FOOD STANDARDS
Australia New Zealand
Te Mana Kounga Kai – Ahitereiria me Aotearoa

15 September 2008
[16-08]

PROPOSAL P1002
HYDROCYANIC ACID IN READY-TO-EAT CASSAVA CHIPS
APPROVAL REPORT

Executive Summary

FSANZ prepared Proposal P1002 to assess the public health risks associated with hydrocyanic acid (hydrogen cyanide) in ready-to-eat cassava chips. As a result of this assessment, FSANZ considers that regulatory measures in the *Australia New Zealand Food Standards Code* (the Code) are required to reduce levels of hydrocyanic acid in ready-to-eat cassava chips to protect public health and safety. This decision is based on the data available and follows a re-assessment of the risks to public health and safety following public comment. The decision also takes into account the issues raised in public submissions.

The food regulatory measures include definitions for 'ready-to-eat cassava chips' and 'hydrocyanic acid, total' and a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips. Compliance with these measures would reduce acute dietary exposure to hydrocyanic acid from ready-to-eat cassava chips and would address the potential public health implications that have been identified with these foods.

While not currently within the scope of this Proposal, additional regulatory and non-regulatory measures may be required as investigations continue into hydrocyanic acid in other foods. The inclusion of a maximum level for total hydrocyanic acid in ready-to-eat cassava chips is considered an appropriate risk management measure while these investigations continue.

Assessing the Proposal

Proposal P1002 – Hydrocyanic Acid in Ready-to-eat Cassava Chips has been assessed under the General Procedure.

In assessing the Proposal and the subsequent development of a food regulatory measure, FSANZ has had regard to the following matters as prescribed in section 59 of the *Food Standards Australia New Zealand Act 1991* (FSANZ Act):

- whether costs that would arise from a food regulatory measure developed or varied as a result of the Proposal outweigh the direct and indirect benefits to the community, Government or industry that would arise from the development or variation of the food regulatory measure;

- there are no other measures that would be more cost-effective than a variation to Standard 1.4.1 that could achieve the same end;

- any relevant New Zealand standards;

- any other relevant matters.

Decision

Approve the variations to Standard 1.4.1 – Contaminants and Natural Toxicants of the Code to include a maximum level of 10 mg/kg for 'hydrocyanic acid, total' in 'ready-to-eat cassava chips' and to facilitate compliance monitoring, a definition of 'hydrocyanic acid, total' for 'ready-to-eat cassava chips'.

Reasons for Decision

- with proper preparation or processing, cassava and cassava-based foods are safe for human consumption, even though whole, unprocessed cassava contains naturally occurring cyanogenic substances[1];

- the composition of some 'ready-to-eat cassava chips' is such that existing food regulatory measures in the Code are not considered sufficient to reduce levels of total hydrocyanic acid in ready-to-eat cassava chips and to protect public health and safety;

- following a revised risk assessment, a food regulatory measure in the Code for total hydrocyanic acid in ready-to-eat cassava chips is considered necessary to minimise acute dietary exposure to hydrocyanic acid and thereby protect public health and safety;

- a maximum level in Standard 1.4.1 is considered to be an appropriate risk management measure while additional information is gathered about other cassava based foods and other foods containing cyanogenic substances;

- a maximum level specifically for 'ready-to-eat cassava chips' only is considered necessary at this time. FSANZ considered the views in some submissions that this level should extend to all ready-to-eat foods containing cassava. At this time, FSANZ considers the level should only apply to ready-to-eat cassava chips because there are existing food regulatory measures for managing hydrocyanic acid in some other cassava based products and some other foods (see below);

- a maximum level of 10 mg/kg is considered to be necessary to confidently protect public health and safety. Based on the data available to FSANZ and the revised risk assessment conducted by FSANZ, a maximum level higher than 10 mg/kg is not considered adequate to protect public health and safety;

- the maximum level of 10 mg/kg is also considered to be practical and reasonably achievable with proper processing of cassava or with specific cassava selection. These measures are likely to increase costs for some producers of ready-to-eat cassava chips because the selection of low total hydrocyanic acid cassava may be difficult to guarantee, and additional processing of cassava with higher levels of total hydrocyanic acid will therefore require additional resources.

[1] 'cyanogenic substances' are those substances that produce hydrocyanic acid (hydrogen cyanide) in specific circumstances.

Results provided to FSANZ demonstrate that some ready-to-eat cassava chip producers would already comply with a limit of 10 mg/kg for total hydrocyanic acid. Additional results provided to FSANZ indicate that a number of other cassava containing foods also contain less than 10 mg/kg total hydrocyanic acid. On this basis, FSANZ considers that compliance with the maximum level is achievable and generates the greatest net benefit for the community by protecting public health and safety;

- the maximum level of 10 mg/kg is also consistent with the level in the international (Codex) standard for edible cassava flour (another processed cassava product for direct human consumption). Typical production of cassava flour or starch, especially in the large-scale commercial factories, has ensured that processing steps and parameters are effective in eliminating total hydrocyanic acid from cassava. Based on comments in submissions, edible cassava flour is an ingredient in ready-to-eat cassava chips, although not necessarily the predominant ingredient in all types of chips. Some types of ready-to-eat cassava chips have the potential to contain total hydrocyanic acid above 10 mg/kg. This is because they contain dried raw cassava, which may contain higher levels of total hydrocyanic acid than cassava flour. To comply with a limit of 10 mg/kg, these types of chips need to be produced from low total hydrocyanic acid cassava or the dried cassava used for them must be further processed to adequately reduce the levels of total hydrocyanic acid. Given the potential acute public health implications associated with total hydrocyanic acid in ready-to-eat cassava chips, FSANZ considers that all cassava based ingredients in ready-to-eat cassava chips should be processed to a level equivalent to that of edible cassava flour.

Furthermore, it is considered necessary that the maximum level come into effect upon gazettal and that the usual 'stock in trade' transitional arrangements not apply. FSANZ does not consider that transitional arrangements are appropriate because of the potential acute public health implications associated with the levels reported in some ready-to-eat cassava chips.

FSANZ does not consider that it is practical or necessary to prescribe a method of analysis for hydrocyanic acid in ready-to-eat cassava chips as this will restrict the flexibility of industry and compliance agencies to develop more contemporary methods for monitoring hydrocyanic acid in ready-to-eat cassava chips. To facilitate compliance monitoring, FSANZ has developed a definition of 'hydrocyanic acid, total' for the purposes of ready-to-eat cassava chips (see **Attachment 1A**). This reflects the views in some submissions and is considered a more practical approach to ensuring the appropriate range of substances are measured for compliance than prescribing a specific method.

Some submissions raised issues about existing levels for total hydrocyanic acid in the Code and that the potential public health implications are also relevant to other foods containing cyanogenic glycosides. FSANZ acknowledges these views and intends to discuss these issues with other food regulatory agencies. FSANZ has also noted that the issues associated with certain varieties of cassava are also under discussion at an international level within the Codex Alimentarius Commission.

Some submissions included comments in relation to implementing the maximum level, including the need to validate methods, concerns about variability in results and suggestions in relation to monitoring compliance. FSANZ acknowledges that there are implementation issues associated with the maximum level. However, the role of FSANZ does not extend to developing or validating methods or determining specific arrangements for compliance monitoring. These aspects will need to be implemented by compliance agencies either individually or collectively.

Consultation

This Proposal has been assessed under the General Procedure with one round of public consultation. This consultation occurred from 6 March 2008 until 3 April 2008. The consultation period was subsequently extended to 17 April 2008 to allow submitters more time to prepare their submissions.

FSANZ originally acknowledged that this Proposal would be of interest to a broad range of stakeholders and applied a general communication strategy to this Proposal. This included advertising the availability of the Assessment Report for public comment in the national press and making the reports available on the FSANZ website.

FSANZ received fifteen submissions. These were from individuals, government agencies, including an overseas agency and industry. FSANZ has had regard to the issues raised in submissions and the FSANZ response to them is in **Attachment 2**.

Amendments Following Public Consultation

FSANZ sought public comment on the draft variation at **Attachment 1C**. Following public comment and taking into account submissions on the original draft variation, FSANZ has amended the draft variation (see **Attachment 1A** - unmarked version or **Attachment 1B** - marked version).

The amendment to the draft variation is to include a definition of 'hydrocyanic acid, total' for the purposes of ready-to-eat cassava chips. FSANZ considers that this definition is necessary to facilitate compliance monitoring. This reflects the views in some submissions and is considered a more practical approach to ensuring the appropriate range of substances are measured for compliance than prescribing a specific method.

CONTENTS

INTRODUCTION

Food regulatory agencies in Australia and New Zealand have found that certain cassava based foods (some chips or crackers) contained higher than expected levels of hydrocyanic acid (hydrogen cyanide). These results prompted the national recall of the implicated products in Australia and further investigation by food regulatory authorities. FSANZ has prepared this Proposal to:

- consider the potential public health and safety risks associated with the consumption of ready-to-eat cassava chips; and
- develop appropriate risk management strategies to manage these risks, including the need for a maximum level in the Code for hydrocyanic acid (hydrogen cyanide) and/or its precursors in these foods.

1. The Issue

Cassava naturally contains compounds called cyanogenic glycosides which release hydrocyanic acid (hydrogen cyanide) as a result of enzymatic hydrolysis during processing of the plant tissue. Safe traditional human consumption of cassava is dependent on adequate processing to minimise the cyanogenic glycoside content. If cassava is eaten either raw or after inadequate processing then toxicity in humans may be observed, sometimes resulting in death.

FSANZ has been notified of total hydrocyanic acid levels in ready-to-eat cassava chips manufactured in Australia. These results ranged from less than 10 mg/kg up to 145 mg/kg. The reported levels are much higher than would be expected in cassava based foods which have been adequately processed. As previously stated, if cassava is eaten after inadequate processing then toxicity in humans may be observed, including potentially tragic and irreversible consequences. Therefore, and based on a revised risk assessment, FSANZ considers the elevated[2] levels of total hydrocyanic acid reported in ready-to-eat cassava chips to be of public health and safety concern (See Section 5). FSANZ also noted that the actions of businesses in relation to the elevated levels of total hydrocyanic acid varied with one business recalling implicated products while another business did not. The absence of a specific food regulatory measure has resulted in different responses by manufacturers.

The current regulatory measures do not address the potential for raw cassava containing less than 50 mg/kg of total hydrocyanic acid to be dried, minimally processed and then sold as a ready-to-eat food. This is because, at the time the current measures were developed, it was understood that adequate processing of all raw cassava was occurring. There was no information at that time to indicate that dried, minimally processed cassava was being sold as a ready-to-eat food with elevated levels of total hydrocyanic acid.

Accordingly, this Proposal examines the existing regulatory measures in the Code for managing hydrocyanic acid in ready-to-eat cassava chips and assesses the need for additional risk management measures to ensure the protection of public health and safety. This assessment includes consideration of the need for a maximum level in the Code for total hydrocyanic acid in ready-to-eat cassava chips.

1.1 Terminology

Throughout this Report there are some terms which have the potential to be used ambiguously. To avoid doubt, these expressions have been further explained below.

[2] The levels are elevated in the context of what is understood to be readily achievable.

1.1.1 Hydrocyanic acid

Throughout this report the term 'hydrocyanic acid' will be used instead of the term 'hydrogen cyanide'. This is because the term 'hydrocyanic acid' is currently used in Standard 1.4.1 – Contaminants and Natural Toxicants which is the Standard in which Maximum Levels for substances are included. It is also the term used in Codex Alimentarius Commission standards for processed cassava products.

FSANZ acknowledges that the term 'hydrogen cyanide' is the term recommended by the International Union of Pure and Applied Chemists. It is also the term used in the definition of 'sweet cassava' in Standard 1.1.2 – Supplementary Definitions For Foods, which is based on the Codex Standard for Sweet Cassava.

In this report the term 'hydrocyanic acid' refers to total hydrocyanic acid which includes the hydrocyanic acid which may be enzymatically released from a cyanogenic glycoside as well as any 'free' or unbound hydrocyanic acid in the food. Further detail on this is described in **Attachment 6**.

1.1.2 Ready-to-eat Cassava Chips

The term 'ready-to-eat' is used in the Code and 'ready-to-eat food' is defined in Standard 3.2.2 of the Code as:

> ***ready-to-eat food*** *means food that is ordinarily consumed in the same state as that in which it is sold and does not include nuts in the shell and whole, raw fruits and vegetables that are intended for hulling, peeling or washing by the consumer*

Throughout this Report the term 'ready-to-eat cassava chips' will be used to describe those foods which contain cassava and are 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. This term does not include processed cassava foods which would not be considered snack foods such as desserts e.g. tapioca pudding.

The term 'ready-to-eat cassava chips' has been used to differentiate those snack foods for direct consumption from raw cassava 'chips' which is a form of cassava that is used in trade but which is intended to be further processed before human consumption. The term 'ready-to-eat cassava chips' does not apply to raw cassava unless the raw cassava is represented as a ready-to-eat food with no further preparation before consumption.

2. Current Standards

Cassava (*Manihot esculenta Crantz*) contains naturally occurring substances called cyanogenic glycosides, primarily linamarin. Safe traditional human consumption of cassava is dependent on adequate processing to minimise the linamarin content. Conventional processing usually involves peeling and grating and then soaking in warm water for several days, possibly followed by drying (in the case of cassava with high cyanogenic glycoside content).

2.1 *Australia New Zealand Food Standards Code*

The current and specific food regulatory measures for cassava in the Code were developed primarily as part of Proposal P257 – Advice on the Preparation of Cassava & Bamboo Shoots.

The following link provides information on the assessment that was undertaken at that time, as well as background about cassava and cassava production:

http://www.foodstandards.gov.au/standardsdevelopment/proposals/proposalp257preparationofcassava21august2002/index.cfm

As a result of the consideration of Proposal P257, the Code was amended to:

- include a prohibition on the sale of cassava other than 'sweet cassava' (Standard 1.4.4 – Prohibited and Restricted Plants and Fungi);

- define 'sweet cassava' 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 hydrogen cyanide (fresh weight basis)'. Raw cassava is whole cassava root or unprocessed cassava and the current level applies on a 'fresh weight' basis (i.e. approximately 70% moisture); and

- include a requirement for raw cassava to be labelled or accompanied by a statement indicating that sweet cassava should be peeled and fully cooked before being consumed (Standard 1.2.6 – Directions for Use and Storage).

While not developed as part of P257, the Code also includes the following levels for hydrocyanic acid in the following foods: 25 mg/kg in confectionery; 5 mg/kg in stone fruit juices; 50 mg/kg in marzipan; 1 mg/kg per 1% alcohol in alcoholic beverages. Consistent with the international standard that formed the basis for these levels, these levels only apply to a food to which a flavouring substance has been added. The information on these levels has been included as background to this Proposal.

2.2 Codex Standards

The Codex Alimentarius Commission has developed and published Standards for Sweet Cassava, Edible Cassava Flour and Gari (a product obtained from processing cassava tubers) (also spelled as 'garri').

Throughout this report these Standards are regarded and referred to as 'international standards'. 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; and

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

2.3 Requirements in Other Countries

FSANZ has not identified any specific requirements in other countries for hydrocyanic acid in ready-to-eat cassava chips.

3. Objectives

In the context of this Proposal, the most relevant objective of FSANZ is to assess the need for additional risk management measures to ensure the protection of public health and safety, including the need for a maximum level in the Code for hydrocyanic acid in ready-to-eat cassava chips.

In developing or varying a food standard, FSANZ is required by its legislation to meet three primary objectives which are set out in section 18 of the FSANZ Act. These are:

- the protection of public health and safety;
- the provision of adequate information relating to food to enable consumers to make informed choices; and
- the prevention of misleading or deceptive conduct.

In developing and varying standards, FSANZ must also have regard to:

- the need for standards to be based on risk analysis using the best available scientific evidence;
- the promotion of consistency between domestic and international food standards;
- the desirability of an efficient and internationally competitive food industry;
- the promotion of fair trading in food; and
- any written policy guidelines formulated by the Ministerial Council.

4. Questions to be answered

The key questions to be answered are:

- What is the risk to public health and safety of hydrocyanic acid from ready-to-eat cassava chips?

- Are additional risk management measures required to protect public health and safety?

- Is a Maximum Level (ML) in the Code for hydrocyanic acid from ready-to-eat cassava chips an appropriate risk management measure?

- Could industry comply with any additional risk management measures, including an ML?

- Could compliance with any risk management measures be effectively monitored, including any ML?

RISK ASSESSMENT

5. Risk Assessment Summary

For more details in relation to the peer-reviewed Hazard Assessment see **Attachment 3**. For more details in relation to the peer-reviewed Dietary Exposure Assessment see **Attachment 4**.

5.1 Principal Toxicological Effect of Concern

The primary toxicological endpoint of concern for hydrocyanic acid (HCN) is inhibition of mitochondrial oxidation, which if the level of exposure to HCN exceeds the capacity of normal physiological detoxification mechanisms, may rapidly lead to death. Clinical manifestations of acute cyanide poisoning, especially non-lethal doses, are often non-specific and mainly reflect those of oxygen deprivation of the heart and brain. Typically these effects include headaches, dizziness, stomach pain, or mental confusion.

As these symptoms closely resemble that of over indulgence or mild gastro-intestinal tract disturbance, the dose response curve is steep, and symptoms would not occur until some hours after ingestion of cassava chips, individuals exposed to dangerous levels of HCN may not recognise warning symptoms before consuming a lethal dose. This is likely to be particularly true for young children. Death in humans has been reported from HCN doses as low as 0.58 mg/kg bw. Sub lethal exposures leading to clinical signs of intoxication would not be expected to lead to presentation at hospital emergency departments or general practitioners, and therefore would not be reported in most instances.

5.2 Rationale for the Establishment of an Acute Reference Dose (ARfD)

HCN is a normal component of mammalian physiology, and efficient mechanisms for its detoxification are present. Cyanide clearance is very rapid and its half life is short (14 minutes in rats). For acute toxicity the maximum systemic exposure (C_{max}) is the primary determinant of toxicity rather than the average exposure over a period of time (AUC). An individual consuming a near lethal dose over a few minutes for example, would have normal background levels of blood cyanide within approximately two hours (around six half lives). Thus, the appropriate toxicological reference value must reflect acute rather than cumulative toxicity, and dietary modelling must reflect exposures at a single sitting rather than averages over longer periods. Due to practical limitations of the DIAMOND dietary modelling program, modelling is limited to assessing exposures over a single day. However, for most foods and particularly snack foods, daily consumption may readily be, and often is, in a single sitting.

For acute toxicity the concept of an Acceptable Daily Intake (ADI) or Tolerable Daily Intake (TDI) is not appropriate. Inherent in their establishment, and associated dietary modelling, is that isolated excursions above these values will be of no concern and therefore average intakes over many months may be used to compare population exposures against these reference values. For acute toxicity the appropriate reference value is the acute reference dose (ARfD), the maximum amount that, confidently, can be safely consumed in a single meal or a single day. This value is not a ‘bright line’, exposure above which will predictably result in adverse consequences. Rather, the ARfD represents a limit of confidence above which the risk to consumers increases unacceptably.

The principal cyanogenic glycoside in cassava is linamarin. Toxicity studies have been conducted directly on linamarin so that an extrapolation from HCN studies to the toxicity of linamarin, with associated adjustments for pharmacokinetics, is not necessary. For linamarin an ARfD of 0.7 mg/kg bw was established on the basis of death in hamsters at doses greater than 70 mg/kg bw and applying a 100-fold uncertainty factor to account for intra-species variability and inter-species extrapolation. The available data indicates that no unchanged linamarin is excreted in the faeces following oral ingestion suggesting that there is sufficient enzymatic capacity in the microflora of the caecum to completely hydrolyse large amounts of linamarin. As total HCN levels are more readily determined than linamarin levels, the linamarin ARfD is converted to an ARfD for total HCN measured in cassava, as an analytical convenience.

One mole of linamarin can release one mole of hydrocyanic acid if hydrolysis is complete and on this basis the linamarin ARfD equates to an ARfD for HCN in cassava of 0.08 mg/kg bw. As the lowest reported fatal absorbed dose for HCN is 0.58 mg/kg bw, the ARfD for hydrocyanic acid provides a margin of exposure of seven which, given the steep dose response curve for HCN toxicity, is considered to be appropriate.

5.3 Hydrocyanic Acid Levels in Ready to Eat Cassava Chips

The results reported to FSANZ from the analysis of over 200 samples of cassava based vegetable chips and crackers included levels of total hydrocyanic acid of between 2 - 145 mg/kg with a mean value of 63 $\pm$ 28.6 mg/kg.

5.4 Dietary Modelling

The dietary modelling needed to take two key, variable, parameters into consideration; the range of levels of total hydrocyanic acid likely to be found in these chips, and the range of exposures likely for a single sitting for various age groups.

Consumption of ready-to-eat cassava chips was not separately reported in the 1995 Australian National Nutrition Survey and the 1997 New Zealand National Nutrition Survey but would likely be captured under the 'extruded snacks' or 'other snacks' categories.

Throughout the dietary modelling, the combined consumption of equivalent salty snacks (crisps, extruded and other salty snacks) was used to estimate the amount of ready to eat cassava chips that might be consumed in one day, on the assumption that consumers may substitute any similar salty snack with ready to eat cassava chips. Two dietary modelling approaches were used; deterministic and probabilistic.

The deterministic assessment, based on acute dietary exposures to total hydrocyanic acid from ready to eat cassava chips, estimated for the Australian and New Zealand populations, and for age and gender population sub-groups, showed that the mean concentration of total hydrocyanic acid of 63 mg/kg in cassava chips available for sale in January 2008 (sampled from domestic and imported cassava chips and a variety of products of the major suppliers of these products), could result in exposures above the ARfD for all groups assessed. This modelling identified 2-4 year old children as having the highest risk of exceeding the ARfD, with adults being less at risk. At a concentration of 25 mg/kg, most groups, particularly children, still exceed the ARfD. At concentrations of 10 mg/kg, the lowest considered in the report, 2-4 year old children remained at risk. However, such exposure would most likely only occur if a young child consumed more than 100 g of cassava chips in a single sitting. The risk is increased by the irregular eating patterns and low body weight of this age group.

To better reflect the interplay of the parameters impinging on the risks to the key age group of 2-4 year olds, exposure was also modelled using a probabilistic approach. A distribution of possible dietary exposures in the target age group and a calculation of the probability of exceeding the Acute Reference Dose, were achieved through a simulation carried out by randomly multiplying each point of the distribution of salty snacks consumption with each point of the actual and simulated distributions of HCN concentration ('sampling').

Using probabilistic techniques and assuming consumption patterns for cassava chips over a single day are comparable to that of the combined intake of other salty snacks over a day,, the likelihood of 2-4 year old Australian children exceeding the ARfD from consuming cassava chips with a mean concentration of 63 mg/kg was estimated at 56% i.e. approximately one out of two occasions eating cassava chips with this level of HCN may result in exposure above the ARfD.

The probability of exceeding the ARfD decreases to 17-22% at a mean concentration of 25 mg/kg, and 2-4% at a mean concentration of 10 mg/kg and a standard deviation of 5 or 10 mg/kg. Because of the short half life of HCN however, the consequences of exceeding the ARfD will depend on the time over which the exposure occurs, one sitting or across a day. Consequently the probability of exceeding the ARfD is not necessarily equivalent to the probability of being at risk of an adverse event. Available food consumption data does not distinguish between that eaten in a single sitting and that eaten across the day so refinement of the calculations is not possible. However, the 97.5th percentile consumption for salty snacks is approximately 100g (male and female combined) for 2-4 year olds and this quantity of snack is readily consumable in a single sitting.

For children aged 2-4 years, reducing the mean concentration of hydrocyanic acid in Cassava chips from the *status quo* to 10 mg/kg would reduce the potential incidence of exposures above the ARfD by 93-97%. Reducing the concentration to 25 mg/kg would lead to a lesser reduction of 61-70%.

5.5 Conclusions

HCN is a lethal acute toxin with a steep dose response curve. Doses slightly higher than those producing relatively non specific symptoms can be fatal. Toxicity across species is similar and animal models have clear relevance to estimation of safe human exposures. An ARfD based on death as the primary endpoint in hamsters was determined for linamarin and, by extension, HCN, its principal intestinal metabolite. Dietary modelling identified 2-4 year old children as the highest risk group in the population.

The probability of exposure above the ARfD at total HCN levels of 10 mg/kg of cassava chips was determined to be low with the probability increasing with increasing levels of hydrocyanic acid.

As tragic and irreversible results could potentially, and rapidly, arise from a single instance of a young child consuming a moderate quantity (50 - 100 g) of cassava chips containing a somewhat, but indeterminably, higher level of HCN in a short space of time (a few minutes) without intake-limiting warning symptoms, a degree of conservatism is warranted and has been built into the risk assessment underpinning the proposed maximum level for total HCN.

RISK MANAGEMENT

6. Issues

6.1 Risk to public health and safety

Although raw cassava naturally contains cyanogenic glycosides[2], it has been safely consumed by millions of people around the world for many centuries. It is usually made safe for human consumption following appropriate preparation and processing[3] (See the Food Technology Report in **Attachment 5)**.

Inadequate processing of raw cassava that results in appreciable residual quantities of total hydrocyanic acid may represent a public health risk. A range of total hydrocyanic acid concentrations (under 10 mg/kg and up to 145 mg/kg) in ready-to-eat cassava chips were reported to FSANZ by food regulatory agencies. FSANZ considers this range of total hydrocyanic acid in ready-to-eat cassava chips to be of public health and safety concern.

[3]Cassava processing, FAO Plant Production and Protection Series No. 3, Food and Agriculture Organization.

Based on the current maximum level for total hydrocyanic acid in confectionery in the Code, a guidance level of 25 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips was proposed by FSANZ as part of the initial response to the higher than expected levels in certain ready-to-eat cassava chips. FSANZ has now refined the risk assessment aspects in relation to this Proposal and established an Acute Reference Dose (ARfD) for hydrocyanic acid.

The probability of exposure above the ARfD at total hydrocyanic acid levels of 10 mg/kg of cassava chips was determined to be low with the probability increasing with increasing levels of hydrocyanic acid. Reducing the mean concentration of total hydrocyanic acid from the *status quo* to 10 mg/kg would reduce the incidence of exposures above the ARfD by 93-97%. Reducing the concentration to 25 mg/kg would lead to a lesser reduction of 61-70%. On this basis, FSANZ considers that a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips would confidently protect public health and safety.

7. Options

FSANZ is required to consider the impact of various regulatory and non-regulatory options on all sections of the community, including consumers, food industries and governments. FSANZ considered the non-regulatory options of education and of encouraging the industry to reduce total hydrocyanic acid in ready-to-eat cassava chips as part of the option of maintaining the status quo (Option 1 below).

Labelling regulatory measures may be appropriate where a food has implications for a specific group in the community, especially those, who by the nature of their vulnerability, actively seek out such labelling (e.g. labelling for allergens). However, it would not be appropriate to rely on product labelling to mitigate a potentially serious public health risk for the general community where public awareness of the risk is low.

The acute public health implications will vary according to the level of total hydrocyanic acid in the specific cassava chips and the amount of the product consumed by the consumer. A wide range of total hydrocyanic acid levels have been reported (from less than 10 mg/kg up to 145 mg/kg) and ready-to-eat cassava chips are available in a variety of snack-pack sizes. This variation means that it would be impractical to determine a labelling statement that would be adequate to address the acute public health implications for all potential consumers. The general availability of ready-to-eat cassava chips, including for children and the risk assessment which indicates children are the group at greatest risk of exceeding safe doses, means that it would be inadequate to rely on specific labelling statements to protect public health and safety for products containing elevated levels of total hydrocyanic acid.

A labelling approach would also unfairly disadvantage those producers who currently produce cassava chips with low levels of cyanogenic glycosides.

In summary, FSANZ considered the use of labelling regulatory measures to address the public health implications associated with the elevated levels of total hydrocyanic acid reported in ready-to-eat cassava chips. However, FSANZ does not consider it feasible to expect industry or consumers to manage a potentially serious public health risk such as this, through labelling regulatory measures. This is because the cassava chips are a generally available ready-to-eat food, a wide range of total hydrocyanic acid levels have been reported and these levels have varying public health implications for the general community. For these reasons, the option of labelling regulatory measures is not considered viable and has therefore not been proposed or assessed further.

The options available for this Proposal are:

7.1 Option 1 – To reject the draft variation and not vary the Code to incorporate an ML for total hydrocyanic acid in ready-to-eat cassava chips

This option maintains the *status quo* and the existing regulatory measures for managing hydrocyanic acid in ready-to-eat cassava chips continue to apply, including advice to consumers.

7.2 Option 2 – Vary the Code to incorporate an ML of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips

This option would require an amendment to Standard 1.4.1 to incorporate an ML that would require all ready-to-eat cassava chips to contain 10 mg/kg or less of total hydrocyanic acid.

7.3 Option 3 – Vary the Code to incorporate an ML of 10 mg/kg for total hydrocyanic acid in all ready-to-eat foods containing cassava (except confectionery)

This option would require an amendment to Standard 1.4.1 to incorporate an ML that would require all ready-to-eat foods containing cassava, except confectionery, to contain 10 mg/kg or less of total hydrocyanic acid. An ML already exists for total hydrocyanic acid in flavoured confectionery.

7.4 Option 4 – Vary the Code to incorporate an ML of 25 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips

This option would require an amendment to Standard 1.4.1 to incorporate an ML that would require all ready-to-eat cassava chips to contain 25 mg/kg or less of total hydrocyanic acid.

8. Impact Analysis

8.1 Affected Parties

The affected parties may include the following:

- consumers of food products containing cassava, including raw cassava;
- industry sectors such as:
    - cassava producers;
    - processors and manufacturers of cassava products;
    - food retailers; and
    - importers of ready-to-eat cassava chips;
- Government agencies.

8.2 Benefit Cost Analysis

In analysing the costs and benefits of the options, FSANZ has also noted that raw cassava containing 50 mg/kg or more of hydrogen cyanide and derivatives of this cassava (e.g. dried bitter cassava) must not currently be sold.

8.2.1 Option 1 – To reject the draft variation and not vary the Code to incorporate an ML for total hydrocyanic acid in ready-to-eat cassava chips

FSANZ is unaware of a specific overarching industry association for ready-to-eat cassava chip manufacturers that would promote any voluntary initiatives to address the potential acute public health implications with the levels of total hydrocyanic acid in ready-to-eat cassava chips. As mentioned above, reports of elevated levels of total hydrocyanic acid prompted different actions by manufacturers. NSW Health has advised consumers to only eat moderate amounts of cassava-based vegetable chips/crackers after testing by the NSW Food Authority revealed unacceptable levels of naturally-occurring cyanogenic glycosides in some of these products that could present a health risk[4].

8.2.1.1 Benefits

There are no particular benefits, for consumers, industry or government agencies, in this option, although this option would mean that some businesses would not incur the costs of reducing the levels of total hydrocyanic acid in ready-to-eat cassava chips.

8.2.1.2 Costs

- for consumers, there would be potential adverse and acute public health impacts from ongoing dietary exposure to elevated levels of hydrocyanic acid. There is no guarantee that consumer advice will be disseminated by the mainstream media or that they will be seen, heard, remembered or acted upon by consumers. In addition, this advice may not result in any change in behaviour in the wider community that will reduce the consumption of these chips to a point at which the potentially serious public health implications are adequately addressed. The costs for consumers could include tragic and irreversible consequences in relation to public health and safety;

- for producers and processors of ready-to-eat cassava chips, there have already been significant costs associated with managing responses to the detection of hydrocyanic acid in ready-to-eat cassava chips, including legal costs, consultancy costs, supply interruption costs and staff losses. A typical product recall could cost a company millions of dollars depending on credits and promotional losses incurred by the company. A wide range of levels have been reported and any consumer advice would need to be very conservative to reflect the highest levels reported and worst case scenario in relation to levels reported. In addition, consumers would have no means of differentiating between products containing less than 10 mg/kg and those containing elevated levels. Any consumer advice would therefore need to be generic for all ready-to-eat chip products. This would negatively impact on all producers and importers of ready-to-eat cassava chips, including manufacturers and importers with low levels of total hydrocyanic acid in their chips;

- for Government agencies, there may be, and there have already been, costs associated with managing responses to the detection of total hydrocyanic acid in ready-to-eat cassava chips. To ensure adequate coverage, any consumer advice would need to be substantial and ongoing, and would need to be regularly issued in a variety of media throughout Australia and New Zealand. This would incur substantial advertising costs for the agencies responsible for issuing this advice.

[4] http://www.foodauthority.nsw.gov.au/aboutus/media%2Dreleases/mr%2DFeb%2D08%2Dveg%2Dchip%2Dcracker%2Drecall%2Dresults%2Dwarning/

8.2.2 Option 2 – Vary the Code to incorporate an ML of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips

8.2.2.1 Benefits

- for consumers, the major benefit would be a reduction in dietary exposure to hydrocyanic acid from ready-to-eat cassava chips and consequently a reduced risk of public health implications from the consumption of ready-to-eat cassava chips;

- for producers and processors of ready-to-eat cassava chips, this option would provide minimal if any benefits;

- for Government agencies, this option would enhance community confidence that regulatory authorities are maintaining standards that minimise dietary exposure to hydrocyanic acid in food products.

8.2.2.2 Costs

- for some producers and processors, this option could result in major costs. In the short term this could be in the form of reduced revenue and commercial losses in removing certain products from the market. On an ongoing basis, in order to comply with the new maximum level, such producers may need to limit themselves to certain suppliers, namely those providing sweet cassava with low levels of total hydrocyanic acid. This limitation in supply could result in higher costs for inputs. Alternatively, they may have to adopt the more costly option of changing their production processes; to further process their cassava raw materials to reduce the levels of total hydrocyanic acid (See Section 11 - Conclusion below for more information). FSANZ has not quantitatively estimated the costs of these options as the costs will be highly dependent on the natural and seasonal variation in cassava tubers and processing costs will vary among the different processing establishments.

  Submissions from two producers of ready-to-eat cassava chips have indicated that the higher costs of inputs and processing could negatively impact on the viability of such businesses and may result in the closure of a particular business. FSANZ has not independently verified this claim but has had regard to it, including the worst case scenario that the closure of these businesses would, in turn, affect the families of the employees and directors of the businesses, and other businesses that trade with the implicated businesses. These other businesses would include producers and processors in other countries where cassava is grown and processed; however no additional costs would be incurred by those whose products already comply with the proposed standard. A business has provided information in relation to business closure costs and these have been taken into account as confidential commercial information;

- some consumers may be disadvantaged due to decreased choice resulting from unavailability of ready-to-eat cassava chips containing more than 10 mg/kg hydrocyanic acid. The extent of this reduction in choice will depend on the market share of those chips that currently contain more than 10 mg/kg total hydrocyanic acid. Data on the current market share of ready to eat cassava chips are not available to FSANZ. However, based on the levels reported to FSANZ, there are products currently available that would comply with a maximum level of 10 mg/kg. On this basis, products would still be available to consumers even if a maximum level of 10 mg/kg were to apply;

- for Government agencies, this option may require them to validate analytical methods for measuring total hydrocyanic acid in ready-to-eat cassava chips, and develop strategies for ensuring that businesses comply with the maximum level. This may include costs associated with purchasing specific materials or equipment for implementing analytical methods.

  Based on comments in submissions these costs will vary from jurisdiction to jurisdiction, depending on the location of manufacturer and importers in these jurisdictions and the compliance activities of these jurisdictions.

8.2.3 Option 3 – Vary the Code to incorporate an ML of 10 mg/kg for total hydrocyanic acid in all ready-to-eat foods containing cassava (except confectionery)

8.2.3.1 Benefits

- as for Option 2, for consumers, the major benefit would be a reduction in dietary exposure to hydrocyanic acid from not only ready-to-eat cassava chips, but potentially all food products containing cassava;

- as for Option 2, for producers and processors of foods containing cassava, this option would provide minimal if any benefits;

- as for Option 2, for Government agencies, this option would enhance community confidence that regulatory authorities are advocating standards that minimise dietary exposure to hydrocyanic acid in food products. The broader scope of this regulatory measure could assist compliance agencies since they would not need to differentiate between 'ready-to-eat cassava chips' and other food products containing cassava.

8.2.3.2 Costs

- as for Option 2, for consumers, there may be a short term reduction in the choice of products available;

- for producers and processors that are not adequately processing cassava for use in food products, this option could result in major costs as detailed above (See 8.2.2.2);

- as for Option 2, for Government agencies, this option may require them to validate analytical methods for measuring total hydrocyanic acid in food products containing cassava, and develop strategies for ensuring that businesses comply with the maximum level. This may include costs associated with purchasing specific materials or equipment for implementing analytical methods.

8.2.4 Option 4 – Vary the Code to incorporate an ML of 25 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips

8.2.4.1 Benefits

- for consumers, the major benefit would be a reduction in dietary exposure to hydrocyanic acid from ready-to-eat cassava chips, although under this option, this reduction would not be as protective of public health and safety as a level of 10 mg/kg (Options 2 and 3);

- compared with Option 2, for producers and processors of ready-to-eat cassava chips, this option would provide some manufacturing flexibility to produce ready-to-eat cassava chips from blending cassava flour and tubers;

- as for Options 2 and 3, for Government agencies, this option would enhance community confidence that regulatory authorities are advocating standards that minimise dietary exposure to hydrocyanic acid in ready-to-eat cassava chips.

8.2.4.2 Costs

- for consumers, this option would have costs for consumers, particularly for some consumers such as children, as a level of 25 mg/kg would not be adequate to confidently protect public health and safety. In addition and as with Option 2, there may be a short term reduction in the choice of products available. Based on the information provided to FSANZ, some cassava chip products would continue to be available to consumers as these products have been reported as containing low levels of total hydrocyanic acid;

- for producers and processors of ready-to-eat cassava chips, this option could result in major costs. These costs may be different from Option 2 as the costs of sourcing cassava tubers to comply with a level of 25 mg/kg may be less than sourcing cassava tubers to comply with a level of 10 mg/kg. This is because it is more likely that manufacturers could source cassava tubers with low levels of total hydrocyanic acid and produce chips with less than 25 mg/kg total hydrocyanic acid from these tubers by blending with an appropriate proportion of cassava flour or other ingredients. These costs will be highly dependent on the seasonal availability of cassava tubers containing low levels of total hydrocyanic acid. If such tubers are not available then the option of blending with flour containing low levels of total hydrocyanic acid becomes less viable as a means of complying with a limit of 25 mg/kg in the ready-to-eat cassava chips. However, it should be noted that the use of cassava tubers containing more than 50 mg/kg total hydrocyanic acid is already prohibited.

  The costs associated with processing of cassava to produce ready-to-eat cassava chips with less than 25 mg/kg total hydrocyanic acid are expected to be the same or similar to those for Option 2, even though the proposed level for Option 4 is higher. This is because the typical cassava processing techniques to reduce levels to below 25 mg/kg in the cassava chips would be the same as those used to reduce levels to below 10 mg/kg.

  Therefore, there is not considered to be any reduction in processing costs associated with achieving a higher level of total hydrocyanic acid in processed cassava ingredients, although there may be some cost savings in relation to time for processing. These costs savings will be highly dependent on the total hydrocyanic acid levels in cassava tubers prior to processing which must be less than 50 mg/kg total hydrocyanic acid;

- as for Options 2 and 3, for Government agencies, this option may require them to validate analytical methods for measuring total hydrocyanic acid in ready-to-eat cassava chips, and develop strategies for ensuring that businesses comply with the maximum level. This may include costs associated with purchasing specific materials or equipment for implementing analytical methods.

8.3 Comparison of Options

See Section 11 (Conclusion) for a comparison of the options including the preferred option.

COMMUNICATION AND CONSULTATION STRATEGY

9. Communication

It is proposed that a maximum level of 10 mg/kg for hydrocyanic acid in ready-to-eat cassava chips be included in the Code. FSANZ is applying a basic communication strategy to this Proposal.

This Report will be made available on FSANZ's website and FSANZ will notify the Ministerial Council of its decision. Stakeholders, including the public, will be notified on the gazettal of changes to the Code in the national press and on the FSANZ website.

10. Consultation

FSANZ acknowledged that this Proposal may have impacts on a specific industry sector (cassava chip producers, importers and processors). On this basis the public consultation ensured that these specific sectors of industry had the opportunity to comment on the proposed measures.

This Proposal has been assessed under the General Procedure with one round of public consultation. This consultation occurred from 6 March 2008 until 3 April 2008. The consultation period was subsequently extended to 17 April 2008 to allow submitters more time to prepare their submissions.

FSANZ received fifteen submissions to the Assessment Report. These were from individuals, government agencies, including an overseas agency and industry. Some of these submissions were very comprehensive and provided a substantial amount of information that FSANZ has considered. A range of different views were expressed in the submissions and some included a number of issues.
FSANZ has had regard to the issues raised in submissions and the major issues are addressed below. The FSANZ response to the individual submissions is shown in **Attachment 2**. The major issues were:

- the need for additional risk management measures;
- an ML in the Code for hydrocyanic acid from ready-to-eat cassava chips; and
- effectively monitoring compliance with any ML.

10.1 Additional risk management measures

10.1.1 Assessment

The Code currently includes a range of regulatory measures to limit and manage hydrocyanic acid in cassava and cassava based foods. These are based on limiting access to varieties of cassava that may contain levels of hydrocyanic acid of 50 mg/kg or above, and ensuring adequate information about processing is provided to consumers. The level of 50 mg/kg currently only applies to raw cassava, which is whole cassava root or unprocessed cassava and the level applies on a 'fresh weight' basis (i.e. approximately 70% moisture).

Based on the data published by Cardoso et al[5], it is possible for total hydrocyanic acid levels to be higher in dried cassava products as a result of the removal of moisture from the raw cassava root. If hydrocyanic acid is not lost during this drying process then the hydrocyanic acid in the dried raw cassava may be two and a half times higher than in the raw cassava. For example, 40 mg/kg total hydrocyanic acid in raw cassava could, in theory, be as high as 100 mg/kg in dried raw cassava.

The drying and minimal preparation of cassava into 'raw chip' or 'raw pellet' form is commonplace as these forms are more stable during transport and reduce the bulk of the product resulting in reduced transport costs. These forms are then further processed before being used in food for human consumption.

The existing regulatory measures do not address the potential for raw cassava containing less than 50 mg/kg to be dried, minimally processed and then sold as a ready-to-eat food. This is because, at the time these measures were developed, it was understood that adequate processing of all raw cassava was occurring. There was no information to indicate that dried, minimally processed cassava was being sold as a ready-to-eat food with elevated levels of cyanogenic substances.

The data provided to FSANZ indicates that the processing of certain ready-to-eat cassava chips is insufficient to reduce the levels of total hydrocyanic acid. These data also indicate that these levels are of public health and safety concern. On this basis, FSANZ considered that additional regulatory measures were necessary to ensure that total hydrocyanic acid in ready-to-eat cassava chips was reduced to a level that is as low as reasonably achievable.

Compliance with the proposed maximum level would reduce dietary exposure to hydrocyanic acid from ready-to-eat cassava chips and would address the identified public health implications that had been identified with these foods.

FSANZ considered the non-regulatory option of encouraging the industry to amend their practices to reduce total hydrocyanic acid in ready-to-eat cassava chips. However, given the public health implications, FSANZ was of the view that a regulatory measure in the Code was necessary to ensure that levels of total hydrocyanic acid in ready-to-eat cassava chips were adequately managed.

10.1.2 Submissions

Some submitters did not agree with the FSANZ Assessment and raised a number of issues in relation to this Assessment. The comments included that:

- there was insufficient justification for a food regulatory measure, including that the risk associated with the reported levels did not justify a regulatory measure or was overly conservative;
- there had been no reports of illness associated with the consumption of ready-to-eat cassava chips and that these were safe;
- the non-regulatory option of encouraging industry to implement audited food safety plans may be a more appropriate means of controlling hydrocyanic acid levels;
- sufficient measures already existed to ensure that hydrocyanic acid is reduced to acceptable and safe levels;
- more investigation into hydrocyanic acid in foods should occur before a food regulatory measure is considered; and

[5] Processing of cassava roots to remove cyanogens, Cardoso, A.P.; Mirione, E.; Ernesto, M.; Massaza, F.: Cliff, J.; Rezaul Haque, M.; Bradbury, J.H. Journal of Food Composition and Analysis, 18 (2005) 451-460.

- there are existing Codex Alimentarius Commission standards.

10.1.3 FSANZ response

FSANZ has now refined the risk assessment aspects in relation to this Proposal and established an Acute Reference Dose (ARfD) for hydrocyanic acid. The probability of exposure above the ARfD at total hydrocyanic acid levels of 10 mg/kg of cassava chips was determined to be low with the probability increasing with increasing levels of total hydrocyanic acid. On this basis, FSANZ considers that a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips is considered necessary to protect public health and safety.

