

CYANOGENIC GLYCOSIDES IN CASSAVA CHIPS

Risk Assessment

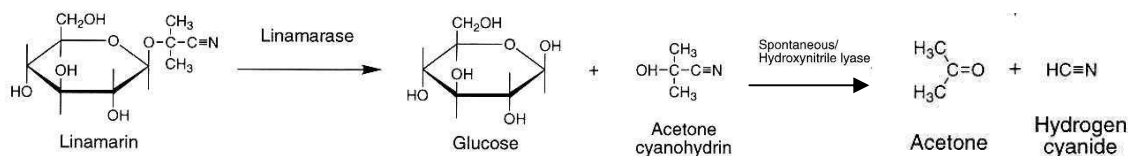
Introduction

Cassava (*Manihot esculanta* Crantz) is grown for its enlarged starch-filled roots and is consumed in a number of forms: flour used for cooking; root slices; root chips; grated root (pan fried, baked or steamed), steamed whole root or tapioca pearls made as a pudding. The focus of these risk assessment considerations is cassava chips made from the root.

Cassava contains potentially toxic compounds called cyanogenic glycosides, primarily linamarin and a small amount of lotaustralin (ethyl linamarin). Linamarin is chemically similar to glucose but with cyanide (CN ion) attached. Cyanogenic glycosides are toxic because they release hydrogen cyanide (HCN) as a result of enzymatic hydrolysis following maceration of the plant tissue. Safe traditional human consumption of cassava is dependent on adequate processing to minimise the linamarin content. If cassava is eaten either raw or after inadequate processing then toxicity may be observed.

Conventional processing usually involves peeling and grating and then soaking in warm water for several days. This allows the cassava's own natural enzymes to convert the linamarin to glucose and hydrogen cyanide gas. The released hydrogen cyanide gas disperses slowly and harmlessly.

The chemical reaction for the formation of hydrogen cyanide gas from linamarin in cassava is shown below (Figure 1).



Cyanogenic glycoside content of cassava

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. Cassava varieties that have high levels of linamarin in the root are not normally traded as food.

Cyanogenic glycoside content of processed products

In 2004, FSANZ identified information on the cyanogenic potential of cassava flour commercially processed cassava chips prepared from root slices and flour. Cassava flour samples obtained from various African countries and Indonesia had a total cyanide content varying from 13 – 131 mg/kg. It is understood that most of the chip samples were available in the United States and all samples had a total cyanide content of less than 10 mg/kg.

In 2008, FSANZ was notified of cassava chips manufactured in Australia from cassava pellets imported from Indonesia having a total cyanide content of 59 mg/kg. Follow up analysis on more batches has revealed even higher quantities (up to 145 mg/kg)

Risk assessment considerations

Both acute and chronic effects occur following exposure to cyanogenic glycoside compounds in cassava based foods. These risk assessment considerations are focussed on:

- estimating the likelihood of acute toxicity as a result of consumption of cassava chips; and
- establishing a guidance level for HCN in cassava chips.

Review of toxicological data

The potential toxicity of cassava chips depends on the likelihood that its consumption will produce a concentration of HCN that is toxic to exposed humans. The factors important in this toxicity are that the cassava may not have been sufficiently detoxified during processing or preparation and, therefore, high concentrations of HCN or linamarin are present. If cassava is consumed raw or insufficiently processed, HCN will be released for absorption by the action of β -glucosidase in the gastrointestinal tract.

Absorption, distribution, metabolism and excretion

Cyanides

Following oral administration, soluble cyanide salts, at the physiological pH of the stomach, form predominantly HCN, which can rapidly penetrate mucous, and cell membranes. Absorbed cyanide is converted in the liver to less toxic metabolites such as thiocyanate. Cyanide has not been shown to accumulate in the blood and tissues following chronic oral exposure.

The metabolism of cyanide in the liver has been studied in animals. The major pathway involves conversion to thiocyanate by rhodanese or 3-mercaptopyruvate sulfur transferase. This major route of detoxification requires sulphur donors, which by different metabolic pathways are provided from dietary sulphur 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 sulfur-containing amino acids and vitamin B12.

Linamarin

Since release of cyanide from linamarin only occurs in the GI tract through microbial fermentation the concentration of cyanide being available for absorption occurs only slowly. This slow rate of absorption allows for more detoxification relative to an equivalent dose of cyanide. This difference in release and detoxification is thought to account for the marked difference in acute toxicity between equivalent doses of cyanide in linamarin and a cyanide salt (see under acute toxicity).

In well nourished humans and rodents approximately 25% of linamarin present in ingested cassava flour is excreted unchanged in urine. Thiocyanate excretion in the urine suggests that a further 10-20% of the linamarin is enzymatically converted in the gastrointestinal tract to cyanide. It is not known how much of the HCN will be absorbed from the gut, but is expected to be less than 100% due to localised detoxification mechanisms. Studies in isolated perfused rat liver have shown that cyanoglycosides require gut microbial flora for their metabolism and that they are not metabolized by mammalian cells.

