint FAO/WHO Expert Committee on Food Additives (JECFA).[2]
2. CAC31 (2008) sent the draft Standard for Bitter Cassava back to CCFFV for further work (step 6) on the labelling and processing of bitter cassava due to the recognized safety concerns if cassava is consumed without adequate processing, with a view to referral to the Committee on Food Labelling (CCFL) for re-consideration.[3]
3. The proposed levels for HCN of the Draft Standard for Bitter Cassava are as follows *in italic*: *[Bitter varieties of cassava are those that contain more than 50 mg/kg but less than 200 mg/kg HCN (fresh weight basis). In any case, cassava must be peeled and fully cooked before being consumed.]*
4. CCCF02 (2008) considered the need for a re-evaluation of cyanogenic glycosides by JECFA.
5. Pivotal to considerations on the safety of bitter and sweet cassava is whether the current preparation instructions are adequate to ensure safe consumption of these foods. It is unclear what level of processing is required for different initial levels of cyanogenic glycosides in bitter cassava. For example, it is not clear to what extent, following peeling and cooking, additional preparation techniques are necessary to adequately reduce the risk for cassava with 50 mg/kg HCN (fresh weight basis) compared with 200 mg/kg HCN (fresh weight basis).
6. The same CCCF noted that potential excessive dietary exposure to cyanogenic glycosides, mainly from cassava but also from other products, was assessed at the 39th Meeting of JECFA (JECFA39) (1992) and that, due to lack of quantitative toxicological and epidemiological information at that time, JECFA could not conclude on a safe level of dietary exposure for this naturally occurring toxicant. However, JECFA (WHO 1993) had also concluded that a level of up to 10 mg/kg HCN in the *Standard for Edible Cassava Flour* (CXS 176-1989) was not associated with acute toxicity. A review of the available data by the European Food Safety Authority in 2004 reached a similar conclusion.
7. There are a few FAO publications addressing good agricultural and manufacturing practices for the growing and processing of cassava, including other ongoing work in this field, to assist countries with the cultivation, processing and handling of this product. This information should be taken into account if a Code of Practice or an ML is considered necessary for cyanogenic glycosides in the future.
8. The CCCF02 agreed[4] that an electronic working group (EWG), led by Australia, prepare a discussion paper which should include an overview of available data on cyanogenic glycosides with a view to possible re-evaluation by JECFA. The EWG in its submission made the following recommendations:
    a. JECFA is requested to re-consider the data available on cyanogenic glycosides and advise on the public health implications of cyanogenic glycosides and their derivatives in food. In particular, whether there are sufficient data to establish an appropriate health standard, such as an acute reference dose or tolerable daily limit, for cyanogenic glycosides or their derivatives present in food.
    b. JECFA to consider whether or not the current level of up to 10mg/kg HCN in the *Standard for Edible Cassava Flour* is still an appropriate level which is not associated with acute toxicity, and whether this level would be applicable to any other HCN-containing food.
    c. JECFA to consider what levels of these cyanogenic glycosides and their derivatives may be appropriate in food, including levels that are appropriate to minimise any risks to public health from the consumption of foods containing cyanogenic glycosides and their derivatives.
    d. JECFA to consider what an appropriate descriptor for total HCN in food could be.

[2] ALINORM 07/30/REP para 92
[3] ALINORM 08/31/REP paras 37-39
[4] ALINORM 08/31/41 para 180

e. Taking into account any assessment by JECFA, that CCCF consider developing a Code of practice for producing, processing and marketing of foods which may contain cyanogenic glycosides or their derivatives. In consultation with CCFL, this would also include whether further information requirements are necessary for these foods to ensure adequate processing of cyanogenic glycoside-containing foods by consumers before consumption.

f. Following receipt of any risk assessment advice from JECFA, CCCF and the Committee on Methods of Analysis and Sampling (CCMAS) should review the current relevant Codex Standards to ensure these standards are consistent in relation to any limit for cyanogenic glycosides and their derivatives in food.

9. At CCCF03 (2009), Australia presented a discussion paper on cyanogenic glycosides. The CCCF agreed[5] to request JECFA to re-consider the data available on cyanogenic glycosides and advice on the public health implications of cyanogenic glycosides and their derivatives in food. 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.

