CYANOGENIC GLYCOSIDES IN CASSAVA AND BAMBOO SHOOTS

A Human Health Risk Assessment

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CONTENTS

SUMMARY

Cassava and bamboo shoots, grown primarily in the tropics, contain potentially toxic compounds called cyanogenic glycosides, linamarin and taxiphillin respectively, which break down upon disruption of the plant cells to form hydrogen cyanide. The toxicity of cyanogenic glycosides can be reduced by appropriate preparation of the plant material prior to consumption as food. Traditional uses of cassava and bamboo shoots as food are dependent on adequate processing prior to human consumption. If either cassava or bamboo shoots are eaten either raw or after inadequate processing, evidence of toxicity may be observed.

For cassava, peeling and slicing disrupts the cell structure of the plant, with subsequent liberation of hydrogen cyanide. Hydrogen cyanide can be removed by further processing such as cooking (baking, boiling, or roasting) or fermentation. For bamboo shoots, slicing into thin strips liberates hydrogen cyanide, which is removed by boiling.

Cyanogenic glycoside content of cassava and bamboo shoots

There are a number of varieties of cassava, each of which has a different cyanide level. Values from 15-400 mg/kg fresh weight of hydrogen cyanide in cassava roots have been reported in the literature. Sweet varieties of cassava (low cyanide content) will typically contain approximately 15-50 mg/kg hydrogen cyanide on a fresh weight basis. Sweet varieties of cassava can be processed adequately by peeling and cooking (e.g. roasting, baking or boiling), whereas bitter varieties of cassava (high cyanide content) require more extensive processing, involving techniques such as heap fermentation which take several days. Bitter varieties are not normally commercially traded.

There are approximately 1200 species of bamboo, although only a small number are used as food. Bamboo shoots may contain as much as 1000 mg/kg hydrogen cyanide, significantly higher than the amounts detected in cassava tubers, however, the cyanide content is reported to decrease substantially following harvesting. The bamboo shoots sold commercially as food can be processed adequately by boiling before consumption. The process of canning bamboo shoots liberates and adequately removes hydrogen cyanide.

Toxicity data

The potential toxicity of a food produced from a cyanogenic plant depends on the likelihood that its consumption will produce a concentration of hydrogen cyanide (HCN) that is toxic to exposed humans. Factors important in this toxicity are: (i) the plant may not be sufficiently detoxified during processing or preparation and, therefore, HCN may remain in the food; (ii) if the plant is consumed raw or insufficiently processed, HCN may be released in the body, until the low pH of the stomach deactivates the β-glucosidase enzyme.

Cyanide ingested by release from a plant containing cyanogenic glycosides, either prior to or following consumption, follows the known cyanide metabolic pathway and toxicokinetics for humans and animals. In humans, cyanide is detoxified by the enzyme rhodanese, forming thiocyanate, which is excreted in the urine. This detoxification requires sulphur donors, which by different metabolic pathways are provided from dietary sulphur amino acids. There are several factors influencing hydrolysis of cyanogenic glycosides, and therefore overall

toxicity, including the confounding influence of nutritional status, particularly with respect to protein, riboflavin, vitamin B12, sodium and methionine.

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.

In animals, the lethal doses of HCN are generally reported to be between 0.66 and 15 mg/kg body weight (bw) for various species. Chronic sub-lethal dietary cyanide has reportedly caused some reproductive effects including lower birth rates and an increased number of neonatal deaths; impaired thyroid function; and behavioural effects including increasing ambivalence and slower response time.

In humans, the symptoms of acute cyanide intoxication from inadequately prepared cassava or bamboo shoots can include: rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhoea, mental confusion, twitching and convulsions. Death due to cyanide poisoning can occur when the cyanide limit exceeds the limit an individual is able to detoxify. The likelihood of cyanide intoxication from consumption of cassava or bamboo shoots is dependent on body weight and it is possible that a child or person of smaller body weight would not be able to detoxify the cyanide resultant from a meal of inadequately prepared cassava or bamboo shoots. The acute lethal dose of hydrogen cyanide for human beings is reported to be 0.5-3.5 mg/kg bw. Approximately 50-60 mg of free cyanide from cassava and its processed products constitutes a lethal dose for an adult man.

Other long-term diseases related to dietary cyanide intake include (i) konzo, an upper motor neuron disease characterised by irreversible but non-progressive symmetric spastic paraparesis with an abrupt onset; (ii) tropical ataxic neuropathy (TAN), a term used to describe several neurological syndromes whose clinical features include optical atrophy; angular stomatitis; sensory gait ataxia; and neurosensory deafness; (iii) goitre and cretinism, which are caused by iodine deficiency, can be considerably aggravated by a continuous dietary cyanide exposure. These diseases occur in countries where there is chronic consumption of cassava as a staple food and dietary intake of protein and/or iodine are inadequate.

Dietary exposure

Consumption of both cassava and bamboo shoots in Australia and New Zealand is considered to be relatively low based on 1995 dietary survey data. More recent data based on trade statistics and information from industry indicates that in New Zealand cassava is the second major imported root crop, but consumption is largely confined to the Polynesian population and, to a more limited extent, the Asian population. In Australia, there is limited use of raw cassava but processed products, such as cassava chips and tapioca, are widely available. Fresh bamboo shoots in significant quantity have been available in Australia only since 2001 but the market is reported to be expanding.

Risk characterisation

Cassava and bamboo shoots contain cyanogenic glycosides that break down to produce hydrogen cyanide, which can cause both acute and chronic toxicity in humans. The symptoms of acute cyanide intoxication include rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhoea, mental confusion, twitching and convulsions. In extreme cases, death due to cyanide poisoning may occur. The chronic effects of cyanide intoxication are linked to regular long-term consumption in individuals with poor nutrition and are unlikely to be a significant concern in Australia and New Zealand.