FSANZ is unaware of any existing specific provisions in the Code that ensure that the level of total hydrocyanic acid is reduced sufficiently in ready-to-eat cassava chips to protect public health and safety. If a level is not stipulated for total hydrocyanic acid in ready-to-eat cassava chips in the Code then products containing unacceptably high levels of hydrocyanic acid could continue to be sold.

Given the public health implications associated with the higher than expected levels of total hydrocyanic acid in ready-to-eat cassava chips, FSANZ considers that there is sufficient justification to amend the Code to limit the amount of total hydrocyanic acid in ready-to-eat cassava chips.

FSANZ acknowledges that there may be a need to consider other cassava containing foods and other foods which may contain cyanogenic glycosides. However, given the public health implications, FSANZ does not consider that a food regulatory measure for total hydrocyanic acid in ready-to-eat cassava chips should be delayed pending the future investigations into these other foods.

10.2 Maximum Level for hydrocyanic acid in ready-to-eat cassava chips

In relation to managing naturally occurring substances in food that may be of public health and safety concern, the usual regulatory measure that is adopted is the incorporation of a Maximum Level (ML) in Standard 1.4.1 – Contaminants and Natural Toxicants. This Standard already includes levels for hydrocyanic acid in certain foods. An ML is established only where it serves an effective risk management function and it is established at a level which is consistent with the protection of public health and safety, and which is reasonably achievable.

10.2.1 Ready-to-eat cassava chips

10.2.1.1 Assessment

MLs are usually applied to a raw food and not normally applied to a processed food. However, MLs can be established for nominated processed foods where the setting of an ML for the primary commodity is judged to be ineffective (e.g. hydrocyanic acid in stone fruit juices), including where the contaminant concentrates in the processed food compared with the raw food. The situation with 'ready-to-eat cassava chips' is unusual and establishing a lower ML for whole unprocessed cassava is not considered to be an effective or practical means of ensuring levels are minimised in ready-to-eat cassava chips. On this basis, an ML for 'ready-to-eat cassava chips' was proposed so that raw cassava intended to be further processed could continue to comply with the existing 50 mg/kg level.

Dried raw cassava is a legitimate product which, with adequate processing, is a safe ingredient in food. There was no intention to prohibit the sale of raw sweet cassava that had been dried and was intended for further processing. In the unlikely event that raw cassava chips were represented in a ready-to-eat form then the proposed ML for ready-to-eat cassava chips was intended to apply.

A level specifically for 'ready-to-eat cassava chips' was proposed to complement existing food regulatory measures for managing hydrocyanic acid in some other cassava based products. FSANZ proposed to describe 'ready-to-eat cassava chips' in the Code to provide clarity about the range of foods to which the level applied.

FSANZ acknowledged that there may be a need to consider other cassava containing foods and other foods which may contain cyanogenic glycosides. However, the data currently available for these other foods are insufficient to consider the need for additional food regulatory measures.

A level for 'total hydrocyanic acid' was proposed as this was consistent with other levels in Standard 1.4.1 and was also consistent with international standards for processed cassava products.

FSANZ proposed that the ML be included in the Table to clause 5 of Standard 1.4.1, rather than in the Table to clause 4 of this Standard. This was because FSANZ considered that the level should apply to all ready-to-eat cassava chips and not only those ready-to-eat cassava chips to which flavouring preparations had been added (See Section 2.1 for information about existing food regulatory measures). This was to ensure that the full range of ready-to-eat cassava chips would need to comply with the proposed ML.

In summary, an ML in Standard 1.4.1 for total hydrocyanic acid in ready-to-eat cassava chips was considered an appropriate risk management measure:

- to minimise dietary exposure to hydrocyanic acid and thereby protect public health and safety; and
- to reduce the levels of total hydrocyanic acid in ready-to-eat cassava chips to as low as reasonably achievable.

10.2.1.1 Submissions

Some submitters did not agree with the FSANZ Assessment and raised a number of issues in relation to this Assessment. The comments included that:

- the need for any level to apply to a range of foods rather than specifically to ready-to-eat cassava chips (e.g. all cassava containing foods or other foods containing cyanogenic glycosides);
- some comment about whether the definition of 'ready-to-eat cassava chips' was adequate for compliance purposes;
- comment that the imposition of a maximum level in ready-to-eat cassava chips effectively imposes end product testing, which is inconsistent with policies of minimum effective regulation and the application of food safety plans; and
- comments about some of the existing levels for total hydrocyanic acid in the Code.

Based on comments in submissions, industry is currently reliant on imported cassava as there is no adequate local industry.

These submissions stated that cassava fluctuates in terms of its cyanogenic glycoside content which depends on the cultivar, season, rainfall and growing conditions. These submissions stated that a maximum level could make it impossible to supply at certain times of the year.

10.2.1.3 FSANZ Response

Some submitters provided comment on the composition of ready-to-eat cassava, including that some ready-to-eat cassava chips may be made from a mixture of 'dried cassava' and cassava flour/starch. FSANZ acknowledges that ready-to-eat cassava chips are made from cassava, either from edible cassava flour or dried cassava or combinations of these cassava based ingredients, along with other ingredients.

Chips and crackers reported as containing higher than expected levels of total hydrocyanic acid were also reported as containing 'cassava' as distinct from 'cassava flour'. From the information reported, it could not be determined if this cassava ingredient was derived from the currently prohibited bitter cassava or from the permitted sweet cassava. Bitter cassava and derivatives of bitter cassava (e.g. dried bitter cassava) are currently prohibited from sale in Australia and New Zealand.

While dried cassava may be the source of the total hydrocyanic acid in ready-to-eat cassava chips, this has not been confirmed. It is possible that other ingredients in these chips may be the source of the total hydrocyanic acid, including the edible cassava flour component. On this basis, applying a level to the final ready-to-eat product is considered the most prudent means of ensuring that public health and safety is protected and that levels are minimised to a level consistent with the international standard for edible cassava flour (another processed cassava product).

The view of FSANZ is that it is appropriate to establish a maximum level for a specific food where the risks to public health and safety justify such a measure. Given that the public health concerns are currently limited to ready-to-eat cassava chips, FSANZ considers that it is appropriate for any regulatory measure to be limited to this food. This will ensure that the measure does not apply more broadly than the existing evidence base for the regulatory 'problem'. This approach has historically been used (e.g. aflatoxins, chloropropanols) and FSANZ is of the view that this approach is appropriate in this circumstance.

FSANZ also considers that a maximum level in the Code provides the most practical means of reducing the amount of total hydrocyanic acid in ready-to-eat cassava chips and protecting public health and safety. FSANZ also considers that the definition of ready-to-eat cassava chips is sufficient to allow compliance agencies to monitor compliance. The level is proposed to apply to ready to eat cassava chips so that the level is independent of the cassava based ingredients used to make the chips or crackers. This will assist compliance agencies by applying a single level, irrespective of the ingredient profile or cassava source. Separate levels based on edible cassava flour or other cassava based ingredients is not considered practical or appropriate. FSANZ therefore considers that this specific food regulatory measure represents minimum effective regulation and is proportional to the problem to be addressed, even though monitoring compliance with the level may include end product testing.

FSANZ acknowledges the comments in a number of submissions about other foods where the presence of cyanogenic glycosides may be an issue, including a concern in one submission about the existing levels relating to foods containing flavourings. FSANZ also noted the view in some submissions that the proposed regulatory measure should extend to all foods containing cassava.

FSANZ has also noted that the issues associated with certain varieties of cassava are also under discussion at an international level within the Codex Alimentarius Commission.

Further consideration of other cyanogenic glycosides in foods will require additional data gathering and further risk analysis to determine whether it is necessary to establish maximum levels for these substances in other foods. Consideration of cyanogenic glycosides in all foods will also address whether the proposed maximum level should be extended in the future to all ready to eat foods containing cassava. As this will take some time and given the public health implications, FSANZ does not consider that a maximum level for total hydrocyanic acid in ready-to-eat cassava chips should be delayed pending future consideration of these issues.

10.2.2 Proposed Level of 10 mg/kg

10.2.2.1 Assessment

An ML is established at a level which is consistent with the protection of public health and safety, and which is considered to be reasonably achievable. In considering an appropriate ML for hydrocyanic acid in ready-to-eat cassava chips, FSANZ noted the following:

- ready-to-eat cassava chips are composed predominantly of cassava, cassava flour or tapioca flour i.e. cassava products that would be expected to be adequately processed;

- the Codex international standard for edible cassava flour includes a level of 10 mg/kg for total hydrocyanic acid in edible cassava flour and this standard has been in existence for over ten years;

- it has long been regarded that proper processing of cassava ensures that hydrocyanic acid and its precursors are removed or destroyed during cassava processing. On this point, FSANZ has noted that there is some compliance monitoring data available on the levels of hydrocyanic acid in cassava products[6]. These data indicate that 10 mg/kg should be readily achievable;

- there is published information indicating that levels of hydrocyanic acid in cassava based ingredients can be reduced with adequate processing[7,8];

- data have been provided to FSANZ indicating that levels of hydrocyanic acid below 10 mg/kg are achievable by some producers of ready-to-eat cassava chips; and

- the risk assessment which indicates that a lower level, if reasonably achievable, would further reduce the dietary exposure to hydrocyanic acid from these foods.

---

[6] Natural Toxins in Food Plants, Report No. 27, Risk Assessment Studies, March 2007, Centre for Food Safety, Food and Environmental Hygiene Department, The Government of the Hong Kong Special Administrative Region. http://www.cfs.gov.hk/english/programme/programme_rafs/programme_rafs_fc_01_17_report.html

[7] http://www.agnet.org/library/pt/2003017/

[8] Processing of cassava roots to remove cyanogens, Cardoso, A.P.; Mirione, E.; Ernesto, M.; Massaza, F.: Cliff, J.; Rezaul Haque, M.; Bradbury, J.H. Journal of Food Composition and Analysis, 18 (2005) 451-460.

Based on the information available, FSANZ was of the view that a level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips was appropriate and achievable. Compliance with this level would minimise dietary exposure to hydrocyanic acid and address the potential public health implications recently identified with these foods. If the cassava based ingredients (e.g. cassava flour) in these chips had been adequately processed to reduce levels of total hydrocyanic acid to a level consistent with the international standard, and taking into account the dilution effect of other ingredients in the chips, FSANZ considered that a level of 10 mg/kg should be achievable by manufacturers.

10.2.2.2 Submissions

Some submitters did not agree with the FSANZ Assessment and raised a number of issues in relation to this Assessment. The comments included that:

- a level of 10 mg/kg was not consistent with the risk to public health and safety and a higher level consistent with public health and safety would be more appropriate. On this point some submitters suggested alternative levels;

- ready-to-eat cassava chips are either not composed of cassava flour or that these chips are made from 'dried cassava';

- the Codex international standard for edible cassava flour was not relevant to ready-to-eat cassava chips;

- a level of 10 mg/kg was not achievable for ready-to-eat cassava chips or was questioned as being achievable given the processing of ready-to-eat cassava chips from dried cassava and the reported variability in hydrocyanic acid levels.

10.2.2.3 FSANZ Response

A number of submissions raised concerns about the achievability of 10 mg/kg and whether this level was required given the public health implications. Some other submitters suggested other higher levels, including 50 mg/kg or 100 mg/kg. Another submitter stated that a level should not apply to ready-to-eat cassava chips.

FSANZ has now refined the risk assessment aspects in relation to this Proposal and established an Acute Reference Dose (ARfD) for hydrocyanic acid. The probability of exposure above the ARfD at total hydrocyanic acid levels of 10 mg/kg of cassava chips was determined to be low with the probability increasing with increasing levels of hydrocyanic acid. Reducing the mean concentration of hydrocyanic acid from the *status quo* to 10 mg/kg would reduce the incidence of exposures above the ARfD by 93-97%. Reducing the concentration to 25 mg/kg would lead to a lesser reduction of 61-70%. On this basis, FSANZ considers that a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips is considered necessary to confidently protect public health and safety.

Through proper processing of cassava, total hydrocyanic acid content can be reduced to a safe level of 10 mg/kg in cassava-based products, such as ready-to-eat (RTE) cassava chips. Further detail about cassava processing and ready-to-eat cassava chips is in **Attachment 5**.

The most important control for ready-to-eat cassava chips to achieve a total hydrocyanic acid level below 10 mg/kg is to use cassava ingredients containing less than 10 mg/kg. Total hydrocyanic acid levels in dried raw cassava ingredients can be reduced through various processing techniques.

The manufacturing process of ready-to-eat puffed cassava chips may have the potential to reduce hydrocyanic acid in the chips. However, if the total hydrocyanic acid level in the raw cassava ingredients is greater than 40 mg/kg, then it is very unlikely that the final cassava chips will contain total hydrocyanic acid below 10 mg/kg.

Basic puffed cassava chips made from cassava flour or starch, using properly processed cassava starch or flour should enable manufacturers to achieve a level of total hydrocyanic acid below 10 mg/kg. Some puffed cassava chips may contain ingredients such as 'cassava' in addition to cassava flour. This 'cassava' component could consist of milled or pulverised dried cassava chips or pellets, and is claimed by the manufacturers to provide a special cassava flavour and texture to the puffed chips. Other types of cassava chips are not puffed and are raw (and/or dried) sliced cassava which is fried directly in hot oil until crisp; a product similar to potato chips. These types of chips have much higher potential for containing total hydrocyanic acid above 10 mg/kg because the less processed dried raw cassava may contain higher levels of total hydrocyanic acid than cassava flour. To comply with a limit of 10 mg/kg, these types of chips need to be produced from low total hydrocyanic acid cassava or the cassava must be processed sufficiently to reduce the levels of total hydrocyanic acid.

A number of submitters questioned the relevance of the Codex standard for edible cassava flour in relation to ready-to-eat cassava chips. FSANZ remains of the view that all cassava in a ready-to-eat form should have been adequately processed to an equivalent standard as edible cassava flour. FSANZ acknowledges that some submissions do not agree with this view. FSANZ does not accept that a lower standard of cassava processing should be accepted for 'dried cassava', particularly given the potential acute public health implications of the levels that have been recently reported to FSANZ. FSANZ therefore considers that it is appropriate to apply the same level for edible cassava flour and ready-to-eat cassava chips. If adequate processing of edible cassava flour can achieve a level of 10 mg/kg then it should be possible for all processed cassava in a ready-to-eat form to be adequately processed such that it would comply with this level.

In summary, the level of total hydrocyanic acid in ready-to-eat cassava chips can be less than 10 mg/kg through the use of starch or flour as the primary raw material, appropriate selection of low total hydrocyanic acid cassava or through adequate processing of cassava tubers. These measures are likely to increase costs for producers of certain types of cassava chips because the selection of low total hydrocyanic acid cassava may be difficult to guarantee and additional processing will require additional resources.

A level of 10 mg/kg is considered necessary to protect public health and safety and it is therefore essential that ready-to-eat cassava chips comply with this level. FSANZ acknowledges that this may require changes in processing or production and that there may be costs associated with implementing these changes for some producers or manufacturers. Data on the current market share of ready-to-eat cassava chips are not available to FSANZ. However, FSANZ has also noted that the information in some submissions included data indicating that some cassava based chips would already comply with the 10 mg/kg level.

10.3 Effective Compliance Monitoring

10.3.1 Assessment

Levels for hydrocyanic acid in foods have been in the Code for many years and no compliance monitoring problems with these levels have been raised with FSANZ. In addition, the international standards for cassava related products have also been in existence for some time.

At Assessment, FSANZ was of the view that monitoring compliance with any level should be possible and that it was not considered necessary to prescribe a method of analysis for hydrocyanic acid in ready-to-eat cassava chips.

FSANZ does not ordinarily prescribe methods as this can inhibit method development and require the prescribed method to be used even though better, cheaper or more sophisticated methods may be developed in the future.

Prescribing a method may also prevent the use of equivalent methods for monitoring purposes and restricts the flexibility of industry and compliance agencies. In the absence of a prescribed method, analysts would still need to develop and use methods that are 'fit for purpose' and suitably validated.

Notwithstanding this, FSANZ acknowledged that ready-to-eat cassava chips are a unique product and that there may be specific analysis aspects that may justify the prescription of a method. FSANZ therefore invited comment on the need to prescribe a method for the analysis of hydrocyanic acid in ready-to-eat cassava chips and the details of the method that should be prescribed.

FSANZ also acknowledged that the establishment of a new ML may mean that compliance agencies need to request appointed analysts to develop new methods for regulatory analysis. For this reason, FSANZ invited comment on any transitional arrangements that compliance agencies or industry consider may be necessary.

10.3.2 Submissions

Some submitters did not agree with the FSANZ Assessment and raised a number of issues in relation to this Assessment. The comments included:

- information on what were regarded by some submitters as shortcomings with a certain test kit used to measure hydrocyanic acid;

- support from some submitters for the prescription of a method to provide certainty as to the methods to use;

- information from other submitters about the methods for the analysis of total hydrocyanic acid which have been in existence for many years and that accredited laboratories for this analysis already exist;

- concerns that time was needed to validate methods, source essential analytical consumables (reference standards, enzymes) and address issues associated with variable results;

- suggestions for a transitional period to alleviate the difficulties with the issues above, including the suggestion that the proposed maximum level should be delayed pending resolution of these issues; and

- comments from other submitters that did not support a transitional period and did not include any concerns with their current ability to monitor compliance.

10.3.3 FSANZ Response

While FSANZ is not a compliance agency, compliance monitoring aspects are taken into account by FSANZ when developing food regulatory measures.

This consideration includes comments from compliance agencies and industry as to the availability of suitable compliance monitoring mechanisms, as well as the need for any transitional arrangements considered necessary to implement suitable compliance monitoring mechanisms. See **Attachment 6** for a more detailed assessment of the compliance monitoring aspects.

Based on the submissions, the main issues with monitoring compliance with the proposed food regulatory measure are the specific substances that should be measured and the current analytical capability available for measuring these substances. By stipulating the substances to measure, FSANZ considers that compliance with the proposed food regulatory measure could be effectively monitored, as methods are available to monitor these substances in food.

Following the assessment of the comments and based on other aspects considered in this Proposal, FSANZ considers that:

1. a definition of total hydrocyanic acid in the context of ready-to-eat cassava chips should be included in the Code to identify those substances which should be measured in determining compliance with the proposed ML. FSANZ considers that this will facilitate compliance monitoring and the following definition is considered appropriate:

   Hydrocyanic acid, total means any hydrocyanic acid including hydrocyanic acid evolved from linamarin, lotaustralin, acetone cyanohydrin and butanone cyanohydrin during or following either enzyme hydrolysis or acid hydrolysis, expressed as milligrams of hydrocyanic acid per kilogram of ready-to-eat cassava chips; and

2. methods should not be prescribed for measuring total hydrocyanic acid in foods, including ready-to-eat cassava chips. This is because submissions have indicated there are published methods that could be effectively used for measuring total hydrocyanic acid. By defining the substances in the definition of 'total hydrocyanic acid', FSANZ considers that the potential use of inappropriate methods is sufficiently minimised. This approach is considered more practical than prescribing a specific method of analysis. FSANZ also considers that the prescription of a particular method would be unnecessary and impractical, and is likely to prevent method innovation and restrict the use of equivalent, appropriate methods for compliance purposes.

FSANZ acknowledges that there are implementation issues associated with the ML. However, the role of FSANZ does not extend to developing or validating methods or determining specific arrangements for compliance monitoring. These aspects will need to be implemented by compliance agencies either individually or collectively.

FSANZ also acknowledges the view of some submitters that this Proposal and the maximum level be delayed while compliance agencies, industry and individual laboratories develop and validate methods. FSANZ is concerned about this specific food regulatory measure being delayed, particularly given the potential public health implications. On this basis and as methods for measuring total hydrocyanic acid are available, FSANZ considers that the food regulatory measures should progress in accordance with the FSANZ Act and that transitional arrangements should not apply. This will allow compliance agencies and any laboratories with existing capability to institute testing, while remaining laboratories develop and validate methods in accordance with their own individual circumstances.

Transitional arrangements include measures such as delaying when a food regulatory measure comes into effect (e.g. 12 months after gazettal) and/or allowing existing food products to continue to be sold after a food regulatory measure comes into effect (known as 'stock in trade'). Currently, subclause 1(2) of Standard 1.1.1 applies a 'default' twelve month period to stock in trade for all food regulatory measures unless otherwise stated.

At Assessment and on the basis of the public health concerns, FSANZ did not propose any transitional arrangements for the proposed maximum level i.e. the level was proposed to come into effect upon gazettal. It was also proposed that subclause 1(2) of Standard 1.1.1 not apply. Notwithstanding this, FSANZ invited comment on whether any transitional arrangements would assist industry in complying with the proposed level or would assist compliance agencies in developing or implementing compliance arrangements (e.g. developing suitable confirmatory methods for compliance purposes). The need for transitional arrangements to implement compliance monitoring must be considered against the objectives of food regulatory measures, including the protection of public health and safety.

The costs for industry may be mitigated to a certain degree with a transitional period. Manufacturers of ready-to-eat cassava chips specifically requested transitional periods in their submissions as this would allow existing shelf and distribution stock to be sold and not require recalls or disposal of manufactured product. It would also allow industry time to liaise with overseas suppliers to develop consistently low linamarin cultivars and optimised production and processing regimes.

Ordinarily, transitional arrangements for food regulatory measures are possible. However, the situation with total hydrocyanic acid in ready-to-eat cassava chips is unusual as it relates to acute dietary exposure concerns for substances that have been reported in a food. A transitional arrangement or delay in the maximum level coming into effect would therefore have costs for consumers in relation to prolonging the acute dietary exposure to total hydrocyanic acid from ready-to-eat cassava chips. Given the potential public health implications of this ongoing exposure, FSANZ considers that the greatest net benefit for the community would result from the maximum level coming into effect on gazettal. On this basis, FSANZ considers that transitional arrangements should not apply. For this reason FSANZ considers that the maximum level should come into effect upon gazettal and that subclause 1(2) of Standard 1.1.1 should not apply.

In conclusion, FSANZ considers that:

- a definition of total hydrocyanic acid is required to facilitate effective compliance monitoring;
- methods for measuring total hydrocyanic acid should not be prescribed; and
- transitional arrangements should not apply, given the potential public health implications and that methods have been published for measuring total hydrocyanic acid.

10.4 World Trade Organization (WTO)

As members of the World Trade Organization (WTO), Australia and New Zealand are obligated to notify WTO member nations where proposed mandatory regulatory measures are inconsistent with any existing or imminent international standards and the proposed measure may have a significant effect on trade.

There are not any specific international standards for ready-to-eat cassava chips, although there are relevant international standards for some other cassava products i.e. edible cassava flour. Amending the Code to include a maximum level for total hydrocyanic acid in ready-to-eat cassava chips was originally considered to be unlikely to have a significant effect on international trade as:

- the level would not apply to raw cassava for further processing;
- producers of processed ready-to-eat cassava chips should already be able to comply with the level if they are adequately processing cassava for human consumption; and
- the proposed level is the same as the level in the relevant international standard for edible cassava flour (another processed cassava product).

Given the trade in raw and processed cassava based products and that no specific international standard exists for ready-to-eat cassava chips, notification was made to the agencies responsible in accordance with Australia's and New Zealand's obligations under the WTO Sanitary and Phytosanitary Measures (SPS) Agreements. This notification was made to enable other WTO member countries to comment on proposed changes to standards where they may have a significant impact on them. No comments were received from member countries of the WTO. However, during the submission period, the Director for Food Product Standardization in Indonesia commented in his submission that:

- the maximum level for hydrocyanic acid should not include cassava chip (keripik singkong) which is produced from fresh cassava directly; and
- processing of cassava chip (keripik singkong) is less intensive than cassava flour and proposed that the maximum level for hydrocyanic acid in cassava chip (keripik singkong) be more than 10 mg/kg.

FSANZ has taken this comment into consideration and considers that a level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips is necessary to protect public health and safety.

CONCLUSION

11. Conclusion and Decision

In assessing Proposals, FSANZ considers the impact of various regulatory (and non-regulatory) options on all sectors of the community, including consumers, food industries and governments in Australia. The options considered were:

- Option 1 – to reject the draft variation and not vary the Code to incorporate a maximum level for total hydrocyanic acid in ready-to-eat cassava chips;
- Option 2 – to vary the Code to incorporate a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips;
- Option 3 – to vary the Code to incorporate a maximum level of 10 mg/kg for total hydrocyanic acid in all ready-to-eat foods containing cassava (except confectionery); and
- Option 4 – to vary the Code to incorporate a maximum level of 25 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips

FSANZ considered the option of rejecting the draft variation and retaining the *status quo*, including education and encouraging voluntary reductions in total hydrocyanic acid in ready-to-eat cassava chips (Option 1). In this case, FSANZ does not consider this option (including education) as appropriate or viable. This is because of the potential acute public health implications of the elevated levels reported in ready-to-eat cassava chips, and the lack of an

overarching industry association for ready-to-eat cassava chip manufacturers that would promote adequate voluntary initiatives to address these public health implications.

In addition, while consumer advice may well assist consumers in relation to product choice, FSANZ considers that it is not appropriate to rely on this approach, other than as a short-term interim measure. This is because consumer advice complements food regulatory measures and on its own is an inadequate measure for protecting public health and safety for the general community, particularly in the longer term.

Option 1 (*status quo*) is therefore considered unacceptable because of the potential and unmanaged risks to public health and safety associated with the reported levels of total hydrocyanic acid in ready-to-eat cassava chips. FSANZ considers that additional regulatory measures (i.e. Options 2, 3 or 4) are necessary to minimise total hydrocyanic acid in ready-to-eat cassava chips. This is because the current food regulatory measures in the Code are not considered sufficient to minimise dietary exposure to total hydrocyanic acid in ready-to-eat cassava chips to protect public health and safety.

Based on comments in submissions from two manufacturers of ready-to-eat cassava chips, the introduction of a maximum level for total hydrocyanic acid in ready-to-eat cassava chips would have a significant financial impact on these manufacturers. These impacts may include:

- removal of stock from the marketplace;
- permanent and irreparable loss of business with major retail clients;
- reduction in goodwill among consumers;
- significant costs in developing cultivars with low levels of total hydrocyanic acid and optimised watering regimes;
- significant modification of the product in relation to structure, texture and appearance with possible consumer rejection; and
- loss of trade to a point where these businesses would be unable to continue.

FSANZ has not quantitatively estimated the costs of these impacts because of the absence of specific data to calculate these costs. The costs for such businesses must be balanced against the need to protect public health and safety. Given the refined assessment of the risk to public health and safety, FSANZ considers that a maximum level for total hydrocyanic acid in ready-to-eat cassava chips is necessary to protect public health and safety. FSANZ considers that the public health benefits of this measure outweigh the costs for business of complying with a maximum level.

On the basis of the refined assessment of the risk to public health and safety, Option 4 (a maximum level of 25 mg/kg) is now no longer considered an acceptable option for minimising the dietary exposure to hydrocyanic acid from ready-to-eat cassava chips. This is because a level of 25 mg/kg is not considered to be sufficient to confidently protect public health and safety. Options 2 and 3 are considered viable options in that they apply measures that would minimise the dietary exposure to hydrocyanic acid from ready-to-eat cassava chips to a level that is sufficient to confidently protect public health and safety.

Option 3 of applying a maximum level of 10 mg/kg to all ready-to-eat cassava containing foods is unlikely to have a major impact if products are made from adequately processed cassava. However, extending the maximum level to all cassava containing foods is not consistent with minimal effective regulation on the data currently available, as only cassava chips have been identified as containing higher than expected levels of hydrocyanic acid. For this reason, Option 3 is not preferred and no risk assessment has specifically been undertaken for this option.

Option 2 (incorporating a maximum level of 10 mg/kg for hydrocyanic acid in ready-to-eat cassava chips in the Code) is the preferred option because:

- it protects public health and safety and minimises acute dietary exposure to hydrocyanic acid from ready-to-eat cassava chips;
- it is specific to the products for which the issues have been raised and does not extend the impacts to non-implicated businesses and products. For example, it does not impact on raw cassava which will be further processed;
- based on the data available, it should be achievable for industry although there are likely to be costs for at least two manufacturers of ready-to-eat cassava chips whose products do not currently conform to the proposed standard. For a manufacturer that cannot comply, the costs may include closure of their business; and
- it is not considered to unnecessarily impede trade because it aligns with a relevant international standard for edible cassava flour, which is an ingredient, but not necessarily the predominant ingredient in the ready-to-eat cassava chips.

The conclusion is that the public health and safety benefits of Option 2 would outweigh the costs for complying with this level. FSANZ recognises that while this option is considered to provide public health and safety benefits to the community, the costs for some businesses of having to comply with it may be significant.

The proposed food regulatory measure is the inclusion in the Code of a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips. The purpose of this measure is to minimise total hydrocyanic acid to a level that is considered to confidently protect public health and safety. Compliance with the proposed food regulatory measure would reduce the acute dietary exposure to hydrocyanic acid from ready-to-eat cassava chips and would address the potential public health implications that have recently been identified with these foods.

While not currently within the scope of this proposal, additional regulatory and non-regulatory measures may be required in the future as investigations continue into hydrocyanic acid in other foods.

Decision

Approve the variations to Standard 1.4.1 – Contaminants and Natural Toxicants of the Code to include a maximum level of 10 mg/kg for 'hydrocyanic acid, total' in 'ready-to-eat cassava chips' and to facilitate compliance monitoring, a definition of 'hydrocyanic acid, total' for 'ready-to-eat cassava chips'.

11.1 Reasons for Decision

- with proper preparation or processing, cassava and cassava-based foods are safe for human consumption, even though whole, unprocessed cassava contains naturally occurring cyanogenic substances[9];

- the composition of some 'ready-to-eat cassava chips' is such that existing food regulatory measures in the Code are not considered sufficient to reduce levels of total hydrocyanic acid in ready-to-eat cassava chips and to protect public health and safety;

[9] 'cyanogenic substances' are those substances that produce hydrocyanic acid (hydrogen cyanide) in specific circumstances.

- following a revised risk assessment, a food regulatory measure in the Code for total hydrocyanic acid in ready-to-eat cassava chips is considered necessary to minimise acute dietary exposure to hydrocyanic acid and thereby protect public health and safety;

- a maximum level in Standard 1.4.1 is considered to be an appropriate risk management measure while additional information is gathered about other cassava based foods and other foods containing cyanogenic substances;

- a maximum level specifically for 'ready-to-eat cassava chips' only is considered necessary at this time. FSANZ considered the views in some submissions that this level should extend to all ready-to-eat foods containing cassava. At this time, FSANZ considers the level should only apply to ready-to-eat cassava chips because there are existing food regulatory measures for managing hydrocyanic acid in some other cassava based products and some other foods;

- a maximum level of 10 mg/kg is considered necessary to confidently protect public health and safety. Based on the data available to FSANZ and the revised risk assessment conducted by FSANZ, a maximum level higher than 10 mg/kg is not considered adequate to protect public health and safety.

- the maximum level of 10 mg/kg is also considered to be practical and reasonably achievable with proper processing of cassava or with specific cassava selection. These measures are likely to increase costs for some producers of ready-to-eat cassava chips because the selection of low total hydrocyanic acid cassava may be difficult to guarantee, and additional processing of cassava with higher levels of total hydrocyanic acid will therefore require additional resources. Results provided to FSANZ demonstrate that some ready-to-eat cassava chip producers would already comply with a limit of 10 mg/kg for total hydrocyanic acid. Additional results provided to FSANZ indicate that a number of other cassava containing foods also contain less than 10 mg/kg total hydrocyanic acid. On this basis, FSANZ considers that compliance with the maximum level is achievable and generates the greatest net benefit for the community by protecting public health and safety;

- the maximum level of 10 mg/kg is also consistent with the level in the international (Codex) standard for edible cassava flour (another processed cassava product for direct human consumption). Typical production of cassava flour or starch, especially in the large-scale commercial factories, has ensured that processing steps and parameters are effective in eliminating total hydrocyanic acid from cassava. Based on comments in submissions, edible cassava flour is an ingredient in ready-to-eat cassava chips, although not necessarily the predominant ingredient in all types of chips. Some types of ready-to-eat cassava chips have the potential to contain total hydrocyanic acid above 10 mg/kg. This is because they contain dried raw cassava, which may contain higher levels of total hydrocyanic acid than cassava flour. To comply with a limit of 10 mg/kg, these types of chips need to be produced from low total hydrocyanic acid cassava or the dried cassava used for them must be further processed to adequately reduce the levels of total hydrocyanic acid. Given the potential acute public health implications associated with total hydrocyanic acid in ready-to-eat cassava chips, FSANZ considers that all cassava based ingredients in ready-to-eat cassava chips should be processed to a level equivalent to that of edible cassava flour.

Furthermore, it is considered necessary that the maximum level come into effect upon gazettal and that the usual 'stock in trade' transitional arrangements not apply.

FSANZ does not consider that transitional arrangements are appropriate because of the potential acute public health implications associated with the levels reported in some ready-to-eat cassava chips;

FSANZ does not consider that it is practical or necessary to prescribe a method of analysis for hydrocyanic acid in ready-to-eat cassava chips as this will restrict the flexibility of industry and compliance agencies to develop more contemporary methods for monitoring hydrocyanic acid in ready-to-eat cassava chips. To facilitate compliance monitoring, FSANZ has developed a definition of 'hydrocyanic acid, total' for the purposes of ready-to-eat cassava chips (see **Attachment 1A**). This reflects the views in some submissions and is considered a more practical approach to ensuring the appropriate range of substances are measured for compliance than prescribing a specific method.

11.2 Transitional Arrangements

Given the potential public health implications, FSANZ considers that transitional arrangements should not apply. For this reason FSANZ considers that the maximum level should come into effect upon gazettal and that subclause 1(2) of Standard 1.1.1 should not apply.

12. Implementation and Review

A maximum level of 10 mg/kg for hydrocyanic acid in ready-to-eat cassava chips in Standard 1.4.1 is proposed as an appropriate risk management measure while additional information is gathered about other cassava based foods and other foods containing cyanogenic substances.

ATTACHMENTS

1A. Draft variations to the *Australia New Zealand Food Standards Code* (at Approval)
1B. Draft variations to the *Australia New Zealand Food Standards Code* (Changes Marked)
1C. Draft variations to the *Australia New Zealand Food Standards Code* (at Assessment)
2. Summary of Submissions
3. Hazard Assessment
4. Dietary Exposure Assessment Report
5. Food Technology Report
6. Compliance Monitoring

Attachment 1A

Draft variations to the *Australia New Zealand Food Standards Code* (at Approval)

Section 87(8) of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

To commence: on gazettal

[1] ***Standard 1.4.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in subclause 5(1) –*

Hydrocyanic acid, total means all hydrocyanic acid including 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 of ready-to-eat cassava chips.

Ready-to-eat cassava chips means the product containing sweet cassava that is represented as ready for immediate consumption with no further preparation required including crisps, crackers or 'vege' crackers.

[1.2] *inserting after subclause 5(3) –*

(4) Subclause 1(2) of Standard 1.1.1 does not apply to ready-to-eat cassava chips for the purposes of the Table to clause 5.

[1.3] *inserting in the* Table to clause 5 –

Hydrocyanic acid, total Ready-to-eat cassava chips	10 mg/kg

Attachment 1B

Draft variations to the *Australia New Zealand Food Standards Code* (Changes Marked)

Section 87(8) of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

To commence: on gazettal

[1] ***Standard 1.4.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in subclause 5(1) –*

Hydrocyanic acid, total means all hydrocyanic acid including 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 of ready-to-eat cassava chips.

Ready-to-eat cassava chips means the product containing sweet cassava that is represented as ready for immediate consumption with no further preparation required including crisps, crackers or 'vege' crackers.

[1.2] *inserting after subclause 5(3) –*

(4) Subclause 1(2) of Standard 1.1.1 does not apply to ready to eat cassava chips for the purposes of the Table to clause 5.

[1.3] *inserting in the* Table to clause 5

Hydrocyanic acid, total Ready to eat cassava chips	10 mg/kg

Attachment 1C

Draft variations to the *Australia New Zealand Food Standards Code* (at Assessment)

Section 87(8) of the FSANZ Act provides that standards or variations to standards are legislative instruments, but are not subject to disallowance or sunsetting

To commence: on gazettal

[1] ***Standard 1.4.1*** *of the Australia New Zealand Food Standards Code is varied by –*

[1.1] *inserting in subclause 5(1) –*

Ready to eat cassava chips means the product containing sweet cassava that is represented as ready for immediate consumption with no further preparation required including crisps, crackers or 'vege' crackers.

[1.2] *inserting after subclause 5(3) –*

(4) Subclause 1(2) of Standard 1.1.1 does not apply to ready to eat cassava chips for the purposes of the Table to clause 5.

[1.3] *inserting in the* Table to clause 5

Hydrocyanic acid, total Ready to eat cassava chips	10 mg/kg

Attachment 2

Summary of Submissions

Submitter	Summary of Comments	FSANZ Response to Issues
Government		
Department of Health (WA) (Sophe Williamson)	Supports a maximum residue for HCN in ready-to-eat (RTE) dried cassava products (which may or may not be limited to cassava chips).	Noted.
	Further information on correct processing techniques to reduce the level of HCN in dried cassava should be considered.	Prepared additional information on the processing of cassava.
	Data provided on preliminary sampling of cassava products indicating some cassava chip/cracker products contain approximately 80 mg/kg, data appear to clear tapioca starch and flour as sources of high HCN, only products that included dried cassava as an ingredient had levels of HCN above 10mg/kg, with the exception of one frozen cassava sample that had a level of 15 mg/kg.	Notes the further data provided and that only products containing 'dried cassava' were found to have levels above 15 mg/kg.
	If other RTE cassava products are processed from dried cassava pellets then maximum HCN level be extended to cover all RTE cassava products made from dried cassava. Further clarification on how dried cassava (pellets) is processed (overseas) and what RTE products other than cassava chips/crisps it is added to as an ingredient would be useful.	No data currently available to FSANZ that indicates that these other cassava-based foods contain higher than expected levels of hydrocyanic acid. Currently, the evidence base to justify the food regulatory measure is limited to RTE cassava chips.
	Expects enforcement costs to be minimal. Costs to industry will include supply interruption.	Notes this information - agrees that there will be costs to industry.
	Suggests risk assessment consider the milder symptoms of HCN exposure.	Risk assessment revised.
	A transitional period may be inappropriate if this is a significant public health and safety matter. Not opposed to considering transitional provisions to assist industry compliance but consider it appropriate for industry to have a HCN management plan in place to monitor/manage the HCN levels.	Potential acute public health implications associated with the levels reported and therefore transitional arrangements are not appropriate.

Submitter	Summary of Comments	FSANZ Response to Issues
	Some subjection in relation to variations in HCN results but it is not clear whether this is due to the method, performance, or batch variation. Prescribing a method of HCN analysis with equivalence recognition would alleviate much of subjection in relation to results of analyses but does not have a preferred method of HCN analysis. Any prescribed method should be based on total HCN and consistent with international methodologies.	A definition of 'total hydrocyanic acid' will ensure that appropriate substances are monitored. Analytical laboratories would develop and validate methods suitable for monitoring compliance of these substances.
	Can it be confirmed that the dried cassava pellets being imported into Australia are not of the prohibited (bitter) cassava variety?	Unaware of any means of differentiating between sweet or bitter cassava other than on the basis of total hydrocyanic acid levels.
	Could the risk categorisation of dried cassava be elevated to 'high risk' requiring post border testing and clearance by the Australian Quarantine and Inspection Service? If the processing issue is limited to imported dried cassava, could a certification scheme similar to the existing Bovine Spongiform Encephalopathy arrangements be considered.	Understands that these issues could be undertaken as part of any compliance strategy but may need to be practical and targeted to RTE cassava chips to ensure the supply of cassava for further processing was not unnecessarily interrupted.
	Industry education and consumer awareness related to the consumption of RTE cassava products should complement any regulatory measures implemented.	Considers that industry should be aware of this hazard and the means to ensure it is adequately controlled. In relation to consumer education, RTE cassava chips should be safe for human consumption and that on this basis, no education would be necessary.
DHS Victoria (Erica Clifford)	Does not support the proposal proceeding on the grounds set out in the Food Regulation Agreement that it is difficult to enforce or comply with in both practical and resource terms; and it does not protect public health and safety. Proposal P1002 should either be withdrawn or held in abeyance until the following matters are addressed: 1. establishment of an effect level on population groups; 2. determination as to which foods are at risk of reaching that effect level; 3. determination of an appropriate safe level for hydrocyanic acid across all foods; and 4. validation of testing methodology.	Noted.

Submitter	Summary of Comments	FSANZ Response to Issues
	Raised issues with the risk assessment including the hypothetical consumption of 200 g of cassava chips by a 20 kg child over a two hour period and that this is not a reasonable and possible dietary intake. Based on the information provided in the assessment does not appear that a maximum level of 10 mg/kg is justified. DHS seeks a more detailed explanation for the setting of this limit.	Based on the conclusions of the revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety. More detail on the basis for a limit of 10 mg/kg provided as part of the revised risk assessment.
	Notes that the risk assessment on which this proposal is based was prepared rapidly, where a rapid response was required to address a potential public safety issue. A much more detailed risk assessment and analysis is required to support a proposed change to the law. Notes that the current risk assessment does not include a toxicological report or a food technology report.	A more detailed, revised risk assessment provided. This revised risk assessment supports an ML of 10 mg/kg.
	There has been no cost benefit analysis and therefore the proposal does not comply with the COAG guidelines and principles for standard setting and regulatory action.	Cost/benefit analysis in accordance with COAG requirements included (was in Assessment Report). The public health benefits of the ML are considered to outweigh the costs of complying with this limit.
	No documented instances of symptoms of cyanide poisoning occurring as a result of consumption of cassava chips at any quantity. There are mechanisms in the body which can inactivate cyanide and when cyanide poisoning occurs, it only happens when the dose exceeds the capacity of these body systems to detoxify the cyanide.	Based on a revised risk assessment, an ML of 10 mg/kg is necessary to protect public health and safety.
	In order to establish a safe limit it is first necessary to determine when symptoms would realistically occur. It appears that further work is required to establish a theoretical threshold. Only then can a limit be set.	An acute reference dose has been established and this has been used in determining an appropriate maximum limit.
	A standard should be applied to all RTE products that contain cassava, not just cassava chips. Notes that almond meal, currently used in many food products, has a higher level of hydrocyanic acid than cassava.	Unaware of any other RTE cassava containing foods with unexpectedly or unacceptably high levels of total hydrocyanic acid. Extending a food regulatory measure to all cassava based foods would be beyond the evidence base for the 'problem'. Almonds for human consumption are derived from 'sweet' almond varieties.

Submitter	Summary of Comments	FSANZ Response to Issues
	There are other foods in the marketplace that contain much higher levels of naturally occurring hydrocyanic acid than RTE cassava chips, such as apricot kernels. However, it is not proposed to create a standard setting a maximum level of hydrocyanic acid for these products.	The scope of this Proposal is RTE cassava chips and considering other foods would have to be the subject of another Proposal and another risk assessment.
	Development of this standard is premature. It is undesirable from both a 'good legislation' and efficiency perspective to develop food standards on a product by product basis.	Reported findings for total hydrocyanic acid in RTE cassava chips are of potential public health significance and therefore a specific food regulatory measure is needed.
	Maximum level of total hydrocyanic acid in RTE cassava chips at 10 mg/kg is not consistent with Codex. Codex sets the level in cassava flour rather than RTE cassava chips. Extending the limit to RTE chips is not valid. Any proposal to set a maximum level should be based upon the risk associated with the final product.	The limit of 10 mg/kg is necessary to protect public health and safety. Applying a limit to an RTE cassava based food in accordance with the international standard for another RTE cassava based food, as all cassava should be processed to the same degree as cassava flour.
	Not possible to assess, on the information provided, whether industry could comply with the level proposed as no information is provided on the current methods of processing cassava, the methods of manufacturing cassava chips or the hydrocyanic acid levels each process is likely to produce.	The Assessment Report included a reference to a comprehensive Food and Agriculture Organization document on cassava processing. A link was provided to the previous assessment of cassava to minimise duplication. Additional information has been provided in this report to address this issue.
	Understands that the level of cassava can vary from 10% to 60% in products. This should be considered as part of a cost/benefit analysis in relation to the proposal.	The amount of total hydrocyanic acid in RTE cassava chips is the specific issue and not the proportion of individual cassava based ingredients in a food. All cassava should be adequately processed prior to use in RTE chips to protect public health and safety.
	Concerned that there appears to be no validated method of laboratory analysis for hydrocyanic acid in food in Australia that would be likely to withstand court scrutiny. In order to enforce this proposed standard, regulators require a validated analytical method that is reliable and that will stand up in court.	Methods are readily available for monitoring hydrocyanic acid in foods. Laboratories or compliance agencies validate their own specific methods of analysis that are suitable for their specific compliance purposes.