10. JECFA72 (2010) JECFA conducted a risk assessment of cyanogenic glycosides in foods. 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.

11. 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.

12. 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 HCN in foods.

13. CCCF06 (2012) 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.[6]

14. At CCCF07 (2013), the EWG which worked on the review of the MLs, development of a code of practice (COP) and identifying of analytical methods suitable for analysis of HCN in foods made the following recommendations that were adopted:[7]

    a. Revision or establishment of new MLs for HCN in cassava and cassava products

        i. 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.

        ii. Currently there are no MLs for HCN in cassava in the *General Standard for Contaminants and Toxins in Food and Feed* (CXS 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.

[5] ALINORM 09/32/41 para 108
[6] REP12/CF paras 165-168
[7] REP13/CF paras 89-92

iii. In the absence of a Codex ML 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.

iv. 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.

v. 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.

vi. Other risk management strategies, particularly the development and implementation of a COP, 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.

vii. 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.

b. Methods of analysis

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

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

c. Development of a COP

i. Appropriate code of practices for effective reduction of HCN in the following processed cassava products: gari, fufu and fufu powder, dried cassava chips and other cassava products: Lafun, Atteke, Chikwangue, Bila, Farinha, Bikedi and NtobaMbodi; were recommended.

ii. Recommended practices based on GAP and GMP for reduction in HCN in various cassava products were also given.

15. Current Codex and international standards and texts

a. Currently there are no provisions in the GSCTFF for HCN MLs in cassava and its products.

b. CAC has developed and published standards for sweet cassava, bitter cassava, edible cassava flour and gari (a product obtained from processing cassava tubers) (also spelt as "garri"). The key aspects of these standards are:

i. Sweet cassava is defined as a raw product containing less than 50 mg/kg of "hydrocyanic acid".

ii. 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.

iii. For gari, another product for direct human consumption, the "total hydrocyanic acid" must not exceed 2 mg/kg as free hydrocyanic acid.

iv. 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).

v. In the absence of a Codex maximum level for hydrogen cyanide for bitter cassava in the GSCTFF 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.

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

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

i. cassava must not be eaten raw

ii. cassava shall be peeled, de-pithed, cut into pieces, rinsed and fully cooked before consumption

iii. cooking or rinsing water must not be consumed or used for other food preparation purposes.

e. CAC36 (2013), based on recommendations from CCCF07, adopted a Code of Practice for the Reduction of Hydrocyanic Acid in Cassava and Cassava Products, aimed at assisting primary producers and processors on best practices which will ensure elimination or reduction to the lowest possible level of Hydrocyanic acid in cassava.[8]

f. 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)**

General Information on Cassava and Fermented Cassava Products

16. Cassava (*Manihot esculanta* Crantz) is a shrub with tuber root containing high starch which belongs to the Euphorbiaceae family. The shrub which is mainly grown for its starch was domesticated in West-Central Brazil by 4600 BC (Pope et al. 2001) and was introduced to Africa from Brazil in the 16th Century by Portuguese traders. There are many cultivars and species of cassava but are all classified into bitter and sweet varieties according to the cyanogenic glucoside contents of their roots. The bitter and sweet varieties have high (≥ 100/mg/kg) and low (≤ 50 mg/kg) HCN content respectively.

17. It is the sixth most produced and consumed crop in the world after sugar cane, maize, rice, wheat and potatoes (FAOSTAT, 2017). It is cultivated in 103 countries covering a total of 23, 482.052 hectares of land in the world. World production of cassava are from 40 African, 12 South American, 16 Asian and 13 Oceania countries with Africa producing more than 50% of the crop. The ten biggest producers of cassava in 2016, in decreasing order, are Nigeria, Thailand, Brazil, Indonesia, Ghana, Democratic Republic of Congo, Vietnam, Cambodia, Angola and Mozambique. According to FAOSTAT (2014), the world net production value of cassava in 2014 was $26.1 billion US dollars.