By adequate processing (peeling, slicing and cooking), both the cyanogenic glycosides and hydrogen cyanide can be removed or reduced prior to consumption, thus significantly reducing the potential health risk. On the basis of information on cassava and bamboo products currently available in Australia and New Zealand and the levels of consumption, the likelihood of cyanide intoxication from consumption of cassava or bamboo shoots is low, but due care in preparation remains necessary. While the current users have adequate knowledge regarding the risks associated with consumption of cassava and bamboo shoots, more widespread use in the community would increase the public health risks.

CYANOGENIC GLYCOSIDES IN CASSAVA AND BAMBOO SHOOTS

A Human Health Risk Assessment

INTRODUCTION

Cassava grows well in a tropical climate and is an important root crop in Pacific Island countries, Latin America, Africa and regions of Asia. Bamboo shoots grow in a variety of climates and are commonly used in Asian cuisine. However, if either are eaten raw or processed inadequately, their consumption may be potentially harmful due to the presence of cyanogenic glycosides, linamarin in cassava and taxiphillin in bamboo shoots, which break down to produce hydrogen cyanide.

The potential toxicity of cassava and bamboo shoots can be significantly reduced by adequate processing to break down the cyanogenic glycoside and remove the resulting hydrogen cyanide. Traditional preparation techniques reflect the need for removal of the natural toxicants prior to consumption.

Cassava is grown for its enlarged starch-filled roots, which contain about 30% starch and very little protein. It is consumed in a number of forms: flour used for cooking; root slices; root chips; baked grated root; steamed grated root; pan fried grated root; steamed whole root; and tapioca pearls made as a pudding. Cassava leaves are also eaten in some countries after extensive boiling.

Cassava flour can be produced by either sun drying or heap fermentation. Cassava flour produced by heap fermentation has lower cyanide content than cassava flour produced by sun drying. Sweet varieties of cassava (low cyanide content) are adequately processed by peeling and then fully cooking (e.g. roasting, baking or boiling). Bitter varieties of cassava (high cyanide content) require further processing, involving techniques such as heap fermentation.

Bamboo shoots are a traditional component of Asian cuisine. There are approximately 1200 species of bamboo, although only a limited number of these are viable as food. Bamboo shoots may contain significantly higher levels of hydrogen cyanide than cassava tubers, however, the cyanide content is reported to decrease substantially following harvesting. Preparation generally involves boiling for an extended period.

Botanical characteristics of cassava and bamboo shoots

Cassava

The botanical name for cassava is *Manihot esculanta* Crantz and it is a member of the Euphorbiaceae (Surge) family. Cassava is also known by the other common names: manioc, manihot, and yucca. Cassava originates in Latin America and was later introduced into Asia and Africa.

Cassava is a perennial woody shrub that grows 1-3 m tall. It grows well between 30 degrees north and 30 degrees south of the equator, from sea level to an altitude of 2,000 m. Deep soil

and regular rains are needed and the ideal growing temperature is about 20 degrees. Cassava is propagated by 20-30 cm long cuttings of the woody stem and plants are usually spaced at 1 to 1.5 m. Cassava is often planted with other crops such as beans or maize.

The plant parts used are the storage root and leaves. The starchy tuber is most commonly consumed and very large tubers may reach the size of 0.5 m long and 10 cm in diameter.

There are a number of varieties of cassava that range from low cyanide content (referred to as 'sweet cassava') to higher cyanide content (referred to as 'bitter cassava'). Bitter cassava requires more extensive processing (sometimes more than one day) to remove the cyanogenic potential than sweet cassava.

Bamboo shoots

There are approximately 1200 species of Bamboo. Bamboo species can be categorised into two groups: the clumping types with short rhizomes (underground stems) botanically referred to as sympodial; and the running types with long rhizomes, referred to as monopodial. In general, the clumping types are adapted to sub-tropical and tropical climates and produce shoots after mid-summer, while the runners are adapted to cooler climates and produce shoots in spring. Clumping types have larger shoots (up to 5 kg each) than those of running types (usually not greater than 1.5 kg each) and are harvested when shoots are above ground, in contrast to shoots of running types which are mainly below soil surface at harvest (Midmore, 1988).

While there are approximately 1200 species of bamboo, only a few have been used as human food in Asia and Australia. Those currently used in Australia include:

- *Dendrocalamus asper;*
- *Dendrocalamus latiflorus;*
- *Bambusa oldhamii; and*
- *Phyllostachys pubescens.*

Dendrocalamus apser is the most important species in for shoot production in Thailand (Fu et al., 1987), while *Dendrocalamus latiflorus* and *Bambusa oldhamii* are the most important in Taiwan (Tai, 1985). The different bamboo species also have different levels of cyanide. The characteristics of the above varieties are described by Midmore (1998) below:

Dendrocalamus asper: Shoots can reach 30 cm in diameter, 30 cm in length and weigh 4-7 kg.
Dendrocalamus latiflorus: Mature plantations give shoots up to 60 cm in length and weigh 3-5 kg.
Bambusa oldhamii: Small diameter shoots (approximately 10 cm) weighing approximately 0.5 kg. This variety has good eating quality.
Phyllostachys pubescens: Shoots range from 7.5 to 15 cm in diameter and weigh on average 1.5 kg.

A period of three to seven years is required between establishing a bamboo plantation and the harvesting of commercial-sized shoots (Midmore, 1988).

Cyanogenic glycosides in plants

The cyanogenic glycosides may be defined chemically as glycosides of the α-hydroxynitriles and belong to the secondary metabolites of plants. They are amino acid-derived plant constituents. The biosynthetic precursors of the cyanogenic glycosides are different L-amino acids, which are hydroxylated, then the *N*-hydroxylamino acids are converted to aldoximes and these are converted into nitriles and hydroxylated to α-hydroxynitriles and then glycosylated to Cyanogenic glycosides (Vetter, 2000). All known cyanogenic glycosides are β-linked, mostly with D-glucose.