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

Cyanides

In humans, the clinical signs of acute cyanide intoxication are constriction of the throat, nausea, vomiting, stomach pains, giddiness, headache, palpitations, hyperpnoea and dyspnoea, bradycardia, unconsciousness and violent convulsions followed by terminal coma. Death due to cyanide poisoning occurs when the quantity of ingested cyanide exceeds the ability to detoxify it.

In humans an average fatal dose of cyanide has been calculated from case report studies of intentional or accidental poisonings to be 1.52 mg/kg bw (range 0.56-3.4 mg/kg bw and corresponding to 1-7 mg/kg bw of KCN). The lowest fatal oral dose reported in humans was estimated in 1938 as being 0.56 mg/kg bw cyanide (form not specified) but the analytical measurements of the time lack the precision of current technology.

Laboratory animals such as mouse, rat, rabbit and dog appear to be similarly sensitive. The median oral lethal doses (50% death) for sodium cyanide was calculated to be 3-4 mg CN/kg bw in rats and rabbits. In dogs the median lethal dose was 2 mg CN/kg bw whereas in mice it was 6 mg CN/kg bw with potassium cyanide.

Linamarin

In rats the median lethal dose of linamarin was reported to be 450 mg/kg bw but clinical signs (ataxia, apnoea and paraparesis) and a death were observed at a dose as low as 250 mg/kg bw. This suggests that like classical cyanide intoxication the dose which will result in clinical signs is not much different from that which also causes death.

In hamsters a single oral dose of 120 or 140 mg/kg bw linamarin (by stomach tube) caused deaths and clinical signs (deep and laboured breathing, uncoordinated movement, tremors and

hypothermia) were observed in most animals after one hour. No deaths or clinical signs were observed at either 70 or 100 mg/kg bw.

There are no available data on the acute toxicity of linamarin in humans. However, the likelihood of cyanide intoxication from consumption of linamarin in cassava 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.

Long-term toxicity

There have been no adequately reported long term controlled studies in laboratory animals with HCN or linamarin. However, adverse effects noted in humans from long-term cassava consumption include neurological diseases. 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. 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. This situation is not expected to occur in Australia.

Tropical Ataxic Neuropathy is used to describe several neurological syndromes attributed to toxico-nutritional causes. 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. The cause is attributed to dietary cyanide exposure from the chronic monotonous consumption of foods processed from cassava. As cassava products are not a major component of the diet, this situation is not expected to occur in Australia.

Because of the nature of the toxicity of cyanide compounds, no suitable data on subchronic oral exposure to cyanide in humans is available. Reported cases of survival after oral exposure to cyanide typically involve individuals who ingested cyanide in lethal dose range but who received emergency supportive medical treatment.

International Risk Assessments

In July 2006, the Agency for Toxic Substances and Disease Registry (ATSDR), US Department of Health and Human Services, published a comprehensive risk assessment for cyanide. It cites a 1993 NTP study in which rats were dosed with cyanide in their drinking water for 3 months. It shows a no effect level of 5 mg/kg bw/day for male fertility. Based on this study, a minimum risk level for an intermediate duration (15-364 days) oral exposure to cyanide is 0.05 mg/kg bw/day which represents an exposure of 3 mg/day cyanide for a 60 kg adult, or 1 mg/kg/day for a 20 kg child.

The US EPA oral reference dose for chronic exposure to cyanide is 0.02 mg/kg bw/day (from drinking water). 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 along with a modifying factor of 5. The modifying factor is employed because cyanide has a higher tolerance when ingested with food than by drinking water. The NOAEL was based on a 2-year dietary study in rats completed in 1955 where the critical effects associated with the lowest observed adverse effect level (LOAEL) were weight

loss, thyroid effects and myelin degeneration. For a 60 kg adult, this equates to 1.2 mg. However, the tolerable daily intake associated with food would not include this modifying factor. The tolerable daily intake associated with food consumption would be 0.108 mg/kg bw/day (10.8 mg/kg bw/day, divided by a 100-fold safety factor).

No safe level of intake of cyanide derived from linamarin in cassava and other plant-based foods has been established. JECFA in 1993 and EFSA in 2004 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/kg HCN in the Codex Standard for cassava flour was not associated with acute toxicity.

The Australian and WHO Drinking Water Guideline levels are based on an effect level (LOAEL) of 1.2 mg/kg bw/day from a 6-month feeding study in pigs. The effects observed at 1.2 mg/kg bw/day were on behavioural patterns and serum biochemistry. Using the LOAEL from this study and applying a safety factor of 100 to reflect inter- and intra-species variation (no additional factor for a LOAEL was considered necessary because of doubts over the biological significance of the observed changes); a tolerable daily intake (TDI) of 0.012 mg/kg bw/day was calculated. 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 results in a guideline value of 0.08 mg/L (rounded figure but 0.07 mg/L for WHO) which is considered to be protective for both acute and long-term exposure.