18. The tuber root plant is drought resistance and therefore thrives and is grown in arid soil of tropical and subtropical regions of the world. Its high water management efficiency during drought are hinged on the plant's capacity to develop thin deep roots that extract subsoil water, close stomata before water stress signs are elicited in the plant and reduce the production of osmolytes which would have otherwise increase water loss from cells during drought (Daryanto et al. 2016). The authors also reported increased synthesis of abscisic acid, a compound that lowers leaf area by leaves shedding, limit formation of new leaves and only allows for production of small leaves which concomitant reduction in water loss.

19. The crop is grown for its high starch content (38%) and because it is one of the cheapest source of energy (Howeler *et al*. 2013). Although cassava root contains small amount of calcium (16mg/kg), phosphorus (27mg/kg) and vitamin C (20.6mg/kg), it is low in protein (1%) and other nutrients (Olumide, 2004) such that fortifications with minerals and vitamins and supplementation with proteins is required for balanced animal diet. However, the high amylopectin content (83%) of cassava starch gives it a digestibility of over 75% (Olumide, 2004). Like other crops, cassava has two toxic antinutritional factors. The toxic principles are cyanogenic glucosides; linamarin and lotaustralin which are hydrolysed to hydrocyanic acid that has been associated with acute cyanide intoxication, goiters and chronic pancreatitis.

20. The chemical composition of Cassava (*Manihot esculanta* Crantz) makes it an important food energy supply for human beings and animals. It is the staple food and source of nourishment for more than one billion people worldwide (FAO, 2011) especially in Africa, Asia and South America.Of the 254, 999, 000 tons of cassava produced in 2013 in the world, 39.5% of it (100, 637, 000 tons) was consumed by human beings (FAOSTAT). In Africa, as much as 70% of the cassava produced is for human consumption while between 35 and 40%, and 41% of cassava produced in Latin America and Caribbean, and Asia respectively are used for direct human consumption (Anyanwu *et al*. 2015). It is consumed by human beings in these continents in boiled, fried, roasted, grilled and baked forms as sandwiches, snacks, recipes of soup, desserts and bread (Table 1). The popular fermented products of cassava as shown in same table are alcoholic beverages, *gari, lafun* and *fufu* (*mwanga* or *ugali*)

[8] REP13/CAC para 32

21. Large quantities of cassava starch are mash with protein concentrates and mineral salts for livestock feeding. FAOSTAT estimates that 34.1% (87,059, 000 tons) of world cassava production of 2013 was used for feed production and 67130 tons of cassava valued at 39 billion USD was exported to various countries for livestock product trade in 2012.

22. The paradigm shift from being only a food crop to an export commodity for use as an energy crop and global industrial raw material has led to almost 400% increase in world cassava production from 71,259,839 tons in 1961 to 277,102,564 tons in 2016with 15.1% and 14.1% involved in import and export trade respectively (FAOSTAT). Same source of information estimates that as of 2014, the world net production value of cassava was over six billion US dollars. About 15% of world production of 2013 was used for industrial purposes. Cassava starch is used in the manufacture of food, adhesives, thickening agents, paper and pharmaceutical. It is processed to sweeteners mainly glucose, high fructose syrup and sorbitol. Cassava starch and waste (peels) are used in production of ethanol and animal feeds. Cassava waste are increasingly digested to biogas and the waste product of the process used as fertilizer.

23. The increasing need for cassava as both a food crop that is drought resistant and therefore future crop against hunger in adverse climate change conditions, and its current high industrial profile, coupled with the threats to its production and utilization due to hydrocyanic acid and probably mycotoxins has drawn more attention to the crop. Since there is an existing ML for gari and none for other fermented cassava foods, and the limited information on mycotoxins in cassava products; makes the need to establish MLs for fermented cassava foods; (fufu and fufu powder, dried cassava chips and other cassava products: Lafun, Atteke, Chikwangue, Bila, Farinha, Bikedi and NtobaMbodi) and to determine whether mycotoxins are of health concern in consumption of these food commodities an imperative.

Methodology Adopted by EWG

24. This discussion paper therefore intends to generate current data on the incidence and concentrations of HCN and mycotoxins in the fermented cassava products, and the dietary exposure to these contaminants with a view to making appropriate recommendations to CCCF 12 on possibility of establishing MLs for other fermented food products apart from gari and adverse public health effects of mycotoxins in the cassava products.