There are at least 2650 species of plants that produce cyanogenic glycosides and usually also a corresponding hydrolytic enzyme (beta-glycosidase), which are brought together when the cell structure of the plant is disrupted by a predator, with subsequent breakdown to sugar and a cyanohydrin, that rapidly decomposes to hydrogen cyanide (HCN) and an aldehyde or a ketone (Hosel, 1981; Moller and Seigler, 1999). The glycosides, cyanohydrins and hydrogen cyanide are collectively known as cyanogens. This combination of cyanogenic and hydrolytic enzyme is the means by which cyanogenic plants are protected against predators (Moller and Seigler, 1999).

There are approximately 25 cyanogenic glycosides known with the major cyanogenic glycosides found in the edible parts of plants being: amygdalin (almonds); dhurrin (sorghum); linamarin (cassava, lima beans); lotaustralin (cassava, lima beans); prunasin (stone fruit); and taxiphyllin (bamboo shoots).

The toxicity of a cyanogenic plant depends primarily on the potential concentration of hydrogen cyanide that may be released upon consumption. If the cyanogenic plant is inadequately detoxified during processing or preparation of the food, the potential hydrogen cyanide concentration which may be released can still be high. Upon consumption of a cyanogenic plant, β-glycosidase will be released during digestion and remain active until deactivated by the low pH of the stomach. This enzyme will hydrolyse the cyanogenic glycoside and release at least part of the potential hydrogen cyanide content of the plant (WHO, 1993).

GLYCOSIDE CONTENT OF CASSAVA AND BAMBOO SHOOTS

The actual level of cyanogenic glycosides of a cyanogenic plant is influenced by various factors, both developmental (endogenous) and ecological (exogenous). The development cycle of cyanogenic plants shows characteristic changes in cyanogenic glycoside (and HCN) content (Vetter, 2000).

Cassava

In cassava, the major cyanogenic glycoside is linamarin, while a small amount of lotaustralin (methyl linamarin) is also present, as well as an enzyme linamarinase. Catalyzed by linamarinase, linamarin is rapidly hydrolysed to glucose and acetone cyanohydrin and lotaustralin hydrolysed to a related cyanohydrin and glucose. Under neutral conditions,

acetone cyanohydrin decomposes to acetone and hydrogen cyanide. This reaction for linamarin is shown below in Figure 1.

Figure 1: Reaction of linamarin with linamarinase in cassava
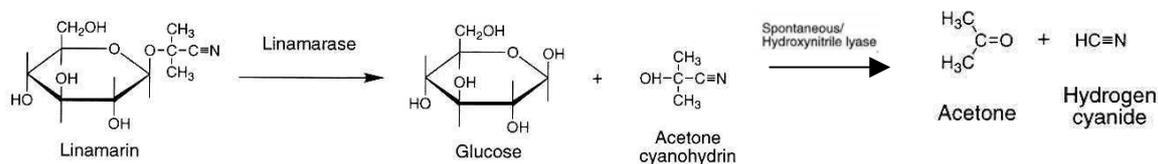
If the cassava plant is not adequately detoxified during the processing or preparation of the food, it is potentially toxic because of the release of this preformed hydrogen cyanide. The hydrogen cyanide is readily removed during processing of cassava, however, the presence of residual linamarin and its acetone cyanohydrin in cassava-based food products has the potential to cause adverse health effects.

There are many varieties of cassava and the cyanide content differs as well as the suitability for different growing and consumption conditions. Usually higher cyanide content is correlated with higher yields. During periods of drought the cyanide 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).

Values from 15 to 400 mg/kg of hydrocyanic acid in cassava roots on a fresh weight basis have been mentioned in the literature. Sweet varieties of cassava will typically contain approximately 15 to 50 mg/kg of hydrogen cyanide on fresh weight basis. Cassava leaves contain approximately 10% more linamarin than cassava roots.

Bamboo shoots

Bamboo shoots contain the cyanogenic glycoside taxiphyllin, which is a p-hydroxylated mandelonitrile tiglochinin. Taxiphyllin is hydrolysed to glucose and hydroxybenzaldehyde cyanohydrin. Benzaldehyde cyanohydrin then decomposes to hydroxybenzaldehyde and hydrogen cyanide.

A WHO report (1993) states that the concentration of cyanide in the immature shoot tip of bamboo is 8000 mg/kg of hydrogen cyanide, whereas Ferreira et al. (1990) report that bamboo shoots contain as much as 1000 mg/kg of hydrogen cyanide in the apical part. A sample of *Dendrocalamus giganteus* contained, on average, 894 mg/kg of hydrogen cyanide (Ferreira et al., 1995). The discrepancy between the two reported levels likely reflects the large number of varieties and their varying cyanide contents. It is likely that the varieties normally eaten contain, on average, closer to 1000 mg/kg of hydrogen cyanide. This total amount of cyanide appears quite high in comparison with the concentrations of cyanide in cassava however, the cyanide content in bamboo shoots decreases substantially following harvesting.

Processed cassava and bamboo shoot products

Cassava flour and other products

The safe level of cyanide in cassava flour has been set by the WHO as 10 ppm (FAO/WHO, 1991), while the acceptable level in Indonesia is 40 ppm (Damardjati et al., 1993).

The total cyanide content of cassava flour has been determined in African countries including different provinces of Mozambique, Tanzania, and the Central African Republic. Djazuli and Bradbury (1999) also surveyed the total cyanide content of cassava roots, cassava flour, cassava chips and gaplek in various cassava growing provinces of Indonesia. These results are summarised in Table 1.