Submitter	Summary of Comments	FSANZ Response to Issues
Sri Irawati Susalit Director for Food Product Standardization, NADFC, Indonesia	In principle, agrees that maximum level for hydrocyanic acid is 10 mg/kg but should not include cassava chip (keripik singkong) which is produced from fresh cassava directly.	Noted.
	Processing of cassava chip (keripik singkong) is not more intensive than cassava flour. Proposes the maximum level for hydrocyanic acid in cassava chip (keripik singkong) is more than 10 mg/kg.	All cassava in RTE chips should be adequately processed to protect public health and safety.
	The proposal does not mention an analysis method. Indonesia uses colorimetric method after conducted condenses distillation. (Modification of AOAC 18th ed. 2005 Vol. 2 No. 28.1.47 chapter 28 page 15 and standard methods the examination of water and waste water, American public health association, water environment federation, 16th ed., Washington DC, APHA, 1995).	Understands that this method may not measure the hydrocyanic acid from cyanogenic glycosides and may therefore be unsuitable for monitoring total hydrocyanic acid in food. Notes that there is a specific longstanding AOAC method for measuring cyanogenic glycosides in animal feed.
New Zealand Food Safety Authority (NZFSA) (Carole Inkster)	Agrees that a maximum level for hydrocyanic acid in RTE cassava chips should be added to the Code and supports that the maximum level should be set at 10 mg/kg hydrocyanic acid, which is as low as reasonably achievable.	Noted.
	Proposes that the scope be expanded from 'in RTE cassava chips' to 'in ready-to eat foods that contain cassava, including cassava chips' as the standard only captures products currently in the marketplace and product innovation could lead to the marketing of other ready to eat products containing cassava. Expansion of the scope of the proposed regulatory measure would negate the need to revisit the standard if other RTE products containing cassava were marketed.	Acknowledged but currently the evidence base to justify the food regulatory measure is limited to RTE cassava chips and no issues with other cassava based foods have been brought to the attention of FSANZ. As additional information about total hydrocyanic acid becomes available, FSANZ will continue to monitor whether the proposed ML should extend to all cassava based foods.
	Proposes a nomenclature review to ensure that the standard covers free hydrocyanic acid and substances which can release hydrocyanic acid. Considers the term 'hydrocyanic acid, total' as used in the Code is misleading because the substance that is present in cassava is not what is usually termed hydrocyanic acid, but the cyanogenic glycoside linamarin.	Acknowledged and in the context of RTE cassava chips, considers that a definition of 'hydrocyanic acid, total' will assist in clarifying this. Notes that the existing limits in the Code reflect the relevant international standards that formed the basis for the existing limits in the Code.

Submitter	Summary of Comments	FSANZ Response to Issues
	Notes that Codex Committee on Contaminants in Foods (CCCF) meets in the Hague in the week commencing 31 March 2008 where the Proposed Draft Standard for Bitter Cassava will be discussed. Any relevant discussion on cassava/ hydrocyanic acid levels at CCCF should be taken into account when FSANZ prepares its Approval Report.	Acknowledges that there are international developments occurring in relation to cassava.
Whole of Queensland Government response (Gary Bielby)	Supports the development of a standard for a Maximum Level (ML) for hydrocyanic acid in RTE cassava chips but does not want this Proposal progressed until a number of issues are fully addressed.	Noted.
	Complete understanding required by all stakeholders about how the RTE cassava chips are manufactured and what is reasonably obtainable by industry.	Additional information provided about cassava chips. Considers that cassava should be adequately processed before being used in RTE foods. Acknowledges that this may require some members of industry to investigate their practices to ensure that their cassava based ingredients are adequately processed.
	Expressed concern that this Proposal is attempting to develop a standard for a particular product, in this case RTE cassava chips. Considers resources across government, industry and other stakeholders could be better utilised if the issue of hydrocyanic acid in a broad range of foods was addressed at one time rather than by a 'case-by-case' type basis.	Notes this concern but considers that the reported findings justify a specific response in relation to RTE cassava chips while additional information is gathered. Gathering information for other foods may take some time and given the potential public health implications, the regulatory measure for hydrocyanic acid in RTE cassava chips should not be delayed pending the availability of that information.
	Concern that methodology being used by the two jurisdictions appears to be producing different results on the same batches of chips being tested. It has not been established whether this is due to sample variability or application of the Bradbury test kit. This is a major issue to be resolved in the setting of any standard and cannot be ignored by FSANZ.	Variability may be as a result of sample variability. Issues around variability of results would need to be addressed as part of method validation studies. Acknowledges that jurisdictions may need to consider the implementation aspects of the ML.

Submitter	Summary of Comments	FSANZ Response to Issues
	An analytical methodology, which is robust and reliable, must be agreed upon. Without this, the standard will clearly not meet the criteria for review in that it is difficult to enforce or comply with in both practical and resource terms. This matter requires resolution before the setting of a standard.	Given the potential public health implications, the ML should not be delayed pending method development and validation procedures by individual laboratories. Acknowledges that laboratories will need to develop and validate methods.
	The end result from the FSANZ Risk Assessment is a margin of safety 8 times higher than the original FSANZ risk assessment which concluded an unlikely risk of adverse health effects in a 20 kg child eating an exceptionally large amount of chips in a short time frame.	Based on a revised risk assessment, an ML of 10 mg/kg is necessary to protect public health and safety. An acute reference dose has been established and this has been used in determining an appropriate maximum limit. More detail on the basis for this limit has been provided as part of this Approval Report. This assessment supports an ML of 10 mg/kg to protect public health and safety.
	Unaware of any cases of suspected or demonstrated adverse health effects from consumption of RTE cassava chips. No reports even after the issue of cyanide in RTE cassava chips was raised in the public eye by the recall that occurred and the press releases and media articles that emanated from that.	Notes that Queensland Health is unaware of any reported adverse health effects from the consumption of RTE cassava chips.
	Risk assessment is in the context of margins of safety, rather than responses to documented adverse health effects. Considers there needs to be greater linkage between the issues of analytical methods, what is reasonably achievable by industry and health risk assessment in determining a final ML.	The limit is based on protecting public health and safety and is consistent with a Codex limit for another processed cassava product. Considers that the limit is based on both a risk assessment basis and an achievability basis.
	Proposal based on the Codex ML for edible cassava flour but advice from the Queensland manufacturer of RTE cassava chips indicates that their chips contain cassava flour as a minor ingredient whilst the major ingredient is the cassava root which has a different chemical composition to cassava flour.	Acknowledges that some manufacturers are producing RTE cassava chips from ‘dried cassava’. To be suitable for use in a RTE product and to protect public health and safety, cassava should have been adequately processed to an equivalent level as edible cassava flour.

Submitter	Summary of Comments	FSANZ Response to Issues
	Proposal also appears to be predicated on the argument that the perceived ease of processing of raw cassava, rather than dietary exposure risks, should be the driver for extending the 10 mg/kg Codex ML for cyano-glycosides in cassava flour to RTE cassava chips.	Considers that the limit is based on protecting public health and safety and is consistent with a limit in an international standard for another processed cassava product.
	Given that cassava flour is a staple food for much of Africa's rural population as well as for many Asian-Pacific communities and thereby consumed on a daily basis, application of the same standard to RTE cassava chips may not be sustainable from a risk argument.	Considers that based on the best available scientific evidence, an ML of 10 mg/kg is necessary to protect public health and safety for consumers in Australia and New Zealand.
	Without a detailed understanding of the agriculture and processing of raw cassava, it is difficult to comment on the additional cost and quality control for suppliers although it is recognised that only sweet cassava should be processed into cassava pellets for trade.	Substantial information on this was provided in the references in the Assessment Report, as well as part of the Previous Proposal that considered this issue. Additional information has been provided.
	Recent testing undertaken by the Queensland Health Forensic & Scientific Services and results from the NSW Food Authority have not reported any cassava chip samples as low as 10 mg/kg.	Results indicating that products can comply with 10 mg/kg are available. Acknowledges that some chips do not currently comply. The ML is considered necessary to protect public health and safety.
	Unclear whether the higher results are due to a lack of adequate processing on cassava imports or due to the lack of local availability of varieties of cassava with minimal levels of linamarin or to the variations with analytical methodologies that are not validated.	Higher results may be as a result of inadequate processing of cassava before its use in RTE cassava chips.
	The test kit has not undergone a rigorous collaborative study to establish inter-laboratory reproducibility and the lack of ready availability of linamarin standard and suitable reference materials provides laboratories with minimal quality assurance on monitoring method accuracy.	Considers that there are potential acute public health implications associated with the levels reported and therefore considers that transitional arrangements to implement the ML are not appropriate.
	Only method currently employed is the Bradbury test kit for the detection of cyanides in cassava which was primarily designed for an 'in-field' determination of gross levels of cyano-glycosides in bitter varieties of cassava. Should it be proposed to apply the Bradbury test kit for monitoring and possible regulatory purposes, there are several short-comings with the kit.	Understands that there are laboratories with capability for measuring cyanogenic glycosides in plant tissues. The prescription of any specific test kits has not been proposed and methods of analysis would need to be developed or validated by appropriately accredited and appointed analysts.

Submitter	Summary of Comments	FSANZ Response to Issues
	Without additional method validation, and in the absence of calibration standards, interlaboratory study data or Certified Reference Materials, it is unlikely that the method would comply with NATA/ISO 17025 accreditation standards and would unlikely be acceptable for regulatory purposes.	The role of FSANZ does not extend to conducting proficiency studies, providing certified reference materials or validating published methods of analysis.
	Key point made by the Vege Chip Company to Queensland Health includes the FSANZ preferred approach to vary Standard 1.4.1 would have a devastating impact on the business.	Considered this impact in the context of the public health implications of the levels being reported in RTE cassava chips.
	Key point made by the Vege Chip Company to Queensland Health includes cassava chips undergo a very different production process than cassava flour.	Noted this and considers that all cassava based ingredients in RTE chips should be adequately processed to protect public health and safety.
	Key point made by the Vege Chip Company to Queensland Health includes a 10 mg/kg ML for total hydrocyanic acid is not reasonably achievable or necessary, given the history of this product over some 18 years of production in Australia.	Based on a revised risk assessment, an ML of 10 mg/kg is necessary to protect public health and safety.
NSW Food Authority and NSW Health (David Miles)	Supports Option 3 to vary Standard 1.4.1 to include a maximum level (ML) of 10 mg/kg for total hydrocyanic acid in RTE foods containing cassava. At a minimum, NSW supports Option 2.	Noted.
	Considers there is merit in extending the proposed ML of 10 mg/kg to all RTE foods containing cassava, to reduce the potential public health risk associated with the exposure of consumers to sources of hydrocyanic acid.	Acknowledged but the evidence base to justify the food regulatory measure is limited to RTE cassava chips and no issues with other cassava based foods have been brought to the attention of FSANZ.
	Of the opinion that the proposed level of 10 mg/kg is achievable by industry without significant additional cost, and industry should be aiming to keep levels of hydrocyanic acid as low as reasonably achievable in all RTE products containing cassava.	Considers that a ML of 10 mg/kg is necessary to protect public health and safety.

Submitter	Summary of Comments	FSANZ Response to Issues
	Does not support a transitional period for the proposed level, as it has been identified as a potential public health risk. Willing to consider the adoption of an interim level of 25 mg/kg as a transitional period to aid manufacturers with compliance.	Given the potential public health implications, a transitional arrangement is not appropriate.
	Does not support prescribing a method for the analysis of hydrocyanic acid in RTE cassava chips in the Code. Notes that an Australian Standard for such a determination does not exist and has written to Standards Australia proposing that a standard development process is considered. Proposes that a definition for total hydrocyanic acid is included in the Code to ensure the future use of appropriate methodology.	Acknowledged and in the context of RTE cassava chips, considers that a definition of 'hydrocyanic acid, total' will assist in clarifying this. Notes that the existing limits in the Code reflect the relevant international standards that formed the basis for the existing limits in the Code.
	Does not believe that any other regulatory or non-regulatory measure could achieve the equivalent level of public health and safety as the establishment of an ML in the Code.	Agreed.
South Australian Department of Health (Joanne Cammans)	The preferred approach as set out by FSANZ in the Assessment Report - to set a limit for hydrocyanic acid at the lowest achievable level - is generally supported.	Noted.
	Concerned as to whether industry is able to consistently achieve a level of 10 mg/kg industry in their cassava products, especially given the FSANZ Risk Assessment Report conclusion in relation to 25 mg/kg.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety.
	Some doubt about the reliability and validity of analytical methods and while not the FSANZ role to set analytical methods, it is an enforcement concern that there is no validated method for testing hydrocyanic acid.	Acknowledges this implementation issue and recognises that methods of analysis will need to be developed or validated by appropriately accredited and appointed analysts.
	Recommended that a level of 10 mg/kg is only set if industry can consistently achieve this level. Alternatively, it is suggested that a maximum level of 25 mg/kg is set for 12 months and dropped to 10 mg/kg after this time depending upon results of samples taken over this period and further investigation into dietary exposure and public health and safety issues.	Considered this option and a revised risk assessment now indicates that a level of 10 mg/kg is considered necessary to protect public health and safety.

Submitter	Summary of Comments	FSANZ Response to Issues
	Due to enforcement considerations, it is suggested that any proposed amendments to the Standard are not introduced on gazettal but given an introductory period of 12 months.	Considers that there are potential acute public health implications associated with the levels reported and therefore considers that transitional arrangements are not appropriate.
Industry		
Australian Food and Grocery Council (AFGC) (Kim Leighton)	Rejects the FSANZ risk assessment that regulating the maximum permitted level of hydrogen cyanide in Cassava Chips is necessary to protect public health and safety, and rejects all four FSANZ risk management options as being ineffective and inappropriate.	Noted.
	Does not support Option 1, given that there is an identified food hazard. However, without proper characterisation of the risk to the Australian and New Zealand population it is inappropriate to incorporate an ML for hydrocyanic acid in RTE cassava chips.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety. An acute reference dose has been established and this has been used in determining an appropriate maximum limit.
	Recommends that it would be appropriate to develop a non-regulatory option of encouraging the industry to implement audited food safety plans and to take account of the need to control the amount of hydrocyanic acid and cyanogenic glycosides in RTE cassava chips.	Does not consider that this will result in the protection of public health and safety, primarily because there is no requirement to reduce the levels to a level that will protect public health and safety.
	There is no substantial body of evidence which allows a confident estimate of the levels at which cyanogenic glycosides represent a significant health and safety risk in food products. Imposing an ML for hydrogen cyanide in cassava chips is not in response to a well defined public health threat and will impose an unnecessary regulatory burden on the industry.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety.
	RTE Cassava Chips are NOT made from edible cassava flour, and their preparation and manufacture is inconsistent with the definition of Edible Cassava Flour. Rather chips are made from dried cassava.	Does not agree and notes that this is contrary to the advice provided by other submitters.
	Rejects the proposal to apply the Codex limit on the basis that RTE Cassava Chips do not undergo the same processing as is applied to Edible Cassava Flour and does not fit the definition of the product in this Standard.	Considers that all cassava should be adequately processed before being used in RTE food.

Submitter	Summary of Comments	FSANZ Response to Issues
	Inappropriate to apply the Codex limit for edible cassava flour to dried cassava chips given that the Codex Standard for Sweet Cassava specifies a level of 50 mg/kg hydrogen cyanide (fresh weight basis). The Codex limit is not based on toxicological evidence but rather on empirical data and evidence demonstrates that populations in developing countries are able to safely tolerate significantly higher levels of cyanogenic glycosides in cassava than the limits imposed in the 1995 Codex standard.	Considers that it is appropriate to apply a limit to a RTE cassava based food in accordance with the international standard for another RTE cassava based food. The limit for sweet cassava is a limit for a food that will be further processed and applying this same limit to a RTE food is not considered appropriate. In addition, applying a 50 mg/kg limit to a RTE food would not be adequate to protect public health and safety. Based on a revised risk assessment, considers that a limit of 10 mg/kg is considered necessary to protect public health and safety.
	The imposition of the limit on Cassava Chips alone fails to consider dietary exposure of other sources of cyanogenic glycosides from major staple foods, such as edible cassava flour and tapioca, as well as the Linseed and Sorghum, and is therefore indiscriminate in the application of a limit, potentially creating a technical barrier to trade.	The scope of P1002 is limited to RTE cassava chips. If problems become apparent with other foods then this may prompt further action. Based on information currently available, the levels of total hydrocyanic acid in these other cassava based foods have not been found to be a problem. Acknowledges that this may change as more data is gathered.
	The imposition of a maximum limit in RTE Cassava Chips effectively imposes end product testing, which is inconsistent with policies of minimum effective regulation and the application of food safety plans.	An ML is a practical and effective means of ensuring that total hydrocyanic acid in RTE cassava chips is adequately managed.
Unique Food Group Ltd. (John McDonald)	Agrees with the proposal but would like more consideration given to the methods used in the analysis, as this can alter the results quite considerably.	Notes this view.
	Supports the inclusion of a maximum level of 10 mg/kg for hydrocyanic acid for RTE cassava chips, in the Code. Aware of the existing World Health Organization maximum level of 10 mg/kg for hydrocyanic acid in cassava products and has already been voluntarily managing its own 'RTE cassava chip' products within this guideline.	Notes this view.

Submitter	Summary of Comments	FSANZ Response to Issues
	Agrees that given the potential public health implications, the level come into effect upon gazettal and with no 'stock in trade' transitional arrangements. It is very likely that RTE cassava chip manufacturers already are familiar with the existing WHO standard and should already be in voluntary compliance with this standard.	Notes this view.
	Opposes any special labelling requirements on packaging if the maximum level of 10 mg/kg for hydrocyanic acid standard is adopted.	No specific labelling requirements are proposed.
	Concerned about the lack of a test method for hydrocyanic acid and would propose that it would provide a standard method on behalf of the industry through Crop and Food Research. This approach would not only standardize the test method but would also provide a credible and independent facility for testing.	Notes this concern but does not consider it practical or necessary to prescribe a specific method of analysis. Considers that the total hydrocyanic acid should be measured as this is the most appropriate means to protect public health and safety.
	The Free HCN method is the preferred option, as opposed to the Total HCN method.	Appears to be the methods that some in industry are using. The definition of 'total hydrocyanic acid' will assist in clarifying which methods are considered adequate for monitoring total hydrocyanic acid in RTE cassava chips.
Samoa Association of Manufacturers & Exporters Gaosia mo Samoa (Grant Percival)	Objects to the proposal for a standard for Cassava Snack food on the basis that it fails to address the issues and is a protectionist measure for local industry and would increase costs for Exporters to Australia and increase the costs of these products for consumers when the issue has been generated by the importation of dried cassava with high levels of cyanide used by an Australian manufacturer for the manufacture of Cassava Chips.	Acknowledges that introducing an ML of 10 mg/kg to protect public health and safety would increase costs for those manufacturers using inadequately processed cassava.
	The focus on cassava snacks the end product is invalid.	Considers that applying a limit to RTE cassava chips to protect public health and safety is appropriate while additional information is gathered about other foods.

Submitter	Summary of Comments	FSANZ Response to Issues
	CODEX has adequate guidelines for the importation of edible flours and this could be extended to cover dried Cassava pellets intended for human consumption. This is set at 10 mg/kg and would appear to adequately address the issue and protect the public from the dangers of Cassava cyanide consumption.	Considers that it is currently unnecessary to impose food regulatory measures on product for further processing and that it is impractical to impose food regulatory measures based on the 'intentions' for the use of a product. Agrees that a limit of 10 mg/kg is appropriate to protect public health and safety.
	Putting the ban on the end product would not stop the importation of cassava pellets that would exceed this guideline and would allow the continued manufacture of potentially hazardous cassava snacks in the Australian market.	There is no intention to prevent the importation of cassava pellets for further processing. Further processing of cassava may reduce the levels. The limit applies to the RTE cassava chips.
	Dried cassava pellets have been an animal feed for years. Misuse of this product could cause the issues raised above and therefore the current focus may be too narrow as there may also need to be an analysis for animal feed.	Regulatory measures in the Code do not apply to stock food or animal feed.
	The blanket approach assumes that all practitioners are guilty and negligent which is not in line with the approach of most Food Standards Authorities and shall put up a barrier for imports without addressing the reason for the problems as identified in the notice. Fails to address the issues of other cassava-based products that may be based on the imported pellets or the use of pellets with high cyanide levels.	The proposed food regulatory measure applies equally to all producers and manufacturers of RTE cassava chips, both for importers and for manufacturers in Australia and New Zealand. The measure does not apply to cassava pellets for further processing unless they are in a RTE form.
	Fails to minimize the dietary exposure to hydrocyanic acid if it fails to put in place the CODEX standard for Cassava flour and expands it to dried pellets. Agrees that a maximum level in Standard 1.4.1 is considered an appropriate risk management measure BUT for the right product. Cassava Flour is not mentioned in the Standard.	Based on submissions, cassava based ingredients other than edible cassava flour are used in RTE cassava chips. At this time, considers that the food regulatory measure should only apply to RTE cassava chips and not to ingredients that may or may not be present in the chips.
	No definitive study that would show the acceptable levels for cyanide in Cassava and throws doubt on the whole process as the cyanide in Cassava is based on a different chemical to that used in most of the tests, and extrapolations are used to arrive at the 10 mg/kg level when 25 mg/kg was considered safe.	Considers that an ML of 10 m/kg is necessary to protect public health and safety. Risk assessment revised and an acute reference dose established that has been used in determining an appropriate maximum limit.

Submitter	Summary of Comments	FSANZ Response to Issues
	There are little differences in the outcomes for all the other considerations. In the consumer's interest it would be best that the code level be increased to 25 mg/kg and a standard introduced for pellets intended for processing for human consumption that would achieve a lower outcome for consumers.	Based on the revised risk assessment, considers that a Maximum Level of 10 m/kg is necessary to protect public health and safety.
	The primary reason for the development of this standard appears to be the need to protect public health and safety based on an analysis of Australian produced snack foods from suspect dried cassava pellets. There are no other manufacturers identified and the development of this standard goes beyond the protection of public health and safety.	Considers that the food regulatory measure is necessary to protect public health and safety and is based on a risk assessment.
	There are no standards proposed for the other primary objectives of section 18 of the FSANZ Act and no relevance and it is therefore extending into the area of protection of Australian industry.	Considers that an ML of 10 m/kg for total hydrocyanic acid in RTE cassava chips is required at this time to protect public health and safety.
	Extrapolating standards that are not even consistent between the various foods with naturally occurring hydrocyanic acid as the standards range from 5 mg/kg to 50 mg/kg. In fact at levels of 8 times the restricted levels FSANZ found that this was unlikely to result in any short-term toxicity in a 20 kg child.	Considers that an ML of 10 m/kg is necessary to protect public health and safety. It is reasonable to apply the limit for edible cassava flour – a processed cassava product – to RTE cassava chips – another processed cassava product. With proper processing all processed RTE cassava based ingredients should be able to comply with a limit of 10 mg/kg.
	The Cost Benefit analyses of the two standards indicate that the only difference in the outcomes is greater certainty of greater cost for the standard of 10 mg/kg. If there were additional benefits accruing to the higher standard than it would be reasonable to apply the higher standard of 10 mg/kg.	Notes that there are greater costs with a limit of 10 mg/kg compared to 25 mg/kg but a limit of 25 mg/kg is not considered to be sufficiently protective of public health and safety.

Submitter	Summary of Comments	FSANZ Response to Issues
	Application of international codex standards would address many of the problems particularly extended to dried pellets intended for human consumption. There may then be a need for an AQIS notice about concerns at the level of hydrocyanic acid in ready to eat cassava snacks and the need for random testing to ensure compliance with safe standards and these be promulgated through the cassava and snack importers so as to ensure compliance. This would achieve the required safety needs without creating a problem for the industry.	Acknowledges that there are implementation issues associated with the ML and considers that any testing should be focussed on the RTE product and apply to imported product and product manufactured in Australia and New Zealand. An ML in the Code would be required before any compliance action could be taken, including random testing.
Flour Mills of Fiji Ltd (FMF)(Anuj Patel)	As per the Options given by FSANZ it is better to amend the current Standard 1.4.1 (Option 2 & 4) and it will help lots of cassava producers, manufactures etc.	Noted.
	States that ingested and absorbed linamarin is rapidly excreted in the urine and the glucoside itself does not appear to be acutely toxic. The generation of cyanide from linamarin is usually enzymatic and occurs when linamarin is exposed to linamarase, an enzyme normally expressed in the cell walls of cassava plants.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety. An acute reference dose has been established and this has been used in determining an appropriate maximum limit. More detail provided on the basis for this limit as part of this Approval Report. This assessment supports an ML of 10 mg/kg to protect public health and safety.
	The Processing of Cassava before consumption reduces the cyanide content and cassava cultivars vary widely in their cyanide content with most in a range from 2 mg to 40 mg/ 100 g peeled root. South pacific cultivars of Cassava have been thought to be low in cyanide and cyanide levels in Fiji cultivars of cassava are provided with details of processing of cassava and a process flow chart was provided.	Notes this information.
	In Fiji 80% of the local people's staple food is Cassava and until now there has been no cases reported concerning hydrogen cyanide poisoning or no case studies conducted on the same.	Notes that there have been no cases of cyanide poisoning reported in Fiji. Considers that based on the best available scientific evidence, an ML of 10 mg/kg is necessary to protect public health and safety for consumers in Australia and New Zealand.

Submitter	Summary of Comments	FSANZ Response to Issues
	In current processing and cooking cyanide can be eliminated to a very low level (Analysis in progress). Due to the lack of analytical tools and methods it is very difficult to give an accurate result analysis at the moment.	Notes the processing steps that can be undertaken to reduce or eliminate hydrocyanic acid in RTE cassava chips.
	Currently many agricultural commodities are facing problems due to incomplete or insufficient studies which will adversely affect the poor people who are fully dependent on these types of crops for their very survival.	Notes this information.
Food Technology Association of Australia (David Gill)	The majority of the Committee agreed with Option 1 – to retain the *status quo* to not vary the Code to incorporate an ML for hydrocyanic acid in RTE cassava chips.	Noted.
	The high levels of hydrocyanic acid found in the RTE cassava chips was TOTAL hydrocyanic acid and not FREE hydrocyanic acid. The body does not possess the enzyme necessary to release the BOUND hydrocyanic acid. The majority of the total hydrocyanic acid in cassava is unavailable to the human digestive system.	Does not agree with this view and considers that it is the total hydrocyanic acid content of RTE cassava chips that is of public health relevance.
	The method of analysis used is often but not always a method that employs an enzyme whose function is to release the BOUND hydrocyanic acid. This method although rapid is not the only accepted method of analysis available.	Notes this and also notes that methods for determining total hydrocyanic acid in cassava, based on enzyme hydrolysis, have been published.
	If a level of hydrocyanic acid is to be set it should be for a group of foods or for all foods (with nominated exceptions such as those in clause 4 of Standard 1.4.1). It is considered that a Standard should not contain a level for a single food.	There are existing MLs for specific foods in the Code. In addition, considers that extending the limit to other foods is not currently justified, given the evidence base.
	A food with a somewhat different shape to the current cassava chips and a different name would become exempt from the restriction imposed on RTE cassava chips. Chips, although mentioned in the User Guides, etc are not, *per se*, defined anywhere in the Code as such would create problems with interpretation.	Notes this concern but considers the definition is sufficiently precise to encapsulate cassava chips in a RTE form.

Submitter	Summary of Comments	FSANZ Response to Issues
	Until an acceptable method of processing the cassava to reduce to the lower level of 10 mg/kg is found, it may be more realistic to set the level at the higher safe level of 25 mg/kg.	Considers that appropriate methods for processing cassava are currently available and have been available for many years. Considers that based on the best available scientific evidence, an ML of 10 mg/kg is necessary to protect public health and safety.
	Industry must know what is going to be analysed, i.e. total hydrocyanic acid or free hydrocyanic acid. Industry must know what the limit refers to, otherwise monitoring of ML is useless. Industry may be able to comply with a ML as long as what they need to comply with is explicitly spelt out, including acceptable testing methods. Monitoring compliance effectively can also be done once everybody knows what is being testing for and by what method.	Notes this concern and by indicating the substances for which the ML applies, analysts can develop and validate appropriate methods for their determination. For this reason, a definition of total hydrocyanic acid has been developed. Notes that there are many MLs in the Code now and methods are not prescribed for these.
	The current Code has sufficient restrictions to ensure that all RTE foods are made from cassava that has been treated in the prescribed manner that ensures the hydrocyanic acid is reduced to acceptable and safe levels. If products made from cassava are not treated in the prescribed manner, then surely it can be assumed that such products should not be offered for sale.	Not aware of any specific provisions in the Code that would limit the amount of total hydrocyanic acid in RTE cassava chips. Given the public health implications, considers that the Code should include an ML for total hydrocyanic acid in RTE cassava chips to protect public health and safety.
	Noted that certain maximum levels of hydrocyanic acid were permitted in particular foods (confectionery, stone fruit juices, marzipan and alcoholic drinks) that contain a flavouring substance. It is considered that this aspect and interpretation of the Code requires further investigation.	Notes this point. The limit in clause 4 of Standard 1.4.1 applies to the food to which a flavouring substance has been added. Acknowledges that further consideration will need to be given to cyanogenic glycosides in food, including in relation to existing MLs in the Code.

Submitter	Summary of Comments	FSANZ Response to Issues
Vege Chip Company (VCC) (Carter Newell Lawyers)(James Plumb)	Submits that there is no need to introduce an ML in respect of total hydrocyanic acid in RTE cassava chips.	Noted.
	In 18 years of trade in Australia, has never received report of an illness related to consumption of its product. The proposed introduction of the Maximum Limit is not a response to any actual reported illnesses from consumption of cassava chips.	Notes this information and considers that on the basis of the revised risk assessment a limit of 10 m/kg for total hydrocyanic acid in RTE cassava chips is considered necessary to protect public heath and safety.
	Cassava is fried to produce chip products. Exposure to high temperatures denatures the linamarase enzyme, significantly reducing the levels of hydrocyanic acid released during human consumption.	Processing may denature the enzyme in the chip but enzymes in the human body can produce hydrocyanic acid from cyanogenic glycosides in the chip.
	Current production practices by VCC and industry are sufficient to ensure that consumption of RTE cassava chips is safe, and that any residual hydrocyanic acid released during consumption is adequately detoxified by the human body.	On the basis of the levels being reported in some cassava chips and the revised risk assessment, considers that a limit of 10 mg/kg for total hydrocyanic acid in RTE cassava chips is necessary to protect public heath and safety.
	The introduction of the proposed ML would be a commercially prohibitive measure that will not materially enhance the safety of the public; it is likely that an introduction of the ML would result in VCC being forced to cease trade.	Acknowledges this significant cost impact. However, also considers that a ML of 10 mg/kg is considered necessary to protect public health and safety. Notes that the information provided to FSANZ indicates that some manufacturers can achieve 10 mg/kg.
	States that any limit should be at a level of 50 parts per million as 80 ppm total hydrocyanic acid is a demonstrated safe level of consumption in RTE cassava chips and 50 ppm is reasonably achievable and will not be so low as to be cost prohibitive in production or result in severe impediment to industry.	Does not consider that a limit of 50 mg/kg in ready-to-eat cassava chips is sufficient to protect public health and safety. Acknowledges that there will be costs associated with reducing levels to 10 mg/kg.

Submitter	Summary of Comments	FSANZ Response to Issues
	FSANZ must undertake further investigations into the production processes, levels of consumption and toxicity associated with RTE cassava chips, must undertake a review and provide guidance on the appropriate methodology to be used to test for total hydrocyanic acid in RTE cassava chips, and must allow for a 2 year transitional period to prevent damaging disruption to industry.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety. An acute reference dose has been established and this has been used in determining an appropriate maximum limit. More detail provided on the basis for this limit which supports an ML of 10 mg/kg to protect public health and safety. Considers that by defining total hydrocyanic acid for RTE cassava chips, sufficient guidance is provided to allow analysts to develop or validate methods. Given the potential public health implications, does not consider that a transitional arrangement is appropriate.
Tixana Pty Ltd (Alex Sapurmas)	Tixana believes that the proposed regulatory measures under the Assessment Report of Proposal P1002 take an extremely conservative approach and do not adequately consider the practicality of compliance by the industry.	Noted.
	The proposed maximum level of 10 mg/kg cannot be achieved by the industry upon such short notice and without substantially changing the current nature of the product.	Acknowledges this impact. However, also considers that a ML of 10 mg/kg is considered necessary to protect public health and safety. Notes that the information provided to FSANZ indicates that some manufacturers can achieve 10 mg/kg.
	The proposed regulatory measures under Proposal P1002 are based on a Risk Assessment conducted by FSANZ based on the assumption that a 20 kg child can consume 200g of cassava chips in 1-2 hours. It can be clearly demonstrated that this level of consumption is an overestimation by up to 400%. Tixana have sought expert opinion by two independent toxicologists Dr. Simon Brooke – Taylor and Dr Ian Spence, both of whom deem the current RTE cassava chips to be safe.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety. An acute reference dose has been established and this has been used in determining an appropriate maximum limit. More detail provided on the basis for this limit which supports an ML of 10 mg/kg to protect public health and safety.
	FSANZ's own risk assessment deemed 80 mg/kg total CN to be safe even at excessive consumption levels and calculations show that the same reasoning by FSANZ can allow 100 mg/kg total CN to be considered a safe level.	Risk assessment revised which supports an ML of 10 mg/kg to protect public health and safety.

Submitter	Summary of Comments	FSANZ Response to Issues
	Applying a limit of 10 mg/Kg for total CN is unnecessarily restrictive and would devastate the Australian manufacturers and wholesale distributors with no demonstrated increased public health benefit.	Acknowledges this significant cost impact. However, also considers that a ML of 10 mg/kg is considered necessary to protect public health and safety.
	Believes P1002 does not take into account the manufacturing capabilities, agricultural crop variability, ability to comply with 10 mg/Kg total CN and the potential cost to industry.	The ML is necessary to protect public health and safety but acknowledges that there will be costs in instituting adequate processing or specific crop selection.
	Believes that the term 'Hydrocyanic Acid' has been used loosely by FSANZ to refer to free cyanide, potential cyanide from Linamarin and total cyanide and believes that such loose use of the term may cause confusion.	Notes this and has developed a specific definition for total hydrocyanic acid for RTE cassava chips.
	Believes that introducing a Standard for total CN without an official and fully accredited method of testing for total CN will cause confusion and will make compliance with the Standard impossible.	Considers that prescribing specific methods is not practical or necessary. By indicating the substances for which the ML applies, analysts can develop and validate appropriate methods for their determination.
	Questions P1002 because it does not address the potential toxicity of other foods with up to 500 mg/Kg total CN currently in the marketplace.	P1002 is in relation to total hydrocyanic acid in RTE cassava chips. The need for MLs for other foods would be the focus of another Proposal or Application.
	Recommends a maximum limit of 100 mg/kg total CN.	Considers that a Maximum Level of 10 m/kg is necessary to protect public health and safety.
Other Submitters		
Tony Hazzard (Private)	Advocates that Australia's public would be better protected in the short term by FSANZ working with industry, exporters and competent authorities in the country of origin to reduce the hydrocyanic acid content of ready to eat cassava chips rather than taking immediate action to reduce the level in a standard below that necessary to protect health.	Does not agree with this view because products containing unacceptable levels of hydrocyanic acid are currently available. Non-regulatory liaison with industry, exporters and competent authorities in the country of origin is not considered a practical or appropriate response to ensure that public health and safety is protected.

Submitter	Summary of Comments	FSANZ Response to Issues
	Not necessarily a predetermined step that because Codex sets a standard for one form of cassava flour that it would set the same limits for hydrocyanic acid content of a cassava product of a different form. Codex might first assess what are the levels which are both as low as reasonably achievable with the specific product and which protect health and safety of consumers.	An ML of 10 m/kg is necessary to protect public health and safety. If a level of 10 mg/kg is achievable for adequately processed RTE edible cassava flour then it should be appropriate for all other processed cassava based ingredients.
	There is a flaw in the logic that says Australia is importing food for which no current standard exists and for which high levels of hydrocyanic acid content have been found in some samples and therefore there is a need to set a standard set at a lower level than other product standards in Australia and that this would ensure the standard is met.	The limit applies to RTE cassava chips to account for the fact that the processing of cassava results in a moisture loss. This moisture loss can increase the level of total hydrocyanic acid if the processing is ineffective in eliminating the total hydrocyanic acid.
	Establishing a standard would not affect the product entering Australia unless the country of origin is required to provide a certificate of analysis or unless there is an intensified program of testing. If this occurred and the level is not changed in the Code the public health in Australia would be protected.	An ML in the Code is a mandatory standard that applies to all sources of RTE cassava chips. Without an ML in the Code, there is no legal mechanism to require the level of hydrocyanic acid to be no more than a prescribed level.
	The risk assessment discusses the likelihood of toxicity as a result of consuming 200 g of chips over a 1-2 hour period and suggests that if the standard was set at less than 80 mg/kg or such that product not exceed 80 mg/kg there would be no public health concern. Yet a level is identified in a Codex Standard which is much lower and which is set for a different product.	Based on a revised risk assessment, considers that an ML of 10 mg/kg is necessary to protect public health and safety. An acute reference dose established and this has been used in determining an appropriate maximum limit. More detail on the basis for this limit as part of this Approval Report which supports an ML of 10 mg/kg to protect public health and safety.
	The assessment report argues that industry would not be affected but industry and the associated communities could be affected in the countries where this industry is located.	Acknowledges this cost impact. However, considers that an ML of 10 m/kg is necessary to protect public health and safety.
	A lower standard in the Code would have no impact on the health and safety of consumers in the region while a collaborative effort would not only be beneficial to Australian consumers but also to consumers in the Pacific.	It is not the role of FSANZ to develop food regulatory measures to protect public health and safety in other countries.

Submitter	Summary of Comments	FSANZ Response to Issues
	The Pacific and other markets for such products would be better protected by Australia transferring expertise to the neighbouring countries in the region to enable them to meet levels which are as low as reasonably achievable and which are protective of human health and safety.	It is not the role of FSANZ to provide food processing expertise to organisations or businesses in other countries. There are other mechanisms that can be used to address this.

Attachment 3

Hazard Assessment

CYANOGENIC GLYCOSIDES IN READY-TO-EAT CASSAVA CHIPS

SUMMARY

1. Cassava root, like many other food plants such as bamboo shoots or lima beans, contain variable amounts of naturally occurring cyanogenic glycosides. In cassava root the major cyanogenic glycosides are linamarin (93%) together with a small amount of lotaustralin (7%). All cyanogenic glycosides are intrinsically non-toxic unless they come into contact with the appropriate enzymes that can liberate very toxic hydrocyanic acid from the glycoside. This release of hydrocyanic acid can occur during cassava root maceration or through fermentation by microflora in the gastrointestinal tract following ingestion.

2. Ready-to-eat cassava chips available in Australian supermarkets have recently been found to contain levels of total hydrocyanic acid which ranged from undetectable to 145 mg/kg. The hydrocyanic acid is thought to be due to inadequate processing of cassava resulting in cyanogenic glycosides being present in the ready-to-eat chips. This hazard assessment considers whether there are sufficient data to establish an appropriate health standard for acute intake of cyanide or cyanogenic substances in foods such as cassava chips.

3. Following oral administration, a proportion of ingested linamarin is absorbed and excreted unchanged in the urine. The remainder is enzymatically converted to hydrocyanic acid by micro-organisms in the gastrointestinal tract. The hydrocyanic acid absorbed from the gut is metabolically converted to the less toxic thiocyanate. Other detoxification pathways include combination with vitamin B12 or some sulphur-containing amino acids. Acute toxicity results when the rate of absorption of hydrocyanic acid is such that the metabolic detoxification capacity of the body is exceeded.

4. Cases of human intoxication and chronic neurological effects have occurred from the ingestion of improperly processed cassava. This is particularly apparent when processing practices change as a result of an uncertain food supply in countries where cassava is a staple food.

5. The cyanide ion inhibits enzymes associated with cellular oxidation and causes death through energy deprivation. The symptoms, which occur within a few minutes, may include headache, nausea, vomiting, giddiness, palpitations, hyperpnoea then dyspnoea, bradycardia, unconsciousness and violent convulsions, followed by death. The available toxicity data show that ingested linamarin in cassava can produce acute toxic effects.

6. The chronic uptake of hydrocyanic acid, in sub-acutely toxic doses, may be involved in the pathogenesis of certain conditions including disturbance of thyroid function and neuropathies. The thyrotoxic effects of cyanide depend on its conversion to the iodine antagonist, thiocyanate. There is some recent evidence that linamarin may be taken up by active transport processes into human neural cells. However, the long-term effects of daily linamarin exposure remain unclear.

7. Human cassava-eating populations showed ophthalmological and neurological symptoms which are associated with exposure to hydrocyanic acid, though it is likely that other nutritional or metabolic deficiencies affecting the cyanide detoxification mechanism are also involved (e.g. sulphate and zinc deficiencies).

8. Several epidemiological studies in cassava-eating populations, which established an association between cyanide exposure and spastic paraparesis, amblyobia ataxia or tropical ataxia neuropathy (TAN) and possibly goitre have also been considered. However, the data are highly confounded by other nutritional and environmental factors. Suitable long-term toxicity studies in animals fed a diet containing hydrocyanic acid or linamarin could not be located.

9. The database for the toxicity of hydrocyanic acid and cyanogenic glycosides is incomplete and limited particularly with respect to chronic intake although there is sufficient data to establish an acute reference dose (ARfD). In hamsters a no-observed-adverse-effect level (NOAEL) of 70 mg/kg bw was reported in a single dose developmental study. Applying a 100-fold safety factor to the NOAEL gives an ARfD of 0.7 mg/kg bw. Since it is conventional to express the cyanogenic glycoside content in cassava in terms of total hydrocyanic acid the ARfD for linamarin can be calculated to be equivalent to 0.08 mg hydrocyanic acid/kg bw.

REVIEWS BY OTHER REGULATORY AGENCIES

Joint FAO/WHO Meeting on Pesticide Residues (JMPR; 1965)

At its second meeting in 1965 the JMPR established an Acceptable Daily Intake (ADI) for hydrocyanic acid intake of 0-0.05 mg HCN/kg bw. It was based on a no-effect level in a two-year rat study of 100 ppm expressed as residue in the diet after fumigation with hydrocyanic acid (Howard & Hanzal, 1955). JMPR concluded that the 'long-term experiment in rats in the course of their whole life-span can be taken as a basis for determining the acceptable daily dose for man. This dose (100 ppm) does not increase the level of thiocyanate in the blood to the same extent as reported in smokers, which is about three-fold of that of non-smokers, so that effects of thiocyanate produced in the organism from the consumption of food treated with HCN are improbable'.

US Environmental Protection Agency (US EPA; 1993)

The US EPA oral reference dose (RfD) associated with food consumption is 0.108 mg HCN/kg bw/day (i.e. 10.8 mg/kg bw/day, divided by a 100-fold safety factor). This is derived from a no observed adverse effect level (NOAEL) of 10.8 mg/kg bw/day of cyanide, applying an uncertainty factor of 100 to account for intra- and inter-species variability. The NOAEL was based on a 2-year dietary study in rats in which there were no treatment-related effects on growth rate, no gross signs of toxicity, and no histopathologic lesions at the highest tested dose (Howard & Hanzal, 1955; see US EPA, 1993).

Joint FAO/WHO Expert Committee on Food Additives (*JECFA; 1981, 1993) & European Food Safety Authority (2004)*

In 1981 JECFA reviewed cyanide which occurs naturally in certain flavouring agents, particularly those derived from the fruits and other parts of *Prunus* species, in their 25th report (JECFA, 1981). The Committee decided that hydrocyanic acid or its salts should not be used as food additives, and that the amount of hydrocyanic acid in finished foods as the result of using natural flavouring agents containing hydrocyanic acid should be kept to the lowest level necessary to achieve the desired organoleptic effect (taste). JECFA did not produce a toxicological monograph, but referred to an earlier one prepared by JMPR in 1965 which they had evaluated hydrocyanic acid as a food fumigant.

In 1993, JECFA reviewed the toxicology of cyanogenic glycosides from cassava and other plant based foods and concluded that a safe level of intake could not be estimated because of a lack of quantitative toxicological and reliable epidemiological information. However, JECFA concluded that a level up to 10 mg hydrocyanic acid/kg in the Codex Standard for cassava flour was not associated with any acute toxicity. A review of the available data by European Food Safety Authority (EFSA Journal) in 2004 reached a similar conclusion.

European Committee of Experts on Flavourings (2000 & 2005)

In 2000 the Committee of Experts on Flavourings of the Council of Europe evaluated the safety of cyanogenic glycosides (CoE, 2000). Based on an overall toxicological assessment, but in particular based on the data of studies on a Konzo-affected (a distinct form of a tropical myelopathy) population in the Democratic Republic of Congo, with a daily intake equivalent to 0.19-0.37 mg CN^-/kg bw/day, a Temporary Maximum Daily Intake (TMDI) of 0.02 mg CN^-/kg bw/day was set.