    If the sourced information on levels of HCN in the fermented cassava products and estimates of their dietary exposure indicates exceedance of ARfD or PMTDI, there will be need to recommend MLs for the contaminant in such product(s). The Provisional Maximum Tolerable Daily Intake (PMTDI) is 0.02 mg/kg body weight while the ML of 2.00mg/kg free hydrocyanic acid is for gari.

25. Similarly, any mycotoxin found at levels above set ML in the fermented products and will therefore lead to exceedance of ARfD and PMTDI will be termed to be of public health concern.

General Information on Hydrocyanic Acid

26. Hydrogen cyanide is a colourless or pale blue liquid or gas with a faint bitter almond-like odour, which is released into the atmosphere from natural biogenic processes either from higher plants, bacteria, and fungi (Orjiekwe *et al*., 2013).

27. Hydrogen cyanide can be produced following the hydrolysis of cyanogenic glucosides; linamarin and a small amount of Lotaustralin present in cassava. The Linamarin is readily hydrolysed to glucose and acetone cyanohydrin in the presence of the enzyme Linamarase, which is also produced by the plant (Orjiekwe *et al*., 2013). The acetone cyanohydrin decomposes rapidly in neutral or alkaline conditions liberating hydrogen cyanide and acetone as shown below

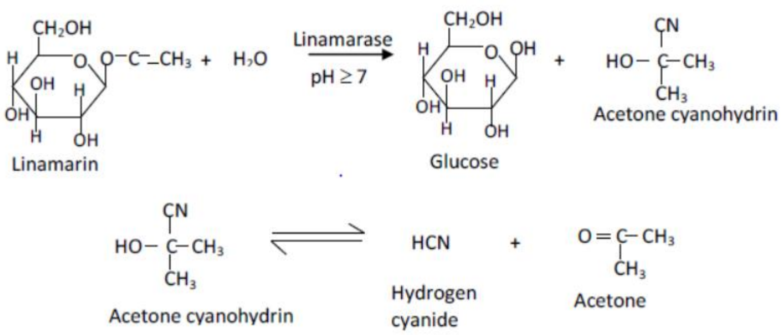

Figure 1: Enzymatic hydrolysis of linamarin. Source: Orjiekwe *et al*., 2013

28. The mechanism involves a two-step reaction which are; the penetration of cyanide into a protein crevice, with initial binding of cyanide to protein while the second step is the binding of cyanide to heme iron. Cyanide exerts its toxic effects by binding to the ferric ion of cytochrome c oxidase, an enzyme that accounts for about 90 percent of the total oxygen uptake in most cells via the electronic transport chain (Baskin et al., 2004). Cyanide acts as an inhibitor of the electron transport chain by binding non-competitively to cytochrome c oxidase (complex IV) and altering the shape of its active site. Consequently electrons cannot be released to oxygen and the electron transport chain shuts down (Garett and Grisham, 2005) thereby resulting in decrease in the utilization of oxygen in the tissues. It also causes an increase in blood glucose and lactic acid levels and a decrease in the ATP/ADP ratio indicating a shift from aerobic to anaerobic metabolism (WHO, 1993).

29. Three main factors determine the degree to which HCN exerts its effect; amount of exposure, route of exposure, and the length of time of the exposure (MDOCH, 2004). HCN poisoning occurs in two forms; acute and chronic poisoning. In acute poisoning short-term exposures to low levels of cyanide through inhalation, skin absorption or ingestion results in rapid breathing and heart rate, restlessness, dizziness, weakness, headache, and nausea/vomiting in few minutes (Mburu, 2013).While in chronic conditions following long term exposure to low levels of cyanide may result in breathing difficulties, eye irritation, chest and/or heart pain, vomiting, loss of appetite, headaches, nosebleeds, enlargement of the thyroid gland (goiter) and death. Tropical Ataxic Neuropathy (TAN) also is a syndrome attributed to dietary cyanide exposure from inadequately prepared cassava. TAN is a progressive disorder that mainly affects older adults (CCDN News, 2008). Survivors of chronic cyanide exposure may develop damage to the brain and the heart and in some cases injury to the central nervous system due to protracted oxygen deprivation to the organ system (Baskin *et al*., 2004).An occurrence of ataxic polyneuropathy was recorded in Ososa, Southwest Nigeria with 22 per 1000 in 1969, 60 per 1000 in 1998 and 64 per 1000 in 2003 (Oluwole *et al*., 2013) which is been attributed to cyanide poisoning from cassava food products. A case was reported in Malaysia due to cassava (ubikayu) poisoning following its consumption amongst a mother and three daughters with the loss of one daughter with symptoms such as nausea, abdominal cramp, diarrhea, vomiting, drowsiness (Arriffin *et al*., 1992). Poor /coloured vision was recorded due to consumption of small amounts of cyanide over a long period of time due to inadequate processing of cassava roots to gari in Zaria metropolis town Nigeria, which contributes to high prevalence of blindness and severe visual impairment (Yusuf *et al*., 2014).