A document entitled *Cassava based foods: A step towards safer consumption*[1] provides the cyanogenic potential of a number of commercially processed cassava-based products prepared from cassava roots and flour. These values are shown in Table 2.

Table 1: Total cyanide content of cassava flour

Source of flour	Year obtained	Number of samples surveyed	Author(s)	Total cyanide content (ppm)
Mujocojo (Nampula, Mozambique)	1996	32	Cardoso et al., 1998	49(±29)
Mujocojo (Nampula, Mozambique)	1997	30	Ernesto et al., 2000	26(±23)
Terrene A (Nampula, Mozambique)	1996	22	Cardoso et al., 1998	43(±30)
Terrene A (Nampula, Mozambique)	1997	30	Ernesto et al., 2000	13(±19)
Acordos de Lusaka	1997	30	Cardoso et al., 1998	67(±39)
Indonesia (East Java, Central Java, West Java and Lampung in Sumatra)	1996	59	Djazuli and Bradbury, 1999	22 samples of starch and 8 other products: 5(±4) 14 samples of cassava flour, 9 samples of cassava chips and six samples of gaplek: 54(±51)
Tanzania	-	-	Mlingi et al., 1992	131(±71)
Central African Republic	-	-	Tylleskar et al., 1992	32

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[1] sourced from: http://staff.science.nus.edu.sg/~dbsyhh/web%20cassava%20food%20safety%202.htm

Table 2: Cyanogenic potential of commercially processed cassava-based products prepared from cassava roots and flour

Generic name	Preparation	Cyanogenic potential (ppm)
Chilli tapioca	Root slices	2.8(±0.8)
Tapioca crisps	Root slices	3.2(±1.1)
Tapioca sticks	Root chips	4.5(±0.8)
Tapioca chips	Root slices	6.6(±2.7)
Tapioca crackers	Flour	1.5(±0.7)
Opak sambal	Flour	2.1(±0.5)
Tapioca crisps		4.1(±1.5)
Tapioca chips (BBQ)	Flour	4.6(±1.3)
Tapioca chips (seaweed)	Flour	5.3(±1.2)
Tapioca chips (chilli chicken)	Flour	6.1(±1.4)

Canned bamboo shoots

Ferreira et al. (1995) assessed the optimal cooking conditions for the reduction in the initial HCN levels. Bamboo shoots were cooked (one part bamboo shoots, 4 parts water) for 20, 100 and 180 minutes at 98°C/ambient pressure, 110°C/14.5 x 10^4 kPa, and 122°C/21.12 x 10^4 kPa. The shoots were then cooled in water, canned and sterilized. The maximum removal of HCN was about 97% leaving a residue level of about 27 mg/kg HCN in the canned sterilized product. The optimum conditions that resulted in this reduction of HCN were 98-102°C for 148-180 minutes.

Traditional preparation of cassava and bamboo shoots

Cassava

Cassava roots are highly perishable and deteriorate in air at ambient temperature in 3-4 days. In subsistence agriculture the plants are left in the ground until needed for food processing.

Cassava is consumed in a number of forms: flour used for cooking; root slices; root chips; baked grated root; steamed grated root; pan fried grated root; steamed whole root; and tapioca pearls made as a pudding. There are many traditional methods for the preparation of cassava used around the world and some of these are discussed below.

In East Africa either sun drying or heap fermentation are used. Sun drying involves peeling the roots, followed by drying the whole roots or large pieces cut longitudinally in the sun. The brittle dry material is then pounded in a wooden mortar and pestle and sieved to remove fibrous material, which produces white flour. Heap fermentation is more work intensive and time consuming. It involves peeling and cutting the roots and leaving them in a small heap for 3-5 days during which some fermentation takes place with liberation of hydrogen cyanide. The roots are then sun dried, pounded and sieved to produce white flour. Flour produced by heap fermentation is slightly dark coloured and contains only about one half of total cyanide content of the white flour produced by sun drying (Cardoso et al., 1998). In times of drought when the total cyanide content of roots is high, heap fermentation is preferred over sun drying (Ernesto et al., 2002).

In Western Africa a roasted product called *gari* is made. *Gari* is prepared by storing ground cassava in a bag for 2-3 days, then squeezing out excess water out in a press. The damp product is then roasted in a metal disch over a wood fire; this dries it and removes the hydrogen cyanide.

Cassava leaves are also an important part of the diet in countries such as Sierra Leone, Guinea, the Democratic Republic of Congo and the Central African Republic where they are consumed as a basic vegetable. For example, *mpondu* is prepared by grinding young cassava leaves, usually after blanching, and extensive boiling (Simons et al., 1980).

In the South Pacific including Papua New Guinea and Fiji, the introduced cassava varieties are virtually all sweet and, after peeling, the roots may be safely boiled and eaten. In some parts of the Pacific, cassava roots are placed in shallow pits surrounded by damp sawdust and the pit is then covered with soil, preserving the roots for several months[2].

In Indonesia, a product called gaplek is used which is the peeled dried cassava tuber (Djazuli and Bradbury, 1999).

Bamboo shoots

The following steps are typically taken in the preparation of bamboo shoots: (i) fresh bamboo shoots are cut in half lengthwise; (ii) the outer leaves are peeled away and any fibrous tissue at the base is trimmed; (iii) the bamboo shoot is thinly sliced into strips; (iv) the shoots are boiled in lightly salted water for 8-10 minutes.

In Thailand and Vietnam some shoots are finely grated and used in salads. In Japan, shoots are sometimes boiled whole for in excess of 2 hours. The most common preparation involves boiling the shoots in stocks, soups or salted water for use in assorted dishes.