However, in 2005 the Committee of Experts on Flavourings reconsidered the TMDI in the light of confounding factors, such as malnutrition, associated with Konzo-affected populations (CoE, 2005). They established a new TMDI of 0.023 mg/kg bw/day based on a NOAEL of 4.5 mg/kg bw/day for minor changes in the testis observed in a rat sub-chronic study in which animals were exposed through their drinking-water (NTP, 1993). A 200-fold safety factor was applied to take into account the small but measurable effect on reproduction in male rats, which were insufficient to affect the fertility and also the recognized higher sensitivity of humans, as compared with rats, to these effects.
The TMDI is 10-fold lower than the estimated intake level of 0.19 mg CN^-/kg bw/day leading to neurological disorders in cassava-consuming populations as observed in the Konzo study.

UK Food Safety Authority (2006)

The UK Committee on Toxicity established a 'nominal' acute reference dose (ARfD) based on reported lethality in humans. The range for the acute lethal dose in humans is 0.5 to 3.5 mg/kg bw. A 100-fold uncertainty factor (10 to account for inter-individual variability and 10 to extrapolate from an effect level to a no effect level, taking into account the steep dose-response relationship) could be applied to the lowest lethal dose. This would indicate that a dose of 0.005 mg/kg bw would be unlikely to cause acute effects (UK Committee on Toxicity, 2006).

Agency for Toxic Substances and Disease Registry (2006)

In 2006 the Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services, published a risk assessment for cyanide (ATSDR, 2006). For the purpose of establishing a minimum risk level (MRL) of
0.05 mg/kg bw/day for oral exposure of up to one year they cited a 1993 NTP study in which rats were dosed with sodium cyanide in their drinking water for three months. The ATSDR considered that the reproductive effects observed in males, namely reduced left epididymis weight, left cauda epididymis weight, left testis weight, spermatid heads, and spermatid counts at 12.5 mg CN^-/kg bw/day, as being adverse. The NOAEL was considered to be 5 mg/kg bw/day for the observed effects in male fertility.

No minimum risk level was derived for chronic cyanide exposure because of the limitations of the toxicological database. The human studies of cassava eaters lacked quantitative information on exposure, and the only available chronic oral study in rats (Howard & Hanzal, 1955) was considered unsuitable because it 'found no treatment related effects'.

WHO Guidelines for Drinking-water Quality (2003 & 2007)

In 1996 a WHO Review Group noted that 'there are a very limited number of toxicological studies suitable for use in deriving a guideline value', and that there was some indication 'that pigs might be more sensitive than rats'. The LOAEL of 1.2 mg CN/kg bw/day observed for changes in behaviour and serum biochemistry after six months of dosing (Jackson, 1988) and an uncertainty factor of 100, consisting of factors of 10 each for inter- and intra-species variation, was used to derive a TDI of 0.012 mg/kg bw/day. The top dose was described by the Review Group as a 'clear effect level' but 'because of doubts over the biological significance of the observed changes', the additional factor of 10 normally used when basing a TDI on a LOAEL rather than a NOAEL was not considered necessary. An allocation of 20% of the TDI to drinking-water is made because exposure to cyanide from other sources is normally small and because exposure from water is only intermittent. This guideline value of 0.07 mg/L was considered to be protective of both short- and long-term exposure (WHO, 2003).

In 2007, the WHO Drinking Water Guideline level was revised upwards from 0.07 mg/L to 0.3 mg/L because the pig study which formed the basis of the 2003 guideline value was considered to be unreliable. The study, with group sizes of only three, suffered from a lack of statistical power, and there was doubt as to whether the effects observed have a relevance to humans.

The 2007 Guideline level was based on a NOAEL of 4.5 mg/kg bw/day for minor changes in the testis observed in a rat sub-chronic study in which animals were exposed through their drinking-water (NTP, 1993). In view of the minor nature of the changes observed in the sub-chronic study it is not considered necessary to include an additional uncertainty factor to allow for the short duration of the study. The application of an uncertainty factor of 100 to the NOAEL of 4.5 mg/kg bw/day gives a TDI of 0.045 mg/kg bw/day.

Assuming a 60-kg adult drinking 2 litres of water per day and allowing 40% of the TDI to come from water because of the potential for exposure to cyanogenic glycosides in food, the guideline value for long-term exposure is 0.6 mg/L (WHO, 2007).

Background

Cassava (*Manihot esculenta Crantz*) is grown for its edible starchy tuberous root and is consumed in a number of forms: flour; slices; chips; grated (pan fried, baked or steamed) or tapioca pearls made as a pudding. Cassava is a major source of carbohydrate for a large number of people especially in the tropics. Cassava roots are rich in starch, and contain minerals such as calcium and phosphorus.

Ready-to-eat cassava deep-fried chips available in Australian supermarkets have recently been found to contain levels of hydrocyanic acid which ranged from undetectable to 145 mg/kg. The hydrocyanic acid is thought to be present because of inadequate processing to remove linamarin, a naturally occurring cyanogenic glycoside in cassava.

Chemistry

Cassava contains two hydroxynitrile glucosides, namely linamarin (93%) and a small amount of lotaustralin (7%) (Nartey, 1968; Barrett *et al.,* 1978). There are essentially two steps involved in the enzymatic degradation of linamarin and lotaustralin to form hydrocyanic acid. Initially, a ß-glucosidase enzyme hydrolyzes the glycosidic bond linking the glucose to the α-hydroxynitrile (cyanohydrin); then hydrocyanic acid (or hydrogen cyanide) is dissociated from acetone cyanohydrin (or to butanone cyanohydrin for lotaustralin) either non-enzymatically or through action of another enzyme, a hydroxynitrile lyase.

The chemical reaction for the formation of hydrocyanic acid from linamarin (2-methyl-2-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy -propanenitrile; IUPAC) is shown below (Figure 1). The non-enzymatic pathway is pH-dependent. At pH > 6 high rates of dissociation occur; but at pH 5, the yield of hydrocyanic acid is much lower (Cooke, 1978). In spite of the relative instability of cyanohydrin it co-exists with intact glucoside and hydrocyanic acid in differently processed cassava products.
It is therefore clear that the cyanide in cassava products may exist in three forms: (i) the glucosides (linamarin and lotaustralin), (ii) the cyanohydrin and (iii) the free hydrocyanic acid (HCN).

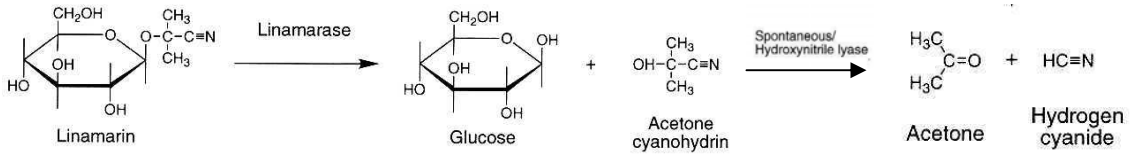


Figure 1: Chemical Reaction for the Formation of Hydrocyanic Acid from Linamarin

Since 1 mole of linamarin could theoretically release 1 mole of hydrocyanic acid if hydrolysis is complete it follows that one gram of linamarin (MW = 247) could liberate a maximum of 109.3 mg of hydrocyanic acid (MW = 27).

Review of toxicological data

This review considered a number of monographs on cyanogenic glycosides which have been prepared but relies mainly on the ones prepared by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 1993, the Concise International Chemical Assessment Document 61**:** Hydrogen cyanide and cyanides: Human Health Aspects and the Agency for Toxic Substances in 2004 and Disease Registry (ATSDR) in 2006. Only brief summaries of toxicological effects are reported in this review but studies of the effects critical for the risk assessment are evaluated in more detail.

Absorption, metabolism and excretion

Linamarin

In a toxicokinetics study 10 male rats were dosed with 50 mg linamarin and six male rats were given water alone by stomach tube. Seven rats dosed with 50 mg linamarin died within 4 h. In a second trial 6 male rats (100-120 g) were dosed with 30 mg of linamarin. Following dosing, urine and faeces were collected after 24, 48 and 72 hours, and heparinised blood samples were taken from the optic vein or lateral tail vein. Blood was taken after 30, 40, 60, 80 and 100 min and at 2, 4, 8, 24 and 48 hours. No intact linamarin was detected in faeces or blood of the rats dosed with 30 mg (~300 mg/kg bw). No linamarin in the faeces of the three surviving rats dosed with 50 mg (500 mg/kg bw) was detected, however blood and urine were not examined in these animals. Linamarin (5.65 mg; ~19%) was excreted in the urine over 72 hours along with 0.823 mg of thiocyanate. These findings suggest that some of the administered linamarin was absorbed and excreted unchanged and some was hydrolysed to form cyanide (Barrett *et al.*, 1977).

In a toxicokinetics study linamarin, at a dose of 300 mg/kg bw, was administered to groups of Wistar rats (10/group) in the feed. Each diet was adjusted to maintain either a vitamin B12-deficient, -sufficient or -excess diet for five weeks or in a fourth group of protein deficient (kwashiorkor) rats.

Free and total cyanide, intact linamarin and thiocyanate levels were estimated in urine and faeces obtained at 0, 24, 48 and 72 hour periods and in blood samples obtained 72 hours after the compound had been administered. No cyanide or intact linamarin could be detected in the faeces samples. Rats on vitamin B12-sufficient and B12-excess diets excreted higher levels of total and free cyanide in the urine than the vitamin B12-deficient group. Most of the linamarin was degraded after 24 hours. The rate of breakdown of the glycoside within the first 24 hours was slowest for zero and half normal vitamin B12 status rats as evidenced by appearance of the glycoside in large quantities in the urine. The kwashiorkor rats, on the other hand excreted less thiocyanate than the controls. In addition, their control group excreted most of the thiocyanate in the first 24 hours, whilst the kwashiorkor rats excreted most of the thiocyanate in the first 48 hours. Dietary protein deficiency prolongs the time of metabolism and hence increases the toxicity of cyanogenic glycosides in the body (Umoh *et al*, 1986).

In a study which investigated the fate of linamarin in 15 healthy adults (9M/6F) following the oral ingestion of a cassava-based porridge containing 243-574 µmol linamarin (60-142 mg cyanide equivalents; 4-10 µmol linamarin/kg bw; 0.99-2.47 mg linamarin/kg bw; 0.1-0.27 mg cyanide equivalents/kg bw), 22% (range, 1-47%) of the ingested linamarin was excreted in the urine within 48 hours. Serum thiocyanate, the main cyanide metabolite, increased in all subjects from a mean (+/- SD) of 34 (+/- 26) to 78 (+/- 28) µmol/L 48 hours after dosing. In a second group of seven subjects ingestion of porridge containing a mean concentration of 431 µmol of linamarin (4-9 µmol linamarin/kg bw (0.99-2.22 mg linamarin/kg bw; 0.1-0.24 mg cyanide equivalents/kg bw) resulted in a mean linamarin excretion of 127 µmol/L and an excess thiocyanate excretion of 118 µmol/L in pooled urine over 48 hours with the remaining 216 µmol of ingested linamarin being unaccounted for. Based on these results approximately 50% of orally ingested linamarin is converted to cyanide, absorbed and metabolized and then excreted as thiocyanate in urine, while another 25% is absorbed and excreted unchanged in urine. The remaining 25% which had been ingested could not be unaccounted for and was thought to be metabolised to an unknown compound (Carlsson *et al.*, 1999). However, an alternative explanation may be that all the thiocyanate which had been formed had not been excreted in urine because the mean elimination half-life of thiocyanate in healthy adults is estimated to be around 48-65 hours (Schulz *et al.,* 1979; Bodigheimer *et al.,* 1979). In this study urine was only collected over 48 hours.

In another study that investigated the fate of linamarin and its metabolite, hydrocyanic acid in five volunteers following ingestion of 0.5 kg of boiled fresh roots of sweet cassava which contained 105 µmol linamarin (26 mg) and 8 µmol free hydrocyanic acid (216 µg) 28% of the ingested linamarin was excreted unchanged in the urine during the following 24 hours (Hernandez *et al.*, 1995)

In a comparative metabolism study the rate of cyanide liberation resulting from hydrolysis of the cyanogenic glycosides linamarin, amygdalin and prunasin by a crude ß-glucosidase prepared from hamster caecum contents were studied *in vitro*. In addition, hamster blood cyanide and thiocyanate concentrations were determined at 0.5, 1, 2, 3, and 4 hours after an oral dose of 0.44 mmol linamarin/kg bw (108 mg/kg bw) or amygdalin (201 mg/kg bw).

Graphical plots of cyanide liberated versus time for linamarin and prunasin yielded straight lines, whereas for amygdalin the plot was curvilinear suggesting that for amygdalin the rate of cyanide release increased with time. At a substrate concentration of 10^{-3} M, the averaged rates of hydrolysis of prunasin, amygdalin and linamarin were 1.39, 0.57 and 0.13 nmol/min/mg protein, respectively. Lineweaver-Burk plots yielded apparent K_m and V_{max} values of 3.63×10^{-2} mM and 0.13 nmol/min/mg protein, respectively for amygdalin, and 7.33 mM and 1.04 nmol/min/mg protein, respectively for linamarin. Blood cyanide concentrations following amygdalin treatment of the hamster reached their highest level (130 nmol/mL) 1 hour after dosing and remained elevated until 3 hours after treatment.

Blood cyanide concentrations following linamarin treatment reached their highest level (116 nmol/mL) after 3 hours and then declined immediately. Area under the blood cyanide concentration-time curve was 395 nmol h/mL for amygdalin and 318 nmol h/mL for linamarin. The results suggest a faster rate of enzymatic hydrolysis and cyanide absorption for amygdalin than for linamarin (Frakes *et al.*, 1986a).

Significant amounts of unchanged linamarin are observed in the urine after consumption of insufficiently processed cassava as well as after consumption of other plants containing linamarin. These results indicate that linamarin, if not metabolised in the gut, will be absorbed and excreted in the urine without causing exposure to hydrocyanic acid. About 80% of ingested cyanide will be turned into thiocyanate and is excreted in the urine after a short period (Rosling, 1987).

In a study with isolated perfused rat liver, Strugala & Elbers (1984) demonstrated that cyanogenic glycosides, such as amygdalin, prunasin, and linamarin, require gut microbial flora for their metabolism and that they are not metabolised by mammalian cells. As a consequence cyanogenic glycosides are relatively non-toxic in germ-free animals.

Biochemical effects

In a study to investigate whether unmetabolised linamarin might be responsible for spastic paraparesis (konzo) in humans, neural cells were incubated with linamarin *in vitro*. At a concentration of 0.1 mM linamarin caused death in pheochromocytoma cells (PC) at a rate of 13.3% (SD 2.07) which was significantly different from that in controls (3.2%; SD 0.92). Cytochalasin B at 10 mM, an inhibitor of glucose transportation, prevented cell death; the percentage of dead cells significantly decreased to 6.06% (SD 1.98). Furthermore, glucose also prevented cell death. These results strongly suggest that linamarin competes with cytochalasin B and glucose for binding to a glucose transporter and enters into cells via glucose transporter (Sreeja *et al.*, 2003).

Cyanides

The metabolism of cyanide in the liver has been studied in animals. The major pathway involves conversion to thiocyanate by a liver mitochondrial enzyme, rhodanese (thiosulphate sulphotransferase; E.C.2.8.1.1). This major route of detoxification requires sulphur donors, which by different metabolic pathways are provided from dietary sulphur containing amino acids.
Three other minor pathways converting less than 20% of the total cyanide involve conversion to 2-aminothiazoline-4-carboxylic acid, incorporation into a 1-carbon metabolic pool or combining with hydroxocobalamin to form cyanocobalamin (vitamin B12). Detoxification is therefore affected by the presence of nutritional factors, such as sulphur-containing amino acids and vitamin B12 (Askar, 1983; Ludwig *et al*., 1975; Freeman, 1988).

It has been reported that other species have lower rhodanese activity than the rat and hence the rat may be able to convert cyanide to thiocyanate more easily than other species such as dog, rhesus monkey, and rabbit (Himwich & Saunders, 1984).

Biochemical effects

Hydrogen cyanide inactivates the enzyme cytochrome oxidase in the mitochondria of cells by binding to the Fe^{3+}/Fe^{2+} contained in the enzyme. This causes a decrease in the utilization of oxygen in the tissues.

Cyanide 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.

Cyanide activates glycogenolysis and shunts glucose to the pentose phosphate pathway decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle. Hydrogen cyanide will reduce the energy availability in all cells, but its effect will be most immediate on the respiratory system and heart.

Acute toxicity

Linamarin

In rats the median lethal dose of linamarin was reported to be 450 mg/kg bw (Oke, 1979). However, a dose of 25 mg linamarin (250 mg/kg bw) fed to rats (100-120 g bw) caused death and clinical signs of toxicity, including apnoea, ataxia and paraparesis. These symptoms were very marked in the absence of methionine supplementation; fifty percent of the rats died within 4 h. In the presence of adequate methionine supplementation, 10% of the rats died and about 60% showed clinical signs of toxicity. The activity of Na+K+ dependent ATPase was reduced in much the same way as it was by the glycoside, digitalis (reviewed by Oke, 1980).

In a developmental study linamarin was administered once to presumed pregnant hamsters (10-13/group) by oral gavage at 0, 70, 100, 120 or 140 mg/kg bw on day 8 of gestation. The day after mating was considered to be day 1 of gestation. The number of actually pregnant hamsters that survived to the day of sacrifice (day 15) was 11/11, 8/11, 9/10, 9/10 and 10/11 at 0, 70, 100, 120 or 140 mg/kg bw respectively.

Clinical signs that included dyspnoea, hyperpnoea, ataxia, tremors, and hypothermia were observed within 1 hour after dosing. The signs of poisoning were greatly reduced or absent three hours after treatment. No relationship between the duration of clinical signs and dose was observed.

Clinical signs occurred at an incidence of 1/11 (9%), 4/10 (40%), 5/11 (45%) and 9/13 (69%) at 70, 100, 120 and 140 mg/kg bw respectively. One hamster at 120 mg/kg bw (9%) and two at 140 mg/kg bw (15%) died within 2 hours of dosing. Dams at 120 mg/kg bw but not 140 mg/kg bw had reduced bodyweight gain (20%) between day 8 and 15 of gestation. A dose of 120 or 140 mg/kg bw of linamarin was associated with an increased incidence of vertebral and rib anomalies. Linamarin treatment had no effect on fetal body weight, ossification of fetal skeletons, embryonic mortality, or litter size (Frakes *et al.*, 1985).

In a toxicokinetic study, in which 22 female hamsters were dosed once with 0.44 mmol/kg bw linamarin (108 mg/kg bw) by oral gavage, four (18%) died at least two hours after treatment. The majority of the hamsters (incidence not reported) treated with linamarin or amygdalin showed signs of cyanide poisoning, such as dyspnoea, hyperpnoea, ataxia, tremors and hypothermia. For amygdalin 4/20 (20%) dosed hamsters died (Frakes *et al.*, 1986a).

Cyanides

Acute toxicity

Following oral administration to rats, LD_{50}s of hydrogen cyanide, sodium cyanide, and potassium cyanide are very similar: 0.156, 0.117, and 0.115 mmol/kg bw, respectively, i.e., 3–4 mg cyanide/kg bw (Ballantyne, 1983). In mice, an LD_{50} of 15.8 mg potassium cyanide (corresponding to 6 mg cyanide)/kg bw has been reported (Ferguson, 1962).

In rabbits, hydrogen cyanide, potassium cyanide, and sodium cyanide appear equitoxic on a molar basis (LD_{50}s of 0.092, 0.104, and 0.090 mmol/kg bw for hydrogen cyanide, sodium cyanide, and potassium cyanide, respectively); rabbits appeared to be somewhat more susceptible to cyanides than mice or rats (Ballantyne, 1983).

Administration of 1 mg KCN/kg bw to male Sprague-Dawley rats resulted in a C_{max} of 6.2 nmol CN^-/mL blood and an elimination half life of 14 min and no clinical signs (Leuschner *et al*., 1991). In contrast a lethal dose in rats corresponds to a cyanide concentration in blood of 100-112 nmol CN^-/mL blood (Egekeze & Oehme, 1979).

In dogs the median lethal dose was 2 mg cyanide/kg bw whereas in mice it was 6 mg cyanide/kg bw with potassium cyanide (Conn, 1979). Three dogs who were orally treated with 20, 50 or 100 mg of potassium cyanide (doses expressed as HCN) died 155, 21 and eight minutes later respectively. Gettler & Baine (1938) estimated that a lethal oral dose in dogs to be 1.06-1.4 mg/kg bw.

Short-term toxicity

Mouse

In a 13-week toxicity study, ten B6C3F1 mice/sex/group were dosed with 0, 3, 10, 30, 100 or 300 mg/L sodium cyanide (NaCN)/day in their drinking water.

The achieved doses were 0, 0.5, 1.8, 5.1, 16.2 and 45.9 mg NaCN/kg bw/day respectively in males and 0, 0.6, 2.1, 6.2, 19.1 and 54.3 mg NaCN/kg bw/day respectively in females. The final mean body weights of males and females in the 3 mg/L groups and males in the 30 mg/L group were slightly greater than those of the controls, the final mean body weight of females exposed to 300 mg/L was reduced relative to the control. No treatment related clinical signs were noted. Water consumption by males and females in the 100 and 300 mg/L groups was lower than that of the controls. Differences in absolute and relative organ weights of male and female mice were sporadic and not considered related to treatment. A few changes in haematology or clinical chemistry were observed; these were minimal and were not considered to be biologically significant. There were no treatment-related gross or histopathological lesions in mice of either sex. Cyanide treatment caused a slight reduction in the cauda epididymal weight of mice in the 300 mg/L group compared with the controls and sperm motility was marginally reduced in the treated mice (NTP, 1993).

In male mice the only effect with toxicological significance was slightly reduced cauda epididymal weight and reduced sperm motility at the highest dose level of 46 mg NaCN/kg bw/day. Based on these effects, a NOAEL of 16 mg NaCN/kg bw/day can be derived, corresponding to 8.5 mg CN^-/kg bw/day. In a 13-week study potassium cyanide (KCN) was administered to groups of male Sprague-Dawley rats (30/group) in their drinking water. The nominal dose levels were 40, 80, or 160/140 mg KCN/kg bw/day. Three control groups, one given normal drinking water *ad libitum*, a 'paired drinking' group (parallel to the high dose level KCN) and a third group receiving drinking water with 10% ethyl alcohol were used. In addition another group received drinking water with KCN (80 mg/kg bw/day) and 10% alcohol. Behaviour, external appearance, body weight, food consumption (daily) and drinking water consumption (twice weekly) were recorded frequently. Extensive haematological, clinical chemical (in serum) and urine analyses were carried out in five animals per group in week 6 and week 13. Autopsy and macroscopy were performed after 13 weeks (20/group) and 11 organs were weighed. Histopathological examination was performed in brain, kidneys, heart, liver and testes of these animals. In addition, thyroids of the control, the 'pair drinking' control and the high dose group (160 mg/kg bw/day for 11 weeks, 140 mg/kg bw/day from week 12 because of observed reduced body weight gain, reduced water consumption and mortality) were examined.

There was a clear indication that reduced food consumption and body weight in the KCN groups were caused by a decrease in water consumption due to a decreased palatability. Urinalyses revealed a dose related higher level of protein for the animals receiving KCN. Several changes in absolute organ weights were seen in the 160/140 mg KCN/kg bw group. Relative weights of organs were very slightly increased in the 40 mg, slightly increased in the 80 mg and clearly increased in the 160/140 mg KCN/kg bw groups. The thymus weight was, however, reduced in the high dose group. Histopathological examination revealed no indication of damage to the brain, heart, liver, testes, thyroids or kidneys due to treatment with KCN (Leuschner *et al*., 1989 as reported in JECFA, 1993). In the study described above the actual administered doses were 38.4, 78.3 and 143.9 mg/kg bw (Leuschner *et al*., 1991). At the highest dose, blood cyanide concentrations were between 16 and 26 nmol CN^- /mL blood and thiocyanate ranged between 341 and 877 nmol SCN^-/mL plasma.

In another 13-week study that was similar to the previous NTP study in mice ten F344/N rats/sex/group were dosed with 0, 3, 10, 30, 100 or 300 mg/L sodium cyanide (NaCN)/day in their drinking water.

The achieved doses were 0, 0.3, 0.9, 2.7, 8.5 and 23.6 mg NaCN/kg bw/day respectively in males and 0, 0.3, 1.0, 3.2, 9.2 and 23.5 mg NaCN/kg bw/day respectively in females. The final mean body weights and body weight gains of males in the 10 and 30 mg/L groups were slightly less than those of the controls. There were no apparent differences between the final mean body weights and body weight gains of exposed and control females. No clinical signs attributable to NaCN exposure were observed. In the 100 and 300 mg/L dose groups of both males and females, water consumption was reduced by more than 10% compared with the control groups. Decreases in urine volume and increases in urine specific gravity occurred in supplemental rats in the 300 mg/L group at all time points and in the 100 mg/L group on day 8. These changes were consistent with the observed decreases in water consumption and with subsequent decreases in urine output, suggesting a palatability problem. Changes in haematology were minor and sporadic and were not considered to be clinically significant. Increases in urinary thiocyanate occurred in treated rats at all but the 3 and 10 mg/L exposure levels on days 22 and 88 and all but the 3 mg/L exposure level on day 43.

Changes in urinary pH, sorbitol dehydrogenase, and N-acetyl-β-D-glucosaminidase were minor and not exposure related; these changes were not considered to be clinically significant. There were no treatment-related gross or histopathological lesions in rats of either sex. Sodium cyanide treatment caused a slight reduction in the cauda epididymal weight of treated male rats (up to 13%). The number of sperm heads per testis was reduced by 13.6% in the 300 mg/L group compared with the controls and sperm motility was marginally reduced in treated animals. The authors suggest that sub-chronic exposure to low dose of cyanide may produce mild, but potentially biologically significant, adverse effects on the male reproductive tract (NTP, 1993).

Forty-six male adult inbred Wistar rats were used in four experimental groups and one control group and treated with 0, 0.3, 0.9, 3, or 9 mg potassium cyanide/kg bw/day in the drinking-water for 15 days. This was equivalent to 0, 0.12, 0.36, 1.2, and 3.6 mg cyanide/kg bw/day. The high-dose group exhibited a 70% lower body weight gain than the control animals. In qualitative histological analysis, without statistical treatment or morphometric analysis, changes were observed in the kidney, liver, and thyroid. Cytoplasmic vacuolation, considered to reflect hydropic degeneration of proximal tubular epithelial cells, was noted in animals treated at doses of 3 and 9 mg potassium cyanide/kg bw/day and in hepatocytes of those animals treated at a dose of 9 mg potassium cyanide/kg bw/day. A dose-dependent increase in the number of re-absorption vacuoles on follicular colloid in the thyroid gland was noted in all animals of the experimental groups. No changes were observed in serum tri-iodothyronine (T3), thyroxine (T4), creatinine, or urea levels; a decrease was observed in serum alanine aminotransferase (ALAT) activity at the two lowest exposure levels.

Serum aspartate aminotransferase (ASAT) was elevated by 30% at the two lowest dose levels and by 21% at the 3 mg potassium cyanide/kg bw/day dose; it was decreased by 29% at the highest dose level (Sousa *et al.,* 2002; ATSDR, 2006).

However, the reliability of the hepatic, renal, and body weight changes are questionable because of the lack of incidence data for the histopathological lesions and because no body weight changes were observed in other rat studies with exposures for longer durations and at higher doses.

Long-term toxicity

Two groups of 20 rats (10 males and 10 females) were fed a diet fumigated with hydrocyanic acid, containing residual hydrocyanic acid in the concentration of 100 and 300 ppm, for two years. Another group of 20 rats was fed a control diet. Because of the volatility of hydrocyanic acid, the diets were prepared every other day, and analysed at the beginning and end of each two-day period. Growth, food consumption and survival in both groups were comparable. Haematological values determined initially and at the end of the experiment appeared to be within normal limits. Organ-body-weight ratios for the liver, kidneys, spleen, brain, heart, adrenals and testes or ovaries did not show any substantial differences from controls. Histological examination of tissues was carried out for the heart, lung, liver, spleen, stomach, small and large intestines, kidneys, adrenals, thyroid, testes or uterus and ovary, and the cerebrum and cerebellum of the brain. In the tissues examined no changes due to hydrogen cyanide feeding were found. At the end of the experiment the amount of free cyanide and thiocyanate in blood, liver and kidney was determined.

In the group fed 100 ppm hydrocyanic acid, free cyanide was found only in red blood cells with an average of 5.40 µg per 100 ml, thiocyanate was found in plasma with an average of 936 µg per 100 ml, in the liver and kidney 719 and 1023 µg per 100 g of tissue, respectively.

In the group fed 300 ppm hydrocyanic acid, free cyanide was found in the liver of one rat and in the erythrocytes of less than 50% of animals (average 1.97 µg per 100 g tissue). Average values for thiocyanate in plasma and erythrocytes were 1123 and 246 µg per 100 mL, respectively, in the liver and kidney 665 and 1188 µg per 100 g tissue, respectively. The average thiocyanate values in the controls were as follows: plasma 361 µg, red blood cells 73 µg per 100 mL; liver 566µg, kidney 577 µg per 100 g (Howard & Hanzal, 1955; reported in JMPR, 1965; US EPA, 1993).

Genotoxicity

Cyanide

Two negative and one marginally positive bacterial mutagenicity studies have been reported. Hydrocyanic acid was not mutagenic in *Salmonella typhimurium* strains TA 1535, TA 1537, TA 1538, TA 98, and TA 100 with or without metabolic activation (De Flora, 1981, cited in USEPA, 1993).

One study reported marginal mutagenic activity of hydrocyanic acid to *S.typhimurium* strain T 100 in the absence of metabolic activation but no mutagenic activity to strain TA 98 with or without metabolic activation (Kushi *et al*., 1983, cited in USEPA, 1993).

Potassium cyanide did not induce gene mutations at the HGPRT-locus in cultured Chinese hamster V79 cells both in the presence or absence of metabolic activation up to high, cytotoxic concentrations (Leuschner *et al*, 1989).

One *in vivo* chromosomal aberration assay was carried out in Chinese hamsters treated orally by gavage with a single dose of 0.4 mg hydrocyanic acid/kg bw, with three sampling times of 6, 24 and 48 hours after the treatment. There was no indication of clastogenic activity relative to structural chromatid or chromosome damage (Leuschner *et al*, 1983).

Reproductive and developmental toxicity studies

Rat

In a one generation reproduction study, albino female rats (10/group) were fed *ad libitum* respectively: a normal laboratory diet (5% carbohydrate, 21% protein, 4% fat, 3.5% fibre, 0.098% calcium, 0.0025% iron), a 50 % gari diet (Nigerian preparation of cassava), a raw cassava diet (content: 35.7% carbohydrate, 1.2% protein, 0.2% fat, 1.1% fibre, 0.0068% calcium, 0.0019% iron, 62% moisture, 0.36-2.5% hydrogen cyanide), a diet containing 5 g KCN/100 g laboratory diet and a diet containing 10 g KCN/100 g laboratory diet. After two weeks each group was mated with five adult males fed on normal diet.

Pregnant rats from each group were maintained on their respective diets. After littering the newborn rats were studied for postnatal development. After 21 days, F1 rats were put for another 4 weeks on their respective diets. The offspring of the rats fed the 50 % gari diet had significantly lower birth weights and brain weights and never attained the same adult weights as those of the controls. The adult female rats fed a diet consisting entirely of raw cassava had significantly reduced haematological and biochemical parameters, haemoglobin, packed cell volume, serum protein and thyroxine concentration). An increased incidence of cannibalism was observed together with a significant reduction in the frequency of pregnancy, in the averaged number of the litter and in birth weights. In addition there was an increased incidence of neonatal deaths among the offspring, which also had poor development, reduced brain weights and an increased tendency of aggression towards their littermates. Adult female rats fed diets containing 5 and 10 g KCN/100 g laboratory diet survived for more than three months but never became pregnant. They developed enlarged thyroid glands and tumours of the large intestine. The usual content of cyanide in cassava varies from 70 to 500 mg /kg which is much less than the levels used in these experiments. According to the authors the rats were able to cope with the 50% gari diet and detoxify the glycoside present (Olusi *et al*., 1979).

A short-term reproduction study (49 day study in adults and 28 day study in pups) was performed to evaluate the effects in pregnant rats of adding 500 mg KCN/kg to a cassava root flour-based diet prepared from a low-HCN cassava variety (21 mg HCN/kg feed). A control group received only the basal. The high dietary level of KCN did not have any marked effect on gestation and lactation performance of female rats. No carry-over effect of high cyanide-containing diet fed during gestation was observed on lactation performance.

The high cyanide-containing diet, however, significantly reduced feed consumption and daily growth rate of the offspring when fed during the post-weaning period. Protein efficiency ratio was not only reduced by the cyanide diet during the post-weaning growth phase but there was a carry-over effect from gestation. Serum thiocyanate was significantly increased in lactating rats and their offspring during lactation and in the post-weaning growth phase of the pups. No apparent carry-over effect was noticed on this parameter. Rhodanese activity in liver and kidneys was unaffected by feeding the high cyanide diet during gestation, lactation, and or during post-weaning growth (Tewe & Maner, 1981).

Hamster

In a teratogenic study groups of pregnant hamsters (eight dams/group) were fed diets consisting of cassava meal:laboratory chow (80:20) during days 3-14 of gestation. One low cyanide (sweet) cassava meal and one high cyanide (bitter) cassava meal were studied. An additional group was fed a diet which resembled cassava in nutritional value, but which lacked cyanogenic glycosides. Thiocyanate concentrations in the urine and blood of dams fed cassava diets increased significantly. Increased tissue thiocyanate concentrations were observed in foetuses recovered from cassava-fed dams. Cassava-fed dams gained significantly less weight than did control animals and their offspring showed evidence of fetotoxicity. Reduced foetal body weight and reduced ossification of sacrocaudal vertebrae, metatarsals and sternebrae were associated with cassava diets.

High cyanide cassava diets were also associated with a significant increase in the numbers of runts compared to litters from dams fed either low protein or laboratory stock diets (Frakes et al., 1986b).

Pregnant golden hamsters (LKV strain) were exposed to sodium cyanide on days 6 to 9 of gestation by infusion via subcutaneously implanted osmotic mini-pumps of NaCN at doses equivalent to 0, 6.17, 6.25 or 6.34 mg NaCN/kg bw/h. Cyanide induced high incidences of resorption and malformations in the offspring. Although signs of toxicity (e.g. weight loss, ataxia, dyspnoea) were apparent in some animals, the occurrence of malformations in the offspring was not statistically correlated either with weight loss or with poor health of the dams. The most common abnormalities observed were neural tube defects (Doherty *et al*.,1982).

Human data

Acute toxicity

Linamarin

Cyanide poisoning by ingestion of foods containing cyanogenic glycosides, such as linamarin, seems to occur very rarely in countries where such foods are not a major component of the diet, but it is reported more frequently for children in tropical countries, where such foods form an important part of the diet.

Numerous cases of acute cyanide poisoning after ingestion of inadequately processed cassava have been reported in children in tropical countries (Dawood, 1969; Cheok, 1978; Akintonwa, 1992; Arrifin, 1992; Espinoza *et al.*, 1992; Ruangkanchanasetr *et al.*, 1999).

Cassava poisoning in adults has also been reported, but paediatric poisoning may be more frequent and severe. Children seem to be more susceptible than adults to poisoning by ingestion of cyanogenic foods including cassava often developing more severe toxicity than adults concurrently ingesting cassava. The apparently greater vulnerability of children to poisoning by cyanogenic foods has been attributed to children's lower body mass (Geller *et al.*, 2006).

Cyanides

In humans an average fatal dose of hydrocyanic acid has been calculated from case report studies of intentional or accidental poisonings to be 1.4 mg/kg bw (range 0.58-3.4 mg/kg bw and corresponding to 1-7 mg/kg bw of potassium cyanide) (Montgomery, 1969; Gosselin *et al*., 1976).

Based on analyses of hydrocyanic acid content in tissues and in gastrointestinal tract contents among fatal (oral) poisoning cases (and comparative kinetics with dogs), it was estimated that death occurred after absorption of an average of 1.4 mg hydrocyanic acid/kg bw (Gettler & Baine, 1938); the lowest fatal absorbed dose was 0.58 mg hydrocyanic acid/kg bw. In most poisoning cases, a large part of the ingested cyanide remained in the gastrointestinal tract (thus, using the dose ingested as an indicator of the lethality of hydrocyanic acid can be misleading). Some individuals ingesting between 1-3 g (16-50 mg/kg bw) of cyanide salts have survived. The clinical signs and symptoms of acute cyanide toxicity include headache, dizziness, mental confusion, stupor, cyanosis with twitching and convulsions, followed by terminal coma.

Epidemiology

There have been no adequately reported long term controlled studies in laboratory animals with hydrocyanic acid or linamarin. However, adverse effects noted in humans from long-term exposure to cyanide by dietary intake is estimated to be potentially of major significance for cassava-consuming populations; cassava has been estimated to be the staple food for 500 million people. However, data on the concentrations of cyanides in the total diet are lacking; hence, the daily cyanide intake from food cannot be calculated. For human consumption, cassava can be eaten raw, cooked, or grated and roasted into flour and eaten as 'gari,' which is the common form in Nigeria (Kendirim et al., 1995). In Mozambique, it was estimated that in families affected by the 'mantakassa' disease (spastic paraparesis), the daily intake of cyanogens was 14-30 mg (as cyanide) at the time of a mantakassa epidemic in 1981 (Ministry of Health, Mozambique, 1984). In Nigeria, it was estimated that the intake of hydrogen cyanide in the tropical ataxia-endemic areas may be as high as 50 mg/day (Osuntokun, 1981).

Urinary excretion of thiocyanate has been applied in the biological monitoring of exposure to cyanogenic glycosides, especially among cassava-consuming populations. The average urinary thiocyanate concentration among children in the Bandundu region of the Democratic Republic of the Congo (formerly Zaire) was 757 µmol/L in the south and 50 µmol/L in the north (both populations consumed cassava as their staple diet, but the cassava was well processed in the north and inadequately processed in the south). These concentrations can be compared with an average of 31 µmol/L in a non-smoking Swedish reference population (Banea-Mayambu *et al.,* 2000). In the same Bandundu region, it was shown that there was a marked seasonal variation in urinary thiocyanate concentrations in the villages with a high 'konzo' (spastic paraparesis) incidence (563- 627 µmol/L in the dry season and 344-381 µmol/L in the wet season), while the average in non-konzo areas was 241 µmol/L (Banea-Mayambu *et al.,* 1997). In Mozambique, the average urinary thiocyanate levels among healthy children from areas with epidemic spastic paraparesis varied between 33 and 1175 µmol/L, whereas levels in areas with no paraparesis were between 18 and 400 µmoL/litre (Casadei *et al.,* 1990). In Nampula province in Mozambique, where spastic paraparesis epidemics had been observed in 1981-1982 and during the civil war in 1992-1993, average urinary thiocyanate concentrations among schoolchildren in five areas were between 225 and 384 µmol/L in October 1999 (Ernesto *et al.,* 2002). In Malawi, in an area where cassava was typically soaked for 3-6 days for processing to flour, urinary thiocyanate concentrations were between 2 and 410 µmol/L, with a median of 32 µmol/L (Chiwona-Karltun *et al.,* 2000).

Iodine deficiency and goitre, hypothyroidism, and cretinism are endemic in many areas of Africa. Several surveys in the endemic areas have demonstrated that there is also a strong correlation between cassava consumption and the thyroid effects (JECFA, 1993; Abuye *et al.,* 1998).

A study in rural Mozambique found that in a population suffering from endemic spastic paraparesis, adequate iodine intake mitigated against the development of hypothyroidism or goitre, and high levels of dietary cyanogenic glycosides from cassava could be tolerated (Cliff *et al.,* 1986).

Originally based on a geographical link between the prevalence of diabetes and cassava consumption (McGlashan, 1967), dietary exposure to cyanides has been linked to the malnutrition-related diabetes mellitus (WHO, 1985), also known as the 'type-J' or 'type-Z' diabetes (Hugh-Jones, 1955; Zuidema, 1959). The very existence of this third type of diabetes (in addition to the juvenile-onset and maturity-onset types) has been controversial (Gill, 1996), and not all studies have detected a relationship between cassava consumption and diabetes prevalence (Cooles, 1988; Swai *et al.,* 1992). The results of the standard glucose tolerance test were no more often abnormal among 88 Nigerian patients with tropical neuropathy than among 88 referents (Famuyiwa *et al.,* 1995).

Discussion

Cyanogenic glycosides, such as linamarin, are not particularly hazardous when administered intravenously (LD_{50} = 20,000 mg/kg bw; Oke, 1979) because no cyanide is liberated. However, once ingested the LD_{50} is reported to be around 450 mg/kg bw in rats (or 51 mg/kg bw hydrocyanic acid equivalents) (Oke, 1979).

Cyanogenic glycosides such as linamarin are relatively non-toxic in germ free animals.

Following oral administration, a proportion (20-30%) of ingested linamarin is readily absorbed and eliminated unchanged from the body in urine (Barrett *et al.*, 1977; Hernandez *et al.*, 1995; Carlsson *et al.*, 1999). There is some indirect evidence that the uptake of linamarin from the GI tract may be mediated through the glucose transporter receptor (Sreeja *et al.*, 2003). The remaining, unabsorbed, linamarin (70-80%) then undergoes enzymatic hydrolysis by micro-organisms in the digestive tract, predominantly the colon (Frakes *et al.,* 1986a; Carlsson *et al.*, 1999). In a two step process ß-glucosidase from the colonic micro-organisms hydrolyzes the glycosidic bond linking the glucose to the α-hydroxynitrile (cyanohydrin) in linamarin; then hydrocyanic acid is dissociated from acetone cyanohydrin either non-enzymatically or through the action of another enzyme, a hydroxynitrile lyase.

When simple cyanide salts such as potassium and sodium cyanide are ingested, free cyanide ion can rapidly bind hydrogen ion to form hydrocyanic acid in the highly acidic medium of the stomach. The lower the pH in the stomach, the more hydrocyanic acid is released as gas from ingested salts. Essentially all cyanide ingested as cyanide salts will form hydrocyanic acid and will be quickly absorbed. However, only part of the dose reaches the blood due to first-pass metabolism by the liver (ECETOC, 2004). Hence, the form of cyanide to which exposure occurs, the salt or the free acid, does not influence distribution, metabolism, or excretion from the body (ECETOC, 2004). The major portion of cyanide in blood is sequestered in the erythrocytes, and a relatively small proportion is transported via the plasma to target organs. Low levels of cyanide are found in normal blood plasma (<140 µg/L) and other tissues (<0.5 mg cyanide/kg) in humans without any known occupational cyanide exposure (Feldstein & Klendshoj, 1954).

Hydrocyanic acid toxicity is mediated primarily by its high affinity for the ferric moiety of cytochrome c oxidase in mitochondria, a key component in oxidative respiration.
This reversible interaction blocks the last stage in the electron transfer chain, resulting in cellular hypoxia and a shift of aerobic to anaerobic cellular respiration, leading to cellular ATP depletion, lactic acidosis and cell and tissue death.

Metabolism of hydrocyanic acid primarily involves its conversion to soluble thiocyanate (SCN^-) by the enzyme rhodanese (thiosulphate sulphotransferase), with about 80% of cyanide detoxified by this route. This requires sulphane-sulphur as a co-factor i.e. one sulphur atom bonded to another sulphur atom such as in a thiosulphate salt (e.g. sodium thiosulphate). This conversion is irreversible and the thiocyanate ion may then be readily excreted in the urine. Cyanide is therefore unlikely to accumulate in human tissues after chronic oral exposure. Acute toxicity results when the rate of absorption of hydrocyanic acid is such that the metabolic detoxification capacity of the body is exceeded; typically this occurs following ingestion of a single dose.

Clinical manifestations of acute cyanide poisoning, especially non-lethal doses are often non-specific and mainly reflect oxygen deprivation of the heart and brain. Typically these effects include headaches, dizziness, stomach pain, or mental confusion.

The acute toxicity of hydrocyanic acid in mouse, rat, rabbit and dog are quite similar with the median oral lethal doses (50% death) estimated to be 3-4 mg cyanide/kg bw in rats and rabbits. In dogs the median lethal dose was 2 mg cyanide/kg bw whereas in mice it was 6 mg cyanide/kg bw with potassium cyanide (Conn, 1979). Based on analyses of cyanide contents in tissues and in gastrointestinal tract contents from fatal poisoning cases (and comparative kinetics with dogs), Gettler & Baine (1938) estimated that death in cases of suicide occurred after absorption of an average of 1.4 mg hydrocyanic acid/kg bw; the lowest fatal absorbed dose was 0.58 mg hydrocyanic acid/kg bw. However, the oral lethal dose of hydrocyanic acid in the four cases of suicide reported by Gettler & Baine which were calculated from the total amount of hydrocyanic acid absorbed in the body at the time of death, and from the amount of hydrocyanic acid found in the digestive tract, differed considerably (calculated as mg hydrocyanic acid): 1450 (62.5 kg bw), 556.5 (74.5 kg bw), 296.7 (50.7 kg bw), and 29.8 (51 kg bw). This corresponds to doses varying from 0.58 mg/kg bw to 23 mg/kg bw

Indications of teratogenicity in offspring from hamsters treated with 120 or 140 mg/kg bw linamarin (equivalent to 13.1 and 15.3 mg hydrocyanic acid/kg bw, respectively) on day 8 of gestation were only observed at maternally toxic doses.