30. The level of hydrocyanic acid (HCN) in cassava limits the use of cassava and its products for livestock feed/food and hence requires additional supplementation of cassava-based diets with methionine and lysine either in its pure form or as animal protein supplements, particularly fish meal(FAOSTAT, 2013).

31. Cyanide levels can be significantly reduced and in some cases eliminated depending on the processing methods employed (FOA, 2004).The processing methods generally adopted include a combination of procedures, such as peeling, slicing, fermentation, boiling, drying, pounding or milling and sieving. Fermentation has shown to reduce and in some cases totally eliminated HCN in cassava products (loop fermentation) (Egwim *et al*., 2013).

    The cassava is first peeled (as about 60-70% of the poison is in the peel) and then soaked in stagnant water or fermented in sacks for about three days. It is sometimes grated or rasped as this helps to speed up the fermentation process (Milena *et al.,* 2013). At the beginning of the fermentation, *Geotricum candidia* acts on the cassava. This helps to make the product acidic, which finally kills off the microorganisms as they cannot exist in such a medium. A second strain of microorganisms (*Corynebacterium lactis*) which can tolerate the acidic environment then take over and by the third day 90-95% of the dangerous chemicals would have been hydrolyzed. The cassava also develops its characteristic flavour. The product is then sieved and the fine starch particles are fried in an iron pan over a flame or with some oil. During this process most, if not all the remaining HCNis released. The liquor from a previous fermentation is used as a starter, thereby reducing the period of fermentation to about 6- 8hours (Egwim *et al*., 2013).

32. Loop fermentation is achieved by using starter culture from already fermented product to inoculate a fresh barge of fermentation process. In this case organisms are “trained” to further utilize the compounds in the fermenting substrate and acidified by squeezing lime (citrus) juice into it before inoculation begins (Egwim*et al*., 2013).

Concentrations of and Dietary Exposure to HCN Hydrocyanic Acid in Fermented Cassava

33. The concentrations of HCN in *gari*, *fufu* and *akpu* from Nigeria and Sierra Leone (Table 2) available in nine publications were mostly above the ML of 2.00 mg/kg in the products. Literature search by the EWG did not reveal data on dietary exposure to HCN.

Evaluation of Existing MLs in Relation to Dietary Exposure to HCN in Fermented Cassava Products

Submissions still expected from members of EWG and other CCCF member nations on existing MLs of HCN in Fermented Cassava

Need and Feasibility to Establish ML for HCN in All Fermented Cassava Products

This is deferred to JECFA based on the response received on its data request call

Harmonizing the Expression of HCN Levels-

Suggestions with justification on harmonizing the expression of HCN levels i.e. free or total HCN are outstanding.

Levels of Mycotoxins in Some Fermented Cassava Products

Gari

The data obtained for gari showed that total aflatoxins were found in 22.64% (106 / 404) of samples analysed, within the range 0.05-13.8 µg/kg (Table 3). Aflatoxin B1 was found in 72.2% (13 / 18) of analysed samples at the mean concentrationof 0.25 µg/kg. Deoxynivalenol (DON) was present in 37.5% (9/24) samples were contaminated within 35-99 µg/kg and at a mean of 57 µg/kg, Fumonisin B1 was implicated in 25% (6/24)of samples within 45-80 µg/kg and mean value of 6 µg/kg. Zearalenone (ZEN) contaminated 16.7% (4/24) of the samples within 11-17µg/kg. The other mycotoxins reported are DON-3G, Fumonisin B2 (FB2), Diacetoxyscirpenol (DAS) and T-2.