REVIEW OF TOXICOLOGICAL DATA

Introduction

The potential toxicity of a food produced from a cyanogenic plant depends on the likelihood that its consumption will produce a concentration of HCN that is toxic to exposed animals or humans. The factors that are important in this toxicity are:

- The plant may not be sufficiently detoxified during processing or preparation and, therefore, HCN may remain in the food;

- If the plant is consumed raw or insufficiently processed, HCN may be released in the body, until the low pH of the stomach deactivates the β-glucosidase enzyme.

Cyanide ingested by release from a plant containing cyanogenic glycosides, either prior to or following consumption, follows the known cyanide metabolic pathway and toxicokinetics for animals and humans. Cyanide is detoxified by the enzyme rhodanase, forming thiocyanate, which is excreted in the urine. There are several factors influencing hydrolysis of cyanogenic

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[2] Cassava Leaflet No. 5, South Pacific Commission, 1995

glycosides including the confounding influence of nutritional status, particularly blood levels of riboflavin, vitamin B12, sodium and methionine. Because of this, human cases studies and epidemiological studies of the chronic toxicological effects have shown variable results and are rarely of a quantitative nature.

The following information is largely summarised from a WHO Report (1993), unless referenced otherwise. The WHO Report discusses the toxicity of cyanide and cyanogenic glycosides separately. The toxicity of cyanide is discussed first as a basis for understanding that of cyanogenic glycosides. This report summarises both cyanide and cyanogenic glycosides together.

Metabolism and toxicokinetics

Hydrogen cyanide after oral ingestion or administration is readily absorbed and rapidly distributed in the body through the blood. It is known to combine with iron in both methaemoglobin and haemoglobin present in erythrocytes. The cyanide level in different human tissues in a fatal case of HCN poisoning has been reported gastric content, 0.03; blood, 0.5; liver, 0.03; kidney, 0.11; brain, 0.07; and urine, 0.2 (mg/100g).

The major defence of the body to counter the toxic effects of cyanide is its conversion to thiocyanate mediated by the enzyme rhodanese (discovered by Lang, 1933). The enzyme contains an active disulfide group which reacts with the thiosulphate and cyanide. The enzyme is localized in the mitochondria in different tissues and is relatively abundant, but in sites, which are not readily accessible to thiosulphate, the limiting factor for the conversion of cyanide is thiosulphate. This detoxification requires sulphur donors, which by different metabolic pathways are provided from dietary sulphur amino acids (Bradbury and Holloway, 1988; Rosling, 1994). If the dietary intake of sulphur amino acids is adequate, the sulphur containing amino acids methionine and cysteine, which are not required for protein synthesis, are degraded to inorganic sulphate and excreted in the urine.

There are also several minor reactions that detoxify ingested cyanide. Firstly, cystine may react directly with the cyanide to form 2-imino-thiazolidine-4-carboxylic acid, which is excreted in the saliva and urine. Secondly, a minor amount may be converted into formic acid, which may be excreted in urine.

Thirdly, cyanide may combine with hydroxycobalamine (vitamin B12) to form cyanocobalamine, which is excreted in the urine and bile (it may be reabsorbed by the intrinsic factor mechanism in the ileum allowing effective recirculation of vitamin B12). Fourthly, methaemoglobin effectively competes with cytochrome oxidase for cyanide, and its formation from haemoglobin, effected by sodium nitrile or amylnitrite, is exploited in the treatment of cyanide intoxication.

Studies involving cassava

A part of ingested linamarin in cassava products has been found to pass through the human body unchanged and it is excreted in the urine within 24 hours in both humans (Brimer and Rosling, 1993; Carlsson et al., 1995; Hernandez et al., 1995) and rodents (Barrett et al., 1977). Remaining cyanohydrins are assumed to break down to cyanide in the alkaline environment of the gut (Tylleskar et al., 1992). Carlsson et al. (1999) investigated the metabolic fate of linamarin in cassava flour when consumed as a stiff porridge, which is the

most common staple food in southern Tanzania, and found that less than one-half of orally ingested linamarin is converted to cyanide and hence thiocyanate, about one-quarter is excreted unchanged and another quarter is metabolized into an as yet unknown compound.

Effects on enzymes and other biochemical parameters

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.

Cyanide can inhibit several other metalloenzymes most of which contain iron, copper or molybdenum (e.g. alkaline phosphatase) as well as enzymes containing Schiff base intermediates (e.g. 2-keto-4-hydroxyglutarate aldolase).

Hydrogen cyanide will reduce the energy availability in all cells, but its effect will be most immediate on the respiratory system and heart. The lethal dose for an adult depends on body weight and nutritional status and is somewhere between 30 and 210 mg of hydrogen cyanide. If the hydrogen cyanide exceeds the limit an individual is able to detoxify/tolerate, death may occur due to cyanide poisoning. Smaller, non-fatal amounts of cyanide cause acute intoxication with symptoms of rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting and diarrhoea.

Toxicological studies in animals

Acute toxicity studies

The lethal doses of HCN in mg/kg bw are generally reported to be between 0.66 (rabbit, i.v.; EPA, 1990) to 10-15 for various species (rat, oral; WHO, 1965), although much larger values have been reported for mouse (oral, 598 and i.v. 484; WHO, 1965). Lethal doses of HCN in mg/kg bw were reported for mouse, 3.7; dog, 4.0; cat, 2.0; and for cattle and sheep, 2.0 (summarised by Conn, 1979a).

The acute toxicity studies of linamarin (the cyanogenic glycoside present in cassava) are summarised in Table 3.