Experimental data on chronic toxicity and carcinogenicity are not available. Overall, the mutagenicity tests conducted with hydrocyanic acid and cyanides at gene and/or chromosome level did not reveal a genotoxic potential.

Cyanide poisoning by ingestion of foods containing a cyanogenic glycoside such as cassava seems to occur very rarely in regions where they do form major components of the diet, but it is reported more frequently in children in tropical countries, where such foods are more important parts of the diet. Numerous cases of acute cyanide poisoning after ingestion of cassava have been reported in children in tropical countries (Dawood, 1969; Cheok, 1978; Akintonwa, 1992; Arrifin, 1992; Espinoza *et al.*, 1992; Ruangkanchanasetr *et al.*, 1999). Children seem to be more susceptible than adults to poisoning by ingestion of cyanogenic foods such as cassava and often developing more severe toxicity than adults concurrently ingesting cassava. The apparently greater vulnerability of children to poisoning by cyanogenic foods is likely to be due to their lower body mass.

Long-term consumption of cassava containing high levels of cyanogenic glycosides, usually when constituting the principal source of calories, and associated with malnutrition and protein and vitamin deficiencies, has been associated with neurological diseases involving tropical ataxic neuropathy and endemic spastic paraparesis. In areas with low iodine intake, development of hypothyroidism and goitre, sometimes accompanied by the neurological diseases, has also been linked to cassava.

While daily cyanide exposure has been estimated to be 15–50 mg/day in endemic areas, owing to the limitations of data on exposure, which is likely to be quite variable, and the potential impact of confounders, such as general malnutrition, low protein content of the diet, and iodine status, the available data do not provide meaningful information on a dose-response for cyanide.

The available evidence on Konzo indicates that there are many confounding factors, and whilst cyanide intake may contribute it is likely to be one of a number of possible causal factors specific to a high cassava diet.

Toxicological evaluation

The toxicological database to characterize the toxicity of linamarin and its metabolite, hydrocyanic acid, in laboratory animals and humans is very limited. While there are numerous studies which assess the acute or short term toxicity of linamarin and hydrocyanic acid there are no suitable quantitative studies which examine the effects of long-term repeat dosing or its carcinogenic potential. There is no available evidence in adequately nourished humans to show that chronic intake of cyanogenic glycosides causes a cumulative hazard above that of repeated acute toxicity. *In vitro* and *in vivo* genotoxicity tests were negative. There are no human or animal data available on the carcinogenicity of cyanide. The International Agency for Research on Cancer (IARC) has not considered hydrocyanic acid. The US EPA considers that it is not classifiable as a carcinogen (US EPA, 1993). Teratogenic and adverse reproductive effects attributable to linamarin and hydrocyanic acid were seen only at doses that also caused maternal toxicity.

Acute Reference Dose

In a developmental study a single dose of linamarin administered by gavage to hamsters on day 8 of gestation identified a possible NOAEL of 70 mg/kg bw (Frakes *et al.*, 1985). This study investigated the teratogenic potential after a single dose of 70, 100, 120 or 140 mg/kg bw linamarin on day 8 of gestation. Although no deaths were observed at the next higher tested dose of 100 mg/kg bw in the teratogenicity study a follow-up toxicokinetic study by the same investigators using a larger number of non-pregnant hamsters revealed that deaths and clinical signs occurred at 108 mg/kg bw (Frakes *et al.*, 1986a). This information casts doubt on whether 70 mg/kg bw may also not be 'true' NOAEL because a larger number of hamsters per group may reveal a significant incidence of clinical signs.

Using a NOAEL of 70 mg/kg bw and applying of 100-fold safety factor gives an acute reference dose (ARfD) of 70/100 = 0.7 mg linamarin/kg bw. A 100-fold safety factor was applied to account for intra-species variability in sensitivity and an inter-species extrapolation.

Characteristics of the toxicological profile of linamarin which may support the use of a chemical specific adjustment factor (*e.g.* a safety factor of 3-5 instead of 10 for interspecies variability) (IPCS, 2005) include; concordance of the toxicological endpoint with the maximum concentration (C_{max}) of hydrocyanic acid in plasma. The maximum concentration (C_{max}) varies less within species than the AUC (area under the curve).

Although the toxicokinetics of linamarin indicated that the released hydrocyanic acid which resulted in clinical signs and death was related to C_{max} in plasma a reduction in the safety factor was not considered appropriate because of the uncertainty that 70 mg/kg bw was a true NOAEL and the closeness of the NOAEL to a lethal dose (108 mg/kg bw).

Additional support for use of the hamster as a relevant surrogate for human risk assessment comes from the observation that, in adult humans, the blood cyanide level which is regarded as 'toxic' and causing clinical signs following acute exposure is generally considered to be ≥ 1 mg/L (39 µmol/L), whereas a 'fatal' concentration is generally considered to exceed 2.6–3 mg/L (100–115 µmol/L) (Geller *et al.*, 2006). These concentrations which are considered lethal in humans show remarkably good agreement with the levels which caused death in hamsters after an oral dose of 0.44 mmol linamarin/kg bw. The cyanide concentration in plasma following linamarin treatment reached a maximum of 116 µmol/L.

The ARfD of 0.7 mg linamarin/kg bw is also supported by the absence of any adverse effects in volunteers following the dietary ingestion of linamarin in a cassava-based porridge at doses ranging between 1–2.5 mg linamarin/kg bw (Carlsson *et al.*, 1999). This metabolic fate study in humans was not suitable to establish an ARfD because the inadequate range of clinical parameters measured and reported. Moreover the use of the highest tested dose in this volunteer study would result in a lower ARfD once a 10-fold safety factor for intra-species variability has been applied. Clinical signs and symptoms of acute cyanide toxicity in humans are subtle and studies designed to monitor effects need protocols which include the monitoring of headaches, dizziness, stomach pain, or mental confusion. The Carlsson *et al.*, study does not indicate if such monitoring took place.

Conversion of linamarin to hydrocyanic acid concentration equivalents

The available data indicates that no unchanged linamarin is excreted in the faeces following oral ingestion suggesting that there is sufficient enzymatic capacity in the microflora of the caecum to completely hydrolyse large amounts of linamarin. Since 1 mole of linamarin can release 1 mole of hydrocyanic acid if hydrolysis is complete it follows that one gram of linamarin (MW = 247) could liberate a maximum of 109.3 mg of hydrocyanic acid (MW = 27). Using this conversion factor the calculated linamarin ARfD would be equivalent to 0.08 mg hydrocyanic acid/kg bw. Gavage administration in laboratory animals has often been questioned as to its relevance for human exposure because it results in a bolus dose in the GI-tract, whereas human exposure through the diet will show a more gradual exposure within a certain time frame. For most compounds, however, gavage administration might be considered as a 'worst case' acute exposure relative to dietary exposure.

Reviews by other regulatory agencies

Acute Reference Dose

In order to establish a health standard to determine a safe dose for bitter apricot kernel consumption the UK Committee on Toxicity reported that the database for the toxicity of cyanide and cyanogenic glycosides in humans was incomplete. It acknowledged that the reported acute lethal oral dose for cyanide in humans was in the range 0.5-3.5 mg/kg bw. They applied a 100-fold safety factor (10 to account for inter-individual variability and 10 to extrapolate from an effect level to a no effect level, taking into account the steep dose-response relationship) to the lowest lethal dose (0.5 mg/kg bw). This indicated that a dose of 0.005 mg/kg bw would be unlikely to cause acute effects, i.e. a 'nominal' acute reference dose (ARfD).

It is worth noting that the estimated lethal hydrocyanic acid dose (0.5 mg/kg bw) used to establish this 'nominal' acute reference dose is based on only one individual who ingested an unspecified cyanide salt preparation to commit suicide (Gettler & Baine, 1938).

Nevertheless, there is little doubt that this health standard would be protective but it may be overly conservative because it takes no account of the different toxicokinetics for amygdalin (the cyanogenic glycoside present in apricot kernels) that involves bacterial enzymatic conversion to hydrocyanic acid once ingested. In contrast ingested hydrocyanic acid is rapidly absorbed unchanged from the GI tract.

In a toxicokinetic study by Frakes *et al.,* (1986a) 4/20 hamsters orally dosed with 0.44 mmol/kg bw amygdalin (201 mg/kg bw) died. The blood cyanide concentrations following amygdalin treatment reached their highest level (130 nmol/mL) 1 hour after dosing and remained elevated until 3 hours after treatment. This cyanide concentration in blood is similar to that achieved following oral administration of 0.44 mmol/kg bw linamarin (108 mg/kg bw), namely 116 µmol/L and also corresponds closely with concentrations in blood known to be lethal in humans i.e. 100–115 µmol/L (Geller *et al.*, 2006). This suggests that the dose of hydrocyanic acid considered to be protective for linamarin exposure, namely 0.08 mg/kg bw, would also be protective for any exposure to amygdalin as well.

Provisional Tolerable Daily Intake

Table 1 shows PTDI levels which have been established by several regulatory agencies. The PTDI values range from 0.02 mg/kg bw/day to 0.108 mg/kg bw/day.

Table 1: Health Standards (PTDI) established by other regulatory agencies

Organisation*	Year	NOAEL study	NOAEL (mg HCN/kg bw/day)	PTDI (mg HCN/kg bw/day)
JMPR	1965	Two-year rat study; (Howard & Hanzal,1955)	5	0.05
US EPA	1993	Two-year rat study; (Howard & Hanzal,1955)	10.8	0.108
JECFA	1993			No suitable data
EFSA	2004	-	-	available to establish
UK COT	2006			PTDI
CoE	2000	Several epidemiological studies	0.19	0.02
CoE	2005	Three-month rat study; (NTP, 1993)	4.5	0.023
ATSDR	2006	Three-month rat study; (NTP, 1993)	5	0.05
WHO	2004	Six-month study in pigs. (Jackson, 1988)	1.2	0.012
WHO	2007	Three-month rat study; (NTP, 1993)	4.5	0.045

* JMPR, Joint FAO/WHO Meeting on Pesticide Residues; US EPA, US Environmental Protection Agency; JECFA, Joint FAO/WHO Expert Committee on Food Additives; EFSA, European Food Safety Authority; UK COT, UK Committee on Toxicity; CoE, Committee of Experts on Flavourings of the Council of Europe; ATSDR, Agency for Toxic Substances and Disease Registry; WHO, World Health Organization.

Although there have been several epidemiological studies performed among cassava-eating populations, which identify an association between cyanide exposure and spastic paraparesis, amblyobia ataxia or tropical ataxia neuropathy and possibly goitre, none are considered adequate to establish a numerical NOAEL for chronic exposure in humans. This is because they are highly confounded by other nutritional and environmental factors. However, in 2000 the Committee of Experts on Flavourings of the Council of Europe did establish a Temporary Maximum Daily Intake (TMDI) based on consumption estimates for a Konzo-affected (a distinct form of a tropical myelopathy) population in the Democratic Republic of Congo.

Their daily intake was estimated to be equivalent to 0.19-0.37 mg CN-/kg bw/day. Applying a 10-fold safety factor for intra-individual variability, a TMDI of 0.02 mg CN^-/kg bw/day was established.

The basis of the TMDI was re-considered by the Committee in 2005 and it was changed to a three month rat study in which rats were dosed with sodium cyanide in their drinking water (NTP, 1993). This same study was also the basis of the PTDI established by the WHO Drinking Water Quality Guideline Committee in 2007 and the 'minimal risk level' established by the Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services in 2006.

However, unlike the WHO and ATSDR who applied a 100-fold safety factor to a NOAEL for minor reproductive effects in male rats the Committee of Experts on Flavourings applied an additional 2-fold safety factor so that the 'revised' TMDI of 0.023 mg/kg bw/day approximately matched that established in 2000
(0.02 mg/kg bw/day) although the basis had now been changed.

JECFA, EFSA and the UK Committee on Toxicity concluded that there were no suitable quantitative long-term toxicity studies in animals treated with either HCN or cyanogenic glycosides and so were unable to establish a PTDI. This review concurs with this conclusion despite the presence of a two-year study in rats which formed the basis of the PTDI for JMPR in 1965 and the US EPA in 1993. This two-year study in rats which was performed by Howard & Hanzal in 1955 was considered to be an inadequate study because of the small group size (10/sex/dose) and it was not clear how many rat tissues underwent a histopathological examination. Furthermore, there was considerable uncertainty over the exact dose of hydrocyanic acid delivered to the animals due to its volatility in the feed. The group fed the feed fumigated with 100 ppm hydrocyanic acid apparently had detectable cyanide in the blood whereas only around 50% of those receiving feed fumigated at 300 ppm had detectable cyanide in the blood. JMPR considered that only rats at 100 ppm were adequately treated and assigned a PTDI of 0.05 mg/kg bw/day whereas the US EPA considered that the NOAEL for the study was at the highest tested dose of 300 ppm and allocated a PTDI of 0.105 mg/kg bw/day.

Conclusions

Estimate of tolerable daily intake for humans

Not able to estimate due to paucity of suitable data. However, there is no available evidence in adequately nourished humans to show that chronic intake of cyanogenic glycosides causes a cumulative hazard above that of repeated acute toxicity.

Estimate of acute reference dose

0.08 mg hydrocyanic acid/kg bw

Studies that would provide information valuable for continued evaluation of the compound

Quantitative data on chronic exposure with either hydrocyanic acid or a cyanogenic glycoside, such as linamarin.

References

Abuye C, Kelbessa U, & Wolde-Gebriel S (1998) Health effects of cassava consumption in south Ethiopia. East African Medical Journal, 75: 166–170.

Akintonwa A, & Tunwashe OL. Fatal cyanide poisoning from cassava-based meal. Hum Exp Toxicol. 1992;11 :47 –49

Ariffin WA, Choo KE, & Karnaneedi S. (1992) Cassava (ubi kayu) poisoning in children. Med J Malaysia. 47: 231–234.

Askar, A. & Morad M.M. (1983). Lebensmittelvergiftigung 1. Toxine in natürlichen Lebensmittel. Alimentia. 19, 59-66.

ATSDR (2006) Toxicological Profile for Cyanide; Chapter 3 - Health Effects. Available at http://www.atsdr.cdc.gov/toxprofiles/tp8.html

Ballantyne B (1983) The influence of exposure route and species on the acute lethal toxicity and tissue concentrations of cyanides. In: Hayes AW, Schnell RC, Miya TS, eds. *Developments in the science and practice of toxicology.* New York, NY, Elsevier Science Publishers, pp. 583–586.

Banea-Mayambu JP, Tylleskar T, Gitebo N, Matadi N, Gebre-Medhin M, & Rosling H (1997) Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease konzo in former Zaire. Trop. Med. Internat. Health. 2: 1143–1151.

Banea-Mayambu JP, Tylleskar T, Tylleskar K, Gebre-Medhin M, & Rosling H (2000) Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). Annals of Tropical Paediatrics, 20:34–40.

Barrett M.D., Hill D.C., Alexander J.C. & Zitnak A. (1977). Fate of orally dosed linamarin in the rat. Can. J. Physiol. Pharmacol. 55, 134-136.

Barrett, M. D., Alexander, J. C. & Hill, D. C. (1978). Effect of linamarin on thiocyanate production and thyroid activity in rats. J Toxicol Environ Health 4(5-6): 735-40.

Bödigheimer K, Nowak F, & Schoenborn W.(1979) Pharmacokinetics and thyrotoxicity of the sodium nitroprusside metabolite thiocyanate. Dtsch Med Wochenschr. 104(26): 939-43.

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

Casadei E, Cliff J, & Neves J (1990) Surveillance of urinary thiocyanate concentration after epidemic spastic paraparesis in Mozambique. J. Trop. Med. Hyg. 93: 257–261.

Cheok SS. Acute cassava poisoning in children in Sarawak. (1978) Trop Doct. 8: 99 –101

Chiwona-Karltun L, Tylleskar T, Mkumbira J, Gebre-Medhin M & Rosling H (2000) Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. Internat. J. Food Sci. Nutrition. 51: 33–43.

Cliff J, Lundquist P, Rosling H, Sobro B, Wide L (1986) Thyroid function in a cassava-eating population affected by epidemic spastic paraparesis. Acta Endocrinol. 113: 523–528.

(CoE) Council of Europe (2000). Committee of Experts on Flavouring Substances 46th meeting - RD 4.13/1-46. Datasheet on HCN.

(CoE) Council of Europe (2005). Active principles (constituents of toxicological concern) contained in natural sources of flavourings. Available at http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/flavouring_substances/Active%20principles.pdf

Conn E.E. (1979) Cyanide and cyanogenic glycosides. In Rosenthal, G.A. & Janzen, D.H. (eds). Herbivores: Their interaction with secondary plant metabolites, Academic Press, Inc., New York - London, pp 387-412.

Cooles P (1988) Diabetes and cassava in Dominica. Trop. Geograph. Med., 40: 272–273.

Cooke R. D. (1978) An enzymatic assay for the total cyanide content of cassava *(Manihot* esculenta Crantz.). J. Sci. Food Agric. 29:345

Dawood MY. Acute tapioca poisoning in a child. (1969) J Singapore Paediatr Soc. 11: 154 –158

Doherty P., Ferm V. & Smith R. (1982). Congenital malformations induced by infusion of sodium cyanide in the Golden Hamster. Toxicol. Appl. Pharmacol. 64: 456-464.

ECETOC (2004) *Hydrogen cyanide, sodium and potassium cyanides and acetone cyanohydrin*. Brussels, European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC Joint Assessment of Commodity Chemicals)

EEC (1988). Council Directive 88/388/EEC of 21 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production. Official Journal of the European Communities, 15.7.1988, L184/61-67.

EFSA Journal (2004) Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food (AFC) on hydrocyanic acid in flavourings and other food ingredients with flavouring properties. 105, 1-28

Egereze, J. 0. & Oehme F.W. (1979) Blood and liver cyanide concentrations in rats poisoned with oral doses of potassium cyanide. Toxicol. Lett. (Amst.) 3: 243-247.

Ernesto M, Cardoso AP, Nicala D, Mirione E, Massaza F, Cliff J, Haque MR, & Bradbury JH (2002) Persistent konzo and cyanogen toxicity from cassava in northern Mozambique. Acta Tropica, 82: 357–362.

Espinoza OB, Perez M, Ramirez MS. (1992) Bitter cassava poisoning in eight children: a case report. Vet Hum Toxicol. 34: 65

Famuyiwa OO, Akanji AO, & Osuntokun BO (1995) Carbohydrate tolerance in patients with tropical ataxic neuropathy — A human model of chronic cyanide intoxication. African J. Med. Med. Sci. 24:151–157.

Feldstein M & Klendshoj NC (1954) The determination of cyanide in biologic fluids by microdiffusion analysis. J Lab. Clin. Med., 44: 166–170.

Ferguson HC (1962) Dilution of dose and acute oral toxicity. Toxicol. Appl Pharmacol, 4: 759–762.

Frakes R.A., Sharma R.P. & Willhite C.C. (1985) Development toxicity of the cyanogenic glycoside linamarin in the golden hamster. Teratology 31: 241-246.

Frakes R.A., Sharma R.P., & Willhite C.C. (1986a) Comparative metabolism of linamarin and amygdalin in hamsters. Food Chem. Toxicol. 24: 417-420

Frakes R.A., Sharma R.P., Willhite C.C. & Gomez 0. (1986b) Effect of Cyanogenic glycosides and protein content in cassava diets on hamster prenatal development. Fund.Appl. Toxicol. 7: 191-198.

Freeman A. (1988). Optic neuropathy and chronic cyanide intoxication: a review. Arch. J. Royal Soc. Med., 81, 103-106.

Geller R.J., Barthold C., Saiers J.A. & Hall A.H. (2006) Pediatric cyanide poisoning: causes, manifestations, management, and unmet needs. Pediatrics 118; 2146-2158.

Gettler AO, & Baine JO (1938) The toxicity of cyanide. Am. J. Med. Sci., 195:182–198

Gill G (1996) Tropical diabetes? Tropical Doctor, 26: 1–3.

Gosselin R.E., Gleason M.N. & Hodge H.C. (1976). 'Clinical Toxicology of Commercial Products'., 4th Ed. Williams & Wilkins, Baltimore , Maryland.

Hernández T, Lundquist P, Oliveira L, Pérez Cristiá R, Rodriguez E, & Rosling H. (1995) Fate in humans of dietary intake of cyanogenic glycosides from roots of sweet cassava consumed in Cuba. Nat Toxins. ; 3(2):114-7.

Himwich W.A. & Saunders I.P. (1984) Enzymatic conversion of cyanide to thiocyanate. Am. J. Physiol., 153, 348-354.

Howard, J.W. & Hanzel, R.F. (1955) Chronic toxicity for rats of food treated with hydrogen cyanide. Agric. and Food Chem. 3: 325-329.

Hugh-Jones P (1955) Diabetes in Jamaica. Lancet, 2: 891–897.

IPCS (International Programme of Chemical Safety) 2005. Harmonization project document no. 2. Chemical-specific adjustment factors for interspecies differences and human variability guidance document for use of data in dose/concentration–response assessment.

Jackson LC (1988) Behavioral effects of chronic sublethal dietary cyanide in an animal model: implications for humans consuming cassava (*Manihot esculenta*). *Human Biology* 60 597 -614.

JECFA (1981) Joint FAO/WHO Expert Committee on Food Additives. Evaluation of Certain Food Additives, 25th Report, WHO Technical Report Series No 669, WHO, Geneva.

JECFA (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.

JMPR (1965) Report of the second joint meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues, FAO Meeting Report No. PL/1965/10; WHO/Food Add./26.65.

Kendirim OC, Chukwu OA, & Achinewhu SC (1995) Effect of traditional processing of cassava on the cyanide content of gari and cassava flour. *Plant Food for Human Nutrition*, 48:335–339.

Leuschner F., Neumann B.W. & Liebsch N. (1983). Mutagenicity study of hydrocyanic acid in Chinese hamster (chromosome aberration) by oral administration. Unpublished study, Laboratory of Pharmacology and Toxicology, Hamburg, august 1983, submitted by Detia Freyberg GmbH.

Leuschner F. & Neumann B.W. (1989). *In vitro* mutation assay of KCN in Chinese hamster cells. Unpublished study, Laboratory of Pharmacology and Toxicology, July 1989, submitted by Detia Freyberg GmbH.

Leuschner J, Winkler A, & Leuschner F. (1991) Toxicokinetic aspects of chronic cyanide exposure in the rat. Toxicol. Lett. 57(2):195-201.

Ludwig R. & Lohs K. (1975). Akute Vergiftungen. P116. Gustav Fischer Verlag

McGlashan ND (1967) Geographical evidence on medical hypotheses. Trop. Geographical Med., 19: 333–344.

Ministry of Health, Mozambique (1984) Mantakassa: An epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 2. Nutritional factors and hydrocyanic acid content of cassava products. *Bulletin of the World Health Organization*, 62: 485–492.

Montgomery R.D. (1969). In 'Toxic Constituents of Plant Foodstuffs'. I.E. Liener, ed., pp 143-157, Academic Press, New York.

Nartey, F. (1968). Studies on cassava Manihot utilissima Pohl I Cyanogenesis: the biochemistry of linamarin and lotaustralin in etiolated seedling. Phytochem. 7: 1307-1312

NTP (1993) National Toxicology Program. *Technical Report on Toxicity Studies of Sodium Cyanide (CAS No 143-33-9) Administered in Drinking Water to F344/N Rats and B6C3F1 Mice*, NTIS No PB94-194693, US Department of Health and Human Services, Public Health Service, National Institutes of Health.

Oke, O.L. (1979) Some aspects of the role of cyanogenic glycosides in nutrition. Wld. Rev. Nutr. Diet, 33: 70-103.

Oke O.L. (1980). Toxicity of cyanogenic glycosides. Food Chem. 6, 9-109.

Olusi, S.O., Oke, O.L. & Odusote, A.C. (1979). Effects of cyanogenic agents on reproduction and neonatal development in rats. Biol. Neonate, 36: 233-293.

Oluwole OSA, Onabolu AO, Cotgreave IA, Rosling H, Persson A, & Link H (2002) Low prevalence of ataxic polyneuropathy in a community with high exposure to cyanide from cassava foods. J. Neurol., 249: 1034–1040.

Osuntokun BO (1981) Cassava diet, chronic cyanide intoxication and neuropathy in the Nigerian Africans. *World Review of Nutrition and Dietetics*, 36:141–173.

Rosling, H. (1987). Cassava toxicity and food security. Ed. Rosling. Tryck Kontakt, Uppsala, Sweden, 3-40.
Ruangkanchanasetr S, Wananukul V & Suwanjutha S. (1999) Cyanide poisoning, 2 case reports and treatment review. J Med Assoc Thai. 82 (suppl 1):S162 –S171Schulz V, Bonn R, & Kindler J. (1979) Kinetics of elimination of thiocyanate in 7 healthy subjects and in 8 subjects with renal failure. J Mol Med 57(5):243-247.

Seigler, D. S**.** 1981. Cyanogenic glycosides and lipids: structural types and distribution. In: B. Vennesland et al. (Ed.) Cyanide in Biology. Academic Press, New York.

Sousa, A.B., Manzano, H., Soto-Blanco, B. & Górniak, S.L. (2003) Toxicokinetics of cyanide in rats, pigs and goats after oral dosing with potassium cyanide. Arch Toxicol 77: 330-334.

Sreeja V.G., Nagahara N., Li Q., & Minami M. (2003) New aspects in pathogenesis of konzo: neural cell damage directly caused by linamarin contained in cassava (Manihot esculenta Crantz). Brit J Nutr, 90, 467–472

Strugala G.J. & Elbers R. (1984) Metabolism of amygdalin and prunasin in the isolated perfused rat liver. Naunyn-Schmiedeberg's Archs. Pharmac. 325 (Suppl). R33

Swai AB, McLarty DG, Mtinangi BL, Tatala S, Kitange HM, Mlingi N, Rosling H, Howlett WP, Brubaker GR, & Alberti KG (1992) Diabetes is not caused by cassava toxicity. A study in a Tanzanian community. Diabetes Care, 15: 1378–1385.

Tewe O.O. & Maner I.H. (1981). Long-term and carry-over effect of dietary inorganic cyanide (KCN) in the life cycle performance and metabolism of rats. Toxicol. Appl. Toxicol. 58, 1-7.

UK Committee on Toxicity (2006) Chemicals in food, consumer products and the environment. Statement on cyanogenic glycosides in bitter almonds. available at http://www.food.gov.uk/multimedia/pdfs/cotstatementapricot200615.pdf

Umoh, I.P.; Maduagwu, E.N. & Amole, A.A. (1986): fate of ingested linamarin in malnourished rats. Food Chem. 20, 1-9

US EPA (1993) Hydrogen cyanide (CASRN 74-90-8). US Environmental Protection Agency, Integrated Risk Information System. Available at http://www.epa.gov/iris/subst/0060.htm.

WHO (1985) *Diabetes mellitus*. *Report of a WHO Study Group.* Geneva, World Health Organization, 131 pp. Available at http://whqlibdoc.who.int/trs/WHO_TRS_727.pdf (WHO Technical Report Series 727).

WHO (2003) Water quality guidelines http://www.who.int/water_sanitation_health/dwq/chemicals/cyanide.pdf

WHO (2007) Water quality guidelines http://www.who.int/water_sanitation_health/dwq/chemicals/second_addendum_cyanide_short_term%20_4_.pdf.

Zuidema PJ (1959) Cirrhosis and disseminated calcification of the pancreas in patients with malnutrition. Trop. Geograph. Med., 11: 70–74.

Attachment 4

Dietary Exposure Assessment

Proposal P1002 – Hydrocyanic Acid in Ready-To-Eat Cassava Chips

EXECUTIVE SUMMARY

Consumers of cassava chips are at risk of exceeding the Acute Reference Dose for hydrocyanic acid if consuming cassava chips with concentrations above 10 mg/kg. Limiting hydrocyanic acid concentrations to a mean of 10 mg/kg substantially reduces the probability of exposures above the Acute Reference Dose for all population groups.

Acute dietary exposures to hydrocyanic acid from ready to eat cassava chips were estimated for the Australian and New Zealand populations, and for age and gender population sub-groups. Food consumption data used were from the 1995 Australian and the 1997 New Zealand National Nutrition Surveys. The NSW Food Authority provided concentration data for cassava chips sold in Australia.

Following best practice, where significant uncertainties in the data existed conservative assumptions were used to ensure that the dietary exposure assessment did not underestimate exposure. The probabilities of exceeding the Acute Reference Dose reported here reflect the conservatism inherent in the exposure assessment and the Acute Reference Dose.

Based on estimates of consumption of cassava chips, 2-4 year old children have the highest risk of dietary exposures to hydrocyanic acid above the Acute Reference Dose, whilst adults are less at risk. The deterministic assessment showed that the mean concentration of hydrocyanic acid of 63 mg/kg in cassava chips available for sale in January 2008 could result in acute dietary exposures above the Acute Reference Dose for all population groups assessed.

At a hydrocyanic acid concentration of 25 mg/kg, most population groups still exceed the Acute Reference Dose for hydrocyanic acid, with small children particularly at risk. Even at concentrations of 10 mg/kg, the lowest considered in the report, some 2-4 year old children were at risk of acute dietary exposure exceeding the Acute Reference Dose. However, such exposure would most likely only occur in the unlikely event of a young child consuming more than a bag (100 g) of cassava chips in a day. The potential risk is increased by the irregular eating patterns and low body weight of this age group.

Using probabilistic techniques, the likelihood of 2-4 year old Australian children exceeding the Acute Reference Dose from consuming cassava chips with a mean concentration of hydrocyanic acid of 63 mg/kg was estimated at 56%, i.e. approximately one out of two occasions eating cassava chips may result in exposure above the Acute Reference Dose. The probability of exceeding the Acute Reference Dose decreases to 17-22% at a mean concentration of 25 mg/kg, and 2-4% at a mean concentration of 10 mg/kg.

Based on an estimated 1.1 million annual occasions of eating cassava chips by Australian children aged 2-4 years, reducing the mean concentration of hydrocyanic acid from the *status quo* to 10 mg/kg would reduce the incidence of acute dietary exposures above the Acute Reference Dose by 93-97%. Reducing the concentration to 25 mg/kg would lead to a lesser reduction of 61-70%.

1. Background

FSANZ has prepared a Proposal (P1002) to assess the public health risks associated with hydrocyanic acid in ready-to-eat cassava chips. FSANZ considers that regulatory measures may be required to reduce levels of hydrocyanic acid in ready-to-eat cassava chips to protect public health and safety. The food regulatory measures may include a maximum level (ML) for total hydrocyanic acid in ready-to-eat cassava chips. The options available for this Proposal include establishing an ML that would require all ready-to-eat cassava chips to contain 10 mg/kg or less of total hydrocyanic acid or 25 mg/kg or less of total hydrocyanic acid.

The dietary assessment exposure report sets out the findings of the dietary exposure assessment for hydrogen cyanide in cassava chips, necessary due to the establishment of an Acute Reference Dose for hydrocyanic acid of 0.08 mg/kg body weight/day. The details of the Proposal, including the basis for establishing the Acute Reference Dose are outlined in the Assessment Report at http://www.foodstandards.gov.au/standardsdevelopment/proposals/proposalp1002hydrocy3848.cfm

It should be noted that in practice a chain of events must occur for an individual to experience an exposure above the Acute Reference Dose, and possibly the clinical symptoms of exposure to hydrocyanic acid. The body weight of the individual, the amount of food eaten, the level of cyanide in the specific batch of food consumed, and whether the food is consumed in a single sitting or throughout the day, may contribute towards the possibility of dietary exposure exceeding the Acute Reference Dose that may lead to an observable adverse exposure event. For more details, please refer to the Hazard Assessment (Attachment 3).

In considering an appropriate ML for hydrocyanic acid in ready-to-eat cassava chips, FSANZ has noted that the Codex international standard for edible cassava flour includes a limit of 10 mg/kg for hydrocyanic acid in edible cassava flour and that this standard has been in existence for over ten years. A level of 10 mg/kg is considered to be practical and reasonably achievable with proper processing of cassava or with specific cassava selection.

2 Dietary exposure assessments

2.1 What is dietary modelling?

Dietary modelling is a tool used to estimate dietary exposure to food chemicals from the diet, as part of the FSANZ risk assessment process. To estimate dietary exposure to food chemicals, records of what foods people have eaten are needed along with reports of how much of the food chemical of interest is in each food.

The accuracy of these dietary exposure estimates depends on the quality of the data used in the dietary models. Sometimes, not all of the data needed are available or their accuracy is uncertain so assumptions have to be made, either about the foods eaten or about chemical levels, based on previous knowledge and experience. The models are generally set up according to international conventions for food chemical dietary exposure estimates.

However, each modelling process requires decisions to be made about how to set the model parameters and what assumptions to make. Different decisions may result in different answers. Therefore, FSANZ documents clearly all such decisions, model assumptions and data limitations to enable the results to be understood in the context of the data available and so that FSANZ risk managers can make informed decisions.

2.2 'Chronic' versus 'acute' exposure assessment

There are two basic, fundamentally different types of exposure assessments: *chronic* and *acute*. What type of exposure assessment is appropriate depends on the toxicological properties of the chemical that forms the basis of the assessment. In the case of hydrocyanic acid, *acute* effects following exposure to cyanogenic glycoside compounds in cassava-based foods are the primary concern and an acute exposure assessment is therefore required (World Health Organisation 2008).

FSANZ typically performs dietary exposure assessments for long-term, chronic exposures to a food chemical or a contaminant. In a chronic exposure assessment, the risk assessor estimates dietary exposure over the long-term i.e. several months to a lifetime. Consequently, the use of average and 90th percentile consumption amounts for each food commodity, average chemical concentrations and market share weightings are appropriate.

The relevant reference health standard for a chronic dietary exposure assessment typically is the *Acceptable Daily Intake*, i.e. the amount of a chemical that can be consumed every day for a lifetime without appreciable health risk. It is expressed in milligrams of the chemical per kilogram body weight of the consumer.

In contrast, in acute dietary exposure assessment, the risk assessor estimates the exposure that 'high-end' consumers could experience on a single eating occasion or over a single day, where 'high-end' is a plausible estimate of exposure for those individuals at the upper end of the consumption distribution, usually the 97.5th percentile.

The risk assessment is focussed on estimating the likelihood of single day exposure above the reference health standard that results from the consumption of the food in question, often based on the highest concentration of a residue or contaminant.

The relevant reference health standard is the *Acute Reference Dose*, i.e. an estimate of the amount of the chemical in food, expressed on a bodyweight basis, that can be ingested over a short period, usually during one meal or one day, without appreciable health risk to the consumer.

This risk assessment is focussed on estimating whether a single day exposure above the reference health standard could result from consumption of the food in question (a deterministic acute assessment) and how likely this would be (a probabilistic acute assessment).

To evaluate acute dietary exposure, many regulatory organisations (e.g. the Canadian Pest Management Regulatory Agency, the European Food Safety Authority, and the US Office of Pesticide Programs) consider it best practice to use probabilistic exposure modelling techniques, an example of which is *Monte Carlo analysis*. Monte Carlo methods are a class of computational algorithms that rely on repeated random sampling to compute their results. In a software program such as @Risk, a Monte Carlo simulation randomly generates values for uncertain variables over and over (iterations) to simulate a model.

The resulting distribution of exposures can be represented graphically as a probability density function (Figure 1; reproduced from: Office of Pesticide Programs, 2000) and can be used to provide refined estimates of acute dietary exposure to a chemical such as hydrocyanic acid.

2.3 Approach taken to assess acute dietary exposure

Consumption of cassava chips was not reported in the Australian 1995 and New Zealand 1997 NNS. Instead, data of the consumption ready to eat cassava chips was estimated using FSANZ's dietary modelling computer program, DIAMOND from combined food consumption for consumers of crisps, chips, extruded snacks, and other salty snacks (see Tables 1 and 2 below[10]) as a proxy for consumption of ready to eat cassava chips. The same data were used for the deterministic as well as the probabilistic assessments.

In the absence of data on the actual consumption of cassava chips, this approach gave the best possible estimate of the consumption of ready to eat cassava chips because:

1. Ready to eat cassava chips are very similar to other salty snacks, such as vegetable crisps and chips
2. It is a reasonable assumption that a consumer of one bag of salty snacks (i.e. approximately 100 g) would instead consume one bag of cassava chips.
3. While consumption of cassava chips is estimated from the combined consumption of a range of proxy foods, individual consumers did not necessarily report consuming more then one of the proxy foods. High-end consumers of salty snacks typically consumed large amounts of a single type of snack, even though the particular food reported may have been a crisp, chip, extruded snack or other salty snack.

The approach used for the dietary exposure assessment of hydrocyanic acid is summarised in Figure 2. Deterministic assessments of acute dietary exposure to hydrocyanic acid from ready to eat cassava chips were conducted separately for the Australian population segments of the 1995 Australian NNS and for the New Zealand population segments of the 1997 New Zealand NNS. In addition, a probabilistic assessment of acute dietary exposure to hydrocyanic acid was carried out for 2-4 year old Australian children.

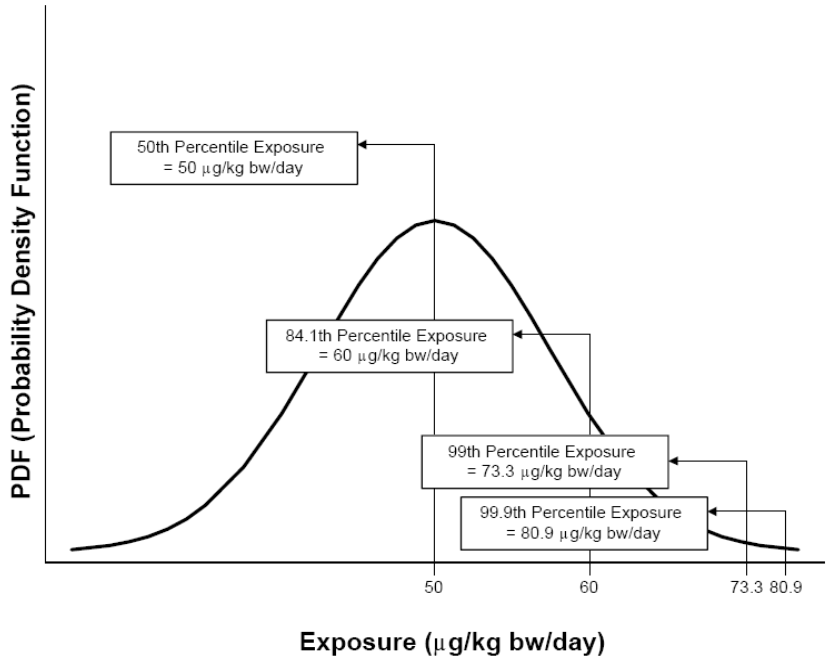


Figure 1: Example of a probability density distribution

[10] It should be noted that the New Zealand and Australian NNS use different coding for these types of foods.

To estimate acute dietary exposure dose to hydrocyanic acid using deterministic techniques, for each scenario and population group the concentration in each scenario was multiplied by the 97.5th percentile food consumption amount (expressed per kg of body weight) based on the 1995 and 1997 NNS data, for consumers only of salty snacks. Scenarios considered cassava chips with hydrocyanic acid levels of 10, 25, 63 and 145 mg/kg. Dietary exposure was also expressed as a percentage of the Acute Reference Dose of 0.08 mg hydrocyanic acid per kg of bodyweight per day. The outcome of this assessment informed the approach taken for the probabilistic exposure assessment.

The probabilistic exposure assessment was used to estimate the probability of 2-4 year old Australian children exceeding the Acute Reference Dose from the consumption of cassava chips. This group was chosen because they had the highest estimated dietary exposure as a percentage of the Acute Reference Dose. Distributions of consumption and hydrocyanic acid concentrations were used as inputs to construct probabilistic models for several scenarios using @Risk software[11] utilising the Monte Carlo sampling technique (Rubinstein 1981).

Finally, the number of children potentially at risk of exposure above the Acute Reference Dose each year within the total Australian population of children between 2 and 4 years was estimated based on the estimated number of eating occasions of cassava chips by these children.

2.4 Population groups assessed

A deterministic acute dietary exposure assessment was conducted for both the Australian and New Zealand populations. Dietary exposure assessments were conducted for the whole population (Australians aged two years and above; New Zealanders aged 15 years and above). Assessments were also conducted for the population sub-groups:

- Australians aged 2-4 years
- Australians aged 5-12 years
- Australians aged 13-18 years
- New Zealanders aged 15-18 years
- Australians and New Zealanders aged 19 years and above

An acute dietary exposure assessment was conducted for children because it is highly likely that many children consume ready to eat cassava chips. Children generally have higher dietary exposures to food chemicals when expressed on a body weight basis because they consume more food per kilogram of body weight than adults due to their increased energy needs for growth and development. It is important to note that, while children aged 2-4 years, 5-12 years and 13-18 years have been assessed as separate groups, these groups have also been included in the whole population's dietary exposure assessment.

2.5 Dietary survey data

DIAMOND contains dietary survey data for both Australia and New Zealand; the 1995 NNS from Australia that surveyed 13,858 people aged two years and above, and the 1997 New Zealand NNS that surveyed 4,636 people aged 15 years and above. Both of the 1995 and 1997 NNS used a 24-hour food recall methodology.

It is recognised that these survey data have some limitations (see Section 4).

[11] Palisade Corporation

2.6 Additional food consumption data or other relevant data

2.6.1 Foods used to estimate ready to eat cassava chips consumption amounts

Consumption of ready to eat cassava chips was not reported in the 1995 and 1997 NNS. Such products may however have been reported under the 'extruded snacks' or 'other snacks' categories. It is assumed that consumers may substitute any similar salty snack with ready to eat cassava chips.

Therefore, the combined consumption of equivalent salty snacks was used to estimate the amount of ready to eat cassava chips that might be consumed in one day (Tables 1 and 2). These foods were chosen because:

- they are presented and sold as a packaged salty snack in a similar manner to cassava chips;
- the organoleptic properties of these products are similar to cassava chips; and
- cassava chips are likely to be consumed in a similar manner.