Lafun

There was no report on aflatoxin in lafun however, DON, FB1and ZENcontaminated 36 samples of lafun by 27.8% (31-91 µg/kg), 44% (44-256 µg/kg) and 5.6% (13-16µg/kg), respectively. Thus in comparison with gari it turns out being lower incidence of DON in lafun, higher incidence but lower level of FB1 in lafun, and lower incidence and levels of ZEN in lafun. Other mycotoxins reported in lafun are 15ADON, FB2, DAS, FUS-X and a-zearalenol.

Health Implications of Incidence and Levels of Mycotoxins found in the Fermented Cassava Products

The incidence of aflatoxins in gari and lafun consumed across Nigeria is indicative of the possibility of chronic or acute toxicological impacts. Liver cancer, liver cirrhosis, immunosuppression, growth impairment, mutagenesis, and death have been attributed to ingestion of aflatoxin contaminated food or food product. Aflatoxin contamination of over 200µg/kg in maize led to the death of over 106 Western Indians and 125 Kenya natives (Bhumi and Chinnam, 2007; Azziz-Baumgartner et al, 2005). Also, Immune suppression in the Gambia (Turner et al., 2003) along with have child stunting and underweight in children inTogo, Benin and Tanzania (Gong et al, 2004) have also been reported.

While dietary exposure to mycotoxins is yet to be examined, however from the values of the toxins found mostly in Nigeria, it is unlikely that acute effects could occur from a sole source of cassava, although a long term health impacts may be fueled.

FAO food security data of 2012 reveals that an average Nigerian, based on 2005 - 2007 survey consumes cassava at 321.89 g/person/day (FAOSTAT, 2012 - http://nso.nigeria.opendataforafrica.org/bpgqoxe/fao-food-security-data-by-food-groups-items-june-2012) making up 10% of food item consumed.

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Annex I

Table 1: Cassava Based Food Products from Various Countries of the World

Continent	Country	Name of non-fermented food products	Name of Fermented food product
South America	Bolivia	Boiled and fried Yuca	
	Brazil	Cooked root to paste-*Vaca-atolada* *Pirão*- gravy-like gruel prepared by cooking fish bits (such as heads and bones) with cassava flour. *Farofa*-lightly roasted cassava Cassava cake Boiled cassava into sweet pudding	
	Colombia	*Sancocho*-Soup *Pandebono* bread from *Yuca* dough *Bollo de yuca*-boiled dough served with butter and cheese. *Enyucados*-dessert from ground boiled *yuca* Boiled, fried and roasted cassava	
	Suriname	*Telo*-steamed and deep fried cassava withed salted fish.	
	Ecuador	Boiled, *Yuquitos*-fried chips, *Bolitos de yuca*-bread, yuca dough, baked yuca dough.	*Chicha*-traditional fermented drink
	Paraguay	Boiled and *Chipa*-bagel-shaped cheesy bread	
	Peru	Boiled and fried *yuca*	
	Venezuela	Boiled, fried and grilled yuca *Plancha*-roasted ground cassava spread as pancake. *Casabe*-pancake	
Central America	Belize	*Bammy*-fried cassava cake. Cassava pane-dessert recipe Component of *Bile up.* *Ereba*-Cassava bread	
	El Salvador	Yuca used in soup and sandwiches *YucaFrita con Chicharrón*-deep fried yuca	
	Costa Rica	Boiled, fried and as snacks	
	Panama	*Carimanolas*-boiled, mashed cassava dough	
	Nicaragua	As soup and recipe of *vigoron* and *vaho* dishes	
Caribbean	Cuba	*Casabe*-round shaped flat bread *Yucafrita*-similar to French fries. Ingredient of Cuban vegetarian stew (*Ajiaco*) and Cuban *Buñuelos,* a local variation of a traditional Spanish fritter	
	Haiti	Eaten as bread, flour boiled into a meal named *Moussa*, various soups referred to as *joumou* and cookies called *BonBonLamindon*	
	Dominican Republic	Used as cassava bread (*casaba*), french fries (*arepitas de yuca),* dough made from cassava flour (*catibía*) and fried grated (*(chulos).*	
	Puerto Rico	Used to cook stew (*Sancocho),* eaten as boiled, in form of paste (*masa),* and other dishes (*pasteles* and *alcapurrias*)	
	Jamaica	Cassava cake (*Bammy*)	
	Bahamas	Eaten boiled, cooked in soup with okra and baked into cakes	
	Eastern	Boiled and served with flour dumplings and	