Table 3: Acute toxicity studies of linamarin

Species	Route	LD_{50} (mg/kg bw)	References
Rat	i.v.	20 000 linamarin	Oke, 1979
Rat	oral	450 linamarin	Oke, 1979

The following studies were also described in the WHO Report (1993):

A dose of 25 mg linamarin (250 mg/kg bw) fed to rats (100-120 g bw) caused clinical signs of toxicity, including apnoea, ataxia and paresis. In the absence of methionine supplementation, 50% of these rats died within 4 hours. With adequate methionine

supplementation, 10% of rats died and about 40% showed no signs of toxicity (reviewed by Oke, 1980).

In a toxicokinetic study 7 out of 10 rats (100 g bw) died after administration of 50 mg linamarin by stomach tube (Barrett et al., 1977).

Oral doses of 100, 120 and 140 mg linamarin/kg bw given by stomach tube to hamsters produced signs of cyanide intoxication in a large percentage. Two animals dosed with 140 mg/kg bw and one animal dosed with 120 mg/kg bw died within 2 hours of dosing (Frakes et al., 1985).

Short-term toxicity studies

Albino female rats fed a raw cassava diet showed: a decrease in haemoglobin, PCV, total serum protein concentration and T_4 concentration; and an increase in serum thiocyanate levels compared with a control group.

One-day old broiler chickens were fed a diet containing 0, 10, 20 or 30% cassava. No changes in haematological parameters due to cassava were seen, however, decreased weight gain, decreased feed efficiency and increased blood serum thiocyanate concentrations were associated with cassava feeding.

Reproduction studies, embryotoxicity and teratogenicity studies

A short-term reproductive study in rats (49 day study in adults and 28 day study in pups) was performed to evaluate the cumulative effects of adding 500 mg KCN/kg to cassava root flour-based diet in pregnant rats. High dietary level of cyanide did not have any marked effect on gestation or lactation performance. The high cyanide-containing diet, however, significantly reduced feed consumption and daily growth weight of the offspring when fed during the post-weaning period. Protein efficiency rate was not only reduced by the cyanide diet during post-weaning growth phase but there was an additional 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.

Pregnant golden hamsters exposed to sodium cyanide (0.126-0.1295 mmol/kg/hour) on days 6-9 of gestation, had a high incidence of resorptions and malformations in offspring, the most commonly observed being neural tube defects.

A study on albino female rats fed a 50% gari diet (Nigerian preparation of cassava), a raw cassava diet, a diet containing added KCN or a control diet investigated effects on reproduction. 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 females fed the raw cassava diet had a significant reduction in the frequency of pregnancy, the average number of pups per litter and birth weights among these pups. In addition, there was an increased incidence of neonatal deaths among the offspring that also had poor development, reduced brain weights and an increased tendency of aggression towards their littermates. The female rats that received additional KCN in their diet never became pregnant and survived no more than 3 months.

Reproductive effects were studied for cassava in combination with added cyanide in a 110-day feeding experiment with 18 pregnant pigs (Yorkshire gilts). Cyanide fed during gestation did not affect performance during lactation. Milk thiocyanate and colostrums iodine concentrations were significantly higher in the group fed additional cyanide. No effects of cyanide were reported on indices of reproduction performance (Tewe and Maner, 1981a).

A dose of 120 or 140 mg linamarin/kg bw in pregnant hamsters was associated with an increased incidence of vertebral and rib abnormalities as well as the production of encephaloceles in the offspring. Linamarin feeding had no effect on fetal body weight, ossification of skeletons, embryonic mortality, or litter size.

The offspring of pregnant hamsters fed cassava meals showed evidence of: fetotoxicity; increased tissue thiocyanate concentrations; reduced fetal body weight; and reduced ossification of sacrocaudal vertebrae, metatarsals and sternebrae. There were an increased number of runts compared to litters of hamsters fed control diets.

Studies on the thyroid gland

A group of 10 male rats were fed a diet containing potassium cyanide (1500 mg/kg feed) for almost one year. Compared with a control group not receiving cyanide, there was depression of body weight, plasma thyroxine and thyroxine secretion rate suggestive of depressed thyroid function. At autopsy, the animals were found to have enlarged thyroids.

In a study cited by Oke (1980), the influence of a 100% cassava diet on the thyroid gland was investigated in a 7-day experiment with rats. A significant decrease in glandular stores of stable iodine, significantly higher thyroid weight and higher thyroidal ^{131}I uptake were observed. Each effect was attributed to a synthetic block in the conversion of monoiodothyronine to diiodothyronine.

Genotoxicity

The genotoxicity studies that have been performed on *Salmonella* and *Bacillus* strains and hamsters cells have been negative with the exception of one marginally positive study (*Salmonella typhimurium* strain TA100).

An *in vivo* mutagenicity study was conducted in Chinese hamsters to detect chromosomal aberrations following oral administration of cyanide. There was no indication of mutagenic properties relative to structural chromatid or chromosome damage (Leuschner et al., 1983b).

Studies on the nervous system

A special study on the behavioural effects of chronic, sub-lethal dietary cyanide in juvenile swine mimicked the situation of free cyanide intake in Liberia from eating cassava-based foods. There were two clear behavioural trends: 1) increasing ambivalence and slower response time in reacting to various stimuli; and 2) an energy conservation gradient influencing which specific behaviours would be modified in treated animals.

Neuronal lesions including myelin damage in several animal species have been produced by chronic cyanide. The neuropathological changes include areas of focal necrosis especially

around the centrum ovale, corpus striatum, corpus callosum, substantia nigra, anterior horn cells, and patchy demyelination in the periven-tircular region.

Observations in humans

Acute effects

Symptoms of acute cyanide intoxication include: rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhoea, mental confusion, twitching and convulsions. If the hydrogen cyanide exceeds the limit an individual is able to detoxify/tolerate, death may occur due to cyanide poisoning.