Table 1: 1995 NNS (Australia): Food codes used to estimate ready to eat cassava chips consumption

Code	Food	Comments
2511	Potato crisps	plain and flavoured potato crisps
2521	Corn chips	plain and flavoured crisps and chips
2531	Extruded Snacks	prawn, tapioca-based chips or crisps; vegetable or tapioca-based crisps; cheese flavour, extruded snacks; non-cheese flavour extruded snacks
2541	Pretzels	pretzels; oriental snack mix; bhuja snack mix
2542	Other Snacks	soy chip or crisps, noodle-based snacks, pea-based snacks

Table 2: 1997 NNS (New Zealand): Food codes used to estimate ready to eat cassava chips consumption

Code	Food	Comments
233	Potato crisps	potato and sweet potato crisps
24110001	Crisp, corn/nacho	corn chips and crisps
24110002	Nachos, chips only	excludes taco shells
2431	Extruded snacks	grain based crisps; shrimp crisps; vegetable crisps; flavoured and non flavoured extruded snacks
24410001	Snack, pretzels	pretzels only
24410004	Snack, mix, bhuja	oriental snack mix
24410005	Snack mix, rice crackers	oriental snack mix
2441006	Snack, mix, Bombay	oriental snack mix

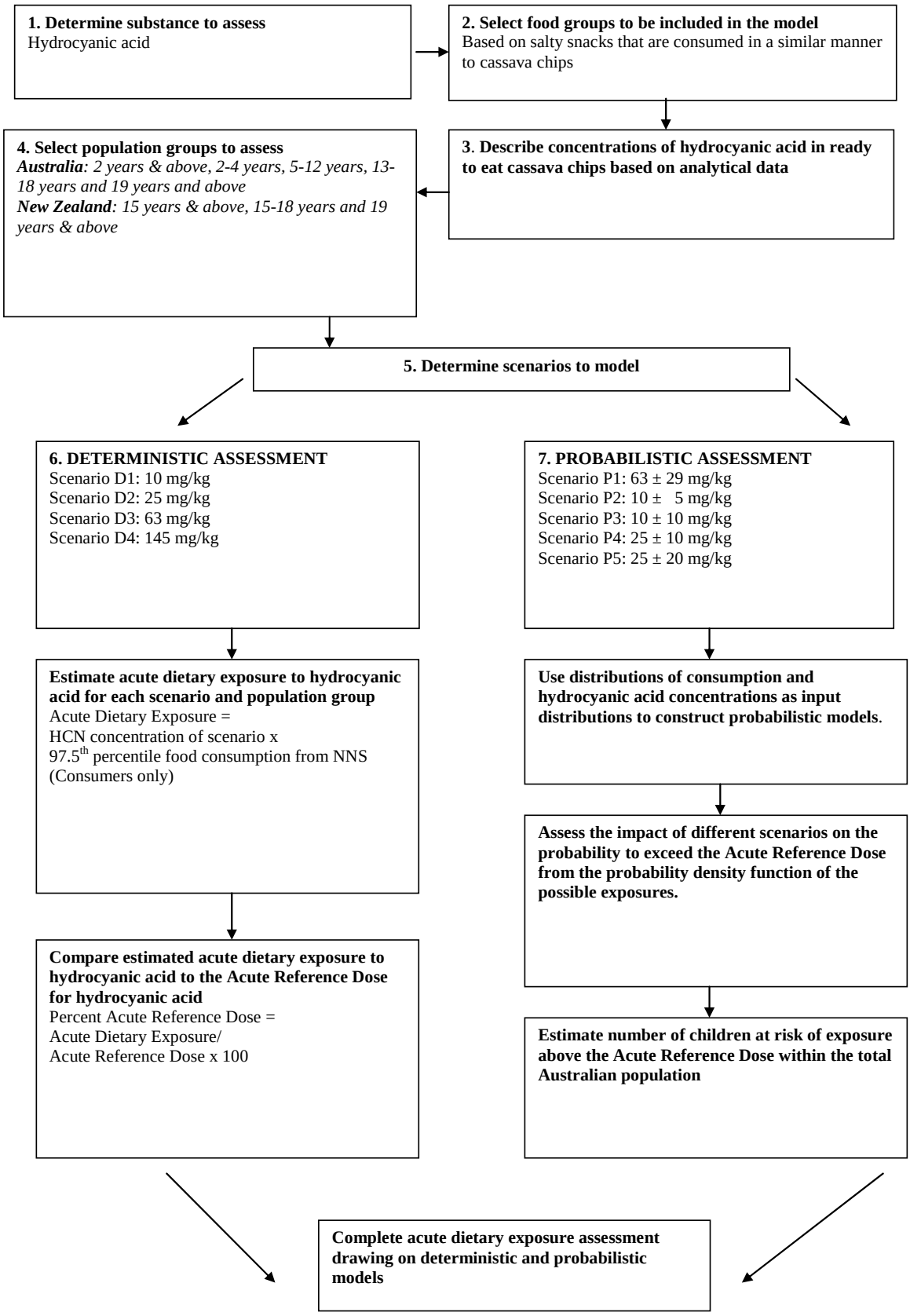


Figure 2: Acute dietary exposure assessment approach used for the Australian and New Zealand populations

2.6.2 Estimated market share of ready to eat cassava chips

In order to calculate the size of the population at risk of exceeding the Acute Reference Dose using a probabilistic exposure assessment, the total number of eating occasions of cassava chips was estimated. Estimates are based on population and consumption data and on the estimated market share of ready to eat cassava chips. Data on the current market share of ready to eat cassava chips are not available to FSANZ. However, market share can be estimated from data on the category shares of salty snacks sold by major Australian retailers.

The total production of salty snacks in Australia is approximately 48,628 tonnes per year. Based on the shares of salty snacks category the value share of other salty snacks[12] is 3.0% and the volume share 1.9% (approximately 972 tonnes/year, Retail Media, 2006). The volume share of cassava chips can therefore was estimated at around 2% of salty snacks, although the actual current market share may be somewhat different to this.

2.7 Hydrocyanic acid concentration in cassava chips

Three data sets were available for determining the concentration of hydrocyanic acid in ready to eat cassava chips sold in Australia.

The first data set was supplied by industry under the confidential commercial information provisions of the FSANZ Act. This data set was not suitable for carrying out an exposure assessment.

The second data set was made available by the Department of Health in Western Australia. It contained analytical data on nine cassava chip samples. The data showed that cassava chips had a minimum hydrocyanic acid level of 24 mg/kg and a maximum level of 83 mg/kg (mean of 48 mg/kg). This data set was not included in the data set because of its small sample size and lack of supporting documentation.

The third, most comprehensive, data set was made available to FSANZ by the NSW Food Authority in April 2008 and consisted of 222 measurements following the method proposed by Bradbury *et al.* (2006) of the total hydrocyanic acid levels in cassava based vegetable chips and crackers sold in Australia. Samples were collected as a follow-up to product rejection after export, and the sample set was not based on a statistical sampling plan. However, the samples included domestic and imported cassava chips and a variety of products of the major suppliers of these products were represented in the sample set. The sample set included samples that had been recalled from sale (n = 188) and samples that remained available for sale (n= 34).

Sample sizes available for analysis were 100 g and 28 g, based on the packaging of the product. The limit of reporting for hydrocyanic acid was 3 mg/kg for 100 g samples and 10 mg/kg for 28 g samples.

All the 100 g samples were reported to contain detectable levels of hydrocyanic acid. Six of the 28 g samples were reported to contain levels of hydrocyanic acid of less than 10 mg/kg.

These samples were assigned a value of 10 mg/kg (i.e. the level of reporting for this kind of sample) because for contaminant data it is prudent to take a conservative approach and assume that low levels of the chemical are present in the food.

[12] This excludes the following foods: nuts, potato chips, corn chips, cereal based chips, pretzels and mixes. ‘Other snacks’ would include snacks other than cassava chips (e.g. prawn crackers) and therefore may be a conservative estimate of the amount of cassava chips present in the marketplace

188 samples were from cassava chips that were voluntarily withdrawn from the market and 34 from products still available for sale. The values ranged from 3-145 mg/kg. The mean hydrocyanic acid concentration for all the samples was 63±28.6 mg/kg. For the available and withdrawn products, the mean was 53±13.6 mg/kg and 65±30.2 mg/kg respectively (Table 3).

The data set was normally distributed (Figure 3). StatGraphics® statistical analysis software was used to test for normality. The standardised skewness and kurtosis values were within the range expected for data from a normal distribution.

Table 3: Descriptive statistics of hydrocyanic acid concentrations in ready to eat cassava chips in Australia calculated for products available for purchase and withdrawn from the market following a voluntary recall

[HCN] mg/kg			
	Available for purchase	Recalled from sale	All
Sample size (n)	34	188	222
Mean	53	65	63
Standard Error	2.3	2.2	1.9
Median	52	67	64
Standard Deviation	13.6	30.2	28.6
Minimum	13	3	3
Maximum	86	145	145

Source: New South Wales Food Authority 2008.

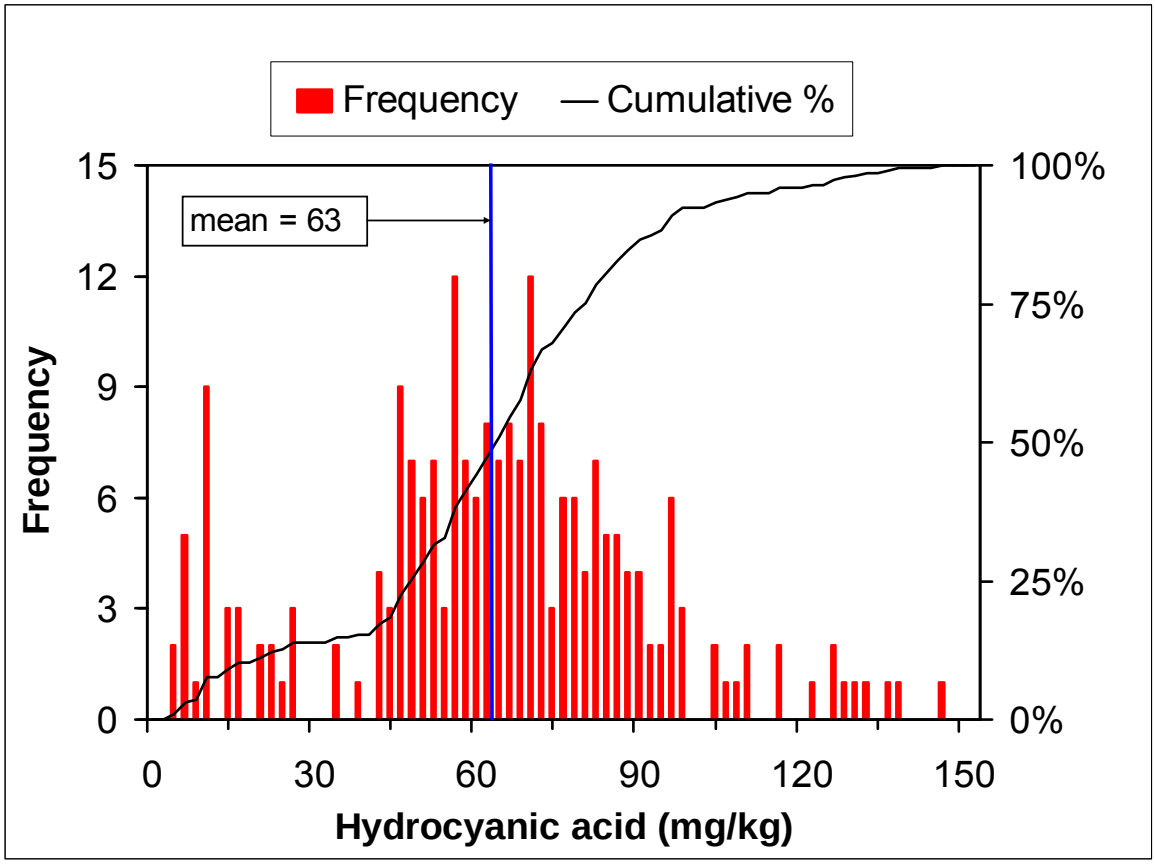


Figure 3: Distribution of hydrocyanic acid concentration in cassava chips

2.8 Scenarios for deterministic dietary modelling

For carrying out the assessment, four scenarios were considered: hydrocyanic acid levels of 10, 25 and 63 and 145 mg/kg ready-to-eat cassava chips.

- *Scenario D1 : 10 mg/kg*

This exposure level was selected based on the Codex Standard for edible cassava flour and is the preferred option for a Maximum Level (ML) put forward in the Assessment Report for Proposal P1002.

- *Scenario D2: 25 mg/kg*

Scenario 2 was based on Option 4 of the Assessment report. In submissions to the Assessment report, this option received some support and was promoted as a feasible interim Maximum Level by some stakeholders.

- *Scenario D3: 63 mg/kg*

This level was chosen because it represents the mean concentration of hydrocyanic acid in cassava chips from the most relevant data set available.

- *Scenario D4: 145 mg/kg*

This level was chosen because it represents the maximum hydrocyanic acid concentrations measured in ready to eat cassava chips sold in Australia.

2.9 Scenarios for probabilistic dietary modelling

For carrying out the probabilistic assessment of acute dietary exposure of Australian children between 2 and 4 years, several scenarios were considered: the current situation, mean concentrations of 10 mg/kg with two different standard deviations and a mean concentration of 25 mg/kg with two different standard deviations.

- *Scenario P1: Current situation*

This scenario was based on the distribution of hydrocyanic acid in all 222 samples analysed by the NSW Food Authority, with a mean and standard distribution of 63±28.6 mg/kg (See Figure 3).

- *Scenario P2: 10±5 mg/kg*

This scenario assumed a normal distribution with a mean of 10 mg/kg and a standard deviation of 5 mg/kg to represent a mean hydrocyanic acid concentration of 10 mg/kg with small variability in analytical controls.

- *Scenario P3: 10±10 mg/kg*

This scenario assumed more variation in the levels of concentrations represented by a normal distribution with a mean of 10 mg/kg and a standard deviation of 10 mg/kg.

- *Scenario P4: 25±10 mg/kg*

This scenario assumed a normal distribution with a mean of 25 mg/kg and a standard deviation of 10 mg/kg to represent a mean hydrocyanic acid concentration of 25 mg/kg with small variability in analytical controls.

- *Scenario P5: 25±20 mg/kg*

This scenario assumed a normal distribution with a mean of 25 mg/kg and a standard deviation of 20 mg/kg to represent a mean hydrocyanic acid concentration of 25 mg/kg with more variability in the distribution.

2.10 Deterministic exposure assessment: calculations and methods

Consumption of salty snacks that may be substituted by cassava chips[13] was calculated for each individual in the relevant NNS using his or her individual food records from the dietary survey. Each individual's total dietary consumption was divided by his or her own body weight, the results ranked, and the 97.5th percentile of consumption per kilogram body weight was determined. A small number of NNS respondents did not provide a body weight. These respondents were not included in calculations of estimated dietary exposures that were expressed per kilogram of body weight.

Estimated acute dietary exposure to hydrocyanic acid for each scenario and population group was calculated by multiplying the concentration of each scenario by the 97.5th percentile food consumption per kg of body weight (consumers only of salty snacks) from the relevant NNS.

Where estimated dietary exposures were compared to the Acute Reference Dose, the estimated acute dietary exposure was divided by the Acute Reference Dose and expressed as a percentage.

2.11 Probabilistic exposure assessment: calculations and methods

The deterministic approach tends to result in an overestimation of actual dietary exposure, particularly for an acute dietary exposure assessment, as it is unlikely that a consumer will always choose a bag of cassava chips with the scenario concentration of hydrocyanic acid and that they will also consume a large amount of the food (97.5th percentile consumption) on a single eating occasion, or over a single day.

For this reason, the probabilistic approach is appropriate to use when an Acute Reference Dose has been established (WHO, 2006). It assesses how likely it is that a high consumer will choose a bag of cassava chips with a high concentration of hydrocyanic acid resulting in an exposure over the Acute Reference Dose, based on the distributions of consumption and chemical concentration as input distributions to construct the probabilistic models.

From the deterministic models, Australian children aged 2-4 years had the highest proportion of the population with dietary exposures exceeding the Acute Reference Dose, and this age group was therefore used in the probabilistic calculations.

Distributions of consumption and hydrocyanic acid concentrations were used as input distributions to construct probabilistic models. The distribution of the consumption for 2-4 year old children reported in the 1995 NNS (derived using DIAMOND, expressed as gram of chips per kg body weight per day, Figure 3) were alternatively combined with:

- the analytical results for hydrocyanic acid concentration in cassava chips (Scenario 1, Figure 3); or
- simulated distributions of hydrocyanic acid concentrations (Scenarios 2-5).

The analytical data were checked for normality before creating the simulated distributions used in scenarios 2-5. None of the simulated distributions allowed concentrations below zero to be used (i.e. the distributions were censored at zero to avoid negative values). StatGraphics® statistical analysis software was used to test for normality and to create the distributions.

[13] i.e., consumers of crisps, chips, extruded snacks, and other salty snacks

To produce a distribution of possible dietary exposures and calculate the probability of exceeding the Acute Reference Dose a simulation was carried out by randomly multiplying each point of the distribution of food consumption with each point of the actual and simulated distributions of hydrocyanic acid concentration ('sampling'). The impact of different scenarios on the probability of exceeding the Acute Reference Dose can then be derived from the distribution of the possible exposures. Sampling of input distributions was carried out using @Risk software utilising the Monte Carlo sampling technique with 10,000 sample iterations.

Finally, the number of children at risk of exposure above the Acute Reference Dose within the total Australian population of children between 2 and 4 years was estimated based on the estimated number of eating occasions of cassava chips over one year by children between 2 and 4 years old.

2.12 Assumptions made in the acute dietary exposure assessment

One of the principles for exposure assessment, in particular in the case of acute exposure, is that the underlying data should be conservative. Conservatism incorporated into the analysis is determined by the data and assumptions that are used in calculating the estimate.

Following best practice for acute dietary exposure assents, where significant uncertainties in the data existed (such as the lack of consumption data from cassava based chips *per se* and the concentrations of hydrocyanic acid in cassava chips used in the model), conservative assumptions were generally used to ensure that the dietary exposure assessment did not underestimate exposure. The assumptions made in the dietary exposure assessment are listed below, broken down into several categories.

In the assessment, the Acute Reference Dose of 0.08 mg/kg body weight has been used as the comparator to determine the safety of consuming cassava chips over one day. Modelling shorter durations of exposure, such as that occurring in a single meal or snack, was not possible due to the lack of suitable consumption data. However, such modelling is considered unnecessary because the Acute Reference Dose covers consumption of food in a single sitting or over periods of up to 24 hours. A full day's exposure therefore includes a quantity of food consumed either over multiple meals or as a single snack of cassava chips.

General

- The relevant reference health standard is the Acute Reference Dose.
- The Acute Reference Dose for hydrocyanic acid is 0.08 mg/kg body weight/day. All calculations were based on this value.

Concentration data

- Concentrations of hydrocyanic acid in cassava chips used in the model are representative of the occurrence of hydrocyanic acid in the market place under different scenarios. This is a conservative assumption, as it is likely that following the recall the mean concentration of hydrocyanic acid in cassava chips currently on the shelf is lower.
- A concentration of 10 mg/kg is as low a reasonably achievable in these products.
- Australian data are representative of hydrocyanic acid concentration in cassava chips available in New Zealand.

Consumption data

- It the absence of data on the consumption of cassava chips, it is reasonable to assume that the foods used to estimate ready to eat cassava chips consumption are foods that could be substituted by cassava chips. However, this is a conservative assumption because not all consumers will substitute salty snacks with cassava chips.
- Consumption of foods as recorded in the 1995 and 1997 NNS reflect current food consumption patterns.
- Consumption data for Australian children 2-4 years reflect consumption patterns for New Zealand children of the same age.
- No other significant sources of hydrocyanic acid are consumed at the same time as cassava chips. This assumption may result in an underestimate of exposure to hydrocyanic acid.

Consumer behaviour

- Consumers do not alter their food consumption habits besides substituting salty snack products with cassava chips.
- Consumers do not increase or decrease the amount of salty snacks consumed upon switching to cassava chips.

3 Results

3.1 Estimated Australian consumption of salty snacks as a proxy for ready to eat cassava chips[14] by gender and age.

The estimated mean, median and 97.5th percentile consumption of cassava chips is set out in Table 4. The distribution of consumption was used in the deterministic assessment and as an input into the probabilistic assessment.

For the whole population (i.e. all consumers including children, Table 4a), 11% of respondents (or 1572 individuals) consumed salty snacks equivalent to cassava chips with a mean food consumption of 40 g/day (0.9 g/kg body weight/day) and 125 g/day (3.1 g/kg body weight/day) at the 97.5th percentile. Mean consumption of the food was slightly higher for men, although the consumption per kg body weight was very similar between the genders.

The median consumption of salty snacks was very similar across the population groups (Table 4). For the whole population, 2-4 year old children, and 5-12 year old children the median consumption of salty snacks was 25 g/day (Table 4a-c). Median consumption was slightly higher in teenagers (29 g/day, Table 4d) and highest in adults (32 g/day, Table 4e).

For all children between 2-4 years the mean consumption amounts for consumers of salty snacks (32 g/day, Table 4) were slightly lower than for adults and higher for boys compared to girls.

Considering the low mean body weight of this population group (17 kg, boys and girls) this meant that mean food consumption per kg body weight per day for consumers of salty snacks was double that of the general population (2.0 g/day compared to 0.9 g/kg body weight/day, Table 4a-b).

[14] the combined consumption of equivalent salty snacks was used to estimate the amount of ready to eat cassava chips that might be consumed in one day

The number of consumers in the age and gender subgroups (66 for boys and 60 for girls) were sufficient to estimate consumption levels at the 97.5th percentile. The high percentile consumption amounts for all 2-4 year olds expressed per kilogram of body weight were more than double that for the general population and more than triple for 2-4 year old boys. This clearly identifies this population sub-group as the most likely to exceed the Acute Reference Dose at the 97.5th percentile of consumption.

In this context, it should be noted that in the 1995 NNS very few children consumed more than 80 g per day of salty snacks that may be substituted with cassava chips, over 24 hours. Figure 3 clearly shows the outliers of the distribution of consumption of salty snacks per kg of body weight in this age group. There were five out of 1572 children that reported high consumption of the foods in question, with two children reporting that they consumed more than 100 g (250 g at 13 kg of body weight and 126 g at 21 kg body weight, see Figure 4). Three children reported consuming 80-100 g (100 g with 20 kg body weight, and 90 and 85 g with a bodyweight of 12 kg).

It is acknowledged that these high levels of consumption are extreme cases well outside the usual consumption pattern of salty snacks. However, after examining the total consumption of all foods reported for these individuals, it appeared unlikely that the consumptions reported were the result of misreporting or faulty coding. Therefore, there was no reason to exclude these data when deriving estimates of acute dietary exposure to hydrocyanic acid.

While these high consumption amounts represent uncommon events (less than 5% of salty snack eating events) they, however, form part of eating pattern typical of this age group: eating behaviours of children may vary, ranging from picky eating to irregular eating, overeating, and disinhibited or binge eating (Lewinsohn *et al* 2005, Marcus and Kalarchian 2003). This type of behaviour may further increase the likelihood of consuming more than 100 g of cassava chips and therefore the risk for 2-4 year olds exceeding the Acute Reference Dose.

For 5-12 year old children (Table 4c) mean food intakes for consumers of salty snacks were lower than for the younger age group, with the consumption figures slightly higher than for the general population. While the total mean consumption for 13-18 year old consumers of salty snacks was higher than for the general population, in particular for boys (50 g/day, Table 4d), the consumption per kg body weight at the 97.5th percentiles was very similar to the general population, and somewhat lower for girls.

Finally, for the adult population groups (Table 4e) consumer mean consumption of salty snack foods was slightly higher than the whole population but the consumption per kg body weight at the 97.5th percentiles was lower than in the whole population or for any of the other age differentiated population sub-groups. Adults are therefore the population group least likely to exceed the Acute Reference Dose.

3.2 Estimated New Zealand consumption of salty snacks approximate to cassava chips by gender and age.

The 1997 New Zealand NNS did not collect data for children under the age of 15 years. Therefore, consumption of salty snacks approximate to cassava chips could not be estimated for young New Zealand children[15]. For the general population surveyed 9% of the respondents (416 individuals) ate these foods.

[15] The codes against which foods were reported in the 1997 New Zealand NNS vary from those of the 1995 Australian NNS. However, the actual foods used as proxies for cassava chips are similar for both countries. Refer to Table 2 for details.

The mean consumption for the whole population was 51 g per day (Table 5a), 11 g per day above the estimated Australian consumption. However, consumer mean consumption per kg of body weight was lower in the New Zealand population. This most likely reflects the exclusion of young children from the survey, and the resultant higher mean body weight of the New Zealand survey population.

Children in the 15-18 year age bracket had a higher mean consumption of salty snacks than the general population (Table 5b); this was also reflected in the consumption at the 97.5th percentile. The values were similar to the comparable Australian age group, however at 3.8 g per kg bodyweight per day at the 97.5th percentile girls in this age group consumed more of these products than their Australian equivalents (2.4 g per kg body weight per day, Table 4d) and more than any other New Zealand population subgroup.

Therefore, on the data available, 15-18 year old girls are considered the most likely New Zealand subgroup to exceed the Acute Reference Dose for hydrocyanic acid. However, it is likely that children younger than 15 years of age would have higher dietary exposure per kg body weight as the Australian children do.

The 2002 New Zealand National Children's Nutrition Survey (CNS) surveyed 3275 New Zealand children aged 5-14 years. FSANZ does not currently hold food consumption data in DIAMOND in the correct format to enable dietary intake assessments to be conducted; however, it is possible to derive some trends. The data show that 11 children consumed vegetable chips or crisps[16]. The average amount consumed was 39 g, with a minimum consumption of 5 g and a maximum of 150 g. This is similar to comparable Australian age groups (see Table 4).

The New Zealand adult population who consumed salty snacks had lower mean cassava chip consumption (50 g/day, Table 5c) than the younger age group (56 g/day, Table 5b). However, consumption was higher than the equivalent Australian age group at the mean level of consumption and at the 97.5th percentiles.

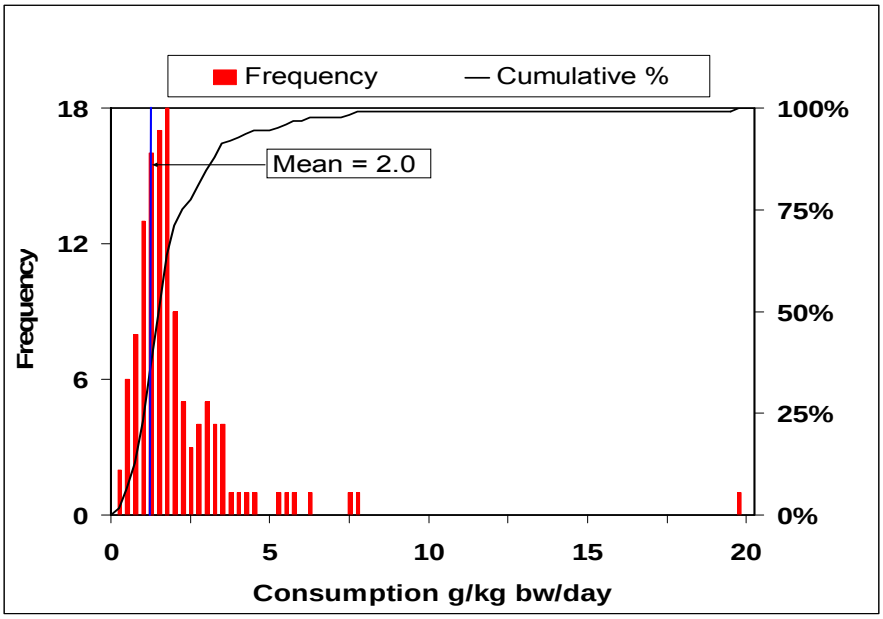


Figure 4: Distribution of consumption of salty snacks by 2-4 old Australian children (excludes those who did not consume salty snacks)

[16] Includes: Crisps non specific as to type, Vegetable crisps non specific as to type; kumara/sweet potato crisp, non specific as to type; Crisp, tapioca non specific as to type

Table 4: Estimated Australian consumption of salty snacks equivalent to cassava chips by gender and age

(a) 2 years and above

	Gender all	Male	Female
Number of consumers	1572	774	798
Percent of consumers to respondents	11	12	11
Mean body weight kg	67	72	62
Consumer mean food consumption (g)	40	43	37
Consumer median food consumption (g)	25	29	25
Consumer P97.5 food consumption (g)	125	150	100
Consumer mean food consumption g per kg bw	0.9	0.9	0.9
Consumer median food consumption g per kg bw	0.7	0.7	0.6
Consumer P97.5 food consumption g per kg bw	3.1	3.1	3.2

(b) 2-4 years

	Gender all	Male	Female
Number of consumers	126	66	60
Percent of consumers to respondents	22	23	20
Mean body weight kg	17	17	16
Consumer mean food consumption (g)	32	34	29
Consumer median food consumption (g)	25	25	25
Consumer P97.5 food consumption (g)	98	166	95
Consumer mean food consumption g per kg bw	2.0	2.1	1.9
Consumer median food consumption g per kg bw	1.5	1.5	1.5
Consumer P97.5 food consumption g per kg bw	7.2	10.4	7.5

(c) 5-12 years

	Gender all	Male	Female
Number of consumers	438	224	214
Percent of consumers to respondents	29	29	29
Mean body weight kg	32	32	33
Consumer mean food consumption (g)	34	33	35
Consumer median food consumption (g)	25	25	25
Consumer P97.5 food consumption (g)	90	100	85
Consumer mean food consumption per kg bw	1.1	1.1	1.2
Consumer median food consumption g per kg bw	0.9	0.9	0.9
Consumer P97.5 food consumption g per kg bw	3.4	3.2	3.9

(d) 13-18 years

	Gender all	Male	Female
Number of consumers	237	117	120
Percent of consumers to respondents	26	24	27
Mean body weight kg	62	65	59
Consumer mean food consumption (g)	44	50	38
Consumer median food consumption (g)	29	42	25
Consumer P97.5 food consumption (g)	176	200	125
Consumer mean food consumption g per kg bw	0.8	0.8	0.7
Consumer median food consumption g per kg bw	0.6	0.6	0.5
Consumer P97.5 food consumption g per kg bw	3.1	3.2	2.4

Table 4 (continued)

(e) 19 years and above

	Gender all	Male	Female
Number of consumers	771	367	404
Percent of consumers to respondents	7	7	7
Mean body weight kg	74	82	68
Consumer mean food consumption (g)	44	49	38
Consumer median food consumption (g)	32	42	28
Consumer P97.5 food consumption (g)	134	164	117
Consumer mean food consumption g per kg bw	0.6	0.6	0.6
Consumer median food consumption g per kg bw	0.5	0.5	0.4
Consumer P97.5 food consumption g per kg bw	2.0	2.2	2.0

Table 5: Estimated New Zealand consumption of salty snacks approximate to cassava chips by gender and age

(a) 15 years and above

	Gender all	Male	Female
Number of consumers	416	166	250
Percent of consumers to respondents	9	9	9
Mean body weight kg	71	79	65
Consumer mean food consumption (g)	51	60	45
Consumer median food consumption (g)	40	40	40
Consumer P97.5 food consumption (g)	200	200	157
Consumer mean food consumption g per kg bw	0.7	0.8	0.7
Consumer median food consumption g per kg bw	0.5	0.5	0.5
Consumer P97.5 food consumption g per kg bw	2.5	3.2	2.4

(b) 15-18 years

	Gender all	Male[17]	Female
Number of consumers	73	25	48
Percent of consumers to respondents	30	23	35
Mean body weight kg	65	69	61
Consumer mean food consumption (g)	56	67	51
Consumer median food consumption (g)	50	54	40
Consumer P97.5 food consumption (g)	225	-	198
Consumer mean food consumption g per kg bw	0.9	0.9	0.8
Consumer median food consumption g per kg bw	0.7	0.7	0.7
Consumer P97.5 food consumption per g kg bw	3.2	-	3.8

[17] sample size too small to reliably calculate 97.5th percentile

Table 5 (continued)

(c) 19 years and above

	Gender all	Male	Female
Number of consumers	343	141	202
Percent of consumers to respondents	8	8	8
Mean body weight kg	71	79	66
Consumer mean food consumption (g)	50	59	44
Consumer median food consumption (g)	40	40	36
Consumer P97.5 food consumption (g)	194	200	159
Consumer mean food consumption per kg bw	0.7	0.8	0.7
Consumer median food consumption per kg bw	0.5	0.5	0.5
Consumer P97.5 food consumption per kg bw	2.5	3.2	2.4

3.3 Estimated acute dietary exposure to hydrocyanic acid of the Australian population – Deterministic assessment

The acute dietary exposure to hydrocyanic acid was calculated based on the 97.5th percentile of consumption per kilogram body weight, which was derived from the ranked total dietary consumption of each individual consumer divided by his or her own body weight as follows:

$$Acute\ Dietary\ Exposure = (P97.5\ consumption\ g/kg\ bodyweight \times 1000) \times [hydrocyanic\ acid]\ mg/kg$$

Acute dietary exposure was calculated for four different scenarios: concentrations of 10, 25, 63 and 145 mg hydrocyanic acid per kg of cassava chips. Dietary exposure was also expressed as a percentage of the Acute Reference Dose of 0.08 mg hydrocyanic acid per kg of bodyweight per day (Table 6):

$$\%ARfD = \left(\frac{Acute\ Dietary\ Exposure\ mg/kg\ bodyweight/day}{ARfD\ mg/kg\ bodyweight/day} \right) \times 100$$

For 145 mg/kg, the maximum concentration reported, the Australian population consuming cassava chips could be potentially exposed to 0.46 mg of hydrocyanic acid per kg of body weight per day, or close to six times the Acute Reference Dose (Table 6). 2-4 year old children could be exposed to 1.04 mg/kg/bodyweight/ day, approximately 13 times the Acute Reference Dose.

In a scenario that assumes hydrocyanic acid concentrations of 10 mg/kg, the adult population consuming cassava chips was estimated to be exposed to 0.02 mg/kg body weight/day, or 25% of the Acute Reference Dose. The general population and older children consuming cassava chips were potentially exposed to 0.02-0.04 mg/kg body weight/day or 25-50% of the Acute Reference Dose. However, as predicted from the consumption data, 2-4 year old children consuming cassava chips were exposed to higher levels. Even at 10 mg/kg, girls were potentially exposed to 100% of the Acute Reference Dose, while boys exceeded the Acute Reference Dose by 25% (0.10 mg/kg body weight/day).

It should be noted that the mean body weight of 2-4 year old consumers was 17 ± 3.3 kg, with a minimum body weight of 10 kg. This compounds the risk of exposure above the Acute Reference Dose because the body weight of the individual and the amount of salty snacks consumed in a single in this age group day are not correlated and low and high body weight individuals are equally likely to be high-end consumers of salty snacks.

3.4 Estimated acute dietary exposure to hydrocyanic acid of the New Zealand population

The approach taken to estimate acute dietary exposure to hydrocyanic acid in the New Zealand population followed the approach described above.

For the maximum concentration of 145 mg/kg reported, the complete New Zealand population (15 years and over) consuming cassava chips would be potentially exposed to 0.36 mg of hydrocyanic acid per kg of body weight per day, or 446% of the Acute Reference Dose (Table 7).

Concentrations of hydrocyanic acid at 10 mg/kg resulted in acute dietary exposures of 0.02-0.04 mg/kg body weight/day, or 25-50% of the Acute Reference Dose (Table 7). However, concentrations of 25 mg/kg led to exposure exceeding the Acute Reference Dose in some population groups, namely men 15 years and above, all 15-18 year olds (and in particular girls) and adult men. Similar to Australia, at 63 mg/kg, all New Zealand population groups exceeded the Acute Reference Dose, in some cases by more than 200%.

Table 6: Estimated acute dietary exposure to hydrocyanic acid from ready to eat cassava chips of the Australian population at the 97.5th percentile of consumption

		Dietary Exposure to HCN in mg/kg bw/day				**Dietary Exposure to HCN as a % Acute Reference Dose**			
	Scenario:	10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg	10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg
2 years and above	Gender all	0.02	0.08	0.20	0.46	25	**100**	**247**	**569**
	Male	0.03	0.08	0.20	0.46	38	**100**	**247**	**569**
	Female	0.03	0.08	0.20	0.47	38	**100**	**254**	**585**
2-4 years	Gender all	0.07	0.18	0.45	1.04	88	**225**	**564**	**1297**
	Male	0.10	0.26	0.66	1.51	**125**	**325**	**822**	**1892**
	Female	0.08	0.19	0.47	1.09	**100**	**238**	**591**	**1360**
5-12 years	Gender all	0.03	0.08	0.21	0.49	38	**100**	**267**	**615**
	Male	0.03	0.08	0.20	0.47	38	**100**	**253**	**582**
	Female	0.04	0.10	0.24	0.56	50	**125**	**303**	**698**
13-18 years	Gender all	0.03	0.08	0.20	0.45	38	**100**	**243**	**560**
	Male	0.03	0.08	0.20	0.46	38	**100**	**250**	**576**
	Female	0.02	0.06	0.15	0.35	25	75	**189**	**436**
19 years and above	Gender all	0.02	0.05	0.13	0.29	25	63	**160**	**368**
	Male	0.02	0.06	0.14	0.32	25	75	**174**	**401**
	Female	0.02	0.05	0.12	0.28	25	63	**155**	**356**

Table 7: Estimated acute dietary exposure to hydrocyanic acid from ready to eat cassava chips of the New Zealand population at the 97.5th percentile of consumption

		Dietary Exposure to HCN in mg/kg bw/day				Dietary Exposure to HCN as a % Acute Reference Dose			
	Scenario:	10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg	10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg
15 years and above	Gender all	0.02	0.06	0.16	0.36	25	75	**194**	**446**
	Male	0.03	0.08	0.20	0.46	38	**100**	**248**	**572**
	Female	0.02	0.06	0.15	0.35	25	75	**190**	**437**
15-18 years	Gender all	0.03	0.08	0.20	0.47	38	**100**	**254**	**584**
	Male	0.03	0.07	0.19	0.43	38	88	**234**	**538**
	Female	0.04	0.09	0.24	0.55	50	**113**	**298**	**687**
19 years and above	Gender all	0.02	0.06	0.15	0.36	25	75	**193**	**445**
	Male	0.03	0.08	0.20	0.47	38	**100**	**256**	**588**
	Female	0.02	0.06	0.15	0.35	25	75	**191**	**439**

3.5 Predicted dietary exposure to hydrocyanic acid from consumption of one whole bag of cassava chips

To estimate the dietary exposure to hydrocyanic acid from consuming a typical package of cassava chips, a 100 g portion of chips was divided by the individual body weights for each consumer of salty snacks. These values were averaged for each consumer subgroup to provide a mean estimated consumption of chips per kg of bodyweight.

Based on the assumption that:

1. a consumer may eat a whole bag of cassava chips (i.e. 100 g), and
2. the maximum hydrocyanic acid concentration of the chips is 10 mg/kg,

the estimated Acute Dietary Exposure would be no more than 0.06 mg/kg body weight/day, or no more than 75% of the Acute Reference Dose for all the population groups assessed, including 2-4 year old children (Table 8).

However, consumption of one whole bag of cassava chips with a maximum hydrocyanic acid concentration of 25 mg/kg would result in an acute dietary exposure above the Acute Reference Dose for a child between the ages of 2-4 and 5-12 years assuming mean body weights for these ages. At 63 mg/kg, eating one whole bag of cassava chips would result in exposure above the Acute Reference Dose for anyone in the population, with a child aged 2-4 being exposed to close to four times the Acute Reference Dose.

Table 8: Predicted dietary exposure to hydrocyanic acid from consumption of one whole bag of cassava chips (100 g)

		Acute Dietary Exposure to HCN (mg/kg bw/day)				Acute Dietary Exposure to HCN (% Acute Reference Dose)			
	Scenario:	10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg	10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg
2 years and above	Gender all	0.02	0.06	0.14	0.33	25	75	**180**	**414**
	Male	0.02	0.06	0.14	0.33	25	75	**180**	**414**
	Female	0.02	0.06	0.14	0.32	25	75	**175**	**402**
2-4 years	Gender all	0.06	0.15	0.39	0.89	75	**188**	**482**	**1108**
	Male	0.06	0.16	0.39	0.90	75	**200**	**489**	**1125**
	Female	0.06	0.15	0.38	0.88	75	**188**	**476**	**1095**
5-12 years	Gender all	0.03	0.08	0.21	0.49	38	**100**	**267**	**614**
	Male	0.03	0.09	0.22	0.50	38	**113**	**272**	**625**
	Female	0.03	0.08	0.21	0.48	38	**100**	**262**	**602**
13-18 years	Gender all	0.02	0.06	0.15	0.35	25	75	**188**	**434**
	Male	0.02	0.06	0.15	0.35	25	75	**189**	**434**
	Female	0.02	0.06	0.15	0.35	25	75	**188**	**434**
19 years and above	Gender all	0.02	0.04	0.11	0.25	25	50	**138**	**318**
	Male	0.02	0.04	0.11	0.24	25	50	**132**	**304**
	Female	0.02	0.05	0.12	0.26	25	63	**144**	**331**

3.6 Distribution of dietary exposures of 2-4 year old Australian children and probability of exceeding the Acute Reference Dose

Based on the probability density functions, the likelihood of 2-4 year old Australian children exceeding the Acute Reference Dose from the consumption of cassava chips could be estimated for the five scenarios of interest (Figures 5 and 6). The results are summarised in Table 10. As shown below, the variability of the distribution (i.e., the standard deviation) has an impact on the probability of exceeding the Acute Reference Dose.

In the current situation (Scenario P1, mean hydrocyanic acid concentration: 63 ± 28.6 mg/kg, Figure 5), the mean dietary exposure to hydrocyanic acid from cassava chips for children between 2 and 4 years was estimated to be 0.12 mg/kg bodyweight per day, corresponding to 150% of the Acute Reference Dose. The 50^{th} and 97.5^{th} percentiles of the distribution were 0.09 and 0.43 mg/kg body weight per day, respectively. Assuming a representative input distribution, the probability of exceeding the acute reference dose was estimated to be 56%, which is approximately one out of every two eating occasions of these chips.

Assuming a normal distribution of 10 ± 5 mg/kg (Scenario P2) representing a concentration limit of 10 mg/kg with small variability in analytical controls, the mean dietary exposure to hydrocyanic acid from cassava chips for children between 2-4 years was 0.02 mg per kg body weight per day, corresponding to about 25% of the Acute Reference Dose. The 50^{th} and 97.5^{th} percentiles of the distribution would be respectively 0.01 and 0.07 mg per kg body weight per day. The probability of exceeding the acute reference dose was estimated to be 1.6%, which is on approximately one out of every 62 eating occasions.

In case of greater variability (i.e. a larger standard deviation, Scenario P3) of the hydrocyanic acid concentration represented by a normal distribution of 10±10 mg/kg the mean dietary exposure to hydrocyanic acid from cassava chips for children between 2 and 4 years was 0.02 mg/kg, corresponding to 31% of the Acute Reference Dose. The 50^{th} and 97.5^{th} percentiles of the distribution were 0.02 and 0.10 mg/kg body weight.

The probability of exceeding the acute reference was 4.2%, which is on approximately one out of every 24 eating occasions of these chips.

Assuming a normal distribution of 25 ± 10 mg/kg to represent a concentration of 25 mg/kg with small variability in analytical controls (Scenario P4), the mean dietary exposure to hydrocyanic acid was 0.05 mg/kg bodyweight/day, or 62% of the Acute Reference Dose. The 50th and 97.5th percentiles of the distribution were 0.04 and 0.17 mg/kg body weight. The probability of exceeding the acute reference was 17%, which is on approximately one out of every six eating occasions of these chips.

In case of greater variability of the concentration represented by a normal distribution of 25 ± 20 mg/kg (Scenario P5) the mean dietary exposure to hydrocyanic acid from cassava chips for children between 2 and 4 years was 0.06 mg/kg bodyweight/day corresponding to about 70% of the Acute Reference Dose. The 50th and 97.5th percentiles of the distribution were 0.04 and 0.22 mg/kg body weight. The probability of exceeding the acute reference dose was 22%, which is on approximately one out of every five eating occasions of these chips.