Continent	Country	Name of non-fermented food products	Name of Fermented food product
	Caribbean	other root vegetables	
	Bermuda	Cassava pie and cassava chips (for export)	
Africa	Nigeria and Sierra Leone	Boiled cassava	*Eba or garri, lafun and fufu*
	Central Africa	Eaten as boiled, mashed and cooked and snack.	
	Tanzania and Kenya	Fried, roasted and cassava flour made into *ugali* or *mwanga* or *fufu.*	*ugali* or *mwanga* or *fufu.*
	Central African Republic	Fries, boiled, snacks and bread	
Asia	China	Production of ethanol/export trade	
	India	Boiled, deep fried to make crisps and used to sweet milk puddings.	
	Indonesia	Eaten as boiled, fried or baked.	It is fermented to make *peuyeum* and *tape,* a sweet paste which can be mixed with sugar and made into a drink, the alcoholic (and green) *es tape.*
	Philippines	Steamed and eaten plain, as dessert	
	Sri Lanka	Eaten as supplementary diet in boiled form	
	Vietnam	Tapioca	

Annex II

Table 2: Occurrence and Concentrations of Hydrocyanic Acid in Fermented Cassava Products

Fermented Cassava Product	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration	Mean ± SD	No of samples above ML for HCN of 2mg/kg	Limit of detection of method	Reference
Garri	Nigeria Okada town, Edo State,	12/12	5-10mg/kg	5±0.10	All		Orjiekwe*et al*, 2013
Fufu	Nigeria Okada town, Edo State	12/12	5-10mg/kg	10±0.13	All		Orjiekwe*et al*, 2013
White garri (processed by researcher) White gari (purchased from market)	Nigeria Osusu, IsialaNgwa, Abia State	1/1 20/20	3.8-32.2mg/kg 3.70-65.8g/kg	3.8±0.6 24.21±17.55	all All		Odoemelam, 2005
Yellow gari Yellow gari (purchased from market)	Nigeria Osusu, IsialaNgwa,Abia State	6/6 20/20	0.62-20.3mg/kg 1.8-52.1mg/kg	0.62±0.6 14.35±13.85	5/6 19/20		Odoemelam, 2005
Gari	Nigeria Ekiti State	6/6	2.10-15.30mg/kg	8.41±4.75	All		Babalola, 2014
Gari (urban areas) Rural areas	Nigeria Ekiti,Oyo, Lagos, Ondo and Osun states	10/10 10/10	0.03-0.11mg/kg 0.01-0.08mg/kg	0.07±0.03 0.03±0.02	0/6 0/6		Abimbola, 2012
Akpu	Nigeria Karu, Nasarawa state	2/2	2.04-8.54mg/kg	2.04±0.64	All		Ojo*et al*, 2013
Gari	Nigeria Karu, Nasarawa state	2/2	2.04-8.54mg/kg	8.54±0.30	All		Ojo*et al*, 2013
Gari	Nigeria Oshodi, Lagos	4/154	3-200 mg/kg	39.15±38.75	All		NAFDAC, 2017
Soaked cassava	Fiji Islands Tonga,Vanuatu and Fiji	10/10	<0.1	<0.1	0/10		Dolodolotawake*et al*, 2011
Fermented cassava mash Fufu	Nigeria Uyo, Akwa Ibom state	Cassava tubers	8.43-10.73mg/kg	8.43±2.03 10.73±2.03	All all		Uyoh*et al*, 2009
Foofoo	Sierra Leone	51/51		28.2±21.2	All		Blanshard*et*

Fermented Cassava Product	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration	Mean ± SD	No of samples above ML for HCN of 2mg/kg	Limit of detection of method	Reference
Gari	Freetown,	36/36		8.6±3.45	all		*al*, 1993