Regulatory limits on HCN in food or water

Food

In the Australia New Zealand Food Standards Code (the Code) the following limits apply:

- 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.

In the EU the maximum permitted levels of hydrocyanic acid (HCN) are as follows; 1 mg/kg in foodstuffs, 1 mg/kg in beverages, with the exception of 50 mg/kg in nougat, marzipan or its substitutes or similar products, 1 mg per percent of alcohol in alcoholic beverages and 5 mg/kg in canned stone fruit.

Water

The Australian Drinking Water Guidelines has a guideline level of cyanide in drinking water of 0.08 mg/L. The WHO drinking water guideline for cyanide is 0.07 mg/L.

Risk/safety assessment

Source (cassava chips) – HCN content measured by NSW

Based on recent measurements the cassava-derived crackers contain linamarin which when exposed to appropriate enzymes released approximately 80 mg/kg of HCN. Since about 20% of the HCN released from linamarin in cassava in the GI tract is absorbed it follows that 200 g of cassava chips will yield approximately 3 mg HCN as a systemic dose.

Furthermore since one gram of linamarin can release a maximum of 109.3 mg HCN it follows that the chips contain a maximum of approximately 800 mg/kg linamarin. So in 200 g of cassava chips there will be 160 mg of linamarin.

Acute toxicity

1. Humans – lethal dose (HCN)

A lethal oral dose of HCN is reported to be about 1.52 mg/kg bw (range 0.56-3.4 mg/kg bw). These data are not directly comparable because the HCN released from cassava is likely to be slow which, in turn, would permit detoxification in the liver. These data are included for comparative purposes.

2. Rat – median lethal dose (LD₅₀)

Linamarin – 450 mg/kg bw

HCN – 4-6 mg/kg bw

Hamster – A single oral dose study with 100 mg/kg bw of linamarin (~2 mg/kg bw available HCN) caused no deaths or clinical signs. Death and clinical signs observed at 120 and 140 mg/kg bw.

Estimated Dietary exposure

Two hundred grams of chips (2 packets) is considered to be a reasonable and possible dietary intake for a 20 kg child in a two hour eating session. Hence 200 g of chips eaten by 20 kg child equates to 160 mg (8 mg/kg bw) linamarin (or 0.15 mg/kg bw available HCN).

Margin of Safety

Exposure “dose”: is 160 mg/kg bw linamarin (or 0.15 mg/kg bw available HCN) in children.
Toxicity: the lethal dose in rats is 450 mg/kg bw linamarin (or 9 mg/kg bw available HCN in humans).

In hamsters the NOAEL was 100 mg/kg bw linamarin (or ~2 mg/kg bw available HCN).

The safety factor applied to the NOAEL in the single dose study in the hamster is 10-fold rather than the usual 100-fold because humans appear to be approximately as sensitive to HCN as laboratory animals as judged from the acute toxicity data. Thus we do not need to account for variability in extrapolation from laboratory animals to humans. The applied 10-fold safety is intended to account for individual variability in sensitivity within the population.

The margin of safety (MOS) is calculated by comparing the likely exposure of 8 mg/kg bw linamarin (or 0.15 mg/kg bw available HCN) in children to the safety factor corrected value in hamsters of 10 mg/kg bw linamarin (or 0.2 mg/kg bw available HCN). Hence the MOS is around 10-fold. A MOS of 10 is considered to be acceptable to ensure that individuals sensitive to the effects of HCN are adequately protected.

Conclusion: The consumption of 200 g of cassava chips in a 20 kg child is without apparent toxic consequence (i.e. the required margin of safety of 10 was achieved. However, if the content of HCN in the cassava chips is greater than 80 mg/kg or other sources of cyanide (eg. marzipan or stone fruit juice) are consumed within 1-2 hours then it may be possible to take children over a safe level. The most recent testing undertaken by the NSW Food Authority indicates that some HCN levels in cassava chips may be as high as 145 mg/kg.

Establishing a guidance level for HCN in cassava chips

Using existing regulatory limits

The Code contains regulatory limits for HCN in certain processed foods: 25 mg/kg in confectionery; 5 mg/kg in stone fruit juice; 50 mg/kg in marzipan; and 1 mg/kg in alcoholic beverages per 1% alcohol content.

FSANZ has conducted a dietary exposure assessment to determine the consumption of chip products relative to confectionery. This was conducted to test the assumption that confectionery consumption may be similar to the consumption of chips. It was proposed that if chip consumption was similar to that of confectionery consumption, the ML of 25 mg/kg in the Code for confectionery may also provide an appropriate level of protection when used as a guidance level for cassava chips.

Comparing reference health standards from long-term safety studies with existing regulatory limits

If a guidance level for cassava chips of 25 mg/kg HCN was applied, then the safety margin would increase to around 30-fold for a 20 kg child. This level could probably be achieved through appropriate quality control measures during cassava processing.

References:

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