Table 10: Dietary exposure assessment of 2-4 years old children and their probability of exceeding the Acute Reference Dose (0.08 mg/kg bw/day) in a 24-hour period: current situation and four simulated scenarios of hydrocyanic acid concentrations

Scenario:	Current Situation	10 ± 5 mg/kg	10 ± 10 mg/kg	25 ± 10 mg/kg	25 ± 20 mg/kg
Median (mg/kg bw/day)	0.09	0.01	0.02	0.04	0.04
Mean (mg/kg bw/day)	0.12	0.02	0.02	0.05	0.06
80th percentile (mg/kg bw/day)	0.18	0.03	0.04	0.07	0.09
97.5th percentile (mg/kg bw/day)	0.43	0.07	0.10	0.17	0.22
Probability of exceeding the Acute Reference Dose	**56%**	**1.6%**	**4.2%**	**17%**	**22%**

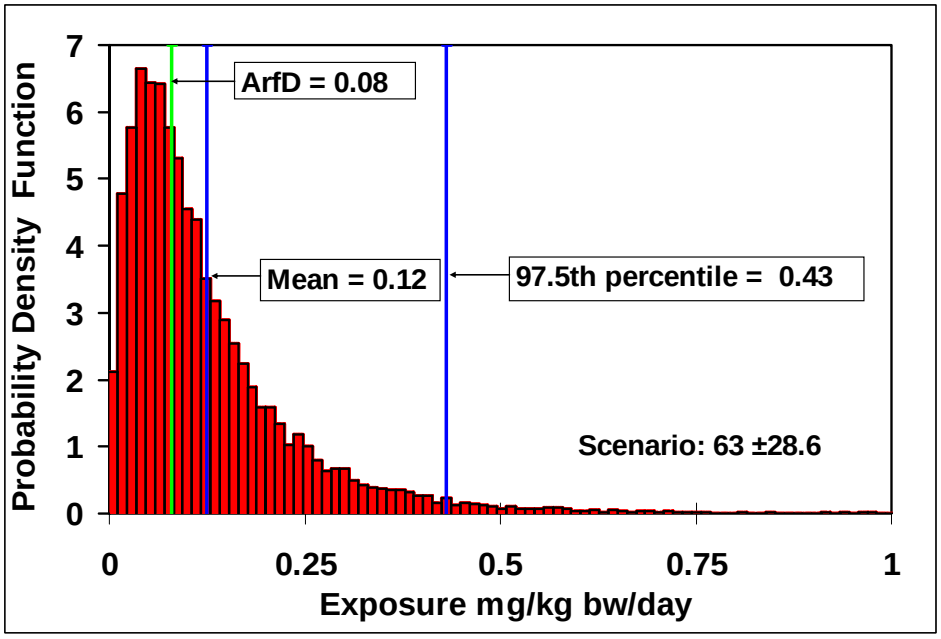


Figure 5: Distribution of exposure (mg/kg body weight/day) to hydrocyanic acid from cassava chips of Australian 2-4 year olds at current concentrations

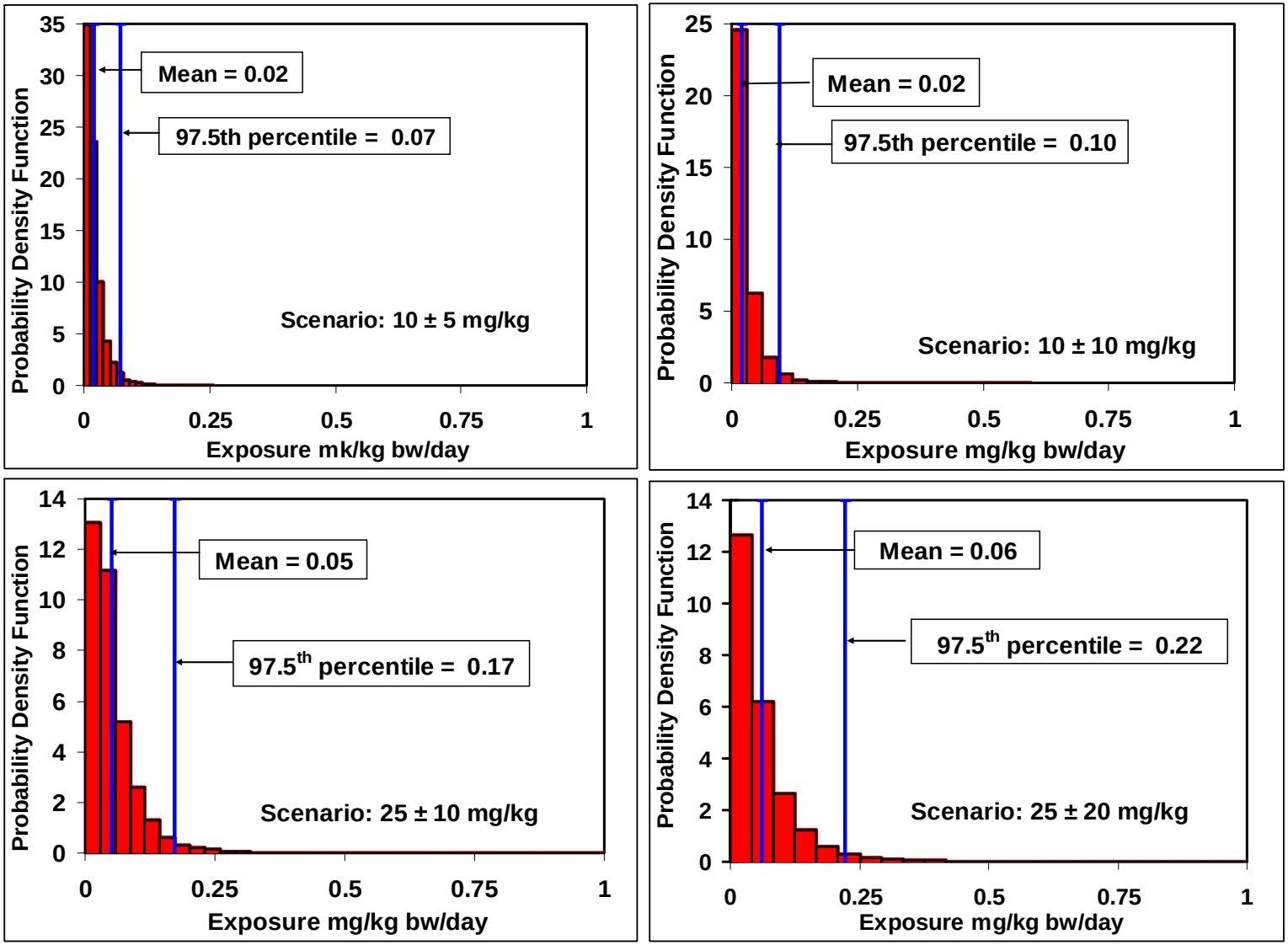


Figure 6: Distribution of exposure to hydrocyanic acid from cassava chips of Australian 2-4 year olds (mg/kg body weight/day) at four different scenarios

3.7 Estimates of the population at risk: number of Australian children likely to exceed the Acute Reference Dose

3.7.1 Estimated number of eating occasions

In order to calculate the size of the population at risk of exceeding the Acute Reference Dose, the total number of eating occasions of cassava chips by 2-4 year olds over one year was estimated. Estimates are based on population data, consumption data and the estimated market share of cassava chips and were calculated as shown in Figure 7.

Based on Australian census data (ABS, 2008), the total number of children between 2-4 years old is approximately 600,000 and, based on the consumption data from the 1995 NNS (see Table 5b), approximately one quarter of 2-4 year old children (i.e. 150,000 children) consume salty snacks on any given day. Therefore, the annual number of eating occasions for salty snacks is estimated to be 54,750,000 (150,000 x 365). The market share for cassava chips represents approximately 2% of the volume of salty snacks sold at retail. Market share can be estimated from data on the category shares of salty snacks sold by major Australian retailers (see section 2.6.2). The number of eating occasions for cassava chips in 2-4 year old children in any given year is therefore predicted to be approximately 1.1 million (54,750,000 x 0.02).

In the absence of any data on age-stratified consumption of cassava chips, this analysis has been based on the assumption that all population groups eat the same proportion of cassava chips in the food category i.e. that within a household the proportion of salty snacks consumed as cassava chips is the same for everyone. The market share data is based on retail purchases and therefore is considered a reasonable estimate of the proportion of cassava chips available to 2-4 year olds, who are unlikely to make their own purchases and therefore have limited choices. In other words, the retail figures reflect the type of salty snacks available to children from the household pantry.

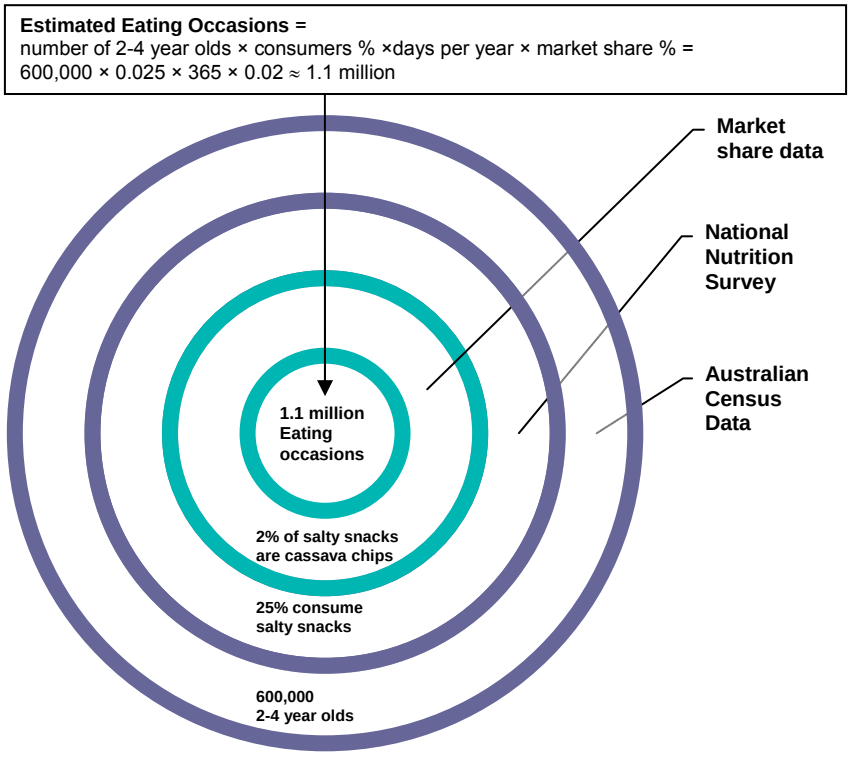


Figure 7: Calculation of estimated eating occasions

3.7.2 Estimated number of eating occasions exceeding the Acute Reference Dose

As noted above, where significant uncertainties in the data existed conservative assumptions were used to ensure that the dietary exposure assessment did not underestimate exposure. Therefore, the probabilities to exceed the Acute Reference Dose reported reflect the inherent conservatism of an acute dietary exposure assessment and the Acute Reference Dose. In addition, it should be noted that in practice a chain of events must occur for an individual to experience an exposure above the Acute Reference Dose, and possibly the clinical symptoms of exposure to hydrocyanic acid. The body weight of the individual, the amount of food eaten, if the food is consumed in a single sitting or throughout the day, other foods consumed, gut microflora and transit time, and many other factors may contribute towards the probability of an observable exposure event. For more details, please refer to the Hazard Assessment (Appendix 3).

Based on a total of 1.1 million annual eating occasions for cassava chips by children between the ages of 2-4 years, and a 56% chance of exceeding the Acute Reference Dose (Table 10), the number of eating occasions predicted to lead to an exposure above 0.08 mg/kg body weight was 616,000 per year for the *status quo*.

If the concentration of hydrocyanic acid in cassava chips was lowered to 10 ± 5 mg/kg (Scenario P2), the predicted number of exposures above the Acute Reference Dose would be reduced to 17,600 per year. This means a reduction in the number of probable exposure events by 97%. Other scenarios considered resulted in less reduction in the occurrence of such events (Table 11).

Table 11: Potential reductions in the number of exposures to hydrocyanic acid levels above the Acute Reference Dose of 2-4 year old Australian Children as a consequence of lowering hydrocyanic acid concentrations in cassava chips

Scenario:	Current Situation	10 ± 5 mg/kg	10 ± 10 mg/kg	25 ± 10 mg/kg	25 ± 20 mg/kg
Probability of exceeding the Acute Reference Dose	56%	2%	4%	17%	22%
Predicted number of annual exposures events above the Acute Reference Dose	616,000	17,600	46,200	187,000	242,000
Potential reduction in number of total exposures events above the Acute Reference Dose		97%	93%	70%	61%

4 Limitations of the dietary modelling

4.1 Consumption data

Dietary modelling based on 1995 or 1997 NNS food consumption data provides the best estimate of actual consumption of a food and the resulting estimated dietary exposure to a food chemical for the population.

However, it should be noted that the NNS data have limitations. These limitations relate to the age of the data and the changes in eating patterns that may have occurred since the data were collected.

Generally, consumption of staple foods such as fruit, vegetables, meat, dairy products and cereal products, which make up the majority of most people's diet, is unlikely to have changed markedly since 1995/1997 (Cook *et al*., 2001).

However, there is uncertainty associated with the consumption of foods that may have changed in consumption since 1995/1997 (such as ready to eat cassava based chips), or that have been introduced to the market since 1995/1997.

Over time, there may be changes to the ways in which manufacturers and retailers make and present foods for sale. Since the data were collected for the 1995 Australian and 1997 New Zealand NNS, there have been significant changes to the Code to allow more innovation in the food industry. Consequently, another limitation of the dietary modelling is that some of the foods that are currently available in the food supply were either not available, or were not as commonly available, in 1995/1997. This includes ready to eat cassava-based chips and crisps.

While the results of the 1995 and 1997 NNS can be used to describe the usual intake of groups of people, they cannot be used to describe the usual intake of an individual (Rutishauser, 2000). In particular, they cannot be used to predict how consumers will change their eating patterns because of an external influence such as the availability of a new type of food.

4.2 Limitations and inherent uncertainties in the assessment of exposure

The limitations and inherent uncertainties in the assessment of acute dietary exposure to hydrocyanic acid were examined following the guidance of the Opinion of the Scientific Committee related to Uncertainties in Dietary Exposure Assessment (EFSA, 2006). In addition, the draft report on *Characterizing and Communicating Uncertainty in Exposure Assessment* which is in preparation to be published as WHO/IPCS monograph has been considered (WHO/IPCS, 2007).

According to the guidance provided by EFSA (2006) the following sources of uncertainties have been considered: Assessment objectives, exposure scenario, exposure model, and model input (parameters). The opinion for okadaic acid in molluscs recently adopted by EFSA (2008) was referred to in drafting the present document.

4.2.1 Assessment objectives

FSANZ has prepared Proposal P1002 to assess the public health risks associated with the presence of hydrocyanic acid in ready-to-eat cassava chips. The objective was to provide risk managers with the assessment of the risks to public health and safety including an acute exposure assessment using data currently available. The uncertainty of the assessment objectives is considered negligible.

4.2.2 Exposure scenario / exposure model

It is uncertain whether the available occurrence data are representative of the contamination of cassava chips in Australia and New Zealand. The 222 data points were from the NSW Food Authority (NSWFA). Samples were collected as a follow-up to product rejection after export, and the sample set was not based on a statistical sampling plan.

However, the samples included domestic and imported cassava chips and a variety of products of the major suppliers of these products were represented in the sample set.

Regarding chips consumption, as no cassava chips were reported as consumed in the 1995 and 1997 NNS, the food groups included in the model were salty snacks including potato crisps and extruded snacks, assuming that if someone chose to eat cassava chips they were likely to eat them in a similar way as these other snacks. The overall uncertainty in the model estimations is considered medium to high.

4.3.3 Model input (parameters)

The analytical data is assumed to be of good quality. Analysis was performed by an official laboratory using a validated method of analysis. Very few samples were reported to be below the limit of detection (5/222), therefore no decision was necessary on how to deal with these samples.

The food consumption amounts reported in the 1995 and 1997 NNS are based on 24-hour recalls. These types of data are expected to adequately reflect the short-term consumption but to underestimate the number of consumers on a long-term basis. The proportion of children reported to eat cassava chips each day is expected to be robust, but it is likely that the consumers are not the same children every day during one year.

5 Summary and conclusion

The assessment shows that the current mean concentration of hydrocyanic acid of 63 mg/kg in cassava chips may result in dietary exposures to hydrocyanic acid above the Acute Reference Dose for almost all population subgroups in Australia and New Zealand.

At half this concentration, acute dietary exposure for most consumer segments still exceed the Acute Reference Dose, with small children particularly at risk. Even at the lowest concentration of 10 mg/kg considered in the dietary exposure assessment, 2-4 year old Australian children were still potentially at risk of exceeding the Acute Reference Dose. It should be noted, that such an exposure would only occur in the event of a young child eating in excess of 100 g of cassava chips on a single eating occasion or over one day. This risk, however, may be exacerbated by the eating patterns and low body weight that are typical for this age group.

Based on the probabilistic techniques, the likelihood of 2-4 year old Australian children exceeding the Acute Reference Dose from the consumption of cassava chips was:

- 56% at a mean concentration of hydrocyanic acid of 63 mg/kg (approximately one in every two eating occasions);
- 17-22% at a mean concentration of 25 mg/kg (approximately one in every 5-6 eating occasions); and
- 2-4% at a mean concentration of 10 mg/kg (approximately one in every 24-63 eating occasions).

Based on a total of 1.1 millions annual occasions of eating cassava chips by children between the ages of 2 and 4, reducing the mean concentration of hydrocyanic acid in cassava chips from the *status quo* to 10 mg/kg would reduce the potential for exposures above the Acute Reference Dose by 93-97%. Reducing the concentration to 25 mg/kg would lead to a reduction of 61-70%.

Where significant uncertainties in the data existed conservative assumptions were used to ensure that the dietary exposure assessment did not underestimate exposure. Therefore, the probabilities to exceed the Acute Reference Dose reported reflect the inherent conservatism of the exposure assessment and of the Acute Reference Dose. In addition, it should be noted that in practice a chain of events must occur for an individual to experience an exposure above the Acute Reference Dose, and possibly the clinical symptoms of exposure to hydrocyanic acid.

In conclusion, consumers of cassava chips are potentially at risk of exceeding the Acute Reference Dose for hydrocyanic acid at concentrations above 10 mg/kg. Limiting the concentrations to a mean of 10 mg/kg substantially reduces the probability of dietary exposures above the Acute Reference Dose.

References

Australian Bureau of Statistics (2008) 3105.0.65.001 - Australian Historical Population Statistics, 2006.
http://www.abs.gov.au/AUSSTATS/abs@.nsf/DetailsPage/3105.0.65.0012006?OpenDoument

Bradbury, J.H. (2006) Simple wetting method to reduce cyanogen content of cassava flour. *J. Food Comp. Anal.* 18:451-60.

Cook, T., Rutishauser, I. and Allsopp, R. (2001) The Bridging Study: comparing results from the 1983, 1985 and 1995 Australian national nutrition surveys. Australian Food and Nutrition Monitoring Unit, Commonwealth Department of Health and Aged Care, Commonwealth of Australia, Canberra.

European Food Safety Authority (2006) Guidance of the Scientific Committee on a request from EFSA related to Uncertainties in Dietary Exposure Assessment. The EFSA Journal 438:1-54.

European Food Safety Authority (2008) Marine biotoxins in shellfish – okadaic acid and analogues. Scientific Opinion of the Panel on Contaminants in the Food Chain. The EFSA Journal 589:1-62.

Lewinsohn P, Holm-Denoma JM, Gau JM, Joiner TE, Streigel-Moore R, Bear P, Lamoureux B: (2005) Problematic eating and feeding behaviors of 36-month-old children. Int J Eat Disord 38(3):208-219.

Marcus M, Kalarchian M (2003) Binge eating in children and adolescents. Int J Eat Disord 34:S47-S57.

Office of Pesticide Programs US Environmental Protection Agency (2000) Choosing A Percentile of Acute Dietary Exposure as a Threshold of Regulatory Concern.

Retail Media (2006) Retail Worlds Australian Grocery Guide 2006. 16th Edition. Retail Media, Parramatta, Australia.

Rubinstein, R.Y. 1981 Simulation and the Monte Carlo Method. John Wiley and Sons, N. Y, USA.

Rutishauser, I. (2000) Getting it right:- how to use the data from the 1995 National Nutrition Survey. Commonwealth of Australia, Canberra.

World Health Organisation (2006) Draft Guidance document on characterising and communicating uncertainty in exposure assessment.
http://www.who.int/ipcs/methods/harmonization/areas/draftundertainty.pdf

World Health Organisation (2008). Project to update the principles and methods for assessment of chemicals in food, Chapter 7 Exposure assessment (draft), WHO, Geneva, Switzerland.

Attachment 5

Food Technology Report

P1002 – Total Hydrocyanic Acid in Ready-to-eat Cassava Chips

Summary

Through proper processing of cassava, total hydrocyanic acid content can be reduced to a safe level of 10 mg/kg in cassava-based products, such as ready-to-eat (RTE) puffed cassava chips.

Some puffed cassava chips have been found to contain more than 10 mg/kg total hydrocyanic acid. Therefore, there is a need to review the process of manufacturing of puffed chips in order to identify those control measures that can achieve end product safety. A number of food technology issues have been identified as a result. These include:

1. The most important control for RTE cassava chips to achieve a total hydrocyanic acid level below 10 mg/kg is to use dried raw cassava ingredients containing less than 10 mg/kg.
2. Total hydrocyanic acid levels in dried raw cassava ingredients can be reduced through various processing techniques, if the processes are undertaken sufficiently and effectively. Some new technologies, such as faster drying in ovens, have to be evaluated for their effect on hydrocyanic acid reduction.
3. The manufacturing process of RTE puffed cassava chips may have the potential to reduce hydrocyanic acid in the chips. However, if the total hydrocyanic acid level in the raw cassava ingredients is greater than 40 mg/kg, then it is very unlikely that the final puffed cassava chips will have a total hydrocyanic acid level below 10 mg/kg.

The total hydrocyanic acid content of cassava products consists of cyanogenic glycosides, cyanohydrins and 'free' hydrogen cyanide. The level of hydrocyanic acid depends on the variety of cassava tuber, the growing conditions and the methods of processing. The relative level of each cyanide 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 reactions do not take place or are interrupted, the final cassava may contain unacceptably high levels of total hydrocyanic acid.

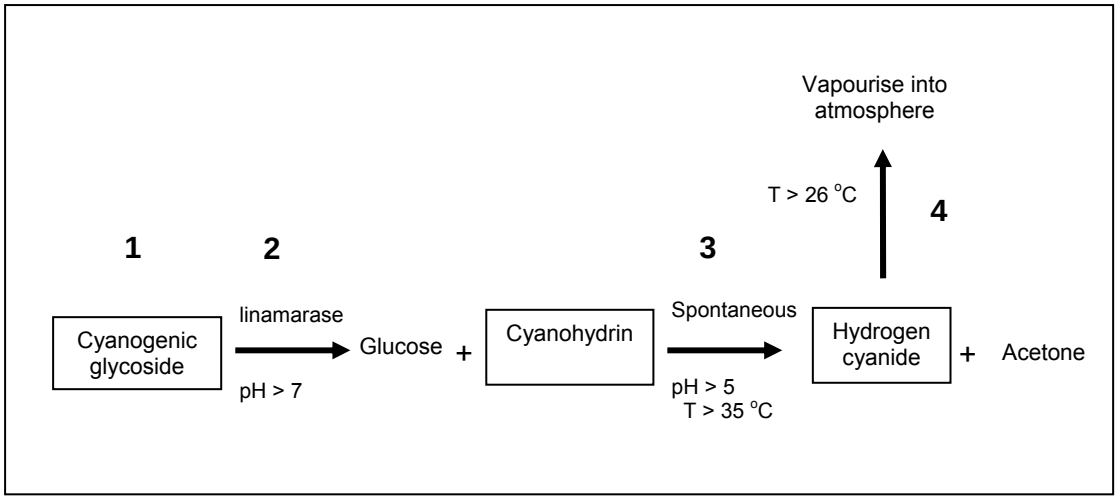


Figure 1: Cyanogenesis reaction pathway and steps in cassava processing. 1: nature of tuber; 2: grating/rasping, soaking, fermentation; 3: sun/oven drying; 4: sun drying, hot manufacturing process (steaming, frying).

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 hydrocyanic acid from cassava. Cassava, 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. Levels of total hydrocyanic acid in some modified starches could be as low as 0.01 mg/kg.

Cassava tubers, once harvested, are usually fermented or dried to inhibit deteriorative physiological changes and microbial growth. General processing steps, as labelled in Figure 1, are discussed in relation to the four stages in the cyanogenesis pathway, examining the parameters and techniques that are most effective in eliminating hydrocyanic acid and maintaining product quality of the final products. When cassava products are found to have unacceptably high levels of total hydrocyanic acid, failures in one of those steps is the most likely cause.

Step 1: Nature of the cassava tuber. Tubers with high levels of cyanogenic glycosides are difficult to reduce to an acceptable level through the typical cassava product processes. Bitter cassava tubers have much higher cyanogenic glycosides than sweet cassava varieties and within the sweet varieties, there is a large range of cyanogenic glycoside levels existing in the tubers. Droughts have been shown to stress cassava plants to produce and accumulate high levels of cyanogenic glycosides.

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 levels of cyanogenic glycoside may remain in the products.

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 levels 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.

Step 3: Sun/oven drying. 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^{o}C. The final product of this cyanogenesis pathway is the volatile hydrogen cyanide, which vaporises at 26^{o}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.

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^{o}C). If the cyanide is still in the cyanogenic glycoside form, a steaming process at a temperature less than 100^{o}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.

Ready-to-eat Cassava Chips

RTE puffed cassava chips have been found to contain total hydrocyanic acids level higher than 10 mg/kg. Given the background on cyanide eliminating steps as discussed above, an additional understanding of the processing of cassava chips is needed. Simplified processing steps in the manufacturing of cassava chips are illustrated in Figure 2.

Briefly, for basic puffed cassava chips (Type A), cassava flour or starch is made into a wet-dough and gelatinised by hot steam. At this stage, the reaction between trapped cyanogenic glycoside and enzymes may be reactivated in the moist heat and release the volatile hydrogen cyanide. It thus reduces the total hydrocyanic acid to an acceptable level if the initial level of the flour is less than 40 mg/kg. The gel is then sliced and dried to become brittle chips containing about 15% moisture. These chips are called ‘half-products’ or ‘intermediates’. They are very stable and light in weight and thus suitable for export. When these chips are fried in hot oil or air, the trapped steam vaporises and expands the gel network formed by the starch (similar to the expansion of bread during baking). The hot oil flashes the moisture out of the chips and the final puffed product has only about 4% moisture and a brittle-dried porous texture.

Some puffed cassava chips (Type B) may contain 50% of the ingredients as ‘cassava’ in addition to cassava flour. The ‘cassava’ could consist of milled or pulverised dried cassava chips or pellets, and is claimed by the manufacturers to provide a special cassava flavour and texture to the puffed chips.

The third type (C) of cassava chips are not puffed and are raw (and/or dried) sliced cassava fried directly in hot oil until crisp, a product similar to potato chips. This is a rather common alternative in South East Asia where potato chips are expensive.

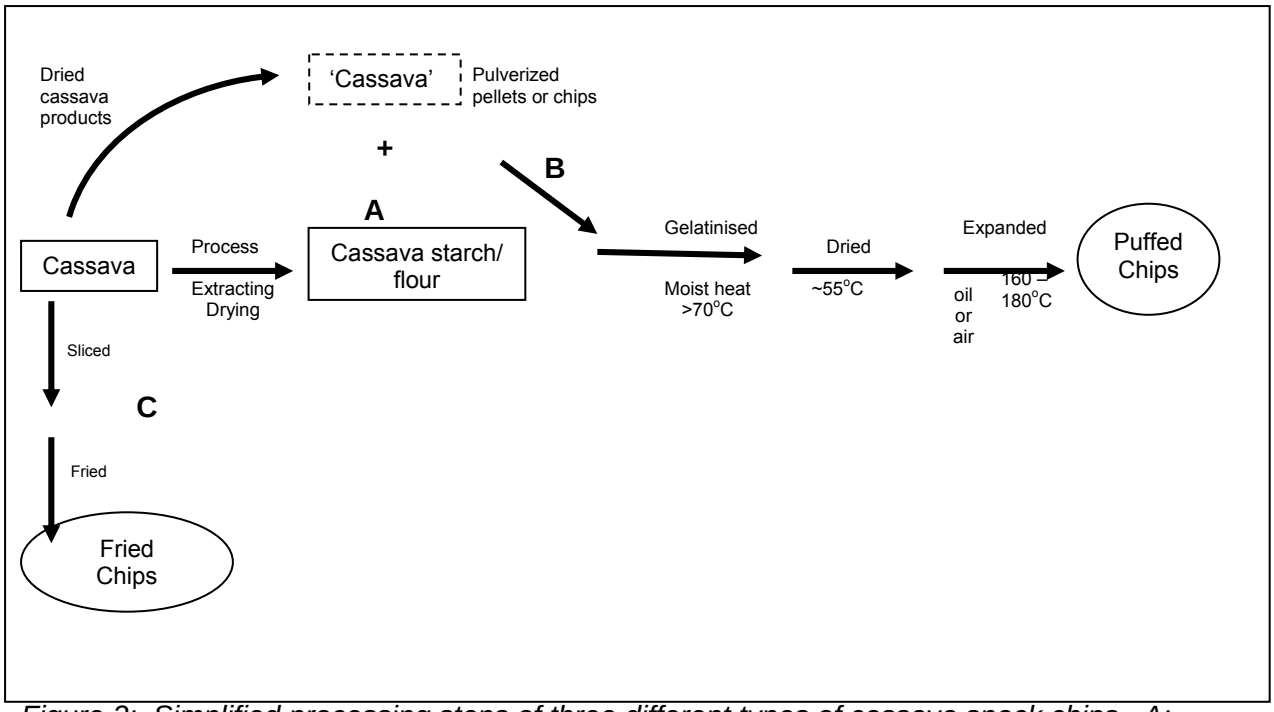


Figure 2: Simplified processing steps of three different types of cassava snack chips. A: Basic puffed cassava chips; B: 'Cassava +' puffed cassava chips; C: Fried cassava chips.

Type A cassava chips, using properly processed cassava starch or flour, should be able to achieve a level of total hydrocyanic acid below 10 mg/kg.

Type B and C cassava chips have much higher potential for containing total hydrocyanic acid above 10 mg/kg because the less processed dried raw cassava may contain higher levels of hydrocyanic acids than the cassava flour. To comply with a limit of 10 mg/kg, these types of chips need to be produced from low total hydrocyanic acid cassava or the cassava must be processed sufficiently to reduce the levels of total hydrocyanic acid.

In summary, the level of total hydrocyanic acid in ready-to-eat cassava chips can be less than 10 mg/kg through use of starch or flour as the primary raw material, appropriate selection of low total hydrocyanic acid cassava or through adequate processing of cassava tubers. These measures are likely to increase costs for producers of ready-to-eat cassava chips because the selection of low total hydrocyanic acid cassava may be difficult to guarantee and additional processing will require additional resources.

1 INTRODUCTION

This Food Technology Report examines the effect of various processing steps and parameters in eliminating total hydrocyanic acid from cassava products, such as dried cassava raw chips, pellets, meals, flour, starch and in particular, ready-to-eat puffed cassava chips. The processing steps are discussed in relation to the cyanogenesis pathway so that the causes for unacceptably high hydrocyanic acid levels found in finished cassava products can be identified and managed.

1.1 Cyanogenesis

Cyanogenesis is initiated in cassava when the plant tissue is damaged. Rupture of the vacuole releases linamarin and lower levels of lotaustralin (cyanogenic glycosides), which are hydrolysed by linamarase (denatured at 100°C), a cell wall-associated beta-D-glycosidase. Hydrolysis of linamarin yields an unstable hydroxynitrile intermediate, acetone cyanohydrin, plus glucose. Acetone cyanohydrin spontaneously decomposes to acetone and hydrogen cyanide (boiling point at 26°C) at pH >5.0 or temperatures > 35°C and can be broken down enzymatically by hydroxynitrile lyase (HNL) found in cassava leaves. However, HNL was found to be absent in the stems and roots of cassava, which could contribute to the presence of acetone cyanohydrin in the roots at low pH and temperature processing conditions (White, 1998).

It had been generally assumed that the total hydrocyanic acid (THA) present in cassava foods was in the form of cyanogenic glycosides (White, 1998). This assumption was based on the observation that acetone cyanohydrin is unstable and the hydrogen cyanide generated from acetone cyanohydrin is readily vaporised during food processing. Recently, however, it was demonstrated that the major hydrocyanic acid present in some poorly processed cassava roots was acetone cyanohydrin, not the glycoside. These results suggested the spontaneous (high pH and/or warm temperature) and enzymatic breakdown of acetone cyanohydrin was reduced or absent in roots. In part, the high residual acetone cyanohydrin levels could be attributed to the low-pH conditions established during the soaking (fermentation) of roots for food preparation (White, 1998).

1.2 Hydrocyanic acid in cassava and cassava products

Total hydrocyanic acid (THA) content of cassava depends on the numerous varieties of cassava plants, growing and processing conditions. During periods of drought, the THA content of both sweet and bitter cassava varieties increases (Bokanga et al., 1994). Bitter cassava varieties are more drought resistant and thus more readily available and cheaper. However, owing to food shortage in times of drought, less time is available for the additional processing required (Akintonwa and Tunwashe, 1992). Therefore higher THA would be consumed. Values from 15-400 mg/kg of fresh weight of THA in cassava roots have been mentioned in the literature.
Sweet varieties of cassava will typically contain approximately 15-50 mg/kg THA on a fresh weight basis. Table 1 provides a general composition of cassava tuber, flour and starch, illustrating the relative level of THA for cassava products.

Table 1: General composition of cassava tuber, flour and starch (Cardoso et al. 2005)

	Water %	Protein %	Fat %	Carbohydrate %	Fibre %	THA (mg/kg)
Raw tuber (peeled)	65	0.8-1.0	0.2-0.5	32	0.8	50-500
Flour	13	0.5-0.7	0.27-0.45	85	0.5	20-40*
Starch	10-14	0.3	0.11-0.22	85	0.1-0.15	<10

* Acceptable THA value in Indonesia is 40 mg/kg (Cardoso et al. 2005)

2 PROCESSING OF CASSAVA

Cassava roots, if left attached to the main stem, can remain in the ground for several months without becoming inedible. However, once the roots have been harvested, they start deteriorating within two to three days. The primary deterioration process is caused by physiological changes that produce discolouration of the tuber.

Also, the tuber would have conversion of starch into sugars, as well as accumulation of cyanogenic glycosides. Wounds and bruises from the harvesting process would also lead to micro-organism deterioration.

The drying process is one of the major preservation processes that can inhibit physiological reactions and microbial growth. Reducing moisture content to 14%, corresponding to a water activity of 0.70, is the critical point. The most common drying method is by sun-drying in rural farms. The cassava chips or granules from a grater are spread on a drying surface exposed to the sun. Fewer chips (low loading rate) on a tray or thinner chips, increase the drying rate, which would lead to higher quality chips (e.g. high starch content and white in colour). However, the THA level of cassava is lower when the drying time is longer. Therefore, drying parameters that affect the drying rate are important in determining and balancing the quality and the residual THA content of the dried cassava.

2.1 Dried cassava products

Cassava can be processed into different dried stable product forms (International Starch Institute, 1999) before marketing, as follows:

2.1.1 Raw chips

This is the most common form in which dried cassava roots are marketed and most exporting countries produce them. The raw chips are dried irregular slices of roots, which vary in size but should not exceed 5 cm in length, so that they can be stored in silos. They are produced extensively in Thailand, Malaysia, Indonesia and some parts of Africa.

- ***Processing of raw chips.*** The present method of processing chips is very simple, consisting of mechanically slicing the cassava roots and then sun drying the slices until their moisture content is only 14%. Dry raw chips can be stored longer and are cheaper to transport. The recovery rate of chips from roots is about 20-40%. When the roots are not sorted, peeled and washed the chips are usually brown in colour and have a high content of fibre, sand and foreign objects as well as THA. Trimming, peeling and washing the roots in a similar manner as for the processing of cassava flour are recommended in order to produce white raw chips of superior quality. The roots are shredded in a special machine, which is usually made locally.

- ***Drying***. Sun drying is used mostly where the sliced roots are spread out on drying areas or concrete. To produce good quality chips the roots must be sliced and dried as quickly as possible after harvest, the chips should be turned periodically in the drying period (usually two or three sunny days) until the moisture content reaches 13-15%. The chips are considered dry when they are easily broken but too hard to be crumbled by hand. The thickness of the slices also has an effect on the quality of chips. Thick slices may appear dry on the surface when their internal moisture content is still high. When rain threatens during the drying process, the chips are collected and put under shelter. Interrupted sun drying affects the quality of the finished chips and pellets. If the semi-dried chips are wet by rain, they become soggy and upon completion of drying lose their firm texture. In rainy regions, where continuous sun drying is difficult, some form of artificial heat drying is required.

2.1.2 Broken Roots

These are similar to chips in appearance, but broken roots are generally thicker and longer. They are often 12-15 cm long.

2.1.3 Pellets

Pellets are obtained from dried and broken roots by grinding and hardening into a cylindrical shape. The cylinders are about 2-3 cm long and about 0.4-0.8 cm in diameter and are uniform in appearance and texture (Figure 3). The production of pellets has recently been increasing as they meet a ready demand on the European markets. They have the following advantages over raw chips: quality is more uniform; they occupy 28-30% less space than raw chips, thus reducing the cost of transport and storage; handling charges for loading and unloading are also cheaper; they usually reach their destination sound and undamaged; while a great part of a cargo of sliced chips is damaged in long-distance shipment because of sweating and heating. Pellets are produced by feeding dried chips into the pelleting machine, after which they are screened and bagged for export. There is usually about 2-3% loss of weight during the process.

2.1.4 Meal

This product is the powdered residue of the raw chips and roots after processing to extract edible starch. It is generally inferior in quality to raw chips, pellets and broken roots; has a lower starch content and usually contains more sand. It is generally used as an animal feed.

2.1.5 Pulp

During the processing of cassava starch, the residual pulp is separated from the starch in the screening process. With effective extraction, the starch content is quite low and this pulp is utilised by ruminants only.

2.2 Cassava Flour

Drying has been identified as the major step for producing high quality cassava flour. The most basic drying technique is dependent on sun drying, especially in rural and domestic levels when capital investment in equipment and energy is not available. However, sun drying has limitations due to product susceptibility to damage caused by bad weather, possible slow rates of drying and contamination. As a result, other sources of more efficient modified drying processes such as heating the air, rotary drying and flash drying are being used. (The process is summarised in Figure 4A).

2.3 Cassava starch

The separation of the starch granules from the tuber in as pure a form as possible is essential in the manufacture of cassava starch. The starch granules are locked in cells together with all the other constituents of the protoplasm (proteins, soluble carbohydrates, fats and fibres), which can only be removed by a purification process in the aqueous phase. Processing of starch can therefore be divided into the following stages and is summarised in Figure 4B:

1. Preparation and extraction. Crushing of the cells and separation of the granules from other insoluble matter (e.g. adhering dirt and cell-wall material) including the preparatory operations of washing and peeling the roots, rasping them and straining the pulp with the addition of water.

2. Purification. Substitution of pure water for the aqueous solution surrounding the starch granules in the mash obtained in the first stage, as well as the operation of sedimentation and the washing of the starch in tanks and on flour tables, silting and centrifuging.

3. Removal of water by centrifuging and drying

4. Finishing. Grinding, bolting (milling) and other finishing operations. Crude dry cassava flour consisted mainly of hard lumps of starch at this stage. It has to be pulverised and followed by dry-screening, to produce free flowing flour. This operation is called bolting.

During the cassava starch production process, cyanogenic glycosides in the root are first converted to hydrocyanic acid and dissolved into the liquid in the rasper. Fresh rasped roots are then pumped through a series of coarse and fine extractors to produce starch slurry. The starch slurry is then passed to separators, dewatering centrifuges, and flash dryers. Therefore the THA in the final starch product is very low.

2.3.1 Modified tapioca starch

The process above describes the processing of native starch. This native starch is sometimes further processed by commercial starch supplying companies. The THA level reduces exponentially along the processing chain. When the native starch is received in the factory, the level of THA is typically below 10 ppm. The starch is then modified via pH adjustment and a reaction time of 10-24 hours at approximately 40^{o}C. These conditions reduce the THA level to around 0.01 ppm.

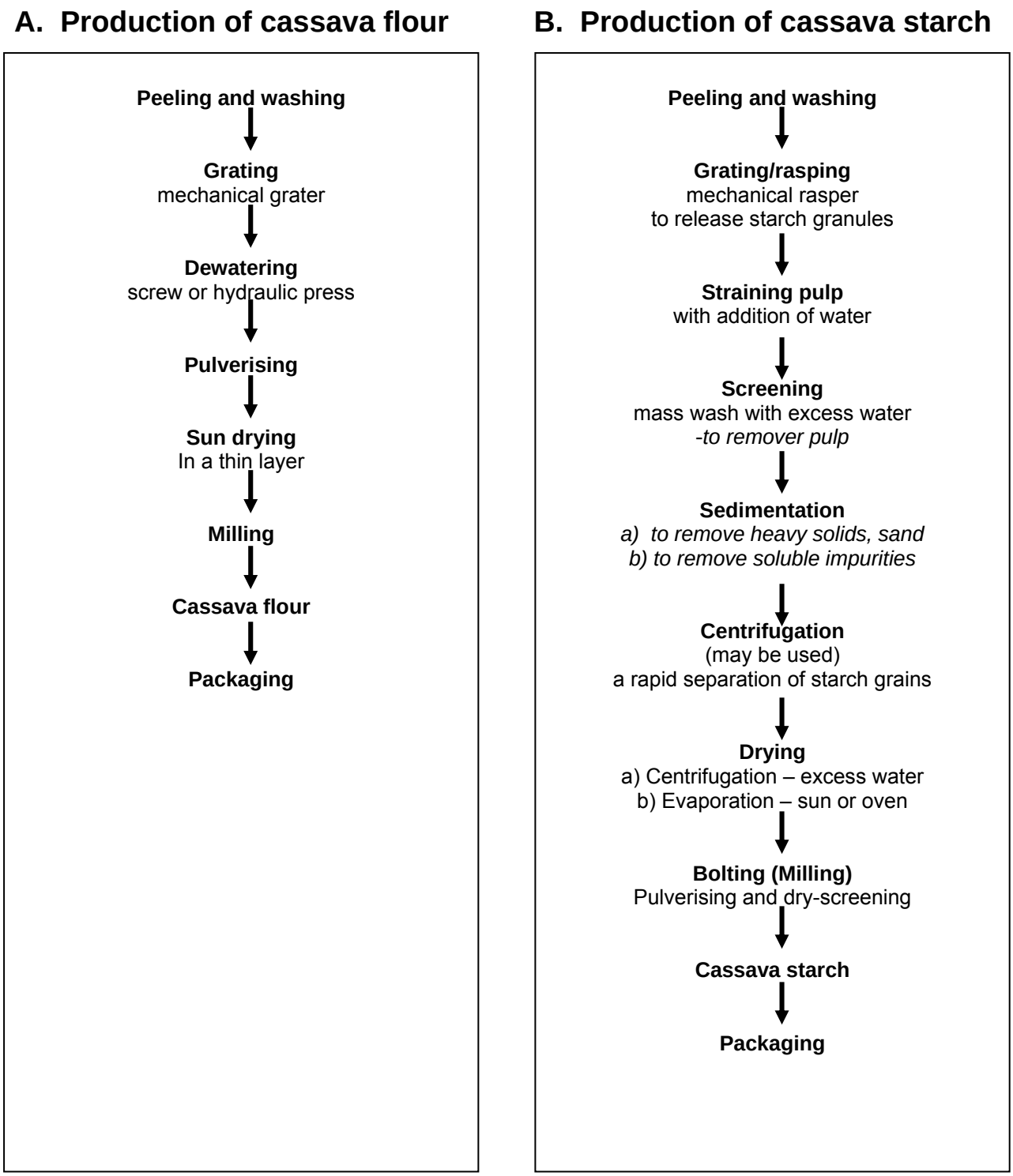


Figure 4: Processing steps for the production of (A) cassava flour and (B) cassava starch (Integrated cassava project, 2005; Grace, 1977)

2.4 Specifications for processed cassava products

Methods and specifications for determining the quality of cassava products are given by the FAO at http://www.fao.org/docrep/X5032E/x5032E09.htm. The cassava products include the following: cassava flour, dextrin, starch, chips, manioc, meal.

3. PRODUCTION OF READY-TO-EAT CASSAVA CHIPS

3.1 Four different product processes

3.1.1 Fried tuber chips

Fried tuber chips are prepared directly from fresh cassava roots. Contrary to those prepared from cassava dough (starch or flour), they do not expand and these tuber chips look like potato chips. Peeled cassava roots are kept in water at room temperature until use in order to reduce oxygen-activated physiological deterioration. The flesh is sliced with a rotary slicer with a thickness of 1.5 mm. The chips are fried in palm oil of 160^{o}C for 70-90 seconds to achieve a final moisture content of 4%. (Vitrac et al., 2000).

3.1.2 Traditional method – fried half-products

Half-products, also known as intermediates, are produced by gelatinisation of starchy dough in steam; the cooled steamed dough is then cut into shapes before drying until a brittle consistency (glassy state) with a moisture content of 15% is achieved. The dried sliced dough (half-product) can be expanded by frying in hot oil and puffed into a low-density, ready-to-eat, porous and crisp product with a moisture content of 4% (Gilbert, 2005). Such products are call *keropok* in Malaysia (Nair et al., 1996), *kroepeck* in Indonesia and *ca-charon* in the Philippines. The nutritional value of these puffed chips can be improved by adding high protein flour such as soy flour, or lower value fish or shrimp.

3.1.3 Extrudates (half-products)

Half-products can also be produce by an extrusion process. The half-products can then be expanded by further processing – frying or baking. The types of ingredients (e.g. starch and protein), feed moisture content and process variables (barrel temperature, screw speed and feed rate) influence physical characteristics of extrudates.

3.1.4 Extruded expanded chip products

Extrusion processing is carried out using an extruder with dough composition similar to the dough used for traditional chip production (Gilbert et al., 2005). Puffed chips are obtained directly after extrusion without further heating steps. During extrusion, molecules are exposed to heat and shear force, causing chemical reactions and consequently changes in component structure. These changes include gelatinisation and dextrinisation of starch.

Starch provides most of the texture and structure of expanded products made from cereals and tubers. The porous, expanded, sponge-like structure of the puffed chip is formed inside extrudates as a result of many small steam bubbles created by the rapid release of pressure after exiting the extruder (Suknark et al., 1997).

3.2 Physico-chemical and sensory properties of cassava chips

3.2.1 Fried tuber chips (Vitrac et al., 2000)

Because of the high initial dry matter content of the cassava used, the frying yield is about 50%, making cassava among the more interesting raw materials for processing by frying. The chips have relatively low oil uptake, between 25% and 40%. By comparison, potato production yield is around 20-30% and an oil content of between 30% and 40% (Vitrac et al., 2000). The lower the initial water content, the higher the yield.

Texture of the chips is independent of starch content and amylose content. However, higher fibre content leads to a less rigid cassava chip product compared to another of same water content at 4%. Higher levels of reducing sugar content in tuber may cause more browning, which may in turn influence the texture and flavour of the chips.

Cultivars with high cyanogenic glycoside content (over 400 mg/kg dry-wt-basis) resulted in bitter chips, indicating that glycoside elimination during frying was poor (Vitrac et al., 2000). Cultivars with a high cyanogenic glycoside must be pre-treated (Almazan, 1988 and Jones et al., 1994) to remove THA before processing.