The acute oral lethal dose of hydrogen cyanide for human beings is reported to be 0.5-3.5 mg/kg bodyweight. Approximately 50-60 mg of free cyanide from cassava and its processed products constitutes a lethal dose for an adult man. Data on the oral lethal dose of cyanide for man in four cases of suicide, calculated from the amount of hydrogen cyanide absorbed in the body at the time of death, and from the amount of hydrogen cyanide found in the digestive tract, differed considerably and corresponded to doses of 0.58-22 mg/kg bw (WHO, 1965).

Long-term studies and cyanide diseases

Konzo

'Konzo' is a local Zairean term for a disease first described in 1938 in the Democratic Republic of Congo (formally Zaire) by Trolli (1938), but has also been observed in Mozambique, Tanzania, Central African Republic and Cameroon (Ministry of Health, Mozambique, 1984; Howlett et al., 1990; Tylleskar et al., 1992, 1994; Lantrum et al, 1988; Ernesto et al., 2002). Konzo is an upper motor neuron disease characterised by irreversible but non-progressive symmetric spastic paraparesis that has an abrupt onset. It mostly affects children and women of childbearing age. Severe cases have a spastic toe-scissor gait, or patients will not be able to walk at all, and the arms and speech may also be affected. A long-term follow-up of konzo patients showed that the neurological signs in konzo patients remained constant, however, functional improvement may occur (Cliff and Nicala, 1997). High urinary thiocyanate concentrations and presence of ankle clonus are also observed.

In all reports of epidemics, konzo has been associated with high and sustained cyanogens intake at sub-lethal concentrations from cassava or cassava flour in combination with a low intake of sulphur amino acids.

Tropical Ataxic Neuropathy (TAN)

TAN is used to describe several neurological syndromes attributed to toxico-nutritional causes. The syndromes grouped as TAN can differ widely in clinical presentation, natural history and response to treatment. TAN has occurred mainly in Africa, particularly Nigeria. The main clinical features of some of the syndromes have included: sore tongue, angular stomatitis, skin desquamations, optical atrophy, neurosensory deafness and sensory gait ataxia (in Oluwole et al, 2000). The cause is attributed to dietary cyanide exposure from the chronic monotonous consumption of foods processed from cassava. The onset of TAN is

usually slow over months or years and the mean age of people affected by TAN is greater than 40 years. TAN affects males and females in all age groups equally.

Goitre and cretinism

Studies in African countries such as Zaire have established that goitre and cretinism due to iodine deficiency can be considerably aggravated by a continuous dietary cyanide exposure from insufficiently processed cassava. This effect is caused by thiocyanate, which is similar in size to the iodine molecule and interferes with uptake of iodine into the thyroid gland. High thiocyanate levels, which can occur after exposure to cyanide from cassava, can only affect the gland when the iodine intake is below 100 micrograms/day, which is regarded minimal for normal function. Populations with very low iodine intake and high thiocyanate levels from consumption of cassava, show severe endemic goitre, but this decreases with iodine supplementation (reviewed by Rosling, 1987).

NUTRITION ASSESSMENT

Nutritional composition

The cassava tuber has a high carbohydrate content, but is not a good source of protein. It contains vitamin C, potassium and dietary fibre. Cassava leaves have higher protein content, contain vitamin C and vitamin A and provide some dietary fibre (Leaflet No. 5 – Cassava, South Pacific Commission).

Bamboo shoots are a good source of thiamine, vitamin B6 and potassium. However, the process of canning greatly reduces the vitamin content. One cup of cooked bamboo shoots provides approximately 14 calories, 0.3 g fat, 1.2 g fibre, 1.8 g of protein and 640 mg potassium[3].

Role of sulphur-containing amino acids in cyanide detoxification

Dietary cyanide exposure from cyanogenic glycosides in insufficiently processed cassava has been implicated as a contributing factor in growth retardation. In the human body, cyanide is detoxified mainly by enzymatic conversion to the much less toxic thiocyanate (SCN). This detoxification requires sulphur donors, which are provided from sulphur-containing dietary amino acids, cysteine and methionine (Bradbury and Holloway, 1988; Rosling, 1994).

In subjects who have an adequate protein component of their diet, excess cysteine and methionine are not required for protein synthesis and are degraded to inorganic sulphate and excreted. Where dietary intake of protein is inadequate, the preferential use of metabolically available sulphur-containing amino acids for cyanide detoxification is also believed to hamper protein synthesis and hence contribute to growth retardation in children exposed to dietary cyanide from cassava.

A deficit in height-for-age index, otherwise referred to as 'stunting' was associated with children who consumed inadequately processed cassava, however, weight-for-height and weight-for-age indices were not significantly different from children who consumed cassava

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[3] Sourced from http://www.wholehealthmd.com/print/view/1,1560,FO_233,00.html

which was adequately processed (Banea-Mayambu et al., 2000). This indicates that because of the preferential use of sulphur amino acids for cyanide detoxification in the human body, dietary cyanide exposure may be a factor aggravating growth retardation.

Iodine deficiency diseases

Iodine deficiency diseases, which include goitre (enlargement of the thyroid gland) and, in its most severe form, cretinism (shortness of stature and severe mental impairment) are due to a low dietary intake of iodine, and are exacerbated by intake of cyanogenic plants such as cassava (Ermans et al., 1983; Simons et al., 1980). Ingested cyanide from cyanogenic plants is converted in the body to thiocyanate, which is removed in the urine. The thiocyanate inhibits the uptake of iodine by the thyroid gland.

DIETARY EXPOSURE ASSESSMENT

The extent of consumption of both cassava and bamboo shoots in Australia and New Zealand is assessed to be relatively small although there are few recent and reliable statistics available. Most of the information available is specific to cassava.