Annex III

Table 3: Occurrence and concentrations of mycotoxins in fermented cassava products

Fermented Cassava Product	Type of mycotoxin	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration	Mean ± SD	No of samples above ML for Myco toxin	Limit of detection of method	Reference
Gari	DON	Nigeria	9/24	35-99	57±19		14.5	Chilaka et al., 2017
	DON-3G	Nigeria	3/24	12-20	16±5		3.2	Chilaka et al., 2017
	FB1	Nigeria	6/24	45-80	6±13		15.0	Chilaka et al., 2017
	FB2	Nigeria	5/24	29-65	40±15		10.5	Chilaka et al., 2017
	ZEN	Nigeria	4/24	11-17	14±7		3.6	Chilaka et al., 2021
	DAS	Nigeria	2/24	5-10	8±3		2.0	Chilaka et al., 2017
	T-2	Nigeria	3/24	17-22	19±3		4.5	Chilaka et al., 2017
	Total Aflatoxin	Nigeria	11/24	0.05-3.3	0.74± 1.03		1	NAFDAC, 2015
	Total Aflatoxin	Nigeria	18/46	1.1-13.8	1.10± 2.26		1	NAFDAC, 2016
	Total Aflatoxin	Nigeria	24/34	1.0-5.4	1.73± 1.43		1	NAFDAC, 2017
	AFB1	Nigeria Benin City,	3/10	1500-2000				Ibeh et al., 1991
	Ochratoxin	Nigeria Niger state,	18/18	3.28-22.73	7.63± 4.07	10	0.001	Makun et al., 2013
	Total Aflatoxin	Nigeria Anambra	6/30	0.44–3.69				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Cross River	5/30	0.32–4.57				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Delta	3/30	0.26–3.64				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Edo	4/30	0.13–4.46				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Enugu	5/30	0.37–5.71				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Imo	7/30	0.14–3.16				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Lagos	9/30	0.12–2.54				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Ogun	3/30	0.25–1.66				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Ondo	2/30	0.18–2.41				Ogiehor et al., 2007
	Total Aflatoxin	Nigeria Rivers	8/30	0.17–4.14				Ogiehor et al., 2007
	AFB1		13/18	nd-0.69	0.25	0	0.5 ng/ml	Adejumo et al., 2013
Lafun	DON	Nigeria	10/36	31-91	62±19		14.5	Chilaka et al., 2017

Fermented Cassava Product	Type of mycotoxin	Country / Location	Number of contaminated sample/total number of samples analyzed	Range of concentration	Mean ± SD	No of samples above ML for Myco toxin	Limit of detection of method	Reference
	15ADON	Nigeria	3/36	21-36	30±8		8.5	Chilaka et al., 2017
	FB1	Nigeria	16/36	44-256	110± 71		15.0	Chilaka et al., 2017
	FB2	Nigeria	22/36	30-392	116± 89		10.5	Chilaka et al., 2017
	ZEN	Nigeria	2/36	13-16	15±2		3.6	Chilaka et al., 2017
	DAS	Nigeria	11/36	7-22	14±6		2.0	Chilaka et al., 2017
	FUS-X	Nigeria	3/36	128-159	143± 16		54.5	Chilaka et al., 2017
	a-zearalenol	Nigeria Lagos,	1/1	11			6	Rubert et al., 2013
Cassava chips/ flour	Total Aflatoxin	Kenya Nairobi and Mombasa,	3/36	2.84-8.89	6.11± 3.05	2	1	Gacheru Patrick et al., 2015
	Total Aflatoxin	Nigeria Ogun,	3/4	0.07-0.07	0.05			Adejumo et al., 2013
Cassava chips	Total Aflatoxin	Cameroun	18/72	5.2- 14.5				Essono et al., 2009
		Uganda /	4/15	0-3.5	0.633±			Kaaya and Eboku, 2010
		Ngora						
		Uganda / Kumil	6/17	0-2.5	0.412±			
		Uganda / Bukedea	8/28	0-4.5	0.500±			
		Rwanda	0/15	nd				Matsiko et al., 2017
Cassava bread					0.03			Adegoke et al., 1993

APPENDIX II

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