3.2.2 Expanded or puffed snacks (Quintero-Fuentes et al., 1999)

Puffed snacks (traditional or extruded) consist of a starch gel matrix with numerous trapped air cells, which is then expanded through the use of pressure and/or heat. The number of air cells, the thickness of the cell wall and the composition of the gel matrix influence the texture of the RTE chips.

The texture may be modified by the addition of other materials to the cassava starch, or the more common practice of including less processed cassava products, such as ground dried raw cassava.

3.2.3 Difference between traditional puffed chips versus extruded puffed chips

One of the major differences between traditional puffed chips and extruded puffed chips is that despite having equivalent initial composition, the moisture content in the final product may be more than doubled in the extruded chips, about 4% and 10% respectively. It has been suggested that more fat is absorbed and more water is 'flashed off' during deep-fat frying. Extruded chips should be dried to below a target moisture content to achieve the desired crispy texture and storage stability (Gilbert et al., 2005).

It has been shown that a major portion of THA in the cassava flour dough was removed during the steaming step in the traditional process (Almazan, 1988). However, in the extrusion process, the gelatinisation step of the dough is in an enclosed barrel(s) and extruder temperatures could go up to 150-170^{o}C in a very short time. Therefore, the linamarase could be denatured and unable to convert glycosides to free hydrogen cyanide that could be flash off at the exit of the extruder.

4. PUBLISHED REPORTS ON THE EFFECT OF PROCESSING ON THA CONTENT IN CASSAVA PRODUCTS

4.1 THA reduction during the manufacturing of cassava products/snacks

During the preparation of the traditional puffed chips, it was reported that THA concentration decreased during processing into chips (Figure 4) (Almazan, 1988). Drying of the fresh tuber chips at 55^{o}C to less than 10% moisture content during flour preparation reduced THA considerably. Steaming of the dough removed a major portion of the hydrogen cyanide in all the varieties studied. A slight reduction occurred during drying of the cooked chips. After frying, THA concentrations of cassava varietals B and C decreased further to values of 0.4-3 mg/kg. In varietal A, an apparent increase in concentration was noticed, probably as a result of the decrease in moisture content without the simultaneous increase in the release of free hydrogen cyanide from the cyanogenic glycosides in the gel matrix. The initial heat treatment of the dough had inactivated linamarase and therefore no further conversion of cyanogenic glycoside proceeded; only the trapped hydrogen cyanide was removed by subsequent steps in the preparation of the chips.

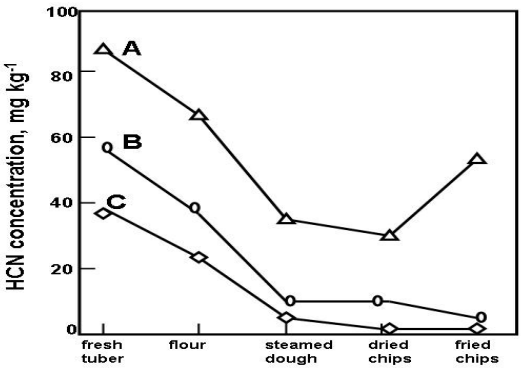


Figure 4: Total HCN concentration in fresh tuber, flour, steam dough, dried and fried chips of three varieties of cassava (A, B, C) (Almazan, 1988)

4.2 Effect of soaking techniques on removal of THA

Different techniques are used in Africa in reducing THA levels during the production of some cassava products (Agbor-Egbe et al., 2006). One example of such process is baton de manioc (Figure 5). The processing techniques are highly effective, in reducing the THA from 197-952 mg/kg to low levels of 1-27 mg/kg. Despite the different cassava varieties used for processing, reduction levels in excess of 97% of THA were obtained in the different cassava products. Analysis of the processing steps showed that maximizing the disruption of the root tissue caused the maximum reduction in cyanogenic glycosides and pH levels, which coincide with significant increase in cyanohydrin. The residual THA was in the form of cyanohydrin, which was partially removed during post-fermentation processes (Figure 6).

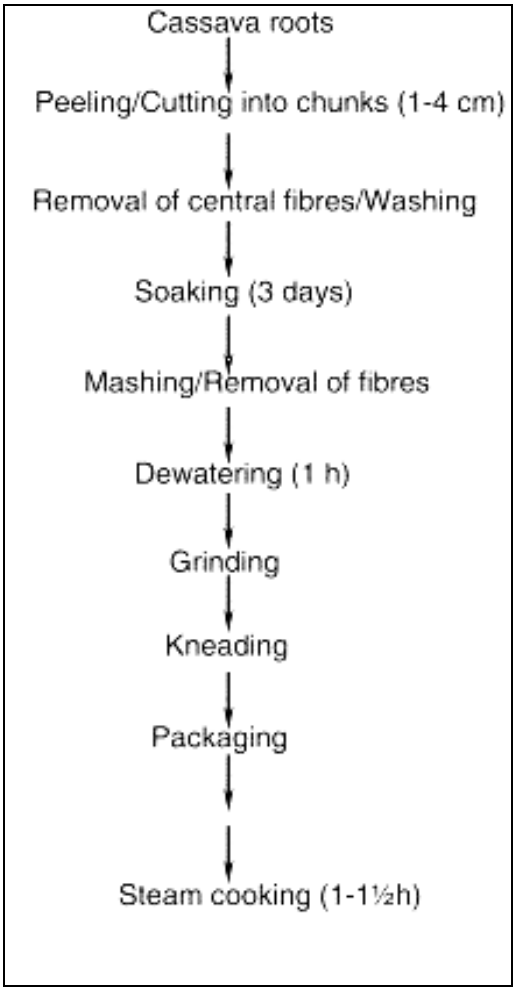


Figure 5: Flow diagram showing the process techniques used in the production of baton de manioc (an African cassava product) (Agbor-Egbe et al., 2006)

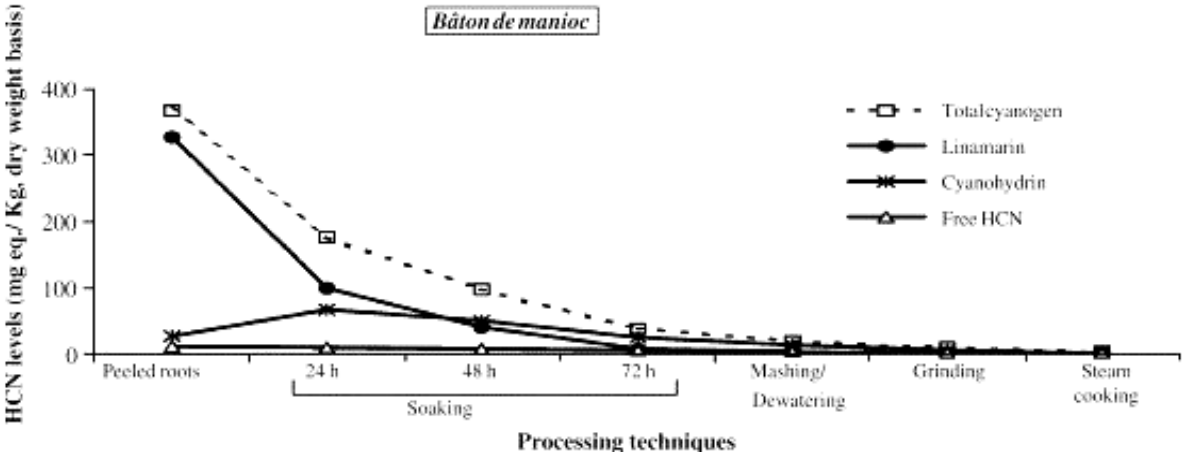


Figure 6: Cyanogen levels during processing for the production of Baton de manioc. (Agbor-Egbe at el., 2006)

4.3 Processing steps and parameters influencing the reduction of THA

Effect of processing steps and parameters in eliminating cyanide from cassava were examined (Jones et al., 1994). Approximately 50% THA of the mechanically prepared chips and 25% of manually prepared raw chips was eliminated by drying at 50°C. Mincing of whole roots completely degraded the glycosides; while rasping and mechanical chipping degraded 70-80% and 30% of the initial glucosides, respectively. The results indicate that the method of size reduction and particle size materially impacts the rate of glycoside degradation. The effect of the drying step on the quantity of glucosides was also established. Details of the results are in the following four tables (Table 3-6).

Table 3: Effect of chipping method on THA elimination from peeled and whole root chips (mean ± SD, n = 6)

	Cyanogen concentration (*mg/kg* (db)), *equivalent HCN*				*Moisture content (g/kg* (wb))
	Total	*Glucoside*	*Cyanohydrin*	*Free HCN*	
Mechanically prepared peeled chips					
Fresh chips	848 ± 27	647 ± 33	158 ± 17	16 ± 5	671
Dried chips	467 ± 28	455 ± 26	7 ± 2	5 ± <1	74
Mechanically prepared, whole root chips					
Fresh chips	1019 ± 98	841 ± 53	166 ± 22	12 ± <1	653
Dried chips	497 ± 20	466 ± 23	23 ± 4	8 ± <1	63
Manually prepared, peeled chips					
Fresh chips	734 ± 23	710 ± 25	16 ± <1	8 ± <1	682
Dried chips	590 ± 51	569 ± 50	16 ± <1	5 ± <1	213
Manually prepared, whole root chips					
Fresh chips	1143 ± 49	1063 ± 47	68 ± 4	12 ± 1	714
Dried chips	828 ± 69	695 ± 77	80 ± 9	10 ± <1	174

[a] db, dry-weight basis
[b] wb, wet weight (as is) basis
[c] Chips dried for 8 hours at 50°C

Table 4: Effect of raw chip size on cyanide elimination during drying of mechanically prepared whole root chips (mean ± SD, n = 6)

	Cyanogen concentration (*mg/kg* (db)), *equivalent HCN*				*Moisture content (g/kg* (wb))
	Total	*Glucoside*	*Cyanohydrin*	*Free HCN*	
Fresh roots	1186 ± 37	1133 ± 34	42 ± 3	11 ± 1	692
Unscreened chips					
Fresh chips	1042 ± 39	801 ± 29	172 ± 6	70 ± 3	682
Dried chips	508 ± 27	424 ± 32	89 ± 5	8 ± <1	41
Chips retained on 13 mm screen					
Fresh chips	988 ± 48	812 ± 50	119 ± 15	57 ± 14	677
Dried chips	626 ± 53	538 ± 43	81 ± 14	7 ± <1	42
Chips through 13 mm screen, retained on 10 mm screen					
Fresh chips	1090 ± 82	864 ± 84	160 ± 20	66 ± 17	681
Dried chips	419 ± 33	330 ± 20	81 ± 18	8 ± <1	41
Chips through 10 mm screen					
Fresh chips	1038 ± 91	644 ± 54	293 ± 44	101 ± 14	__[b]
Dried chips	430 ± 52	307 ± 33	112 ± 38	11 ± <1	41

[a] Chips dried for 22 hours at 50°C
[b] Not recorded

Table 5: Effect of disintegration method and holding time on cyanide elimination from whole roots (mean ± SD, n = 6)

	Cyanogen concentration (*mg/kg* (db)), *equivalent HCN*				*Moisture content (g/kg* (wb))
	Total	*Glucoside*	*Cyanohydrin*	*Free HCN*	
Fresh roots	1136 ± 67	1073 ± 57	56 ± 6	7 ± 1	694
Minced roots					
After mincing	890 ± 9	ND[a]	825 ± 15	65 ± 12	637
After 4 h	756 ± 32	ND	391 ± 10	365 ± 22	636
After 8 h	768 ± 37	ND	295 ± 20	473 ± 17	636
Rasped roots					
After rasping	911 ± 18	215 ± 33	675 ± 47	21 ± 3	649
After 4 h	679 ± 21	39 ± 11	613 ± 16	27 ± 1	639
After 8 h	612 ± 25	33 ± 14	544 ± 36	35 ± 2	633
Mechanically prepared chips, 8 mm x 8 mm					
After chipping	963 ± 73	718 ± 54	234 ± 35	11 ± 3	641
After 12 h	798 ± 53	405 ± 38	243 ± 21	150 ± 8	641
After 24 h	748 ± 72	392 ± 47	292 ± 17	64 ± 7	630
Pressed, mechanically prepared chips, 8 mm x 8 mm					
After chipping	975 ± 118	611 ± 91	341 ± 29	23 ± 3	633
After 12 h	1018 ± 111	610 ± 101	324 ± 65	84 ± 19	633
After 24 h	918 ± 19	518 ± 21	518 ± 29	123 ± 32	622

[a] ND, not determined

Table 6: Effect of disintegration, holding and drying on cyanide elimination from whole roots (mean ± SD, *n* = 6)

	Cyanogen concentration (*mg/kg* (db)), *equivalent HCN*				*Moisture content (g/kg* (wb))
	Total	*Glucoside*	*Cyanohydrin*	*Free HCN*	
Fresh roots	1136 ± 85	1073 ± 69	56 ± 6	7 ± 1	694
Rasped roots					
Fresh pulp	1141 ± 61	306 ± 42	802 ± 48	33 ± 12	669
After 4 h	913 ± 42	92 ± 17	677 ± 67	144 ± 67	641
After drying[a]	87 ± 7	18 ± 6	62 ± 3	7 ± 1	73
Mechanically prepared chips, 8 mm x 8 mm					
Fresh chips	1152 ± 82	728 ± 93	395 ± 38	29 ± 5	643
After 12 h	909 ± 81	585 ± 55	247 ± 15	77 ± 15	637
After drying[a]	451 ± 58	285 ± 31	145 ± 24	21 ± 2	67
Pressed mechanically prepared chips, 8 mm x 8 mm					
Fresh chips	1049 ± 39	614 ± 39	395 ± 22	40 ± 3	641
After 12 h	773 ± 45	486 ± 40	189 ± 40	98 ± 21	636
After drying[a]	395 ± 45	248 ± 29	126 ± 17	21 ± 3	54

[a] Drying for 9 hours at 50°C

4.4 New technologies that may impact THA reduction in cassava processing

4.4.1 Cassava flour

A simple wetting method (Bradbury, 2006 and Cumbana 2007). This method was trialled to reduce THA content of cassava flour. It was found that, providing that there was a reasonable amount of linamarase left in the flour, the THA content reduced about 16-45% if the flour had been wetted with water for over five hours. Addition of linamarase greatly increased the breakdown of cyanogenic glycosides. This method may be a feasible solution for cooking cassava flour or processing of cassava snacks since adequate amount of water has to be added to flour for hydration prior to processing.

4.4.2 Cassava Starch

Ozone in starch processing (Somboonchai et al., 2008). In a large-scale processing plant, the total amount of THA content entering the process ranges from 28 to 43 kg THA equivalent per day, based on a production capacity of 100 tons starch per day and 280-430 mg THA per kg root.

To minimise water usage in the starch production process, water from the dewatering centrifuge is normally recycled from the separators to the root washer, and forms the coarse extractor to the rasper. The THA content of the recycled water ranged from 10 to 50 mg/L, causing the accumulation of THA in the process and, subsequently, in the finished products

Research was carried out by Somboonchai et al. (2008) to reduce THA in the recycled water by using ozone in large scale starch production plants. The results showed that the cyanide content was sharply decreased to about one fourth of the initial concentration. Cyanide oxidation with ozone is rapid, and complete decomposition of cyanide can be obtained without harmful residue. However, they found that cyanohydrin that was not completely converted to hydrogen cyanide and some portions of bound cyanide remained in the process.

4.5 Modernisation of processing methods

Even though there are many reports supporting that the long soaking and drying of the cassava roots is the most efficient way of reducing the THA content, these steps are considered by some to be too time consuming (Iwuoha et al., 1997). With the modernisation of processing methods, low pH preservatives such as acetic acid and sodium metabisulphites may be added to inhibit microbial growth and colour changes. It is possible these would affect the conversion of cyanogenic glycosides (Jyothi et al., 2007).

The sun drying step may also be replaced by oven drying, possibly resulting in an increase in THA retention. For example, unpeeled roots from a high-cyanide cultivar of cassava were chipped and dried at 25^{o}C for 24 hours (at a loading rate of 8 kg/m^2) contained a total cyanide level of 482 mg/kg, while slow-dried product (72 hours, at a loading rate of 14 kg/m^2) was shown to contain a total cyanide level of 38 mg/kg (Panigrahi et al., 1992).

4.6 Estimating maximum THA in cassava for some processing techniques

Cardoso et al. (2005) have developed a simple equation to estimate the maximum amount of THA the initial cassava could have when a certain processing method is being used. For example, as illustrated in Table 7, if sun dried cassava chips are to be used in a product or further processed into flour, then sweet cassava tubers should not contain more than 12-32 mg THA/kg as a starting material in order to produce cassava chips or flour with 10 mg/kg or less. With this method of estimation, they found that traditional sun drying or heap fermentation by itself does not adequately remove THA when cassava is grown in a normal year and would definitely be inadequate in a drought year.

Table 7: Calculations of maximum initial THA levels (mg/kg) in cassava tuber, using a particular processing method that will lead to safe cassava products at 10 mg/kg (Cardoso et al., 2005)

Processing Method	Name of Product	% retention	Maximum initial root THA levels for final product to contain less than 10 mg/kg
Sun drying	Flour	25-33	12-16
Heap fermentation	Flour	12.5-16.5	24-32
Soaking & sun drying	Lafun / fufu	1.3-2.2	181-308
Soaking, fermentation & roasting	Farinha / gari	1.8-2.4	167-222
Crushing & sun drying	Flour	1.5-3.2	125-267

Overall, the soaking process seems to be an important part of detoxification step in the processing of some native cassava products (Agbor-Egbe et al., 2006); however that requires time and introduces risks of microbial contamination and biochemical deterioration that leads to darkening of the tuber. Bainbridge et al (1998) showed that crushing cassava root pieces prior to drying was found to improve the efficiency of THA removal by about 12% and that traditionally pounding and crushing cassava prior to sun-drying provides 90% removal of THA. Essers et al. (1996) concluded that the faster the rate of dehydration the lesser the amount of cyanogenic glycoside is degraded and furthermore, the cyanohydrin removal is improved by prolonged drying. Therefore, it again stresses the longer the drying time, the more effective is the THA removal.

References

Agbor-Egbe, T., Mbome, I. Lape. (2006). The effects of processing techniques in reducing cyanogen levels during the production of some Cameroonian cassava foods. *Journal of Food Composition and analysis,* 19 (4): 354-363

Almazan, A. M. (1988). Influences of Sugar and Cyanide Concentrations and Paste Viscosities of Cassava Flour on Fried Cassava Chip Quality. *Journal of Science, Food and Agriculture,* 42: 67-75

Bainbridge, Z., Harding, S., French, L., Kapinga, R., Westby,A. (1998). A study of the role of tissue disruption in the removal of cyanogens during cassava root processing. *Food Chemistry,* 62 (3): 291-297

Bokanga, M.(1994). Cassava - Post-harvest Operations. FAO http://www.fao.org/inpho/content/compend/text/ch12-01.htm. Accessed on 26 May 2008

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

Cardoso, A. P., Mirione, E., Ernesto, M., Massaza, F., Cliff, J., Haque, M. R., Bradbury, J. H. (2005). Processing of cassava roots to remove cyanogens. *Journal of Food Composition and Analysis,* 18: 451-460

Cassava based foods: A step towards safer consumption. http://staff.science.nus.edu.sg/~dbsyhh/web%20cassava%20food%20safety%202.htm Accessed on 23 May 2008

Codex Standard for Edible Cassava Flour (1989). *Codex Stan,* 176 (1): 1-4

Cumbana, A., Mirione, E., Cliff, J., Bradbury, J. H. (2007). Reduction of cyanide content of cassava flour in Mozambique by the wetting method. *Food Chemistry,* 101: 894-897

Essers, A. J. A., Van der Grift, R. M., Voragen, A. G. J. (1996). Cyanogen removal from cassava roots during sun-drying. *Food Chemistry,* 55(4): 319-325

Gilbert, O., Rakshit, S. K. (2005). Cassava starch snack formulation using functional shell fish by-products: mechanical, sorption and geometric properties. *Journal of the Science of Food and Agriculture,* 85: 1938-1945

Grace, M. R. (1977). Cassava processing – Cassava flour and starch. FAO http://www.fao.org/docrep/X5032E/x5032E02.htm Accessed on 20 May 2008

Integrated cassava project (2005). Processing steps for cassava flour. http://www.cassavabiz.org/postharvest/hqcf01.htm Accessed on 23 May 2008.

International Starch Institute (1999). Cassava. http://www.starch.dk/isi/starch/cassava.htm Accessed on 23 May 2008

Iwuoha, C. I., Banigo, E. O. I., Okwelum, F. C. (1997). Cyanide content and sensory quality of Cassava (*Manihot esculenta Crantz*) root tuber flour as affected by processing. *Food Chemistry,* 58(4): 285-288

High Quality Cassava Flour. Integrated Cassava Project. www.cassavabiz.org/poshydrocyanic acidrvest/hqcf01.htm Accessed on 20/05/2008

Jones, D. M., Trim, D. S., Bainbridge, Z. A., French, L. (1994). Influence of Selected Process Variables on the Elimination of Cyanide from Cassava. *Journal of Science Food and Agriculture,* 66: 535-542

Nair, C. K. V., Seow, C. C., Sulebele, G. A. (1996). Effects of frying parameters on physical changes of tapioca chips during deep-fat frying. *International Journal of Food Science and Technology,* 31: 249-256

Jyothi, A. N., Kiran, K S., Wilson, B., Moorthy, S N., Nambisan, B. (2007). Wet Storage of Cassava Starch: Use of Sodium Metabisulphite and Acetic Acid and the Effect on Starch Properties. *Starch,* 59: 141-148

Panigrahi, S., Rickard, J., O'Brien, M., Gay, C. (1992). Effects of different rates of drying cassava root on its toxicity to broiler chicks. *British Poultry Science,* 33: 1025-1042

Somboonchai, W., Nopharatana, N., Songkasiri, W. (2008). Kinetics of cyanide oxidation by ozone in cassava starch production process. *Journal of Food Engineering,* 84: 563-568

Standards for cassava products. Food and Agriculture organization of the United Nations. http://www.fao.org/docrep/X5032E/x5032E09.htm. Accessed on 20 May 2008

Vitrac, O., Dufour,., Trystram, G., Raoult-Wack, A. (2000). Deep-fat frying of cassava: influence of raw material properties on chip quality. *Journal of the Science of Food and Agriculture,* 81: 227-236

White, W. L. B., Arias-Garzon, D. I., McMahon, J. M., Sayre, Richard T. (1998). Cyanogenesis in Cassava. *Plant Physiol,* 116: 1219-1225

Attachment 6

Compliance Monitoring

Summary

While FSANZ is not a compliance agency, compliance monitoring aspects are taken into account by FSANZ when developing food regulatory measures. This consideration includes comments from compliance agencies and industry as to the availability of suitable compliance monitoring mechanisms, as well as the need for any transitional arrangements considered necessary to implement suitable compliance monitoring mechanisms.

In the Assessment Report of this Proposal, FSANZ proposed that an ML of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips be included in the Code. FSANZ initially identified effective compliance monitoring as a potential issue for this proposed food regulatory measure. On this basis, FSANZ invited comment on the need to prescribe specific compliance procedures and the need for any transitional arrangements in relation to the proposed food regulatory measure.

A variety of comments were received from submitters about effective compliance monitoring and FSANZ has considered these comments. The comments included:

- information on what were regarded by some submitters as shortcomings with a certain test kit used to measure hydrocyanic acid;
- support from some submitters for the prescription of a method to provide certainty as to the methods to use;
- information from other submitters about the methods for the analysis of total hydrocyanic acid which have been in existence for many years and that accredited laboratories for this analysis already exist;
- concerns that time was needed to validate methods, source essential analytical consumables (reference standards, enzymes) and address issues associated with variable results;
- suggestions for a transitional period to alleviate the difficulties with the issues above, including the suggestion that the proposed maximum level should be delayed pending resolution of these issues; and
- comments from other submitters that did not support a transitional period and did not include any concerns with their current ability to monitor compliance.

Based on the submissions, the main issues with monitoring compliance with the proposed food regulatory measure are the specific substances that should be measured and the current analytical capability available for measuring these substances. By stipulating the substances to measure (see below), FSANZ considers that compliance with the proposed food regulatory measure could be effectively monitored, as methods are available to monitor these substances in food. Following the assessment of the comments and based on other aspects considered in this Proposal, FSANZ considers that:

1. a definition of total hydrocyanic acid in the context of ready-to-eat cassava chips should be included in the Code to identify those substances which should be measured in determining compliance with the proposed ML. FSANZ considers that this will facilitate compliance monitoring and the following definition is considered appropriate:

Hydrocyanic acid, total means any hydrocyanic acid including hydrocyanic acid evolved from linamarin, lotaustralin, acetone cyanohydrin and butanone cyanohydrin during or following either enzyme hydrolysis or acid hydrolysis, expressed as milligrams of hydrocyanic acid per kilogram of ready-to-eat cassava chips.

2. methods should not be prescribed for measuring total hydrocyanic acid in foods, including ready-to-eat cassava chips. This is because submissions have indicated there are published methods that could be effectively used for measuring total hydrocyanic acid. By defining the substances in the definition of 'total hydrocyanic acid', FSANZ considers that the potential use of inappropriate methods is sufficiently minimised. This approach is considered more practical than prescribing a specific method of analysis. FSANZ also considers that the prescription of a particular method would be unnecessary and impractical, and is likely to prevent method innovation and restrict the use of equivalent, appropriate methods for compliance purposes.

FSANZ acknowledges that there are implementation issues associated with the ML. However, the role of FSANZ does not extend to developing or validating methods or determining specific arrangements for compliance monitoring. These aspects will need to be implemented by compliance agencies either individually or collectively.

FSANZ also acknowledges the view of some submitters that this Proposal and the maximum level be delayed while compliance agencies, industry and individual laboratories develop and validate methods. FSANZ is concerned about this specific food regulatory measure being delayed, particularly given the potential public health implications. On this basis and as methods for measuring total hydrocyanic acid are available, FSANZ considers that the food regulatory measures should progress in accordance with the FSANZ Act and that transitional arrangements should not apply. This will allow compliance agencies and any laboratories with existing capability to institute testing, while remaining laboratories develop and validate methods in accordance with their own individual circumstances.

In conclusion, FSANZ considers that:

- methods for measuring total hydrocyanic acid should not be prescribed as a definition of total hydrocyanic acid is sufficient to facilitate effective compliance monitoring; and
- transitional arrangements should not apply, given the potential public health implications and that methods have been published for measuring total hydrocyanic acid.

1. Introduction

This report focuses on certain compliance monitoring aspects that were identified during the Assessment phase of this Proposal and which have been commented on in specific submissions to the Assessment Report.

FSANZ is not a compliance agency and is therefore unable to institute compliance arrangements such as sampling and testing of foods for compliance with the Code. However, whenever developing food regulatory measures, FSANZ takes into account comments on compliance monitoring aspects, including methods. In considering these comments, FSANZ will assess:

- whether specific compliance monitoring procedures should be prescribed in the Code (e.g. methods); and
- whether transitional arrangements should apply to food regulatory measures to enable compliance agencies or industry to develop and institute compliance arrangements.

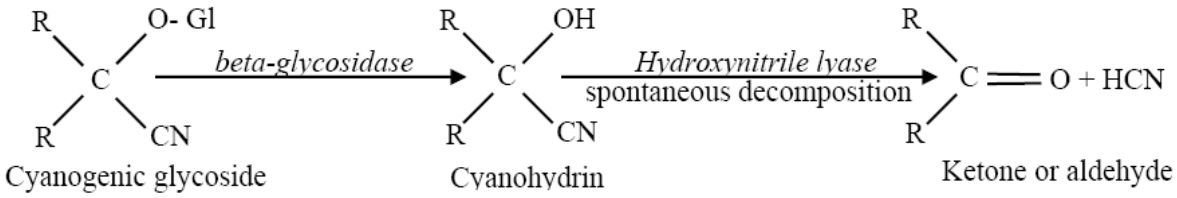
2. Background

In relation to Proposal P1002, FSANZ proposed that a maximum level of 10 mg/kg for total hydrocyanic acid in ready-to-eat cassava chips be included in the Code. As part of the Assessment, FSANZ initially identified effective compliance monitoring of total hydrocyanic acid in ready-to-eat cassava chips as a potential issue. In the Assessment Report for Proposal P1002, FSANZ proposed not to prescribe a method of analysis for total hydrocyanic acid in ready-to-eat cassava chips. This was on the basis that methods have been published and the prescription of a method would restrict the flexibility of industry and compliance agencies to develop more contemporary methods for monitoring hydrocyanic acid in ready-to-eat cassava chips. However, FSANZ invited comment on:

- the need to prescribe methods of analysis and if so, full details or references of these methods so they could be considered for prescription in the Code; and
- the need for any transitional arrangements for the proposed level and the reason for such transitional arrangements.

Levels for 'hydrocyanic acid, total' in certain foods have been in the Code for many years and no compliance monitoring problems with these levels have been raised with FSANZ. In addition, the international standards for certain foods, including processed cassava foods have also been in existence for some time. Notwithstanding these longstanding requirements, the food regulatory measures proposed as part of Proposal P1002 have raised some issues about the compliance monitoring for 'total hydrocyanic acid' in ready-to-eat cassava chips.

Cyanogenic glycosides are converted by β-glycosidases into sugars and a cyanohydrin and these cyanohydrins can then be converted into a ketone or aldehyde and hydrocyanic acid, either through spontaneous decomposition or enzyme action.[18] This can be represented by the following diagram from the European Food Safety Authority 'Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to Cyanogenic Compounds as Undesirable Substances in Animal Feed':



Linamarin and to a lesser degree, lotaustralin are the major cyanogenic glycosides in cassava. In the case of linamarin the 'R' in the diagram above represents two methyl groups. In the case of lotaustralin the 'R' in the diagram above represents one methyl and one ethyl group.

Under appropriate conditions, linamarin is converted to acetone cyanohydrin and glucose, and the acetone cyanohydrin decomposes to form acetone and hydrocyanic acid. Under appropriate conditions, lotaustralin is converted to butanone cyanohydrin and glucose, and the butanone cyanohydrin decomposes to form butanone and hydrocyanic acid.

3. Submission Issues

Submissions raised a number of issues of relevance to compliance monitoring including:

[18] Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to Cyanogenic Compounds as Undesirable Substances in Animal Feed

- the substances included in the term ‘total hydrocyanic acid’;
- the availability and prescription of methods for measuring total hydrocyanic acid in ready-to-eat cassava chips; and
- implementation aspects for instituting compliance monitoring.

3.1 Substances included in the term ‘total hydrocyanic acid’

FSANZ explained its understanding of the term ‘total hydrocyanic acid’ in the Assessment Report and at that time did not consider it needed further definition. This was based primarily on the longstanding existence of this term in the Code and the international standard for edible cassava flour. However, some submissions advocated further definition of this term to ensure clarity as to the substances to be measured for compliance with the proposed ML.

In general terms, and as stated in Section 1.1.1 of the Assessment Report, the term ‘total hydrocyanic acid’ is the hydrocyanic acid which may be released from cyanogenic glycosides as well as any ‘free’ or unbound hydrocyanic acid in the food. In the case of ready-to-eat cassava chips, the ‘total hydrocyanic acid’ is the existing hydrogen cyanide in the chips and any hydrogen cyanide evolved from these chips following enzyme or acid hydrolysis. This hydrolysis results in the conversion of cyanogenic glycosides to cyanohydrins and then to hydrogen cyanide. The ‘total’ hydrogen cyanide is then measured.

One submitter advocated the use of the Association of Official Analytical Chemists (AOAC) method for cyanide in water. Another submitter also referred to an AOAC method for cyanide, which FSANZ understands is the method for measuring cyanide in water. FSANZ does not consider that this method is adequate for measuring the hydrocyanic acid from cyanogenic glycosides in ready-to-eat cassava chips because it is not known whether it includes the hydrocyanic acid from the hydrolysis of the glycosides. However, FSANZ has noted that the AOAC has published measured methods for measuring cyanogenic glycosides in animal feed. FSANZ has not determined whether this method will determine ‘free’ or ‘total’ hydrocyanic acid.

One submission indicated their preference for the ‘free HCN’ method as opposed to the ‘Total HCN’ method. In considering this submission and the methods referred to in it, FSANZ is of the view that the ‘free HCN’ referred to in this submission was actually ‘total hydrocyanic acid’ as stated in the Assessment Report. These comments indicate some confusion about ‘free’ versus ‘total’ hydrocyanic acid. On this basis and taking into account some of the comments in other submissions, FSANZ considers that it would appropriate to define the term ‘total hydrocyanic acid’ for ‘ready-to-eat cassava chips’. This will ensure that the appropriate range of substances are measured for compliance with the proposed ML and avoid any potential confusion about the substances to be determined in any analyses.

The intention of the definition would be for the term ‘hydrocyanic acid, total’ to include any hydrocyanic acid[19] in ready to eat cassava chips, including any hydrocyanic acid evolved from linamarin[20], lotaustralin[21], acetone cyanohydrin[22], butanone cyanohydrin[23] during or following hydrolysis (enzyme or acid hydrolysis).

[19] Hydrogen cyanide. CAS No. 74-90-8.
[20] 2-methyl-2-[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy-propanenitrile. CAS No. 554-35-8.
[21] (2*S*)-2-methyl-2-{[(2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yl]oxy}butanenitrile. Sometimes known as ‘methyl linamarin’. CAS No. 534-67-8.
[22] 2-hydroxy-2-methyl-propanenitrile. CAS No. 75-86-5.
[23] 2-hydroxy-2-methylbutanenitrile. CAS No. 4111-08-4.

The limit would apply to the sum of the hydrocyanic acid in the ready-to-eat cassava chips and any hydrocyanic evolved from these substances and expressed on a milligram of hydrocyanic acid per kilogram of ready-to-eat cassava chips basis.

The following definition is considered appropriate for 'hydrocyanic acid, total' for ready-to-eat cassava chips:

> **Hydrocyanic acid, total** means any hydrocyanic acid including hydrocyanic acid evolved from linamarin, lotaustralin, acetone cyanohydrin and butanone cyanohydrin during or following either enzyme hydrolysis or acid hydrolysis, expressed as milligrams of hydrocyanic acid per kilogram of ready-to-eat cassava chips.

FSANZ considered whether the definition should be restricted to enzyme hydrolysis or include both enzyme and acid hydrolysis. From the submissions and information available to FSANZ it would appear that the enzyme hydrolysis methods are more readily available and reflect contemporary use. However, so as not to unnecessarily limit the use of appropriate methods which determine the substances in the definition; FSANZ considers that the definition should refer to both enzyme and acid hydrolysis.

3.2 The availability and prescription of methods for measuring total hydrocyanic acid in ready-to-eat cassava chips

In the Assessment Report, FSANZ stated that the prescription of methods can inhibit method development and would require the prescribed method to be used for regulatory purposes even though better, cheaper or more sophisticated methods may be developed in the future. FSANZ also stated that prescribing a method may also prevent the use of equivalent methods for monitoring purposes and restricts the flexibility of industry and compliance agencies. In the absence of a prescribed method, analysts would still need to develop and use methods that are 'fit for purpose' and suitably validated.

Notwithstanding this view, FSANZ acknowledged that ready-to-eat cassava chips are a unique product and that there may be specific analysis aspects that may justify the prescription of a method. The Assessment Report stated that if a method were to be prescribed then there would need to be agreement on the method that should be prescribed, and full details of the method would be needed so this detail could be included in the Code. On this basis, FSANZ invited comment on the need to prescribe a method for the analysis of hydrocyanic acid in ready-to-eat cassava chips and the details of the method that should be prescribed.

Analyses for total hydrocyanic acid involve determination of the total amount of cyanide that can be formed from cyanogenic glycosides, the cyanohydrins and cyanide. According to the European Food Safety Authority[24], a wide range of relatively simple assays have been developed for this purpose. These are based on:

- hydrolysis of the glycosides present;
- degradation of cyanohydrins to form cyanide; and
- subsequent measurement of total cyanide by one of several available methods.

Based on the comments in submissions and other information available to FSANZ, methods for determining total hydrocyanic acid in cassava based foods are available and have been available for many years.

[24] Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to Cyanogenic Compounds as Undesirable Substances in Animal Feed

FSANZ has also noted that one submission referred to the potential for Standards Australia to develop a method, and that another submission referred to the establishment of an analysis test method funded by Crop & Food Research at Lincoln University in New Zealand.

Among the submitters there appeared to be more references to methods based on enzyme hydrolysis and there are enzyme based methods which do not rely on linamarase[25]. However, there are other methods based on acid hydrolysis which may also be appropriate if appropriately validated[26,27]. From the information available to FSANZ, these methods appear capable of monitoring total hydrocyanic acid as defined above.

Some submitters advocated method prescription to ensure 'acceptable' methods were used or to 'provide guidance on the appropriate methodology to be used'. One submitter in particular advocated that 'the establishment of a Maximum Limit must be accompanied by a prescribed methodology of testing to deliver certainty for industry and to maintain industry and public confidence in the consistency of enforcement'.

It should be noted that method prescription does not ensure that the result is accurate or that analysis is performed correctly. Results, their accuracy and the conduct of analyses, including method validation, is the responsibility of analysts, their accreditation bodies and if relevant, analyst appointment or approval arrangements. For these reasons and given the range of methods available, FSANZ does not consider that it is necessary or practical to prescribe a particular method for determining total hydrocyanic acid in ready-to-eat cassava chips.

In relation to 'certainty for industry' and 'consistency of enforcement', FSANZ considers that defining the substances in the definition of 'total hydrocyanic acid' provides sufficient certainty as to the substances to be measured. This approach is the same approach used for many other limits in the Code where methods are not prescribed. On this basis, FSANZ considers that it should therefore be adequate in relation to total hydrocyanic acid in ready-to-eat cassava chips.

In summary, FSANZ considers that a range of methods are available for suitably determining the substances in the definition of 'total hydrocyanic acid'. For this reason and based on the comments in submissions, FSANZ does not consider it necessary, practical or appropriate to prescribe or 'recommend' methods that should be used to monitor compliance with the proposed ML. By defining the substances in the definition of 'total hydrocyanic acid', FSANZ considers that the potential use of inappropriate methods is sufficiently minimised. FSANZ considers that accredited, authorised, approved or appointed analysts should determine and validate the method which determines the substances stipulated in the definition and which best suits their individual facility and result reporting requirements.

3.3 Implementation aspects for instituting compliance monitoring

In the Assessment Report, FSANZ acknowledged that the establishment of a new maximum level may mean that compliance agencies need to request analysts to develop new methods for regulatory analysis. FSANZ also acknowledged that industry may need to update or develop quality assurance methods to monitor compliance.

---

[25] P.N. Okafor. Determination of the hydrolytic activity of *Achatina achatina* β-glucosidease toward some cyanogenic glycosides of some tropical plants. Process Biochemistry. 40 (5), April 2005, 1579-1582.

[26] Haque, R. H and Bradbury, J.H. Total Cyanide Determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry 77 (2002) 107-114.

[27] Bradbury, J.H. Egan S.V., Lynch M J. Analysis of Cyanide in Cassava Using Acid Hydrolysis of Cyanogenic Glycosides. *J Sci Food Agric* 1991, 55, 277-290.

This may require the purchase of specific analytical standards, reference materials or analytical equipment, as well as time for the validation of any analytical methods. For this reason, FSANZ invited comment on any transitional arrangements that compliance agencies or industry consider may be necessary.

Some submitters raised a number of implementation aspects including:

- the availability of materials to institute the methods e.g. Enzymes, standards etc
- the time required for laboratories to validate methods and if relevant seek accreditation for this analysis;
- concerns from some submitters about variable results or issues with test kits that have recently been used to monitor total hydrocyanic acid; and
- suggestions about approaches to use for monitoring compliance.

In considering these implementation aspects, it should be noted that FSANZ cannot specifically implement compliance arrangements. FSANZ can only consider whether a transitional period may be appropriate to allow others time to address the implementation issues.

A submitter provided a comprehensive amount of data on analyses that they had performed and also expressed concern about the variability of results from these analyses. From the information available, FSANZ is not able to determine the reason for this variability. In any case, this variability can only be addressed by analysts as their method development and validation progresses.

A submitter expressed concern that an ML to protect public health and safety was being considered when concerns had been expressed about methods used to generate results and that there were variations in results reported by some laboratories. The submitter further stated that these issues should be resolved and that analytical methodology 'must be agreed upon' before the setting of a standard.

One submitter requested a specific transitional period of 12 months on the basis that there is no validated method for testing hydrocyanic acid and a lack of laboratories with the analytical capability. Another submitter stated that certain essential substances to validate methods were not currently available to them and a further submitter commented that methods would need to be validated by analysts to withstand court scrutiny.

One submitter was under the impression that the only method currently employed is the Bradbury test kit and listed some 'shortcomings' with this test kit. Another submitter provided substantial comment on the 'Bradbury test kit' and questioned its use for compliance monitoring.

FSANZ is of the view that many methods are available for monitoring total hydrocyanic acid in foods and as stated above, FSANZ does not propose to prescribe a method for determining total hydrocyanic acid in ready-to-eat cassava chips. However, FSANZ acknowledges that other existing laboratories, including laboratories appointed or approved for regulatory analysis, may not have validated the methods for their facility or may be awaiting materials in order to validate published methods for their facility.

Submitters provided a range of comments with some supporting transitional arrangements to allow time to address the implementation aspects and some submitters not being supportive of transitional arrangements. Some submissions appeared to advocate an indefinite delay for the ML pending resolution of analytical issues that laboratories may be experiencing.

FSANZ acknowledges that there are implementation issues associated with the proposed ML.

FSANZ agrees that these implementation issues should be considered in parallel with the development of food regulatory measures and where appropriate include transitional arrangements to facilitate effective compliance monitoring. However, a number of these issues and the approaches to use in relation to monitoring compliance will need to be implemented by compliance agencies either individually or collectively.

While the role of FSANZ does not extend to developing or validating methods or arrangements for compliance monitoring, FSANZ does have the option of delaying the progression of a food regulatory measure while compliance agencies, industry and individual analysts address these issues to their own satisfaction. However, given the potential public health implications, FSANZ does not consider it appropriate to delay the progress of the proposed food regulatory measure in this proposal while these implementation issues are further developed.

In summary, FSANZ acknowledges the view of some submitters that this Proposal be delayed while compliance agencies, industry and individual laboratories develop and validate methods. However, FSANZ is concerned about this specific food regulatory measure being delayed, particularly given the potential public health implications. On this basis and as methods for measuring total hydrocyanic acid are available, FSANZ considers that the food regulatory measure should progress in accordance with the FSANZ Act. This will allow compliance agencies and any laboratories with existing capability to institute testing, while remaining laboratories develop and validate methods in accordance with their own individual circumstances. FSANZ acknowledges that this may require some compliance agencies to appoint, authorise or approve different analysts from those currently appointed but considers that the potential public health implications warrant this approach.

References

Bradbury, J.H., Egan S.V., Lynch M. J. Analysis of Cyanide in Cassava Using Acid Hydrolysis of Cyanogenic Glycosides. *J Sci Food Agric* 1991, 55, 277-290.

Bradbury, J. H., Egan, Sylvia V. Improved methods of analysis for cyanide in cassava and screening for low cyanide varieties in the Pacific. Symposium of the International Society for Tropical Root Crops. Accra (Ghana). 1991. Proceedings. International Society for Tropical Root Crops. Accra, GH. 1994. p. 237-242

Cooke, R.D. An enzymatic assay for the total cyanide content of cassava (Manihot esculenta Crantz). J Sci Food Agric, 1978, 29 345-352.

Cyanide content of cassava and fermented products with focus on attiéké and attiéké Garba. A dissertation submitted to the SWISS FEDERAL INSTITUTE OF TECHNOLOGY ZURICH, Christoph Heuberger. Zurich 2005.

Essers, A.J.; Bosveld, M.; Grift, R. M.; Voragen, A.G.J. Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen.; J Sci Food Agric (United Kingdom), 1993, 63 (3) p. 287-296.

Haque, R. H and Bradbury, J.H. Total Cyanide Determination of plants and foods using the picrate and acid hydrolysis methods. Food Chemistry 77 (2002) 107-114.

O'Brien, G.M., Taylor, A.J., Poulter, N.H. Improved enzymic assay for cyanogens in fresh and processed cassava. J Sci Food Agric. 1991. v. 56 (3) p. 277-289.

Okafor, P.N. Determination of the hydrolytic activity of *Achatina achatina* β-glucosidease toward some cyanogenic glycosides of some tropical plants. Process Biochemistry. 40 (5), April 2005, 1579-1582.

Opinion of the Scientific Panel on Contaminants in the Food Chain on a Request from the Commission Related to Cyanogenic Compounds as Undesirable Substances in Animal Feed. *The EFSA Journal* (2007) 434, 1 – 67.