National Nutrition Survey information

Of the respondents in the Australian National Nutrition Survey 1995 there were two consumers of cassava. In the New Zealand National Nutrition Survey 1997 there were nine consumers of cassava. In these surveys cassava was described as 'cooked', 'boiled' or 'baked'. No cassava flour or cassava chips were recorded as eaten. The Australian National Nutrition Survey recorded 36 consumers of bamboo shoots (either 'cooked'/'canned' or 'raw') and the New Zealand National Nutrition Survey recorded one consumer of cooked bamboo shoots. The dietary consumption data for cassava and bamboo shoots in Australia and New Zealand is summarised in Tables 3 and 4.

Table 3. Dietary consumption of cassava and bamboo shoots for Australia and New Zealand from National Nutrition Survey data

Food	Number of Consumers	Consumers as a % of total respondents (#)	Mean Consumption g/day
Cassava Boiled (New Zealand)	8	2.6	287.85
Cassava, Baked (New Zealand)	1	0.3	145.15
Cassava, Cooked (Australia)	2	0.01	114.96
Bamboo Shoots, Cooked or Canned (Australia)	31	0.2	35.10
Bamboo Shoots, Raw (Australia)	5	0.04	31.27
Bamboo, Cooked (New Zealand)	1	0.02	70.00

Total number of respondents for Australia whole population aged 2 – 100 years = 13 858, Total number of
respondents for New Zealand 15 years and above = 4 636 and total number of respondents for New Zealand Pacific
Islanders 15 years and above = 307.

- All New Zealand consumers of cassava were Pacific Islanders aged between 48 – 80 years, 8 female and 1 male consumer.

- All Australian consumers of cassava were aged between 6 (female) – 44 (male) years.

- Australian consumers of Bamboo shoot included 21 females and 10 males. The youngest consumer of bamboo shoots was a 3 year old female. Table 2 gives a more detailed breakdown of the consumer groups who recorded eating bamboo shoots in the Australian National Nutrition Survey 1995.

Table 4: Dietary consumption data for bamboo shoots in Australia

Food	Consumer group	Number of Consumers	Mean Consumption g/day
Bamboo Shoot, Raw	2-12 yrs	1(3yrs)	42.33
	25 – 64 yrs	4	28.51
Bamboo Shoot, Cooked or Canned	2-12 yrs	2	68.24
	13-24 yrs	5	32.74
	25-64 yrs	22	36.31
	65+ yrs	2	5.67

Availability of cassava and bamboo shoots in Australia and New Zealand

Availability of cassava in New Zealand

Information on the availability of cassava in New Zealand has been obtained from the South Pacific Trade Commission, New Zealand. Cassava is the second major root crop imported into New Zealand, behind taro and ahead of yam. The New Zealand market for tropical root crops is largely confined to the Polynesian population situated in south and west Auckland and in the Wellington suburb of Porirua. There is also a growing market among the Asian population. In 1999, 1400 tonnes of cassava were imported into New Zealand. The fresh imports came mainly from Fiji (approximately 2/3) and Tonga (approximately 1/3). The varieties of cassava grown in the Pacific Island countries have low cyanide contents. About 55% of the tropical root crops imported are sold to specialist fruit and vegetable retailers and large supermarkets.

Cassava is usually available all year round. Some frozen cassava identified in retail outlets in New Zealand reportedly included preparation instructions.

Availability of cassava in Australia

The availability of cassava in Australia is limited and is mostly sold through greengrocers.

The majority of cassava imported into Australia comes from Pacific Island countries (personal communication from Dr Howard Bradbury, Australian National University, June 2003). Cassava chips (e.g. 'Vegechips') made from cassava and/or cassava flour are available from supermarkets, service stations and other retail outlets in Australia, however, this is a processed product and there would be little, if any cyanide present. Tapioca pearls, derived from cassava and consumed as tapioca pudding, is available in supermarkets in both Australia and New Zealand. Like cassava chips, tapioca pearls are a processed product which has a long history of safe consumption.

Availability of bamboo shoots in Australia

The Australian Commercial Bamboo Corporation Ltd (ACBC) sold fresh bamboo shoots for the first time on the Australian domestic market during 2001. The total sale of bamboo shoots through the Sydney and Melbourne markets was 21,850 kg and, of that, 16,630 kg was reportedly produce of the ACBC. Other bamboo shoots sold originate from small operators or plantation owners.

RISK CHARACTERISATION

Cassava and bamboo shoots contain cyanogenic glycosides that break down to produce hydrogen cyanide, which can cause both acute and chronic toxicity in humans. The symptoms of acute cyanide intoxication include rapid respiration, drop in blood pressure, rapid pulse, dizziness, headache, stomach pains, vomiting, diarrhoea, mental confusion, twitching and convulsions. In extreme cases, death due to cyanide poisoning may occur. The chronic effects of cyanide intoxication are linked to regular long-term consumption in individuals with poor nutrition and are unlikely to be a significant concern in Australia and New Zealand.

By adequate processing (peeling, slicing and cooking), both the cyanogenic glycosides and hydrogen cyanide can be removed or reduced prior to consumption, thus significantly reducing the potential health risk. Sweet varieties of cassava (low cyanide content) can be processed adequately by peeling and then fully cooking (e.g. roasting, baking or boiling). Bitter varieties of cassava (high cyanide content) require further processing, involving techniques such as heap fermentation. Currently the commercial trade is only in sweet cassava. The varieties of bamboo shoots sold commercially for food are processed adequately by boiling before consumption.

On the basis of information on cassava and bamboo products currently available in Australia and New Zealand and the levels of consumption, the likelihood of cyanide intoxication from consumption of cassava or bamboo shoots is low, but due care in preparation remains necessary. While the current users have adequate knowledge regarding the risks associated with consumption of cassava and bamboo shoots, more widespread use in the community would increase the public health risks.

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